Acta Medica Hungarica

VOLUME 48, NUMBERS 1–2, 1991

Akadémiai Kiadó, Budapest

ACTA MED. HUNG. 48 (1–2) 1–127 (1991) HU ISSN 0236–5286

ACTA MEDICA HUNGARICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Medica publishes reviews and original papers on clinical and experimental medicine in English.

Acta Medica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences
H-1117 Budapest, Prielle K. u. 19—35

Manuscripts and editorial correspondence should be addressed to the Managing Editor:

Dr. Miklós Papp

Acta Medica
H-1083 Budapest, Szigony u. 43 or H-1450 Budapest 9, P.O. Box 67

Subscription information

Orders should be addressed to

AKADÉMIAI KIADÓ
H–1519 Budapest, P.O. Box 245

"This periodical is included in the document delivery program THE GENUINE ARTICLE of the Institute of Scientific Information, Philadelphia. The articles published in the periodical are available through *The Genuine Article* at the Institute for Scientific Information, 3501 Market Street, Philadelphia PA 19104."

Acta Medica Hungarica is abstracted/indexed in Abstracts of World Medicine Abstracts, Chemical Abstracts, Current Contents–Clinical Medicine, Excerpta Medica, Index Medicus, International Abstracts of Biological Sciences

Acta Medica Hungarica

VOLUME 48

1991

AKADÉMIAI KIADÓ, BUDAPEST 1991

ACTA MEDICA
Volume 48
1991

Numbers 1—2

Numbers 3—4

GASTROENTEROLOGY

METABOLISM

THEMES

AUTHOR INDEX

SUBJECT INDEX

CONTENTS

Acta Medica Hungarica, Vol. 48/1-2, pp. 3-11 (1991)

IMMUNOLOGY

SOLUBLE INTERLEUKIN-2 RECEPTOR IN SERA OF PATIENTS WITH GRAVES' DISEASE

CS. BALÁZS, N.R. FARID

III. Department of Medicine, Teaching Hospital, Debrecen, Hungary and
Thyroid Research Laboratory, Health Science Centre,
St. John's Hospital, Newfoundland, Canada

(Received: December 20, 1990)

Recently, in vitro production of interleukin-2 receptor induced by mitogens have been shown to be impaired in autoimmune disorders including organo-specific autoimmune diseases. The aim of this study was to investigate serum levels of soluble interleukin-2 receptor in 20 untreated patients with Graves' disease and to follow up their changes in relation to clinical picture and TSH-receptor-, anti-thyroglobulin-, anti-microsomal as well as anti-eye muscle antibodies. Soluble interleukin-2 receptor level was significantly increased in newly-diagnosed Graves' patients compared to controls (667±270 vs. 205±45 U/ml) ($P < 0.001$). Among the patients sera those with active infiltrative ophthalmopathy had higher soluble interleukin-2 receptor levels than those without eye symptoms (810±313 vs. 525±180 U/ml). Soluble interleukin-2 receptor level was normalized in Methimazole-treatment-induced remission in the majority of patients except those with ophthalopathy. In five patients the soluble interleukin-2 receptor levels were studied after interruption of thyrostatic therapy; an increase was observed in three patients; thereafter hyperthyrosis relapsed in two cases. Furthermore, a correlation was found between soluble interleukin-2 receptor levels and TSH-receptor antibodies, however, the association with other immune parameters examined was not significant. In conclusion, an enhanced level of soluble interleukin-2 receptor was detected in patients with untreated Graves' disease. This finding might play a significant role in regulation of impaired cell-mediated immune mechanism and has a prognostic value for relapse of autoreactive processes.

Keywords: Graves' disease, soluble interleukin-2 receptor, TSH-receptor antibodies, Graves' ophthalmopathy, antimicrosomal antibodies, anti-thyroglobulin antibodies, anti-eye muscle antibodies.

Offprint requests should be sent to: Cs. Balázs, III. Department of Medicine, Teaching Hospital, H-4043 Debrecen, Bartók B. u. 4, Hungary

Akadémiai Kiadó, Budapest

Introduction

The importance of autoimmunity in the pathogenesis of Graves' disease has been well established, and a dysfunction in both humoral and cellular immunity has been extensively reviewed /26, 28/. TSH receptor (TSH-R) antibodies have been shown to be involved in induction and perpetuation of thyrotoxicosis and have presented diagnostic as well as predictive values /17/. Despite the indisputable the importance of TSH-R antibodies, the concept of imbalance of immunoregulatory T lymphocytes as primary aetiologic factor in the pathogenesis of this disease has been assumed /12/. Reduction of TSH-R-specific T suppressor activity and activation of T lymphocytes in both the periferal and thyroid gland have been published /2, 7, 11/. Recent advances in immunology have provided evidence for a role of cytokines in cell-mediated immunity. Interleukin-1 (IL-1) produced by macrophages and various other cells, including thyrocytes, might play an important role in activation of autoreactive clone(s) in organ-specific autoimmune diseases /8, 13/. Furthermore, it has become clear that the key factors in activation processes are interleukin-2 (IL-2) and the expression of IL-2 receptor on the T cell surface /23/. IL-2 production is confined to CD4 T cells and it exerts pleiotropic effect via an up-regulated specific receptor. The interaction of IL-2 with IL-2 receptor (IL-2R) having been assumed to be involved in the pathogenesis of autoimmune diseases, has attracted the attention of immunologists /9/. Conflicting data have been published on production of IL-2 by mitogen-induced peripheral mononuclear cells and on the density of IL-2R on the surface of lymphocytes in various autoimmune disorders /18, 19/. Recently, IL-2 in vitro production and IL-2-R expression have been shown to be impaired in Graves' disease and insulin-dependent diabetes mellitus /4, 6/. On the contrary, other authors have found enhanced levels of soluble IL-2R (sIL-2R) in supernatant of activated mononuclear cells as well as sera of patients with thyrotoxicosis /3, 10, 14/.

This study was undertaken to determine whether sIL-2R could be changed due to impaired T cell function in Graves' patients in relation to thyroid function, immunological parameters and Methimazole therapy.

Patients and Methods

Subjects: twenty patients (19 female and 1 male) with untreated hyperthyroid Graves' disease (aged 23-54 years, on the average 44.1 years) were diagnosed. All patients presented with cardinal features of Graves' hyperthyroidism, seven had infiltrative ophthalmopathy according ATA (American Thyroid Association) criteria. Thyrotoxicosis was supported by a high level triiodothyronine (12.3±3.2 nmol/l, normal range 1.5-3.0 nmol/l) and thyroxine (211±22 nmol/l, normal range 66-169 nmol/l) and suppressed TSH (1.1±0.3 mU/l, normal range 1.5-3.0 mU/l) measured by radioimmuno-assay. All patients were treated with Methimazole (30-40 mg/day) for 12 months and steroid therapy (Prednisolone average dose 50.0 mg/day) introduced in seven patients with infiltrative ophthalmopathy. Prednisolone treatment was tapered in relation to the activity of ophthalmopathy. Serum sIL-2R, TSH-R-, anti-microsomal-, anti-thyroglobulin- and anti-eye muscle antibodies were measured before and at 3-month intervals until discontinuation of treatment. Five patients were followed up after stopping Methimazole therapy until 18 month and tested for teh above-mentioned parameters every third month.

TSH-R antibodies: were determined by radioreceptor assay (TRAK assay, Henning, Chemie und Pharmawerk, Berlin) and expressed in U/l. The values above 10.0 U/l were considered positive.

Anti-thyroglobulin- and anti-microsomal antibodies: were tested by a passive haemagglutination technique (Thymune-T, Thymune-M, Wellcome).

sIL-2R: was measured by enzyme immunosorbent assay (ELISA). Briefly, 96-well microtiter plates were coated overnight with 100 µl of purified anti-tac in PBS (pH 7.4). After washing, 50 µl of sample was added to antibody-coated wells in triplicate. Standard wells were prepared with soluble human IL-2R at 0, 100, 400 and 1600 U/ml concentrations. 1000 U was defined as the amount of relesed sIL-2R in 1.0 ml of preparation of supernatant from PHA-stimulated peripheral lymphocytes (T Cell Science Inc. Cambridge, MA. USA). After 2 h incubation at 37°C the plates were washed with Tween-PBS and 100 µl horseradish peroxidase--conjugated 7G7B6 antibody was added to each well. After 2 h incubation 100 µl of o-phenylenediamine resuspended in buffer containing citric acidmonohydrate, Na_2HPO_4, 30% H_2O_2 (pH 6.3) was incubated for 30 min at room temperature. The average absorbance at 490 nm of each well and each patient' sample were read using Biorad microtiter plate reader. The detection limit of sIL-2R in our laboratory was 50 U/ml, intra-assay variation ranged between 3.8 and 4.9; interassay coefficient of variation was between 11.0 and 12.8%.

Anti-eye muscle antibodies: were determined by modified method of Faryna et al. /1, 5/. AEMA was calculated as follows: ^{125}I-protein-A counts bound by eye muscle membrane in the presence of patients' serum per counts bound by muscle membrane in the presence of control sera. AEMA index of 1.2 was considered positive.

Statistical analyses: the results were expressed as mean ±SD. The data were analysed with Student's t-test and linear correlation coefficient (r).

Results

The majority of the untreated Graves' patients showed high sIL-2R levels (667±270 U/ml) compared to controls (205±45 U/ml) ($P < 0.001$). Interestingly, remarkably higher values were observed in patients with infiltrative eye symptoms (810±313 U/ml) than in absence of ophthalmopathy (Fig. 1). In euthyroid stage induced by Methimazole, mean value of sIL-2R was found to be higher (273±72 U/ml) than that of controls, however, this

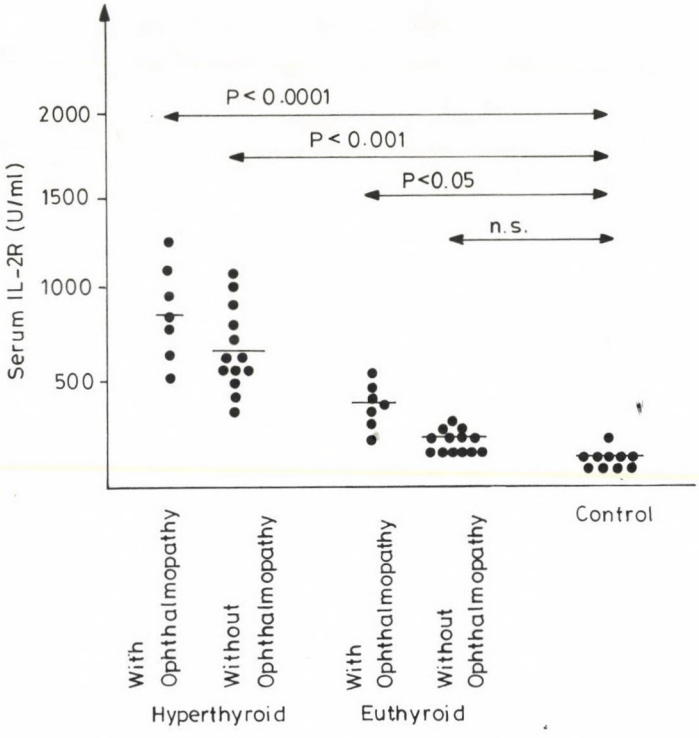


Fig. 1. Investigation of soluble interleukin-2 receptor (sIL-2R) in sera of hyperthyroid and euthyroid patients with Graves' disease. sIL-2R was measured by ELISA technique in 20 patients before and after 12 weeks treatment with Methimazole (30-40 mg/day). In control group consisted of 20 healthy volunteers sIL-2R was 205±45 U/ml. In sera of hyperthyroid patients with ophthalmopathy as well as without ophthalmopathy sIL-2R was significantly increased: 810±313 U/ml ($P < 0.001$) and 605±210 U/ml ($P < 0.01$), respectively. After Methimazole treatment in euthyroid patients without ophthalmopathy the sIL-2R was normal it remained elevated with ophthalmopathy (394±78) ($P < 0.05$) despite therapy with Prednisolone (average dose 50 mg/day for 4-12 weeks)

difference was not significant mathematically. When seven patients with ophthalmopathy were studied separately significantly enhanced levels of sIL-2R were found at the end of the period of observation compared to patients without ophthalmopathy despite their euthyroid stage ($P < 0.05$). No correlation was found between anti-microsomal-, anti-thyroglobulin antibodies and sIL-2R. Undoubtedly, the circulating antibodies to human eye muscle antigen were higher in patients with active eye disease, the correlation between sIL-2R and anti-eye muscle antibodies proved to be low ($r=0.21$) (Fig. 2). Thereafter, we studied the time-dependent changes of TSH-R antibodies and sIL-2R at three-month intervals. We found that the de-

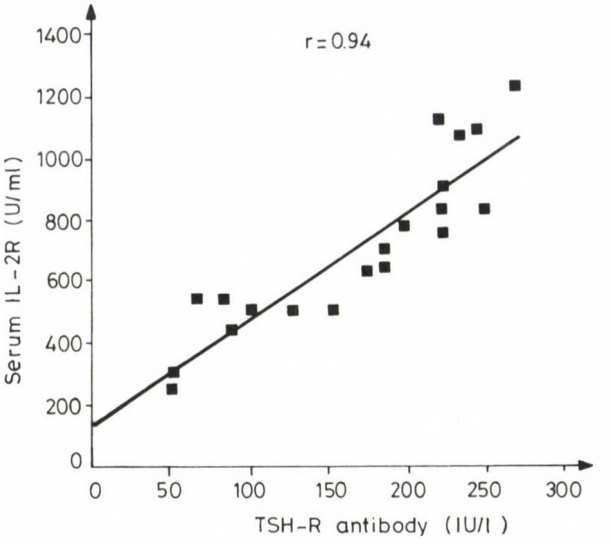


Fig. 2. Correlation between soluble IL-2R and TSH-R antibody in patients with Graves' disease (r=0.94)

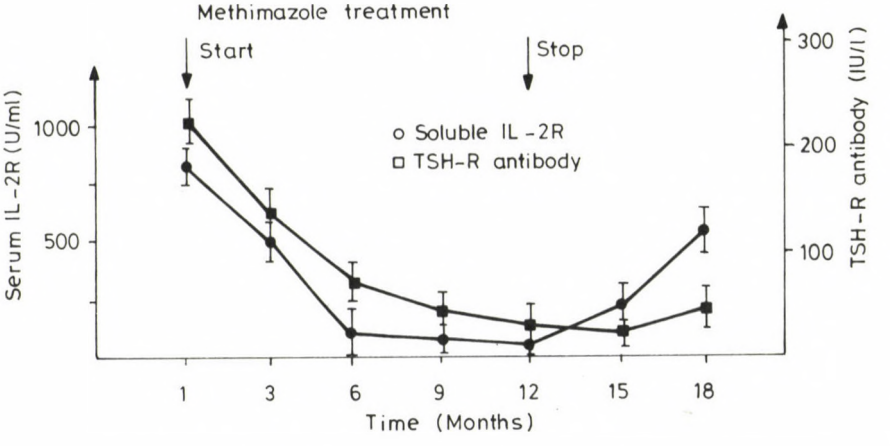


Fig. 3. Serum level of IL-2R and TSH-R antibodies in five patients

crease of sIL-2R was more rapid than that of TSH-R antibodies. In addition, the titre of sIL-2R normalized earlier than other immunological and hormonal parameters. In five patients we made follow-up study for 18 months. The tendency of decrease of sIL-2R and TSH-R antibodies was similar to that found in all patients, however, 6 months after Methimazole treatment had been ceased, an apparent rapid increase of sIL-2R levels was observed in three patients succeeded by elevation of TSH-R antibodies, and at the end of period of study the hyperthyrosis relapsed in two cases (Fig. 3).

Discussion

In the present report we studied the sIL-2R sera of untreated Graves' patients and found it to be significantly higher than in control samples. Previously, sIL-2R had been shown to be elevated in lymphoproliferative and systemic autoimmune diseases /7, 14/, therefore, it seems to be not a disease-specific marker, instead, it reflects the activation of autoreactive lymphocytes involved in the pathogenesis of Graves' disease /2, 26/. Our findings appear to contradict some in vitro observations concerning autoimmune diseases, for an impaired proliferation reponse to T-cell-specific mitogens in systemic lupus erythematosus, rheumatoid arthritis and Sjögren's syndrome has been published, and it was explained by a decreased capacity of helper T cells to produce IL-2, since exogenous IL-2 reversed in part the deficient lymphocyte reactivity /9, 20, 22/. At first glance the well-known findings that autoimmunity results from general hyperactivity of the immune system is incompatible with deficiency of IL-2 production. This contradiction could be explained by various mechanisms based on experimental data, however, a precise proof is missing. The first possibility is that peripheral T lymphocytes are in transient exhaustion of IL-2 secretion. This is supported by in vivo observations: glycocorticoids and cyclosporin A improve IL-2 secretion /27/. The alternative possibility might be an increased level of sIL-2R and IL-2R-positive cells which result in a decrease of IL-2 concentration. Eisenstein et al. /3/ have found significant deficiency in mitogen-induced in vitro cultures of Graves' mononuclear cells and concluded that production and response to IL-2 by mononuclear cells were poor due to an impaired receptor expression which was restored by thyrostatic treatment. In contrast, Lai et al. have observed an increased IL-2 production in response to pokeweed mitogen-stimulated cul-

tures of Graves' patients with ophthalmopathy /10/. Nevertheless, these conflicting data on in vitro IL-2 production in Graves' desease imply that reasonable caution should be necessary in an extrapolation to the in vivo situation. The elevated sIL-2R reflects the activation of autoreactive lymphocytes due to impaired TSH-R-specific suppressor function 2, 24/. This finding is in connection with our observation concerning the presence of the TSH-R antibodies is a characteristic humoral immune parameter of Graves' disease these antibodies could have been in correlation with sIL-2R as a sensitive and early marker of lymphocyte activation. In addition, the fact that the highest sIL-2R has been observed in patients with active infiltrative ophthalmopathy is in accordance with concept of association of two entities of Graves' disease, i.e. thyrotoxicosis and ophthalmopathy, consequently, with higher number of activated autoreactive lymphocytes /26, 28/. This assumption is supported by other findings, that neither anti-eye muscle-, anti-thyroglobulin-, nor anti-microsomal antibodies have showed correlation with sIL-2R level, for these parameters have lower predictive values than TSH-R antibodies /17, 21, 29/. Although we failed to detect serum IL-2 during Methimazole treatment, the progressive and more rapid decrease of sIL-2R level than of TSH-R antibodies serve an indirect evidence for the gradual inhibition of cellular overactivity an autoimmune mechanism. Taken together, we have found a remarkable increase in the sIL-2R level in untreated Graves' patients and a rapid decrease in relation to Methimazole treatment and enhance before relapse of thyrotoxicosis. Therefore, sIL-2R seems to be a sensitivie marker of autoimmune cellular processes of Graves' disease and might be a valuable parameter for prediciton of recurrence of thyrotoxicosis.

REFERENCES

1. Balázs, Cs., Bokk, A., Molnár, I., Stenszky, V., Farid, N.R.: Graves' ophthalmopathy, eye muscle antibodies and HLA antigens. Expl. Clin. Immunogenetics 6, 190-192 (1989)

2. Balázs, Cs., Stenszky, V., Kozma, L., Farid, N.R.: Specific suppressor T cell function in a patient with Graves' diseese. Clin. Endocrinol. 20, 683-688 (1984)

3. Chow, C.C., Lai, K.N., Leung, J.C.K., Chan, J.C.N., Cockram, C.S.: Serum soluble Interleukin 2 receptor in hyperthyroid Graves' disease and effect of Carbimazole therapy. Clin. Endocrionol. 33, 317-321 (1990)

4. Eisenstein, Z., Engelsman, E., Weiss, M., Kalechman, Y., Sredni, B.: Production and response to interleukin 2 in Graves' disease. J. Clin. Immunol. 8, 349-355 (1988)

5. Faryna, M., Nauman, J., Gardas, A.: Measurement of antibodies against human eye muscle plasma mebrane in Graves' ophthalmopathy. Brit. Med. J. 290, 191-192 (1985)

6. Giordano, C., Panto, F., Caruso, C., Modica, M.A., Zambito, A.M., Sapienza, N., Amato, M.P., Galuzo, A.: Interleukin 2 and soluble interleukin 2-receptor secretion defect in vitro in newly diagnosed type I diabetic patients. Diabetes Research 38, 310-315 (1989)

7. Herzog, Ch., Herzog, A., Aeschlimann, A., Gerber, M., Muller, W.: Soluble interleukin-2 receptor (sIL-2R) in rheumatoid arthritis. Zeitschrift für Rheumatologie 49, 8-10 (1990)

8. Kawabe, Y., Eguchi, K., Shimoura, C., Mine, M., Otsubo, T., Ueki, Y., Tezuka, M., Nakao, M., Kawakami, A., Migita, K., Yamashita, S., Matshunga, M., Ishikawa, N., Ito, K., Nagataki, S.: Interleukin-1 production and action in thyroid tissue. J. Clin. Endokrinol. Metab. 68, 1174-1183 (1989)

9. Kroemer, G., Wick, G.: The role of interleukin-2 in autoimmunity. Immunology Today 10, 246-251 (1989)

10. Lai, K.N., Leung, J.C.K., Chow, C.C., Cockram, C.S.: T-lymphocyte activation in euthyroid Graves' ophthalmopathy: soluble interleukin-2 receptor release, cellular interleukin-2 receptor expression and interleukin production. Acta Endocrinol. (Copenh.) 120, 602-609 (1989)

11. Londei, M., Bottazzo, G.F., Feldmann, M.: Human T-cell clones from autoimmune thyroid glands: specific recognition of autologous thyroid cells. Sicence 228, 85-89 (1985)

12. Mariotti, S., Chiovato, L., Vitti, P., Marcocci, C., Fenzi, G.F., Del Prete, G.F., Tiri, A., Ricci, M., Pinchera, A.: Recent advances in the understanding of humoral and cellular mechanisms implicated in thyroid autoimmune disorders. Clin. Immunol. Immunopathol. 50, 873-884 (1989)

13. Kiyotaki, C., Yoshizaki, K.: Demonstration of interleukin-1 beta on perifollicular endothelial cells in the thyroid gland of patients with Graevs' disease. J. Clin. Endocrinol. Metab. 69, 738-744 (1989)

14. Nakanishi, K., Taniguchi, Y., Onji, M.: Increased levels of soluble interleukin-2 receptors in Graves' disease. 8th Int. Congr. Endocrinol. Kyoto, Japan. Abstr. 12-18-011 (1988)

15. Pui, C.H.: Serum interleukin-2 receptor: clinical and biological implications. Leukemia 3, 323-327 (1989)

16. Rees, R.C., Wiltout, R.N.: The biology and clinical application of interleukin-2. Immunology Today 11, 36-38 (1990)

17. Rees Smith, B., McLachlan, S., Furmaniak, J.: Autoantibodies to the thyrotropin receptor. Endocrine Review 9, 106-120 (1988)

18. Rubin, L.A., Jay, G., Nelson, D.L.: The released interleukin-2 receptor binds interleukin-2 efficiently. J. Immunol. 137, 3841-3944 (1986)

19. Rubin, L.A., Kurmann, C.C., Fritz, M.E., Biddison, W.E., Boutin, B., Yarchoan, R., Nelson, D.L.: Soluble interleukin-2 receptors are released from activated human lymphoid cells in vitro. J. Immunol. 135, 3172-3177 (1985)

20. Sakane, T., Murakawa, Y., Suzuki, N., Yuji, U., Tsuchida, T., Takada, S., Yamauchi, Y., Tsunematsu, T.: Familial occurrence of impaired interleukin-2 activity and increased peripheral blood B cells actively secreting immunglobulins in systemic lupus erythematosus. Amer. J. Med. 86, 385-390 (1989)

21. Scherbaum, W.A.: On the clinical importance of thyroid microsomal and thyroglobulin antibody determination. Acta Endocrinol. (Copenh.) Suppl. 281, 325-329 (1987)

22. Semenzato, G., Bambara, L.M., Biasi, D.: Increased serum levels of soluble interleukin-2 receptor in patients with systemic lupus erythematosus and rheumatoid arthritis. J. Clin. Immuol. 8, 447-452 (1988)

23. Smith, K.A.: Interleukin-2, inception, impact and implications. Science 240, 1169-1176 (1988)

24. Topliss, D., How, J., Lewis, M., Row, V., Volpé, R.: Evidence for cell-mediated immunity and specific suppressor T lymphocyte dysfunction in Graves' disease and diabetes mellitus. J. Clin. Endocrinol. Metab. 57, 700-705 (1983)

25. Uchiyama, T., Broder, S., Waldmann, T.A.: Production of anti-tac monoclonal antibody and distribution of tac(+) cells. J. Immunol. 126, 1396-1397 (1981)

26. Volpé, R.: Autoimmune thyroid disease - A perspective. Mol. Biol. Med. 3, 25-51 (1986)

27. Waldmann, Th.: The multichain interleukin-2 receptor. A target for immunotherapy in lymphoma, autoimmune disorders and allografts. JAMA 263, 272-274 (1990)

28. Weetman, A.P.: Autoimmunity in Graeves' ophthalmopathy: a review. J. Royal Soc. Med. 82, 153-158 (1989)

29. Zimmermann, Cr.W., Eblen, F., Herzau, V., Reinauer, K.: The diagnostic value of serum autoantibodies in endocrine orbitopathy and idiopathic myositis. Graefe's Arch. Clin. Exp. Ophthalmol. 227, 521-525 (1989)

Acta Medica Hungarica, Vol. 48/1-2, pp. 13-21 (1991)

COMPARATIVE STUDY ON IgG AND IgA ANTIBODIES AGAINST HUMAN THYROID AND EYE-MUSCLE ANTIGENS IN GRAVES' OPHTHALMOPATHY

ILDIKÓ MOLNÁR, CS. BALÁZS

IIIrd Department of Medicine of Kenézy Hospital, Debrecen, Hungary

(Received: April 18, 1991)

Circulating IgG and IgA anti-thyroid and anti-eye muscle antibodies were investigated in 87 patients with Graves' disease (60 cases with ophthalmopathy). The ELISA method was used. Both IgG and IgA antibodies were demonstrated against human thyroid and eye-muscle membrane or cytosol antigens. Anti-eye-muscle antibodies of the IgA type were observed more frequently than those of the IgG type (25 cases vs. 18 were demonstrated with membrane antigens and 37 cases vs. 23 with cytosol antigens). The respective distributions for thyroid antigens the cytosol fraction were 55 cases vs. 13 and 18 cases vs. 36. A significant difference was observed in the anti-thyroid IgG levels and the anti-eye-msucle membrane or cytosol levels between the patients with Graves' disease and those in control group ($P < 0.001$). The difference in the IgA antibody to thyroid and eye-muscle antigens was significant between the patients with and without ophthalmopathy ($P < 0.002$). The strong correlation between the levels of IgA antibodies to thyroid and those to the eye-muscle cytosol fractions might be connected with the theory of the common aetiology of the thyroid and eye diseases in Graves' ophthalmopathy ($P < 0.001$). Circulating IgA anti-human thyroid and eye-muscle antibodies seemed to have a diagnostic relevance in the development of ophthalmopathy in Graves' ophthalmopathy.

Keywords: IgG antibodies, IgA antibodies, human eye muscle antigen, human thyroid antigen, Graves' ophthalmopathy

---

Abbreviations: GD: Graves' disease, GO: Graves' ophthalmopathy

Offprint requests should be sent to Ildikó Molnár, IIIrd Department of Medicine of Kenézy Hospital, H-4043 Debrecen, Dorottya u. 2, Hungary

Akadémiai Kiadó, Budapest

Introduction

Graves' ophthalmopathy (GO) was generally considered to be an autoimmune disorder /1, 3/. Many studies have been aimed at detect autoantibodies against eye-muscle membrane and cytosol in Graves' disease (GD) /6, 10, 13/. High levels of anti-eye-muscle autoantibodies were attributed to B cell proliferation that was observed in GO /19/. The role of the eye disease in the development of IgG and IgM antibodies to extraocular muscles was studied /14/. An enhanced level of serum immunoglobulins was described in GO and other autoimmune diseases (myasthenia gravis, systemic lupus erythematosus, insulin-dependent diabetes mellitus) /8/. The elevation in tears of Graves' patients was in good correlation with the severity of the eye symptoms /11/.

In the present study it was investigated whether circulating IgA antibodies to human thyroid and eye-muscle antigens are involved in the pathogenesis of GO and might have a diagnostic relevance.

Patients and Methods

1. Patients

Serum samples from 87 treated patients (16 males, 71 females; from 19 to 72 years, mean age 44±14 years) with GO were examined: Sixty of the patients had ophthalmopathy (average duration 4.5±3.8 years, from the onset of hyperthyroidism till 13 years of disease). Ophthalmopathy was classified according to the NOSPECS criteria of the American Thyroid Association /22, 23/. Soft tissue involvement (class 2) was found in 7 patients, proptosis (class 3) in 29 patients and an impaired motility of extraocular muscles (class 4) in 21 patients. Only two patients with corneal involvement (class 5) and one patient with sight loss (class 6) were given. The current thyroid stage was the following: 36 patients were hyperthyroid, 45 euthyroid and 5 hypothyroid. Forty-six had elevated TSH receptor antibody levels (51.9±90.76 U/l, range 0.3 to 405 U/l) at the time of the investigation. A lymphocyte infiltration was confirmed by fine-needle biopsy in 23 patients.

The levels of immunoglobulins (IgG, mean ±S.D., 12.43±3.42 g/l, range 5.4 to 20.03 g/l; normal 10-15 g/l) and IgA 2.17±1.11 g/l, range 0.46 to 5.76 g/l; normal 1.7±2.5 g/l) were measured by Hyland Laser PDQ TM nephelometer. High levels of IgG (16.7±1.6 g/l, range 15.4 to 20.03 g/l) and IgA (3.29±1,02 g/l, range 2.54 to 5.76 g/l) were found in 7 and 9 patients, respectively.

Twenty-five healthy subjects (11 males, 14 females, from 30 to 62 years of age; mean age: 46±9 years) without autoimmune endocrine, infectious and cancer disorders were tested as controls.

Methods

1. Preparation of human thyroid and eye-muscle fractions

The human thyroid tissue was obtained at surgery from two patients with GD. The human eye-muscle tissue was removed within 4-6 h after death from patients who had not suffered from tumour, endocrine and infectious disease. The tissue homogenates were centrifuged at 800 x g and fractions were prepared with centrifugation to separate the different subcellular compartments. The pellet of 3000 x g, 10 000 x g or 100 000 x g were called fractions I, II or III, respectively. Fraction II consisted of mainly mitochondrial compartments (established by electronmicroscope) and fraction III was remarked as a microsomal fraction. The supernatant of the 100 000 x g gave fraction IV. The protein concentration was measured by Lowry's method /15/. The fractions (without protease inhibitors) were stored at -40°C.

2. Indirect enzyme-linked immunosorbent assay

Anti-thyroid and anti-eye-muscle antibodies were measured by the indirect ELISA technique: a modification of Voller's method with antigen-coated enzyme-plate (Propylene GM, Pécs), 96 wells/plate /21/. The plates were coated with 100 µl of thyroid fractions (I-IV, 30 µg/ml) and eye-muscle fractions (I-IV, 50 µg/ml) by overnight incubation at 4°C. 100 µl serum (diluted 1:100) was added to each well for 2 h at room temperature. Goat antihuman IgG and IgA antibodies conjugated with horseradish peroxidase (Human Institute, Hungary) were chosen for detection of antibody binding (dilution 1:1000). The results were given as an index, the ratio of sera O.D. of triplicated patient's samples to the controls' mean O.D. value. An antibody level was defined elevated if the ELISA index exceeded the (mean ±S.D.) value for the control group tested concurrently.

3. Determination of the levels of thyroid hormone and TSH receptor antibodies

The levels of thyroid hormones were determined with radioimmunoassays (T3-RIA (normal 1.2-3.0 nmol/l), T4-RIA (normal 52-154 nmol/l) (Isotope Institute, Budapest)). The TSH hormone and TSH receptor antibodies were determined by Byk Mallincrodt (normal 0.6-3.8 mU/l) and TRAK (normal < 14 U/l) (Henning, Berlin, Germany) kits, respectively. T3-uptake (normal 0.8-1.15) was measured by a test developed in our laboratory.

Statistical Analysis

Statistical analysis was performed by means of Student's paired t-test and the chi-square test. Linear correlation was used to assess the relationship between the levels of IgG and IgA antibodies to the thyroid and eye muscle cytosol fractions.

Results

The IgG and IgA antibodies against human thyroid and eye muscle fractions were investigated in GO. The sera of the patients with GD were reacted with two fractions of eye muscle tissue (Table I) and all fractions of thyroid tissue (Table II). The differences in the levels of IgG anti-

Table I

IgG antibodies to human eye-muscle fractions (I-IV)

in Graves' disease (ELISA)

Groups		ELISA index[a] of anti-eye muscle IgG antibodies Fraction[b] I	II	III	IV
GO[1]	A[c]	0.6 ± 0.27	1.89 ± 0.48*	0.56 ± 0.21	1.4 ± 0.75**
	B[d]	0	17	0	16
GD[2]	A	0.71± 0.21	2.17 ± 0.44*	0.73 ± 0.22	1.45± 0.51**
	B	0	14	0	6
C[3]	A	1 ± 0.25	1 ± 0.56	1 ± 0.33	1.02± 0.41
	B	0	1	0	0

GO[1] : Graves' disease with ophthalmopathy (n=50)

GD[2] : Graves' disease without ophthalmopathy (n=25)

C[3] : Control group (n=25)

A[c] : mean ± S.D.

B[d] : number of patients with circulating IgG anti-eye-muscle antibodies

ELISA Index[a]: the ratio of mean O.D. of triplicated patient's samples to the controls' mean O.D. value. Elevated index means a value exceeding the mean ± 2 S.D. for the control group tested concurrently.

Fraction[b]: I - pellet of 3000 x g; II - pellet of 10 000 x g; III - pellet of 100 000 x g; IV - supernatant of 100 000 x g

*, **$P < 0.001$ compared to control

Table II

Detection of IgG antibodies to human thyroid fractions (I-IV)

in Graves' disease by ELISA

Groups		ELISA Index[a] of anti-thyroid IgG antibodies Fraction[b] I	II	III	IV
GO[1]	A[c]	1.93± 1.72*	1.05 ± 0.81	1.06 ± 0.46	1.59± 0.72**
	B[d]	17	6	22	26
GD[2]	A	2.27± 2.98*	0.76 ± 0.57	0.99 ± 0.66	1.85± 0.4**
	B	8	3	5	24
C[3]	A	1 ± 0.39	1 ± 0.33	0.99 ± 0.06	1 ± 0.23
	B	1	1	1	0

See footnote to Table I; *, **$P < 0.001$ compared to control

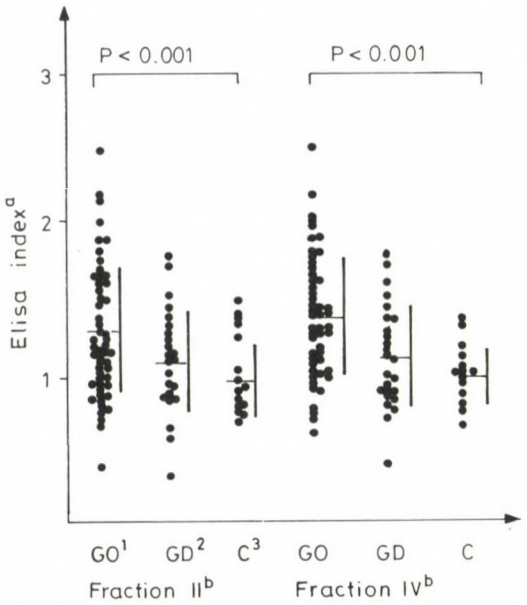


Fig. 1. IgA antibodies to human eye-muscle membrane (fraction II) and cytosol (fraction IV) (ELISA)
GO^1: Graves' disease with ophthalmopathy (n=60)
GD^2: Graves' disease without ophthalmopathy (n=27)
C^3: Control group (n=17)
ELISA Index[a]: the ratio of mean O.D. of triplicated patient's samples to the controls' mean O.D. value. Elevated index means a value exceeding the mean $\pm$ 2 S.D. for the control group tested concurrently
Fraction[b]: II - pellet of 10 000 x g; III - pellet of 100 000 x g; IV - supernatant of 100 000 x g

bodies against eye-muscle-membrane (31 cases) and cytosol (22 cases) antigens were significant between the GD patients and the control group ($P < 0.001$).

Circulating IgG antibodies against human thyroid tissue were detected in fractions III and IV. High levels of IgG antibodies against thyroid cytosol (fraction IV) were observed in 24 (96%) patients without eye disease and in 26 (52%) cases with ophthalmopathy. Twenty-two patients with GO showed circulating IgG antibodies against thyroid fraction III.

Significant differences were found in the presence of IgA antibodies against eye-muscle fractions II and IV between the patients with and without ophthalmopathy ($P < 0.001$) (Fig. 1). IgA antibodies against eye-muscle cytosol were found in 31 patients and IgG antibodies in only 16 patients with GO.

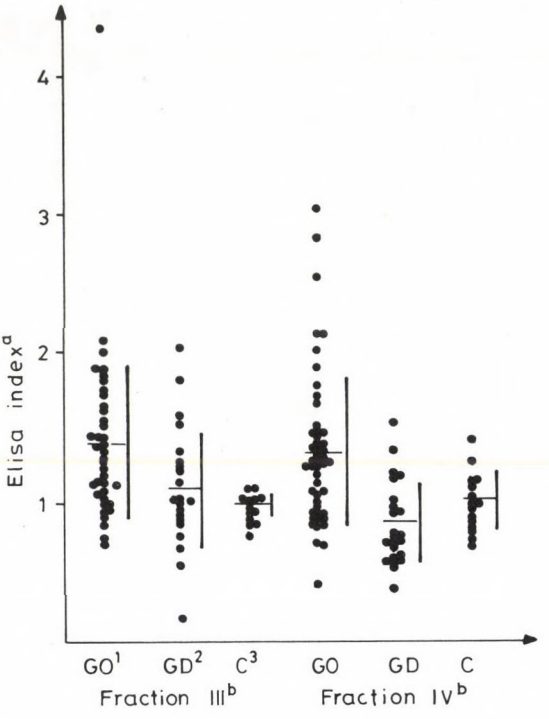


Fig. 2. Detection of IgA antibodies to human thyroid membrane (fraction III) and cytosol (fraction IV) antigens by ELISA.
See footnote to Fig. 1

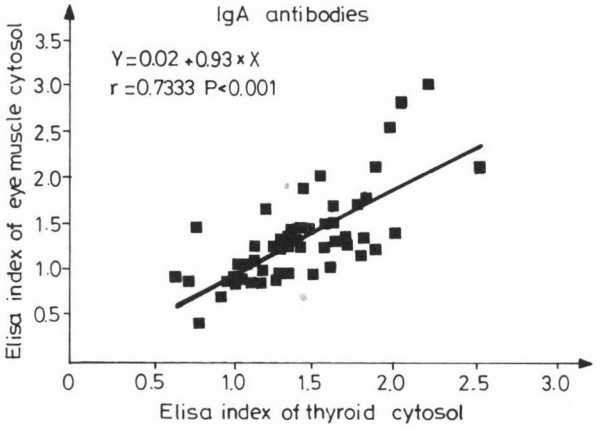


Fig. 3. Linear correlation between the ELISA indices of anti-thyroid and anti-eye-muscle IgA antibodies against cytosol antigens in Graves' ophthalmopathy.
See footnote to Fig. 1

IgA antibodies to thyroid fraction III were detected in 43 patients vs. 16 cases with the presence of anti-thyroid fraction IV antibodies (Fig. 2). The difference in the levels of these anti-thyroid IgA antibodies was significant between the patients with GO and the control group ($P < 0.001$). The occurrence of anti-thyroid and anti-eye-muscle IgA antibodies in GO was higher than that of IgG. Significant differences were found in the levels of IgA antibodies to thyroid and eye-muscle antigens between the patients with and without ophthalmopathy ($P < 0.002$).

An association was confirmed in the study of linear regression of IgA antibodies to thyroid and eye-muscle antigens (Fig. 3). There was a significant correlation between the thyroid and eye-muscle IgA antibodies ($r=0.7333$, $P < 0.001$), but no signficance was found ($r=0.0029$, N.S.) with IgG antibodies.

Discussion

The results reported in this study indicate that IgA anti-eye-muscle antibodies might have relevance in the pathogenesis of GO. Conflicting data on IgG antibodies against extraocular muscle antigens have been published /12, 20/. No significant difference was detected in the levels of IgG anti-eye muscle antibodies between the patients with and without infiltrative ophthalmopathy /16/. Surprisingly, the IgA anti-eye-muscle and anti-thyroid antibodies showed signficant differences between the groups with and without eye disease ($P < 0.002$).

Van der Gaag et al. /19/ described alterations in the serum IgA levels that were depended on the degree of the eye involvement and the thyroid function. A common aetiology between the thyroid and eye disease might be demonstrated the strong correlation that was found between the anti-thyroid and anti-eye-muscle cytosol IgA antibodies ($P < 0.001$).

There is much evidence for existence of Fc-receptor (FcαR) of IgA on lymphocytes, monocytes and polymorphonuclear leukocytes /2, 4, 5/. Synergistic effects between secretory IgA and IgG antibodies were published in ADCC and phagocytosis /9, 17, 18/. Recent data on the role of IgA has suggested that IgA antibodies might be of more importance in the immunoregulation of the development of autoimmune diseases than its local protective role /6/. Our findings established a diagnostic relevance of high levels of IgA anti-thyroid and anti-eye muscle antibodies in GO. Further

investigations have to be carried out to clarify the mechanism of IgA antibodies in the ophthalmopathy.

REFERENCES

1. Ahmann, A., Baker, J.R., Weetman, A.P., Wartofsky, L., Nutman, T.B., Burman, K.D.: Antibodies to porcine eye muscle in patients with Graeves' ophthalmopathy: Identification of serum immunoglobulins directed against unique determinants by immunoblotting and enzyme-linked immunosorbent assay. J. Clin. Endocrinol. Metab. 64, 454-460 (1987)
2. Albrechtsen, M., Yeaman, G.R., Kerr, M.A.: Characterization of the IgA receptor from human polymorphonuclear leucocytes. Immunology 64, 201-205 (1988)
3. Bahn, S.B., Gorman, C.A., Woloschak, G.E., David, C.S., Johnson, P.M., Johnson, C.M.: Human retroocular fibroblasts in vitro: a model for the study of Graeves' ophthalmopathy. J. Clin. Endocrinol. Metab. 65, 665-670 (1987)
4. Fanger, M.W., Shen, L., Pugh, J., Bernier, G.M.: Subpopulations of human peripheral granulocytes and monocytes express receptors for IgA. Proc. Natl. Acad. Sci. USA 77, 3640-3647 (1980)
5. Fanger, M.W., Goldstine, S.N., Shen, L.: Cytofluorographic analysis of receptors for IgA on human polymorphonuclear cells and monocytes, and the correlation of receptor expression with phagocytosis. Mol Immunol. 20, 1019-1024 (1983)
6. Faryna, M., Nauman, J., Gardas, A.: Measurement of autoantibodies against human eye muscle plasma membranes in Graves' ophthalmopathy. Br. Med. J. 290, 191-192 (1985)
7. Flores-Romo, L., Millsum, M.J., Gillis, S., Stubbs, P., Sykes, C., Gordon, J.: Immunoglobulin isotype production by cycling human B lymphocytes in response to recombinant cytokines and anti-IgM. Immunology 69, 342-347 (1990)
8. French, M.A.H., Dawkins, R.L.: Central MHC genes, IgA deficiency and autoimmune disease. Immunology Today 11, 271-274 (1990)
9. Geffner, J.R., Minnucci, F., Isturiz, M.A.: Neutrophilmediated cytotoxicity induced by secretory IgA. Immunology Letters 24, 113-116 (1990)
10. Kadlubowski, M., Irvine, W.J., Rowland, A.C.: Anti-muscle antibodies in Graves' ophthalmopathy. J. Clin. Lab. Immunol. 24, 105-111 (1987)
11. Khalil, H.A., De Keizer, R.J.W., Bodelier, V.M.W., Kijlstra, A.: Secretory IgA and lysozyme in tears of patients with Graeves' ophthalmopathy. Documents Ophthalmol. 72, 329-334 (1989)
12. Kendall-Taylor, P., Jones, D., Weightman, D., Atkinson, S.: The diagnostic value of antibody tests in Graves' ophthalmopathy. Akt. Endokr. Stoffw. 9, 30-32 (1988)
13. Kodama, K., Sikorska, H., Bandy-Dafoe, P., Bayly, R., Wall, J.R.: Demonstration of a circulating autoantibody against a soluble eye-muscle antigen in Graves' ophthalmopathy. Lancet ii, 1353-1356 (1982)
14. Kuroki, T., Ruf, J., Whelan, L., Miller, A., Wall, J.R.: Antithyroglobulin monoclonal and autoantibodies cross-react with an orbital connective tissue membrane antigen: a possible mechanism for the association of ophthalmopathy with autoimmune thyroid disorders. Clin. Exp. Immunol. 62, 361-370 (1985)
15. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the polyphenol reagent. J. Biol. Chem. 193, 265-275 (1951)

16. Molnár, I., Németi, K., Bokk, A., Stenszky, V., Balázs, C., Farid, N.R.: Cellular and humoral autoimmune responses against human eye muscle membrane antigen in Graves' disease. Acta Med. Hung. 47/1-2, 43-51 (1990)

17. Russell, M.W., Reinholdt, J., Kilian, M.: Anti-inflammatory activity of human IgA antibodies and their Fabα fragments: inhibition of IgG-mediated complement activation. Eur. J. Immunol. 19, 2243-2249 (1989)

18. Shen, L., Fanger, M.W.: Secretory IgA antibodies synergize with IgG in promoting ADCC by human polymorphonuclear cells, monocytes and lymphocytes. Cellular Immunol. 59, 75-79 (1981)

19. Van der Gaag, R., Broersma, L., Koornneef, L., Wiersinga, W.M.: B-lymphocyte subset distribution and serum immunoglobulin levels in Graves' ophthalmopathy. Orbit 5, 7-14 (1986)

20. Van der Gaag, R., Vernimmen, R., Fiebelkorn, N., van Dierendonck, M.C., Kijlstra, A.: Graeves' ophthalmopathy: What is the evidence for extraocular muscle specifi autoantibodies. Intern. Ophthalmol. 14, 25-30 (1990)

21. Voller, A., Bartlett, A., Bidwell, D.E.: Enzyme immunoassays with special reference to ELISA techniques. J. Clin. Pathol. 31, 507-520 (1978)

22. Werner, S.C.: Classification of eye changes of Graves' disease. J. Clin. Endocrinol. Metab. 29, 982-984 (1969)

23. Werne, S.C.: Modification of classification of eye changes of Graves' disease: recommendations of the Ad Hoc Committee of American Thyroid Associaton. J. Clin. Endocrinol. Metab. 44, 203-204 (1977)

Acta Medica Hungarica, Vol. 48/1-2, pp. 23-31 (1991)

AUTOANTIBODY AGAINST LIVER CELL MEMBRANE AND KILLER CELL ACTIVITY IN CHRONIC LIVER DISEASES

R. SCHNABEL, L. DALMI*, S. SIPKA*, M. BOKOR, GY. MALATINSZKY, T. GARAM

2nd Department of Medicine of the Róbert Károly Hospital and Polyclinic, Budapest, *University Medical School, Debrecen

(Received: March 21, 1991)

The aim of our present study was to examine the ADCC reaction against liver cell in various chronic liver diseases on the basis of indirect evidence. Forty-nine liver patients and one hundred and twenty-three healthy controls were examined. Anti-LSP autoantibody was determined on rat liver membrane by using the indirect immunofluorescent method. On the other hand, Killer-cell activity against human erythrocyte target cells was established in the lymphocytes of peripheral blood. Anti-LSP autoantibodies were demonstrated in seven patients and were associated with the high Killer-cell activity in six cases. Specific ADCC reaction to liver cell membrane can be assumed if anti-LSP autoantibody presence is topped with increased Killer-cell activity.

Keywords: Liver immunology, ADCC, LSP, anti-LSP autoantibody

Introduction

Ample evidence is available that cell injury induced by antibody dependent cellular cytotoxicity (ADCC) reaction to liver cell membrane may play an important role in the process and etiopathogenesis of chronic liver diseases /4, 5, 6, 11/.

In 1972 Meyer zum Büschenfelde and Miescher isolated the liver specific plasma membrane lipoprotein (LSP) /15/. LSP dispersion on paren-

---

Abbreviations: ADCC: antibody dependent cellular cytotoxicity, LSP: liver specific membrane lipoprotein

Offprint requests should be sent to R. Schnabel, H-1137 Budapest, Pozsonyi út 7, Hungary

Akadémiai Kiadó, Budapest

chyma cells is diffuse. It can be found simultaneously on the cytoplasmic and plasma membrane, or solely on the plasma membrane /8/. In fact LSP is an antigen complex, numerous determinants of which have been recognized, e.g. it contains approximately 0.25 per cent hepatic lectin, called also asialoglycoprotein /12, 24/. This component was also identified on the hepatocellular membrane of various species. Accordingly, animal derived - rat, rabbit - hepatocytes can be used for LSP studies /18, 20/. Anti-LSP autoantibody production can be induced by hepatocellular destruction developing on the effect of some damaging agent, since liver cell membrane fragments that enter circulation become immunogens or, in cases of viral infection, when expression of the virus antigen by hepatocytes occurs near the LSP antigen determinant, furthermore, if antibodies in response to some antigen challenge incidentally cross-react with LSP. Characteristics of LSP have been reported in detail among others by Japanese authors, Morichika et al. and will not be discussed in the present paper /17/. Anti-LSP autoantibodies were demonstrated first by Hopf et al. /9/. The anti-LSP autoantibodies are not species specific, as cross-reaction with LSP of other species occurs /18/. The appearance of anti-LSP autoantibodies is a precondition of the specific ADCC reaction against liver cell. The Fab fragment of anti-LSP binds to the LSP antigen determinant, while the Fc fragment binds the K-cells, the actual causers of cytolysis. The power of ADCC reaction substantially depends on the cytotoxic activity of K-cells and degree of activation.

In the present paper we report on our studies concerning the assumed role of ADCC reaction by the demonstration of anti-LSP autoantibodies and the cytotoxic capacity of K-cells based on indirect evidence.

Patients and Methods

Patients

Forty-nine patients (30 males and 19 females) with various liver diseases have been examined. The mean age amounted to 53,3 years (27-81 years). Distribution according to the diagnosis was as follows:

Liver cirrhosis	18
Fatty liver	12
Acute alcoholic hepatitis	8
Postnecrotic fibrosis	4
Chronic active hepatitis	3
Chronic persistent hepatitis	2

Drug (INH) induced toxic hepatitis	1
Chronic reactive hepatitis	1

The diagnoses were based on complex laboratory examinations (SGOT, SPGT, gamma-GT, alkalic phosphate, electrophoresis, LDH, LE-cell, serum bilirubin, complete blood count, complete urine analysis, antinuclear antibody, anti-DNA, alpha-fetoprotein, serum cholinesterase, HB_sAg), as well as on abdominal ultrasonography and histology, in addition to clinical examinations. Occasionally isotope scintigraphy and computer tomography have also been performed.

K-cell cytotoxicity was determined in 123 healthy volunteers (55 males and 68 females) with a mean age of 56.2 years. In addition the anti-LSP autoantibody examination was carried out on 16 (2 males and 14 females) healthy controls.

Indirect immunfluorescent study of autoantibodies against liver cell plasma membrane

a) Target cells were obtained from young (12-week old) healthy male CFY rats. Hepatocytes were separated according to Schaffer and Kessler /19/. Rats were killed by decapitation. To obtain total ischemia the exposed liver was perfused with isotonic solution, then removed and placed into Parker's solution. Homogenisation was performed in Ca- and Mg-free PBS solution and larger liver fragments were removed by filtration. The filtrate was then washed 4x in PBS solution at 600 g by centrifugation and incubated in a 5 per cent CO_2 environment for 10 min at 37°C. After repeated centrifugation the sediment was homogenized in Parker's solution by mild magnetic stirring. From this cell suspension the integrity and vitality of cells was established by tryptan blue staining. Had results not been satisfactory the procedure was repeated from PBS washing through until success. The cell-suspension was adjusted to $3x10^6$/ml cell count. 30 µl were removed and the hepatocytes placed by means of a cytocentrifuge on a slide and dried at +4°C. Suspension of cells was carried out under sterile conditions.

b) After the 1:10 dilution with PBS of the sera the hepatocytes were placed on slides and incubated in thermostat for 30 min. The aspecific bounds were removed by repeated washing with PBS. The preparate was incubated again with FITC-labelled anti-humang IgG rabbit serum for 30 min and was followed by repeated PBS-washing.

c) Evaluation: The preparations were studied by fluorescence microscopy. Results were considered positive (anti-LSP-positive) if fluorescence appeared on the surface of the hepatocytes.

K-cell activity of lymphocytes in peripheral blood

Antibody dependent cytotoxic activity was studied in anti-human eryhtrocyte test. Urbaniak's basic method modified by Garam and Bakács /7, 22/ has been used. It is based on the enzyme kinetic model of Zeijlemaker et al. and Thorn and Hanney /20, 25/. According to the latter lymphocyte cytotoxicity also depends on the number of target cells, i.e. the cytotoxic mechanism is similar to certain enzyme reactions.

Separation of lymphocytes was carried out from 20 ml heparinized blood. The mononuclear cells were separated by Ficoll-Uromiro gradient centrifugation according to Böyum /3/. "O" (R_1, R_2) human erythrocytes served as target cells. The target cells were washed, incubated with 2 per cent papain for 10 min at room temperature, then labelled with 200 µCi ^{51}Cr isotope (Na-chromate, Amersham, England) at 37°C for 120 min. After incubation the cells and the required concentrations were placed in RPMI-FCS nutrient fluid. Anti-D-immunglobulin (National Institute of Haematology, Budapest) at 6000x dilution was used as antibody. Serial dilutions of $1x10^6$ to $14x10^6$/ml concentration were prepared from the target cells. From these dilutions of 50 µl were pipetted into the "V" shaped openings of the microdish. 50 µl lymphocyte of the $2x10^6$/ml concentration were pipetted into each culture and 50 µl anti-D serum was added and the total volume replenished with 50 µl medium to 200 µl. The test mixture was incubated in a 5 per cent CO_2 environment thermostat at 37°C for 18 h.

Calculation of cytotoxicity. The specific release per cent was calculated on the basic of following formula:

$$\frac{\text{Supernatant "cpm" - spontaneous release "cpm"}}{\text{Total incorporated activity "cpm" - spontaneous release "cpm"}} \times 100$$

From this cytotoxic capacity, which is epxressed by the maximum of target cells destroyed by a unit of lymphocyte can be calculated by the formula:

$$\frac{\text{Number of saturating target cells x related specific-release \%}}{100}$$

The saturating target cell number is the smallest number of target cells at which the peak activity of lymphocytes is measured. In each of the lymphocyte and target cell combinations two parallel samples were used. Their mean represented the basic of the calculation, which was performed with a program from the University of California Los Angeles Biomedical Department.

The Student's two sample t-test was used for the calculation of significance between the two groups.

Results

Anti-LSP autoantibody studies

Anti-LSP autoantibody positivity has not been found in the group of 16 healthy controls.

From the 49 patients with liver diseases findings were highly positive in 4 cases and moderately positive in 3 patients. Data and K-cell activity of lymphocytes in the peripheral blood for these 7 patients are shown on the Table I.

K-cell activity studies

K-cell activity was determined in all the patients with liver disease and mean values were calculated for the group (mean $\bar{x}$ 10^6 $\pm$S.D.) ($2.38 \times 10^6 \pm 1.47 \times 10^6$). Based on detectable anti-LSP autoantibody two sub-groups were formed and mean K-cell activity for latter calculated. K-cell cytotoxicity was $1.95 \times 10^6 \pm 1.43 \times 10^6$ in the 123 healthy volunteers. Results can be seen on Table II.

K-cell activity in patients with liver disease and in the anti-LSP autoantibody negative group ($2.09 \times 10^6 \pm 0.82 \times 10^6$) showed no substantial difference from the findings in the control group.

K-cell activity in the anti-LSP autoantibody positive group ($3.85 \times 10^6 \pm 1.32 \times 10^6$) was significantly higher when compared to controls, as well as to the findings in the anti-LSP autoantibody negative group ($P < 0.001$).

Table I

Main data of patients with anti-LSP positive liver diseases

Patient	Age	Diagnosis	History	Anti-LSP	Cytotoxicity x 10^6
1. B.L.	57	Chronic persistent hepatitis	Alcohol:Ø HB_sAG:Ø	++	3.95
2. SZ.S.	57	Liver cirrhosis (Signs of activity: piecemeal necrosis)	Alcohol:+ HB_sAG:Ø	++	4.20
3. F.GY.	60	Liver cirrhosis (Signs of activity: piecemeal necrosis)	Alcohol:+ HB_sAG:Ø	+++	4.66
4. V.CS.	54	Drug induced hepatitis (INH)	Alcohol:Ø HB_sAg:Ø	++	0.92
5. P.GY.	59	Chronic active hepatitis	Alcohol:+ HB_sAg:Ø	+++	4.07
6. V.J.	51	Chronic active hepatitis	Alcohol:Ø HB_sAG:Ø	+++	4.55
7. M.L.	58	Reactive haptitis (virus infection)	Alcohol:+ HB_sAg:Ø	+++	4.70

$^{+++,(-)}$Meaning of the symbole

Table II

K-cell cytotoxicity in patients with liver diseases based on detectable anti-LSP autoantibody

Groups	N^o of cases	Cytotoxicity mean x 10^6 ± S.D.
1. Total of patients	49	2.38 ± 1.47
2. Anti-LSP negative patients	42	2.09 ± 0.82
3. Anti-LSP positive patients	7	3.85 ± 1.32
4. Control group	123	1.95 ± 1.43

Significant differences: Between group 2 and 3: $P < 0.001$;
between group 3 and 4: $P < 0.001$;
between group 2 and 4: N.S.

Discussion

As already mentioned in the introduction, an important role was attributed to the ADCC reaction, as well as to the anti-LSP autoantibody as the specific antibody of the ADCC reaction in the pathogenesis of the various chronic liver diseases considered presently of autoimmune origin.

According to Ventoi et al. autoreactivity in hepatitis A, B, and non-A, non-B virus infection might be of etiological importance /23/. Mondelli et al. found that ADCC played a significant part in the development of non-A, non-B virus hepatitis-induced chronic hepatitis /16/. In a substantial part of alcoholic liver diseases an increased non-T-cell cytotoxicity against autologous hepatocytes - presumably K-cell cytotoxicity - has been confirmed /1/. Experimental studies confirmed that circulating antibodies against liver cell membrane appear on the effect of ethanol /2/. Cell injury on the effect of ADCC reaction occurs primarily in the portal region and was confirmed in various liver diseases by Vergagni et al. /23/. In autoimmune chronic active hepatitis (CAH) detection of anti-LSP autoantibodies and their change of titer showed close correlation with the remission or relapse of the disease /13/. IgG liver cell membrane autoantibodies were found at a high per cent (LMA) in various liver diseases: e.g. in autoimmune CAH 83%, cryptogenic chronic active liver disease 47%, primary biliary cirrhosis 42%, HB_sAg positive and HB_sAg negative but anti-HB_c positive chronic active liver diseases 11% were LMA positive /14/. By means of the monoclonal antibody technique Kakumu et al. demonstrated anti-LSP antibodies in acute and chronic liver diseases. It seems noteworthy that latter authors found anti-LSP autoantibodies in 13 of the 16 patients with active liver cirrhosis /10/.

Results of our anti-LSP autoantibody studies are in line with the mentioned reports. From the 7 positive cases 2 corresponded to autoimmune CAH (Table I, case Nos 5 and 6), in which, to our knowledge, the occurrence of anti-LSP autoantibodies is high. Anti-LSP antibody positivity in patient No. 1 with chronic persistent hepatitis was neither surprising. In this case it may be assumed that the process has been induced by a former viral infection, eventually non-A, non-B virus infection, though no direct evidence was available. Positivity in the two cirrhotic cases showing activity (patients Nos 2 and 3) was in agreement with data reported in literature. Anti-LSP autoantibody production in patient No. 4 (drug induced INH hepatitis) was probably induced by liver injury. In patient No.7

antibodies elicited by a common virus infection did probably cross-react incidentally with the LSP antigen determinant.

It should be emphasized that K-cell activity essentially exceeded normal values in 6 of the 7 patients revealing anti-LSP autoantibody positivity. Contrariwise, no such increase (2-fold of normal) occurred in the anti-LSP autoantibody negative and control groups. Although the present is only indirect evidence on the role of ADCC reaction against liver cell membrane, correlations may serve as additional data. In patient No. 4 the anti-LSP autoantibody positivity has not been associated to high K-cell activity. Because of the early diagnosis of a mild liver injury (detected during a regular check-up) we assume that the autoreactive process, i.e. ADCC reaction had not been induced besides anti-LSP autoantibody production, which might be an interpretation of the low K-cel activity.

Although the number of patients according the different diagnoses was relatively low, we believe that the 65 cases (49 patients and 16 controls) permit conclusions, the more so, because in almost each of the 7 anti-LSP autoantibody positive cases (in 6 from 7 K-cell activity has been extremely high. This correlation (also statistically of high significance) served as indirect evidence on the role of ADCC reaction. We assume that our results, i.e. the indirect evidence of ADCC reaction might promote knowledge on immune processes in liver diseases and will be helpful in the decision of therapy.

REFERENCES

1. Actis, G., Mieli-Vergagni, G., Portmann, B., Eddleston, A.L.W.F., Davis, M., Williams, R.: Lymphocyte cytotoxicity to autologous hepatocytes in alcoholic liver disease. Liver 3, 8-12 (1983)

2. Anthony, R.S., Farguharson, M., MacSween, R.N.: Liver membrane antibodies in alcoholic liver disease. II. Antibodies to ethanol-altered hepatocytes. J. Clin. Pathol. 36, 1302-1308 (1983)

3. Böyum, A.: Separation of lymphocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. 21, 77-89 (1968)

4. Cohrane, A.M.G., Thomson, A.D., Moussours, A., Eddleston, A.L.W.F., Williams, R.: Antibody-dependent cell-mediated (K-cell) cytotoxicity against isolated hepatocytes in chronic active hepatitis. Lancet 1, 441-444 (1976)

5. Eddleston, A.L.W.F.: Immunology of chronic active hepatitis. Quart. J. Med. 55, 191-198 (1985)

6. Eddleston, A.L.W.F., Mondelli, M.: Immunpathological mechanism of liver cell injury in chronic hepatitis B virus infection. J. Hepatol. 3, 17-23 (1986)

7. Garam, T., Bakács, T., Varga, M., Petrányi, Gy.: Emberi lymphocyták humán vörösvérsejtek elleni cytotoxicus kapacitásának vizsgálata. Orv. hetil. 121, 1705-1708 (1980)

8. Helin, H., Uibo, R., Paronen, I., Krohn, K.: Immunohistochemical localization of human liver specific protein using rabbit antisera and the avidin-biotin complex technique. Clin. Exp. Immunol. 59, 371-376 (1985)

9. Hopf, U., Meyer Zum Büschenfelde, K.H., Arnold, W.: Detection of a liver-membrane autoantibody in HB_sAg-negative chronic active hepatitis. N. Engl. J. Med. 294, 578-582 (1976)

10. Kakumu, S., Yoshioka, K., Tsubouchi, A.: Antibody to liver-specific lipoprotein in acute and chronic liver diseases. Its quantitative assay with monoclonal antibody, but without the use of liver-specific lipoproteins. Gastroenterol. Jpn. 20, 216-221 (1986)

11. Jensen, D.M., McFarlane, I.G., Portmann, B., Eddleston, A.L.W.F., Williams, R.: Detection of antibodies directed against a liver-specific membrane lipoprotein in patients with acute and chronic active hepatitis. N. Engl. J. Med. 229, 1-9 (1978)

12. McFarlane, I.G., McFarlane, B.M., Major, G.N., Tolley, P., Williams, R.: Identification of the hepatic asialo-glycoprotein receptor (Hepatic lectin) as a component of liver specific membrane lipoprotein (LSP). Clin. Exp. Immunol. 55, 347-354 (1984)

13. McFarlane, I.G., Hegarty, J.E., McSorley, C.G., McFarlane, B.M., Williams, R.: Antibodies to liver-specific protein predict outcome of treatment withdrawal in autoimmune chronic active hepatitis. Lancet 2, 954-956 (1984)

14. Meliconi, R., Stancari, M.V., Garagnani, M., Baraldini, M., Stefanini, G.F., Miglio, F., Gasbarrini, G.: Occurrence and significance of IgG liver membrane autoantibodies (LMA) in chronic liver disease of different aetiology. Clin. Exp. Immunol. 51, 565-571 (1983)

15. Meyer zum Büschenfelde, K.H., Miescher, P.A.: Liver specific antigens: purification and characterization. Clin. Exp. Immunol. 10, 89-102 (1972)

16. Mondelli, M., Alberti, A., Tremolada, F., Williams, R., Eddleston, A.L.W.F., Realdi, G.: In vitro cell-mediated cytotoxicity for autologous liver cells in chronic non-A, non-B hepatitis. Clin. Exp. Immunol. 63, 147-155 (1986)

17. Morichiaka, S., Arima, T., Nagashima, H.: Purification and characterization of a liver cell membrane specific antigen. Gastroenterol. Jpn. 21, 471-478 (1986)

18. Perperas, A., McFarlane, B.M., McSorley, C.G., McFarlane, I.G., Williams, R.: Species cross-reactivity of anti-LSP antibodies in acute viral hepatitis. J. Clin. Lab. Immunol. 19, 25-30 (1986)

19. Schaffer, W.J., Kessler, D.J.: In Methods in Cell Biology. Acad. Press. New York-San Francisco-London, 21/B, 457-469 (1980)

20. Sípos, J., McFarlane, B.M., McSorley, C.G., Gove, C.D., Williams, R., McFarlane, I.G.: Immunhistochemical demonstration of the asialoglycoprotein receptor in rat liver by a sensitive avidin-biotin technique. J. of Pathology 158, 247-252 (1989)

21. Thorn, R.M., Hanney, C.S.: Kinetics analysis of target-cell destruction by effector T cells. J. Immunol. 119, 1973-1979 (1977)

22. Urbaniak, S.J.: Lymphoid cell dependent (K-cell) lysis of human erythrocytes sensitised with Rhesus alloantibodies. Brit. J. Haematol. 33, 409-413 (1976)

23. Ventoi, S., McFarlane, B.M., McSorley, C.G., Ranieri, S., Giuliani-Piccari, G., Dal-Monte, P.R., Verruchi, G., Williams, R., Chiodo, F., McFarlane, I.G.: Liver autoreactivity in acute virus A, B and non-A and non-B hepatitis. J. Clin. Lab. Immunol. 25, 1-7 (1988)

24. Vergagni, D., Mieli-Vergagni, G., Modelli, M., Portmann, B., Eddleston, A.L.W.F.: Immunglobulin on the surface of isolated hepatocytes in associated with antibody-dependent cell-mediated cytotoxicity and liver damage. Liver 7, 307-315 (1987)

25. Zeijlemaker, W.F., Van Oers, R.H.J., De Boede, R.E.J., Schellenens, P.Th.: Cytotoxic activity of human lymphocytes. Quantitative analysis of T cell and K-cell cytotoxicity, revealing enzymlike kinetics. J. Immunol. 119, 1507-1513 (1977)

Acta Medica Hungarica, Vol. 48/1-2, pp. 33-43 (1991)

ENDOCRINOLOGY

BIOLOGIC BLOOD MARKERS REFLECTING THYROID HORMONE EFFECT AT PERIPHERAL TISSUE LEVEL IN PATIENTS RECEIVING LEVOTHYROXINE REPLACEMENT FOR HYPOTHYROIDISM

J. FÖLDES, G. TARJÁN, CS. BÁNOS, J. NÉMETH, F. VARGA and B. BÜKI

First Department of Medicine, Semmelweis University Medical School and Radioisotope Laboratory of János Hospital, Budapest, Hungary

(Received: April 8, 1991)

Plasma fibronectin, serum procollagen-III-peptide and sex-hormone-binding globulin as non-specific markers of thyroid hormone effect at peripheral tissue level were determined and their values were related with serum levels of TSH, free-thyroxine and triiodothyronine during levothyroxine sodium replacement therapy for hypothyroidism. Low levels of biologic markers characteristic of hypothyroidism were normalized in consequence of hormone replacement and a negative correlation between their serum levels, and TSH concentration was demonstrated in most subjects. However, in some patients a discrepancy in the response to levothyroxine between the pituitary and other target organs was revealed. Additional evidence was disclosed that the pituitary thyrotroph sensitizes a minor decrease in serum thyroxine level, which would not be recognized by other target organs. Furthermore, it was revealed that during L-T_4 replacement therapy in a large fraction of patients with subnormal serum TSH concentration blood levels of the measured markers often exceeded the upper limit of the normal range indicating a possibility of "tissue" thyrotoxicosis, besides the pituitary, in other target organs, too. According to the present study, which takes into consideration markers reflecting end-organ responsiveness to thyroid hormones, it is recommended to adjust the dose of levothyroxine to maintain serum TSH in the normal range. For patients with subnormal TSH concentration a close follow-up is obligatory and in case of concomitantly raised free-thyroxine level the reduction of the levothyroxine dosage is proposed.

Keywords: Thyroid hormones, peripheral tissue level, levothyroxine, hypothyroidism

Introduction

The choice of levothyroxine sodium (L-T_4) as the most appropriate form of thyroid hormone replacement therapy for hypothyrodism has been well documented. However, the fine adjustment of the L-T_4 replacement doses frequently presents a challenging problem. If the preparation

Offprint requests should be sent to: J. Földes, H-1083 Budapest, Korányi S. u. 2/a, Hungary

Akadémiai Kiadó, Budapest

is administered in an inadequate dosage, subclinical hypothyroidism may be left over or subclinical hyperthyroidism may be induced. In the latter case TSH secretion is already suppressed, while serum thyroid hormone level is still within the normal range and the patient seems to be clinically euthyroid /14, 19/.

Measurement of serum TSH concentration applying a sensitive method which can distinguish normal from subnormal levels is considered the most accurate test for optimizing long-term L-T_4 replacement therapy /6, 9, 16-18/. It is widely accepted that normal TSH concentration is an indicator of appropriate hormone replacement, while a subnormal level refers to excessive L-T_4 dosage. However, according to some observations each subject has a unique TSH setpoint, thus, the attainment of a "normal range" TSH value during replacement therapy may not reliably denote a state of euthyroidism in every patient /16/. Furthermore, experimental data /7/ indicate that the pituitary is more sensitive to serum T_4 than other target organs and this has led to the assumption that serum TSH level does not represent the assessment of overtreatment with L-T_4. Thus, a suppressed TSH secretion would not indicate the induction of "tissue"-thyrotoxicosis reliably.

Lately some sensitive but not specific methods have been developed which reflect the effect of thyroid hormones at peripheral tissue levels. Plasma fibronectin (pFn), serum procollagen-III-peptide (P-III-P) and sex-hormone binding globulin (SHBG) determinations are included in the category of these tests /2, 3, 8, 10-12/. Assessment of these biologic markers in blood may point to the various peripheral tissue responses to thyroid hormones. The present study was undertaken to detect by these markers the peripheral responses to L-T_4 replacement in patients with hypothyroidism and to reveal a possible relationship between their serum levels and TSH concentration. Furthermore, from the results of this investigation we intended to provide evidence of the appropriateness of L-T_4 dosage.

Patients and Methods

Two groups of patients with primary hypothyroidism were studied. Into the first group 18 untreated female patients (age 20-60 years) were enrolled. The thyroid gland failure resulted from Hashimoto's thyroiditis and ^{131}I therapy or surgical treatment of Graves' hyperthyroidism or toxic nodular goiter. After the establishment of the diagnosis levothyroxine replacement treatment was started (L-Thyroxin, Henning) with an initial dose of

25-50 µg/day. Blood samples were taken at the end of each month and if it was necessary the $L\text{-}T_4$ dose was increased by 25 ug/day increments each month till the maintenance dosage was attained. Before the initiation of the hormone substitution and after administration of a constant maintenance $L\text{-}T_4$ dose at least for 3 months, serum levels of free-thyroxine (FT_4), free-triiodothyronine (FT_3), TSH, P-III-P, SHBG and pFn were determined. Having received $L\text{-}T_4$ for replacement, each patient became clinically euthyroid and the serum level of TSH was within the normal range.

Patients in the second group were referred to the thyroid outpatient clinic of the First Department of Medicine of our University. This group comprised of 113 hypothyroid female subjects (age: 20-60 years) who had received Thyranon (Organon) or Eltroxin (Glaxo) for substitution therapy and later these preparations were changed to L-Thyroxin (Henning). At the time of the investigation all patients were clinically euthyroid and had been receiving a constant maintenance dose of L-Thyroxin for at least 3 months. According to their serum TSH level that patients were classified into 3 subgroups: a) normal serum TSH: 0.3-3.0 mU/l; b) subnormal TSH concentration: < 0.15 mU/l; c) high serum TSH: > 3.0 mU/l. Patients with an intermediate serum TSH value (0.15-0.3 mU/l, so-called "gray zone") were dropped out of this study.

For the measurement of serum hormone levels commercially available kits were used: Free-thyroxine (FT_4; Amerlex M FT_4; Amersham; norm. range: 9.5-24.5 pmol/l); free-triiodothyronine (FT_3; Amerlex M FT_3; Amersham; norm. range: 3.5-9.3 pmol/l); TSH (IRMA-mat TSH; Byck-Sangtek; norm. value: 0.3-3.0 mU/l). For the assessment of thyroid hormone effect at peripheral tissue level the following methods were applied: 1. plasma fibronectin: Blood was taken off into EDTA containing tubes and pFn was determined with the Boehringer turbidimetric test kits adapted to Hitachi 704/c; norm. value: 300-550 ug/ml; 2. Procollagen-III-peptide: RIA-gnost P-III-P (Böhring); norm. value: 0.3-0.8 U/ml; 3. SHBG: sex-hormone binding globulin assay kit (Farmos); normal value: 30-90 nmol/l (untreated females; age: 20-60 years).

Student's unpaired t-test and one way analysis of variance were used in statistical analysis. Mean $\pm$ S.D. are presented in the Tables.

Results

Data for 18 patients with primary hypothyroidism are demonstrated in Table I. It is noteworthy that prior to initiation of replacement $L\text{-}T_4$ therapy among the markers related to the peripheral responses to thyroid hormones the pFn and SHBG levels were low, while the mean value of P-III-P approached the lower limit of the normal range. During hormone replacement therapy the mean serum or plasma levels of TSH, FT_4, FT_3, pFn, P-III-P and SHBG were unanimously normalized.

Table II shows the serum TSH, FT_4, FT_3, pFn, P-III-P and SHBG levels of 113 patients with hypothyroidism receiving $L\text{-}T_4$ for hormone replacement. The subjects were classified into 3 categories on the basis of their serum TSH concentration. The test results of the groups with subnormal or high TSH levels were correlated with the data obtained in the group with normal TSH level. In the subnormal TSH group the mean FT_4 level was slightly raised while in the high TSH group its value was lower than that found in the group with normal serum TSH concentration ($P < 0.001$); how-

Table I

Test results (means ± S.D.) of patients (n=18) with primary hypothyroidism prior to treatment and following levothyroxine sodium replacement therapy (means ± S.D.). In brackets are given the reference ranges of the tests. Asterisks denote significant differences from values prior to therapy

(Student's unpaired t-test)

	Prior to treatment	During $L\text{-}T_4$ replacement treatment
TSH mU/l (0.3-3.0)	51.8 ± 28.4	1.3 ± 0.9***
FT_4 pmol/l (9.5-24.5)	4.8 ± 3.0	17.7 ± 2.8***
FT_3 pmol/l (3.5-9.3)	2.8 ± 1.2	5.6 ± 0.9***
Fibronectin µg/ml (300-550)	252.3 ±59.6	415.8 ±93.7***
Procollagen III peptide U/ml (0.3-0.8)	0.33± 0.09	0.64± 0.12***
SHBG nmol/l (30-90)	27.7 ±10.3	51.9 ±17.1***

***P < 0.001

every, their values were still within the wide normal range. In all 3 groups FT_3 levels were equally normal, though, a significant difference was demonstrated between their mean values. In the group with subnormal serum TSH content the mean values of pFn, P-III-P and SHBG compared to those in the group with normal TSH, were significantly ($P < 0.001$) elevated, but only the mean pFn level exceeded the upper limit of the normal range. At last it is shown that in the group with a still raised TSH concentration the mean serum levels of P-III-P and SHBG became already normal, and this is valid also for pFn, but its mean level remained significantly lower than that in the normal TSH group ($P < 0.01$).

Figures 1/a, 1/b, 1/c show the individual pFn, P-III-P and SHBG values for $L\text{-}T_4$-treated patients recruited to groups with normal, subnormal or still high serum TSH concentration and cases with normal or raised FT_4 levels are presented separately. FT_3 values were elevated only in 4 cases in the group with low TSH level. Conversely, the FT_4 levels were higher

Table II

Serum levels (means ± S.D.) of TSH, free-T_4, free-T_3 and biologic markers from 113 hypothyroid patients receiving a constant dose of levothyroxine sodium and categorized according to serum TSH concentrations. Asterisks denote significant differences from group with normal TSH (one way ANOVA)

	Subjects classified by serum TSH level		
	TSH subnormal (n=32)	TSH normal (n=63)	TSH high (n=18)
TSH mU/l	0.15***	1.80 ± 1.21	15.18 ± 7.14***
FT_4 pmol/l	20.54 ± 3.94***	16.78 ± 3.81	12.42 ± 1.56***
FT_3 pmol/l	6.97 ± 2.71**	5.34 ± 1.09	4.39 ± 0.98*
Fibronectin µg/ml	590.5 ±183.0***	426.4 ±100.0	322.5 ±88.9**
Procollagen-III-peptide U/ml	0.78 ± 0.28***	0.59 ± 0.9	0.48 ± 0.18
SHBG nmol/l	75.6 ± 35.5***	48.6 ± 20.6	36.1 ± 13.4

*P < 0.05; **P < 0.01; ***P < 0.001

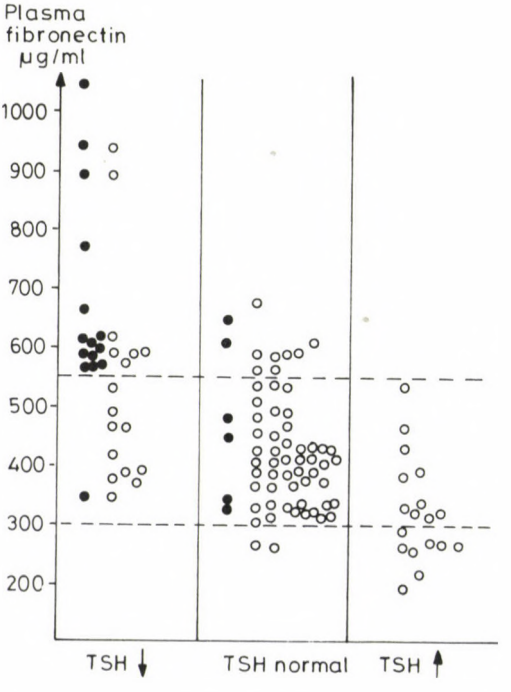


Fig. 1/a.

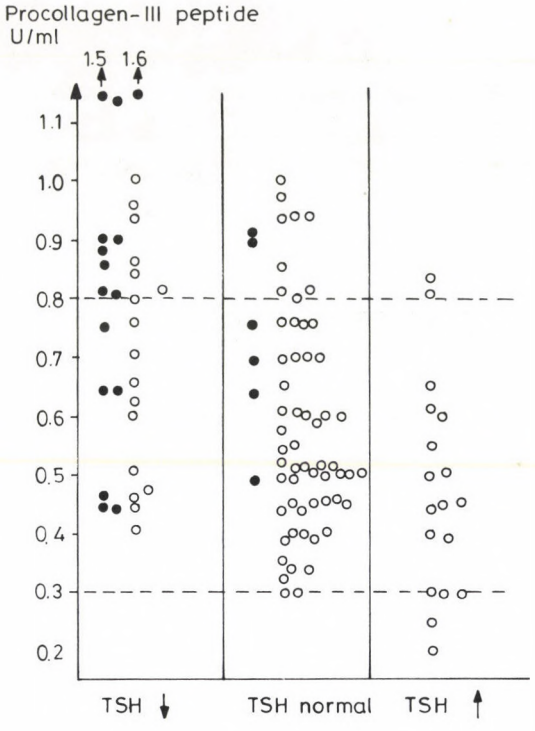


Fig. 1/b.

than normal in 15 subjects of the 32 patients with undetectable TSH concentrations and in 6 cases of the 63 patients with normal TSH levels.

Of the 32 patients with subnormal TSH concentrations in 21 cases the pFn, in 16 subjects the P-III-P and in 9 patients the SHBG levels exceeded the upper limit of the normal range. This refers especially to patients with concomitant raised FT_4 levels. It is noteworthy, however, that out of 63 patients with normalized TSH level in 10 cases the pFn, in 11 subjects the P-III-P and in 9 cases the SHBG levels were higher than normal. On the other hand, in the group with elevated TSH concentration (n=18) in 8 cases the pFn, in 5 the P-III-P and in 6 subjects the SHBG values were lower than normal.

Figure 2 demonstrates that in 5 cases of the group with subnormal serum TSH concentration the high pFn values decreased significantly following the reduction of the L-T replacement dose, when the serum TSH level returned to the normal range.

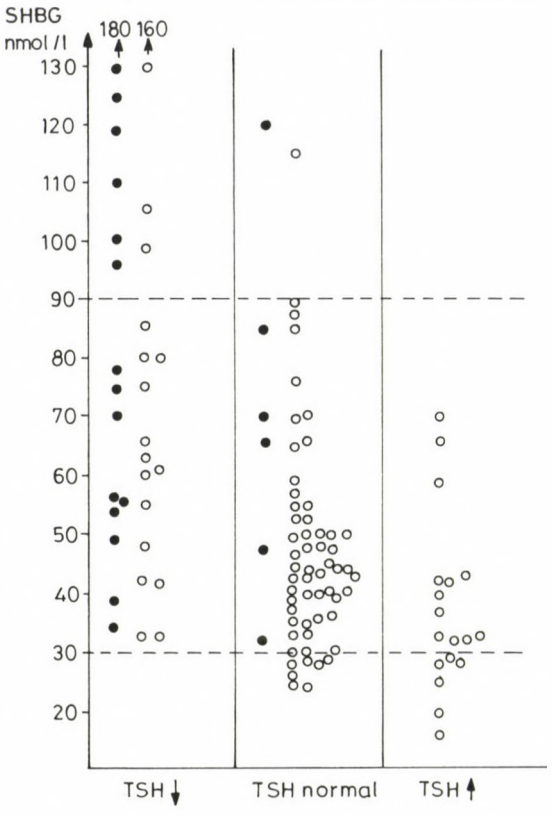


Fig. 1/a, 1/b, 1/c. Plasma fibronectin, serum procollagen-III-peptide and sex-hormone binding globulin levels of patients receiving a constant levothyroxine sodium replacement dose for hypothyroidism (n=113). The subjects are categorized by their serum TSH levels. Classification marks; ↓ TSH subnormal; ↑ TSH high; O FT_4 within the normal range, ● raised FT_4 level. Broken lines indicate the reference range of the biologic markers

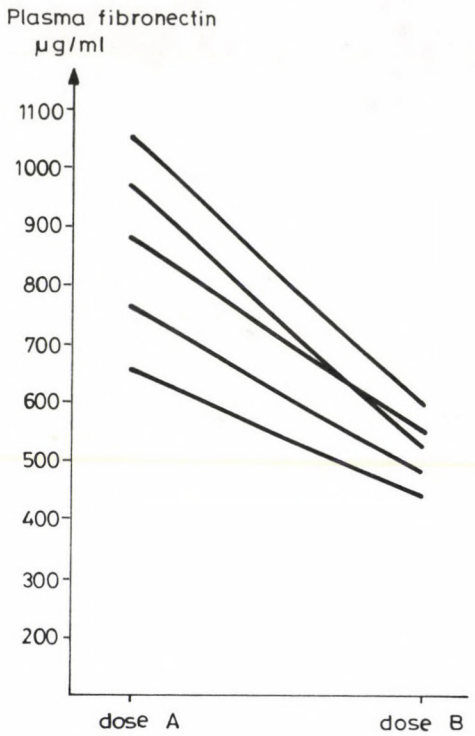


Fig. 2. Plasma fibronectin level before and after a reduction of levothyroxine sodium dose. Patients had a subnormal serum TSH concentration on dose A and a normal TSH value on dose B

Discussion

In the presence of thyroid gland failure signs indicative of "tissue"-hypothyroidism might be discovered. In accordance with this observation blood levels of the biologic markers reflecting thyroid hormone effect at peripheral tissue level are usually lower than normal. In this regard present findings are corroborating previous data /2, 3, 8, 11/. The goal of our further studies was to reveal a change in the blood levels of these markers during L-T_4 replacement therapy and to find out a relationship between their serum levels and TSH concentration.

In most patients receiving hormone substitution normal serum TSH levels have been detected and the value of FT_4 occasionally exceeded the upper limit of the normal range. In this group of patients hormone replacement with L-T_4 seemed to be successful, as for the most part of these

subjects the blood levels of the biologic markers were within the normal range. It is remarkable, however, that in some cases the serum levels of the measured markers were slightly higher than normal, indicating a discrepancy in the response to L-T_4 between the pituitary and other target tissues.

Some clinically euthyroid subjects who were on a stable dose of L-T_4 had a high TSH level, apparently due to under-replacement or non-compliance. Our observations indicate that serum T_4 level gains an access to the wide normal range after a lower dose of hormone replacement than it is needed to normalize TSH secretion. This finding proves that for the fine adjustment of the replacement dose a single FT_4 determination is not enough. In the majority of these patients serum levels of the measured biologic markers were already within the normal range in contrast with the still elevated TSH concentration. This observation is in agreement with earlier data suggesting that the pituitary thyrotroph sensitizes even a minor decrease in serum T_4 to a value that is still within the normal range, which would not be recognized by other target organs /15, 16/. Conversely, in other subjects the serum levels of the biologic markers remained still low, indicating subsistent "tissue"-hypothyroidism. This, taken together with high serum TSH concentration, recommends an increase of the hormone-replacement dose.

The evaluation of patients with subnormal serum TSH concentration during L-T_4 replacement therapy is still debatable. To find out whether doses of L-T_4 sufficient to suppress TSH secretion cause "tissue"-thyrotoxicosis also in other organs, some authors have recently used additional methods for the estimation of peripheral tissue responses to thyroid hormones /4, 5, 12, 13/. In the present study based upon the measurement of some biologic markers, we demonstrated a high individual variability of tissue sensitivity to thyroid hormones. Nevertheless, it was shown that in a large fraction of patients with subnormal serum TSH concentration the blood levels of biologic markers reflecting thyroid hormone effect at peripheral tissue level exceeded the upper limit of the normal range. In this group of patients, however, a strict correlation between serum FT_4 level and the development of "tissue"-thyrotoxicosis could not be revealed, as the serum levels of the biologic markers were elevated in some patients with "normal" range FT_4 level, too. It seems plausible that in some individuals serum FT_4 levels which are still within the broad normal range, but are already capable of suppressing pituitary TSH secretion may induce

changes in other target organ functions similar to, but less marked than, those recorded in overt hyperthyroidism. At the same time, it can be stated that pFn and P-III-P values were frequently elevated in those patients with subnormal TSH concentrations who had concomitantly slightly raised serum FT_4 levels, referring to a generalized tissue overexposure to thyroid hormones. In this group of patients such a relationship between the biologic markers and serum FT_3 was not detectable. At last, our data indicate that in some patients with subnormal serum TSH content the high pFn level truly reflected "tissue"-thyrotoxicosis, as its plasma level decreased significantly after a reduction of the replacement hormone dose.

Present observations which take into consideration the markers reflecting end-organ responsiveness to thyroid hormones are in accordance with earlier studies and show the superiority of a sensitive TSH assay over FT_4 or FT_3 measurement in identifying patients receiving excessive L-T_4 doses /4, 5, 12, 13/ and to all probability in a large fraction of patients subnormal serum TSH level may denote the development of "tissue"-thyrotoxicosis beside the pituitary in other target organs, too. In the event of undetectable serum TSH as a second-step the determination of serum FT_4 is justified and if its value is raised a reduction of the L-T_4 dose is necessary. Furthermore, patients who have suppressed TSH secretion despite "normal"-range serum FT_4 value should be closely followed-up in the long-term, too.

REFERENCES

1. Carr, D., McLeod, D.T., Parry, G., Thornes, H.M.: Fine adjustment of thyroxine replacement dosage. Clin. Endocrinol. 28, 325-333 (1988)

2. Faber, J., Horslev-Petersen, K., Perrild, H.: Circulating levels of procollagen-III-peptide and hyaluronic acid in thyroid disease. Amer. Thyr. Ass. 64 Meeting, San Francisco, 1989, Abstr. 145

3. Földes, J., Bános, Cs., Lakatos, P., Tarján, G.: Szérum "sex-hormone binding globulin" tartalom vizsgálata pajzsmirigybetegségekben. Orv. Hetil. 131, 1579-1584 (1990)

4. Gow, S.M., Caldwell, G., Toft, A.D., Seth, J., Hussey, A.J., Sweeting, W.M., Becket, G.J.: Relationship between pituitary and other target organ responsiveness in hypothyroid patients receiving thyroxine replacement. J. Clin. Endocrinol. Metab. 64, 364-370 (1987)

5. Gow, S.M., Caldwell, G., Toft, A.D., Sweeting, V.M., Becket, G.J.: Restoration of normal TSH secretion reduces the abnormally high serum glutathione-S-transferase levels found in patients receiving thyroxine replacement therapy. Clin. Endocrinol. 29, 249-256 (1988)

6. Helfand, M., Crapo, L.M.: Monitoring therapy in patients taking Levothyroxine. Ann. Int. Med. 113, 450-454 (1990)

7. Larsen, P.R.: Thyroid-pituitary interactions: feedback regulation of TSH secretion by thyroid hormones. New Engl. J. Med. 306, 23-32 (1982)

8. Lervang, H.H., Ditzel, J.: Serum procollagen-III-peptide in thyroid diseases before and after therapy. 18. ETA Meeting, Copenhagen, 1989 (Abstr. 98) Ann. d'endocrinologie 1989

9. Leslie, P.J., Toft, A.D.: The replacement therapy problem in hypothyroidism. In: Bailliere's Clinical Endocrinology and Metab. Lazarus, J.H., Hall, R. (eds). Bailliere Tindall, London, 1988, pp. 653-669.

10. Limouris, G., Karkavitsas, N.: Aminoterminal propeptide of type III procollagen and its evaluation in thyroid disturbances. Eur. J. Nucl. Med. 16, 490A (1990)

11. Németh, J., Büki, B., Földes, J.: Plazma fibronectin tartalom pajzsmirigybetegségben. Orv. Hetil. 131, 2361-2364 (1990)

12. Nyström, E., Caidahl, K., Fager, G., Wikkelso, C., Lundberg, P.A., Lindstedt, G.: A double-blind cross-over 12-month study of L-thyroxine treatment of women with subclinical hypothyroidism. Clin. Endocrinol. 29, 63-76 (1988)

13. Nyström, E., Lundberg, P.A., Petersen, K., Bengtsson, C., Lindstedt, G.: Evidence for a slow tissue adaptation to circulating thyroxine in patients with chronic l-thyroxine treatment. Clin. Endocrinol. 31, 143-150 (1989)

14. Ross, D.S.: Subclinical hyperthyroidism: possible danger of overzelous replacement L-thyroxine therapy. Mayo Clin. Proc. 63, 1223-1229 (1988)

15. Snyder, P.J., Utiger, R.D.: Inhibition of thyrotropin response to TRH by small quantities of thyroid hormones. J. Clin. Invest. 51, 2077-2084 (1972)

16. Spencer, C.A., Nicoloff, J.T.: Serum TSH measurement: a 1990 status report. Thyroid Today 13, 1-12 (1990)

17. Utiger, R.D.: Therapy of hypothyroidism. Brit. med. J. 323, 126-127 (1990)

18. Watts, N.B.: Use of a sensitive thyrotropin assay for monitoring treatment with Levothyroxine. Arch. Int. Med. 149, 309-312 (1989)

19. Wenzel, K.W.: Does suppressive therapy with levothyroxine induce tissue-thyrotoxicosis? Ann. d'Endocrinologie 1988 17. ETA Meeting, Montpelier. (Abstr. 4.) (1988)

Acta Medica Hungarica, Vol. 48/1-2, pp. 45-49 (1991)

NEONATAL SEX RATIO IN PREGNANCIES OF ADOLESCENT MOTHERS

Á.A. JAKOBOVITS and L. ZUBEK

Department of Obstetrics and Gynecology, Toldy Ferenc Hospital, Cegléd, Hungary

(Received: January 3, 1991)

The authors have reviewed the sex ratio in 353 offspring of adolescent parturients based on a 5-year material of their department. The 174 males and 179 females provided a sex ratio of 97.2; less than the usual ratio of 106. Interestingly, females are prevalent not only among low-birth-weight but also among average-birth-weight babies of adolescents. The hypothesis is presented that the anatomy of the cervix and the chemical composition of the cervical mucus may affect the sex ratio in some manner.

Keywords: Adolescent mothers, sex ratio

Introduction

The question of sex ratio in relation to maternal age has been a controversial subject in the literature during the past several decades. In many obstetric complications and also in cases involving perinatal death, the number of males appears to exceed that of females. Noting the opinion that teenage gestations involved increased perinatal risks, we felt that the sex ratio of the offspring in these gestations deserved investigation.

Offprint requests should be sent to Á.A. Jakobovits, H-5600 Békéscsaba, Berzsenyi u. 1, Hungary

Akadémiai Kiadó, Budapest

Patients and Methods

We investigated the 5-year material of our department between January 1, 1985, and December 31, 1989. We sought to clarify the total number of adolescent mothers, the proportion of such mothers in our entire obstetric population and the sex ratios (number of boys per 100 girls) in the investigated material. The sex ratio of newborn babies of adolescent mothers was compared with that of all other pregnancies. The significance of the differences was investigated statistically by the χ^2 test.

Results

During the quoted study years, 351 parturients were less than 18 years old at the time of the delivery. Some adolescent girls gave birth more than once during the 5 study years. Since during the same time 7567 women delivered in the department, the ratio of adolescents was 4.64 percent. Two adolescents gave birth to twins. Each pair belonged to the same sex. Thus, the total number of neonates was 353, including 174 males and 179 females sex ratio: 97.2. The sex ratio in the total population was 106. The difference (χ^2 = 0.66) was not significant.

In the adolescent group, the most frequent age was 16-17 years (Table I). The youngest parturient was 12 years old.

Table I

Sex ratio of neonates according to maternal age

Maternal age (years)	Number of cases	Per cent	Boys	Girls	Sex ratio
12-13	3		2	1	
14-15	46		25	21	119.0
16-17	304	86.12	147	157	93.6
Total	353		174	179	97.2

There were no characteristic differences of sex ratio according to the gestation length at the time of the delivery (Table II). The sex ratio was relatively high when delivery occurred between 31 and 32 and between 37-38 weeks. The ratio was the lowest between 35 and 36 weeks.

The birth weights of neonates ranged between 500 and 4850 grams (Table III). The sex ratio tended to increase with increasing birth weights of the neonates.

Table II

Sex ratio of neonates according to gestation length at birth

Gestations age (weeks)	Number of cases	Per cent	Boys	Girls	Sex ratio
23-24	3		2	1	
25-26	1		1	0	
31-32	6		4	2	
33-34	7		3	4	
35-36	17		7	10	70.0
37-38	64	18.13	35	29	120.7
39-40	212	60.06	104	108	96.3
41-42	43		18	25	72.0
Total	353		174	179	97.2

Table III

Sex ratio of neonates according to birth weights

Body weight (g)	Number of cases	Per cent	Boys	Girls	Sex ratio
500- 999	4		3	1	
1000-1499	3		1	2	
1500-1999	6		4	2	
2000-2499	34		13	21	61.9
2500-2999	103	29.18	45	58	77.6
3000-3499	137	38.81	68	69	98.6
3500-3999	53	15.01	32	21	152.4
4000-4499	12		7	5	140.0
4850	1		1	0	
Total	353		174	179	97.2

Two stillbirths and six neonatal deaths within two days of the delivery occurred during the five years. Of these fetuses and neonates, seven were males and one was female.

Discussion

According to our current understanding, the sex of the offpsring is determined by the fertilizing spermatozoon. The Y-chromosome-carrying androspermatozoa have smaller head, longer tail, and move faster than X-carrying gynospermatozoa /9/. Furthermore, it appears that the androspermatozoa are more vulnerable than gynospermatozoa, insofar as in con-

nection with cryopreservation, the sex ratio is as low as 75-77; considerably lower than the usual 105-107. After artificial insemination resulting from the use of fresh sperm, the noted sex ratio is 91.6-92 /1, 8/. Some authors have found that vaginal inflammations decreased the sex ratio because the gynospermatozoa were more resistant of vaginitis on spermatozoa /6, 7/. We did not found any case of colpitis in the documentation of our relevant material. In contrast, in cases of in vitro fertilization, when the spermatozoa do not need to pass through the genital canal, the sex ratio is as high as 183, presumably due to the fact that the spermatozoa escape the vicissitudes associated with passage through the female genital tract /10/. A different interpretation was presented by James /5/ who attributed the high male ratio to the induced high estrogen levels associated with the assisted reproduction.

In previous publications, we reported a high proportion of female offspring among low-birth-weight neonates /3, 4/. A similar observation, involving a sex ratio of 92.2 of low-birth-weight neonates was reported from Aberdeen, Scotland, for the years 1961-1979. Interestingly, in Soctland at large, between 1973 and 1979, the sex ratio showed close relationship and decreased with gestation length at birth (136.6 before the 27th week and 104.6 by the 40th week). This conclusion was based on an extensive review that involved a total of 169 631 births /2/.

In our material, the sex ratio was low between 2.500 and 3.499 g birth weight. One may speculate that in the adolescent age group the progress of androspermatozoa along the genital tract is hindered by some as yet unclarified mechanism.

In our experience, neither parental age nor birth order is a significant factor, per se, with regard to the determination of the gender of the offspring. However, it is conceivable, that the mechanism of transportation plays a limited role by hindering in adolescents the passage of Y carrying spermatozoa. It is understood that the transportation of spermatozoa is influenced by a variety of factors, which are hormonal, rheologic and, perhaps, also mechanical or chemical in nature. Relevant factors appear to be the patency of the cervical canal, the chemical composition of the cervical mucus, vaginal fluid and, possibly, some other factors that could conceivably be related to previous childbirths.

REFERENCES

1. Amuzu, B., Laxova, R., Shapiro, S.S.: Pregnancy outcome, health of children, and family adjustment after donor insemination. Obstet, Gynecol. 75, 899-905 (1990)

2. Hall, M.N., Carr-Hill, R.: Impact of sex ratio on onset and management of labour. Br. Med. J. 285, 401-403 (1982)

3. Jakobovits, Á.A., Zubek, L.: Sex ratio in twin pregnancies. (A letter) Am. J. Obstet. Gynecol. 156, 1360 (1987)

4. Jakobovits, Á.A., Zubek, L.: Premature rupture of the membranes and fetal sex. (A letter) Am. J. Obstet. Gynecol. 159, 1307-1308 (1988)

5. James, W.H.: Sex ratio of infants following assisted reproduction. Lancet II, 446 (1989)

6. McGregor, J.A., Leff, M.: Does maternal genital herpes infection influence fetal gender? Am. J. Obstet. Gynecol. 162, 1346-1347 (1990)

7. Minkoff, H., Grunebaum, A., McCormack, W.M., Schwartz, R.H.: Relationship of vaginitis to sex of conceptuses. Obstet. Gynecol. 66, 239-240 (1985)

8. Mortimer, D., Richardson, D.W.: Sex ratio of birth resulting from artificial insemination. Br. J. Obstet. Gynecol. 89, 132-135 (1982)

9. Shettles, L.B.: How sperm commence movement and their isolation for in vitro fertilization and sex selection. (A letter) Am. J. Obstet. Gynecol. 163, 270 (1990)

10. Thatcher, S.S., Restrepo, U., Lavy, G., DeCherney, A.H.: In vitro fertilisation and sex ratio. Lancet I, 1025-1026 (1989)

Acta Medica Hungarica, Vol. 48/1-2, pp. 51-60 (1991)

OUTCOME PREDICTION IN ADULT RESPIRATORY DISTRESS SYNDROME USING DISCRIMINANT ANALYSIS OF CARDIORESPIRATORY DATA

T. GONDOS [1], K. SZABÓ [2], G. JOKKEL [3], I. PÉNZES [4]

[1]Postgraduate Medical University, Cardiovascular Clinic, [2]Central Military Hospital, Burn Center, [3]Experimental Research Institute, and 2nd Department of Physiology of Semmelweis University Medical School, [4]National Korányi Institute for Pulmonology, Surgical Intensive Care Unit, Budapest, Hungary

(Received: February 22, 1991)

In order to examine the prognostic value of different cardiopulmonary variables in adult respiratory distress syndrome the data of 30 patients with this illness were studied retrospectively. The patients were divided into 3 groups: Group A: survivors (9 cases, 40 examinations), Group B: early stage nonsurvivors (8 cases, 37 examinations), Group C: late stage nonsurvivors (19 cases, 89 examinations). In 6 nonsurvivor patients a few measurements were done in the early and late stage, too. There were highly significant differences between Groups A and C (mean pulmonary arterial pressure, pulmonary arterial diastolic pressure minus pulmonary capillary wedge pressure, left ventricular stroke work index, systemic and pulmonary vascular resistance, inspired oxygen fraction, arterial oxygen tension per inspired oxygen fraction, mixed venous oxygen saturation, pulmonary shunt fraction, and oxygen delivery, but the differences in relation to other groups were less prominent. Using a stepwise discriminant analysis, it was found that the oxygenation parameters alone determined the outcome correctly in 68-75%. Extending the analysis to haemodynamic variables the result improved (72-80%). Similar prediction was obtained when parameters potentionally measurable by noninvasive methods were analysed (69-80%). These results suggest that it is possible to predict the outcome of ARDS correctly without any invasive monitoring technique.

Keywords: Prognosis prediction, ARDS, haemodynamics, oxygenation, discriminant analysis

---

Abbreviations: ARDS: adult respiratory distress syndrome, CI: cardiac index, CVP: central venous pressure, DO_2: oxygen delivery, FiO_2: fraction of inspired oxygen, LVSWI: left ventricular stroke work index, MPAP: mean pulmonary arterial pressure, MSAP: mean systemic arterial pressure, O_2ER: oxygen extraction ratio, PADP-PCWP: pulmonary arterial diastolic - pulmonary capillary wedge pressure, PaO_2: arterial oxygen tension, PCWP: pulmonary capillary wedge pressure, PEEP: positive end expiratory pressure, PVR: pulmonary vascular resistance, Qs/Qt: reight to left shunt, RPP: rate pressure product, RVSWI: right ventricular stroke work index, SV: stroke volume, SvO_2: mixed venous oxygen saturation, SVR: systemic vascular resistance, VO_2: oxygen consumption

Offprint requests should be sent to: T. Gondos, H-1135 Budapest, Szabolcs u. 35, Hungary

Akadémiai Kiadó, Budapest

Introduction

Outcome prediction has a great importance in the treatment of critically ill patients. Shoemaker et al. /2, 17, 19, 20/ developed a predictive index based on nonparametric multivariance analysis of the cardiorespiratory data of critically ill surgical patients. In patients with ARDS Smith and Gordon /24/ described a simple index to predict outcome, involving only 3 parameters (age, airway pressure, alveolo-arterial oxygen gradient) to determine the "ventilator score". The disadvantage of this method was that a real discrimination between survivors and nonsurvivors became possible only from the 9th day of the ventilator therapy. Hankeln et al. /9/ used a complex cardiorespiratory prognostic index in ARDS, resulting in a useful way to predict outcome but only in the late stage. For outcome prediction several other studies /4, 6, 22, 29/ proposed that the VO_2 is the main parameter of the prognosis, because its level depends on the function of different organs. Recently, however, this statement has been disproved /10, 27/. The prognostic value of VO_2 has not improved even by its entering into the SAPS (Simplified Acute Physiologic Score) system. Knaus et al. /11/ developed the Acute Physiologic and Chronic Health Evaluation (APACHE II) system containing also results on the oxygenation. It is a useful and sensitive index to graduate the severity of the illness and thus predict the outcome. Much of the score relies upon time-consuming laboratory data. It has a limited use as a tool for repetitive, hour to hour evaluation. The aim of the present retrospective study was to evaluate the prognostic role of the different cardiorespiratory data in patients with ARDS of different aetiology.

Patients and Methods

Thirty patients (16 females, 14 males, aged 14-71, mean 48 years) with ARDS of various origins were included in the study. Each patient met the criteria for the diagnosis of ARDS: 1) an acute, progressive respiratory failure without pre-existing lung and heart diseases, 2) FiO_2 higher than 0.4 necessary to maintain PaO_2 above 60 mmHg at zero level of PEEP, 3) necessity of more than 72 h of mechanical ventilation, 4) diffuse, bilateral pulmonary infiltrates on chest X-ray, and 5) without evidence of left ventricular failure (PCWP less than 15 mmHg).

Associated conditions were: sepsis in 12, thoracic surgery in 10, abdominal surgery in 9, pulmonary embolism in 4, multiple injury in 2 and paraquat intoxication in one cases.

For the study the patients were divided into 3 groups: Group A: survivors (9 cases, 40 measurements) who had been weaned from respiratory support and recovered, Group B: nonsurvivors, early stage group (8 cases, 37 measurements) who died during the treatment, but

the measurements were done up to 5 days before death, and Group C: nonsurvivors, late stage group (19 cases, 89 measurements) where the measurements were done during the last 5 days of the life. In 6 patients the measurements were repeatedly performed before and after the limit time (5 days) between Groups B and C, so the sum of cases of the groups exceeded the number of the studied patients. During the examination period each patient was given mechanical ventilation and the same therapeutical protocol was applied. Pulmonary haemodynamics were measured with Swan-Ganz catheters (7F Edwards). Cardiac output was determined by the thermodilution method (Sirecust 404, Siemens, FRG). Each measurement was performed at least in triplicates with the mean taken as cardiac output. To characterize the haemodynamic status of the patients the following parameters were used: MSAP, MPAP, PCWP, CVP, PADP-PCWP, CI, LVSWI, RVSWI, PVR, SVR and RPP.

Arterial and mixed venous oxygenation parameters were measured with a blood gas analyser (Radiometer ABL 300, Copenhagen, Denmark). To characterize the oxygenation status of the patients, the following parameters were used: SvO_2, PaO_2/FiO_2, DO_2, O_2ER, VO_2, Qs/Qt. At derived parameters we used conventional equations /8/.

Statistical analysis used means $\pm$S.D., multiple t-test, and the discriminant analysis test of the SPSS statistical software package. This analysis was a stepwise variable selection with the selection rule minimized by Wilk's lambda value.

Results

The summarized cardiorespiratory data of the patients are shown in Table I. There are significant differences between Groups A and C in MPAP, PADP-PCWP, CI, SV, LVSWI, SVR, PVR, FiO_2, PaO_2/FiO_2, SvO_2, Qs/Qt, and DO_2. The data of Group B take an intermediate position between survivors and late-stage nonsurvivors. Clinically important differences between Groups A and B are only in CI, PVR, and DO_2, and between Groups B and C in PaO_2/FiO_2, SvO_2 and Qs/Qt.

To determine the prognostic role of oxygenation parameters in ARDS, the following variables were entered into the discriminant analysis: VO_2, DO_2, O_2ER, SvO_2, Qs/Qt and PaO_2/FiO_2. Taking Groups A and B, DO_2 and PaO_2/FiO_2 were included into the analysis with mean classification value of 68% (Table II). Using the same parameters, the discrimination between Groups A and C was higher: 75%. The result was similar in Groups B and C (mean classification value: 74%), but the involved parameters changed for Qs/Qt, PaO_2/FiO_2. In the next step, all of the examined parameters were entered into the analysis (Table III). Analysing the data of Groups A and B the discriminating variables (CI, PVR, PaO_2/FiO_2) changed and their average discriminant function increased to 74%. The best result was achieved between Groups A and C (Fig. 1). The involved CI, PADP-PCWP and PaO_2/FiO_2 variables correctly classified the data in 80%. The discrimination between Groups B and C was 72% and the included parameters changed for SVR, VO_2 and PaO_2/FiO_2. Finally, the prognostic function of the different potentianally

Table I

Summarized cardiorespiratory data of survivors (Groups A), early stage nonsurvivors (Group B), and late stage nonsurvivors (Group C)[a]

Variables	Units	Group A n=40	Group B n=37	Group C n=89
MSAP	mmHg	108 ± 15	102 ± 16	100 ± 19*
MPAP	mmHg	23 ± 8	26 ± 7*	28 ± 8***
PCWP	mmHg	11 ± 5	12 ± 4	11 ± 4
CVP	mmHg	5 ± 2	5 ± 3	5 ± 3
PADP-PCWP	mmHg	3 ± 4	5 ± 3**	6 ± 4$^{***}_{o}$
CI	l/min/m^2	4.3 ± 1.2	3.4 ± 0.8***	3.5 ± 1.1***
SV	ml	78 ± 20	68 ± 14*	57 ± 23$^{***}_{o}$
LVSWI	gxm/m^2	58 ± 15	50 ± 20*	43 ± 20***
RVSWI	gxm/m^2	9 ± 3	9 ± 4	9 ± 4
SVR	dynxsec/cm^5	1146 ± 358	1361 ± 305**	1395 ± 452**
PVR	dynxsec/cm^2	150 ± 102	233 ± 110***	272 ± 150$^{***}_{o}$
RPP	mmHg/min/1000	15.1 ± 3.5	13.6 ± 3.5	13.4 ± 3.6*
FiO_2		0.5 ± 0.1	0.5 ± 0.2	0.7 ± 0.2$^{***}_{oo}$
PaO_2/FiO_2	mmHg	326 ± 92	339 ± 99	216 ± 106$^{***}_{ooo}$
SvO_2	%	81 ± 9	81 ± 7	76 ± 10$^{***}_{ooo}$
Qs/Qt	%	18 ± 10	18 ± 10	28 ± 15$^{***}_{ooo}$
DO_2	ml/min/kg	17.2 ± 5.0	13.5 ± 4.0***	13.9 ± 4.0***
O_2ER	%	20.5 ± 8.0	20.6 ± 7.0	23.3 ± 9.0
VO_2	ml/min/kg	3.3 ± 1.3	2.7 ± 1.1*	3.2 ± 1.2

[a]Data are expressed as mean ±S.D. Asterisks (*) indicate significant differences between Group A and Group B, or Group A and Group C. Circles (o) indicate significant differences between Group B and Group C.

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{o}P < 0.05$; $^{oo}P < 0.01$; $^{ooo}P < 0.001$

Table II

Classification results of oxygenation parameters

Actual groups	n	Predicted group membership /%/ (1)	(2)	Parameters included
Group A (1)	40	26 /65/	14 /35/	DO_2
Group B (2)	37	11 /30/	26 /70/	PaO_2/FiO_2
Group A (1)	40	31 /78/	9 /22/	DO_2
Group C (2)	89	23 /26/	66 /74/	PaO_2/FiO_2
Group B (1)	37	26 /70/	11 /30/	Qs/Qt
Group C (2)	89	22 /25/	67 /75/	PaO_2/FiO_2

Table III

Classification results of cardiorespiratory parameters

Actual groups	n	Predicted group membership /%/ (1)	(2)	Parameters included
Group A(1)	40	29 /73/	11 /27/	CI, PVR
Group B (2)	37	9 /24/	28 /76/	PaO_2/FiO_2
Group A (1)	40	33 /83/	7 /17/	CI, PADP-PCWP
Group C (2)	89	19 /21/	70 /79/	PaO_2/FiO_2
Group B (1)	37	25 /68/	12 /32/	SVR, VO_2
Group C (2)	89	23 /26/	66 /74/	PaO_2/FiO_2

Table IV

Classification results of potentionally noninvase parameters

Actual groups	n	Predicted group membership /%/ (1)	(2)	Parameters included
Group A (1)	40	24 /60/	16 /40/	CI, VO_2
Group B (2)	37	8 /22/	29 /78/	PaO_2/FiO_2
Group A (1)	40	33 /83/	7 /17/	CI
Group C (2)	89	19 /21/	70 /79/	PaO_2/FiO_2
Group B((1)	37	25 /68/	12 /33/	Qs/Qt
Group C (2)	89	23 /26/	66 /74/	PaO_2/FiO_2

Standardized Canonical
Discriminant Function
Coefficients:

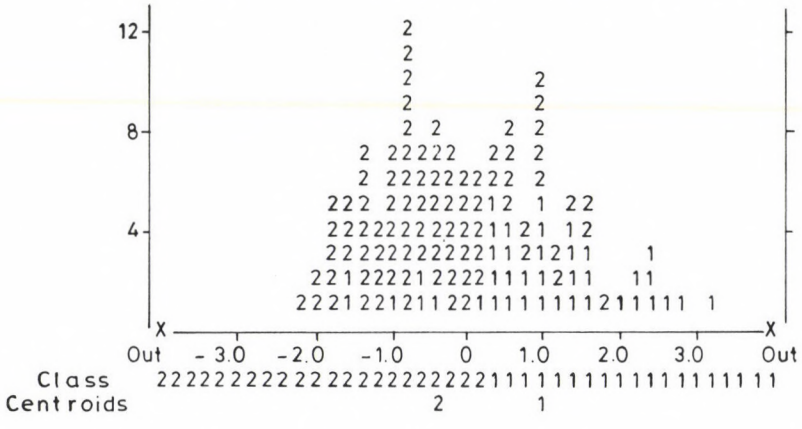
	FUNC 1
CI	56750
PaO2/FiO2	.74736
PADP-PCWP	-.18552

Group Centroids:

Group 1	.960
Group 2	-.431

All-groups stacked Histogram

Canonical Discriminant Function 1



Group 1 = Survivors, /Group A/
Group 2 = Late stage nonsurvivors /Group C/

Fig. 1. The result of the discriminant analysis in respect of survivors and late-stage nonsurvivors involving all of the examined cardiorespiratory parameters

noninvasive measurable parameters was analysed (Table IV). The VO_2, CI, Qs/Qt and PaO_2/FiO_2 were taken as noninvasive parameters. The ratio of correctly classified data was 69% between Groups A and B and the included parameters were CI, VO_2, and PaO_2/FiO_2. Between Groups A and C, CI and PaO_2/FiO_2 correctly discriminated the data in 80%. The result of the discriminant analysis between nonsurvivors (Groups B and C) was correct in 72%, included Qs/Qt and PaO_2/FiO_2 into the analysis.

Discussion

Several cardiorespiratory variables of survivors and nonsurvivors were found to be significantly different in the patients with ARDS. Due to the random sampling, we failed to continuously follow the changes of the different parameters, in contrast with temporal prognostic analysis of others /2, 24/. However, assessment of the patients into three groups (survivors, early and late stage nonsurvivors) resulted a good selection of the nonsurvivors, too. In both groups of nonsurvivors, the flow related parameters changed primarily (CI, SV, LVSWI, DO_2 decreased and PVR, SVR increased). This is a typical feature of nonsurvivors in ARDS and other diseases /3, 5, 9, 16, 27, 28/. In the late stage of nonsurvivors, a significant pulmonary hypertension and an impaired oxygenation developed which were characterized by decreased PaO_2/FiO_2, SvO_2 and increased FiO_2, Qs/Qt. High PAPD-PCWP gradient was measured in Group C, similarly to the results of Sibbald et al. /21/, that may indicate a fatal outcome. The VO_2 was maintained by an increase in O_2ER. These changes are universal hallmarks of ARDS /15, 28, 30/, nevertheless their prognostic role is ambiguous because they discriminate survivors and nonsurvivors only in late stage. Our discriminant analysis, using inhomogeneous distribution of data base, resulted in a detachment of 70-80% probability, which is closely comprable to the results of others /2, 13, 20, 25/. The most important variable was PaO_2/FiO_2 which took part in the discrimination of each group. Langhi et al. /12/ emphasize the importance of a similar respiratory index (alveoloarterial oxygen gradient normalized by PaO_2) in the prognosis of posttraumatic ARDS. Their study demonstrated that the relationship between respiratory index and Qs/Qt was significantly increased in cases of fatal ARDS, compared with those who did not develop ARDS, or with those whose ARDS resolved. In our study Qs/Qt played a discriminant role only in the data of early and late stage nonsurvivors. To discriminate between survivors and nonsurvivors, in addition to PaO_2/FiO_2, CI or its closely related parameter DO_2 was included into the analysis, both reinforcing the early prognostic role of CI /3, 16, 27, 28/. The best result was achieved by analysing the data of survivors and late stage of nonsurvivors. The prognostic probability was 80% when either of the noninvasively measurable parameters alone or the extended cardiorespiratory variables were used. In the latter case the PAPD-PCWP and, analysing the data of survivors and early stage nonsurvivors, the PVR were involved into the discrimination

with regard to the significant role of pulmonary circulation in the development of ARDS /21, 25, 28/.

We should like to emphasize that the results of the applied discriminant analysis based on an occasionally pooled inhomogeneous data base, were similar to the conclusions drown by others, when the regularly measured parameters /2, 9, 13, 17/ were applied. Namely, Qs/Qt, PVR, PAPD-PCWP gradient, CI and PaO_2/FiO_2 were the most important variables in view of the outcome.

Contrary to the early suggestion that VO_2 was an important discriminator between survivors and nonsurvivors /2, 4, 6, 17, 22, 29/, in our discriminant analysis VO_2 was entered only in two occasions: in discriminating data of early- and late-stage nonsurvivors using potentionally noninvasive parameters, and in making discrimination between survivors and early-stage nonsurvivors using all cardiorespiratory parameters. In an earlier study we compared the tissue oxygenation parameters (VO_2, DO_2, O_2ER) of survivors and nonsurvivors /8/. Using 3-dimensional representation method /7/ we could separate survivors and late stage nonsurvivors at 73% probability, which is equivalent to the present result of the discriminant analysis of the oxygenation parameters.

It was an interesting observation that data, potentially measurable with noninvasive techniques, did not decrease the prognostic value of survival, compared to the all invasively measured cardiorespiratory variables. Monitoring VO_2 by direct measurement in respirated patient, the PaO_2/FiO_2 by blood gas analysis, the Qs/Qt by inert gas method or blood gas analysis, the CI by several noninvasive method (bioimpedence /1, 15, 18/, echocardiography /14/, oesophageal Doppler /23/, systolic time intervals /26/) may allow a continuous supervision without any risk of the right heart catheterization. The PC-processed data provide enough information for early interventions to correct dysfunctions and to correctly estimate the prognosis in 80% of the cases. Application of this method may decrease the frequency of the indwelling pulmonary arterial catheters.

REFERENCES

1. Appel, P.L., Kram, H.B., Mackabee, J., Fleming, A.W., Shoemaker, W.C.: Comparison of measurements of cardiac output by bioimpedance and thermodilution in severely ill surgical patients. Crit. Care Med. 14, 933-935 (1986)

2. Bland, R.D., Shoemaker, W.C.: Probability of survival as a prognostic and severity of illness score in critically ill surgical patients. Crit. Care Med. 13, 91-95 (1985)

3. Bland, R.D., Shoemaker, W.C., Abraham, E., Cobo, J.C.: Hemodynamic and oxygen transport patterns in surviving and nonsurviving postoperative patients. Crit. Care Med. 13, 85-90 (1985)

4. Carlsson, M., Nordenström, J., Hedenstierna, G.: Clinical implications of continuous measurement of energy expenditure in mechanically ventilated patients. Clin. Nutr. 3, 103-112 (1984)

5. Czer, L.S.C., Appel, P., Shoemaker, W.C.: Pathogenesis of respiratory failure after hemorrhage and trauma: II. Cardiorespiratory patterns after development of ARDS. Crit. Care Med. 8, 513-518 (1980)

6. Dhan, M.S., Lange, P.: Hormonal changes and their influence on metabolism and nutrition in the critically ill. Intensive Care Med. 8, 209-213 (1982)

7. Gondos, T.: 3-dimensional presentation of tissue oxygenation parameters. Acta Physiol. Hung. 76, 301-304 (1990)

8. Gondos, T., Kiss, P., Pénzes, I., Kulka, F.: Estimation of the optimal tissue oxygenation parameters in ARDS. Acta Physiol. Hung. 77, 121-127 (1991)

9. Hankeln, K.B., Senker, R., Schwarten, J.U., Beez, M.G., Engel, H.J., Laniewsky, P.: Evaluation of prognostic indices based on hemodynamic and oxygen transport variables in shock patients with adult respiratory distress syndrome. Crit. Care Med. 15, 1-7 (1987)

10. Houtchens, B.A., Eestenskow, D.R.: Oxygen consumption in septic shock: collective review. Circ. Shock 13, 361-384 (1984)

11. Knaus, W.A., Zimmerman, J.E., Wagner, D.P., Draper, E.A., Lawrence, D.E.: APACHE-acute physiology and chronic health evaluation: A physiologically based classification system. Crit. Care Med. 9, 591-596 (1981)

12. Laghi, F., Siegel, J.H., Rivkind, A.I., Chiarla, C., DeGaetano, A., Blevins, S., Stoklosa, J.C., Borg, U.R., Belzberg, H.: Respiratory index/pulmonary shunt relationship: Quantification of severity and prognosis in the post-traumatic adult respiratory distress syndrome. Crit. Care Med. 17, 1121-1128 (1989)

13. van Lanschot, J.J.B., Feenstra, B.W.A., Vermeij, C.G., Bruining, H.A.: Outcome prediction in critically ill patients by means of oxygen consumption index and simplified acute physiology score. Intensive Care Med. 14, 44-49 (1988)

14. Mitaka, C., Naugra, T., Salianish, N., Tsunoda, Y., Amaha, K.: Two-dimensional echocardiographic evaluation of inferior vena cava, right ventricle, and left ventricle during positive-pressuve ventilation with varying levels of positive end-expiratory pressure. Crit. Care Med. 17, 205-210 (1989)

15. Salandin, V., Lussa, C., Risica, G., Michielon, C., Paccaquella, A., Lipolotto, G., Simini, G.: Comparison of cardiac output estimation by thoracic electrical bioimpedance, thermodilution and Fick methods. Crit. Care Med. 16, 1157-1158 (1988)

16. Shoemaker, W.C., Appel, P.L.: Pathophysiology of adult respiratory distress syndrome after sepsis and surgical operations. Crit. Care Med. 13, 166-172 (1985)

17. Shoemaker, W.C., Appel, P.L., Bland, R., Hopkins, J.A., Chang, P.: Clinical trial of an algorithm for outcome prediction in acute circulatory failure. Crit. Care Med. 10, 390-397 (1982)

18. Shoemaker, W.C., Appel, P.L., Kram, H.B., Nathan, R.C., Thompson, J.L.: Multicomponent noninvasive physiologic monitoring of circulatory function. Crit. Care Med. 16, 482-490 (1988)

19. Shoemaker, W.C., Chang, P., Bland, R., Czer, L., Shabot, M.M., Clifton, J.F.: Cardiorespiratory monitoring in postoperative patients: II. Quantitative therapeutic indices as guides to therapy. Crit. Care Med. 7, 243-249 (1979)

20. Shoemaker, W.C., Chang, P., Czer, L., Bland, R., Shabot, M.M., State, D.: Cardiorespiratory monitoring in postoperative patients: I. Prediction of outcome and severity of illness. Crit. Care Med. 7, 237-242 (1979)

24. Sibbald, W.J., Paterson, N.A.M., Holliday, R.L., Anderson, R.A., Lobb, T.R., Duff, J.H.: Pulmonary hypertension in sepsis: Measurement by the pulmonary arterial diastolic-pulmonary wedge pressure gradient and the influence of passive and active factors. Chest 73, 583-591 (1978)

22. Siegel, J.H., Greenspan, M., del Guercio, L.R.M.: Abnormal vascular tone, defective oxygen transport and myocardial failure in human septic shock. Ann. Surg. 165, 504-517 (1967)

23. Singer, M., Clarke, J., Bennett, E.D.: Continuous hemodynamic monitoring by oesophageal Doppler. Crit. Care Med. 17, 447-452 (1989)

24. Smith, P.E.M., Gordon, I.J.: An index to predict outcome in adult respiratory distress syndrome. Intensive Care Med. 12, 86-89 (1986)

25. Stürm, J.A., Lewis, F.R., Trenzt, O., Oestern, H.J., Hempelman, G., Tscherne, H.: Cardiopulmonary parameters and prognosis after severe multiple trauma. J. Trauma 19, 305-316 (1987)

26. Szabó, K., Takács, S., Pick, R., Siposs, T., Kempler, P., Novák, J.: Systolic time intervals in burn injury. Acta Physiol. Hung. 69, 51-69 (1987)

27. Tuchschmidt, J., Fried, J., Swinney, R., Sharma, O.M.P.: Early hemodynamic correlates of survival in patients with septic shock. Crit. Care Med. 17, 719-723 (1989)

28. Villar, J., Blazquez, M.A., Lubillo, S., Quintana, J., Manzano, J.L.: Pulmonary hypertension in acute respiratory failure. Crit. Care Med. 17, 719-723 (1989)

29. Wilson, R.F., Christensen, C., Le Blanc, L.P.: Oxygen consumption in critically ill surgical patients. Ann. Surg. 176, 801-804 (1972)

30. Zapol, W.M., Snider, M.T.: Pulmonary hypertension in severe acute respiratory failure. N. Engl. J. Med. 296, 476-480 (1977)

Acta Medica Hungarica, Vol. 48/1-2, pp. 61-72 (1991)

99-m TECHNETIUM (Dupont Cardiolite) INVESTIGATIONS IN POSTINFARCTION PATIENTS WITH HOLTER-CHECKED SILENT ISCHAEMIA

M. HORVÁTH, E. BÖSZÖRMÉNYI, M. KÁRMÁN, I. BERÉNYI, J. MOLNÁR, Á. PSZOTA, M. SZALAI, L. NÉMETH

State Hospital for Cardiology, Balatonfüred, Hungary

(Received: February 20, 1991)

Sixteen middle-aged, normotensive, slightly overweight male patients with previous myocaridal infarction were studied during Holter-checked silent myocardial ischaemia. As reference, stress and late 201-Tl scintigraphy served for comparison with Cardiolite-MIBI silent ischaemic perfusion scan, both carried out in planar mode. The circumferential profiles differed in 9 cases, on region of interest basis the segment number difference was 10, but the late distribution segment number was near to both ischaemic numbers. The quantitative scores were distinctive (ratio 133-128/103) indicating the silent ischaemia appeared in the peri-infarct area. The silent ischaemic MIBI and stress 201-Tl ischaemic score difference was reduced by means of repeated SPECT investigation. With gated radionuclide ventriculography there was -4.3% difference between the left ventricular ejection fractions, measured with first pass MIBI technique during silent ischaemia and afterwards in basal state. The impairment of the left ventricular function was reflected on the stroke pattern of our Holter-based radiocyclogram, as well. Taking the 43.7-48.0=-4.3% "ischaemic shift" into consideration it was a close correlation (r=0.90) between the two kinds of ejection fraction determination. The major rhythm failures (occurring during the 24 h Holter monitoring) decreased to a higher degree the left ventricular ejection fraction than silent ischaemia or silent ischaemia and minor rhythm failure together (38-42-50%).

Keywords: Holter-controlled silent ischaemic periods on postinfarction patients, 99m-Tc methoxy-isobutyl-isonitrile investigations, myocardial perfusion, left ventricular ejection fraction, radiocyclographic contraction dynamic analysis

---

Abbreviations: MIBI: Cardiolite methoxy-isobutyl-isonitrile; RNV: 99m-Tc radionuclid ventriculography; LV-EF: left ventricular global ejection fraction; p-MI SMI: silent ischaemia on postinfraction patients; Tl: thallium

Offprint requests should be sent to: M. Horváth, H-8231 Balatonfüred, P.O. Box 13, Hungary

Akadémiai Kiadó, Budapest

Introduction

It is a fundamental fact that the (painful) angina is preceeded by myocardial perfusion imbalance producing electrophysiological events, i.e. ECG-changes /14/. Holter monitoring shed light yet on more types of ischaemia episodes. The painless form is called silent myocardial ischemia (SMI) /22/.

SMI can occur in asymtomatic individuals, in patients with angina pectoris and after myocardial infarction (p-MI SMI) /4/. The hypothesis that the p-MI SMI is a special entity has been refuted /26/.

One of the main trends of the SMI research is to compare the ratio of the ST-segment changes during stress and Holter monitoring. It is very important to define the SMI criteria from the clinical as well as technical point of view correctly, for SMI diagnosis depends on the correct performance of the test (> 0.1 mV ST-depression 80 ms after the point J for more than 30 S /25/.

The diagnosis is supported by the VEST-like nuclear probes combined with Holter-monitoring, estimating together with the ST-change the reduction of the stroke volume and of the LV-EF during SMI /19, 27/. Convincing evidence can be a new or extending myocardial perfusion defect during SMI, but the simultaneous determinations are rarely feasible. What can be performed, it is the detection of the perfusion defect at all, carried out by different radiopharmaca. 201-Tl is the classical radiopharmacon. More recently the new isonitrile derivative RP-30 Cardiolite methoxy-isobutyl isonitrile (MIBI) has proved to be useful, too /15/. MIBI gives two preferences: 1) being non-redistributive radiopharmacon, it holds "frozen" the myocardial perfusion situation prevailing the SMI period /8/, 2) MIBI is suitable for measuring at the same time the myocardial perfusion and the left-ventricular function /13/. If PET (=positron emission computer tomograph) is available, 18-F deoxyglucose may be considered the best ischaemic marker /2, 21/ and important information can still be got about the aerobe metabolism of the heart by 11-C acetate as well /1/.

Our clinical goal was to detect SMI myocardial perfusion of a small Holter-cheked postinfraction group with MIBI in comparison with 201-Tl stress myocardial perfusion distribution, moreover, to compare the SMI LV-EF with basal LV-EF as well, in planar presentation.

Patients and Methods

Sixteen middle-aged patients were selected from our postinfarction rehabilitation cataster and studied with their informed consent after permission of the competent ethical commission. The patients aged between 36 and 58 years (average, 51 years), they were slightly overweight (body mass excess: 10-12 kg), and normotensive. Infarct localisations: non-Q:2, posterior:3, inferior:4, anteroseptal:5 and extensive anterior:2. All had undergone previous combined Holter-monitoring and 113m-In radiocardio-cyclography at about 15 min lasting silent ischaemia.

The injected radioactivities were: 25mCi 99m-Tc MIBI, 12mCi 99m-Tc RNV, 2mCi 201-Tl. The labelling yield of 99m-Tc MIBI was 95%. Clinical side effect of MIBI was not observed and at all.

We used 24 h Holter monitor of the type Marquette Laser 8000/T.

On the casette of this device we developed a special combined beat-to-beat ECG and radiocyclogram analogue technique /5/ to synchronize the informations got by the radiopharmacon, injected even in the SMI period. The Holter tape continued after the SMI event served for further analysis, beside the ST changes it checked rhythm failures. The SMI lasted about 15 min, presenting more or less ST-changes and 1 monofocal and 1 polymorphous ventricular extrasystole as well. Minimal SMI criterion was ST depression somewhat more than 0.1 mV, but three major 0.2mV ST-depressions also occurred at the end of observation, joined with slight oppression. Major arrhythmia criterion meant short runs, ventricular tachycardia or, in 1 case, fibrillation, polymorphous ventricular extrasystole, while minor arrhythmias were Lown I-II grade rhythm failures, mostly monomorphous, unifocal extrasystole. Except the two extrasystoles during the SMI period, the rhythm failures were fixed on the 24 h Holter tape.

The MIBI myocardial perfusion examination followed 1 h post injection, the ergometric stress Tl-scan and gated 99m-red blood cell RNV in the subsequent week.

For SMI MIBI LV-EF determination, we used necessarily first pass LV-EF technique /20/.

Nuclear cardiological imaging was carried out on a computer-assisted scintillation gamma camera (Gamma Works, Budapest MB 9100-9101), using mostly the softwares elaborated by The Medical School of Nuclear Medicine, Szeged (including circumferential profiles of myocardial perfusion MIBI and Tl, moreover the gated RNV programme). For more precise quantification we used region of interest (ROI) sporing: 4 most severe hypoperfusion defects, 0 normal situation. From each of AP, LAO 45° and LAO 70° directions 4 segments were separated, but because of 2 common regions in 2 directions, the individual segment numbers were only 10, instead of 3 directions x 4 segment = 12 individual character. Typical left coronary supply dominance being considered, the anterior, the antero-lateral, the infero-apical, the proximal and distal septal segments belong to the left anterior descending (LAD), the septal+inferior, the infero-diaphragmatic and the posterobasal to the right coronary supply, while the posterobasal is a circumflex (CX) area. This topography helped us to fasten the ischaemic reaction to the infarct-related culprit artery.

For clarification of MIBI and TL myocardial perfusion segment and score discrepancies, we requested 7 patients for SPECT examination, which has carried out with Gamma Works MB 9300 gantry and with Ketronic-Siemens Medax N tomo-programme.

Statistical evaluation was not possible because of the small number of the patients.

Results

For demonstration of our technical facilities, we present Figs 1, 2, 3.

The myocardial perfusion data of the 16 p-MI SMI patients are summarized with MIBI and Tl, during SMI and stress (Table I), moreover for comparison the late 3 h Tl redistribution data.

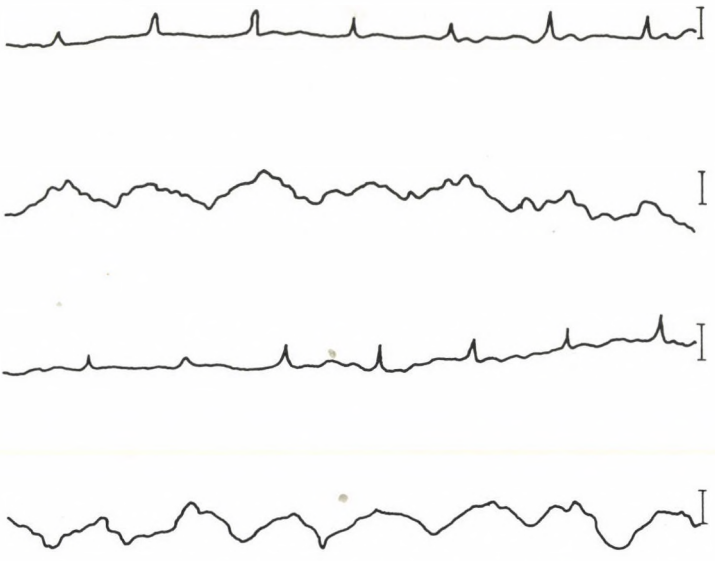


Fig. 1. Holter completed with beat to beat radiocyclographic programme, developed in Balatonfüred. Top: J. Caunt miniaturized CsI/Tl detector with preamplifier and without photomultiplier tube(PMT); bottom: our old RCG-system with collimated NaI/Tl scintillation crystal

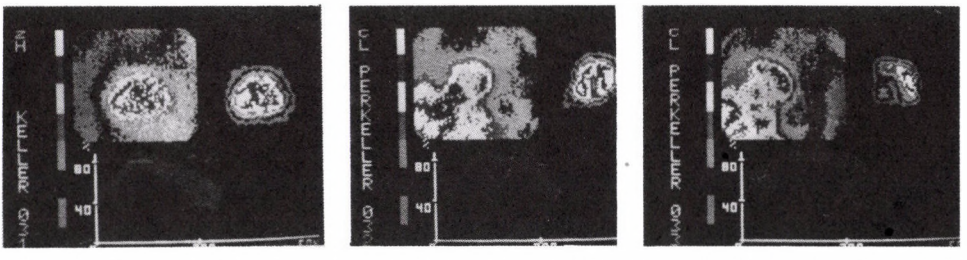


Fig. 2. 99m-Tc Cardiolite myocardial perfusion scan. The back part of the circumferential profile 033 on low level under the normal scatter corresponds largerly to inf. post. region. The defect arises partly from postinfarction scar, partly from ischaemic hypoperfusion

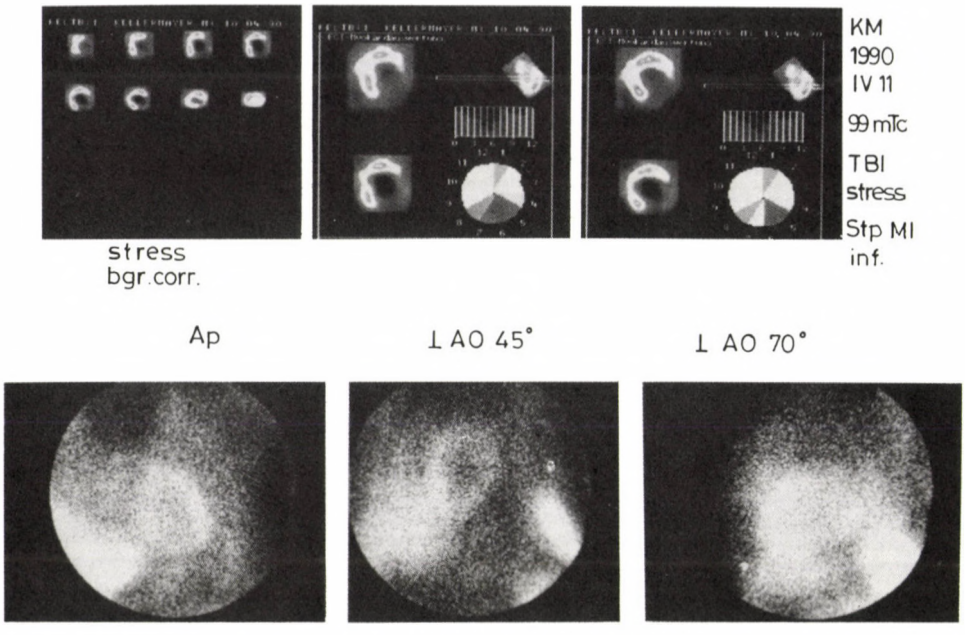


Fig. 3. Comparative planar (bottom) and SPECT (top) pictures on the same patient as on the Fig. 2 with TBI (tertiary butyl isonitrile). The transaxial short slices, their histograms and circle-segments unequivocally present the large defect in the inf. post, region

Table I

Summarized myocardial perfusion data of 16 postinfarction PTS

(2 non-Q, 3 post., 4 inf., 5 ant. sept., 2 ext. ant.)

	Hypoperf. segment Nr.	Hypoperf. defect score
Holter-controlled SMI Cardiolite	49	133
Ergometric stress 201-Tl	44	128
3 h late redistributive 201-Tl	42	103

SMI MIBI and stress Tl segment number difference - 10 but because of 8 MIBI>Tl and 2 Tl > MIBI cases the final result is only 49/44

The direction of the SMI ST change was elevation only in 1 case, it was depression in all others (both in major and minor cases). Major ST depressions occurred in ext. post., post. and inf. patients, major arrhytmias in 1 post., 1 inf., 3 ant., septal and 1 ext. ant. patients. The majority of the massive defects came from the ant. sept. and ext. ant. group. Discrepancies between the SMI MIBI and stress Tl segment numbers were 10, including 1 ext. ant., 3 ant. sept., 1 non-Q, 3 post. and 2 inf. localisations. Being the affectued segments MIBI > Tl 8, Tl > MIBI 2 cases, therefore, is only 49/44 ratio.

133-128=5 SMI and stress Tl score differences spread among the segments. The MIBI incongruence diminished on SPECT in the inferior localisation and, what is more, with tertiary-butyl-isonitrile (TBI). The disturbing factor by the isonitrile investigations is the liver-activity overlap on the lower heart regions. The SPECT correction with TBI is convincing because the TBI liver kinetics is slower than that of MIBI.

The non-ischaemic Tl segment number, 42, is very near to the ischaemic value and, at the same time, there exists a significant score difference: 128-128/103, which means that the two kinds of ischaemia appeared mostly in the peri-infarct area.

The evaluation of the LV-EF followed two aspects: a) according to the Holter-ischaemia and rhythm data, b) according to the p-MI localizations. The average LV-EF during SMI detected with the first pass technique was lower than in non-ischaemic "basal state" detected with gated RNV. Otherwise, the LV-EF was mainly affected in patients who had major arrhythmia during continuous Holter monitoring. Their localizations was mostly ext. ant. and ant. sept. (Table II).

The infarct localization of the major arrhythmic patients was mostly ext. ant. and ant. sept. The LV-EF of mixed minor ischaemic and minor arrhythmic group shared between severe arrhythmia and the major ST-depression group (38.5-42-50%). The minor ST-depressions occurred, surprisingly, among the post. inf. patients (true, their LV-EF was only slightly diminished).

In each localisation group, there existed an "ischaemic shift", with overall -4.3% SMI Δ . Regression equation was obtained with r=0.90 (Table III).

$$EF_{SMI,MIBI-FP} \cdot 0.88 + 9.5 = EF_{REST\ GATED\ RNV}$$

Table II

Left ventricular ejection fraction (EF%)

	SMI first pass MIBI	Gated RNV basal
SMI ST↓< 0.1 mV minor rhythm failure	42	47
SMI ST- ↓ 0.2 mV+	50	55
Maior rhythm failure 24 h	38.5	42

+To this groups belonged post. inf. localization

Table III

Left ventricular ejection fraction (EF%)

		SMI first pass MIBI	Gated RNV basal
Infarct localization			
2	Non - Q	57	61
3	Posterior	47	50
4	Inferior	49	53
5	Anteroseptal	36	42
2	Extensive anterior	29	35.5
		- 4.3%	
		43.75 ± 9.5	48.0 ± 8.6
		Controls 66.62%	

$EF_{FB-MIBI}$. 0.88 + 9.5 = Gated RNV basal

correl. coeff. r = 0.90

In other group of lo p-MI SMI patients who belonged to NYHA III-IV severity, as markers of the left ventricular function, the fast and mean ejection, the fast and mean filling velocities were found significantly decreased already at rest and their radiocyclographic pattern was similar to those detected during SMI monitoring.

We tested also milder p-MI SMI cases (NYHA I-II severity groups) during ergometric dynamic, handgrip isometric and arithmetic psychological stress. Being our radiocyclographic technique quite individual, we do not give absolute values, only scatter grade: $P < 0.05$ (Table IV).

Our different exercise data are in congruence with the observations carried out on coronary heart disease patients and control subjects with VEST /12, 24/.

Table IV

Postinfarction silent myocardial ischaemic patients radiocyclographic (RCG) contraction dynamics times and interval ratios

	Stress	Handgrip	Psychological test
Cycl. time	s	s	s
Systole p.c.	s	s	ns
Diastole p.c.	s	s	s
Fast filling/diast.	ns	ns	ns
Fast/slow ejection	ns	ns	ns
Blood pressure	s	s	ns
Patients belonged to the:	1st severity NYHA I-II	2nd severity NYHA II	3rd severity NYHA II

Postinfarction silent myocardial ischaemic patients

10 - 10 - 10 were selected in 3 groups

Ergometric stress	forced handgrip	bearing 1x1
30 - 60 - 90 V	endurance	psychological test
1st severity NYHA I-II	2nd severity NYHA II	2nd severity NYHA II

Radiocyclographic (RCG) contraction dynamics velocities

	Stess	Handgrip	Psychological test
Blood pressure	s	s	
Mean ejection	s	ns	ns
Maximal ejection	s	s	ns
Mean filling	ns	ns	ns
Maximal filling	s	s	ns
Fast filling	ns	s	ns
Slow filling	ns	ns	ns
Cycle time	s	s	s

Contraction dynamics analysis of p-MI SMI patients at t rest. and during different multi-tools.

s: significant $P < 0.05$

ns: non-significant

Discussion

The feasibility of the first-pass LV-EF determination has been convincingly verified /10, 13/. Our data about the derangement of the left-ventricular function agree with the literature, mostly with non-imaging technique-detected impairment during short-ischaemic periods /24, 27/. The examination of the left-ventricular function during SMI can be extended, of course, on the regional wall motion analysis, too /8/. Our observation that the LV-EF was mostly affected in p-MI SMI patients with major rhythm disorder, correlates with less favourable prognosis of SMI patients with ventricular ectopy /22/.

The situation with the myocardial perfusion analysis is a more complicated problem. First of all, in MIBI publications the time relation of the injection to SMI, and the character of SMI as grade, duration, etc. are not discussed. Comprehensive study was dealing with the methodological aspects of symptomatic and silent myocardial ischaemia, including high-resolution surface electrocardiography among others /6/.

The conclusion is that both Tl and MIBI SPECT can be used. With planar scan the scatter is wider, compared to the more objective SPECT distribution images /23/. Only concordant positive or negative SPECT and ST-changes can be accepted in scientific publications. In our study the 16 positive Tl-scans served for reference, major ST-changes were 3, minor ones 12 and stress ST-depressions were more than 0.2 MV in number 2. It is worthy of note that in normal subjects the perfusion increment in exercise is at least 2.1 times that of rest perfusion /18/. The perfusion scan can be carried out also in combination with dipyridamole infusion /16/, helping to a better understand the underlying by means of dilatative and, in severe stenotic cases, steal-producing effect.

The myocardial perfusion determining factor in the peri-infarct area, in the region of infarct-related culprit artery is, without doubt, the collateral circulation. It depends rather on the absence of collaterals and duration of occlusion than on the extent of the ischaemic myocardium /17/. In patients with good collaterals, stress-induced transient enlargement of the perfusion defect occurs in those with poor collaterals, however, the enlargement did not occur /7/.

99m-Tc MIBI and 201-Tl correlated well in both planar and SPECT images /11/, in respect to the identification of patients with coronary artery disease affecting the individual arteries, the presence and severity

of perfusion defects and the assessment of defect reversibility. The precise quantification of confluent scar and ischaemic areas in patients with prior myocardial infarction is a hard task and it needs special bull's eye technique and mathematical-statistical apparatus as receiver operation characteristic (ROC) method /3/. ROC improved cut specificity for detection in the LCX (40%) and in RCA (20%) territories, having no effect in 12% LAD. The 201-Tl SPECT was more accurate for detection of CAD in the noninfarct region than the clinical and/or stress ECG responses.

Our planar scans were fixed in 64x64 matrix and evaluated circumferentially from 3 directions and 4 segment/directions. In 128x128 matrix and SPECT, the segment scores could be somewhat different.

As we also found, some plus information for MIBI (in contrast with Tl) could be exist independently of detection problem, since the reversible hypoperfused segments were detected 104 with 201-Tl and 134 with MIBI SPECT.

That the myocardial perfusion can assume during long-lasting SMI in the peri-infarct zone ischaemic character is logical on the base of several common electrophysiological issues. The question remains, however, statistically open.

Acknowledgement

We have to thank the Dupont for the free-of-charge Cardiolite MIBI kits.

REFERENCES

1. Allen, R., Pike, V., Maseri, A., Selwyn, A.: Myocardial metabolism of 11-C acetate. Experimental and patients studies. Circulation 64, IV-75. (1981)

2. Ahonen, A., Viopio-Pulkki, L.M., Ruotsalainen, U., Parviainen, S., Haaparanta-Solin, M., Korkeila, P., Vurkki, R., Wegelius, U.: Assessment of myocardial viability in patients with severe angina. A comparative study using Tc-99m MIBI and SPECT and F-18 FDG PET. Eur. J. Nucl. Med. 15, 447. (1989)

3. Chouraqui, P., Maddahi, J., Ostrzega, E., Van Train, K., Charuzi, Y., Pringent, F., Berman, D.S.: A new approach to quantitate thallium-201 rotational tomography (SPECT) for evaluation of patients with prior myocardial infarction. 3rd Intern. Conf. on Non-invasive Cardiology, Tel Aviv, p. 28. (1990)

4. Deedwania, P.C.: Transient myocardial ischaemia in survivors of acute myocardial infarction. In: Bramah, N. Singh: Silent myocardial ischaemia and angina. Chapter 14. Pergamon Press (1988)

5. Horváth, M., Németh, L., Kántor, E., Kiss, J., Bócsa, Z., Kármán, M., Szieberth, P.: New contribution to the development of nuclear stethoscope technics. 3rd Internat. Conference on Non-invasive Cardiology, Tel Aviv, p. 24. (1990)

6. Hombach, V., Clausen, M., Osterhues, H.H., Göller, V., Grossmann, G., Peper, A., Eggeling, T., Höher, M., Ost, W., Kochs, M., Henze, E., Adam, W.E.: Methodological aspects of detecting patients with symptomatic and silent myocardial ischaemia. In: Kellermann, J. J., Braunwald, E. (eds) Advances in cardiology 37. pp. 76-95. Silent myocardial ischaemia: a critical appraisal, 1970

7. Imamura, T., Araki, H., Fukuyama, T., Maruoka, Y., Ootsoubo, H., Nakamura, N., Koiwayo, Y., Tanaka, K.: Significance of collateral circulation on periinfarction zone. Assessment with stress thallium-201 scintigraphy. Clin. Card. 9, 137-144 (1986)

8. Iskandrian, A.S., Heo, J., Kong, B., Lyons, E., Marsch, S.: Use of technetium-99m isonitrile (RP30a) in assessing left ventricular function at rest and during exercise in coronary artery disease and comparison with coronary arteriography and exercise thallium-201 SPECT imaging. Amer. J. Cardiol. 64, 270-275 (1989)

9. Kahn, J.K., McGhie, Akers, M., Sills, M.N., Faber, T.L., Kulkarni, P.V., Willerson, J.T., Corbett, J.R.: Quantitative rotational tomography with 201-Tl and 99m-Tc 2-methoxy-isobutyl-isonitrile. A direct comparison in normal individuals and patients with coronary artery disease. Circulation 79, 1282-1292 (1989)

10. Kaul, S., Boucher, C.A., Okada, R.D., Newell, J.B., Strauss, H.W., Pohost, G.M.: Sources of variability in the radioangiographic assessment of ejection fraction a comparison of first pass and gated equilibrium techniques. Amer. J. Cardiol. 53, 823-828 (1984)

11. Kiat, H., Maddahi, J., Roy, L.T., van Train, K., Friedman, J., Resser, K., Berman, D.S.: Comparison of Tc-99m methoxy-isobutyl-isonitrile and Tl-201 for evaluation of coronary artery disease by planar and tomographic methods. Amer. Heart J. 117, 1-11 (1989)

12. Kiess, M.C., Dimsdale, J.E., Moore, R.H., Liu, P., Newell, J., Barlai-Kovách, M., Boucher, C., Strauss, W.H.: The effects of stress on left ventricular ejection fraction. Eur. J. Nucl. Med. 14, 12-16 (1988)

13. Martin, P., Paperstraete, B., Paternot, J., Verhas, M.: Interest of single intravenous injection of 99m-Tc MIBI in the evaluation of myocardial wall perfusion and left ventricular ejection fraction. Comparison with equilibrium RNV and echography. Eur. J. Nucl. Med. 15, abstr. 42. (1989)

14. Maseri, A., Mimmo, K., Chierchia, S., Marchesi, C., Pesola, A., Abbate, A.: Coronary artery spasm as a cause of acute myocardial ischaemia in man. Chest 68, 625-633 (1975)

15. McKusick, K., Beller, G., Berman, D., Boucher, C., Camin, L., Delaloye, A., Delaloye, B., Maddahi, J., Manspeaker, P., Strauss, W.H., Wackers, F., Watson, D.: Initial clinical results with 99m-Tc methoxy-isobutyl-isonitrile. J. Amer. Coll. Cardiol. 9, Abstr. 457. (1987)

16. Mester, J., Kósa, I., Gaál, N., Gruber, N., Láng, J., Kovács, G., Csanády, M., Csernay, L.: Dipyridamol stress Cardiolite follow up of patients after coronary by pass surgery (CBS). Eur. J. Nucl. Med. 15. Abstr. 461. (1989)

17. Müller-Brand, J., Pfisterer, M., Schmitt, H.E.: Which factors determine ischaemia detection with 99-m Tc MIBI. Eur. J. Nucl. Med. 15. Abstr. 415. (1989)

18. Rautio, P., Vaino, P., Helin, M., Kuikka, J., Laensimies, E.: 99m-Tc methoxy-isobutyl-isonitrile MIBI SPECT in the diagnosis of coronary heart disease (CHD). Eur. J. Nucl. Med. 15. Abstr. 487 (1989)

19. Regenass, S., Pfisterer, M., Bassigna, V., Müller-Brand, J.: Ambulatory detection of left ventricular dysfunction, using a portable scintillator detection system. Validation of the method. Xth Congr. Eur. Cardiol. Soc., Vienna (1988)

20. Schelbert, H., Verba, J.W., Henning, H., Ashburn, W.L.: Left ventricular ejection fraction assessed non-traumatically from high frequency data left ventricular activity curves. Nuc Compat 7, 156-160 (1976)

21. Selwyn, A.P.: Using positron tomography to validate ischaemia. J. angina and silent Ischaemia 1, 10-12 (1986)

22. Stern, A., Tzivoni, D.: Early detection of silent ischaemic heart disease by 24 h ECG monitoring of active subject. Br. Heart J., 36, 481-486 (1974)

23. Stirner, H., Büll, U., Kleinhaus, E., Bares, R., Grisse, W.: Myocardial kinetics of 99m-Tc methoxy-isobutyl-isonitrile (MIBI) in patient with coronary heart disease. A comparative study versus 201-Tl with SPECT. Nucl. Med. Comm. 9, 15-23 (1988)

24. Tamaki, N.T., Yasuda, T., Moore, R.H., Gill, J.B., Boucher, C.A., Hutter, jr. A.M., Gold, H.K., Strauss, W.J.: Continuous monitoring of left ventricular function by an ambulatory radionuclide detector in patients with coronary artery disease. J. Amer. Coll. Cardiol. 12, 669-679 (1988)

25. Tzivoni, D., Stern, S.: Prognostic significance and therapeutic implications of silent myocardial ischaemia. Eur. heart J. 11, 288-293 (1990)

26. Van der Wall, E.E., Cats, V., Blokland, J.A.P., Arndt, J.W., Bosker, H.A., Pauwels, E.K. J., Bruschke, A.V.G.: Silent ischaemia after myocardial infarction: a particular functional disease entity? Eur. Heart J. 10, Abstr. 55. (1989)

27. Zaret, B.L., Kayden, D.S.: Ambulatory monitoring of left ventricular function. A new modality for assessing silent myocardial ischaemia. In: Kellerman, J.J., Braumwald, E. (eds) Advances in cardiology 37. Silent myocardial ischaemia: a critical appraisal, pp. 105-106. (1990)

Acta Medica Hungarica, Vol. 48/1-2, pp. 73-78 (1991)

THE EFFECTS OF SHORT-TERM VITAMIN C ON PLASMA BUN, URIC ACID, CHOLESTEROL AND TRIGLYCERIDE LEVELS

E. BESER

Department of Public Health, School of Medicine,
Karadeniz Technical University, Trabzon, Turkey

(Received: March 12, 1991)

The effects of daily 0.5 g vitamin C on plasma urea nitrogen, uric acid, cholesterol and triglyceride levels were recorded over a period of one month. There was a significant reduction in plasma cholesterol level ($P < 0.05$). There was no significant effect of vitamin C on plasma urea nitrogen, uric acid and triglyceride levels ($P > 0.05$). It was a placebo-controlled trial. The research and control groups were formed of 105 and 47 volunteer university students, respectively. In these groups the mean ages were 20 $\pm$ 0.33 (mean $\pm$ S.E.M.) and 20 $\pm$ 0.49 years, respectively. Mean body mass indices were 22.2 $\pm$ 0.13 and 22.3 $\pm$ 0.19 kg/m^2, respectively.

Keywords: Ascorbic acid, cholesterol, triglycerides, uric acid, urea nitrogen

Introduction

Very high doses of vitamin C ($\geq$ 1 g) have been claimed to prevent or to be efficacious in treating hypercholesterolaemia and atherosclerosis /17/.

Ginter /6/ found that acute vitamin C deficiency in guinea pigs led to an increase in cholesterol synthesis and a reduction in conversion of cholesterol to bile acid and that these changes led to an increase in carcass cholesterol. An increase in plasma and liver cholesterol levels was detected, concomitant with a decrease in the fractional rate of turn-

Abbreviations: BMI = Body mass index (weight/(height)^2) as kg/m^2; F = Female; M = Male; NS = Not significant; Vit. C = Vitamin C

Offprint requests should be sent to: Erdal Beser, Karadeniz Technical University, TR-61080 Trabzon, Turkey

Akadémiai Kiadó, Budapest

over of total body cholesterol levels, indicating a diminished bile acid synthesis /6/. A positive correlation between rate of bile acid synthesis in and concentration of ascorbic acid in the liver was also reported /10/. These possibly indicate an indirect effect on microsomal 7-alpha-hydroxylase /3, 7/. Evidence of atherogenesis in scorbutic animals and its partial prevention by vitamin C administration was also presented /26, 27/.

Dobson et al. recorded a 14% reduction of plasma cholesterol when 1 g of vitamin C per day was given for 6-12 months to each of 4 men and 6 women of mean age 58 years /4/.

Since the prevalence of low ascorbic acid concentrations increase with age, the possibility that an age-related rise in plasma cholesterol may be secondary hypovitaminosis C has been examined /11, 14/.

Spittle recorded that in healthy people under the age of 25 years, cholesterol levels tended to fall when 1 g of vitamin C per day was added to their otherwise normal diet /22/. Ginter reported a significant reduction of plasma cholesterol when hypercholesterolaemic patients were given 1 g of vitamin C per day for twelve months /9/.

When high daily doses of vitamin C ($\geq$ 1 g) are ingested regularly over a period of months or years, adverse effects may occur such as uricosuria, reduced bactericidal activity of leukocytes, secondary hyperoxalaemia in hemodialysis patients, enhanced mobilization of bone calcium, impaired blood coagulation time, lowered plasma B-12 levels, interruption of pregnancy, reduced insulin production and interference with anticoagulant therapy /1, 2, 12, 15, 17, 19-21, 23, 24/.

The purpose of this study was to investigate the effects of short-term, small daily doses of vitamin C (0.5 g/day, for one month) on plasma BUN, uric acid, cholesterol and triglyceride levels.

Subjects and Methods

The research population consisted of 105 volunteer university students. They took daily, for one month, 0.5 g of vitamin C orally. The control population consisted of 47 volunteer university students. They took placebo for one month. It was a placebo-controlled trial. Plasma BUN, uric acid, cholesterol and triglyceride levels were measured twice before and after taking vitamin C and placebo in respective groups.

All the participants were under the age of 24 years, healthy non-smokers who had not had any infection in the previous two months and had no familial history of atherosclerotic vascular disease /5/. None of them had taken any drug or vitamin preparation for at least one month prior to the investigation. None of the participants took regular exercise. All participants consumed a normal diet (that is, no extra food containing vitamin C, uric acid,

cholesterol and triglycerides). All were living in dormitory accommodation and, therefore, were consuming the same kind of food in the student's cafeteria (table d'hôte).

Biochemical Tests

Plasma urea nitrogen, uric acid, total cholesterol and triglyceride levels were determined by routine biochemical enzymatic methods (urease, uricase, cholesterol oxidase and lipase, respectively).

It was reported /25/ that an oral dose of some 2 g vitamin C does not affect cholesterol values.

Statistical Analysis

The chi-square test, significance test for the difference of two means and the paired observation significance test were used.

Results

The characteristics of the control and research groups are shown in Table I. There was no difference between the two groups ($P > 0.05$) (except in vitamin C levels at the end of the study).

Table I

Characteristics of the control and the research groups[o]

Group	No. of subjects	Sex of subjects		Mean age (years)	Mean BMI*		Plasma level of Vit. C (mg/dl)	
		M	F		Start of study	End of study	Start of study	End of study
Control	47	25	22	20±0.49[o]	22.3±0.19	22.2±0.19	1.20±0.05	1.23±0.05
Research	105	57	48	20±0.33	22.2±0.13	22.1±0.13	1.19±0.03	2.31±0.01

[o] mean ± S.E.M.

* BMI = body mass index (weight/(height)^2) as kg/m^2; M = male; F = female

The comparison of double measurements of plasma urea nitrogen, uric acid, cholesterol and triglyceride levels, between the measurement recorded at the start of the study and that recorded at the end of the study (that is at the beginning and end of the one-month period) for respective groups are shown in Table II. There was no difference in the plasma levels of four biochemical determinations between the control and the research groups at the start of the study (for both sexes) ($P > 0.05$). There was no

Table II

Comparison of double measurements of plasma urea nitrogen, uric acid, cholesterol and triglyceride levels at the start and at the end of the study

Biochemical Determinations (normal ranges)	Start of subjects	Control Group (M=25, F=22) Start of study	Control Group (M=25, F=22) End of study (placebo)	P	Research Group (M=57, F=48) Start of study	Research Group (M=57, F=48) End of study (Vit C)	P
UREA NITROGEN	M	12.93 ± 0.91	12.90 ± 0.95	NS	12.88 ± 0.69	12.15 ± 0.64	NS
	F	12.97 ± 0.95	12.91 ± 0.96	NS	12.78 ± 0.74	12.20 ± 0.69	NS
(7-21 mg/dl)	M+F	12.91 ± 0.71	12.92 ± 0.71	NS	12.99 ± 0.48	12.11 ± 0.43	NS
URIC ACID	M	4.63 ± 0.15	4.67 ± 0.15	NS	4.95 ± 0.11	4.74 ± 0.11	NS
	F	4.58 ± 0.17	4.63 ± 0.16	NS	4.50 ± 0.11	4.70 ± 0.12	NS
(3-8 mg/dl)	M+F	4.69 ± 0.11	4.68 ± 0.11	NS	4.65 ± 0.09	4.71 ± 0.08	NS
CHOLESTEROL	M	167.41 ± 6.83	168.99 ± 6.21	NS	168.45 ± 4.39	151.88 ± 4.49	< 0.05
	F	169.41 ± 7.29	170.21 ± 7.09	NS	170.00 ± 4.91	153.10 ± 4.97	< 0.05
(112-270 mg/dl)	M+F	168.98 ± 4.93	169.01 ± 4.33	NS	169.72 ± 3.12	152.17 ± 3.43	< 0.05
TRIGLYCERIDES	M	126.01 ± 7.35	125.87 ± 7.05	NS	126.19 ± 4.67	125.99 ± 4.56	NS
	F	124.32 ± 7.57	123.99 ± 7.35	NS	125.01 ± 4.91	124.98 ± 4.77	NS
(25-170 mg/dl)	M+F	125.87 ± 5.23	125.03 ± 5.11	NS	125.99 ± 3.39	125.34 ± 3.28	NS

All values are mean ± S.E.M.
NS = Not significant
M = male; F = female

difference (i) in the plasma levels of four biochemical determinations on the measurements recorded at the beginning of the study and on the measurements recorded at the end of the study for the control group (that is at the beginning and the end of the one-month period) (for both sexes) ($P > 0.05$) and (ii) in the plasma levels of urea nitrogen, uric acid and triglycerides in the measurements recorded at the beginning of the study and in the measurements recorded at the end of the study for the research group (for both sexes) ($P > 0.05$). However, there was a significant difference in the plasma cholesterol levels recorded at the beginning of the study and those recorded at the end, in the research group (for both sexes) ($P < 0.05$). There was no difference between females' and males' biochemical determinations in the control and research groups.

Discussion

We observed that taking low doses of vitamin C (0.5 g/day) for one-month significantly decreases the level of plasma cholesterol ($P < 0.05$). We found no significant effect of vitamin C on plasma BUN, uric acid and triglyceride levels ($P > 0.05$).

It has been suggested that taking high doses of vitamin C (such as 1 g) over a period of months decreases plasma cholesterol level /4, 9, 22/. Ingestion of high doses of vitamin C ($\geq$ 1 g) over a period of months or years is not recommended /1, 2, 12, 15, 17, 19-21, 23, 24/. In our study the research group took a small daily dose of vitamin C (0.5 g/day) over a short-term period (1 month). Ginter's results are similar to ours. The reported mean reductions of 15-70 mg/dl when three groups of hypercholesterolaemic subjects (n=11-24) with initial mean cholesterol levels of 255-355 mg/dl were given, 0.3-0.45 g/day vitamin C /8/.

Conversely, some investigations indicate continuing increase in cholesterol levels in the group receiving the higher ascorbic acid supplement /13, 16/. When Peterson et al. administered 4 g/day to 9 hypercholesterolaemic persons for two months, plasma cholesterol levels remained unchanged /18/.

These results are perhaps the most rewarding in this rather confusing field and, at least, they provide some encouragement for further clinical trials.

REFERENCES

1. Balcke, P., Schmidt, P., Zazgornik, J., Kopsa, H., Haubenstock, A.: Ascorbic acid aggravates secondary hyperoxalemia in patients on chronic hemodialysis. Ann. Intern. Med. 101, 344-345 (1984)

2. Barness, L.A.: Safety considerations with high ascorbic acid dosage. Ann. NY. Acad. Sci. 258, 523-528 (1975)

3. Bjorkhem, I., Kallner, A.: Hepatic 7-alpha-hydroxylation of cholesterol in ascorbate-deficient and ascorbate-supplemented guinea pigs. J. Lipid Res. 17, 360-365 (1976)

4. Dobson, H.M., Muir, M.M., Hume, R.: The effect of ascorbic acid on the seasonal variations in serum cholesterol levels. Scot. Med. J. 29, 176-182 (1984)

5. Gallin, J.I., Kaye, D., O'Leary, W.M.: Serum lipids in infection. N. Eng. J. Med. 281 (20), 1081-1086 (1969)

6. Ginter, E.: Ascorbic acid in cholesterol and bile acid metabolism. Ann. NY. Acad. Sci. 258, 410-421 (1975)

7. Ginter, E.: Marginal vitamin C deficiency, lipid metabolism and atherogenesis. Adv. Lipid Res. 16, 167-220 (1978)

8. Ginter, E.: Pretreatment serum-cholesterol and response to ascorbic acid. Lancet ii, 958-959 (1979)

9. Ginter, E.: Vitamin C and plasma lipids. N. Engl. J. Med. 294, 559-560 (1976)

10. Ginter, E., Nemec, R., Cerven, J., Mikus, L.: Quantification of lowered cholesterol oxidation in guinea pigs with latent vitamin C deficiency. Lipids 8, 135-141 (1973)

11. Griffiths, L.L., Brocklehurst, J.C., Scott, D.L., Marks, J., Blackley, J.: Thiamine and ascorbic levels in the elderly. Geront. Clin. 9, 1-10 (1967)

12. Herbert, V., Jacob, E., Wong, K-T.J., Scott, J., Pfeffer, R.D.: Low serum vitamin B_{12} levels in patients receiving ascorbic acid in megadoses: studies concerning the effect of ascorbate on radioisotope vitamin B_{12} assay. Am. J. Clin. Nutr. 31, 253-258 (1978)

13. Johnson, G.E., Obenshain, S.S.: Nonresponsiveness of serum high-density lipoprotein-cholesterol to high dose ascorbic acid administration in normal men. Am. J. Clin. Nutr. 34 (10), 2088-2091 (1981)

14. Koh, E.T.: Selected blood components and urinary B vitamin as related to age and sex of black population in Southwest Mississippi. Am. J. Clin. Nutr. 33, 670-676 (1980)

15. Levey, S., Suter, B.: Effect of ascorbic acid on diabetogenic action of alloxan. Proc. Soc. Exp. Biol. Med. 63, 341-343 (1964)

16. Milne, D.B., Omaye, S.T., Amos, W.H. Jr.: Effect of ascorbic acid on copper and cholesterol in adult cynomolgus monkeys fed a diet marginal in copper. Am. J. Clin. Nutr. 34 (11), 2389-2393 (1981)

17. Olson, J.A., Hodges, R.E.: Recommended dietary intakes (RDI) of vitamin C in humans. Am. J. Clin. Nutr. 45, 693-703 (1987)

18. Peterson, V.E., Crapo, P.A., Weininger, J., Ginsberg, H., Olefsky, J.: Quantification of plasma cholesterol and triglyceride levels in hypercholesterolemic subjects receiving ascorbic acid supplements. Am. J. Clin. Nutr. 28 (6), 584-587 (1975)

19. Samborskaya, E.P., Ferdman, T.D.: The problem of the mechanism of artificial abortion by use of ascorbic acid. Bull. Exp. Biol. Med. USSR. 4, 96-98 (1964)

20. Shilotri, P.G., Bhat, K.S.: Effect of mega doses of vitamin C on bactericidal activity of leukocytes. Am. J. Clin. Nutr. 30, 1077-1081 (1977)

21. Sigell, L.T., Flessa, H.C.: Drug interactions with anticoagulants. J.A.M.A. 214, 2035-2038 (1970)

22. Spittle, C.R.: Atherosclerosis and vitamin C. Lancet ii, 1280-1281 (1971)

23. Stein, H.B., Hasan, A., Fox, I.H.: Ascorbic acid induced uricosuria. A consequency of megavitamin therapy. Ann. Intern. Med. 84, 385-388 (1976)

24. Thornton, P.A., Omdahl, J.L.: Further evidence of skeletal response to exogenous ascorbic acid. Proc. Soc. Exp. Biol. Med. 32, 618-621 (1969)

25. Tietz, N.W.: Clinical guide to laboratory tests. Philadelphia: W.B. Saunders, p. 114-116. (1983)

26. Willis, G.C.: An experimental study of intimal ground substance in atherosclerosis. Can. Med. Assoc. J. 69, 17-22 (1953)

27. Willis, G.C.: The reversibility of atherosclerosis. Can. Med. Assoc. J. 77, 106-109 (1957)

Acta Medica Hungarica, Vol. 48/1-2, pp. 79-85 (1991)

STUDY OF THE EFFECT OF SUPEROXIDE DISMUTASE ON ACUTE RENAL FAILURE IN DOGS

L. KÓNYA, G. SZÉNÁSI, P. BENCSÁTH, J. FEHÉR

2nd Department of Medicine Medical Semmelweis University School, Budapest

(Received: November 15, 1990)

Warm ischaemia was provoked by occlusion of the left renal artery in dogs narcotized with Nembutal and it was studied whether a small (0.5 mg/kg body weight /n=6/) or a large dose (5 mg/kg body weight /n=7/) of superoxide dismutase (SOD) improves renal function during 90 min reperfusion, compared to the control group (n=6). In the first period after release of occlusion (min 0-15) the GFR and cPAH values reached 10-20% of those in the contra lateral kidney with normal circulation. The GFR and cPAH values as well as urine flow, sodium and potassium excretion were not different in the three groups. Renal function significantly improved in all groups during reperfusion. At the early stage of reperfusion the malondialdehyde (MDA) concentration exceeded that before occlusion. Such an increase could not be seen when superoxide dismutase (SOD) treatment was applied. Our results show that SOD treatment does not improve renal function at the early stage of acuterly stage of acute renal failure of ischaemic origin.

Keywords: Acute renal insufficiency, free radical, superoxide dismutase

Introduction

Certain clinical conditions occur in the medical practice when transient tissue ischaemia is followed by reperfusion.

Ischaemia depending on the sensitivity of different organs and lasting for a critical period of time certainly leads to irreversible damage of the tissues, and any intervention carried out in due time to improve circulation serves for the preservation of vital functions.

Abbreviations: MDA: malondialdehyde, FR: Free radical, SOD: superoxide dismutase

Offprint requests should be sent to: L. Kónya, H-1088 Budapest, Szentkirályi u. 46, Hungary

Akadémiai Kiadó, Budapest

Recent data emphasize the risk of restarting tissue blood flow /5/. It seems likely that the degree of tissue damage after reperfusion depends both on ischaemia and the injury caused by reperfusion /7/. More and more workers stress the importance of free radicals of oxygenous origin in this type of tissue damage /1, 8, 10, 12, 17/. These radicals are also formed in the course of natural vital processes; their importance is denoted by that all aerobi organisms have a defence system against them, in which one of the most significant enzymes is SOD /18/. Acute ischaemic renal failure is of great clinical importance because of its frequent occurrence, and increasing mortality. That is why free radicals and the protecting effect of antioxidant treatment are widely investigated in this context /3, 7, 10, 21, 22/. From our trials we got contradictory results, so the question needed further study. Such trials on dogs can be found in small number in the literature, therefore, we performed our experiments on dogs. Reactive free radicals are formed in a large quantity at the early stage of reperfusion first of all due to the activity of xanthin oxidase of endothelial origin; their impact is instantaneous; that is why we followed the changes in the control group and in those treated with different doses of SOD in the same period.

Methods

Experiments were performed on mongrel dogs of either sex with a body mass of 10-34 kg. The animals were narcotized with Nembutal (sodium pentobarbital, 30 mg/kg body mass i.v.), steady narcosis was provided with 3 mg/kg body mass of i.v. maintenance doses. A cuffed endotracheal tube was inserted, continuous artificial respiration was maintained (respirator RO-5, Moscow, USSR) and regular control of blood-gas analysis was performed during the trial. Surgery was performed on a heatable operating table, rectal temperature was maintained between 37 and 38 ^{o}C. With the aim of giving infusion, taking blood and measuring blood pressure, a jugular vein and both femoral arteries and veins were catheterized. Urine was obtained through catheters from ureters by a median laparotomy. Venous blood from the kidney was obtained through the v. spermatica or ovarica with the help of a catheter led into the left v. renalis. The left renal artery was exposed, with the renal nerves left intact. On finishing the operation, saline infusion was administered (20 ml/kg body mass) in 30 min, which was then followed by 10 ml/kg body mass/h until the end of the trial. With the aim of measuring GFR and effective renal plasma flow, inulin and paraaminohippuric acid in saline solution was infused into the v. jugularis to reach 30-50 mg/100 ml and 1-3 mg/100 ml plasma concentration, respectively, twice depending on the animal's body weight (0.21-0.58 ml/min). After the operation, a 60 min equilibration period was maintained, then urine was collected at 15 min intervals. After two control periods the left renal artery was occluded for 45 min and urine was collected from the right kidney alone. During the occlusion period the quantity of the infusion was halved. Following the release of the occlusion urine was collected from both kidenys for further six periods. To measure the MDA concentration in the plasma 5 ml of venous blood was taken from the kidney 5 min before the strangulation of the renal artery and

5, 15 and 30 min after its release. Arterial blood samples were taken in the middles of the periods.

Blood pressure, measured with a Statham P 23 db pressure transducer, was continuously recorded (Rikadenki, B 381, Tokyo, Japan).

The following groups were examined: 1) Control (n=6) Animals given saline alone. 2) Small dose SOD group (n=6) 0.5 mg/kg body mass SOD infusion was started 1 min before reperfusion and given continuously for 10 min. 3) Large dose SOD group (n=7) 5 mg/kg body mass SOD was administered as above.

The inulin and PAH concentration in the plasma and the appropriately diluted urine samples were measured with anthrone and diphenylamine method. Sodium and potassium concentration were measured with a Flapho 4 type flame photometer (Carl Zeiss, Jena, Germany). The MDA concentration in the renal venous blood was determined with spectrophotometry as descibed by Ottolenghi /20/. The Tables contain mean values of kidney parameters expressed for 100 g wet kidney $\pm$ S.E.M. The groups were compared with analysis of variance.

Results

In the course of statistical data processing the parameters of ischaemic kidney and that with intact circulation were compared within one group of animals at different periods of time and the values of ischaemic kidney were compared between the groups.

In Table I the data for three stressed periods of time are shown. They show that 15 min after the release of the renal artery occlusion for 45 min the GFR and cPAH values (the latter approximating effective renal plasma flow) reached 10-20% of the control value and after 1.5 h reperfusion it was significantly lower than those in the intact kidney. Potassium excretion of the ischaemic kidney was lower in all of the three groups at the beginning of reperfusion than in the other one with normal circulation. Diuresis and sodium excretion were significantly lower in the large-dose group after reperfusion. Both diuresis and sodium + potassium excretion of the ischaemic kidney were lower in all of the three groups than those of the intact kidney both at the beginning and in the last periods of reperfusion (75-90 min), but the difference was statistically not significant. However, it can be stated that prolonged reperfusion resulted in better parameters in all groups. At given periods of time the parameters of the ischaemic kidney never differed in the three groups or the degree of decrease in the examined parameters was similar in all groups when compared to those of the control kideny. The MDA concentration values of the venous blood of the kideny are summarized in Table II. The MDA concentration significantly exceeded the baseline value at min 5 and 30 of reperfusion in the control group. No changes in the MDA concentration were observed with any of the two SOD doses.

Table I

The effect of small (0.5 mg/kg body mass) and large dose (5 mg/kg body mass) of superoxide dismutase (SOD) on renal function and arterial blood pressure in narcotized dogs after 45 min unilateral warm renal ischaemia

		before ischaemia			0-15 min after ischaemia			75-90 min after ischaemia		
		Control n=6	small dose of SOD m=6	large dose of SOD n=7	Control n=6	small dose of SOD m=6	large dose of SOD n=7	Control n=6	small dose of SOD n=6	large dose of SOD n=7
V, ml/min	C:	1.8± 0.4	1.9± 0.5	1.8± 0.3	1.7± 0.4	1.0± 0.3	1.7± 0.3	2.3± 0.6	1.4± 0.3	1.7± 0.5
	I:	1.8± 0.4	2.1± 0.6	1.9± 0.3	0.8± 0.2	0.4± 0.1	0.6± 0.2	1.4± 0.3	0.6± 0.1	1.3± 0.3
GFR, ml/min	C:	65 ± 5	77 ± 6	80 ± 5	61 ± 5	65 ± 7	66 ± 5	66 ± 4	82 ± 7	82 ± 6
	I:	63 ± 5	75 ± 6	76 ± 4	13 ± 3^{c}	12 ± 4^{c}	6 ± 2^{c}	39 ± 4^{c}	40 ± 6^{c}	32 ± 5^{c}
cPAH, ml/min	C:	157 ± 8	219 ±13	237 ±22	154 ± 5	145 ±20	172 ±12	160 ±12	189 ±13	213 ±12
	I:	155 ± 6	204 ±14	223 ±17	44 ±11^{c}	35 ±12^{c}	17 ± 5^{c}	91 ±10^{c}	106 ±14^{c}	86 ±11^{c}
U V, µmol/min	C:	276 ±57	365 ±90	302 ±60	175 ±41	149 ±38	214 ±26	253 ±61	211 ±37	205 ±34
	I:	319 ±93	383 ±95	308 ±54	118 ±38	48 ±14	73 ±27^{a}	165 ±46	78 ±10	163 ±34
U V, µmol/min	C:	67 ± 8	79 ±12	50 ± 6	66 ± 6$_{b}$	48 ±12	47 ± 9	76 ± 8	84 ±17	53 ±10
	I:	70 ± 9	77 ±11	49 ± 6	28 ± 6^{b}	14 ± 5^{c}	8 ± 2^{b}	58 ±10	42 ± 6^{a}	44 ± 7
BP, mmHg		130 ± 7	133 ± 7	123 ± 5	150 ± 8	139 ± 7	132 ± 7	139 ± 4	138 ± 6	131 ± 4

Abbreviations: C: control (right), I: ischaemic (left) kidney;
a: $P < 0.05$; b: $P < 0.01$; c: $P < 0.001$
The data (mean ± S.E.M.) are expressed for 100 g wet kidney weight

Table II

The effect of small (0.5 mg/kg) and large (5 mg/kg) dose of superoxid dismutase (SOD) of the malondialdehyde concentration (μM/ml) of the renal venous plasma in narcotized dogs after 45 min unilateral warm ischaemia

	before ischaemia	after ischaemia		
		5 min	15 min	30 min
Control (n=6)	8.2 ± 1.7	29.0 ± 17.0[b]	21.4 ± 3.4	25.7 ± 2.5[a]
Small dose of SOD (n=6)	14.2 ± 2.6	12.1 ± 2.1	13.7 ± 2.7	12.1 ± 2.2
Large dose of SOD (n=7)	21.9 ± 4.8	26.6 ± 5.8	21.4 ± 3.4	25.7 ± 2.5

Significant difference V.S.: control: a = $P < 0.05$, b = $P < 0.01$ compared to control
(mean ± S.E.M.)

Discussion

To approach the pathogenetic role of free radicals we had two alternative ways in our trial: (a) examining whether kidney function improves on antioxidant treatment after reperfusion and (b) following the changes of MDA concentration in the blood. Many workers have studied the effect of different doses of SOD; Baker et al. found that the serum-creatinine level was lower in rats treated for 1 h with 3.25 mg/kg body mass of SOD following ischaemia of the kidney on the first days after operation than in the untreated control group, however, later no difference could be seen in mortality; with double dose (6.5 mg/kg b.m.) of the enzyme all the animals survived /3/. Koyama et al. failed to find any protective effect with 0.2-2 mg of the enzyme given into the renal artery of pigs following a cold-warm ischaemia modelling kidney transplantation, while on administration of 20 mg of the enzyme creatinine-clearance improved /15/. With small dose of SOD, tissue damage was experienced in vitro, this can be explained by that the incomplete transformation of the superoxide radical formed during reperfusion facilitates the production of a more toxic root /14/. In this trial we examined the effect of 0.5 mg/kg and 5.0 mg/kg body mass of SOD enzyme; kidney function parameters showed a slight impairment compared to untreated animals. It is common practice to measure the MDA level in the course of the study of reactions caused by free radicals.

Though MDA is not a specific marker of the peroxidation of membraneous lipids, it is considered to correlate with its degree /9/. Contradictory results were obtained during the examination of the venous blood from the kidney and of the MDA level of homogenized tissue /3, 15, 19, 22/. In our trial the MDA concentration in the blood samples from the renal vein was elevated at min 5 and 30 of reperfusion in the control group, while no increase was observed in treated animals. Our results correspond to those of Joannidis et al., who found a significant increase in the arterio-venous difference at the early stage of reperfusion after 45 min ischaemia, while antioxidant treatment failed to improve renal function during 60 min reperfusion /13/. So one of the indirect evidence in our trial indicates the possibility of reactions caused by free radicals while the ineffectivity of antioxidant treatment speaks against this possibility. On the basis of the results described above, it is not unlikely that the protective effect of applied therapy is manifested at a later period of reperfusion or either large dose of SOD enzyme or combined antioxidant treatment is needed to protect against reperfusional damage.

REFERENCES

1. Ambrosio, G., Becker, L.C., Kretchin, G.M., Weisman, H.F., Weisfeldt, M.L.: Reduction in experimental infarct size by recombinant human superoxide dismutase: insights into pathomorphology of reperfusion injury. Circulation 74, 1424-1433 (1986)
2. Baker, G.l., Corry, R.J., Autor, A.P.: Oxygen free radical induced damage in kidneys subjected to warm ischaemia and reperfusion. Protective effect of superoxide dismutase. Ann. Surg. 202, 628-641 (1985)
3. Bird, J.E., Milhoan, K., Wilson, C.B., Young, S.G., Mundy, C.A.: Ischemic acute renal failure and anti oxidant therapy in the rat. The reaction between glomerular and tubular dysfunction. J. Clin. Invest., 81, 1630-1538 (1988)
4. Braunwald, E., Kloner, R.A.: Myocardial reperfusion: a double-edged sword? J. Clin. Invest. 76, 1713-1719 (1985)
5. Bratell, S., Polmeiz, P., Hansson, R., Jonsson, O., Lundstam, S., Petterson, S., Rippe, B., Scherstein, T.: Effects of oxygen free radical scavengers xanthine oxidase inhibition and calcium entry blockers on leakage of albumin after ischaemia. An experimental study in rabbit kidneys. Acta Physiol. Scand. 134, 35-41 (1988)
6. Bulkely, G.B.: Free radical-mediated reperfusion injury: a selective review. Br. J. Cancer 55, 66-73 (1987)
7. Canavese, C., Stratta, P., Vercellone, A.: The case for oxygen free radicals in the pathogenesis of ischaemic acute renal failure. Nephron 49, 9-15 (1988)
8. Freeman, B.A., Crapo, J.D.: Biology of Disease. Free radicals and tissue injury. Lab. Invest. 47, 417-426 (1982)

9. Gamelin, L.M., Zager, R.A.: Evidence against oxidant injury as a critical mediator of post ischaemic acute renal failure. Am. J. Physiol. 255, F450-460 (1988)

10. Granger, P.N., Rutili, G., McCord, J.M.: Superoxide radicals in feline intestinal ischaemia. Gastroenterology 81, 22-29 (1987)

11. Hansson, R., Johansson, S., Johansson, O., Petersson, S., Scherstein, Z., Waldenström, D.: Kidney protection by pretreatment with free radical scavengers and allopurinol: renal function at recirculation after warm ischaemia in rabbits. Clin. Science 71, 245-251 (1986)

12. Joannidis, M., Gastraunthaler, G., Pfaller, W.: Xanthine oxidase: evidence against a causative role in renal reperfusion injury. Am. J. Physiol. 258, F2323-236 (1990)

13. Kato, M., Kako, J.: Effects of N-/2-mercaptopropionyl/glycine on ischaemic reperfused dog kidney in vivo and membrane preparation in vitro. Mol. and Cell. Biochem. 78, 151-159 (1987)

14. Kaur, A., Sethi, A.K., Ganguly, N.K., Majumdar, S.: Concentration dependent function of superoxide dismutase in oxygen free radicals mediated tissue injury in renal brush border membrane. Biochemistry 19, 385-395 (1989)

15. Koyama, I., Bulkley, G.B., Williams, G.M.: The role of oxygen free radicals in mediating the reperfusion of cold-preserved ischaemic kidneys. Transplantation, 40, No. 6, 590-595 (1985)

16. McCord, J.M.: Oxygen derived free radicals in postischaemic tissue injury. New Eng. J. Med. 312, 159-163 (1985)

17. McCord, J.M., Fridovich, I.: The biology and pathology of oxygen radicals, Ann. Int. Med., 89, 122-127 (1988)

18. McCoy, R.N., Hill, K.E., Agon, M.A., Stein, J.N., Burk, R.F.: Oxidant stress following renal ischaemia: changes in the glutathione redox ratio. Kideny Int., 33, 812-817 (1988)

19. Ottolenghi, A.: Interaction of ascorbic acid on mitochondrial lipids. Arch. Biochem. Biophys. 79, 335-363 (1959)

20. Ouriel, K., Smedira, N.G., Ricotta, J.J.: Protection of the kidney after temporary ischaemia: Free radical scavengers. J. Vasc. Surg., 2, 49-53 (1985)

21. Paller, M.J., Hoidal, J.R., Ferris, T.F.: Oxygen free radicals in ischaemic acute renal failure in the rat. J. Clin. Invest. 74, 1156-1164 (1984)

Acta Medica Hungarica, Vol. 48/1-2, pp. 87-94 (1991)

EXOGENOUS SUPEROXIDE DISMUTASE UPTAKE BY THE MYOCARDIUM AND KIDNEY IN AN ISCHAEMIC REPERFUSION MODEL IN DOGS

L. KÓNYA, A. FÜLÖP[+], ILDIKÓ BÁRTFAI, G. SZÉNÁSI

2nd Department of Medicine, [+]Institute of Biology, Semmelweis University Medical School, Budapest, Hungary

(Received: January 2, 1991)

The renal and cardiac tissues of dogs were examined with autoradiographic method in ischaemic reperfusion trials with the aim of studying the access of exogenous superoxide dismutase (SOD) enzyme to the cells. Penetration of SOD into the cells was demonstrated. The enzyme showed special affinity to the mitochondira and myofilaments in the myocardium and it was seen both in the glomeruli and the tubules in the kideny. In both organs the uptake of the enzyme was most abundant in the ischaemic regions than in tissues with intact circulation.

Keywords: Superoxide dismutase, reperfusion, autoradiography

Introduction

Substances carrying unpaired electrons on their outer orbits are called free radicals. Such highly reactive compounds may appear during natural vital processes mainly during oxygen reduction. Aerobic organisms have established an effective defence system against them /5, 13/. Superoxide dismutase (SOD) seems to be an important enzyme in this system. In ischaemic,reperfusion conditions for free radicals of oxygenous origin are often formed e.g. xanthine oxidase enzyme, during the function of activated leukocytes or the metabolism of arachidonic acid, due to the autooxidation of catecholamines and the abnormal functioning of mitochondria.

Abbreviations: LAD: left anterior descending coronary artery, SOD: Superoxide dismutase

Offprint requests should be sent to: L. Kónya, H-1088 Budapest, Szentkirályi u. 46, Hungary

Akadémiai Kiadó, Budapest

Recently it has been stated by many authors that such radicals play an important role in the tissue damage in reperfusion /12/. Different compounds have been examined in view of their impact on diminishing these damages. The impact lies partly on diminishing the appearance of free radicals, partly on inactivating the developing ones. The effect of SOD has been examined in this context. The investigations gave contradictory results /2, 3, 6, 9, 16/. The question has arisen how the large molecules of SOD — being widely applied in the prevention of reperfusion-caused damages — can penetrate the cells and whether this enzyme has an intracellular protective effect.

Our objective was to study this problem. As reperfusion of the heart and the kidney is of great clinical importance (in the thrombolytic treatment of myocardial infarction, organ transplantation and revascularizational operations), we performed our trials on the heart and the kidney.

Materials and Methods

Experiments were performed on mongrel dogs of both sexes with a body mass of 10-34 kg. The dogs were narcotized with 30 mg/kg sodium pentobarbital i.v., steady narcosis was maintained with 3 mg/kg supplementary i.v. doses. A cuffed endotracheal tube was inserted into the trachea and artificial respiration was applied during the trial (respirator RO-5, Moscow, USSR). Operations were performed on a heatable operating table; rectal temperature was maintained between 37 and 37.5 °C. Haemodynamic balance was maintained with administration of saline infusion and/or Rheomacrodex administration. The left renal artery was dissected from two dogs after median laparotomy without damaging the renal nerves, then it was occluded for 45 min with an atraumatic silk snare. There was an intact circulation in the right kidney. One min before reperfusion SOD enzyme was administered in doses of 0.5 mg or 5 mg/kg body mass (50% in bolus, thereafter the remainder in i.v. infusion over ten min).

Samples were taken from the cortex and medulla of the left (ischaemic) kidney and of the right (non ischaemic) one after 20 min reperfusion. Thoracotomy was performed in two other dogs, where the left anterior descending (LAD) coronary branch under the first septal artery was dissected after pericardiotomy. Regional ischaemia was produced by 30 min occlusion. One min before reperfusion 0.5 mg or 5 mg/kg body mass labelled SOD enzyme was administered into the left atrium through a cannula in a similar way as in the first trial. After 20 min reperfusion, samples were taken from the endo- and epicardial regions of the myocardium with both ischaemic and intact circulation. Exogenous SOD (Peroxinorm, Grünenthal Germany) was iodinized by the special method of Fraker and Spec /4/, in which the reaction occurs at +4 °C in a polypropylene (Eppendorf) well. The process was the following: 500 µg IODO-GEN was dissolved in 500 µl chloroform, then dried on the wall of the reaction well by circulating purified nitrogen. Then 185 MBq (5mCi) ^{124}I-Na (12 µl) (code: IMS. 300, Amersham Int. plc., UK) and 50 mg (500 µl) SOD enzyme dissolved in 0.05 M sodium phosphate buffer (pH: 7.5) were added. The compound was mixed for 5 min in a magnetic mixer. The process was stopped by sucking up the wet phase of the reaction compound from the solid IODO-GEN phase. The latter was washed twice in 200 µl phosphate buffer. The efficiency of iodine intake was determined with thin film chromatography (Kieselgel F_{254}/Merck), eluent: acetone aldehyde (Reanal, Budapest). Labelling efficiency: 95-98%, specific activity of the labelled enzyme: 3.5-3.6 kBq/µg.

Autoradiography was performed as follows. The semi-thin sections were covered with photoemulsion ILFORD G5 with immersion method. Photos were taken after a four-week exposure, grain counting was performed after seven weeks of exposure. Development was performed on paper, then the sections were stained with toluidine blue and covered with Canada balm. The grains were counted in squared frames in 1600-fold enlargement with the help of an immersion lens. In each preparation the grains were counted in every 14-16 field and the groups were characterized with the mean grain-count and standard error. Superthin sections were covered with emulsion ILFORD L4 with immersion, they were developed after four month exposure with the developing method Kodak B19. The photos were taken with a Philips 300 type transmission electronmicroscope.

Statistical evaluation of data was performed by analysis of variance. Mean $\pm$ S.E.M. are shown on the Table I.

Results

Exogenous SOD was detected in the examined tissues partly quantitatively. The data are summarized in Table I. The SOD uptake by ischaemic tissues per unit microscopic (high powerfield 1600 x) area exceeded that by the tissues with intact circulation. In the kidney the uptake was significantly higher than that of the tissues with intact circulation both with low and high doses ($P < 0.001$) compared to the heart, where it was dose dependent. After treatment with the higher SOD dose the enzyme uptake by the cells was higher both in the heart and the kidney than with a low dose ($P < 0.001$). However, the increase does not compare to the tenfold difference in the concentration applied in the treatment (the increase of the enzyme concentration was 70% in the kidney and 60% in the heart).

The qualitative analysis of the autoradiographic examinations done by electronmicroscope gave the following results. Grains were be seen above the basement membrane in the kidney. They were also seen in the cytoplasm and the nuclei of the epithelial cells of the collecting tubules, in the capillary lumen, above the mitochondira of the tubular epithelial cells and in or under the brush border of the epithelial cells of the proximal tubule as well as on the basement membrane and the podocytes of the glomeruli. Grains were also found above the filaments in the cytoplasm of the transient epithelial cells.

In the myocardium, grains frequently occurred on the myofilaments and the mitochondria. In some cases grains were seen above the endoplasmic reticulum, the basement membrane and/or the extracellular region. Some typical sites can be seen on Figs 1-3.

Table I

Exogenous SOD content of the kidney and myocardium

		Ischaemic tissue	Non ischaemic tissue
		Mean ± S.E.M.	Mean ± S.E.M.
SOD	Kidney medulla	13.8(15) ± 0.8	0(10)
0.5 mg/kg b.m.	cortex	8.6(15) ± 0.8	2.9(14) ± 0.2
	Heart-endocardium	12.7(14) ± 1.3	8.6(27) ± 0.9
	-epicardium	13.5(15) ± 1.2	6.2(15) ± 0.6
SOD	Kidney medulla	12.8(14) ± 1.8	6.9(18) ± 0.9
5 mg/kg b.m.	cortex	25.3(15) ± 1.5	21.9(15) ± 1.2
	Heart-endocardium	18.9(30) ± 0.8	13.5(13) ± 0.2
	-epicardium	16.9(14) ± 1.9	12.4(14) ± 0.9

Values are means of counted grains ±S.E.M. numbers in parentheses indicate the number of fields studied.

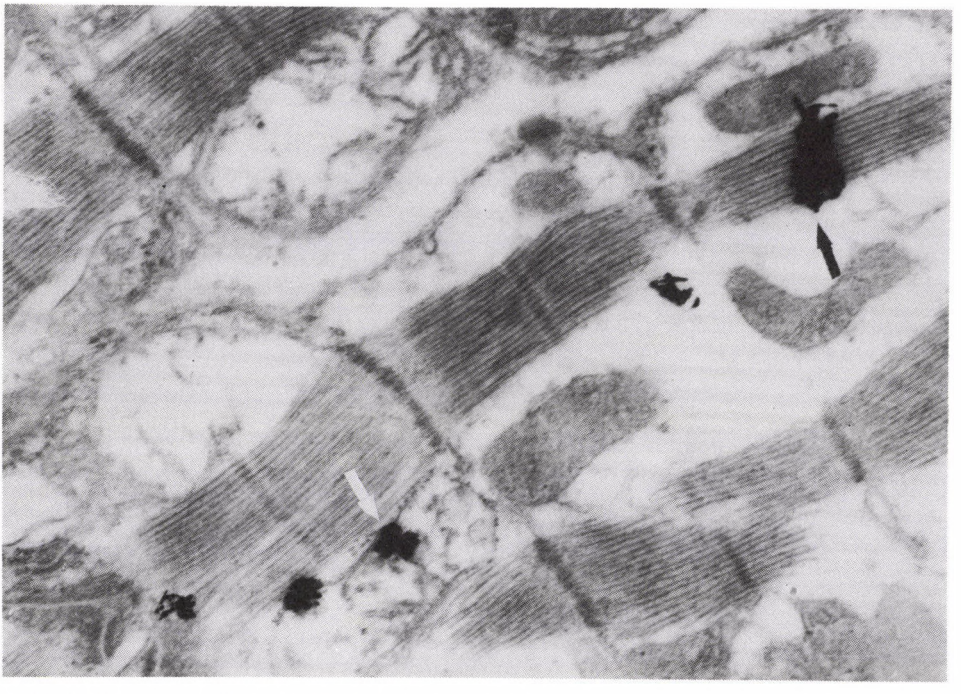


Fig. 1. Myocardium after reperfusion (treated with SOD, 5 mg/kg). There are grains (labelled SOD enzyme) in the myofilaments (⇑) and mitochondria (⇧). (X 30 000)

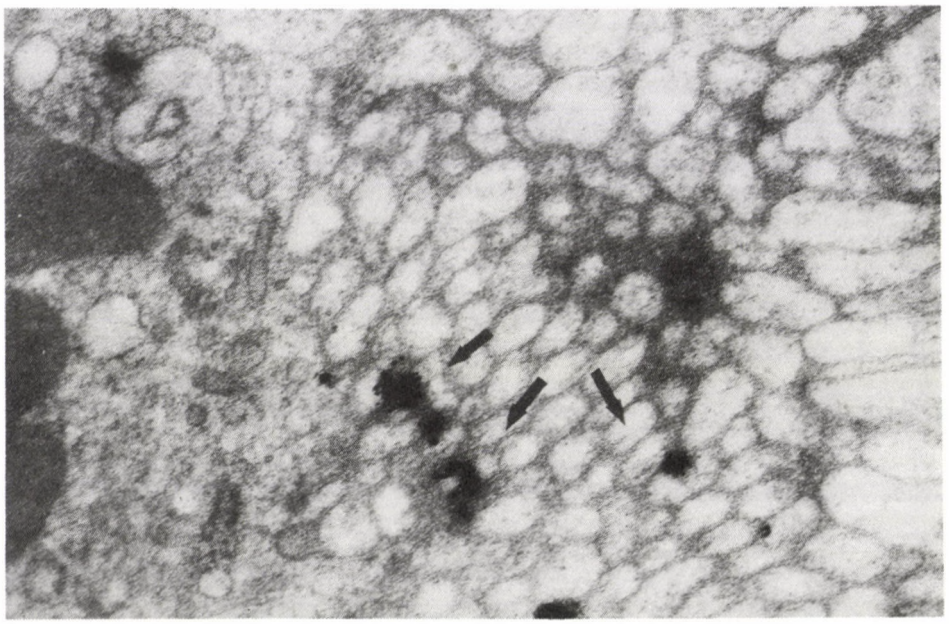


Fig. 2. Kidney after reperfusion (treated with SOD, 5 mg/kg). There are several grains (⬆) in the brush border of epithelial cells. (X 30 000)

Fig. 3. Kidney after reperfusion (treated with SOD 5 mg/kg). There (⬆) is a grain in the basement membrane kof the glomeruli. (X 39 000)

Discussion

Various SOD enzymes can be found in aerobs. Eukaryotes involve an SOD of approximately 33 000 Dalton Cu and Zn content in the cytosol and the mitochondria and a 85 000 Dalton SOD with Mn content in the mitochondria. In the plasma a kind of SOD with significantly larger molecule can be found in a low concentration /11, 13/. The enzyme seems to be an extremely specific transformer of the superoxide radical and it has no other biological function. The protective effect of the enzyme has been examined in the ischaemic-reperfusion trials in tissues of different animals /1, 2, 7, 10/. Recently human recombinant SOD has been investigated in clinical trials too /3, 18/. The pharmacological applicability of SOD is influenced by several factors, such as circulation time, penetration into the cells, intracellular localization and organ specificity. SOD soon disappears from the circulation. In different species it can be shown in large amount in the kidneys within 10-30 min, but it is also accumulated in the pulmonary system and the myocardium /11, 15/. Earlier the access of exogenous SOD to the cells was doubted because of its macromolecular structure /5, 13/. As the permeability of the ischaemic cell membrane can be altered (release of macromolecules from ischaemic not necrotizing cells was verified), the enzyme may penetrated the cells but there its impact on the microvascular system is also possible /1, 8, 14/. Studying the access of exogenous SOD to different cells (monkey's kidney, Ehrlich ascites, hamster fibroblast, human erythrocytes) in vitro Michaelson and Puget found that its 0.04% was fixed after incubation in substances containing SOD on far greater concentration than in the cells with the simultaneous penetration of its 50% through the cell membrane. On examination the intracellular localization of SOD encapsuled in the liposomes it was found that its 25% was bound to the mitochondria or the nuclei /15/.

The aim of our trial was to answer the following questions: Is the exogenous SOD able to penetrate the cells? What is the difference between the SOD uptake by ischaemic tissues and by those with intact circulation? Which are the organelles that the enzyme shows affinity to in the cells? How does the exogenous SOD content of the cells alter in response to treatment with different doses?

On the basis of the results gained with autoradiographic technique and the use of labelled SOD we conclude, that the exogenous SOD can

qucikly penetrate the cells during reperfusion. It was seen in large amount on the basement membrane in the kidney, which can be due to its filtering function: the labelled SOD molecules are retained on this barrier. It also could be detected in large amount in or under the brush border of the epithelial cells in the tubules, which have a reabsorptive function. The enzyme seems to have special affinity to different filaments: most frequently it could be observed above the myofilaments in the myocardium but it also occurred in the filaments of the transient epithelial cells of the kidney. Light-microscope showed that ischaemic tissues contained exogenous SOD in significantly higher quantity than those with intact circulation; the difference may be due to the easier access of the enzyme through the membrane with altered permeability. However, the access is limited. This is proved by the fact that tissues treated with tenfold SOD concentration produced less than tenfold amount of grains. It is known that free radicals can penetrate cells. Thus, it is important that the exogenous antioxidant SOD also enters cells under conditions of ischaemia and reperfusion. Tanaka et al. investigated the fate of exogenously administered SOD in rat myocardium and cultured bovine endothelium. They found (with an immunohistochemical method) a significant amount of SOD in the cells (endothelial uptake by endocytosis) /17/. This is somewhat similar to our results. Our new findings are: we observed that the enzyme specifically binds to certain organelles and that a moderate quantity of the SOD can enter non-ischaemic cell.

Our investigations were performed with bovine SOD enzyme on the basis of the Cu-Zn content. Further studies are needed with other enzyme products (e.g. human recombinant) in other species and organs on ischaemic-reperfusion models in different time-schedules.

REFERENCES

1. Ambrosio, C., Becker, L.C., Hutchins, G.H., Weisman, H.F., Weisfeldt, M.L.: Reduction in experimental infarct size by recombinant human superoxide dismutase: insights into pathophysiology of reperfusion injury. Circulation 74, 1424-1433 (1986)

2. Baker, G.L., Corry, R.J., Autor, A.P.: Oxygen free radical induced damage in kidneys subjected to warm ischaemia and reperfusion protective effect of superoxide dismutase. Ann. Surg. 202, 628.641 (1985)

3. Engler, R., Gilpin, E.: Can superoxide dismutase alter myocardial infarct size: Circulation 79, 1137-1142 (1989)

4. Fraker, P.J., Spec, I.C. jr.: Protein and cell membrane iodination with a sparing soluble chloroamide, 1,3,4-6-tetra-chloro 30, 50, - diphenylglycoluril. Biochem. Biophys. Res. Comm. 80, 849-857 (1978)

5. Freeman, B.A., Crapo, J.D.: Free radicals and tissue injury. Lab. Invest. 47, 412-425 (1982)

6. Gamelin, L.M., Zager, R.A.: Evidence against oxidant injury as a critical mediator of post ischaemic acute renal failure. Am. J. Physiol. 255, F 450-460 (1988)

7. Hansson, R., Johansson, S., Johansson, O., Petterson, S.T., Scherst, A., Waldenström, I.: Kidney protection pretreatment with free radical scavengers and allopurinol: renal function at recirculation after warm ischaemia in rabits. Clin. Science 71, 245-251 (1986)

8. Ichimori, K., Kakazawa, H., Ban, K., Okino, H., Masuda, T., Aoki, N.: SOD Salvages reperfusion injury without entering the myocardium. Possible endothelial protection. Circulation 76, Suppl. IV, 199 (1987) (Abstr.)

9. Jurman, M.J., Schaefers, H.J., Dammenhagn, L., Haverich, A.: Oxygen-derived free radical scavangers for amelioration of reperfusion damage in heart transplantation. J. Thorac. Cardiovasc. Surg. 95, 368-377 (1988)

10. Koyama, I., Bulkley, G.B., Williams, G.M.: The role of oxygen free radicals in mediating the reperfusion injury of cold-preserved ischaemic kidneys. Transplantation 40, 590-595 (1985)

11. Marklund, S.: Distribution of Cu, Zn superoxide dismutase and Mn superoxide dismutase in human tissues and extracellular fluids. Acta Physiol. Scand. Suppl. 492, 19-29 (1980)

12. McCord, J.M.: Oxygen-derived free radicals in post ischaemic tissue injury. New Engl. J. of Med. 312, 159-263 (1985)

13. McCord, J.M., Fridovich, I.: The biology and pathology of oxygen radicals. Annals of Int. Medicine 89, 122-127 (1978)

14. Michael, L.H., Hunt, J.R., Weilbaecher, D., Perryman, M.B., Roberts, R., Lewis, R.M., Entman, M.L.: Creatine kinase and phosphorylase in cardiac lymph: coronary occlusion and reperfusion. Am. J. Physiol. 248, H 350-359 (1985)

15. Michelson, A.M., Puget, K.: Cell penetration by exogenous superoxide dismutase. Acta Physiol. Scand. Suppl. 492, 67-80 (1980)

16. Uraizee, A., Reimer, K.A., Murry, Ch.E., Jennings, R.B.: Failure of superoxide dismutase superoxide dismutase to limit size of myocardial infarction after 40 min of ischaemia and 4 days of reperfusion in dogs. Circulation 75, 1237-1248 (1987)

17. Tanaka, M., Hashimoto, Y., Minczaki, K.K., Shinozaki, Y., Nakazawa, M., Tsukamoto, H., Komatsu, N., Wtanabe, K.: The fate of exogenously administered superoxide dismutase in myocardium and cultured endothelial cells. Free Rad. Biol. Med. 9, 1524 (1990) (Abstr.)

18. Werns, S., Brinker, J., Gruber, J., Rothbaum, D., Hauser, R., George, B., Burwell, L., Kereiakes, D., Mancini, G.B.J., Flaherty, J.: A randomized double-blind trial of human recombinant superoxide dismutase (SOD) in patients undergoing PTCA for acute MI. Circulation 80, II-113 (1989) (Abstr.)

Acta Medica Hungarica, Vol. 49/1-2, pp. 95-102 (1991)

PATHOPHYSIOLOGY

EFFECT OF PULSED MAGNETIC FIELDS ON HEALTHY MICE, A STUDY THROUGH CELLULAR ELECTROPHORESIS OF THYMIC CELLS

A. BELLOSSI

Laboratoire de Biophysique, Faculté de Médecine,
2, Avenue du Pr. Léon Bernard, 35043 Rennes Cedex,
France

(Received: April 8, 1991)

An exposure of adult mice to a pulsed magnetic field (PMF) increases thymus weight. In this work, thymic lymphocytes were studied by cellular electrophoresis because the proliferation and maturation of these cells is linked to an increase in their electrophoretic mobility (EM). Fifteen-week-old female Swiss mice were exposed for 30 min to a 6 mT PMF, 12 or 460 Hz in frequency, according to different modalities. The EM of the thymic cells, suspended in saline were measured from 0 to 96 h after the end of the exposure. For some of the mice the whole body, for others the head only or the body without the head was exposed; the animals were awaken or narcotized, prepared or not with 6-hydroxydopamine. The modifications of the EM are in favour of an action of the PMF on the thymus through the central nervous system.

Keywords: Cellular electrophoresis, pulsed magnetic fields, mice thymus

Introduction

In normal healthy mice thymus involution begins as early as 6 weeks of age and is demonstrable by a decrease in weight /7/. In a previous experiment /1/ we had shown that the exposure of 15-week-old mice to a pulsed magnetic field 0.6 or 6 mT in intensity, 12 or 460 Hz in frequency increased the thymus weight. The variation was the most striking 24 h after a 30 min expopsure. An effect on the thymic cellular content was taken into account.

Abbreviations: EM: electrophoretic mobility, 6-OHDA: 6-hydroxydopamine, PMF: pulse magnetic field

Offprint requests should be sent to: A. Bellossi, 2, Avenue du Pr. Léon Bernard, F-35043 Rennes Cedex, France

Akadémiai Kiadó, Budapest

Sabolovic and Dumont /8/ observed an increase of electrophoretic mobility of thymic lymphocytes during their proliferation and maturation. The EM of cortisone-resistant cells were found to be the fastest. Therefore, a cellular electrophoresis has been undertaken to determine if the exposure to PMF modifies the quality of the thymic cells.

Materials and Methods

Fifteen-week-old female Swiss mice were used. For the exposure they were put into an altuglas parallelopipedic container divided into compartments 29x29x100 mm^3 in size. The mouse, one in each compartment, was unable to move about.

The magnetic generator (Magnobiopulse - Société Atlas, Paris, France) delivered a pulsed magnetic field. The field was created from two independent discs, each 12 cm in diameter. They were put on the top of the container holding the mice. The centre of a compartment for a mouse was placed at a distance of 18 mm from the surface of an emitter disc. The field strength measured at the emitter surface was 6 mT. Twelve and 460 Hz were the frequencies used.

For experiments: the controls and mice to be exposed were taken at random and put in the same conditions, but the controls were not exposed to magnetic field. The exposure lasted 30 min just once. At given times after the end of the exposure, the mice were decapitated, the thymuses were removed and weighed. In each experiment 2 mice were used. The 2 thymuses were torn to pieces together. Then 3 ml of a 0.15 M NaCl solution, pH 7.7 (saline), was added. About 1-min after shaking the cell suspension was put into the measure room of a Zeiss cytopherometer at 25°C. The time of migration of a cell was measured in two ways by the reversal of the electric field. With a few exceptions, 100 cells or more were taken into account in each experiment. The experimenter was not informed about the group of mice the thymuses belonged to.

EM were determined in relation to the EM of erythrocytes. Before and after the thymic cells were measured, measures were done with the erythrocytes of a mouse. Here, in the saline used, the EM of a mouse was considered to be 1.10 $\mu . sec^{-1} . V^{-1} . cm^{-1}$ /5/.

In each case, the values obtained for the exposed mice were compared with the control values. The measuring for each parameter (frequency, and time after exposure) were repeated from 5 to 20 times. Groups of mice were treated as follows:

(1) Whole-body exposure of wide-awake mice; measuring were done 0, 3, 24, 48, 72 or 96 h after the end of the exposure; (2) mice narcotized for the time of exposure by an i.p., injection of 1.5 mg of pentobarbital dissolved in 0.3 ml of saline; (3) exposure of the head of wide-awake mice the rest of the body being shielded by a ferromagnetic tube; measuring 24, 48 and 72 h after the end of the exposure; (4) exposure of the body of wide-awake mice, the head being shielded by a ferromagnetic tube; (5) whole-body exposure of wide-awake axotomized mice. To produce peripheral axotomy, we injected mice with 0.2 ml 6-hydroxydopamine (6-OHDA) (Sigma Chemical Co.) (daily dose: 100 $mg . kg^{-1}$ body mass) for 10 days /6/. 6-OHDA was dissolved in saline containing 0.1 $mg . ml^{-1}$ ascorbic acid. The mice were exposed seven days after the last 6-OHDA treatment. Measuring were done 24 h after the end of the exposure.

Results

(1) Wide-Awake mice

The mean EM ± standard deviation (S.D.) data are given in the Table I. A variance ratio test with the different control groups, from 0 to 96 h, showed no significant heterogeneity. However, there was a slight increase in the EM from the 3rd h after the end of the exposure in relation to the EM of the very controls which had not stayed in the container; the mean EM at the 72nd h was even significantly different from the mean EM of the very controls ($P < 0.02$).

Table I

Awaken mice: mean EM ± S.D. expressed in $\mu \cdot sec^{-1} \cdot V^{-1} \cdot cm^{-1}$ of the thymic cells exposed to the indicated field frequencies

Time of measuring: hours after the end of the exposure. n = sample size

Time of measuring h	Mice having put in container			Controls not put in container
	Controls	12 Hz	460 Hz	
0	0.835 ±0.030 n=10	0.842 ±0.041 n=10	0.848 ±0.032 n=10	0.832±0.032 n = 20
3	0.842 ±0.038 n=11	0.863 ±0.032 n=10	0.862 ±0.032 n=10	
24	0.840 ±0.021 n=13	0.878 ±0.036 n=20	0.862 ±0.052 n=10	
48	0.852 ±0.022 n=10	0.840 ±0.038 n=20	0.862 ±0.035 n=10	
72	0.854* ±0.026 n=14	0.878 ±0.038 n=11	0.872 ±0.043 n=10	
96	0.855 ±0.031 n=10	0.871 ±0.026 n=11	0.867 ±0.046 n=11	

*$P < 0.02$ vs. the controls not put in container

Table II

Awake mice: mean EM ± S.D. expressed in $\mu \cdot sec^{-1} \cdot V^{-1} \cdot cm^{-1}$. Sums of the values obtained for the thymic cells the values at 3, 24, 48, 72 and 96 h after the end of the exposure

(for 12 Hz, the values at the 48th h have been discarded)

n = sample size

Frequency (Hz)	Controls	12 Hz	460 Hz
Mean	0.849	0.873	0.863
±S.D.	0.028	0.033	0.041
n	58	52	51

Variance ratio tests with the different groups exposed either to 12 Hz or to 460 Hz gave significant heterogeneities. All the average EM values from the exposed mice, with the exception of the average EM of mice exposed to 12 Hz 48 h after the end of the exposure, were superior to the average EM values of the controls. But, with the exception of the mice exposed to 12 Hz and measured 24 h and 72 h after the end of the exposure, no significant difference appeared between the exposed mice and the controls. This may be due to the small sample sizes and the heterogeneities of the populations. However, in consequence of the same magnitude of the values, the values from the 3rd to the 96th h of each series were put together, with the exception of the values from the 48th h of the 12-Hz exposed mice. The results are shown in Table II.

A variance ratio test comes to the conclusion that the 3 means are significantly heterogeneous. Student's t-test is in favour of significant differences between the controls and the mice which had been exposed to 12 Hz ($P < 0.001$) or to 460 Hz ($P < 0.05$); there was no significant difference between mice exposed to different frequencies.

(2) Asleep mice

The mean EM ± S.D. values are shown in Table III.

There was no difference between the results. Gathering all the values obtained at different times after the end of the exposure gave the mean values shown on Table IV.

There was no difference between the exposed mice and the controls.

Table III

Narcotized mice: mean EM ± S.D. expressed in $\mu . sec^{-1} . V^{-1} . cm^{-1}$.

Thymic cells exposed to the indicated field frequencies.

Time of measuring: hours after the end of the exposure.

n = sample size

Time of measuring (h after exposure)	Mice put in container: Controls	12 Hz	460 Hz	Controls not put in container
0	0.829 ±0.017 n=5	0.819 ±0.023 n=5	0.826 ±0.016 n=5	0.824 ±0.019 n=5
3	0.830 ±0.035 n=5	0.824 ±0.025 n=5	0.838 ±0.016 n=5	
24	0.844 ±0.019 n=5	0.840 ±0.042 n=5	0.826 ±0.021 n=5	
48	0.835 ±0.046 n=5	0.834 ±0.024 n=5	0.813 ±0.022 n=5	
72	0.825 ±0.022 n=5	0.848 ±0.012 n=5	0.829 ±0.031 n=5	
92	0.844 ±0.027 n=5	0.810 ±0.044 n=5	0.839 ±0.026 n=5	

Table IV

Asleep mice: mean EM ±S.D. expressed in $\mu . sec^{-1} . V^{-1} cm^{-1}$.

Sums of the values obtained for the thymic cells examined at different times after the end of the exposure

n = sample size

Frequency (Hz)	Controls	12 Hz	460 Hz
Mean	0.834	0.833	0.829
±S.D.	0.028	0.033	0.023
n	30	30	30

(3) Body exposure of wide-awake mice

The mean EM ± S.D. values are shown in Table V.

There was no difference between the exposed mice and the controls.

Table V

Body-exposed awaken mice: mean EM ±S.D. in $\mu . sec^{-1} . V^{-1} . cm^{-1}$ for the thymic cells exposed to the indicated field frequencies. Time of measuring: hours after the end of the exposure

n = sample size

Time of measuring (h)	Controls	Exposure to 12 Hz	460 Hz
24	0.846 ±0.017 n=5	0.834 ±0.035 n=10	0.831 ±0.040 n=11
48	0.838 ±0.019 n=5	0.824 ±0.028 n=10	0.837 ±0.027 n=11
72	0.859 ±0.032 n=5	0.838 ±0.022 n=10	0.852 ±0.026 n=10

(4) Head exposure of wide-awake mice

The mean EM ± S.D. values are shown in the Table VI.

Table VI

Head-exposed awaken mice: mean EM ±S.D. expressed in $\mu . sec^{-1} . V^{-1} . cm^{-1}$. Thymic cells exposed to the indicated field frequencies. Time of measuring: hours after the end of the exposure

n = sample size

Time of measuring (h)	Controls	Exposure to 12 Hz	460 Hz
24	0.835 ±0.013 n=5	0.864 ±0.043 n=11	0.863 ±0.039 n=10
48	0.843 ±0.025 n=5	0.869 ±0.025 n=15	0.847 ±0.036 n=17
72	0.825 ±0.018 n=5	0.869 ±0.021 n=5	0.865 ±0.037 n=11

(5) Axotomized mice

The mean EM ± S.D. values are shown in the Table VII.

There was no difference between the exposed mice and the controls.

Table VII

Axotomized mice: mean EM ±S.D. expressed in $\mu . sec^{-1} . V^{-1} . cm^{-1}$. Thymic cells exposed to the indicated field frequencies. 24 h after the end of the exposure

	Exposure to	
Controls	12 Hz	460 Hz
0.828	0.841	0.832
±0.034	±0.030	±0.041
n=19	n=18	n=20

Discussion

The stay of wide-awake mice in the container induced a slight, but obvious, increase in the cellular EM. It appeared as soon as the 3rd h and became more pronounced about the 48th h. This can only be a consequence of the handling of animals and of the restraint stress due to the stay in the container.

The modifications of the EM after the exposure at 460 Hz followed the same line as those of the controls but were more marked than those of the latter and appeared as early as the end of the exposure. The difference between the effects of 460 Hz and the effects of 12 Hz consists of a temporary return to low EM at the 48th h.

As the EM values for the anesthetized mice did not increase, all happened as if the exposure to the PMF had accelerated and amplified the reaction of the organism to the stress. Boranic et al. /2/ reported that in case of stress the CNS, and also the autonomic nerves can influence the lymphatic organs. Barbiturates can produce all sorts of degrees of depression of the CNS and selectively depress the transmission in autonomic ganglia /4/. So, an effect of the PMF via the nervous system may be taken into account.

In fact no effect appeared, if the mice's heads were shielded during exposure. It may therefore be suggested that the encephalon played an essential role in the action of the PMF on the thymic cells. Furthermore,

when only the head was exposed, an increase in the EM was observed. However, the EM at the 48th h for the exposure at 12 Hz and the exposure at 460 Hz were exactly reversed vs. to the values obtained for a whole-body exposure: an absence of acceleration was observed for the exposure to 460 Hz compared to the exposure to 12 Hz.

Axotomy suppressed the increase of EM at the 24th h. According to Felten et al. /3/ the developing thymocytes would be the suspected target cells of the noradrenergic fibers distributed in the thymic cortex. 6-PHDA is a neurotoxic agent that destroys noradrenergic sympathetic nerve terminals. Therefore, the effect of the PMF must be transmitted to the thymus through the noradrenergic fibres in case of both of the frequencies 12 Hz and 460 Hz.

Hence, an exposure of the CNS is absolutely necessary to an effect of the PMF on the thymic cells, and an action on the peripheral nervous system must also be taken into account.

Acknowledgements

We thank Dr. Reyman from the Laboratory of Pharmacology of the Faculty of Medicine of Rennes to have debated these results, M. Ruelloux, C. Rocher and M.C. Hazard from the Laboratory of Biophysics of the Faculty of Medicine of Rennes for their technical collaboration.

REFERENCES

1. Bellossi, A.: Effect of a pulsed magnetic field on healthy mice: a study of the weight of the thymus. In Vivo 3, 29-32 (1989)

2. Boranic, M., Pericic, D., Poljak-Blazi, M.: Immunological reactivity and concentration of neurotransmitters in the brain of mice stressed by overcrowding and treated with haloperiodol. Periodicum Biologorum 82, 1, 13-17 (1980)

3. Felten, O.L., Felten, S.Y., Bellinger, D.L., Carlson, S.L., Ackerman, K.D., Madden, K.S., Olschowski, T.A., Livnat, S.: Noradrenergic sympathetic neural interaction with the immune system: structure and function. Immunological Reviews 100, 225-260 (1987)

4. Goodman, A., Gilman: Barbiturates. In: Goodman, A., Gilman, Goodman, L.I., Rall, R.W., Murad, F. (eds): The Pharmacological Basis of Therapeutics. Macmillan Publishing Companies, New York, pp. 351-355 (1985)

5. Larcan, A., Stoltz, J.F., Streiff, F.: Le Globule rouge. In: Larcan, A., Stoltz, J.F., Streiff, F. (eds): La charge électrique des éléments figurés du sang. Doin, Paris, p. 62 (1974)

6. Miles, K., Chelmicka-Schor, E., Atweh, S., Otten, G., Arnason, B.G.W.: Sympathetic ablation alters lymphocyte membrane property. The Journal of Immunology 135, 2, 7975-8015 (1987)

7. Papiernik, M.: Organes lymphoïdes. In: Bach, J.F. (ed.): Immunologie. Flammarion Médecine Sciences, Paris, 1976, p. 27.

8. Sabolovic, D., Dumont, F.: Separation and characterization of cell subpopulations in the thymus. Immunology 24, 601-606 (1973)

Acta Medica Hungarica, Vol. 48/1-2, pp. 103-109 (1991)

LABORATORY

ALPHA-1-ANTITRYPSIN AND ALPHA-1-ANTITRYPSIN-NEUTROPHIL ELASTASE COMPLEX IN BRONCHOALVEOLAR LAVAGE FLUID OF PATIENTS WITH PULMONARY DISEASES

(Pilot study)

W. PAJDAK[1], M. SIERADZAK-FLEITUCH[2], A. DUBIN[3], J. OWSIŃSKI[4]

[1]Department of Immunology, [2]Department of Bacteriology, Institute of Microbiology, [4]Department of Pulmonology, Institute of Internal Medicine, Nicolas Copernicus Academy of Medicine, Kraków, Poland

[3]Institute of Molecular Biology, Jagiellonian University, Kraków, Poland

(Received: February 16, 1991)

A simple method based on rocket and crossed immunoelectrophoresis was adapted for detection of α_1 AT-HNE complexes in BALF: Agarose gel with anti-α_1AT combined with intermediate gel containing anti-HNE was used for detection and evaluation of the ratio of complexes with HNE to the free form of α_1 AT. BALF samples from 31 patients divided into 5 groups suffering from various respiratory tract diseases were tested. It was found that 3 out of 8 patients with inflammatory pulmonary diseases showed the presence of both forms of α_1 AT, 7 out of 13 patients suffering from cancer showed both forms, whereas the complexed form was not detected in any of 8 patients with asthma bronchiale. It is suggested that the presence of α_1AT-HNE complex may reflect, to some extent, the proteolytic status of pulmonary tissue.

Keywords: α_1-antitrypsin, neutrophil elastase, bronchoalveolar lavage, immunoelectrophoresis, proteinase-antiproteinase complex

Introduction

Alpha-1-antitrypsin (α_1AT), alternatively called alpha-1-proteinase inhibitor (α_1PI), seems to inactivate all the human serine proteinases tested by now. Besides, being responsible for about 90% of the total trypsin inhibitory activity /3, 9, 10, 16/, it is known as the main physiological inhibitor of human neutrophil elastase /5, 6, 16/. The protective

Abbreviations: α_1AT: alpha-1-antitrypsin, α_1PI: alpha-1-proteinase inhibitor, α_2M: alpha-2-macroglobulin, BALF: bronchoalveolar lavage fluid, HNE: human neutrophil elastase, PMN: polymorphonuclear leukocyte

Offprint requests should be sent to: Władysław Pajdak, Department of Immunology, Nicolas Copernicus Academy of Medicine, 18 Czysta Street, 31-121 Kraków, Poland

Akadémiai Kiadó, Budapest

effect of α_1AT against elastolytic activity is known to exist in many organs and tissues, but elastase- α_1AT imbalance is particularly spectacular and dangerous in some pulmonary diseases /5, 6, 14/.

The attraction of leukocytes into air spaces of the diseased lung is regulated by many chemoattractants. Among others, leukotriene B_4, interleukin-1, C5a — (a peptide generated during activation of the complement cascade), cigarette smoke /6, 16/, some peptides -- degradation products of connective tissue /15/ — and also the alpha-1-antitrypsin-human neutrophil elastase (α_1AT-HNE) complex itself /1/. Once initiated, pulmonary accumulation of leukocytes proceeds as a self-perpetuating process.

Leukocyte granules contain a broad spectrum of hydrolytic enzymes, among others elastase, which is a 33-kD single-chain polypeptide containing about 12% carbohydrate /17/. Discharged during phagocytosis or after cell death, it is ready to attack elastin of the surrounding tissues if not inactivated by an appropriate inhibitor.

α_1AT, being small enough (53 kD) to penetrate intracellular spaces, has also been found in the bronchoalveolar fluid. It seems that the proportion of α_1AT-elastase complex to the free form of the inhibitor reflects the important part of proteolytic balance in the respiratory tract.

We present here a simple method — rocket immunoelectrophoresis with intermediate gel containing antibodies to HNE -- to detect both free and complexed forms, of α_1AT. No attempts were made in this work to determine precise concentrations. However, the method is readily adaptable for this purpose.

Materials and Methods

Bronchoalveolar lavage fluid (BALF) was obtained from 31 patients. The patients were divided into 5 groups in respect of their main disease.

Group I — 13 patients (2 women and 11 men) with lung cancer. Mean age: 60.9 years, range: 49-69 years.

Group II — 2 patients with cancer not connected with the respiratory tract (1 woman with cancer of ovary, aged 51 years and 1 man with prostate cancer, aged 67 years).

Group III — 8 patients (2 women and 6 men) with various inflammatory pulmonary diseases, mean age: 47.5 years, range: 35-69 years.

Group IV — 6 patients (3 women and 3 men) with asthma bronchiale, mean age: 49.2 years, range: 31-74 years.

Group V — 2 patients (1 woman aged 55 years and 1 man aged 27 years) with sarcoidosis.

Bronchoalveolar lavage was performed through a fiberoptic bronchoscope with the method of Wilerley & Barlett, with modification of Owsiński /12/ to prevent the samples from being contaminated with oral microorganisms. Within 2 h after collection the samples were centrifuged at $4^{\circ}C$, 20 000 g for 15 min to remove the cells and occasionally present membrane fragments, and stored at $-18^{\circ}C$ until assayed.

Detection of α_1AT and α_1AT-HNE complexes.

We measured the ratio of free to complexed forms of α_1AT by the means of rocket and crossed immunoelectrophoresis using rabbit immunoglobulins to human α_1AT (Difco) and sheep anti-HNE produced in our laboratory by vaccinating sheep with human neutrophil elastase isolated according to Baugh & Travis /2/. Anti-HNE antibodies were purified according to McKinney & Parkinson /11/. In brief, the BALF samples (10 µl each) were run on 1% agarose plates containing 0.1 µl/cm^2 of anti-α_1AT or a combination of 0.2 µl/cm^2 of anti-HNE and anti-α_1AT (Fig. 1).

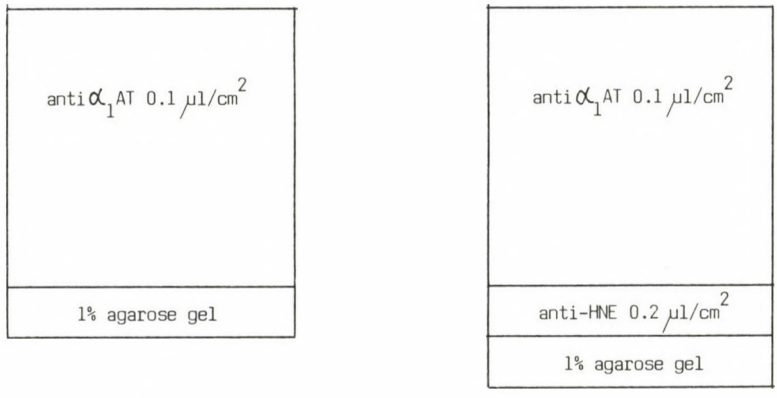


Fig. 1. Agarose plates used for a) crossed and b) rocket immunoelectrophoresis

The separation was performed in 0.1 M Tris-veronal buffer of pH 8.6 at 50V, overnight. In the case of crossed immunoelectrophoresis the BALF samples (20 µl) were first separated on 5% polyacrylalmide gel rods, according to Clarke /4/ and then the gels were cut longitudinally into halves. One of them was stained with Coomassie brilliant blue G-250, and the other was electrophoresed (perpendicularly to the first run) into agarose gel containing the proper concentration of the antibody (antibodies) as described above.

After the run, the agarose plates were pressed, dried, and stained with Coomassie brilliant blue R-250.

Results and Discussion

BALF from 13 patients with lung cancer, 8 patients with inflammatory diseases of the lower respiratory tract and 6 patients suffering from asthma bronchiale were tested for the presence of α_1AT-HNE complexes. A typical results of rocket immunoelectrophoresis of a sample containing both: free and complexed forms of α_1AT is shown in Fig. 2. The lower peak represents α_1AT-HNE complex while the upper one — the free (uncomplexed) form of the inhibitor. Peak heights of the complexed form (lower) and the

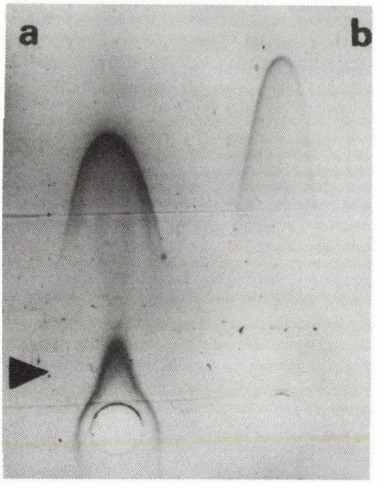


Fig. 2. Rocket immunoelectrophoresis patterns of BALF samples containing a) both forms of α_1AT (uncomplexed and in complex with HNE) and b) only one - uncomplexed form. Arrow indicates the complexed form

free one (higher) of HNE were measured and the proportion α_1AT-HNE/α_1AT was calculated. It is known that HNE liberated from polymorphonuclear leukocytes (PMNs) is immediately complexed and inactivated, mostly by α_1AT. Small amounts of HNE complexed by alpha-2-macroglobulin (α_2M) and other plasma inhibitors /14/ are negligible. The proportion of the lower-to-upper peak (complexed to free inhibitor) varies by patient, ranging form 0 to 0.54 (Table I). Parallelly all samples were analysed by two-directional (crossed) immunoelectrophoresis against anti-α_1AT. The presence of free and complexed forms of α_1AT was clearly shown in each of the materials exhibiting both forms of α_1AT found by means of rocket immunoelectrophoresis with intermediate gel, whereas only the free form was present in samples exhibiting one peak in rocket immunoelectrophoresis (Fig. 3a and 3b). In some patients additional peaks were found representing α_1AT complexes with proteolytic enzymes other than PMN elastase (the most suspected one was leukocytic collagenase), however, the concerning problems were not studied in detail.

None of 8 asthma bronchiale patients exhibited the presence of detectable amounts of complexed forms of α_1AT in BALF, moreover, most of them showed only traces of free α_1AT.

Out of 8 patients with inflammatory diseases 3 showed the presence of both forms of α_1 AT while the remaining 5 showed only the free form.

Table I

The ratio of α_1AT-HNE (alpha-1-antitrypsin-human neutrophil elastase) complex to free α_1AT (alpha-1-antitrypsin) in BALF samples

Patient	Diagnosis (main disease)	$\frac{\alpha_1 AT\text{-}HNE}{\alpha_1 AT}$
WG	Cancer (lung, larynx)	0.09
BK	Cancer (ovary, mamma, pleura)	0.12
BH	Cancer (lung, bronchi)	0.23
PM	Bronchiectasis, purulent pneumonia	0.24
KS	Cancer (lung, bronchi)	0.24
MG	Cancer (lung)	0.32
JG	Cancer (lung, bronchi)	0.37
GJ	Bronchiectasis	0.44
LS	Bronchiectasis, bronchitis	0.45
JD	Cancer (lung)	0.54

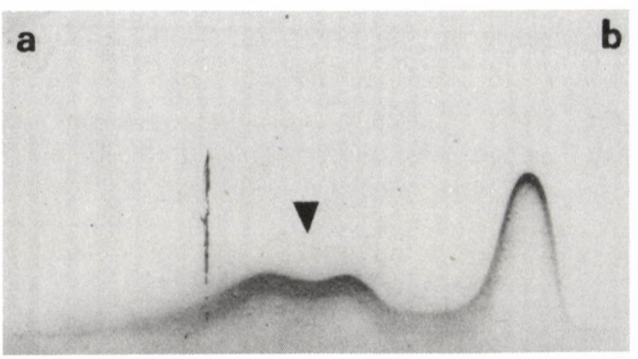


Fig. 3. Crossed immunoelectrophoresis patterns of BALF samples containing a) both forms of α_1AT (uncomplexed and in complex with HNE) and b) only one - uncomplexed form. Arrow indicates the complexed form

It is difficult to say whether the low proportion or even absence of the α_1AT-HNE complex in BALF is a rule in patients with lower respiratory tract inflammatory disease showing almost normal level of white blood cells.

It has been suggested recently that the upper part of the respiratory tract is protected against leukocytic elastase action by a so-called low-molecular bronchial inhibitor, while the lower part mostly by α_1AT /14/.

Seven out of 13 patients suffering from cancer showed clearly the presence of a complexed form of α_1AT in BALF. This high rate is not sur-

prising, while it is well established that PMN infiltration is involved in lung neoplasms. Elastase released from PMNs may penetrate surrounding cancer and healthy tissues and readily enter the BALF. It may enter this compartment either in free or complexed forms, depending on the enzyme-inhibitor dynamic status.

It should be kept in mind, however, that most of the main disease of the investigated patients were complicated by the presence of various bacterial strains in the respiratory tract /7/. The influence of the contaminating microorganisms and the interaction of their proteolytic activities are out of the scope of this study. Nevertheless, we are aware of the possible implications of this fact. An attempt of a preliminary assessment of these interactions will be presented in a separate paper.

It is difficult to say to what extent the ratio of the complexed to free form of α_1AT, when measured only once in a given patient, reflects the status of the disease and would help in diagnosing and monitoring the inflammatory process in the pulmonary tissue. Preliminary works comparing the elastase-α_1AT complex in the circulating blood and in the BALF /8, 13, 18/ seem to be very promising.

However, much more information would be drawn if the progress of the disease had been correlated with the dynamically measured changes of the concentration of free and complexed forms of α_1AT both in BALF and circulating blood.

REFERENCES

1. Banda, M.J., Rice, A.G., Griffin, G.L., Senior, R.M.: The inhibitory complex of human α-1-proteinase inhibitor and human leukocyte elastase is a neutrophil chemoattractant. J. Exp. Med. 204, 1608-1615 (1988)

2. Baugh, R.J.R., Travis, J.: Human leukocyte granule elastase: rapid isolation and characterization. Biochemistry 15, 836-843 (1976)

3. Bermiger, R.W.: Alpha-1-antitrypsin. In: Murano, G. (ed.) Protease Inhibitors of Human Plasma - Biochemistry and Pathophysiology. J. Med. 16, 23-100 (1985)

4. Clarke, T.J.: Simplified "disc" (polyacrylamide gel) electrophoresis. Ann. NY Acad. Sci. 121, 428-436 (1964)

5. Janoff, A.: Investigations into the biochemical mechanisms of pulmonary emphysema: effect of cigarette smoke on enzymes and anti-enzymes in the lung. Respiration 50, Suppl. 1, 13.25 (1986)

6. Janoff, A.: Emphysema: proteinase - antiproteinase imbalance. In: Gallin, J.I., Goldstein, I.M., Snyderman, R. (eds) Inflammation: Basic Principles and Clinical Correlates. Raven Press, New York, 1988, pp. 803-806

7. Kasprowicz, A., Owsiński, J., Białecka, A., Heczko, P.B.: Anaerobic bacteria detected in inflammatory conditions of respiratory tract pulmonary infections. Med. Dośw. Mikrobiol. 42, 108-115 (1991)

8. Klech, H., Rona, G., Kummer, F., Bayer, P.M.: Neutrophil elastase alpha-1-proteinase inhibitor complexes in pleural effusions. Klin. Wochenschr., 66, 346-350 (1988)

9. Koj, A.: The plasma alpha-1-antitrypsin: its biochemical properties, congenital deficiencies and biological functions. Przegl. Lek. 29, 699-705 (1972)

10. Laurell, C.B., Jeppsson, J.O.: Protease inhibitors in plasma. In: Putnam, F.W. (ed.) The Plasma Proteins. Academic Press, London-New York, pp. 229-264 (1975)

11. McKinney, M.M., Parkinson, A.: A simple, nonchromatographic procedure to purify immunoglobulins from serum and ascites fluid. J. Immunol. Meth. 96, 271-278 (1987)

12. Nikodemowicz, E., Owsiński, J., Heczko, P.B., Kasprowicz, A.: The significance of bronchoscopy in bacteriologic diagnosis of penumonias. Pneum. Pol. LII, 535 (1984)

13. Ochnio, J.J., Abboud, R.T., Lam, S., Johal, S.S., Smith, C.E., Johnson, D.A.: Bronchial leukocyte proteinase inhibitor levels in bronchial washings in asthma patients. Chest 93, 1008-1012 (1988)

14. Pajdak, W.: Some aspects of the inactivation of alpha-1-antitrypsin in human pulmonary tissue. Zesz. Nauk. UJ - Seria Biol. Mol. 19, 45-54 (1990)

15. Senior, R.M., Griffin, G.L., Mecham, R.P.: Chemotactic activity of elastin-derived peptides. J. Clin. Invest. 66, 859-862 (1980)

16. Travis, J., Salvesen, G.S.: Human plasma proteinase inhibitors. Ann. Rev. Biochem. 52, 655-709 (1983)

17. Watorek, W.: Heterogeneity of human neutrophil elastase and cathepsin. G. Zesz. Nauk. UJ - Seria, Biol. Mol. 19,15-19 (1990)

18. Yoshida, A., Kuramitsu, K., Aoki, H., Nagata, M., Kikuchi, H.: Study on polymorphonuclear elastase-alpha-1-proteinase inhibitor complex in bronchoalveolar lavage fluid (BALF). Jpn. J. Med. 27, 60-63 (1988)

This work was supported in part by the grant DN-B/WL/29/PKL/9

Acta Medica Hungarica, Vol. 48/1-2, pp. 111-118 (1991)

LETTER TO THE EDITOR

ACTA MEDICA
Akadémiai Kiadó

P.O. Box 24
1363 Budapest
Hungary

1 June 1991

Dear Sir,

We are pleased to send you the enclosed article which we would like to be published in your review.

Yours faithfully

J.P. WILLEM M.D.
President
INTERNATIONAL BIOLOGICAL UNION

Offprint requests should be sent to: J.P. Willem, F-75015 Paris, 222, rue de Vaugirard, France

Akadémiai Kiadó, Budapest

CONTRIBUTION TO THE UNDERSTANDING OF DEGENERATIVE PATHOLOGY AND A PROPOSAL OF A GLOBAL THERAPEUTIC SOLUTION

J.P. WILLEM

The purpose of this paper is to consider the possibility of a global solution to degenerative pathology by means of a simple, unique, ambulatory and totally innocuous procedure.

Degenerative pathology (canceration, atheromasia, multiple sclerosis, schizophrenia and Alzheimer's) constitutes the major agent of morbidity and mortality. In this role, it took the place of viral pathology, suppressed by vaccination, and of infectious pathology, eradicated by treatment with antibiotics. Its progression has been resistant to all efforts and no solution to any of these affections has yet been found.

However, biologically, there are reasons to believe that a common solution resides in the drying up of the growth hormone in the adult. Following are the reasons, all of which are based on established biological data.

First of all, in the subject whose growth has stopped, the growth hormone no longer serves any purpose.

Whether natural and taking place with age or provoked, its drying up is not accompanied in the adult by either immediate or later physiological or pathological effect. Once growth is finished, the growth hormone does not have any more purpose than the other remnants of the organogenesis which is particularly slow in the human species.

With no physiological interest remaining in the adult, the growth hormone presents the inconvenience of maintaining the stimulation of the immature remnants of the organogenesis and the cells which, like cancerous ones, recover a status of immature cells.

This proliferative stimulation of the residual or regressive cells is the major factor in degenerative pathology, for which we will consider the various expressions.

CANCER

One cannot induce cancer in an hypophysiolyzed animal (Courtial)

This inhibition of carcinogenesis results from the fact that the growth hormone is a major factor in the proliferation of the embryonic cells or the cells which, like the cancerous ones, recover an embryonic status.

The sensitivity of the cancerous cells to the growth hormone can be seen as well in vitro as in vivo. Moreover it constitutes the foundation for the indication of hypophysectomy, in painful uncontrollable cancers, by generating a quiescence of the proliferation with a consequent antalgic effect.

Considering the incidence of cancerisation in general mortality, it is better to inhibit carcinogenesis than to fight against cancer. In other words, it is better to proceed with the drying up of the growth hormone as soon as it does not serve anymore purpose than to risk a cancerisation to preserve an organogenetic remnant, which no longer serves any useful purpose at adult age.

AGEING

Genetic programming sets life duration; this maximal life duration is about 120 years in the human species. This programming is fundamentally conditioned by the limitation in the number of possible cellular divisions. In the human species, this "intrinsic kinetic potential" is 70 divisions (in vitro as in vivo) of which 50 are used up during adult maturation of the organism and 20 are available for the rest of the individual life.

The precocious ablation of the hypophysis is accompanied, in animals by an increase of life duration.

The reason for this effect is the same as that which we considered for cancerous cells, i.e. "the growth hormone has a stimulating action on tissular growth in general and on that of tumors in particular" (Sibilly).

By stimulating the maturation of the stock of cells remaining at adulthood, the growth hormone hastens the exhaustion of this "intrinsic kinetic potential" of the cellular populations, and thus reduces longevity. Giants and acromegalics die prematurely; the premature drying up of the growth hormone has an inverse effect.

Of all degenerative processes, the only inescapable one is ageing, which sees the exhaustion of the genetically limited number of cellular divisions, indispensable in replacing the cells used up by the metabolism. The elimination of the stimulating hormone which hastens the exhaustion of this kinetic potential delays the terminal impoverishment of the parenchymas which leads to natural death.

ATHEROMASIA

We have known since 1985 that arterial degeneration fundamentally results from a cancerisation of the arterial wall. This very cancerisation of the vascular muscular cells initiates the development of the atheromatous deposit. All international teams agreed (12th Symposium in Kyoto, 1988) with the new datum which was introduced in France in 1986.

We can already notice two essential points. On the one hand, the most usual manifestations of atheromasia (myocardial infarct, hemiplegia) are both on the list of the states of hypersecretion of the growth hormone; on the other hand, under yet the most atherogenous conditions, the atheromatous evolution is stopped by the drying up of the growth hormone (Sheehan's Syndrome).

Therefore we have all biological reasons to think that, on the one hand, this drying up is able to break the positive correlation between the growth hormone and atheromasia and, on the other hand, to inhibit the atheromatous evolution.

MULTIPLE SCLEROSIS

A French Government inquiry (Salpetrière 1980) seeking to define the correlation between multiple sclerosis and hypophyseal somatotrophic hormone reached the following conclusion: there has never been a case of an hypophysiolyzed individual who contracted multiple sclerosis.

The explanation of this phenomenon is based on the fact that the nervous system is not mature at birth. In effect, the nervous organogenesis is extremely slow; it is completed only at about forty or so and the myelinogenesis which conditions and reflects the accession of the immature neuronal islets to their function becomes discontinuous at the end of its course.

Sclerosis patches are scars of a viral infestation of the myelinic cells (oligodendrocytes) satellites of those islets of tardy maturation. When viral myelinitis occurs in a young adult whose neuronal organogenesis has become insular and discontinuous, the scars become nodular and iterative. By not allowing the maturation of these terminal residual islets from adulthood, the drying up by hypophysiolyzis of the growth hormone does not allow this infestation to be expressed and thus does not allow the disease to develop. That's why an hypophysiolyzed patient cannot get multiple sclerosis, which the research confirms.

Since this drying up does not cost anything biologically, it is clear that it is necessary to proceed with it as soon as the diagnosis of multiple sclerosis is made.

Furthermore, to abstain from drying up of the growth hormone in patients with multiple sclerosis, when one knows that the secretion of somatostatin which inhibits this hormone is failing in these patients, would represent a real problem of conscience.

SCHIZOPHRENIA

It is sufficient to consult the table of the hypersecretion's states of growth hormone to notice that schizophrenia is found at the top of the list, immediately after acromegalia. Furthermore, these two affections encertain close bounds which are manifested by the high frequency of acromegaloid morphotype in schizophrenia.

In addition, there is, in schizophrenia, there is an abnormal secretory response, as much from chemical as from hormonal stimulation, in the cells which secrete the growth hormone (Cilad-Dickerman).

The drying up of the growth hormone, aiming to rupture this corerelation binding the growth hormone and schizophrenia, is absolutely essential as it is in acromegalia.

As in multiple sclerosis, an understanding of the pathogenic mechanism is based on the fact that the nervous system is not mature at birth.

By electively stimulating the tardy immature neuronal remnants of cephalization because of their abnormally low threshold of activation, the growth hormone confers on them a prevalence which finally becomes dominant over the already organized sites of the initial and fundamental cephalization.

Now, the initially organized sites of the cephalization emit information shaped by reality and educated by apprenticeship during two decades whereas terminal sites have no time to be shaped by confrontation with reality. If the information emitted by the terminal sites, which is normally accessory and subordinate, becomes prioritary and dominates over the main information, the hierarchy of the information is ruptured and reversed: that is schizophrenia. It sees the progressive remoteness from reality and prevalence of the uneducated and unbridled signals from the terminal cephalization over the initial signals durably shaped by reality.

This contamination is progressive because it is the activation of the pathways which conditions, anatomically by increasing their calibre, the speed of the signals having access to the frontal integrator. The more, the terminal network is active, the more its fibers are conductive and the more the principal network becomes functionally frustrated and anatomically involutive. The inversion of the hierarchy of information at the frontal leads to the irrevasibility of the process.

ALZHEIMER'S DEMENTIA

Alzheimer's dementia is to the aged adult what schizophrenia dementia is to the young adult. The two processes are fundamentally indentical. They both result from a parasitic and finally exclusive dominance of the information emitted by the terminal sites of the cerebral organogenesis over that emitted by the initial sites.

Now this terminal information is that which has been the least shaped by reality and the least educated by a long apprenticeship of two decades. Under normal conditions, this terminal information is subordinated to the main information, which is prioritary, and limited to influencing it. When this hierarchy in information gets reversed, there is a progressive rupture with reality; that is dementia. Since it is activation which conditions the trophism and conductibility of the networks, as soon as a dominance of the terminal network starts, it tends to become invasive, then irreversible and finally exclusive. The information is transferred from an ancient structure which is shaped by reality towards a recent one which is totally free from experienced contingencies.

To describe schizophrenia and Alzheimer's is to repeat oneself, except the clinical expression differs according to the age. In schizophrenia

in the young adult, the abnormal receptivity of the terminal neuroblasts to the growth hormone induce their hyperplasia with a unreal mental symptomatology which generally is productive, excessive and expansive, whereas in Alzheimer's, in the aged adult, the premature disappearance of the large cells of the initial network makes the ancient and essential memorial data which are fixed in it disappear, giving free rein to the accessory and uneducated information of the terminal network. The fundamental memory regressess with the regresses of the fundamental cortex.

Based on this mechanistic identity the follows a single therapeutical solution which is the drying up of the growth hormone.

In schizophrenia, this drying up prevents the maturation of the terminal parasitic sites.

In Alzheimer's, this drying up implies the same precociousness, that is to say several decades before the appearance of the disease, because it appears at an age when the cerebral organogenesis is totally realized. It seeks to eliminate the subsequent competition of a terminal uneducated structure becoming parasitic as soon as the large initial neurones, which prematurely degenerate in Alzheimer's, disappear. However, since it is the functional activation which maintains the trophicity and survival of the networks, the impoverishment of the primary one is hastened if parasitic pathways exist turning the information aside. Of course, the drying up does not re-establish the prematurely destroyed neurones but, on the one hand, it excludes the competition of an uneducated network carrying factors of dementia and, on the other hand, it prolongs the activation and thence the survival of the fundamental residual neuronal stock carrying the semantic memory.

There is no other solution for Alzheimer's. One cannot effectively count on a neuronal plasticity which does not exist any longer at this age in a structure otherwise impoverished. It is also illusive to consider now the suppression of the multiple causal factors, among which are the neurotropic poisons. They act in minimal and cumulative doses since childhood and result in reducing the longevity of the initial neurones whereas the latecomers of the organogenesis, whose parasitic activity needs to be prevented, escape because of their tardy maturation.

As far as we know, a consultation of the records has not yet been done, which would demonstrate the inexistence of Alzheimer's in aged adults hypophysiolyzed when they were young. But we already know that children

treated with growth hormone run the risk, of which their parents are warned, of contracting a dementia, decades later, at the Alzheimerian age.

* * *

Finally, degenerative pathology consists of a parasitic prevalence of blastic remnants (or cells which recover an embryonic status).

In an adult, they are physiologically as useless as the growth hormone which stimulates them.

Degenerative pathology is in accordance with the Conheim's law, which has remained a standard though unexploited for the past century.

The drying up of the growth hormone in adults will prevail due to simplicity of the theory, experimental evidence, socio-economic necessity and, most of all, the benignity of the procedure.

The effective dose of radiation focused on the hypophysis is well established (13.5 mCi) and is sufficient to inhibit exclusively the growth hormone, the secretion of the other hypophyseal hormones being respected. It is applied using a simple radiotherapeutic flash by means of the helmet of a Gamma Unit apparatus. This apparatus is widely available and the difficulty presented by the procedure is no more than that of a standard radiological examination.

Acta Medica Hungarica, Vol. 48/1-2, pp. 119-124 (1991)

BOOK REVIEWS

Neuroembryology: The Selected Papers of Viktor Hamburger with an Introduction by Ronal W. Oppenheim. pp. XV+420. Birkhäuser, Boston, 1990. Price: DM 138.-

This is an interesting collection of papers celebrating the 90th birthday of their author, a well-known embryologist, Professor V. Hamburger. He began his resourceful research works on amphibian embryos in the early 1920's in H. Speemann's laboratory, and a decade later he switched to chicken embryos in F. Lillie's laboratory to pursue the problems of development on this model during the subsequent 50 years. He is the only living witness of the heroic epoch of experimental neuroembryology initiated by R.G. Harrison and completed to a high perfection by S. Detwiler, G. Coghill, P. Weiss, R. Sperry and himself. He was at the cradle of the Nerve Growth Factor, which earned the Nobel Prize for Rita Levi-Montalcini and S. Cohen in 1986.

The selected papers are classsified in seven groups. The first group contains 8 review papers on developmental neurobiology. In them, Professor Hamburger investigates the development of the nerveless amphibian limb, the principles of neuroembryology in terms of the then fashionable neuronal specificity, and the naturally occurring cell death. In the second group, 4 papers are devoted to the problems of development of motility and behaviour representing a beautiful series of experiments performed mainly on chicken embryos. In the 4 papers of the third group, the author gives comprehensive treaties on the history of neurogenesis, reaching back to the pioneering works of S. Ramón y Cajal and R.G. Harrison. The title of the fourth group is Developmental Genetics and Evolution. The 3 papers on this topic demonstrate Professor Hamburger's wide interest in various aspects of development: as early as 1942, he investigated the mechanisms of hereditary developmental abnormalities. The fifth group contains reviews of 5 books in the field of embryology and evolution. The sixth group comprises 2 biographies of H. Speemann and the reprint of his S. Kuffler lecture that he presented at the Harvard University with the title The Rise of Experimental Neuroembryology. Two papers are listed in the last, Miscellaneous, group. One of them gives an account of his visit to Japan, where he visited Emperor Hirohito's marine zoology laboratory among others. The second paper is a most interesting comment on Goethe's work "Zur Farbenlehre". - Unfortunately, two volumnious works by Professor Hamburger could not be fitted within the limited space of the book. The Manual of Experimental Embryology was, and still is, one of the books most favoured by those who have chosen the rough road of experimental embryology, and the second, A Series of Normal Stages in the Development of the Chick Embryo, is one of the most frequently cited papers in biology.

It is an intellectual pleasure to page through the book and to spend time at selected pages. The book is warmly recommended to neurobiologists and developmental biologists and to students interested in the struggling life of a natural scientist with a strong impact on the growth of our knowledge in biology.

GEORGE SZÉKELY

Akadémiai Kiadó, Budapest

N. Mrosovsky: Rheostasis: The physiology of chance,
Oxford University Press, New York, Oxford, 1990.
Price: £ 40.0

Rheostasis, as defined in Mrosovsky's own words, "refers to a condition or state in which, at any one instant, homeostatic defenses are still present but over a span of time there is a change in the regulated level". Therefore, rheostasis includes a change in set-point, when it is used to indicate a mechanism comprising negative feedback with a reference signal. Rheostasis may apply to both slow (gradual) and sudden changes, but the distinction between slow and sudden changes is not always clear.

Two circumstances in which rheostasis occurs can be distinguished. Changes in defended levels may be obligatory incertain phases of the life cycle and may occur regardless of conditions. These referred to as examples of programmed rheostasis (1), are often though not necessarily cyclic. There also may be developmental, once-in-a-lifetime examples of rheostasis.

Reactive rheostasis (2) occurs in response to stimuli that may or may not be encountered. Mrosovsky gives a set of examples of these two circumstances to explain the two types of rheostasis. Mrosovsky's book is indeed a very interesting review of some exciting new concepts of physiological changes.

JENŐ MAJOR

WHO IARC-Biennal Report: 1988-1989
(For the period 1 July 1987 to 30 June 1989)
IARC, Lyon France 1989

Several successful projects were finished or almost completed in the Agency through June 1989, and the results described in the Biennium Report. Some of them are highlighted. While others are more detailed in the reports. There is a chapter dealing with the epidemiology of cancer to plan the beginning of the sixth Volume of Cancer Incidence on Five Continents. This study includes 30 new centres including registeries in Africa and the USSR. In the present studies the cancer incidence is analysed of Jewish immigrants to Israel from different European, Australian and South American Countries. 17 European countries have been initiated to follow geographic and temporal trends in the incidence of childhood leukaemia from 1980 to the 1990s. This study will allow the evaluation of whether there are changes related to exposures ot the accident at Chernobyl in 1986.

The Agency maintains large interest in the identification of different occupational hazards. Cohort studies are in progress on workers exposed to silica, vinyl chloride and styrene.

The reports contain the epidemiology of the cancer risk following chemotherapy. 14-fold increase on risk for leukaemia was found when therapy of the MOPP type for Hodgkin's disease was continued. The large scale of collaboration between clinicians, epidemiologist and cancer researchers should make possible to identify what specific type and extent of DNA damage induced by the alkylating agents is responsible for the

chemotherapeutic and the long-term adverse effects. The possible relationship between mutation and cancer is also detailed here and the protective effects of vegetables, vitamins and fibres are discussed. The increased risk of cancer in the case of high meat consumption has been confirmed again.

Smoking as a cause of lung cancer is conclusively established and the reports are pointing out to pay more attention to the increasing rate of smokers among young people and among women in developing countries.

SEARCH (Survellance of Environmental Aspects Related to Cancer) programme is an international collaborative programme which was established to provide evidence on the relevant aetiological hypotheses.

The first SEARCH study was conducted on the aetiology of pancreas, gallbladder and bile duct cancer. That tobacco smoking increases the risk of pancreatic cancer was clearly demonstrateds. In the future other studies will be finished on the brain tumour development in children and adults, leukemia and other haematological malignancies will be put on the projects in collaboration with the European Organization for Reserach and Treatment of Cancer. The estimation of risk factors for cervical cancer and the HPV screening is in progress also. Samples and questionnaires are being collected from 20 countries. Another project is aimed to assess the role of N-nitroso compounds themselves or in conjugation with other factors in the origin of human cancer. The Agency has continued to help the evaluation of early detection and cancer prevention programmes for examples in China and in Philippines with regard to stomach cancer and in Czechoslovakia with regard to the lung cancer.

A chemoprevention for precancerous lesions of the stomach by using certain vitamins is under consideration in a high-risk population in Venezuela.

Variation in individual susceptibility to cancer are also being investigated by analysing and measuring individual capacity to metabolize carcinogens by the use of the test drugs as orforin, debrisoquine and antipyrine, which may allow the noninvasive investigation of P-450 isoenzymes.

Measurements of DNA adduct in human tissues are now being assessed as a marker of exposure and their biological significance is being explored. Experimentally, it was shown that levels of DNA adducts after either single or repeated exposure(s) to N-nitrosodimethylamine are similar in the liver and in lymphocytes. This finding supports the more general use of peripheral blood lymphocytes in the human population monitoring studies by DNA-adducts measurement indicating the total body burden and can be used in the biological dosimetry.

ANNA TOMPA

WHO, IARC Cancer: Causes occurrence and control

Editor-in-Chief: L. Tomatis

Co-Editors: A. Aitio, N.E. Day, E. Heseltine, J. Kaldor,

A.B. Miller, D.M. Parkin and E. Riboli

IARC, Scientific Publications, NO 100, Lyon 1990

This Volume presented an easily-readable description of what is known about the incidence of and morbidity from cancer and the actual possibilities of cancer prevention This book gives Marginal picutre about the progress of cancer research and presents an update of the new hopes for the cancer prevention and control.

The book presents the available evidence of the preventability of human cancer and describes its occurrence. It gives information about the agents and risk factors that have been causally associated with the development and early detection of human cancer and provides current information that could lead to preventive intervention at the population level.

Part I contains information on the occurrence of the cancer around the world. The cancer incidence and mortality are described and the geographical differencies in the occurrence of various types of cancer are also shown. Such descriptive epidemiology provides the global dimension to the cancer problem and provides the basis for hypotheses about the aetiology of cancer.

Part II begins with a discussion of how causes of cancer are identified. The major part of this section deals with known environmental determinants of cancer. This chapter describes agents that are recognized as being causally related to human cancer as well as those for which a causal relationship at present are partially verified.

In Part III the authors review the potential benefits of early detection. This is the screening of individuals who have no overt symptom of the disease.

In the final Part IV the book attempts to quantify extent to which primary prevention and early detection are feasible for the most common cancer.

In general, it is a sad reality that in most part ot the world including Hungary the standards of the health care, prevention and treatment of cancer are far from being optimal. For all expert who would like to improve the situation, it is necessary to read this book to renew their information and to compare their situation to this summarized picture.

ANNA TOMPA

Biological and toxin weapons today

Edited by E. Geissler

SIPRI, Oxford University Press, Stockholm

The change in the view of the military value of biological (BW) and toxin weapons (TW), i.e. their use mainly for aggression instead of terrorism and sabotage, their application by the Iraqi troops in the Iraqi-Iranian War against the unarmed population and the threats by the Iraqi leaders in the 1991 Gulf War support the sorrowful actuality of this book.

The 20 contributors follow the history of the use of BWs and TWs from the first BW attack by the Mongols in 1346 to the present and outline the development of the new-generation weapons and particularly the new-generation methods of protection as, e.g., vaccination. The contributors consider the points of view of an aggressor or of a defender in the strategy. They evaluate the theoretically and/or practically available possibilities and methods and also disucss the fallacy even of the defensive BW programmes. The effects of BWs and TWs on the world health and environment are also dealt with in the book.

BWs and TWs and military doctrines of the USA and the role of these weapons in the new US strategy are surveyed in a discrete chapter. It is very important that in this strategy the US policy strictly "prohibits the first use of lethal or incapacitating chemical munitions". It also prohibits any use of BWs.

The contributors also deal with the international conventions regulating and/or prohibiting the use or misuse of BWs and TWs. Among these conventions probably the most important is the 1972 "Convention on the Prohibition of the Development, Production and Stockpilling of Bacteriological (Biological) and Toxin Weapons and on their Destruction", ratified by 103 states as of 1985, the first real internationally-negotiated measure of disarmament. It does not prohibit, however, the work on "microbial or other biological agents or toxins of types and quantities that have justification for prophylactic, protective or other peaceful purposes".

The "Biological and Toxin Weapons Today" is very helpful for those, who are interested in the scientific and legal questions of this threating problem.

JENŐ MAJOR

Control of sexually transmitted diseases

Technical Report Series

Editor is not indicated

World Health Organization (WHO), Geneva

The decrease of the frequency of sexually-transmitted diseases (STDs) after World War II stopped in the late 1960s and a strong increase to a disturbing level has been observed, despite the availability of well-established treatment methods. The complications that may arise at individual, family and community levels, alarmed the WHO to achieve the goal of public health problems including personal patients' care, health education, information and welfare services. Therefore, a scientific meeting was organized by the WHO in Washington (USA) in April of 1982 to examine the problems of the present situation. This book is a brief summary of the meeting.

The "book" deals with a wide selection of technical problems from the initial planning steps, e.g. the estimation of the public health importance of STDs in a given country and the determination of the priority groups, to the programme management. The book also deals with the intervention strategies, the prevention including information and professional training and the laboratory services. Appendices give information about the tests currently available for the early detection of STDs and also contain some prototype patient care protocols, e.g. urethral and vaginal discharge and genital ulcers.

The "Control of Sexually Transmitted Diseases" is useful for the workers of public health services, the physicians of the everyday practice rather than for the specialists of STDs.

JENŐ MAJOR

Acta Medica Hungarica, Vol. 48/1-2, pp. 125-126 (1991)

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

World Health Organization

Lyon - France

FELLOWSHIPS FOR RESEARCH TRAINING IN CANCER 1992-1993

Applications for training fellowships in 1992-1993 are invited from junior scientists wishing to be trained in those aspects of cancer research related to the Agency's own programmes: epidemiology, biostatistics, environmental and viral carcinogenesis and mechanism of carcinogenesis.

Applicants should be engaged in research in medical or allied sciences and intend to pursue a career in cancer research.

Fellowships are awarded for one year and are tenable at the Agency or in another suitable institution abroad. Fellows will, in general, be selected from applicants with some postdoctoral research experience related to cancer in medicine or the natural sciences. Applicants requiring basic training in cancer epidemiology will also be considered. They must have an adequate knowledge, both written and spoken, of the language of the country in which their fellowship is tenable.

Applications cannot be accepted from people already holding fellowships enabling them to study abroad.

Stipends will vary according to the cost of living in the country of study. The cost of travel for the applicant, and in certain circumstances, that of one dependent, will be met.

This programme is partially supported by the Italian Association for Research on Cancer.

VISITING SCIENTIST AWARD
1992-1993

This award is intended for established cancer research workers with a minimum of five years postdoctoral experience who wish to spend up to one year at IARC working on the implementation of a collaborative research project related to the Agency's own programmes: epidemiology, biostatistics, environmental and viral carcinogenesis and mechanisms of carcinogenesis.

Applicants must belong to the staff of a university or a research institution. They must provide a written assurance that they will have a position to return to at the end of the period of award.

Candidates should submit their applications after consultation with an IARC scientific staff member. Applications will be reviewed by the Fellowships Selection Committee each year.

There will be an annual renumeration and the cost of travel will be met.

Fellowship application forms and more detailed information are available from:

Education and Training Programme
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
150 cours Albert-Thomas, 69372 Lyon Cedex 08
France

Applications must reach the Agency no later than 31 December 1991.

Acta Medica Hungarica, Vol. 48/1-2, pp. 127 (1991)

N E W S

Applied Biosystems Ltd.,
7 Kingsland Grange,
Woolston,
Warrington, WA1 4SR,
United Kingdom.
Tel: 0925 825525
Fax: 0925 825378

Contact: **John McQuillian**

Automated Purification of Small Quantities of Genomic DNA Now Possible

APPLIED BIOSYSTEMS INC. LAUNCHES PRODUCTION OF DNA FOR THERAPEUTICS

40-nanomole Polystyrene Columns for DNA Synthesis

ANALYSE A BROAD RANGE OF PROTEINS WITH THE NEW MICRO-COAT™ PROTEIN ANALYSIS KIT FOR CAPILLARY ELECTROPHORESIS

NEW HPLC COLUMNS CATALOGUE AVAILABLE FROM APPLIED BIOSYSTEMS

NEW ANALYTICAL TECHNIQUE FOR THE ELIMINATION OF PROTEIN-WALL INTERACTIONS USING A CAPILLARY-COATING REAGENT

GEL-FILLED CAPILLARIES FOR CAPILLARY ELECTROPHORESIS NOW AVAILABLE FOR OLIGONUCLEOTIDE ANALYSIS

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

INFORMATION FOR AUTHORS

Acta Medica Hungarica is published under the auspices of the Hungarian Academy of Sciences. Manuscripts and editorial correspondence should be sent to the editorial office: H-1450 Budapest 9, P.O. Box 67.

Original articles dealing with clinical and experimental medicine will be accepted with the understanding that they have not been and will not be published elsewhere and are subject to editorial revision.

Form of manuscripts

Two copies of the manuscript typewritten double-spaced with margins at least 4 cm wide should be submitted. Pages should be numbered consecutively. The first page should contain (1) the title of the paper (2) the initials and first name(s) of the author(s), (3) name of the institution where the work was done, (4) name and address of the author to whom correspondence and offprint requests should be addressed — this will appear as a footnote; (5) an abstract not exceeding 250 words which states the purposes of the study, the main findings and principal conclusions. Below the abstract provide 3 to 10 keywords that will assist indexers in cross-indexing the article.

The text of the paper should be divided into sections with the headings: Introduction, Materials (Patients) and Methods, Results, Discussion, References.

Unusual abbreviations should be identified in an alphabetical list typed after the abstract and keywords.

Drugs must be referred to by their WHO code designation (Recommended International Nonproprietary Names); use of proprietary names is unacceptable.

The international system of units (SI) should be used for all measurements.

References

These should be cited in the text as numbers in square brackets. The list of references should contain in alphabetical order of the first authors' names the following: authors' last names with initials; for journal articles the title of the paper (lower case), journal title abbreviated according to the style used in Index Medicus, volume number, inclusive page numbers, year of publication in parentheses; for books the title (upper and lower case), publisher, place and date of publication. Only manuscripts accepted for publication may be included in the reference list.

Examples:

1. Stagg, B. H., Temperly, J. M., Wyllie, J. H.: The fate of pentagastrin. Gut **12**, 825—829 (1971)
2. Falkner, F.: Prevention in Childhood of Health Problems and Adult Life. WHO, Geneva 1980
3. Fishman, A. P.: Dynamics of pulmonary circulation. In: Hamilton, W. F., Dow, P. (eds): Handbook of Physiology. American Physiological Society, Washington 1963, pp. 65—79

Tables

Each table should be typed on a separate sheet. They should be numbered consecutively with Roman numerals and have a brief specific title. The data presented in the table must be logically and clearly organized and should be self-explanatory. Omit internal horizontal and vertical rules. Cite each table in the text and indicate its approximate place on the margin.

Illustrations

Figures should be submitted in duplicate. They must be numbered consecutively with arabic numerals. All figures should bear the name of the first author, the figure number and an arrow indicating the top. Cite each figure in the text and indicate its approximate place on the margin. If a figure has been published, acknowledge the original source and submit written permission from the copyright holder to reproduce the material. Figure captions should be submitted typed double-spaced on a separate sheet.

Proofs and reprints

The first authors will receive (1) comments and suggestions of the Editorial Board for improving their paper; (2) a set of proofs for correction; corrected proofs should be returned without delay to the editorial office; (3) 100 reprints free of charge.

Index: 26.015

Acta Medica Hungarica

VOLUME 48, NUMBERS 3–4, 1991

Akadémiai Kiadó, Budapest

ACTA MED. HUNG. 48 (3–4) 125–237 (1991) HU ISSN 0236–5286

ACTA MEDICA HUNGARICA

A QUARTERLY OF THE HUNGARIAN ACADEMY OF SCIENCES

Acta Medica publishes reviews and original papers on clinical and experimental medicine in English.

Acta Medica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences
H-1117 Budapest, Prielle K. u. 19—35

Manuscripts and editorial correspondence should be addressed to the Managing Editor:

Dr. Miklós Papp

Acta Medica
H-1083 Budapest, Szigony u. 43 or H-1450 Budapest 9, P.O. Box 67

Subscription information

Orders should be addressed to

AKADÉMIAI KIADÓ
H–1519 Budapest, P.O. Box 245

"This periodical is included in the document delivery program THE GENUINE ARTICLE of the Institute of Scientific Information, Philadelphia. The articles published in the periodical are available through *The Genuine Article* at the Institute for Scientific Information, 3501 Market Street, Philadelphia PA 19104."

Acta Medica Hungarica is abstracted/indexed in Abstracts of World Medicine Abstracts, Chemical Abstracts, Current Contents–Clinical Medicine, Excerpta Medica, Index Medicus, International Abstracts of Biological Sciences

CONTENTS

Acta Medica Hungarica, Vol. 48/3—4, pp. 127—136 (1991)

NEUROLOGY

PARKINSON'S SYNDROME: CRANIAL COMPUTED-TOMOGRAPHY FINDINGS. THEIR DEPENDENCE ON SEX AND AGE

W. GRUNDL, R. ZIEGLER, K.-P. WESTPHAL, M. HUFNAGL, J. SZIRTES, H. H. KORNHUBER

Department of Neurology, University of Ulm, Clinic Dietenbronn, Ulm, Germany

(Received: July 10, 1991)

Cranial Computed Tomography (CCT) findings in 123 patients suffering from Parkinson's syndrome were compared with a sex- and age-matched normal control group. Signs of supratentorial atrophy — cortical and subcortical — were more significantly marked in the Parkinson group. In both groups, signs of atrophy were more prevalent in men than in women. Parkinsonian women also more frequently showed limited features, such as lacunar lesions, around the lateral ventricles. Moreover, in these patients atrophy was marked in the frontal cortex.

Keywords: Parkinson's syndrome, computed tomography, sex differences

Introduction

Parkinson's syndrome is an entity characterized by three extrapyramidal-motoric symptoms: hypo- to akinesia, rigidity and tremor may be present to varying degrees. In addition psychic, apractic, vegetative and other disturbances may occur. With increasing age the proportion of parkinsonian patients grows larger — the maximum frequency occurring in the 8th decade of life. In view of primary (idiopathic) and secondary aetiology (within variable fundamental diseases) different forms have to be distinguished. Neuropathological destruction of the ganglia cells, especially in the substantia nigra as well as in the globus pallidus and other regions are demonstrable. Pathophysiological findings show a deficiency in dopamine as a

Offprint requests should be sent to Wolfgang Grundl, University of Ulm, Department of Neurology, Steinhövelstrasse, 7900 Ulm, Germany.

Akadémiai Kiadó, Budapest

neurotransmitter and, accordingly, a preponderance of cholinergic transmitted functions.

Since the development and clinical use of computed tomography (CT), several studies, comparing CCT findings of parkinsonians and normals have been done /1--8, 10--16, 18/. The aim of this study is to check the published results with regard to sex specificity.

Patients and methods

The relevant CCT examinations were carried out from July 1987 to July 1988 at the Neurology Clinic of the University of Ulm at Dietenbronn. A CT apparatus Somatom 2 (Siemens AG) was used. Serial CT scans were performed parallel to the orbitomeatal line. In all cases the thickness of each layer was 2 mm infratentorial and 8 mm supratentorial.

During the above-mentioned period, 59 women and 64 men, diagnosed as suffering from Parkinson's syndrome (but excluding cases caused by neuroleptics) were admitted to the Dietenbronn clinic to undergo a CCT examination. The age- and sex-matched control group consisted of persons whose CCT findings were considered regular (especially patients who complained of headaches, dizziness or uncharacteristic symptoms, and whose neurological examination and laboratory parameters led to normal results) (Table I).

Table I

Age and sex distribution in the Parkinson and the control group

	Parkinsonians		Control persons		
Number of all	114		114		
	men	women	men	women	
6th decade	13	7	13	7	
7th decade	14	13	14	13	
8th decade	23	33	23	33	
9th decade	4	7	4	7	
sum	54	60	54	60	
Average age	68.28	73.08	68.58	73.66	years

The chosen CCT images were evaluated by three persons (evaluators). Each CCT series was classified into three categories: cortical atrophy, width of the inner CSF spaces (supratentorial) and signs of infratentorial atrophy. The two supratentorial categories were disposed into five and the infratentorial category into three degrees. In addition there was a 4th heading for limited features. For the statistical analysis patients as normals were divided into four age-groups (50—59, 60—69, 70—79 and 80—89 years). The statistical evaluation was based on the Wilcoxon Test.

Results

The three evaluators agreed in the estimation of cortical atrophy only slightly better than in the estimation of case ventricular width. There is even greater correspondence after comprehension of degrees 1 with 2 and 3 with 4 to one class each. Accordingly, there was greater disagreement in estimating infratentorial atrophy.

Normal pressure hydrocephalus

Nine patients (5 women and 4 men), who came to our clinic with a diagnosis of Parkinson's syndrome and who were CT scanned while in hospital, were excluded from study. With CCT, the presence of NPH was suspected if there was a difference in width of the inner and the outer CSF spaces. This difference was judged by the three evaluators either > 1.66 (in average of the three raters), or it lay between 1.0 and 1.66, two of the three evaluators reported very small cortical sulci in the upper parietal region. All these patients were furtheron classified according to series cisternography, confirming the suspected diagnosis of NPH. The therapy in each of these nine cases was neurosurgial intraventricular shunt implantation.

Thus it was possible to compare the remaining 114 parkinsonians (54 women and 60 men) with the normal control group.

Men vs. women (overall view)

If parkinsonians and normal persons of both sexes are compared, men in each age group show higher atrophy scores than women -- especially in the supratentorial regions.

Table II

Cortical atrophy — outer CSF spaces (supratent.) — in the Parkinson and the control group (mean of three evaluators, range 3—15)

	6th	7th	8th	9th	decade
Parkinsonians, female	7.4	8.6	9.35	10.65	
Parkinsonians, male	7.9	8.55	10.5	11.2	
Control persons, female	5.2	6.6	6.95	9.3	
Control persons, male	5.9	7.3	8.75	12	

Table III

Ventricular width — inner CSF spaces (supratent.) — in the Parkinson and the control group (mean of three evaluators, range 3—15)

	6th	7th	8th	9th	decade
Parkinsonians, female	7	7.8	9.55	11.1	
Parkinsonians, male	7.7	9.1	11.45	10.75	
Control persons, female	4.7	5.7	7.1	8.5	
Control persons, male	5	6.7	7.9	10.9	

Parkinsonians vs. healthy control group

While there is a steady increase in the atrophy scores in the parkinsonian group, there is a sudden advance in the course of the atrophy process in the normal control group, there is a growing by leap from the 8th to the 9th decade of life. This tendency is more pronounced for cortical atrophy than for ventricular enlargement, and it is more for men than for women (Tables II and III; Figs 1 and 2).

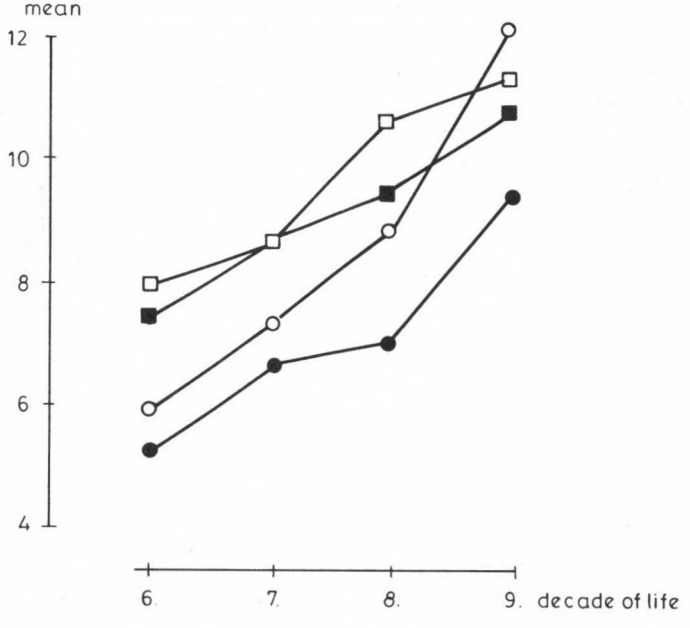


Fig. 1. Cortical atrophy — outer CSF-spaces (supratent.) — of the Parkinson and the control group (mean for three evaluators, range 3—15).
■ Parkinson female, □ Parkinson male, ● control female, o control male

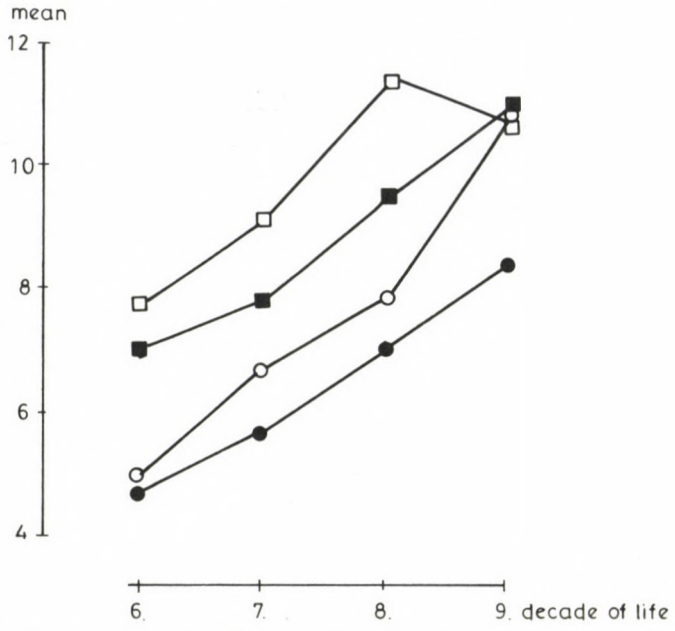


Fig. 2. Ventricular width — inner CSF-spaces (supratent.) — of the Parkinson and the control group (mean for three evaluators, range 3—15). (Symbols as in Fig. 1)

If the Parkinson group and the control group are compared the atrophy scores of the male parkinsonians up to the 8th decade and also of women in the 9th decade of life are higher than in the corresponding control group. The scores for the parkinsonian patients are almost equal to the scores for the control group 10 or in some cases 20 years later. Only the scores for male parkinsonians during the 9th decade of life are the same as those for the control group (Tables II and III; Figs 1 and 2).

Women vs. men (control group)

In the control group, the scores of cortical atrophy and of ventricular width in men clarly correspond to the scores of the female control group one decade later. The results of ventricular enlargement in healthy women in the 9th decade definitely exceed those of healthy men in the 8th decade. The infratentorial atrophy scores are approximately the same (Table IV; Fig. 3).

Table IV

Infratentorial signs of atrophy in the Parkinson and the control group (mean of three evaluators, range 3—9)

	6th	7th	8th	9th	decade
Parkinsonians, female	4.4	4.8	5.3	5.3	
Parkinsonians, male	4.6	5.1	5.78	5.3	
Control persons, female	4	4.3	5.2	5.95	
Control persons, male	4.2	4.7	5.4	6.58	

Women vs. men (Parkinsonians)

There are similar results in both female and male parkinsonians. The ten-year "advantage" of men in the supratentorial region is not recognizable in cases of cortical atrophy in the 7th and 6th decade only; the ventricular width of parkinsonian women within the 9th decade exceeds the scores for men of the same age group. In the infratentorial region, however, there are hardly any differences between the parkinsonian group and the normal control group (Table IV; Fig. 3).

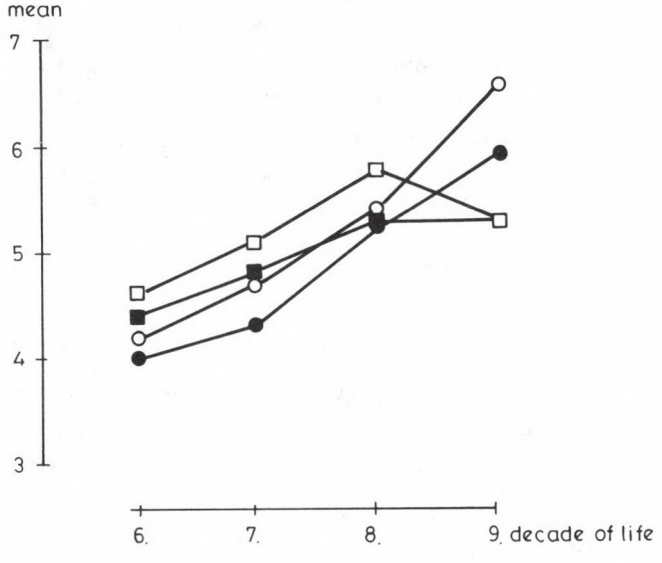


Fig. 3. Infratentorial signs of atrophy of the Parkinson and the control group (mean for three evaluators, range 3—9). (Symbols as in Fig. 1)

Parkinsonians: inner vs. outer CSF spaces

If the three evaluation criteria of parkinsonian women are compared, the scores for cortical atrophy are nearly the same as those for ventricular width. Male parkinsonians show a difference between cortical atrophy and ventricular width score >1 in the 7th decade only (Tables II—IV; Figs 1—3).

Limited features

Limited features were observed in 33 of 114 parkinsonians. (Cerebro-) Vascular-ischaemic lesions were prevalent. Moreover, there was a preponderance of women with the disease (21 women, 12 men). In cases in which cortical atrophy is regional marked (mostly in the frontal regions), there is also a preponderance of parkinsonian women (Table V).

Table V

Limited features CCT of parkinsonians

	Women	Men	Sum
	54	60	114
Periventricular patchy areas of decreased attenuation	11	4	15
Local periventricular lucencies — left hemisphere	6	2	8
— right hemisphere	1	0	1
Fahr's syndrome	6	3	9
Cavum septi pellucidi	0	1	1
	24	10	34
Regional (cortical) atrophic chances — frontal	9	2	
— temporal	1	0	
— parietal	0	1	
	10	3	13

The above-mentioned differences between the Parkinson and the normal control group are significant (in Wilcoxon's Test $P < 0.05$) for the following comparisons: in women for cortical atrophy including the 9th decade, for ventricular width till (and including) the 8th decade; for men with regard to cortical atrophy until the 6th, with regard to ventricular width in the 8th decade, too. — Apart from sex differences between the sexes, the Parkinson and the normal group are significantly different for both supratentorial criteria ($P < 0.05$).

Discussion

The results of this study show that in the normal group as well as in the Parkinson group CCT reveals an increase in the width of inner and outer CSF spaces during aging. Parkinsonians in nearly all decades show higher atrophy scores than the "normal" group. But the difference between the two groups is much more marked in the younger people than in the advanced age groups.

So far these results confirm those of earlier publications /6, 11, 15, 18/. They do, however, differ from those of Lichter et at., who do not mention differences between Parkinson patients and normals as regards CCT signs /12/.

During the last age decade (80—89 years) parkinsonians, especially male ones showed lower supratentorial atrophy levels than the control persons. Perhaps the restricted opportunites that Parkinson patients have to obtain alcohol may help to explain this astonishing phenomenon. In both groups there are higher levels in men than in women.

In our study there are no references to declared differences between general cortical atrophy and ventricular width, which has had been described several times /2, 3, 4, 7/.

If there was a difference between inner and outer supratentorial CSF spaces, and if the level of ventricular width surpassed cortical atrophy signs to a certain extent, suspected NPH could be confirmed. Also in this respect (idiopathic), Parkinson's syndrome is one of the most frequent false diagnoses in case of NPH.

Moreover, our results verify the favoured atrophy of frontal cortex in parkinsonian women /1/. In addition female Parkinson patients frequently show local CCT pecularities, and, to a large extent, vascular lesions. If this point underlines the importance of vascular diseases in the pathogenesis of Parkinson's syndrome, the pecularities in women already mentioned should be a subject of further examination.

From our results it is not very likely, that the duration of Parkinson's syndrome has any influence on cerebral atrophy /6, 7; in contrary to 3/. In younger patients there is a more pronounced difference in the supratentorial atrophy scores between Parkinsonians and normals than in the old age groups. — Age and sex, particularly in the younger age groups are not the most important parameters to explain inner and outer signs of atrophy in parkinsonians compared to normals.

In addition, there may well be another process (or processes) that leads to a loss of brain substance. In view of the sex-differences described, Parkinson's syndrome in each patient may have various causes, which lead to the lack of homogeneity in the Parkinson group.

REFERENCES

1. Adam, P., Fabre, N., Guell, A., Bessoles, G., Roulleau, J., Bes, A.: Cortical atrophy in Parkinson disease: correlation between clinical and CT findings with special emphasis on prefrontal atrophy. AJNR 4, 442—445 (1983)
2. Arcaya Navarro, J., Gutierrez, J. C., Ruiz Ezquerro, J. J., Lopez Albuequerque, J. T., Garcia de Miguel A., Sanchez Navarro, J.: Atrofia subcortical en la enfermedad de Parkinson. Un estudio clinico-TAC controlado y retrospectivo en 29 pacientes. Arch. Neurobiol. (Madr.) 50, 106—116 (1987)
3. Becker, H., Schneider, E., Hacker, H., Fischer, P. A.: Cerebral atrophy in Parkinson's disease — represented in CT. Arch. Psychiat. Nervenkr. 227, 81—88 (1979)
4. Becker, H., Grau, H., Schneider, E., Fischer, P. A., Hacker, H.: Ct examination series of Parkinson patients, in: Lanksch, W. and Kazner, E. (Eds): Cranial Computerized Tomography, Springer, Berlin, 1976, 249—251.
5. Brown, R. T., Polinsky, R. J., Di Chiro, G., Pastakia, B., Werner, I., Simmons, J. T.: MRI in autonomic failure. J. Neurol. Neurosurg. Psychiatry 50 (7), 913—914 (1987)
6. Corthesy, D., Hungerbühler, J. P., Assal, G., Regli, F.: Athropie cérebrale et demence dans la maladie de Parkinson. Arch. Suisse Neurol. Neurochirurgie et de Psych., 136, 75—85 (1985)
7. Fischer, P. A., Jacobi, P., Schneider, E., Becker, H.: Correlation between clinical and CT findings. Cranial computed tomography, 244—248 (1976).
8. Inzelberg, R., Treves, T.: Computed tomography brain changes in Parkinson dementia. Neuroradiology 29, 535—539 (1987)
9. Jellinger, K., Grisold, W.: Zur Frage der Hirnatrophie bei Parkinson-Syndrom, Zbl. allg. Path. Anat., 125, 567—568 (1981)
10. Kitamura, S., Ujike, T., Kuroki, S., Sakamoto, S., Soeda, T., Lio, M., Terashi, A.: Cerebral blood flow and oxygen metabolism in patients with Parkinson's disease. No To Shinkei, 40, 979—985 (1988)
11. Kitani, M., Kobayashi, S., Yamaguchi, S., Tsunematsu, T.: Brain atrophy in Parkinson's disease. Nippon Ronen Igakkai Zasshi, 22, 354—359 (1985)
12. Lichter, D. G., Corbett, A. J., Fitzgibbon, G. M., Davidson, O. R., Hope, J. K., Goddard, G. V., Sharpeles, K. J., Pollock, M.: Cognitive and motor dysfunction in Parkinson's disease. Clinical, performance and CT correlations. Arch. Neurol. 45, 854—860 (1988)
13. Portin, R., Raininko, R., Rinne, U. K.: Neuropsychological disturbances and cerebral atrophy determined by CT in parkinsonian patients with long-term levodopa treatment. Adv. Neurol., 40, 219—227 (1984)
14. Ransmayer, G., Poewe, W., Ploerer, S., Gerstenbrand, F., Leidlmair, K., Mayr, U.: Prognostic implications of the motor symptoms of Parkinson's disease with respect to clinical, CT and psychometric parameters. J. Neural. Transm., 67, 1—14 (1986)

15. Schneider, E., Fischer, P. A., Jacobi, P., Becker, H., Beyer, M.: Cerebral atrophy and long-term response to levodopa in Parkinson's disease. J. Neurol., 222, 37—43 (1979)

16. Schneider, E., Fischer, P. A., Jacobi, P., Becker, H., Hacker, H.: The significance of cerebral atrophy. J. Neurol. Sci. 42, 187—197 (1979)

17. Selby, G.: Cerebral atrophy. J. of the neurolog. sci., 6, 517—559 (1968)

18. Steiner, J., Gomori, J. M., Melamed, E.: Features of brain atrophy in Parkinson's disease. Neuroradiology, 27, 158—160 (1985)

Acta Medica Hungarica, Vol. 48/3—4, pp. 137—144 (1991)

MYASTHENIA GRAVIS: TREATMENT WITH PLASMA EXCHANGE EXPERIENCES OVER 10 YEARS+

L. FORNÁDI, RITA HORVÁTH, A. SZOBOR

Dept. of Neurology, Ferenc Jahn Teaching Hospital, Budapest

(Received: November 5, 1991)

Evaluation of the results of plasmapheresis therapy is reviewed on 160 patients suffering from myasthenia gravis. This new therapeutical procedure is considered very valuable: improvement can be achieved in 63% of the patients during the therapy, a further improvement in 30% after it. Plasmapheresis is especially effective for patients in respiratory crisis-prone state, for drug-resistant cases and for preparation patients for surgery. Plasma exchange therapy may be combined with other forms of immunosuppression with benefit. Thymectomy and plasma exchange are considered to be the main therapeutical procedures in myasthenia gravis.

Keywords: myasthenia, plasma exchange, thymectomy.

Plasma exchange (PEX) has occupied a worthy position among the methods of treating autoimmune disorders in neurology /2—5, 3, 7, 10/. We started with plasmapheresis therapy in our neurological department — which is now a centre for the care of neuroimmune diseases — in 1981, shortly after the first successful applications of PEX in myasthenia gravis (MG) by Pinching et al. /13/, Dau et al. /4/, and Newsom-Davis et al. /11, 12/. Since then, we have gained experience with the method in MG, Lambert—Eaton—Rooke syndrome, acute and chronic forms of demyelinating polyneuropathies and multiple sclerosis. Up to now, we have treated 240 patients (of them 160 with MG) with 450 PEX series.

Abbreviations: myasthenia gravis: MG, plasma exchange: PEX, positive pressure intermittent respirator: PPIR, Disability Status Scale: DSS

+On the basis of the platform presentation at the Third Euro-Myasthenia Conference, Oxford, 1991.

Offprint requests should be sent to L. Fornádi, H-1204 Budapest, Köves Str. 2—4.

Akadémiai Kiadó, Budapest

Treatment of MG by PEX serves the following purposes in our practice: 1. Enhancement of the remaining function by cholinesterase inhibitor medication. — 2. Possible suppression of ACh-receptor antibody production. — 3. Direct removal of circulating antibodies and immunocomplexes. — 4. Preparation of patients with serious MG for surgery. — 5. Treatment after surgery if the efficacy of the operation is not satisfactory. All these aims are realized by circumspect timing and planning in our treatment protocol.

The most convincing improvements in the myasthenic symptoms during PEX are attainable in the severe bulbar crisis-prone cases. For this reason, we installed an automatic cell-separator (Dideco, Italy) in our intensive therapeutic department, thus we can help immediately in respiratory crises, often preventing the use of a respirator (PPIR). So the number of the respirated hours as well as the time spent in intensive care decreases. The need for artificial respiration can be eliminated in general by 2-3 PEX procedures.

Preparation for thymectomy by PEX is advisable not only in crisis-prone cases, but the high-dose demand of cholinesterase inhibitors is also a weighty reason for employing PEX therapy prior to thymectomy. Sometimes benefits can be obtained in cholinergic or mixed crisis, in the so-called oscillating crisis /15/. In this very complicated cases, the detoxicating function of PEX also proves to be effective because there are no other possibilities to solve the antagonistic problem of whether cholinergic and/or anticholinergic medication is needed at the same time /15/. Post-thymectomy ocular and bulbar residual symptoms are also effectively influenced and controlled by PEX, but if thymectomy is performed after a long existence of ocular symptoms, even PEX is ineffective. PEX often has a beneficial effect in immunosuppressive drug-resistant cases /8, 15, 17/, on the other hand, immunosuppression is essential during or after PEX on the account of the risk of rebound phenomenon. A further frequent indication of PEX is preparation of the patient for steroid pulse therapy /1, 16, 17/. After high-dose steroid pulse therapy, however, there is no reason for applying PEX, but if it must still be done because to prevent respiratory crisis, it is unsuccessful in the majority of the cases.

Plasmapheresis has a considerable role in crisis prevention in pregnancy /9/ and before operations other than thymectomy /15, 17/. We usually administer maintaining procedures weekly or monthly, employing corticosteroids, too, if necessary. We have three forms of plasmapheresis protocol from the point of view of the removable plasma quantity: 1. One plasma volume daily for 5-6 days. — 2. One and a half volume every other day, 4 times. — 3. Two volumes every other day 3 times, over a seven day period. The three protocols are similar in efficacy.

Use of immunoglobulin medication is rather restricted on the account of its high cost, though we have found human immunoglobulins very effective in selected cases, by removal of ACh-receptor antibodies, supposedly by binding them and checking their production. In anticholinesterase drug-resistant cases, plasmapheresis is more useful than other immunotherapies, the risk of exchange-dependency, however, is also higher. Aged myasthenic patients tolerate the procedures well, respond to them remarkably, partly because the removal of lipoproteins improves oxygenization and general circulation. Risk of dependency exists in these cases, too, but life can be prolonged and the quality of life can be improved.

MG tends to combine with other diseases or syndromes of immunological or autoimmunological origin, for example, with multiple sclerosis, rheumatoid arthritis, myositis, polymyositis, dermatomyositis, immune thrombocytopenia, psoriasis, anaemia perniciosa, autoimmune diabetes mellitus, Sjögren-sicca syndrome, scleroderma, progressive sclerosis, systemic lupus erythematosus, discoid lupus, etc. In these cases, in addition to MG, the concomitant or associated diseases or syndromes respond to PEX well. We have observed that the resistant cerebellar, brain-stem, sphincter and other symptoms of multiple sclerosis also improve in addition to myasthenic weakness and fatigability, already during PEX treatment. Little or no reaction was seen in the intensity of pyramidal symptoms. Progressive myopathic cases (of course, with myasthenic character) are generally resistant to plasmapheresis, but sometimes the so-called "burnt out cases" of MG resembling to myopathy can be influenced by PEX therapy well.

Plasmapheresis is generally useful in patients after thymectomy (if the result of the operation is not satisfactory). To avoid the rebound phenomenon and to achieve a long-term effect with plasmapheresis, we always use immunosuppressive therapies. As having the first experience with immunosuppression therapy in MG (15), we have been deeply convinced of the beneficial effect of this type of therapies. These methods are usually varied concerning both their types and combinations, as well as timing and duration of their use. Two main drugs have been used in most cases, viz. steroids (in different forms) or azathioprine alone or in combination, sometimes alternatingly used. Levamisol or inosiplex are also used either alone or in various combinations, but never with azathioprine. Azathioprine and, rarely, levamisol are the drugs to be chosen, when a long-term therapeutical procedure seems to be necessary.

Evaluation of the clinical status of the patients have been performed by using the Disability Status Scale (DSS) system /14, 15/. In this score system the higher the number of the points, the worse the patients' condition.

Results achieved by PEX therapy in MG are shown in figures. The correlation of the types of immunosuppressive medication and the measure of

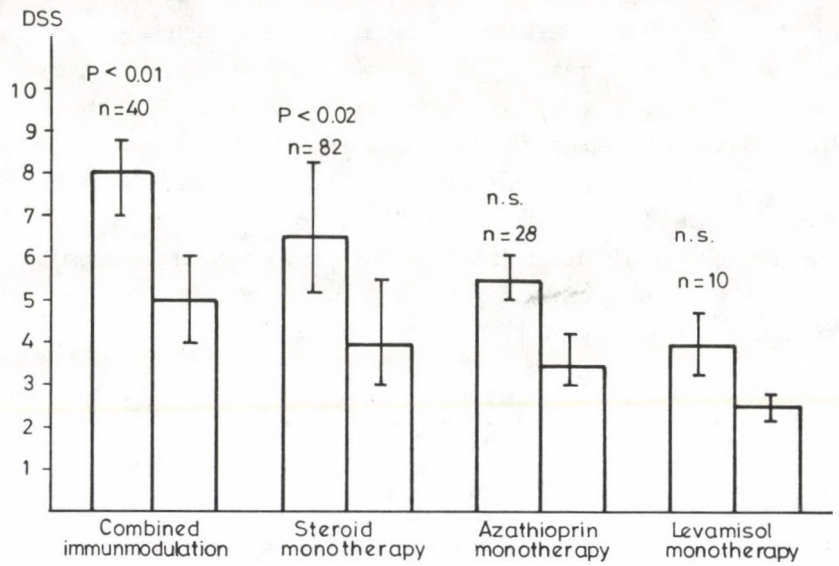


Fig. 1. Clinical improvement of MG patients in relation to the type of immunosuppression during PEX therapy.
DSS = Disability Status Scale, n.s. = not significant, Mean ± S.D. is shown on the Fig.

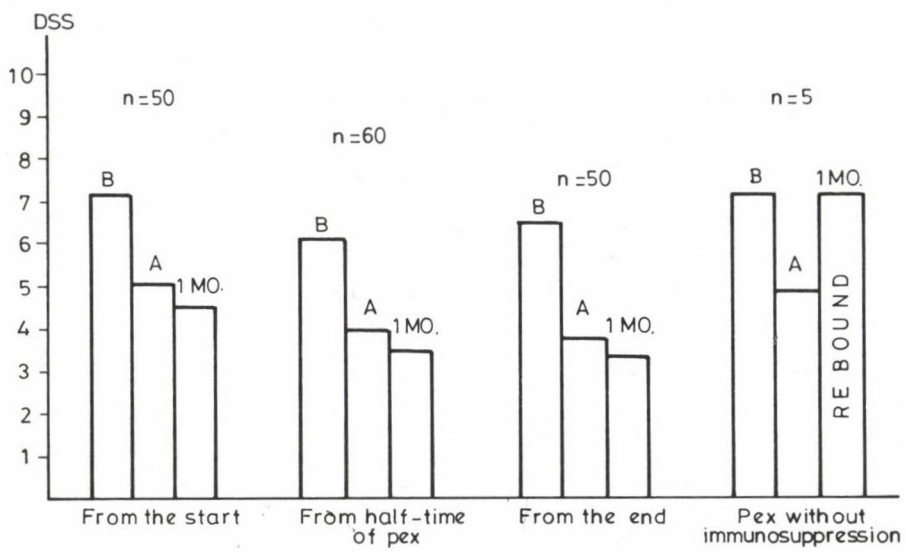


Fig. 2. Clinical improvement in relation to the timing of immunosuppression during PEX.
B = Before PEX, A = Immediately after PEX, 1 MO = One month after PEX

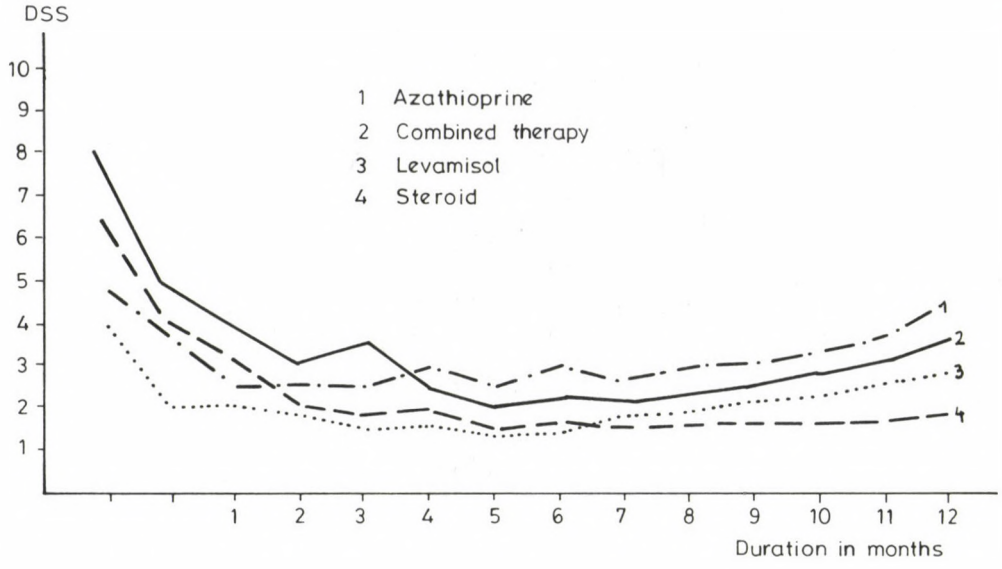


Fig. 3. Duration of the therapeutic effect in a one-year period after PEX, combined with different immunomodulations

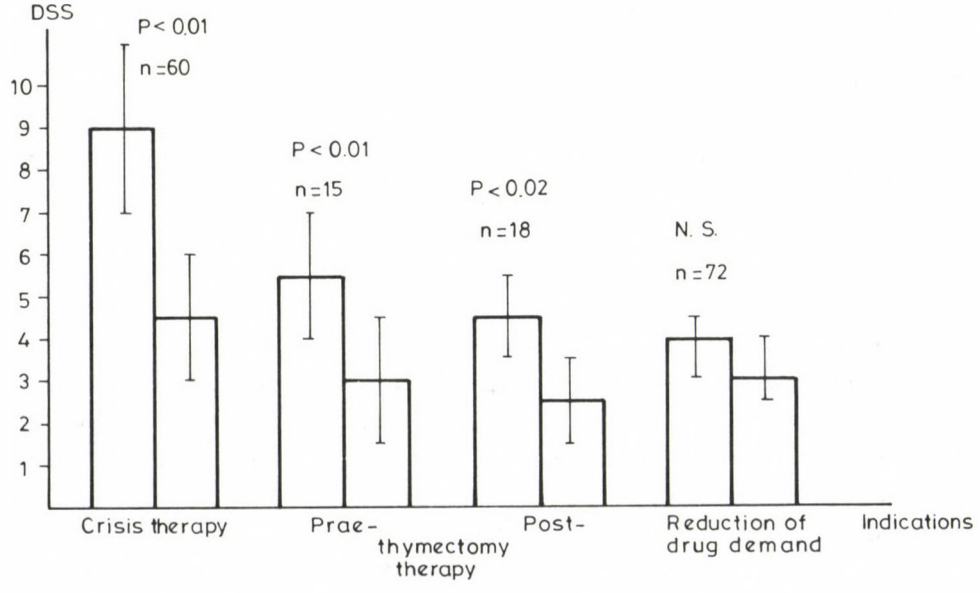


Fig. 4. Efficacy of PEX in patients with different indications of therapy. Mean ± S.D. is shown on the Fig.

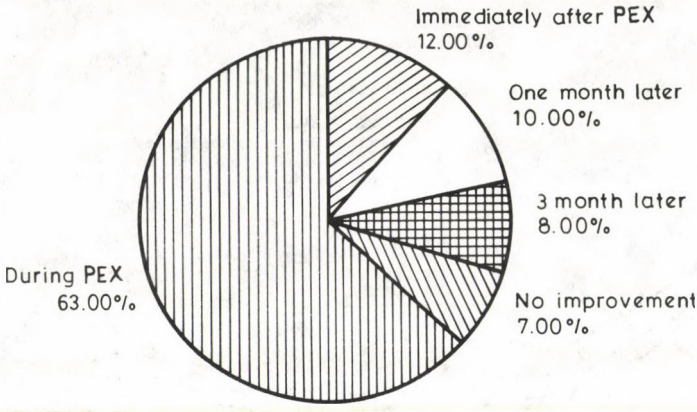


Fig. 5. Improvement of patients in connection with the time elapsed since the start of PEX

clinical improvement according to the DSS system are seen in Fig. 1. The most significant improvement was observed with combined azathioprine-steroid medication.

Relationship between the timing of immunomodulation and the efficacy of plasmapheresis is shown in Fig. 2. We find no difference from the point of view of clinical improvement between the various timings of immunosuppression, but the absence of immunomodulation has usually caused rebound phenomenon.

The longest effect of PEX was achieved in the case of long-term application of combined azathioprine-steroid medication, it sometimes lasted over one year (Fig. 3), but no significant difference was seen concerning the different methods of immunosuppression.

Clinical improvement depends on the condition on which the indication of PEX is based (Fig. 4). The most significant benefits can be obtained in myasthenic or cholinergic crisis. In these cases, PEX is the therapy of choice. The best results after plasmapheresis are achieved on early thymectomized patients, who tolerate immunosuppressive drugs well, continuously for years. Very rarely, surprisingly, long-lasting remission after PEX therapy is achieved even without long-term immunomodulation.

Distribution of temporal manifestation of the therapeutic effect of PEX in percentage of patients is shown (Fig. 5). In 7% there is no improvement after PEX. There are some reasons for this low percentage, out of which we emphasize the late generalized ocular cases with or without thymectomy,

patients with rapid progressive myopathia and the drug-resistant cases. Patients who are susceptible to infections and malignancies can be reckoned to the same group. Improvement was observed in 63% of the patients during PEX therapy, mostly after the first or second treatment; 12% improve immediately after the treatment, 10% one month later.

Apart from the isolated ocular (local) MG cases, PEX has not only a "buy-time role" before thymectomy, but can also better the life prospects and prolong the life of MG patients, using the right method at the right time. PEX is a life-saving method in crisis-therapy. "Qui habet tempus, habet vitam."

REFERENCES

1. Arsura, E., Brunner, N. G., Namba, T., Grob, D.: High-dose intravenous methylprednisolone in myasthenia gravis. Arch. Neurol. 42, 1149—1153 (1985)
2. Dau, P. C.: Plasmapheresis therapy in myasthenia gravis. Muscle Nerve 3, 468—482 (1980)
3. Dau, P. C.: Plasmapheresis: therapeutic or experimental procedure? Arch. Neurol. 41, 647—651 (1984)
4. Dau, P. C., Lindstrom, J. M., Cassel, C. K., Denys, E. H., Shev, E. E., Spitler, L. E.: Plasmapheresis and immunosuppressive drug therapy in myasthenia gravis. New Eng. J. Med. 297, 1134—1140 (1977)
5. Drachman, D. B.: Present and future treatment of myasthenia gravis. New Eng. J. Med. 316, 743—745 (1987)
6. Gracey, D. R., Howaed, F. M., Divertis, M. B.: Plasmapheresis in the treatment of ventilator dependent myasthenia gravis patients. Chest 86, 739—743 (1984)
7. Kornfeld, P., Ambinder, E. P., Papatestas, E. A., Bender, A. N., Genkins, G.: Plasmapheresis in myasthenia gravis. Controlled study. Lancet 2, 1214 (1979)
8. Kornfeld, P., Ambinder, E. P., Mittag, T., Bender, A. N., Papatestas, E. A., Goldberg, J., Genkins, G.: Plasmapheresis in refractory generalized myasthenia gravis. Arch. Neurol. 38, 478—481 (1981)
9. Levine, S. E., Keesey, J. C.: Successful plasmapheresis for fulminant myasthenia gravis during pregnancy. Arch. Neurol. 43, 197—198 (1986)
10. Lisak, R. P., Abramsky, O., Shotland, D. L.: Plasmapheresis in the treatment of myasthenia gravis: preliminary studies in 21 patients. In: Dau, P. C. (ed.): Plasmapheresis and the Immunobiology of Myasthenia Gravis. Houghton Mifflin, Boston, 1979, pp. 209—215.
11. Newsom-Davis, J., Pinching, A. J., Vincent, A., Wilson, S. G.: Function of circulating antibody to acetylcholine-receptor in myasthenia gravis. Investigation by plasma-exchange. Neurology 28, 266—272 (1978)
12. Newsom-Davis, J., Wilson, S. G., Vincent, A., Ward, C. D.: Long-term effects of repeated plasma-exchange in myasthenia gravis. Lancet 1, 464—468 (1979)
13. Pinching, A. J., Peters, D. K., Newsom-Davis, J.: Remission of myasthenia gravis following plasma-exchange. Lancet 2, 1373—1376 (1976)

14. Szobor, A.: Myasthenia gravis: A quantitative evaluation system. Disability Status Scale (DSS) applied for myasthenia gravis. Eur. Neurol. 14, 439—446 (1976)

15. Szobor, A.: Myasthenia Gravis. Akadémiai Kiadó, Budapest, 1990, pp. 147—151, 154—189.

16. Szobor, A., Bárdosi, Z., Klátyik, M., Klein, M.: Myasthenia gravis: Nagydózisú intravénás methylprednisolon életmentő hatása (In Hungarian) (Myasthenia gravis: Life-saving effect of high-dose intravenous methylprednisolone therapy). Orv. Hetil. 129, 495—499 (1988)

17. Szobor, A., Fornádi, L., Vájlok, M.: Plasmapheresis kezelés neurológiai kórképekben (In Hungarian) (Plasmapheresis in neurologic disorders). Orv. Hetil. 129, 119—123 (1988)

18. Szobor, A., Vájlok, M., Jakschitz, G., Fornádi, L.: Myasthenia gravis: a plasmapheresis kezelés hatása (In Hungarian) (Myasthenia gravis: Effect of plasmapheresis treatment). Ideggyógyászati Szemle 36, 385—390 (1983)

19. Toyka, K. V.: Neurologische Indikation zur Plasmapherese. Akt. Neurol. 11, 114—117 (1984)

20. Valbonesi, M., Garelli, S.: Plasma-exchange in neurological diseases. Vox Sang. 44, 65—80 (1983)

Acta Medica Hungarica, Vol. 48/3—4, pp. 145—149 (1991)

FAMILIAL MYASTHENIA GRAVIS: NINE PATIENTS IN TWO GENERATIONS

A. SZOBOR

Dept. of Neurology, Ferenc Jahn Teaching Hospital, Budapest

(Received: October 17, 1991)

In two generations of a family followed up for 15 years, nine patients suffering from myasthenia gravis were observed. The family, being of special genetic importance, is unique in the literature.

Keywords: myasthenia, familial occurrence, thymectomy.

Introduction

We published a study on a family with two patients -- both females -- suffering from apparent myasthenia gravis 15 years age. Further four sisters in the same family proved to have provocable (latent) myasthenia gravis. The oldest sister, however, and four brothers did not suffer from the disease, neither did the parents /1, 8/. Examinations concerning HLA antigens did not prove any specific susceptibility, the individual sex distribution, however, might indicate the role of an unknown factor, suggestive of sexbound susceptibility /1, 8/. No identical or similar reports have been known in the literature, furthermore, we failed to find such a report whilst collecting our familial cases and surveying the literature /2, 3, 5/. There was a further characteristic in this family: the severity of the manifest and provocable myasthenia gravis state increased in a negative correlation with the age of the patients. Thus, the illness of the two youngest sisters were the

Offprint requests should be sent to A. Szobor, H-1204 Budapest, Köves Str. 2—4.

Akadémiai Kiadó, Budapest

most severe, not only generalized, but also crisis-prone, thus, needed relatively urgent thymectomy.

The condition of the operated patients improved to such a degree and at such a rate as it is usual in cases in which thymectomy is performed at the right time, on the basis of proper indication. Initially we were not ready for performing acetylcholine receptor antibody examinations. We could only say that no other immune disease or syndrome — which occurred in 21% in our total clinical material /4/ — associated with myasthenia gravis in this family.

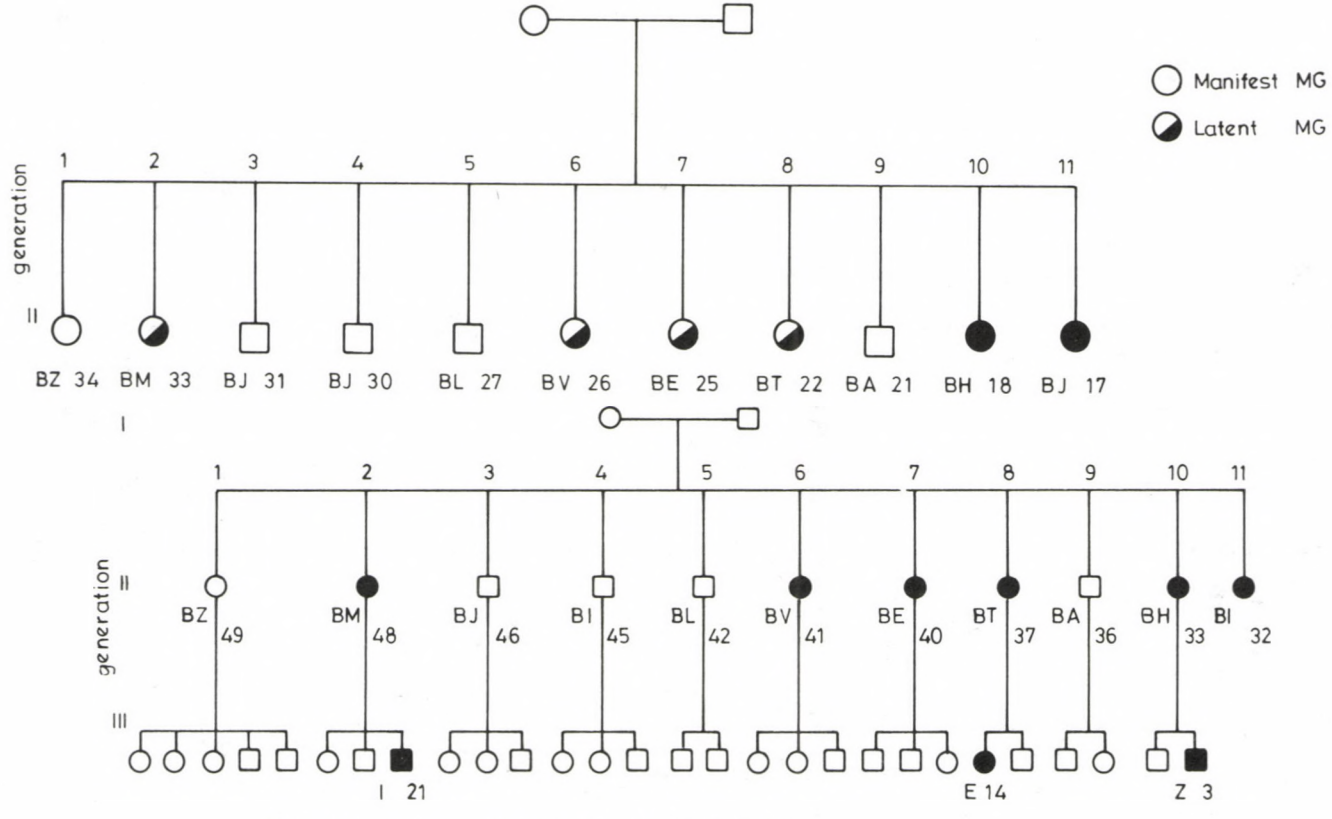
Patients and methods

Every member of the family under study has been monitored, checked and controlled from time to time, in general in every third to sixth month. Their complaints, symptoms, and their general and special state have been followed up and registered. Meanwhile, thymectomy of further four sisters as well as of a young male and a girl of the 3rd generation became necessary. All the thymectomies were performed via median sternotomy (Dr Molnár, Dr Kas), thymic activity was evaluated by the usual method, namely on the basis of the presence, number and size of germinative centres /6, 7/. In the second series of examination we determined acetylcholine receptor antibodies, too. Eleven determinations were carried out on 8 patients, in three cases both prior to operation and thereafter (Dr Szelényi). Treatment and after-care took place in our Department of Neurology in every case, according to the method described in detail elsewhere /6/.

Results

The data for the members of the family are shown in two genealogical tables (Figs 1 and 2). The order and initials of the patients are identical in both tables, the follow-up-time has been 15 years (1976—1991). Myasthenia gravis, which had been only "provocable" (or latent) at first in the 2nd generation, became manifest in a generalized form during the time of observation. Furthermore, the disease proved to be crisis-prone in two cases on the account of respiratory distress. In the change or progression of the illness the role of common factors such as respiratory infections — were recognized. Unequivocal generalized myasthenia gravis became manifest in a young male patient and in a girl in the 3rd generation. These two patients were thymectomized, meanwhile the drug therapy of the third affected member of the 3rd generation, a boy aged 3 years has been continued.

The oldest sister and all brothers in the 2nd generation neither became ill nor needed any therapy during the one and a half decade of obser-



Thymectomy: 2nd generation: cases 2,7,8,10, 11
3 rd generation: cases 2/3, 8/1

AChR-antibody: positive: cases 2,6,7, 8, 10, 11
cases 2/3, 8/1

Fig. 1. and Fig. 2. Members of the family in geneological tables

vation. The other members of the 3rd generation, including children, adolescent and young adults, are healthy, latent myasthenia gravis was not suspected; they have needed no therapy, their somatical development has been normal. Acetylcholine receptor antibodies were found in all the examined cases, but no significant difference was found in the cases in which the test was repeated after operation.

Discussion

The genetic score and the possible background of the family under study was thoroughly discussed at the 3rd Euro-Myasthenia Conference held in Oxford (1991). This family raised interest because it was considered to be unique: no similar observations had been published in the literature or encountered in the personal observation of the participants. In spite of the familial character of myasthenia gravis in this family, the possibility of congenital myasthenia can be excluded, furthermore, there is no reason to suppose any form of specific myasthenia syndrome.

Latent, undetected or only provocable form of myasthenia gravis and its characteristics were discussed in one of our previous papers /7/. This possibility led us in the present case, too, when after the two manifest instances of myasthenia gravis, we applied curare provocation on the other members of the family. We consider the different provocation procedures to be useful diagnostic tools in recognizing uncertain or latent myasthenia cases.

The family has special individual characteristics, therefore, it seems to be worthwhile to study all of its members (including the healthy ones). It would be of interest to get further unexpected experiences in the 2nd or 3rd generation, and to get know whether or not the sex difference would keep to disappear in the further generations.

REFERENCES

1. Gyódi, E., Szobor, A., Onódy, K., Klein, M., Petrányi, G. Gy., Hollán, Zs.: HLA-antigénekhez és nemhez kötött genetikai tényezők szerepe myasthenia gravisban. (The role of factors associated with HLA antigens and sex in myasthenia gravis.) Orv. Hetil. 121, 749—753 (1980)

2. Namba, T., Brunner, N. G., Brown, S. B., Mugurama, M., Grob, D.: Familial myasthenia gravis. Report on 27 patients in 12 families and review of 164 patients in 73 families. Arch. Neurol. 25, 49—60 (1971)

3. Osserman, K. E., Genkins, G.: Studies in myasthenia gravis: Review of a twenty-year experience in over 1200 patients. Mt. Sinai J. Med. 38, 497—537 (1971)

4. Szobor, A.: Myasthenia gravis: Betegség-társulások. Irodalmi adatok és 1000 beteg észlelése alapján. (Myasthenia gravis: Diesase associations on the basis of the literature and on experiences obtained in 1000 cases.) Ideggyógy. Szemle 40, 481—499 (1987)

5. Szobor, A.: Myasthenia gravis: Familial occurrence. A study of 1100 myasthenia gravis patients. Acta Med. Hung. 46, 13—21 (1989)

6. Szobor, A.: Myasthenia Gravis. Akadémiai Kiadó, Budapest 1990, pp. 81—87.

7. Szobor, A., Besznyák, I., Molnár, J., Szende, B.: Myasthenia gravis after removal of mediastinal tumours. Acta Med. Acad. Sci. Hung. 38, 179—188 (1981)

8. Szobor, A., Gyódi, E., Onódy, K., Klein, M., Petrányi, G. Gy., Hollán, S. R.: HLA-Antigene und geschlechtsbedingte genetische Faktoren bei Myasthenia gravis. Aktuelle Neurologie 7, 19—26 (1980)

Acta Medica Hungarica, Vol. 48/3—4, pp. 151—156 (1991)

OBSTETRICS

PRENATAL CYTOGENETIC STUDY OF TRANSLOCATION CARRIERS

A. TÓTH[1], K. RAJCZY[1], K. HAJDU[1], ZS. INTŐDY[2], J. LÁSZLÓ[1]

[1]Department of Obstetrics and Gynaecology, Postgraduate Medical University, Budapest;

[2]Department of Obstetrics and Gynaecology, Uzsoki Hospital, Budapest

(Received: August 5, 1991)

A total of 37 prenatal diagnoses were analysed: 10 observations in which one of the parents carried a Robertsonian translocation and 27 observations in which one a reciprocal translocation was carried by one of the parents. The segregations of the inherited chromosome structural rearrangements were analysed in relation to the methods of ascertainment of the anomaly in the family, and the types of rearrangement. The mode of ascertainment proved to be a very useful indicator of the risk: those cases ascertained through abnormal livebirths had a 44% risk in our series, but there was no unbalanced fetus in the group ascertained through recurrent abortions.

Keywords: prenatal diagnosis, chromosome translocations, cytogenetics.

Introduction

The frequency of balanced chromosome translocations was estimated between 1 and 2 per 1000 in newborns /3, 5/. Approximately half of these are Robertsonian, the others are reciprocal translocations /1/. In the case of balanced chromosomal rearrangements the amount of genetic material is not changed, and usually not accompanied by visible phenotypic effects. However, these abnormalities represent a high risk to reproductive outcome of the balanced carriers. For this reason, parental balanced structural chromosome rearrangements are among the major indications for chromosome analysis in prenatal cytogenetic screening.

Offprint requests should be sent to A. Tóth, H-1389 Budapest, Szabolcs u. 35.

Akadémiai Kiadó, Budapest

Patients and methods

One part of carriers was recognized during the cytogenetic study of infertile couples at our department. The affected parents asked for prenatal investigation at the beginning of the subsequent pregnancy. Major part of the carriers was referred by several genetic laboratories to our prenatal centre. The exact description of karyotype and, in certain cases a picture of the karyotype, was required to get a reliable diagnosis. When the rearrangement included large segments of chromosomes, direct chromosomal preparation of chorionic villi was carried out /4/. In the cases of smaller translocations cultures were set up, frequently from both chorionic villi and amniotic fluid cells. G- and Q-banding were used for evaluation.

Thirty-seven prenatal investigations were carried out on 26 pregnants because of carriership of a chromosome rearrangement. Six affected couples were investigated in successive pregnancies.

Ten observations were analysed in which one parent carried a Robertsonian translocation. The different types of Robertsonian translocation are presented in Table I.

Twenty-seven samplings were carried out on 17 pregnants because of reciprocal translocation in one of the parents. Eight cases were ascertained through a malformed infant with an unbalanced structural rearrangement, nine through a history of spontaneous abortions.

Results

There was no unbalanced offspring in the group investigated for Robertsonian translocation. The fetuses were carriers of a balanced rearrangement in 4 cases (Table I).

Table I

Segregations in Robertsonian translocations

Type of rearrangement	No. of diagnoses	Offspring		
		normal	balanced	unbalanced
Involving chromosome 21	4	2	2	—
Not involving chromosome 21	6	4	2	—

Table II

Risk of reciprocal translocation carriers depending on the ascertainment

Ascertainment	No. of investigations	Offspring		
		normal	balanced	unbalanced
Malformed child	16	5	4	7
Recurrent abortion	11	6	5	—

Investigating reciprocal translocations 7 unbalanced offsprings were found out of 16 samplings in cases of couples ascertained through an unbalanced offspring. There was no unbalanced infant in the group with previous abortions (Table II).

Discussion

Meiotic segregation of chromosome translocations produces gametes carrying different chromosome complements. For a given translocation several constitutions could arise theoretically. According to the cytogenetic studies of sperms of carrier men, most of the sperm complements represent alternate (47%) or adjacent-1 (42%) segregations. Adjacent-2 and 3:1 segregations were much infrequent /9/. Alternate segregation yields normal offspring: theoretically, one-half should have normal chromosomes, and the other half should have the two balanced translocated chromosomes. Results from some studies of liveborn infants and fetuses with prior prenatal diagnosis have indicated an excess of balanced carrier fetuses /12/. Our data show an excess of normal fetuses (18/14). It seems probable that the theoretical 1:1 ratio which was confirmed by the cytogenetic analysis of sperms /9/ is valid in later gestational ages, too. The imbalances observed at birth in several pregnancies of the same carrier nearly always result from the same mode of segregation. This mode can be predicted from the pachytene diagram of the chromosomes involved in the translocation. The meiotic production of imbalances is highly variable according to the translocation type, its incidence ranging from 19% to 77% /11/. The rate of imbalances in sperm is always above (mean value 56%) the one observed at term (10%), and at the prenatal diagnosis for carrier couples (11.6%) /1/. The spermatogenic failure of men heterozygous for a translocation could result from an interaction between the sexual bivalent and the rearranged chromosomes, which can interfere with the XY inactivation /7/, or the pairing disruption around the breakpoints /8/, but a prezygotic selection against unbalanced spermatozoa is unlikely /11/. In contrast, postzygotic selection occurs, depending on the fatality of the imbalance.

90% of the paternal-origin imbalances results from adjacent-1 segregation /6/. A preferential mechanism for this segregation seems to exist. Syntenic disposition of the homologous centromeres could promote an adjacent-1-type segretation. The adjacent-2 and 3:1 segregations are transmitted

almost exclusively maternally. The common characteristic of these two types is the nonsegregation of the homologous centrometers. Out of five families with unbalanced fetuses three translocations were of maternal origin. In contrast to the literature, all of them resulted from adjacent-1 segregation. In general, the maternal meiosis could be more willing to chromosome nondisjunctions.

Though each reciprocal translocation is associated with a particular risk, the efficiency of the natural selection for a given translocation depends on the gene dosage effect /6/. The viability of an unbalanced translocation is related to the size of the translocated segments and their genetic contents. The smaller is the imbalance the greater is the risk of unbalanced live birth /2/. A good rough estimate can also be derived from the manner in which the rearrangement has been ascertained. If there was a previous child with developmental malformations, there is a higher possibility for survival with a certain chromosomal anomaly, and the risk of recurrence tends to be high in about 20%. If the chromosome abnormality has been discovered while investigating for the course of recurrent miscarriages then the majority of the anomalies are usually selected in the first trimester, and consequently, the risk of an unbalanced aberration at the second trimester of the pregnancy is less than 5% /10, 14/. This tendency is well represented in our material, i.e. there was no unbalanced offspring in the infertility group. However, there were seven unbalanced fetuses (44%) in the group ascertained through a previous abnormal child. Cases with previous late spontaneous abortions where the fetus was abnormal and the parents turned out to be carriers of a chromosome translocation, also have a higher chance for survival. This finding calls attention to the importance of a thorough pathological investigation of large fetuses and to the necessity of the cytogenetic analysis of suspected cases.

The rate of carrier mothers was not different from the rate of carrier fathers in case of the reciprocal translocation. The similar role of both parents in transmitting the rearrangements was also found /13/.

The commonest Robertsonian translocation is the t(13q14q) translocation, in which the risk of translocation trisomy 13 (Patau syndrome) in the offspring of carriers is less than 1%. On the other hand, if Robertsonian translocations are involving chromosome 21, the risk of translocation trisomy 21 is about 15% when the mother is the carrier. If this translocation was inherited from the father, then the risk would be much less. These

results underline the importance of cytogenetic investigation of newborns with Down's syndrome.

In conclusion, the study shows a high degree of variability in the incidence of unbalanced karyotypes. The results allow a prediction for the risk in the offspring. According to the literature there is a higher risk for couples with maternal balanced Robertsonian translocations involving chromosome 21, and for couples with reciprocal translocations ascertained through an offspring with unbalanced karyotype. Cytogenetic survey of infertile couples and abnormal infants or fetuses suspected of having a chromosomal anomaly makes it possible to recognize the structural rearrangements. When a family member is found to carry a transmissible chromosome aberration, it is the responsibility of the local genetic services to initiate studies in first degree relatives to ensure that all those who might be at risk of having affected offsprings are identified and counselled. Detection of structural rearrangements in the carrier families and the possibility of prenatal diagnoses will permit the affected couples to avoid the birth of abnormal infants.

REFERENCES

1. Boué, A., Gallano, P.: A collaborative study of the segregation of inherited chromosome structural rearrangements in 1356 prenatal diagnoses. Prenat. Diagn. 4, 45—47 (1984)

2. Daniel, A., Boué, A., Gallano, P.: Prospective risk in reciprocal translocation heterozygotes at aminocentesis as determined by potential chromosome imbalance sizes. Data of the European collaborative prenatal diagnosis centers. Prenat. Diagn. 6, 315—350 (1986)

3. Hamerton, J. L., Canning, N., Roy, M., Smith, S.: A cytogenetic survey of 14,069 newborn infants. Clin. Genet. 8, 223—243 (1975)

4. Intődy, Zs., Gaál, M., Tóth, A., László, J.: Chorionic tissue aspiration: a new possibility in fetal diagnosis. (In Hungarian) Magyar Nőorvosok Lapja, 47, 233—237 (1984)

5. Jacobs, P. A., Melville, M., Ratcliffe, S.: A cytogenetic survey of 11,680 newborn infants. Ann. Hum. Genet. 37, 359—376 (1974)

6. Jalbert, P., Jalbert, H., Séle, B.: Types of imbalance in human reciprocal translocations: risks at birth. In: A. Daniel (ed.): The cytogenetics of mammalian autosomal rearrangements. New York, 1988, pp. 267—291.

7. Lifschytz, E., Lindsley, D. L.: The role of X chromosome inactivation during spermatogenesis. Proc. Natl. Acad. Sci. USA 69, 182—186 (1972)

8. Luciani, J. M., Guichaona, M. R.: L'incidence des anomalies chromosomiques de structure sur la spermatogenese chez l'homme. Reprod. Nutr. Dev. S1, 95—103 (1990)

9. Martin, R. H., McGillivray, B., Barclay, L., Hildebrand, K., Ko, E.. Sperm chromosome analysis in a man heterozygous for a reciprocal translocation 46,XY,t(12;20) (q24.3;q11). Hum. Reprod. 5, 606—609 (1990)

10. Osztovics, M., Tóth, S., Wessely, J.: Cytogenetic investigation on 418 couples with recurrent fetal wastage. Ann. Génét. 25, 232—236 (1982)

11. Pellestor, F., Séle, B., Jalbert, H., Jalbert, P.: Direct segregation analysis of reciprocal translocations: A study of 283 sperm karyotypes from four carriers. Am. J. Hum. Genet. 44, 464—473 (1989)

12. Stene, J.: Comments on estimation of risks to translocation carriers. Am. J. Med. Genet. 33, 54—57 (1989)

13. Szemere, Gy.: Primary and secondary cytogenetic prevention. Theses (in Hungarian), Szeged, 1988.

14. Tóth, A., Gaál, M., Bősze, P., László, J.: Chromosome abnormalities in 118 couples with recurrent spontaneous abortions. Gynecol. Obstet. Invest. 18, 72—77 (1984)

Acta Medica Hungarica, Vol. 48/3—4, pp. 157—166 (1991)

CARDIOLOGY

RETROGRADE (VENTRICULO-ATRIAL) CONDUCTION, PREMATURE BEATS, PSEUDOTRICUSPID REGURGITATION, SYSTOLIC ATRIAL SOUNDS AND PACEMAKER SOUNDS OBSERVED TOGETHER IN TWO PATIENTS WITH VENTRICULAR PACING

D. GELLÉRI

Jász-Nagykun Szolnok Cardiovascular Care Station,
Szolnok, Hungary

(Received: July 20, 1991)

Two patients were found to have retrograde atrioventricular conduction with premature beats during permanent ventricular pacing. In both patients the auscultatory phenomena had been heard and recorded that had been described as independent signs, but all together in the same patient had not been reported before.

In one of the patients pseudotricuspid regurgitation was observed with Doppler echocardiography and the other was suspected having the same. It seems that patients with these symptoms deserve high preventive care and attention.

Keywords: retrograde conduction, premature beat, reciprocal beat, pseudotricuspid regurgitation, systolic atrial sound, pacemaker sound.

Introduction

Inhibited ventricular pacing is the most popular pacing form nowadays, for the implantators have been using the dual chamber pacing modes less frequently than it was predicted in the early '80 s /12/. Nevertheless, in association with ventricular pacing some unfavourable electrophysiologic

---

Abbreviations: AAI: Atrial pacing Atrial sensing Inhibitory mode, cw: cannon wave, DDD: Dual chamber pacing Dual chamber sensing Double response modes, DVI: Dual chamber pacing Ventricular sensing Inhibitory mode, ECG: electrocardiogram, JVP: Jugular Venous Pulse, PCG: Phonocardiogram, PDE: Pulsed Doppler Echocardiography, PMC: Pacemaker Click, PMS: Pacemaker Sound, RP interval is the time lasting from the paced R wave to the P wave of ECG, VVI: Ventricular pacing Ventricular sensing Inhibitory mode.

Offprint requests should be sent to D. Gelléri, Dobó u. 25. I/7., H-5000 Szolnok, Hungary

Akadémiai Kiadó, Budapest

and haemodynamic effects, such as retrograde conduction, premature beats, reciprocal beats and pseudotricuspid regurgitation may arise and some auscultatory phenomena, such as systolic atrial sounds and pacemaker sounds may come into being. The ECG, PCG, JVP tracings and the PD echocardiographic registration of two patients in whom these findings were seen together are described here.

Case reports

Case 1. The patient, a 51-year-old man, experienced dyspnoea, dizziness and tiredness. His heart frequency sometimes decreased to 43 beats min, and premature beats with positive P waves on their ST segments were seen during ECG recording (Fig. 1). Since clinical symptoms and the ECG recordings revealed sinus-node dysfunction, the patient received a Medtronic right-

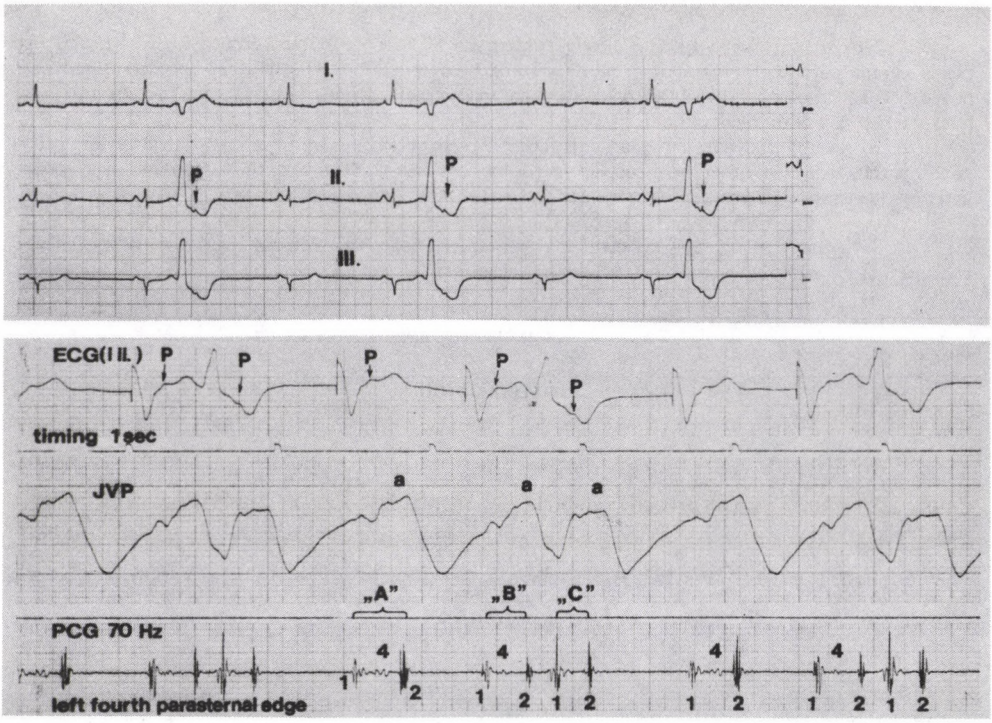


Fig. 1. Upper standard 3-channel ECG registration — Sinus bradycardia (43 beats/min). Every third beat is a ventricular premature beat with positive P wave on the ST segment. Lower 3-channel ECG, JVP and PCG registration — A distinction can be made between "A" and "B" types of systoles produced by pacemaker and between "C" type of systole, which is a premature beat. The "x" descents of JVP are occupied by the large "a" waves. The accentuation of the first heart sound increases from "a" to "c". Abbreviations: 1 4 2 = number of heart sounds (4 = fourth heart sound, systolic atrial sound), ECG (I II) = ECG lead II

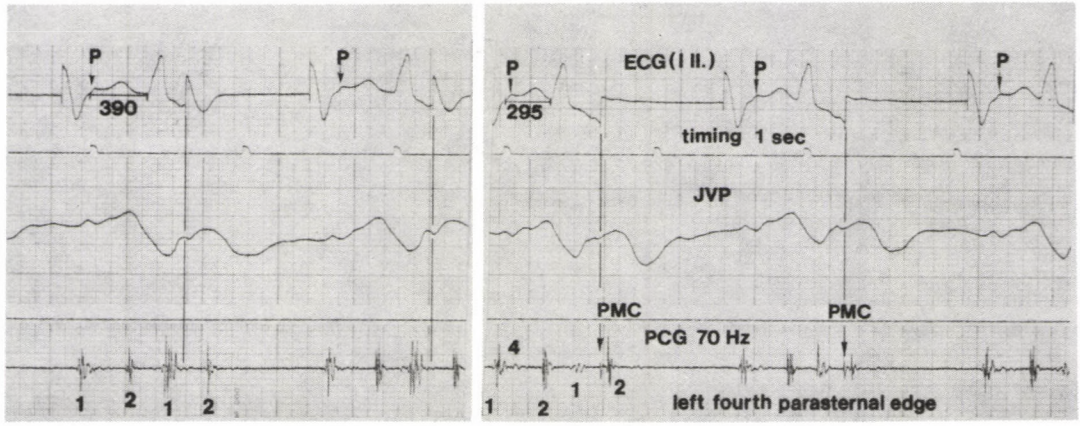


Fig. 2. 3-channel ECG, JVP and PCG registration — Every second nonpaced beat is a premature beat with late systolic click during magnet mode, if the PR interval is 295 ms., so the pacemaker impulse falls in the late systolic region (to the right from the vertical black line). If the PR interval is 390 ms., the pacemaker impulse falls just after the first heart sound and the pacemaker click disappears (to the left from the vertical black line). The pacemaker impulses falling on the systolic cycle can generate no depolarization because of the refractory phase. On the left side of the figure, when the PR interval is long (390 ms.), the pacemaker impulse falls in the middle of electrical systole, thus the electrical movement of the pace increases above the isoelectric level; then it decreases imitating depolarization. On the right side of the figure, when the PR interval is shorter (295 ms.) the pacemaker impulse falls in the negative apex of the T wave, thus the electrical movement of the pace rises only to the isoelectric level, i.e. does not imitate any depolarization. Abbreviations: see Fig. 1.

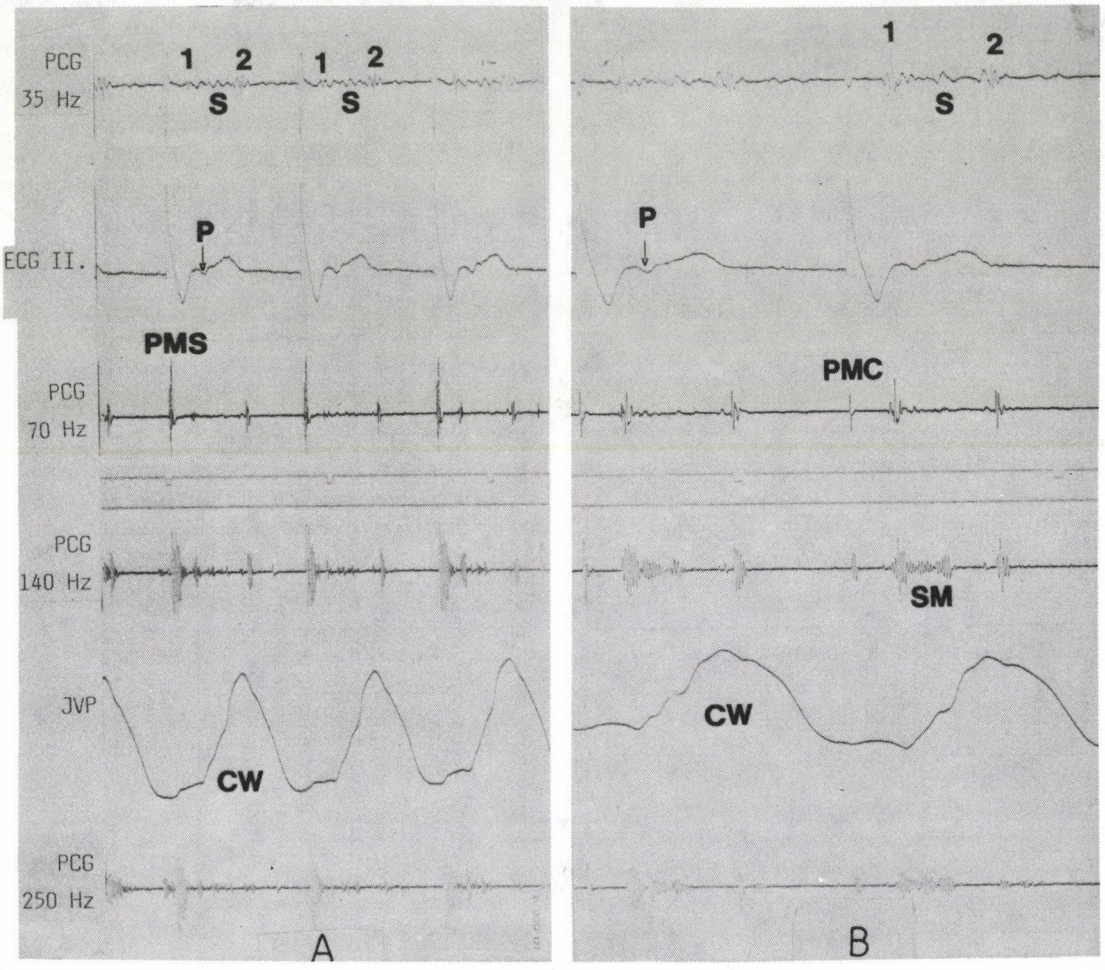


Fig. 3.

ventricular demand pacemaker with Lithium battery at the Department of Cardiovascular Surgery, Semmelweis University Medical School, Budapest (September 1977). After pacemaker implantation every third beat was a premature beat with an accentuated first heart sound, while in the previous two pacemaker beats mesosystolic sounds were heard and recorded (Fig. 1.).

Premature beats could sometimes be registered as every second beat with systolic pacemaker click if the magnet mode was employed (Fig. 2). The dizziness disappeared after pacemaker implantation but the patient developed dyspnoea and tiredness during and after exertion respectively. Although he had been feeling all these for 4 years, he regularly did gardening and drove car. His retrograde conduction and premature beats failed to respond to administration of beta blockers. One evening, watching television, he died suddenly.

At autopsy the heart was enlarged (480 g) with predominant dilatation of the right appendage and ventricle. No thrombi were seen in the chambers. The pacemaker wire was fixed at the apex of the right ventricle and functioned well. In the coronaria vessels moderate sclerosis was found without myocardial infarction. On the viscera the signs of chronic congestive heart failure were observed.

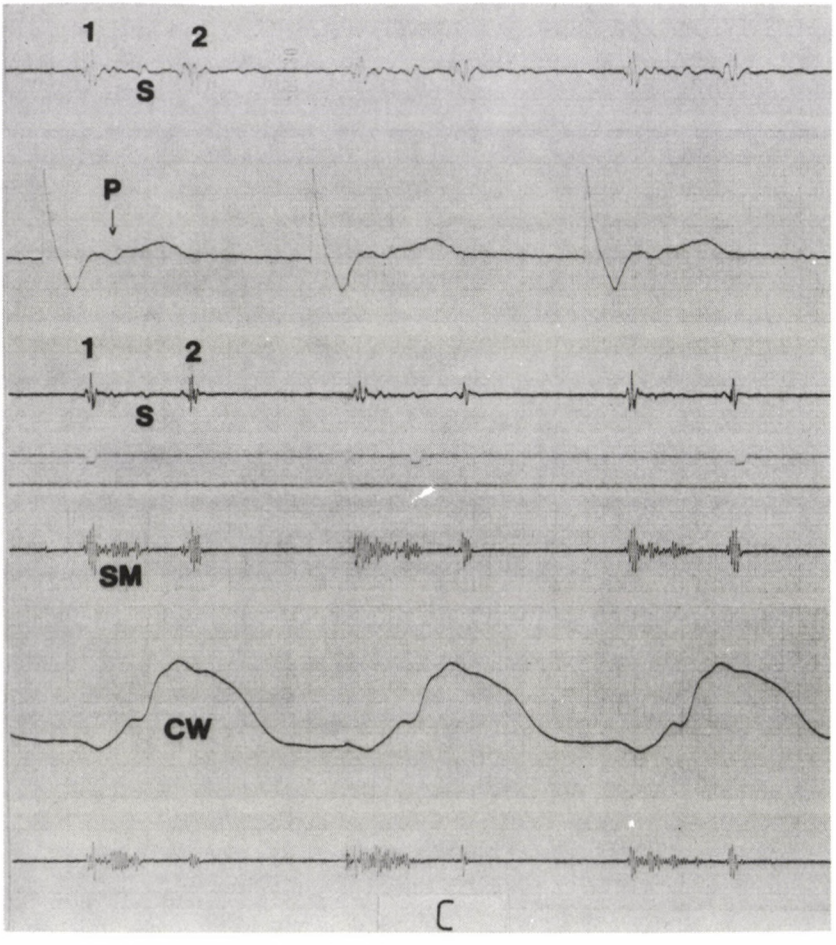


Fig. 3. 6-channel PCG, ECG and JVP registration — (The PCG was recorded over the left lower parasternal border.)
A) at the end of maximal exspiration B) C) and during gentle inspiration. — A) Negative P waves are at the beginning of ST segments on ECG lead II, proving retrograde conduction. Strong pacemaker sounds can be recorded in every frequency-band on PCG. The JVP tracing shows tricuspid regurgitation (Cannon waves). — B) During inspiration the strong pacemaker sounds change to pacemaker clicks. The systolic murmur lasting from the first heart sound to the midsystolic (systolic atrial) sound can be observed better. — C) Later the pacemaker clicks disappear but the systolic murmur and systolic atrial sound do not change.
Abbreviations: ECG = electrocardiogram lead II, see. Fig. 1. Timing: 1 s

Case 2. A 48-year-old woman, having experienced dizziness and anginas underwent electrophysiologic examination and coronarography which revealed sinus-node dysfunction and a moderate coronary heart disease. She was given a Medtronic ventricular demand pacemaker in the Hungarian Institute of Cardiology in June 1982. After pacemaker implantation she developed

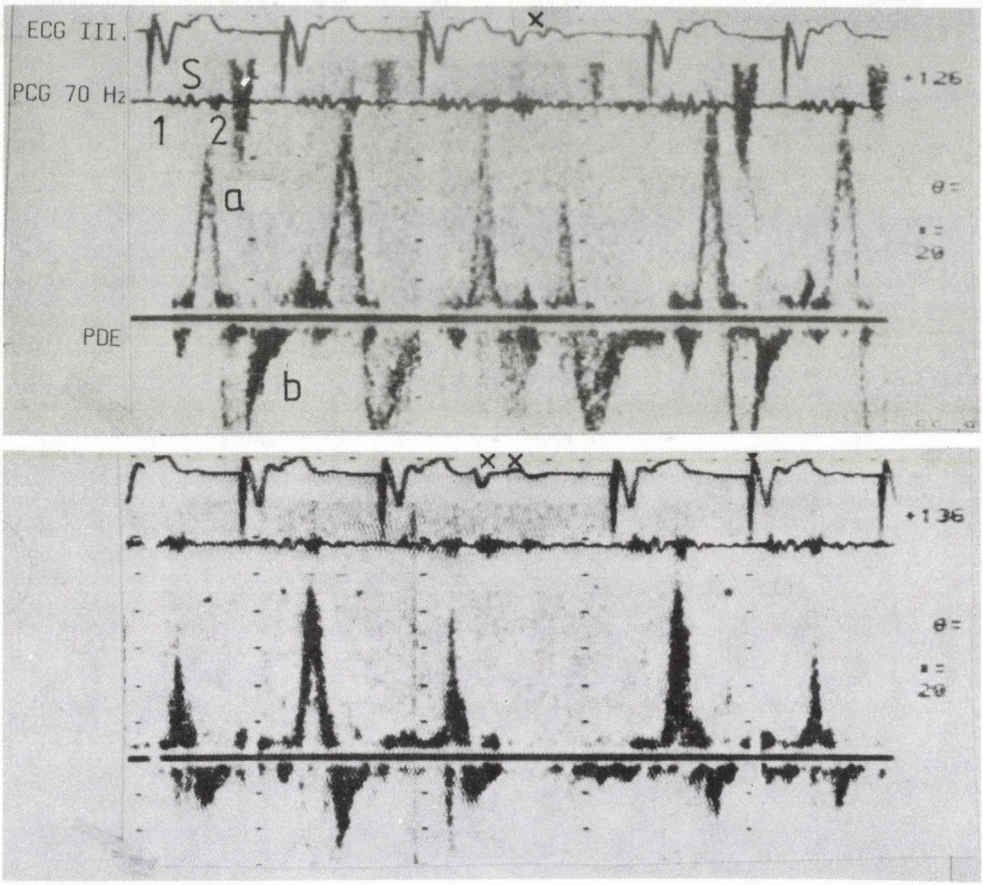


Fig. 4. ECG, PCG recorded over the left lower parasternal border, and the central venous flow velocities in the beginning of inferior vena cava by PDE in subcostal view. After negative P wave of ECG, systolic atrial sound can be recorded on PCG (S) followed by late systolic reverse flow (a) on PDE. The diastolic forward flow (b) is large and occupies the whole diastole. Two types of premature beats can be seen: in the fourth cardiac cycle (×): premature beat with negative P wave and late systolic reverse flow, and in the tenth cardiac cycle (××): premature beat without them. Abbreviations: see Fig. 1, ECG III = electrocardiogram lead III

dizziness only rarely but she needed antiangina drugs longer. By evenings she observed minimal oedema on her legs. Her auscultatory finding was the following: the first heart sound comprised of two components over the apex and the left parasternal border. At the time of the first component a little skeletal muscle contraction was seen around the pacemaker battery and in the left fifth intercostal region, so it was supposed to be a pacemaker sound. (The little skeletal muscle contraction was not felt by the patient.) From the second component of the first heart sound a systolic murmur was heard and recorded to the midsystolic sound. Over the greet vessels the second heart sound was paradox splitted. PDE showed a positive large wave following the systolic atrial sound in the beginning of the inferior vena cava, while there was a trace regurgitation just above the tricuspid valves. These were suggestive of a pseudotricuspid regurgitation (Figs 3, 4). The right atrium was a biten larger (50 x 35 mm) with no anterior movement of tricuspid and bicuspid leaflets during atrial systole.

Despite the above-mentioned minor complaints and the minor signs of pseudotricuspid regurgitation the patient was feeling well, so it was not planned to change her VVI pacing mode to DDD.

Discussion

Sinus-node dysfunction as indication for primary pacemaker implantation made 52% of all cases included in a survey carried out in the USA during 1985 /12/. Treatment with conventional ventricular pacing in patients with sinus-node dysfunction can lead to retrograde conduction in 33-100% of cases in which the anterograde conduction is normal /5/. P waves are typically inverted on the ECG's ST segments in cases with retrograde activation (case 2), but they may be of upright form if rapid retrograde bypass pathways are present /11/ (case 1). Pacemaker-induced retrograde atrial activation has assumed great importance as an inducer of reciprocal or echo beats if the RP interval is long enough so the atrioventricular pathways are not in refractory phase and the atrial depolarization can generate a ventricular depolarization see Johnson's four cases /5/. In our two cases this possibility could be excluded because the P waves were seen at the beginning of the ST segment, namely the RP intervals were short, so the atrial depolarization could not generate ventricular depolarization. Thus a ventricular depolarization just after the paced beat can be interpreted as a ventricular premature beat. Nevertheless, the RP intervals may change /5/, thus reciprocal or echo beats might occur out of the recorded time in our two cases as well. These echo beats may have been dangerous if the impulse ran back to the atrium, then to the ventricle again, making a macro-reentry tachycardia and producing ventricular fibrillation. This possibility could not be excluded in case 1, in which at autopsy there were no signs causing the sudden death. The prominent dilatation of the right appendage and ventricle may contribute to the genesis of a malignant tachycardia, for -- as known for many years -- the dilatation of cardiac chambers is a "hotbed" of rhythm abnormalities /9/. Among 27 patients of Westveer et al. in whom retrograde conduction was present at the time of electrophysiologic study 13 ones displayed retrograde conduction following ventricular premature beat on routine ECG prior to electrophysiologic study /14/. In our case 1 upright P waves were seen on ventricular premature beats' ST segments, predicting that this kind of P waves would be present during ventricular pacing.

Atrial contractions at the time when the mitral and tricuspid valves are closed may produce atrial sounds like those described by Benchimol in 1973 /1/. The synchronously-registered jugular pulse with large and (or tall "cw" would provide a clue to take distinction of other auscultatory phenomena, such as ejection and nonejection clicks, extracardial sounds, etc. The systolic fourth heart sound (atrial sound) can be attributed to either: 1. forceful atrial contraction against a closed mitral and tricuspid valve or 2. a brief movement of the mitral valve apparatus giving rise to audible and recordable vibrations. Benchimol et al. /13/ described a case presenting atrial sound and retrograde conduction with a little anterior movement of the tricuspid valve on M-mode echocardiography. In case 2 anterior movement of the tricuspid and bicuspid valves could not be demonstrated by two-dimensional echocardiography. So one concludes that the systolic atrial sound may be produced by the rapid increase of atrial pressure originating from the forceful atrial contraction.

Pacemaker-induced pseudotricuspid regurgitation was described at the first time in a 83-year-old female patient, by Kay et al. /6/. The evidence of this diagnosis was that the hepatic pulsation and the other peripheral signs of tricuspid regurgitation abolished when the ventricular pacing had been interrupted and the normal atrioventricular sequence of activation was restored. Jacobs et al. found, with inferior vena cava contrast M-mode echocardiography, pseudotricuspid regurgitation in 8 of 20 patients treated with permanent ventricular pacemaker; none of the 8 patients showed clinical symptoms of tricuspid regurgitation /4/. Kay et al. /6/ postulate that the combination of cannon waves superimposed on an already elevated right atrial pressure may be the necessary substrate for producing these findings /4, 6/. In the two cases described here peripheral symptoms of tricuspid regurgitation could not be seen. In case 1 the JVP tracing and the prominent enlargement of right appendage and ventricle at autopsy were considered to be tricuspid regurgitation, whereas in case 2 the high positive wave following the atrial sound proved pseudotricuspid regurgitation. The forceful atrial contraction could produce atrial sound as well as the pseudotricuspid regurgitation rising after the systolic atrial sound.

Harris was the first to state that pacemaker click was an extracardial click /3/. In case 1 the pacemaker click was heard and recorded during the late systole and disappeared when it fell in the mid-systole. One might say that it was a range of systole in which no pacemaker click could come into being. Nager et al., /10/ who had studied the pacemaker

sound/click in detail mentioned the same range. In the case 2, pacemaker click disappeared during inspiration, proving its being changeable. The appearing and disappearing of a pacemaker click or sound may vary by alteration of the body's position, by respiration and, perhaps, by the moving of tricuspid valves and atrioventricular groove /2, 7, 8/. The movement of the tricuspid valves and atrioventricular groove may take the pacemaker wire into a position in which the electric stream towards the thoracic muscle becomes unfavourable, interrupting the rapid skeletal muscle contraction that produced the pacemaker click/sound. This is one of the explanations for being of the zone just following the first heart sound, a zone in which pacemaker sound/click cannot be originated (case 1).

It seems that the retrograde atrioventricular conduction with premature or echo beats and the systolic atrial sounds with pseudotricuspid regurgitation are symptoms that may occur together at the same time, though, in our knowledge these four symptoms had not been reported in the same case. Besides this, if one can hear a pacemaker click/sound it can occur coincidently, but it is of interest that in an attempt to assess the occurrence of pacemaker click was more often found in unfavourable haemodynamic and electrophysiologic situation, such as: retrograde atrioventricular conduction and in the employing of the magnet mode /2/.

In conclusion, treatment with ventricular pacing in patients with sinus-node dysfunction may often lead to retrograde conduction. Thus treated patients deserve careful preventive care and attention, especially systolic atrial sound, pacemaker click/sound and the minor echocardiographic signs of a pseudotricuspid regurgitation are present. The increasing enlargement of the right atrium and peripheral signs of tricuspid regurgitation need changing to atrioventricular sequential pacing. Nevertheless, in the patients with sinus-node dysfunction in whom retrograde conduction exists prior to permanent pacemaker implantation physiological pacing modes, such as AAI, DVI and DDD may be advisable. The mentioned pacing modes could protect also our patients against the described complaints as well as, perhaps, against sudden death.

Acknowledgement

The author wishes to thank Prof. Attila Naszlady M.D., D.Sc., Head of Cardiopulmonary Department NTL. INST. of PULMONOLOGY Budapest, Hungary, and Kristóf Karlócai M.D. for their help in echocardiographic examination, and to Department of Cardiovascular Surgery, Semmelweis University Medical School and to Hungarian Institute of Cardiology for pacemaker implantation.

REFERENCES

1. Benchimol, A., Fishenfeld, J., Desser, K. B.: Systolic Atrial Sounds during Atrioventricular Dissociation: The "Wandering" Fourth Heart Sound. Chest 64, 508—510 (1973)
2. Gelléri, D.: On the origin of pacemaker sounds. (In Hung. with an English abstract.) Cardiologia Hungarica 16, 249—254 (1987)
3. Harris, A.: Pacemaker "heart sound". Br. Heart. J. 29, 608—615 (1967)
4. Jacobs, P., Vandenbossche, J. L., Marneffe, M., Wozniak, B., Englert, M.: Pseudo tricuspid regurgitation in ventricular pacing. Amer. Heart J. 110, 886—888 (1985)
5. Johnson, C. D.: Retrograde Atrial Conduction and Reciprocal Beating. Bol. Asoc. Med. P. Rico 78, 109—113 (1986)
6. Kay, R. H., Ambrose, J. A., Schek, L., Blake, J., Rubin, D., Herman, M. V.: Pacemaker-Induced Pseudotricuspid Regurgitation. Chest 83, 282—284 (1983)
7. Kluge, W. F.: Pacemaker Sound and Its Origin. Amer. J. Cardiol. 25, 362—366 (1970)
8. Kramer, D. H., Moss, A. J., Shah, P. M.: Mechanisms and Significance of Pacemaker-Induced Extracardiac Sound. Amer. J. Cardiol. 25, 367—371 (1970)
9. Matsuura, Y., Ishihara, H., Hamanaka, Y., Sueda, T., Ohno, Y., Yamashina, H., Higo, M., Fujii, T., Yamamoto, M.: Clinical Analysis of 110 Postoperative Deaths of the Patients with Permanent Implantable Pacemaker. Hiroshima J. of Med. Sci. 36, 183—190 (1987)
10. Nager, F., Bühlmann, A., Schaub, F. et al.: Auskultatorische und kardiographische Befunde bei Patienten mit implantiertem elektrischem Schrittmacher. Klin. Wschr. 43, 1232—1237 (1965)
11. Narula, O. S.: Retrograde pre-excitation. Comparison of Anterograde and Retrograde Conduction Intervals in Man. Circulation 50, 1129—1143 (1974)
12. Parsonnet, V., Bernstein, A. D., Galasso, D.: Cardiac Pacing Practices in the United States in 1985. Amer. J. Cardiol. 62, 71—77 (1988)
13. Tong Ping Chee, Desser, K. B., Benchimol, A.: Midsystolic Sound Associated with A-V Junctional Rhythm. Chest 81, 637—638 (1982)
14. Westveer, D. C., Stewart, J. R., Goodfleish, R., Gordon, S., Timmis, G. C.: Prevalence and Significance of Ventriculoatrial Conduction. PACE 7, 784—789 (1984)

Acta Medica Hungarica, Vol. 48/3—4, pp. 167—176 (1991)

GASTROENTEROLOGY

PREVALENCE OF ANTIBODY TO HEPATITIS C VIRUS IN BLOOD DONORS, HIGH-RISK GROUPS AND PATIENTS WITH LIVER DISEASES IN HUNGARY

A multicentre study using ABBOTT EIA test and a comparison with an ORTHO ELISA test system

(Received: July 5, 1991)

A. PÁR and ILDIKÓ KÁNTOR (coordinators)

Participants: E. BARCSAY, I. HOLLÓS, I. MEZEY, M. J. BROJNÁS, M. TAKÁCS (Natl. Institute of Hygiene, Budapest)

M. HÉJJAS (Natl. Institute of Haematology, Budapest)

M. ILLÉS, L. SZONTÁGH (Blood Transfusion Centre, Szeged)

M. NIKA (Blood Transfusion Centre, Ajka)

J. SÁNDOR (Blood Transfusion Centre, Péterffy Hospital, Budapest)

M. ABONYI, B. BÜKI, J. NÉMETH, F. SZALAY (Semmelweis University Medical School, Budapest)

Serum samples from 1185 individuals (blood donors, health-care workers, patients on haemodialysis, those from other high-risk groups and those suffering from non-A, non-B hepatitis or other liver diseases) were examinated for antibody to a recombinant HCV antigen. An ABBOTT HCV EIA system was used throughout and in addition a parallel study with ORTHO HCV ELISA was done in 380 of the samples to compare the two anti-HCV tests. A confirmatory neutralizing ABBOTT ELISA probe was also performed in 45 cases. The anti-HCV test was positive in 1.60% of the healthy blood donors and in 9% of subjects excluded from donation for elevated aminotransferase. In patients on haemodialysis 47%, in other high-risk-group subjects 33% anti-HCV prevalence was found. Patients with acute and chronic post-transfusion NANB hepatitis showed 40% and 70% prevalence, respectively. The two ELISA tests revealed 95% agreement in the parallel determinations. Serial end-point-dilution studies of anti-HCV-positive sera suggest that the ABBOTT test was of superior sensitivity. The results of the confirmatory test suggest that reactive (positive) samples of low optical density near to the cut-off value, required a confirmation with the naturalization test. HCV infection seems to be a common aetiological factor in PT-NANB hepatitis in Hungary, therefore, screening of blood donors for anti-HCV may be justified.

Keywords: hepatitis B virus, hepatitis C virus, antibody to hepatitis C virus, non-A, non-B hepatitis, liver diseases, high-risk groups, post-transfusion hepatitis.

Abbreviations: anti-HBc = antibody to hepatitis B virus core, anti-HCV = antibody to hepatitis C virus, ALT = alanine aminotransferase, AST = aspartate aminotransferase, EIA, ELISA = enzyme-linked immunosorbent assay, HBV = hepatitis B virus, HCV = hepatitis C virus, NANB = non-A, non-B, PT = post-transfusion

Offprint requests should be sent to A. Pár, H-7643 Pécs, Ifjúság Str. 13., Hungary

Akadémiai Kiadó, Budapest

Introduction

In the 15-year history of non-A, non-B (NANB) viral hepatitis research (1-3) the discovery of hepatitis C virus (HCV) can be regarded as an important milestone, as the HCV-related recombinant antigen offered an opportunity for the detection of anti-HCV /4, 5/. Thus an aetiological diagnosis of parenterally-transmitted NANB hepatitis has become available. Two international world conferences on HCV /6, 7/ and many reports indicate how much this discovery stimulated the studies on blood-borne hepatitis. From Hungary, in addition to the early researches on NANB hepatitis reported by Hollós et al. /8/ as well as Schaff et al. /9/ further papers have been published on this subject /10-12/. Recently Héjjas et al. /13/ have published the first results of anti-HCV research from Hungary. Detection of anti-HCV is of significance mostly in the research of epidemiology, diagnosis and prevention of parenterally-transmitted NANB hepatitis, now tentatively called hepatitis C, through, several unsolved questions have remained to judge the real value of anti-HCV studies.

The ORTHO Diagnostic System (Raritan, New Yersey, USA) was the first to develop a technique (an ELISA techniqe) for detection of anti-HCV. This test has spread in a wide range on the world. The first Hungarian experiences were obtained by using this method /12, 13/. Soon ABBOTT (North Chicago, III.) introduced another system for the same purpose. Recently a second-generation test is also available from this firm. Last year a nationwide multicenter screening study was designed in Hungary, to evaluate the clinical significance of anti-HCV determination. In this screening the first-generation ABBOTT EIA test was used, partly in comparison with an ORTHO anti-HCV test.

The present study was performed in seven institutions. Serum samples from blood donors, health-care workers, high-risk group of parenterally-transmitted NANB hepatitis and patients with liver diseases were examinated.

Patients and methods

Patients

Serum samples from a total of 1185 subjects were studied. The distribution of the subjects by diagnosis is shown in Table I: of the blood donors, 257 have excluded from donation because of suspicion of chronic hepatitis or an elevated transaminase level. The health-care workers were laboratory technicians or hospital nurses. In the high-risk group 123 pa-

tients were haemodialysed, 35 were polytransfused, and 10 suffered from haemophilia: 14 patients had undergone open-heart surgery and 5 individuals were on plasmapheresis. Out of the patients with acute NANB hepatitis 35 were post-transfusion cases, 64 had sporadic NANB hepatitis, while out of the patients with chronic NANB hepatitis 36 had post-transfusion (PT) hepatitis and 91 had sporadic ("cryptogenic") NANB hepatitis. In the group of the various liver diseases, 41 patients had alcoholic hepatitis or cirrhosis, 13 had HBsAg-positive (8 acute and 5 chronic) hepatitis and 42 patients suffered from different hepatobiliary diseases.

Methods

The blood samples were stored at -20 °C until used.

Biochemical liver tests. The necrosis enzymes aminotransferase (ALT/GOT or AST/GOT) were determined by conventional standard methods or an optimalized kinetic technique.

Hepatitis B virus (HBV) markers. HBsAg and anti-HBc were tested with ELISA (ORGANON Teknika, Oss, The Netherlands) or with radioimmunoassay (ABBOTT Wiesbaden, Germany, or SORIN Biomedica, Hamburg).

Anti-HCV test. The sera were assayed for anti-HCV with the ABBOTT anti-HCV EIA kit (kindly provided by ABBOTT, Wiesbaden); besides 380 of the sera were tested with the ORTHO HCV antibody ELISA test system as well. For confirmation of positive anti-HCV-reaction, an ABBOTT anti-HCV Neutralization EIA test was used in 45 cases by one of us (M.T.). (Briefly, two parallel samples of serum were studied: the one was subjected to the screening test as above while to the other a neutralization reagent — a recombinant HCV antigen — was added. This antigen forms an immune complex with the anti-HCV antibody present in serum, thus blocking the binding of the antibody to the c-100-3 rHCV antigen absorbed on the surface of beads. As a result, the absorption, i.e. optical density of the anti-HCV-containing serum samples will be reduced.)

Statistical analyses

χ^2 test was used for the comparison of frequency of positive sera in different groups.

Results

Frequency of hepatitis markers

The prevalence of the anti-HCV marker and the two HBV markers found in the different study groups is summarized in Table I.

Blood donors. A significant difference was seen between the healthy persons and those who had raised aminotransferase levels or being suspect of viral hepatitis had been excluded from donation. In the latter subgroup all the three viral markers occurred significantly more frequently than in the group of healthy donors ($P < 0.001$ for all three markers).

The high-risk group. Haemodialysis patients need special attention: anti-HBc and anti-HCV were detected with similar frequency in about a half of these patients. The rate of anti-HCV seropositivity was lower in patients who had undergone open-heart surgery, or who had been polytransfused for haematological disease.

Table I

Viral markers in blood donors, health-care workers, high-risk groups and liver disease

Diagnosis	n	HBV		HCV
		HBsAg	anti-HBc	anti-HCV
		positive (%)		
Blood donors				
— healthy	367	3(0.8%)	17/240(7%)	6/(1.6%)
— raised ALT	257	28(11%)	61/247(25%)	23(9%)
Health-care workers	43	0	5(12%)	2(5%)
High-risk groups				
— haemodialysis	123	17(14%)	61(50%)	58(47%)
— other polytransfused	64	3(5%)	17(27%)	21(33%)
Acute NANB				
— post-transfusion	35	0	9(26%)	14(40%)
— sporadic	64	0	18(28%)	17(27%)
Chronic NANB				
— post-transfusion	36	0	10(28%)	28(78%)
— sporadic	91	0	26(29%)	31(34%)
Various liver diseases	96	13(14%)	16(17%)	12(13%)

Out of the 123 haemodialysed patients 38 were positive for both anti-HCV and anti-HBc (30.9%). This number represents 65% of the anti-HCV-positive haemodialysis cases. Out of the 58 anti-HCV-positive haemodialysis patients 9 were HBsAg-positive, that is, 7.3% of the total haemodialysis cases revealed a "double" carriership.

The NANB hepatitis group. The prevalence of anti-HCV was higher in the parenterally-transmitted acute and chronic hepatitis than in the sporadic (cryptogenic) NANB cases, but the difference 77.8 vs. 34.1% was significant ($P < 0.001$) only in the chronic forms.

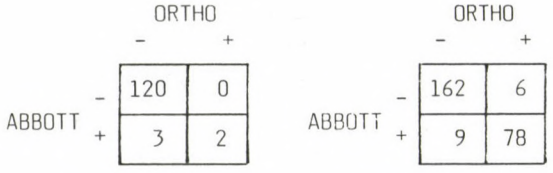
The relationship between the raised aminotransferase level and anti-HCV finding

As mentioned above in the group of blood donors anti-HCV seropositivity rate was significantly higher for subjects with elevated aminotransferase level than for accepted healthy donors. Regarding the haemodialysis patients, a similar relationship was found, namely, 46 of 58 anti-HCV-positive dialysed patients (79.3%) had raised aminotransferase levels while only

22 patients of 65 anti-HCV-negative (33.8%) showed the same. -- In the chronic NANB hepatitis group, 23 of 24 anti-HCV-positive (95.8%) while out of 17 anti-HCV-negative patients 11 (64.7%) had an elevated ALT activity.

Comparison of the ABBOTT EIA and ORTHO ELISA test systems

When 125 serum samples from blood donors with elevated aminotransferase level (and/or with suspicion of hepatitis) were tested simultaneously with the ABBOTT and ORTHO anti-HCV tests, the agreement was 98% (Fig. 1/a).



a) Blood donors with elevated ALT

b) Patients at high risk or with liver disease

Fig. 1. Parallel ABBOTT and ORTHO anti-HCV determinations

In another study, 255 patients belonging to different high-risk groups or with liver disease were examined parallel with both tests: a 94% agreement was found (Fig. 1/b). The concordance for all the parallelly-tested sera was 362/380 (95%). The sensitivity of anti-HCV test systems was compared by the parallel testing of serial end-point-dilution of four anti-HCV-positive sera: in three of those 4 the ABBOTT test proved to be more sensitive than the ORTHO (Table II).

Table II

Anti-HCV EIA endpoint dilution results on seropositive serum samples

Patient	Reciprocal endpoint dilution	
	ABBOTT	ORTHO
1. 0620267	128	48
2. A.B.	128	16
3. V.G.	64	32
4. B.N.	16	64

The results of ABBOTT Neutralization anti-HCV EIA test

A total of 45 confirmatory tests were performed. For this purpose, sera were derived from 12 patients with acute viral hepatitis, from 13 with chronic hepatitis and from two patients with other liver diseases, as well as from 18 blood donors. The results are shown in Table III. Out of the

Table III

Confirmatory studies with ABBOTT Neutralization anti-HCV EIA

Group	Anti HCV				
	Screening test		Neutralization test		
	Reactive	Negative	Positive	Negative	False-p.
Acute hepatitis	12	0	6	3 + 3+	0
Chronic hepatitis	11	2	6	4 + 3+	0
Other liver disease	1	1	0	2	0
Blood donors	18	0	11	4	3
Total	42	3	23	13 + 6+	3

+ after neutralization the absorption decreased

initially anti-HCV-reactive 12 sera of patients with acute hepatitis 6 proved to be positive in the confirmatory test as well. (Serum samples were collected from the patients within one month after the onset of the illness (two sera) or later than one month (four sera). Three of the six negative sera produced absorption values under the cutt-off, but in the neutralization test their optical density values were considerably reduced. Otherwise, all these three patients had undergone surgery, or had got transfusion, two months before the symptoms of hepatitis. Their sera were obtained from the early stage of infection, 5-10 days after the onset of the illness.

Eleven of 13 of chronic hepatitis patients were reactive in the screening test, six of them proved to be positive with the neutralization test and seven were negative. The absorbance value (optical density) of the three negative sera was considerably reduced in the neutralization test.

Sera of patients with other liver disease proved to be negative in the confirmatory test.

Eighteen healthy donors were seropositive in the screening test, 11 of them were positive in the neutralization test. Four were negative and the

remaining three proved to be false positive, that is, the latter sera were strongly reactive both before and after neutralization. This phenomenon might be attributed to a hitherto unknown nonspecific reaction.

Summing up the results of the ABBOTT Neutralization test, 42 of the 45 tested sera were reactive with the ABBOTT screening test and three were negative (the latter sera were positive with the ORTHO screening test). The negative sera were non-reactive in the neutralization test as well. Out of the 42 originally reactive sera 23 were positive in the neutralization test, 3 proved to be false positive and 16 samples produced negative results. The sera, which gave negative results in the neutralization test had low optical density values in the screening test, just above the cut-off value.

Discussion

Our present study with the ABBOTT anti-HCV EIA test resulted in several interesting findings some of which correspond to those obtained with the ORTHO HCV antibody ELISA test in western Europe and Hungary earlier.

We have learned that the anti-HCV seropositivity rate for healthy blood donors (determined only with the sole screening test without neutralization confirmatory probe) was similar to that reported by Héjjas et al. /13/ the first the authors using the ORTHO test in Hungary. (It is conceivable that if we had the checked all the anti-HCV-reactive sera of blood donors and of other study groups with the neutralization test, the true prevalence rate would have been much lower.)

Yet, if we take into account the previous anti-HCV results as well as our recent findings, the frequency of HCV infection in Hungary may be comparable to that found in the Mediterranean area of Europe /6, 7/.

The first nationwide Hungarian multicenter anti-HCV study (using the ORTHO test) /12/ revealed an anti-HCV positivity in 86% of haemophiliacs, 27% of patients undergone open-heart surgery, 20% of haemodialyses cases, furthermore, in 34% of acute NANB hepatitis patients, 87% of chronic PT NANB hepatitis and 48% of chronic sporadic NANB hepatitis patients as well as in 33% of HBsAg-positive chronic hepatitis patients.

Now, with the ABBOTT test, we have found higher seropositivity rates both in polytransfused and haemodialysed patients, and a slightly lower prevalence in chronic NANB hepatitis cases, -- other findings did not differ from the above-mentioned previous result significantly.

It is worth noting that in our haemodialysed patients and elevated aminotransferase (ALT/GPT) together with anti-HBc occurred in about a half of the anti-HCV-positive cases, that is, — corresponding to the earlier suggestion /14/ — the simultaneous determination of these two "surrogate markers" can be able to detect a half of the HCV-infected persons. (Anti-HBc can be regarded as a serologic marker of a previous HBV infection, which presumably might occur coincidentally with the HCV infection.)

The present study revealed a slightly higher anti-HCV positivity rate for health-care workers than for blood donors. The comparative examinations with ABBOTT and ORTHO tests showed an accordance of 95%, while in the end-point dilution study, ABBOTT test appeared to be more sensitive in 3 out of 4 cases. It is no doubt that in the future the really specific, reliable methods in the virological diagnostic producers will be based on direct detection of viral nucleic acids e.g. the polymerase chain reaction. This is true for the HCV testing as well /15/. Until such modern techniques will be introduced in our everyday practice, we can still use the classic serological methods, that is, antibody studies, with suitable confirmatory tests. Concerning the HCV this is of importance, for the false-positive anti-HCV finding reported in chronic autoimmune hepatitis /16/. A correlation was also observed between serum gamma globulin or IgG level and the optical density of anti-HCV reaction, furthermore, the anti-HCV reaction became negative after immunosuppressive treatment, with a decrease of the IgG serum level /17, 18/. It was assumed that the sera of patients with such autoimmune liver diseases might contain a component which can result in false-positive anti-HCV reaction. Although these reports were founded on ORTHO anti-HCV testing, they have master us cautions at the evaluation of the results of the ABBOTT screening test as well.

Our neutralization test studies suggest that any anti-HCV positivity with an absorbance value close to the cut-off, should always be confirmed by a neutralization test. If the absorption is considerably reduced during the neutralization — a new blood sample should be taken for a repeated test. Since the sera of high absorption values (derived from our hepatitis patients) always proved positive in the neutralization test, the confirmation of such samples seems to be unnecessary. Similarly, the sera found to be negative with the screening test do not need further study with neuralization test.

In summary, the ABBOTT anti-HCV EIA together with the neutralization confirmatory test can be really useful for the recognition of the nature of

parenterally-transmitted hepatitis and of "cryptogenic" cirrhosis cases, as well as for epidemiological studies in subjects at high risk of PT hepatitis. If cost/benefit calculations make it justified, an anti-HCV screening of blood donors should be performed.

Although there are controversies in this respect, it was stated /19/ that a 5-12% estimated prevalence of PT-hepatitis could be reduced to 1-2% by screening of blood donors for anti-HCV.

REFERENCES

1. Mosley, J. W.: Hepatitis B and non-B: epidemiologic background. JAMA 233, 967—969 (1975)
2. Alter, H. J., Purcell, R. H., Holland, P. V. et al.: Clinical and serological analysis of transfusion-associated hepatitis. Lancet 2, 838—841 (1975)
3. Bradley, D. W.: The agents of non-A, non-B viral hepatitis. J. Virol. Methods, 10, 307—319 (1985)
4. Choo, Q-L., Kuo, G., Weiner, A. J. et al.: Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244, 359—362 (1989)
5. Kuo, G., Choo, Q-L., Alter, H. J. et al.: An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science, 244, 362—364 (1989)
6. First International Meeting on Hepatitis C Virus. Abstracts Rome, Sept. 14—15, 1989.
7. Second International Symposium on HCV. Abstract Los Angeles, November 8—9, 1990.
8. Hollós, I., László, B., Brojnás, J. et al.: The serological diagnosis of non-A, non-B hepatitis (In Hungarian). Orv. Hetil. 122, 2639—2642 (1981)
9. Schaff, Zs., Tabor, E., Jackson, D. R. et al.: Ultrastructural alterations in serial liver biopsy specimens from chimpanzees experimentally infected with a human non-A, non-B hepatitis agent. Virchows Arch. B. Cell. Pathol., 45, 301—306 (1984)
10. Schaff, Zs., Lapis, K.: "New forms" of hepatitis viruses: HCV, HDV, HEV (In Hungarian). Orvosképzés, 65, 287—293 (1990)
11. Schaff, Zs., Lapis, K.: Hepatitis C Virus-main causative agent of the non-A, non-B hepatitis (In Hungarian). Orv. Hetil. 131, 1903—1906 (1990)
12. Pár, A.: Antibody to hepatitis C virus in Hungary. Lancet, 336, 123 (1990)
13. Héjjas, M., Mihály, I., Tucsok, A. et al.: Report on the first trial with the HCV antibody test in Hungary. First International Meeting on Hepatitis C virus. In abstract. Rome, Sept. 14—15, 1989, p. 16.
14. Alter, H. J., Purcell, R. H., Shih, J. et al.: Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. New Eng. J. Med. 321, 1494—1500 (1989)
15. Weiner, A., Kuo, G., Bradley, D. et al.: Detection of hepatitis C viral sequences in non-A, non-B hepatitis. Lancet, 1, 1-3 (1990)
16. McFarlane, I. G., Smith, H. M., Johnson, P. J. et al.: Hepatitis C virus antibodies in chronic active hepatitis, pathogenetic factor or false positive result. Lancet, 1, 754—757 (1990)

17. Schumpf, E., Elgjo, K., Fausa, O. et al.: The significance of anti-hepatitis C virus antibodies measured in chronic liver disease. Scand. J. Gastroenterol. 25, 1169—1174 (1990)

18. Schwarcz, R., Von Sydow, M., Weiland, O.: Autoimmune chronic active hepatitis: changing reactivity for antibodies to hepatitis C virus after immunosuppressive treatment. Scand. J. Gastroenterol. 25, 1175—1180 (1990)

19. Alter, H. J.: Viral infections of the liver: safety of blood products. In: Bianchy, L., Gerok, W., Maier, K. P., Deinhardt, F. (eds): Infectious Diseases of the Liver. Kluwer Academic Publishers, London, 1990, pp. 353—360.

Acta Medica Hungarica, Vol. 48/3—4, pp. 177—187 (1991)

HEPATOCELLULAR CARCINOMA IN SUB-SAHARAN (TROPICAL) AFRICA

G. A. BALINT[a], H. GHEBREKIDAN[b] and ERICA BALINT[c]

[a]Armed Forces Hospital (R.S.A.F.); King Abdulaziz Airbase;
Dhahran, Kingdom of Saudi Arabia;
[b]Dept. of Pharmacology (S.P.), Addis Ababa University;
Addis Ababa, Ethiopia;
[c]Dept. of Human Anatomy, Albert Szent-Györgyi Medical University;
Szeged, Hungary

(Received: July 5, 1991)

Primary cancer of the parenchymal cells of the liver — hepatocellular carcinoma — is much more common in Africa (and Southeast Asia) than in Europe and North America. The very high incidence of this tumour is one of the outstanding problems of medicine in tropical countries. There is no doubt that it was already widespread in Africa before urbanization and industrialization. In this article the characteristics of hepatocellular cancer in the tropical (sub-Saharan) part of Africa are briefly reviewed.

Keywords: hepatocellular carcinoma, tropics, Africa.

Introduction

In sub-Saharan (tropical) Africa HcC is the most common of all tumours, accounting for 10-30 per cent of all malignancies in men /13, 20, 29, 37/.

In Maputo (Mozambique) it accounts for two-thirds of the tumours in men, i.e. about thousandtimes the incidence in (most parts of) Europe /10/.

A very high rate has also been reported from Senegal /12/.

In much of East Africa and Nigeria, although a common tumour, it does not reach the high rates seen in Mozambique and Senegal.

In Uganda, it constitutes 7.9 per cent of all tumours, thus, it is the fourth commonest malignancy recorded in the Kampala Cancer Registry

---

Abbreviations: aFp: alpha-Fetoprotein, DNA: Deoxyribonucleic acid, HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B virus, HcC: Hepatocellular carcinoma.

Offprint requests should be sent to: G. A. Balint, Semmelweis u. 6., P.O. Box 397, H-6701 Szeged, Hungary.

Akadémiai Kiadó, Budapest

/15/. The overall annual incidence rate is 2.0 cases per 100 000 population. In Kyadondo county (Uganda) — an area best served by medical facilities — the incidence rate is 5.1 cases per 100 000 women and 11.1 cases per 100 000 men /14/.

In Nigeria, the figure is 5.9 per 100 000 population. In Ibadan (Nigeria) 144 cases were seen in a ten-year (1958—68) period. The male/female ratio was 4:1, and death occurred at a mean time of eleven weeks after the first symptom had appeared /21/.

In Zimbabwe Africans, the incidence rate was 20.9 per 100 000 population; among South African Bantus it was 14.0 per 100 000, while in the most affected Mozambique the figure generally quoted is 98.2 per 100 000 /38/.

A high incidence has also been reported from the Sudan. The tumour represented 2.0 per cent of the medical admissions and 40 per cent of all malignancies. The male to female ratio was 6:1 and the disease seems to be more common in Western Sudan than elsewhere in the country /44/.

In Ethiopia 11.0 per cent, and in Kenya about 5 per cent of the tumours are HcCs /51/.

Edington and Thijs gave basically the same figures regarding to Ghana and Zaire, respectively (cited in /12/).

There is apparently a five-fold variation in average within the tropical part of Africa /12, 19/.

Unlike those for more easily biopsied tumours, the true rates are undoubtedly underestimated.

It is worth mentioning that cholangiocellular carcinoma is not more common in Africa than in the western world /1/.

Aetiology of HcC

The reasons for being so common HcC in Africa is probably related mainly to the high carrier rate of HBsAg, but there are further factors possible:

— relationship to macronodular cirrhosis;

— toxic: Aflatoxins from Aspergillus species are hepatotoxic and carcinogenic;

— hormonal: Males are affected more than females;

— nutritional;

— genetic and

— others.

HBV and HcC

There is a strong epidemiological evidence for this association /9, 41, 42/.

HBV is highly endemic in Africa. Most people are infected in childhood, resulting in the highest incidence of chronic carriership in the world /49/.

Clinical studies also have consistently shown that those with HcC have a greatly increased prevalence of carriership of HBV /41, 53/. For example, about 1 in 500 chronic carriers in Gambia developed HcC /38/.

It seems likely that viral hepatitis B is a predisposing factor for HcC and that the intermediate stage is usually cirrhosis /7, 29, 45, 50, 52/.

Duration of chronic liver disease before HcC was found significantly shorter in HBsAg-positive than HBsAg-negative patients /24/.

In a Tanzanian study, performed in Dar-es-Salaam, 21.0 per cent of serum samples from 57 patients with HcC gave positive results for HBsAg, while for a control group (patients without HcC), rates 5.5 and 1.3 per cent for males and females, respectively, were reported /33/.

In Kenya, HBsAg was present in 21.0 per cent of patients with cirrhosis and 14.0 per cent of those with HcC /7/.

It is worth mentioning that 95 per cent of Africans (and Asians) with HcC have antibodies to the hepatitis core antigen (anti-HBc), too /30/.

There is a possibility that the high incidence of HbsAg in patients with HcC is a result of an altered immunological status rendering infection more likely.

It is also possible that a latent HBV infection is activated by the development of HcC /12/.

Moreover, HcC might have a multifactorial aetiology: HBV may be one of a number of co-carcinogenic factors, including genetic, hormonal, immunological and environmental ones /43/. Among these possibilities a possible HBV-DNA (virus-host) interaction needs a special attention /16, 27/.

Relationship to macronodular cirrhosis

In high-incidence areas 60 to 80 per cent of hepatomas arise in cirrhotic livers. (In temperate countries the corresponding figures are 2.5 to 15.0 per cent.)

In Kampala (Uganda), 19.0 per cent of cirrhotic patients (most with macronodular cirrhosis), were found at post-mortem to have HcC /46/. Other reports from Uganda, Ghana and Ethiopia all confirm such an association /12/.

In some areas, although, where cirrhosis is common, HcC is not of high incidence. The explanation for this is not clear /46/.

The underlying cirrhosis is usually macronodular, suggesting that liver cell injury, followed by regenerative hyperplasia is of aetiological importance; further it has been suggested that the greater the degree of hyperplasia, the greater is the danger of the development of HcC /4/. It seems very likely that cirrhosis and hepatoma have similarities in their aetiology /3/.

Alcoholic cirrhosis also predisposes to a high incidence of HcC but alcohol basically seems to be unimportant; hepatoma is common even in Islamic-dominated areas (e.g. Nigeria, Saudi Arabia, etc.) where little or no alcohol is consumed /5, 12/.

Relationship to aflatoxin and other mycotoxins

Aflatoxins are a group of compounds produced by the moulds Aspergillus flavus and Aspergillus fumigatus, which readily grow on nuts and grains in warm, humid conditions, mainly on damaged groundnuts which have been stored for a long time at high temperature and humidity /34/.

In experimental animals (rats, ducklings, turkeys, monkeys), a single dose of aflatoxin is sufficient to induce hepatitis and/or liver cancer /28, 38/.

In Africa, the incidence of cirrhosis and HcC is very high; attempts have been made to explain the distribution in terms of aflatoxins.

In Swaziland, the incidence of HcC was the highest in the hot, low-lying areas, among immigrants from Mozambique. These people ate groundnuts heavily contaminated with aflatoxin /23/.

In Uganda, the incidence was the highest in poor immigrants from Rwanda and Burundi who consumed poor-quality, contaminated grains /2/.

In Kenya, a statistically significant association between the ingested aflatoxin and HcC was observed /6, 28/. Similar observation was made in Nigeria /34/.

However, it is difficult to evaluate the overall role of mycotoxins in HcC in the tropics. Whether or not those agents modify the effect of other factors, e.g. HBV, malnutrition or others, is unknown /43/. Although

some epidemiological and circumstantial evidences point to their being important, the solution in man is still unresolved.

Hormonal factors

It is a well-established fact that males are affected by HcC to a higher degree than females /17, 32, 38, 54/.

Hyperoestrogenism (gynaecomastia) has also been suggested as a possible aetiological factor of HcC in Uganda but it seems probable that the high circulating oestrogen concentrations are a result, rather than the cause, of the liver damage.

Nutritional changes

The role of the diet in the production of HcC is also unclear. HcC is also common in low socio-economic groups where malnutrition is common. It is possible that malnutrition merely potentiates the effect of other potentially carcinogenic substances in man.

Possible role of genetic factors

Family clustering of cases together with hepatitis B carriership has been reported /22, 38/.

Other possible aetiological factors

— Schistosomiasis;

— hydatid disease.

Although HcC frequently occurs in areas where schistosomiasis and/or hydatid disease is a great medical problem, there is no convincing evidence of cause-effect relationship.

Presentation of HcC

Males are involved by the disease four to five times as often as females.

The average age at presentation is 30 to 40 years. Although cirrhosis is often present, the liver is otherwise normal in 15 to 20 per cent.

Excessive alcohol intake is not a consistent feature and no significant relationship to social class seems to exist.

Most patients have a persistent pain in the right hypochondrium, anorexia and weight-loss at presentation.

There is hepatomegaly in approximately 95 per cent.

Interestingly, the majority of the patients are aware of the liver enlargement, and about one quarter of them are jaundiced.

Clinically, the signs of cirrhosis may dominate the picture.

In about 10 per cent of the cases, an acute onset with bleeding into the abdominal cavity occurs. In a further 10 per cent, presentation is associated with metastases. Fever may also be present /51/.

In about half of the patients with hepatoma there is an audible bruit /51/, which leaves no reasonable doubt about the diagnosis /48/.

The presence of (blood-stained) ascites is often a terminal event.

Hypoglycaemic attacks and polycythaemia may also be present in patients with HcC /31/.

The diagnosis is nearly always clear and only rarely presents a differential-diagnostic problem /36/.

The most common problem is to differentiate the tumour from an amoebic liver abscess, or in certain countries (e.g. in Ethiopia), from hydatid cysts.

The clinical condition is usually so obvious that little, if any, investigation is required. Practically an adult African who has upper abdominal pain and enlarged hard liver should be considered a case of HcC.

For confirmation of the diagnosis (if it is needed) aFp estimation might be performed.

In HcC, aFp (which is produced by the tumour cell in abundance) is present at very high concentrations in the serum in 50 to 90 per cent of the patients.

However, one must bear in mind that even with the most sensitive modern methods, aFp cannot be detected in a proportion of the patients with HcC and, on the other hand, there are pathological conditions in which elevated aFp concentrations can be seen without primary hepatoma.

The routinely used liver function tests do not give the diagnosis, nor do they distinguish between some cases of liver abscess and cancer.

It should be noted that the more sophisticated diagnostic techniques (ultrasonography, MR, radionuclide imaging, etc.) usually are not available in most tropical countries.

Death, which is usually a result of cachexia, takes place within a few weeks of diagnosis; longer survivals occur rarely and never last longer than a few months.

Pathology of HcC

The tumours arise from hepatocytes. They may be monocentric or multicentric in origin. The rate of growth is very rapid, and tumours may reach an enormous size.

Necrosis, haemorrhage and fatty change may be present in the liver.

The tumour is superimposed on a macronodular cirrhosis in about three-quarters of the cases.

Metastases are formed mainly by lymphatic spread (porta hepatis, head of the pancreas). Spread to hepatic veins with pulmonary metastases is very common. Other sites of metastases are: bone, brain, gall bladder, diaphragm, peritoneum, etc.

Histologically, there are several types of HcC: adenoid, giant-cell, anaplastic, pseudoendothelial, miscellaneous and mixed types /12/.

On the ultrastructural aspects of HcC, Lapis has recently published an excellent review /26/.

Treatment of HcC

This is uniformly disappointing. Liver cancer is a fatal tumour, it kills rapidly. Survival beyond 3 months after diagnosis is rare.

Methods of treatment include symptomatic care, surgery and drug therapy.

Symptomatic care

In most cases it is advisible to give sedatives and analgetics, including morphine.

Surgery

Most patients with HcC are not fit for surgery. When the tumour is truly localized, partial lobectomy provides the only hope for complete cure. But due to poor facilities there are very few surgeons in tropical countries who will undertake that operation.

Patients fit to stand surgical exploration, whose tumour is found to be non-resectable, can be treated by hepatic artery ligation. This procedure is simple and provides comparatively prolonged palliation /8/.

The vast majority of the tumours are too advanced, have multiple foci or have arisen in a cirrhotic liver, therefore, they are not amenable to surgery. Neither liver transplantation is the solution.

Drug- (chemo) therapy

The use of systemic drug therapy for HcC has so far given extremely low response rates. Many chemotherapeutic agents have been tried but without much success.

The drugs used include 5-fluorouracil, methotrexate, thiotepa, mitomycin-C, percutaneous ethanol injection, etc. All have been disappointing /38, 40/.

Hepatic arterial infusion of cytotoxic agents has also been attempted /18, 47/.

Cochrane et al. obtained some improvement in survival by using multidrug antineoplastic therapy /11/.

Remission can be achieved by doxorubicin ("Adriamycin") in about one third of the patients /25, 35, 39/.

Combination of doxorubicin with other agents did not improve its efficacy but increased its toxicity. The response rate to intra-arterial doxorubicin is higher in average than systemic therapy.

At the present moment, doxorubicin is the only anti-neoplastic drug that has consistently given objective responses in patients with HcC.

It is worth mentioning that in assessing the effectiveness of chemotherapy it is often difficult to separate the real effect of treatment on the tumour from the deterioration in the clinical state associated with the cirrhosis.

Finally, the authors would like to emphasize that they are fully aware of that the problem of HcC is much more complex and has many other aspects than those dealt with in this short review. They wanted to give an account on this question as it is encountered in the tropical part of Africa.

REFERENCES

1. Alpert, M. E., Hutt, M. S. R., Davidson, C. S.: Hepatoma in Uganda. A study in geographic pathology. Lancet, i, 1265—1267 (1968)
2. Alpert, M. E., Hutt, M. S. R., Wogan, G. R., Davidson, C. S.: Association between aflatoxin content of food and hepatoma frequency in Uganda. Cancer, 28, 253—260 (1971)
3. Anthony, P. P.: Primary carcinoma of the liver: A study of 282 cases of Ugandan Africans. J. Pathol. 110, 37—48 (1973)
4. Anthony, P. P., Vogel, C. L., Barker, L. F.: Liver cell dysplasia: A premalignant condition. J. Clin. Path. 26, 217—223 (1973)
5. Arya, S. C.: Type 2 hepatitis B virus (HBV-2) in carriers and patients with malignancy in Saudi Arabia. Infection, 18, 215—218 (1990)
6. Autrup, H.: Aflatoxin exposure measured by urinary excretion of aflatoxin-B_1-guanine adduct and hepatitis B virus infection in areas with different liver cancer incidence in Kenya. Cancer Res. 47, 3430—3433 (1987)
7. Bagshawe, A. F., Parker, A. M., Jindani, A.: Hepatitis associated antigen in liver disease in Kenya. Brit. Med. J. 1, 88—89 (1971)
8. Balasegaram, M.: Complete hepatic dearterialization for primary carcinoma of the liver. Am. J. Surg. 124, 34—44 (1972)
9. Beasley, R. P.: Hepatitis B virus. The major aetiology of hepatocellular carcinoma. Cancer, 61, 1942—1956 (1988)
10. Bjilsma, R.: Malignant tumours in Mozambican Africans with special reference to primary liver carcinoma. Trans. Roy. Soc. trop. Med. Hyg., 75, 451—454 (1981)
11. Cochrane, A. M., Murray-Lyon, I. M., Brinkley, D. M., Williams, R.: Quadruple chemotherapy versus radiotherapy in treatment of primary hepatocellular carcinoma. Cancer, 40, 609—614 (1977)
12. Cook, G. C.: Tropical Gastroenterology. ELBS, Oxford Univ. Press, 1980.
13. Cook, P. J., Burkitt, D. P.: Cancer in Africa. Br. med. Bull. 27, 14—20 (1971)
14. Davies, J. N. P., Elmes, S., Hutt, M. S. R., Mtimavalye, L. A. R., Owor, R., Shaper, L.: Cancer in African community, 1897—1956. An analysis of the records of Mengo Hospital, Kampala, Uganda. Br. med. J., 1, 259—264 and 336—341 (1964)
15. Davies, J. N. P., Knowelden, J., Wilson, B. A.: Incidence rates of cancer in Kyadondo county, Uganda, 1954—1960. J. Nat. Cancer Inst. 35, 789—821 (1965)
16. Davison, F. D.: HBV-DNA sequences in tumour and nontumour tissue in patient with the fibrolamillar variant of hepatocellular carcinoma. Hepatology, 12, 676—679 (1990)
17. Desai, H. N.: Clinical aspects of hepatocellular carcinoma in man. S. Afr. med. J., 50, 1611—1613 (1976)
18. Doci, R., Bignami, P., Bozzetti, F., Boufanti, G., Andidio, R., Colombo, M., Gennari, L.: Intrahepatic chemotherapy for unresectable hepatocellular carcinoma. Cancer, 61, 1983—1987 (1988)
19. Editorial: Hepatocellular cancer: Difference between high and low incidence regions. Lancet, ii, 1183—1184 (1987)
20. Editorial: Hepatocellular carcinoma. Trop. Gastroenterol. 8, 57—58 (1987)
21. Francis, T. I., Smith, J. A.: Hepatocellular carcinoma in Nigerians: A study of 144 autopsy proven cases, 1958—1968. W. Afr. med. J., 21, 37—42 (1972)

22. Gu, J. R.: Molecular aspects of human hepatic carcinogenesis. Carcinogenesis, 9, 697—703 (1988)

23. Keen, P., Martin, P.: The toxicity and fungal infestation of foodstuffs in Swaziland in relation to harvesting and storage. Trop. geogr. Med. 23, 44—53 (1971)

24. Kubo, Y., Okuda, K., Musha, H., Nakashima, T.: Detection of hepatocellular carcinoma during a clinical follow-up of chronic liver disease. Gastroenterology, 74, 578—582 (1978)

25. Lai, E. C.: Doxorubicin for unresectable hepatocellular cancer. A prospective study on the addition of verapamil. Cancer, 15, 1685—1687 (1990)

26. Lapis, K.: Ultrastructural aspects of the human primary liver cancer. Appl. Pathol. 6, 117—127 (1988)

27. Lin, H. H.: DNA measurements in chronic hepatitis, cirrhosis and hepatocellular carcinoma. Liver, 10, 313—318 (1990)

28. Linsell, C., Peers, F. G.: Aflatoxin and liver cell cancer. Trans. Roy. Soc. trop. Med. Hyg., 71, 471—473 (1977)

29. Lygidakis, N. J.: Primary hepatocellular carcinoma. Hepatogastroenterology, 37, 439—440 (1990)

30. Maupas, P., Werner, B., Laronzé, B.: Antibody to hepatitis B core antigen in patients with primary hepatic carcinoma. Lancet, ii, 9—11 (1975)

31. McFadzean, A. J. S., Yeung, R. T. T.: Further observations on hypoglycaemia in hepatocellular carcinoma. Am. J. Med. 47, 220—235 (1969)

32. Nagasue, N.: Specificity of androgen receptors of hepatocellular carcinoma and liver in humans. Hepatogastroenterology, 37, 474—479 (1990)

33. Nantulya, V. M., Lindqvist, K. J., Spencer, S.: Hepatitis B antigen in acute viral hepatitis and primary liver cell cancer in Tanzania. E. Afr. med. J., 52, 430—437 (1975)

34. Nwoloko, C., Okoukwo, P.: Aflatoxin load of common food in savana and forest regions of Nigeria. Trans. Roy. Soc. trop. Med. Hyg., 72, 329—332 (1978)

35. O'Connel, M. J., Hahn, R. G., Rubin, J., Moertel, C.: Chemotherapy of malignant hepatomas with sequential intraarterial doxorubicin and systemic 5-fluoro-uracil and semustine. Cancer, 62, 1041—1043 (1988)

36. Okazaki, N.: Early diagnosis of hepatocellular carcinoma. Hepatogastroenterology, 37, 480—483 (1990)

37. Olebuyide, I. O.: Primary hepatocellular carcinoma in Africans. Trop. Gastroenterol. 7, 43—48 (1986)

38. Olweny, C. L. M.: Cancer. In: Parry, E. H. O. (Ed.): Principles of Medicine in Africa. 2nd Ed. ELBS, Oxford Univ. Press, pp. 973—991 (1984)

39. Olweny, C. L. M., Toya, T., Katongole-Mcbitte, E.: Treatment of hepatocellular carcinoma with adriamycin. Cancer, 36, 1250—1257 (1975)

40. Olweny, C. L. M., Katongole-Mcbitte, E., Bahendeka, S.: Further experience in treating patients with hepatocellular carcinoma in Ungada. Cancer, 46, 2717—2722 (1980)

41. Parry, E. H. O. (Ed.): Principles of Medicine in Africa. 2nd Ed. ELBS, Oxford Univ. Press, 1984.

42. Popper, H.: Relation between hepatocellular carcinoma and persistent hepatitis B infection. Appl. Pathol. 6, 64—72 (1988)

43. Rajagopalan, M. S., Busch, M. P., Blum, H. E., Vyas, G. N.: Interaction of aflatoxin and hepatitis B virus in the pathogenesis of hepatocellular carcinoma. Life Sci. 39, 1287—1290 (1986)

44. Salih, S. Y., Satir, A. A., Suleiman, S. I., Sabba, H. A.: Observations on hepatocellular carcinoma in the Sudan. J. trop. Med. Hyg. 81, 60—62 (1978)

45. Samuel, I., Gebre-Selassie, M., Bocina, B.: Hepatitis associated antigen (HAA) in liver disease in Ethiopia. Ethiop med. J., 12, 53—60 (1974)

46. Shaper, A. G., Kibukamusoke, J. W., Hutt, M. S. R. (Eds): Medicine in a Tropical Environment. B. M. A., London, 1972.

47. Tandon, R. N., Bunnel, I. L., Cooper, R. G.: The treatment of metastatic carcinoma of the liver by the percutaneous selective hepatic artery infusion of 5-fluorouracil. Surgery, 73, 118—121 (1973)

48. Teklu, B.: Hepatic bruits in liver tumours. Lancet, ii, 259—260 (1978)

49. Thomas, H. C., Jewell, D. P.: Clinical Gastrointestinal Immunology. Blackwell, Oxford (1979)

50. Tong, M. J., Sun, S. C., Schaeffer, B. T., Chang, N. K., Lo, K. J., Peters, R. L.: Hepatitis associated antigen and hepatocellular carcinoma in Taiwan. Ann. int. Med. 75, 687—691 (1971)

51. Tsega, E.: Hepatocellular carcinoma in Ethiopia. (A prospective clinical study of 100 patient.) E. Afr. med. J., 54, 281—292 (1977)

52. Vogel, C. L., Anthony, P. P., Sadikali, F., Barker, L. F., Peterson, M. R.: Hepatitis associated antigen and antibody in hepatocellular carcinoma: Results of a continuing study. J. Nat. Cancer Inst., 48, 1583—1588 (1972)

53. WHO Scientific Group's Report: Prevention of primary liver cancer. Lancet, i, 463—465 (1983)

54. Wilkinson, M. L.: Sex steroids and primary hepatocellular carcinoma. Anticancer Res. 7, 1071—1077 (1987)

Acta Medica Hungarica, Vol. 48/3—4, pp. 189—195 (1991)

CHANGES OF MUCOSAL ENDOGENOUS PROSTACYCLIN LEVEL IN HUMAN PEPTIC ULCER DISEASE. THE ROLE OF SMOKING AND SEX DIFFERENCE

G. A. BALINT[a] and J. NAFRADI[b]

[a]Laboratory of Clinical Pharmacology, Dept. of Neurology and Psychiatry;
and
[b]First Dept. of Medicine;
Albert Szent-Györgyi Medical University, Szeged, Hungary

(Received: September 12, 1991)

It seems that in human gastroduodenal mucosal biopsy specimens from healthy persons there is a definite tendency toward a higher endogenous prostacyclin content in favour of women. This discrepancy is present in peptic ulcer disease, too. Smoking exerts an unfavourable effect on the gastric and duodenal mucosa. The target of this action is (among others) the endogenous prostacyclin content. In ventricular as well as duodenal ulceration there is a tendency toward decreased endogenous prostacyclin activity but (moderate) smoking, — as a continuous stimulus — seems to be capable of evoking higher endogenous prostacyclin levels. This phenomenon, most probably, constitutes a part of the reparative reactions against the noxa, i.e. smoking, itself. The results draw attention once again to a possible role of smoking in the development and healing of peptic ulcer disease.

Keywords: peptic ulcer disease, endogenous prostacyclin, smoking, sex difference.

Introduction

Balint et al. reported in a pilot-study /2/ that in PUD there was a definite tendency toward decreased end. PG-I_2 production in human gastric mucosa.

Moreover, they showed that not only among experimental circumstances in rat but in clinical situation as well, in humans, cigarette smoke exerted an unwanted effect on the gastric mucosa. The target of this action was the

---

Abbreviations: End.: Endogenous, mucosal, PG-I_2: Prostacyclin; (PG: Prostaglandin), PUD: Peptic ulcer disease.

Offprint requests should be sent to: G. A. Balint, H-6701 Szeged, P.O. Box 397. (Semmelweis u. 6.) Hungary.

Akadémiai Kiadó, Budapest

end. PG-I_2 content, which significantly decreased under the effect of cigarette smoke /4/.

Taking into consideration that smoking has a positive correlation with PUD, too /10/, it was an intriguing problem to investigate the possible role of end. PG-I_2 in the pathogenesis of PUD, with special reference to smoking.

Methods

Investigations were carried out in gastroduodenal mucosal biopsy specimens. The biopsies were performed in patients who had some gastrointestinal complaints but their careful retrospective clinical check-up failed to show peptic ulceration, inflammatory gastric diseases (gastritis, etc.) or tumour. This group of the patients served as control group.

On the other hand, the presence of active PUD — either its gastric or the duodenal form — was proved by gastroscopy with consecutive histological investigation aimed at excluding malignancy. (Human Investigations' Review Board's Agreement No: 75/1987.)

Before gastroscopy the biopsy donors fasted overnight without systemic premedication. The investigation was carried out with the help of a gastro-duodeno fiberoscope Olympus GIF-Q type.

Aspirin and non-steroidal anti-inflammatory drug therapy, as well as alcohol consumption were excluded for a week before gastroscopy.

The biopsy donors (patients) were divided into groups on the basis of their illness, smoking habit and sex.

The following groups were formed:

— healthy non-smoker males and females;
— healthy smoker males and females;
— non-smokers (males and females) with gastric ulcer disease;
— non-smokers (males and females) with duodenal ulcer disease;
— smokers (males and females) with gastric ulcer disease;
— smokers (males and females) with duodenal ulcer disease.

We considered as "smokers" the persons who had smoked 20 or more cigarettes daily at least over a year.

The biopsy donors' age was 21 to 64 yrs (males), and 27 to 59 yrs (females).

It is worth mentioning that no gastroscopical differences were present between the healthy smokers and non-smokers.

The end. PG-I_2 level was determined by radioimmunoassay throughout.

Since 6-keto-PG-$F_{1\alpha}$ is the stable degradation product of PG-I_2, it is widely accepted that its changes indicate an alteration of the latter (1.15). Therefore, to determine the end. PG-I_2 level we have measured the mucosal level of 6-keto-PG-$F_{1\alpha}$. For the determinations, "RK-16" kits, manufactured by the Inst. of Isotopes of the Hungarian Academy of Sciences (Izinta, Budapest) were used.

The cross-reactivity of the kits used at 50% B/B_0-values was as follows:

6-keto-PG-$F_{1\alpha}$	100.0%		
PG-$F_{2\alpha}$	0.8%	PG-E_2	1.4%
PG-$F_{1\alpha}$	2.0%	PG-E_1	1.2%

The extraction of 6-keto-PG-$F_{1\alpha}$ from the mucosal biopsy specimens was carried out with the help of the acetone-petroleum ether-ethylacetate method /9/.

Samples were taken for protein determination, too /11/.

The recovery of the extraction procedure reached or exceeded 90%, while the detection limit was 5 pg/mg protein.

All results were given in pg 6-keto-PG-$F_{1\alpha}$/mg protein.

Intra- and inter-assay coefficients of variation were less than 5%. The "procedure blank" (buffer sample subjected to exactly the same treatment as biological (biopsy) samples) used showed no detectable "PG-I_2-like" activity, i.e. it was below the detection limit (5 pg/mg protein).

Within each group mean ± S.E.M. was calculated and analysed statistically with the help of analysis of variances. Significant differences were assumed when P was less than 0.05.

Results

The results of the investigation are presented in the Table I.

Table I

Changes of Mucosal Endogenous Prostacyclin Level in Human Peptic Ulcer Disease (mean ± S.E.M.)

Group/Ulcer	n	Antrum	Fundus	Duodenum
		6-keto-PG-$F_{1\alpha}$ pg/mg protein[a]		
MEN, SMOKERS				
1. Control	18	344.6 ± 72.4^{*su}	337.3 ± 68.3^{qv}	369.9 ± 80.9^{xt}
2. Gastric	17	355.9 ± 85.1^{*su}	367.2 ± 79.1^{qv}	440.0 ± 97.6^{t}
3. Duodenal	21	409.4 ± 83.5^{su}	403.1 ± 80.4^{qv}	302.4 ± 77.6^{wxt}
MEN, NON-SMOKERS				
4. Control	17	467.8 ± 87.4^{s}	503.6 ± 95.6^{+}	498.4 ± 94.2
5. Gastric	14	355.4 ± 83.6^{*su}	328.1 ± 81.9^{qv}	406.6 ± 93.2^{xt}
6. Duodenal	18	603.7 ± 109.6^{y§}	583.5 ± 106.7^{+}	330.7 ± 75.1^{wxt}
WOMEN, SMOKERS				
7. Control	15	605.1 ± 103.7^{y§}	587.5 ± 95.4^{+}	461.9 ± 96.1
8. Gastric	11	282.7 ± 70.4^{*su}	299.1 ± 61.1^{qv}	394.4 ± 88.6^{wxt}
9. Duodenal	12	758.4 ± 114.0^{y§}	665.9 ± 104.6$^{+●}$	667.9 ± 108.2^{oz}
WOMEN, NON-SMOKERS				
10. Control	17	680.8 ± 98.7^{y§}	671.2 ± 107.3$^{+●}$	641.7 ± 101.0^{oz}
11. Gastric	12	663.2 ± 108.5^{y§}	596.8 ± 101.4^{+}	670.3 ± 109.9^{oz}
12. Duodenal	10	339.3 ± 55.0^{*su}	355.2 ± 82.2^{qv}	375.8 ± 83.8^{wxt}

[a]Prostacyclin has been estimated in 6-keto-PG-$F_{1\alpha}$ form.

y = P<0.05 vs 8. + = P<0.05 vs 8. o = P<0.05 vs 3.
s = P<0.05 vs 9. q = P<0.05 vs 9. w = P<0.05 vs 9.
u = P<0.05 vs 10. v = P<0.05 vs 10. t = P<0.05 vs 10.
* = P<0.05 vs 11. ● = P<0.05 vs 12. x = P<0.05 vs 11.
§ = P<0.05 vs 12. z = P<0.05 vs 12.

It seems that in the so-called control situation, i.e. in healthy (non-PUD) persons, there is a tendency toward a higher end. PG-I_2 level in favour of women, among both non-smokers and smokers.

Moreover, it is evident that smoking generally decreases the end. PG-I_2 level in both sexes /4/.

In the case of gastric ulceration smoker women showed a significant decrease of the antral end. PG-I_2 content, while in men no change or only a slight decrease was observed. As compared to the non-PUD group in duodenal ulceration on the other hand, regarding to the antral end.PG-I_2 content, there was a non-significant elevation in all groups but the group of non-smoker women.

Interestingly, in the fundic mucosa of men (either smokers or non-smokers), with any form of ulceration the end.PG-I_2 content did not show marked changes but in smoker women with gastric ulcers or in non-smoker women with duodenal ulcer, end.PG-I_2 was significantly low.

In the duodenal region the most important changes are in women, namely, in the case of duodenal ulceration the end.PG-I_2 level showed significantly high values in smokers, while in non-smokers it showed a significant decrease as compared to the control (non-PUD) value.

Discussion

It is well known that several factors, e.g. stress, drugs, may lead to gastrointestinal ulceration.

While the role of some aetiologic factors has been recognized in the initiation and/or activation of gastroduodenal ulcers, quite a number of other, still unknown, factors are most probably present, and the possible interaction of these noxae in the pathogenesis and/or pathomechanism of PUD is still poorly understood.

One of the local factors in these processes might be an imbalance among protective (regenerative) and ulcerogenic reactions in the mucosa.

Taking into consideration that end.PG-I_2 is one of the most important natural protective substances in the gastric mucosa /4, 5/ we considered an investigation of its changes in active PUD to be an interesting problem.

According to the performed investigations in human gastroduodenal mucosal biopsy specimens, it seems that in healthy persons there is a definite tendency toward a higher end.PG-I_2 content in favour of women. This is

in complete agreement with our previous data /7/, i.e. that similar situation was observed in the case of the colonic mucosa. The cause of this sex difference as well as its physiological and/or pathological relevance (if there is any) is not understood yet.

Smoking exerts an unfavourable effect on the gastrointestinal tract as well as on the gastric and duodenal mucosa of humans /13, 14, 16, 17/. One of the targets of this action seems to be the endogenous prostaglandin content of the mucosa. According to McCready /12/, cigarette smoking reduces human gastric luminal PG-E_2 content, while Fedi showed /8/ that the same noxa increases the luminal PG-$F_{2\alpha}$ level in healthy smokers.

In the pertinent literature only scarce data are available regarding to the possible relationship of human gastroduodenal mucosal PG-I_2 content and smoking in PUD /6/.

According to the present investigation it seems that an indisputable damaging effect of smoking was encountered in the end. PG-I_2 content of smoker women with ventricular ulceration. In this group of patients all investigated parts of the mucosa showed a decreased PG-I_2 level compared to the non-PUD (control) values. A similar picture was obtained, also with ventricular ulcers, in the group of non-smoker men.

In the other groups of the investigated persons only partial (antral, fundic or duodenal) damages were noticed. (For example: In men with duodenal ulcer there was a decrease only in the duodenal mucosa; in non-smoker women with duodenal ulcer, damage in the duodenal region; etc.)

The cause of the tendency toward a higher end.PG-I_2 content in smokers (mainly in women) with duodenal ulceration proposes another very interesting question.

According to our previous data /4/ it seems that in certain circumstances smoking -- as a repeated and continuous stimulus -- is capable of producing such a phenomenon. This reaction seems to be a part of the general defensive mechanism(s) in the gastroduodenal mucosa toward noxae. It was established earlier that in the gastroduodenal mucosa the damaging as well as the reparative processes run together simultaneously /3/.

The above-mentioned data draw attention once again to the role of smoking in the development and healing of PUD, particularly if we accept the hypothesis that end.PG-I_2 exerts a cytoprotective effect in the human gastroduodenal mucosa, too.

Moreover, the result that women have a higher end.PG-I_2 level in their gastroduodenal mucosa than men, perhaps enlightens -- at least part-

ly -- the fact that PUD is a more common disease among males than females, i.e. the higher end.PG-I_2 level gives a better protection.

Further investigations seem to be needed to clarify more precisely the role of end.PG-I_2 in the pathomechanism of gastroduodenal ulceration in due course of PUD -- particularly in that case if we take into consideration all of the contradictions and disputes in this matter.

Acknowledgement. Thanks are due to G. Karacsony, M.D., for the histological investigations performed.

REFERENCES

1. Bakhle, Y. S.: Synthesis and catabolism of cyclo-oxygenase products. Brit. Med. Bull. 39, 214—218 (1983)

2. Balint, G. A., Nafradi, J., Varró, V.: A gyomornyálkahártya endogén prostacyclin szintjének változásai gyomorfekélyes megbetegedésben. Magyar Belorv. Arch. 37, 78 (1984) Hung. Abstr.

3. Balint, G. A., Varró, V.: Gastric antral and fundic mucosal protein, DNA and RNA changes in different experimental ulcer models. Agents Actions, 17, 89—91 (1985)

4. Balint, G. A., Varró, V.: The effect of cigarette smoke on gastroduodenal mucosal endogenous prostacyclin level. Experimental and clinical observations. Agents Actions, 19, 224—227 (1986)

5. Balint, G. A., Varró, V.: The role of endogenous and exogenous prostacyclin in the gastric mucosa under physiological and pathological circumstances. Acta Physiol. Hung., 73, 193—197 (1989)

6. Balint, G. A.: Smoking exerts unfavourable effect on human gastroduodenal mucosal endogenous prostacyclin level. E. Afr. med. J., 66, 535—537 (1989)

7. Balint, G. A., Nagy, F.: In healthy humans the colonic mucosal endogenous prostacyclin content shows a sex difference. J. clin. Gastroenterol. In press (1992)

8. Fedi, P.: Cigarette smoking increases gastric luminal Prostaglandin-$F_{2\alpha}$ and Thromboxane-B_2 in helathy smokers. Digestion, 46, 27—34 (1990)

9. Gréen, K., Hamberg, M., Samuelsson, B., Frölich, J. C.: Extraction and chromatographic procedures for purification of prostaglandins, thromboxanes, prostacyclin and their metabolites. In: Fröhlich, J. C. (Ed.): Advances in Prostaglandin and Thromboxane Research. Vol. 5. Methods in Prostaglandin Research. Raven Press, New York, 1978, pp. 1—38.

10. Grossman, M. I.: Peptic ulcer: Pathogenesis and pathophysiology. In: Beeson, P. B., McDermott, W., Wyngaarden, J. B. (Eds): Cecil Textbook of Medicine. 15th Ed. W. B. Saunders, Philadelphia, pp. 1502—1507 (1979)

11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265—275 (1951)

12. McCready, D. R.: Cigarette smoking reduces human gastric luminal prostaglandin-E_2. Gut, 26, 1192—1196 (1985)

13. Miller, G.: Smoking delays gastric emptying of solids. Gut, 30, 50—53 (1989)

14. Müller-Lissner, S. A.: Bile reflux is increased in cigarette smokers. Gastroenterology, 90, 1205—1209 (1986)

15. Myatt, L., Jagee, M., Lewis, P. J., Elder, M. G.: Metabolism of prostacyclin and 6-oxo-PG-$F_{1\alpha}$ in man. In: Lewis, P. J., O'Grady, J. (Eds): Clinical Pharmacology of Prostacyclin. Raven Press, New York, pp. 25—35 (1981)

16. Parente, F., Lazzaroni, M., Sangaletti, O., Baroni, S., Bianchi-Porro, G.: Cigarette smoking, gastric acid secretion and serum pepsinogen I concentrations in duodenal ulcer patients. Gut, 26, 1327—1332 (1986)

17. Read, N. W., Grech, P.: Effect of cigarette smoking on competence of the pylorus. Br. Med. J., 2, 313—316 (1973)

18. Walker, V., Taylor, W. H.: Cigarette smoking, chronic peptic ulceration and pepsin I secretion. Gut, 20, 971—976 (1979)

Acta Medica Hungarica, Vol. 48/3—4, pp. 197—201 (1991)

COLLOIDAL BISMUTH SUBCITRATE EVOKES ADAPTIVE CYTOPROTECTION IN RAT GASTRIC MUCOSA

G. A. BALINT

Laboratory of Clinical Pharmacology, Department of Neurology and Psychiatry,

Albert Szent-Györgyi Medical University, Szeged, Hungary

(Received: September 12, 1991)

The 6-keto-prostaglandin-$F_{1\alpha}$ content (as the stable break-down product of prostacyclin) of rat antral and fundic gastric, as well as of duodenal mucosa, significantly increases after 1, 5 and 10 mg/kg orally administered colloidal bismuth subcitrate treatment. The results indicate that (i) colloidal bismuth subcitrate-induced stimulation of endogenous prostacyclin content ("adaptive cytoprotection") of rat gastroduodenal mucosa may contribute to its therapeutic effect; (ii) the effect of colloidal bismuth subcitrate is not due exclusively to the bismuth content of the molecule, but seems to be connected with the structure of colloidal bismuth subcitrate itself; (iii) the effect seems to be dose-dependent, showing a dose-response relationship.

Keywords: Colloidal bismuth subcitrate, adaptive cytoprotection, prostacyclin, gastric and duodenal mucosa, rat.

Introduction

End.PG-I_2 plays a significant role as one of the natural protective substances in the rat gastroduodenal mucosa /1, 2/. It is known that the H_2-receptor blockers cimetidine and ranitidine enhance the end.PG-I_2 level /3, 4/. Moreover sucralphate also acts (at least partly) via the end.prostaglandin system /5/.

The aim of this study was to establish the possible effect of CBS on gastroduodenal end.PG-I_2 levels in rats.

Abbreviations: BSN: Bismuth subnitrate, CBS: Colloidal bismuth subcitrate, end: Endogenous, mucosal, PG-I_2: Prostacyclin.

Offprint requests should be sent to: G. A. Balint, H-6701 Szeged, P.O. Box 397. (Semmelweis u. 6.) Hungary.

Akadémiai Kiadó, Budapest

Materials and methods

Female Wistar rats of 200 to 220 g body weight were used. The animals were fasted (allowed water ad libitum) in wire-bottomed cages for 24 h prior to investigation.

CBS (Batch No: 08940, Gist-Brocades N.V., Delft, The Netherlands) was given orally (through a gastric tube) as an aqueous suspension in a fixed volume (1 ml/kg) and in various single doses: 1, 5 and 10 mg/kg, respectively.

60, 120, 180 and 240 min following CBS treatment the animals were killed and their stomach and duodenum removed. The handling of mucosal samples as well as the detailed procedure of end.PG-I_2 estimation was published earlier /2/, therefore, a brief account will only be given here.

To determine the end.PG-I_2 level, the mucosal content of 6-keto-prostaglandin-$F_{1\alpha}$ was investigated by radioimmunoassay. For determination "RK-16" kits, manufactured by the Inst. of Isotopes of the Hungarian Academy of Sciences (Izinta, Budapest) were used.

The 6-keto-prostaglandin-$F_{1\alpha}$ from the mucosal samples was extracted with the help of the acetone-petroleum ether-ethylacetate method /6/. Samples were also taken for mucosal DNA determination /7, 9/. The recovery of the extraction procedure reached or exceeded 90%, while the detection limit was 0.5 ng/mg DNA. All results are given in ng 6-keto-prostaglandin-$F_{1\alpha}$/mg DNA.

The intra- and inter-assay coefficients of variation were less than 15%. The "procedure blank" showed no detectable "PG-I_2-like" activity, i.e. it was below the detection limit.

All determinations in each animal were carried out in triplicate, the results were pooled and within each group (n = 5/group) the means $\pm$ S.E.M. were calculated and analysed statistically with the help of analysis of variances. The differences were considered significant when P was < 0.05.

In an additional series of investigations — under the same experimental conditions — the effect of 1, 5 and 10 mg/kg BSN ($Bi(OH)_2NO_3$) was examined.

Results

The experimental results are presented in Tables I and II.

The CBS and the BSN used contains about 35% and 70% bismuth, respectively.

BSN has a physical structure completely different from that of CBS. Therefore, we investigated its effect on the end.PG-I_2 level of rat gastroduodenal mucosa. In this way the effect of bismuth and molecular structure can be separately analysed.

The experimental results showed that:

(i) CBS significantly increases the gastroduodenal mucosal end.PG-I_2 level of rats.

(ii) BSN exerted the same type of effect as CBS.

Table I

The Effect of Colloidal Bismuth Subcitrate (CBS) on Gastroduodenal Mucosal Endogenous Prostacyclin Level of Rat (mean ± S.E.M.)

Group	6-keto-prostaglandin $F_{1\alpha}$ ng/mg DNA		
	Antrum	Fundus	Duodenum
1. CONTROL	15.55 ± 2.46$^{\bullet\S ab}$	9.44 ± 2.01$^{\bullet\S ab}$	2.65 ± 0.57$^{\bullet\S ab}$
2. CBS 1 mg — 60 min	65.68 ± 17.11$^{+\bullet o}$	74.37 ± 20.98^{+}	31.35 ± 6.44^{+o}
3. CBS 1 mg — 120 min	69.54 ± 12.21$^{+\bullet o}$	58.88 ± 7.81^{+}	30.62 ± 7.02^{+o}
4. CBS 1 mg — 180 min	44.18 ± 9.44 $^{+\bullet oab}$	49.95 ± 11.72^{+}	29.97 ± 3.39^{+o}
5. CBS 1 mg — 240 min	39.15 ± 7.07$^{+\bullet\S oab}$	24.10 ± 6.67$^{+\S b}$	18.96 ± 5.33$^{+\S o}$
6. CBS 5 mg — 60 min	38.77 ± 8.58$^{+\bullet\S oab}$	22.90 ± 5.55$^{+\S ab}$	10.89 ± 3.72$^{+\bullet ab}$
7. CBS 5 mg — 120 min	61.88 ± 20.06$^{+\bullet o}$	49.92 ± 7.98^{+}	28.34 ± 6.51^{+o}
8. CBS 5 mg — 180 min	161.47 ± 28.76$^{+\S o}$	49.41 ± 9.71^{+}	34.80 ± 7.31^{+o}
9. CBS 5 mg — 240 min	87.02 ± 18.90^{+o}	95.78 ± 26.39^{+}	54.86 ± 12.36^{+oab}
10. CBS 10 mg — 60 min	14.17 ± 2.78$^{\bullet\S ab}$	25.42 ± 9.32$^{\S ab}$	6.72 ± 0.94$^{+\bullet\S ab}$
11. CBS 10 mg — 120 min	48.65 ± 7.27$^{+\bullet oab}$	31.35 ± 7.63$^{+\S}$	10.73 ± 2.05$^{+\bullet\S b}$
12. CBS 10 mg — 180 min	111.31 ± 23.76^{+o}	110.22 ± 31.78^{+o}	27.40 ± 2.12$^{+\S o}$
13. CBS 10 mg — 240 min	106.00 ± 28.79^{+o}	84.75 ± 35.05^{+}	33.22 ± 9.16^{+o}

+ = $P<0.05$ vs Control ● = $P<0.05$ vs 8. § = $P<0.05$ vs 9.
o = $P<0.05$ vs 10. a = $P<0.05$ vs 12. b = $P<0.05$ vs 13.

(Within antrum, fundus and duodenum, respectively.)

Table II

The Effect of Bismuth Subnitrate (BSN) on Gastroduodenal Mucosal Endogenous Prostacyclin Level of Rats (mean ± S.E.M.)

Group	6-keto-prostaglandin-$F_{1\alpha}$ ng/mg DNA		
	Antrum	Fundus	Duodenum
1. CONTROL	15.94 ± 2.82	10.13 ± 3.24	2.22 ± 0.76
2. BSN 1 mg — 60 min	18.43 ± 4.31	15.34 ± 3.41	5.35 ± 0.97
3. BSN 1 mg — 120 min	23.69 ± 2.96	23.14 ± 7.21	7.03 ± 1.13^{+}
4. BSN 5 mg — 60 min	31.93 ± 7.24	33.27 ± 5.93^{+}	16.48 ± 3.26^{+}
5. BSN 5 mg — 120 min	52.71 ± 11.43^{+}	48.84 ± 11.87^{+}	26.04 ± 4.18^{+}
6. BSN 10 mg — 60 min	70.17 ± 29.85^{+ab}	40.75 ± 2.15^{+a}	24.16 ± 5.61^{+a}
7. BSN 10 mg — 120 min	82.77 ± 17.40^{+abc}	59.80 ± 13.89^{+a}	43.58 ± 13.18^{+ab}

+ = $P<0.05$ vs Control a = $P<0.05$ vs 2. b = $P<0.05$ vs 3. c = $P<0.05$ vs 4.

(Within antrum, fundus and duodenum, respectively.)

Discussion

Several data suggest that different types of anti-ulcer (cytoprotective) drugs exert (at least partly) their mode of action through stimulation of end.prostaglandin synthesis, i.e. via "adaptive cytoprotection".

According to Branski et al. /3/ cimetidine stimulates end.PG-I_2 and PG-E_2 production. Ranitidine has the same effect (Goldin et al. /4/). Tarnawski et al. /5/ showed that end.prostaglandins play a role also in the effect of sucralphate. Konturek et al. /10/ and Hall and van den Hoven /11/ have shown that CBS increases the ability of rat gastric mucosa to synthesize PG-E_2.

CBS precipitates in acid medium; in all probability its bismuth-citrate bond opens, and thus various insoluble compounds are formed. All these structures may bind to proteins, formings diffuse precipitate on the gastric and duodenal mucosa and on the ulcer crater.

A layer of such a precipitate acts as a protective coating against noxae, therefore, the anti-ulcer activity of CBS is very likely due to the formation of the protective bismuth coating on the stomach wall. This coating supports ulcer healing.

Taking into consideration the protein-precipitating effect of CBS — which was found about 25% by Hall (personal communication, unpublished) in an in vitro system containing only bovine serum albumin — we present all results obtained in the present work related to the DNA of the mucosa instead of its protein content.

Our conclusions are as follows:

— In the effect of CBS not exclusively the bismuth content of the molecule is important, for CBS is poorer in bismuth than BSN. The end.PG-I_2-elevating effect of CBS is more pronounced.

— It seems that the end.PG-I_2-elevating effect of CBS may contribute to its therapeutic effect in peptic ulcer disease.

It is worth mentioning that both molecules evoked a dose-dependent effect, i.e. it seems that there is a direct dose-response relationship.

Acknowledgement. We are obliged to the Gist-Brocades N.V., Delft, The Netherlands, for the CBS used in the present work.

REFERENCES

1. Balint, G. A.: Is prostacyclin the natural protective substance of rat gastric mucosa? Dig. Dis. Sci. 30, 367 (1985)

2. Balint, G. A., Varro, V.: The effect of cigarette smoke on gastroduodenal mucosal endogenous prostacyclin level. (Experimental and clinical observations.) Agents Actions, 19, 224—226 (1986)

3. Branski, D., P. Sharon, F. Karmeli, D. Rachmilewitz: Effect of cimetidine on human gastric and duodenal prostanoid synthesis. Scand. J. Gastroenterol. 19, 457—460 (1984)

4. Goldin, E., M. Ligumsky, A. Fich, R. Eliakim, J. Zimmerman, F. Karmeli, D. Rachmilewitz: Effect of drug therapy and ulcer healing on endogenous gastric prostanoid synthesis in duodenal ulcer patients. Dig. Dis. Sci. 30, 377 (1985)

5. Tarnawski, A., D. Hollander, W. J. Krause, H. Gergely: Sucralphate protects the gastric mucosa against ethanol-induced injury via prostaglandin-mediated mechanisms. Dig. Dis. Sci. 30, 403 (1985)

6. Gréen, K., M. Hamberg, B. Samuelsson, J. C. Frölich: Extraction and chromatographic procedures for purification of prostaglandins, thromboxanes, prostacyclin and their metabolites. In: Frölich, J. C. (Ed.): Advances in Prostaglandin and Thromboxane Research. Vol. 5. Methods in Prostaglandin Research. Raven Press, New York, 1978, pp. 1—38.

7. Balint, G. A., Gizella, Karacsony, V. Varro: The effect of long-term prostacyclin-treatment on the protein, DNA and RNA content of rat gastric fundic mucosa. Agents Actions 16, 404—406 (1985)

8. Ceriotti, G.: Determination of nucleic acids in animal tissue. J. Biol. Chem. 214, 59—70 (1955)

9. Giles, K. W., A. Myers: An improved diphenylamine method for the estimation of deoxyribonucleic acid. Nature (Lond.) 206, 93 (1965)

10. Konturek, S. J., T. Radecki, I. Piestucki, T. Brzozowski, D. Drozdowicz: Gastrocytoprotection by bismuth-subcitrate (De-Nol) and sucralphate. Role of endogenous prostaglandins. Gut, 28, 201—205 (1987)

11. Hall, D. W. R., W. E. van der Hoven: Gastric mucosal protection and prostaglandin-E_2 generation in rats by colloidal bismuth-subcitrate (De-Nol). Arch. internat. Pharmacodyn. Therap. 286, 308—319 (1987)

Acta Medica Hungarica, Vol. 48/3—4, pp. 203—210 (1991)

METABOLISM

SERUM ELASTASE-TYPE ENZYMES AND THEIR CORRELATION TO BLOOD LIPIDS IN MALE PATIENTS WITH ATHEROSCLEROSIS

ANNA LANDI[1], MAGDOLNA BIHARI-VARGA[2], L. KELLER[1]

[1]1st Department of Medicine, St. Stephen Hospital, Budapest (Hungary), and
[2]2nd Department of Pathology, Division of Biochemistry, Semmelweis Medical University, Budapest (Hungary)

(Received: June 17, 1991)

Serum elastase-type activity, elastase inhibitory capacity and their relation to lipids were examined in 140 male patients with ischaemic vascular disease (coronary, cerebral, peripheral) and in 60 control subjects. Serum elastase-type activity was found to be significantly lower, inhibitory capacity significantly higher, in the groups of patients than in the controls. HDL- and HDL_2 cholesterol as well as apo A concentration showed significant negative correlation with elastase inhibitory capacity both in atherosclerotic and in control subjects.

Keywords: serum elastase-type proteases, elastase inhibitors, ischaemic vascular disease, blood lipids.

Introduction

Degradation of the elastic fibres of the arterial wall seems to take an important part in the multifactorial events of atherogenesis. Although progressive splitting, fragmentation and lysis of elastic lamellae can be observed in physiological aging as well, the whole process is accelerated in the pathology of atherosclerosis.

Details of the mechanism of elastic tissue destruction are hardly known. The elastic fibres contain two distinct ultrastructural components: a microfibrillar component formed by cross-striated fibrotubules and an amorphous one, elastin. From the earliest phase of atherogenesis plasma low-

Abbreviations: CVD = cerebrovascular disease; IHD = ischaemic heart disease; PVD = peripheral vascular disease.

Offprint requests should be sent to Anna Landi, H-1096 Budapest, Nagyvárad tér 1, Hungary

Akadémiai Kiadó, Budapest

density lipoprotein enter the arterial wall and interact with connective tissue macromolecules, such as glycosaminoglycans, collagen and elastin; this selective interaction is considered to be one of the mechanisms of lipid accumulation in atherosclerotic lesions /10, 16, 19, 23, 30, 33, 34/. The relationship between deposition of lipids and deterioration of elastic fibres is still not clear.

The regulation of elastin metabolism is attributed to humoral and tissue elastases and their inhibitors. Elastases, a group of proteolytic enzymes, are endopeptidases capable of solubilizing and degrading insoluble elastin. Elastases and elastase-type proteases have been isolated from various sources; their structure properties and specificities have been partially characterized /1, 3, 6, 12, 22, 29/ and some of their natural inhibitors identified /13/. There are data indicating that elastases inhibit the progress of experimental atherosclerosis and enhance lipoprotein lipase activity /5, 8, 17, 32, 35/.

The mechanism of action of elastase consists of the selective degradation of lipid-bound elastin, by which elastase promotes the biosynthesis of newly-formed elastin /17/. In the circulation the activity of elastases can be modified by high amounts of elastase inhibitors /9, 14/. It was suggested that the increase in elastinolytic activity may represent a decrease of the level of inhibitors /11/. Disturbance of the equilibrium of humoral and tissue proteases and their inhibitors might lead to connective tissue damage.

Based on the suggestion that changes of vascular elastin metabolism may be reflected in changes of elastase-like activity and elastase-inhibitory capacity of the serum, the aim of our investigations was to get more information about the behaviour of these parameters and their relationship to blood lipids in atherosclerotic patients. Considering that the factors influencing the anatomical localization of atherosclerosis have not yet been elucidated, it seemed to be of interest to subdivide the group of patients according to the clinically dominant areas (coronary, cerebral, peripheral).

Patients and methods

Selection of patients

A total of 140 atherosclerotic male patients (between 42 and 76 years of age, mean age 63.4 years), 60 of them with ischaemic heart disease (IHD), 40 with cerebrovascular disease (CVD) and 40 with peripheral vascular disease (PVD) were studied. The patients in the three groups were practically age-matched. Selection criteria for inclusion were: (i) for patients

with IHD, typical anginal pains and ECG signs of myocardial ischaemia, or myocardial infarction in the case history at least six month prior to the investigation; (ii) for patients with CVD, acute cerebrovascular accident with focal neurological signs in the case history at least 6 months prior to the investigation (the possible intracranial or extracranial site of the pathological vascular process leading to stroke has not been taken into account); (iii) for patients with PVD, intermittent claudication or rest pain with Doppler signs of obliterative arterial disease in the lower extremities. As controls 60 age-matched men without histories, symptoms or findings of ischaemic vascular disease were selected.

Patients with diabetes mellitus, chronic renal or hepatic disease, endocrine disorders, primary hyperlipidaemias, and those treated with drugs affecting lipid metabolism were excluded.

Methods

Venous blood was obtained after an overnight fast. Elastase-type protease activity and pancreas elastase inhibitory capacity were determined using succinoyl-trialanine-paranitroanalide (Suc/Ala/PNA; Precibio, Rueil-Malmaison, France) as substrate, according to the methods of Bieth et al. /2/.

Triglyceride, total cholesterol, HDL-, HDL_2-cholesterol, apo A and apo B contents were determined using Boehringer's Peridochrom and CHOD-PAP test and Sebia's Apofilm plates, respectively. LDL cholesterol levels were estimated by Friedewald's formula /7/ only in patients with triglyceride levels under 4.5 mM/l.

For statistical evaluation of the data Student's t-test, χ^2-test and regression analysis were used.

Results

Ischaemic vascular disease

Average values of serum elastase-type activity and pancreatic elastase inhibitory capacity, as well as lipid levels determined from the same blood samples are summarized in Table I. Elastase-type activity in each of the three groups of ischaemic vascular disease was found to be significantly lower than in the controls. Elastase-inhibitory capacity proved to be significantly elevated in the groups of patients compared to the control subjects. (No age-variation of elastase activity or inhibitory capacity could be demonstrated.) In healthy individuals we found a significant positive correlation between the elastase-type activity and elastase-inhibitory capacity of sera (regression equation: $y = 9,781x + 1120$; $P < 0.001$); similar correlation could not be established in the sera of patients with IVD.

From among the serum lipid and lipoprotein parameters the average HDL- and HDL_2-cholesterol levels in all three groups of patients, the apo A content in IHD and CVD were found to be significantly decreased compared to the controls. LDL-cholesterol levels were higher in IVD groups than in the control, with a significant difference in the case of IHD and of PVD.

Table I

Elastase activity, inhibitory capacity and lipids in the sera of atherosclerotic male patients and in control subjects

Parameters (mean ± S.D.)	Group of patients			
	IHD (n = 60)	CVD (n = 40)	PVD (n = 40)	Control (n = 60)
Elastase activity ($ngml^{-1}$ 24 h^{-1})	7.5 ± 3.4**	8.4 ± 3.7*	7.8 ± 3.8**	10.9 ± 4.1
Inhibitory capacity ($ngml^{-1}$ 24 h^{-1})	44.7 ± 11.5**	41.5 ± 9.4*	40.8 ± 8.9*	32.2 ± 7.9
Cholesterol (mmol/l)	5.4 ± 1.1	5.4 ± 1.3	5.9 ± 1.2	5.1 ± 1.2
HDL cholesterol (mmol/l)	1.2 ± 0.3**	1.1 ± 0.4**	1.3 ± 0.3*	1.7 ± 0.2
HDL_2 cholesterol (mmol/l)	0.8 ± 0.2**	0.7 ± 0.1**	0.8 ± 0.2*	1.2 ± 0.2
LDL cholesterol (mmol/l)	3.2 ± 0.9*	3.0 ± 0.9	3.5 ± 1.0**	2.4 ± 1.1
Triglycerides (mmol/l)	2.3 ± 1.1	2.6 ± 1.3	2.4 ± 1.2	2.3 ± 0.9
Apo A (g/l)	1.3 ± 0.3*	1.3 ± 0.3*	1.4 ± 0.4	1.6 ± 0.4
Apo B (g/l)	1.3 ± 0.4	1.3 ± 0.4	1.3 ± 0.5	1.1 ± 0.4

P value for significance vs. control: *$P < 0.05$; **$P < 0.01$;
IHD = Ischaemic heart disease; CVD = Cerebrovascular disease; PVD = Peripheral vascular disease

Table II

Correlation of elastase inhibitory capacity with the serum HDL-, HDL_2-cholesterol and apo A concentration in male patients with ischaemic vascular disease (IVD) and in control subjects

Group of patients	No. of patients	HDL-cholesterol	HDL_2-cholesterol	Apo A
		regression equation t P	regression equation t P	regression equation t P
IVD	140	y = -0.0014x + 2.09 20.4 < 0.001	y = -0.117x + 46,7 7.37 < 0.001	y = -0.0004x + 1.40 2.89 < 0.05
Control	60	y = -0.0019x + 2.06 14.0 < 0.001	y = -0.387x + 59.6 8.93 < 0.001	y = -0.0068x + 4.27 3.22 < 0.01

HDL-, HDL_2-cholesterol and apo A concentration showed significant negative correlations with elastase-inhibitory capacity, both in IVD and control subjects (Table II). Between elastase-type activity and lipids no significant relationship could be demonstrated.

Discussion

The details of interaction between elastin and lipoproteins are still not clear. The finding of Srinivasan et al. /31/ indicated that certain regions of plaque elastin may have affinity for apo B-containing lipoproteins. According to Kramsch et al. /19, 20, 21/, with the permeation of LDL into arterial elastin a change of the protein composition of arterial elastin takes place in localized areas; the result of this process will be further deposition of lipids attracted by the focally-altered elastin surface. Accumulated lipids are successively phagocytosed by macrophages; after having been destroyed, these release their lipid contents into the intercellular space, causing damage to both to the external and internal elastic membranes of the media. Noma et al. /24, 25, 26/ presented evidence of the in vitro formation of a stable complex between LDL and delipidated arterial elastin, and demonstrated the inhibition of the LDL binding to elastin in the presence of HDL.

Contradictory reports have been published about the serum elastase activity and inhibitory capacity in atherosclerosis. In the experiments of O-hara et al. /27/ administration of elastase inhibited the development of atherosclerosis produced by cholesterol feeding in rabbits. Rabaud et al. /28/ reported that the "serum inhibitory power of elastolysis" was significantly higher in cardiovascular patients than in controls; the highest levels were measured in the acute phase of myocardial infarction. Hornebeck et al. /14/ failed to find statistically significant differences between elastase levels in the sera of atherosclerotic and control subjects. In a previous observation on smaller groups of patients we found the pancreatic elastase inhibitory capacity to have been significantly elevated in persons with ischaemic vascular diseases /4, 18/.

In our investigations a simultaneous determination of elastase-type activity and elastase-inhibitory capacity of the serum was carried out both in atherosclerotic and control subjects. Based on the close metabolic interaction between the vessel wall and the circulating blood it seemed to be

plausible that the observed humoral alteration would also provide some insight into the pathological arterial processes.

Among the investigated lipid and lipoprotein components serum HDL- and HDL_2-cholesterol and apo A concentration showed a significant negative correlation with elastase inhibitory capacity. This finding may be related to the observation of Jacob et al. /15/ that an elastase-type enzyme could be isolated and characterized in close association with apo A of human HDL preparation.

In the groups of patients we found the mean serum elastase-type activity significantly lower and the elastase inhibitory capacity significantly elevated compared to the relevant values of the control subjects. We also demonstrated that in the control group there is a positive correlation between the serum elastase-type enzyme activity and elastase-inhibitory capacity, while in patients with IVD such correlation could not be demonstrated. These findings point to the possibility that the lack of the balanced proportionality of elastase-type enzymes and their inhibitors may be related to the atherosclerotic process.

REFERENCES

1. Baló, J., Banga, I.: Elastase and elastase inhibitor. Nature 164, 491—496 (1949)
2. Bieth, J., Spiess, B., Wermuth, C. G.: The synthesis and analytical use of highly sensitive and convenient substrate of elastase. Biochem. Med. 11, 350—357 (1974)
3. Bieth, J.: Elastases. In: Robert, L. (Ed.): Frontiers of Matrix Biology, Vol. 6, Karger, Basel 1978, pp. 1—8.
4. Bihari-Varga, M., Keller, L., Landi, A., Robert, L.: Elastase-type activity, elastase inhibitory capacity, lipids and lipoproteins in the sera of patients with ischemic vascular disease. Atherosclerosis 50, 273—281 (1984)
5. Butturini, U., Pretolani, E., Gundi, A.: The action of elastase and glucagon on cholesterolemia. Bull. Soc. Ital. Biol. Sper. 35, 27—34 (1959)
6. Franzone, J. S., Cravanzola, C., Reboani, M. C., Torrielli, M. V., Pernigotti, M. L.: Biological properties of pancreatic elastase in relation to atherosclerotic disease. Int. J. Tiss. Res. X (4) 233—243 (1988)
7. Friedewald, W. T., Levy, R. I., Fredrickson, D. S.: Estimation of the concentration of low-density lipoprotein cholesterol in the plasma without use of preparative ultracentrifuge. Clin. Chem. 18, 499—502 (1972)
8. Hayashi, K., Takamizawa, K., Nakamura, T., Kato, T.: Effects of elastase on the stiffness and elastic properties of arterial walls in cholesterol-fed rabbits. Atherosclerosis 66, 259—267 (1987)
9. Heimburger, N., Haupt, H.: Zur Spezifizität der Antiproteinasen des Humanplasmas für Elastase. Klin. Wochenschr. 44, 1196—1199 (1966)

10. Hoff, H. F., Jackson, R. I., Mao, J. T., Goto, A. M.: Localisation of low density lipoproteins in arteriosclerotic lesions from human normolipemics employing a purified fluorescent-labeled antibody. Biochim. Biophys. Acta 351, 407—415 (1974)

11. Hornebeck, W., Brechemier, D., Bellon, G., Adnet, J. J., Robert, L.: Bilogical significance of elastase-like enzymes in arteriosclerosis and human breast cancer. In: Sträuli, P. (Ed.): Proteinases and tumour invasion, Raven, New York 1980, pp. 117—141.

12. Hornebeck, W., Legrand, Y.: Possible implication of two elastinolytic proteases isolated from tissue aorta and blood platelets in atherosclerosis. In: Robert, L. (Ed.): Frontiers of Matrix Biology, Vol. 8, Karger, Basel 1980, pp. 199—215.

13. Hornebeck, W., Bellon, G., Brechemier, D., Godeau, G., Robert, L.: Control of elastic tissue destruction by elastase inhibitors. In: Deyl, Z., Adams, A. (Eds): Connective tissue research, Liss, New York 1981, pp. 233—246.

14. Hornebeck, W., Potazman, J. P., De Cremoux, H., Bellon, G., Robert, L.: Elastase-type activity of human serum. Clin. Physiol. Biochem. 1, 285—289 (1983)

15. Jacob, M. P., Bellon, G., Robert, L., Hornebeck, W., Ayrault-Jarrier, M., Burdin, J., Plonovski, J.: Elastase-type activity associated with high density lipoproteins in human serum. Biochem. Biophys. Res. Comm. 103, 311—318 (1981)

16. Kao, V. C. Y., Wissler, R. W.: A study of immunohistochemical localisation of serum lipoproteins and other plasma proteins in human atherosclerotic lesions. Exp. Mol. Pathol. 4, 465—479 (1965)

17. Katsukuma, H., Shimizu, K., Ebihara, T., Iwamoto, T., Kiyokawa, M.: Antiatherosclerotic action of elastase. Age Ageing 12, 183—194 (1983)

18. Keller, L., Bihari-Varga, M., Landi, A.: Elastinolytic activity, elastase inhibiting capacity and some lipoprotein constituents of the serum of atherosclerotic patients (in Hung.). Magyar Belorv. Arch. 36, 313—319 (1983)

19. Kramsch, D. M., Franzblau, C., Hollander, W.: The protein and lipid composition of arterial elastin and its relationship to lipid accumulation in the atherosclerotic plaque. J. Clin. Invest. 50, 1666—1677 (1971)

20. Kramsch, D. M., Hollander, W.: The interaction of serum and arterial lipoproteins with elastin of the arterial intima. J. Clin. Invest. 53, 236—247 (1973)

21. Kramsch, D. M., Franzblau, C., Hollander, W.: Components of the protein-lipid complex of arterial elastin. Adv. Exp. Med. Biol. 43, 193—210 (1974)

22. Menashi, S., Hornebeck, W., Robert, L., Legrand, Y.: Elastase-like activity in cultured aortic endothelial cells. Thrombos. Res. 53, 11—18 (1989)

23. Noma, A., Takahashi, T., Yarmada, K., Wada, T.: Elastin-lipid interaction in the arterial wall. Atherosclerosis 33, 29—39 (1979)

24. Noma, A., Takahashi, T., Wada, T.: Elastin-lipid interaction in the arterial wall 2., Atherosclerosis 38, 373—382 (1981)

25. Noma, A., Hirayama, T., Yachi, A.: Studies on the binding of plasma low density lipoproteins to arterial elastin. Connect. Tissue Res. 11, 123—133 (1983)

26. Noma, A., Hirayama, T., Yachi, A.: Inhibitory effect of high density lipoprotein subfractions on the in vitro binding of low density lipoproteins to arterial elastin. Connect. Tissue Res. 11, 123—133 (1983)

27. O-hara, M., Esato, K., Mohri, H.: Effect of pancreatopeptidase E (Elastase) on the suppression of intimal fibrous proliferation after arterial reconstruction in high cholesterol fed rabbits. Cardiovasc. Surg. 29, 268—276 (1988)

28. Rabaud, M., Lefebore, F., Lamaziere, J. M. D., Bricand, H.: Serum elastase inhibitors in cardiovascular diseases. Clin. Chim. Acta 90, 53—61 (1978)

29. Robert, L., Robert, A. M.: Elastin, elastase and atherosclerosis. In: Robert, L. (Ed.): Frontiers of Matrix Biology, Vol. 8, Karger, Basel 1980, pp. 130—173.

30. Smith, F.-B.: The relation between plasma and tissue lipids in human atherosclerosis. Adv. Lipid Res. 12, 1—49 (1974)

31. Srinivasan, S. R., Yost, C., Radhakrishnamurty, E. R., Dalferes, B., Berenson, G. S.: Lipoprotein-elastin interaction in human aorta fibrous plague lesions. Atherosclerosis 38, 137—147 (1981)

32. Tono, T.: Elastase therapy for arteriosclerotic disease. Jpn J. Clin. Exp. Med. 53, 1798—1803 (1975)

33. Tsujii, T., Katayama, K., Naito, I., Seno, S.. The circulating antitrypsin-elastase complex attacks the elastic lamina of blood vessels. An immunohistochemical study. Histochemistry 88, 443—451 (1988)

34. Walton, K. W., Williamson, N.: Histological and immunofluorescent studies on the evolution of the human atheromatous plague. J. Athero. Res. 8, 599—624 (1968)

35. Yasui, M., Yano, I., Ota, K., Oshima, A.. Scavenger effect of elastase on calcifications in liver, kidney and central nervous system tissues of rabbits with atherosclerosis induced by cholesterol-rich diet. J. Intern. Med. Res. 17, 350—357 (1989)

Acta Medica Hungarica, Vol. 48/3—4, pp. 211—217 (1991)

MEASUREMENT OF COMPLEMENT COMPONENTS AND ALPHA 1-ANTITRYPSIN DURING PLASMA EXCHANGE

I. CSÍPŐ[1], Z. BEDŐ[1], S. SIPKA[1], J. S. LEBEDIN[2], A. G. CHUCHALIN[2], GY. SZEGEDI[1]

[1]3rd Department of Internal Medicine,
University Medical School of Debrecen, Hungary and
[2]2nd Institute of Medicine, Moscow, USSR

(Received: November 8, 1991)

Therapeutical plasma exchange can be carried out by using blood cell separators based upon centrifugation or in equipments with membranes. We investigated whether activation of the complement system took place in polyvinylchloride tubes used in the blood cell separator. We also examined the changes in the classical and alternative complement pathways by an analysis of functional haemolytic titers, as well as the levels of C4, C3D, C4, albumin and alpha 1-antitrypsin. No measurable activation of the complement system was found. The decreased levels of the C3, C3d, C4, albumin and alpha 1-antitrypsin in the sera after PE could be a consequence of the haemodilution.

Keywords: plasma exchange, complement activation, PVC tubes.

Introduction

In the last twenty years therapeutical plasma exchange (PE) has been used in the treatment of more than 90 diseases /6/. Two forms of PE are applied: (1) that based upon centrifugation using blood cell separator, (2) membrane plasmapheresis /7/ requiring membranes with larger pore size than used in haemodialysis.

The centrifugation method is more time-consuming, and more expensive, but safer than the membrane method. Using artificial membranes -- as it is well known in haemodialysis -- an activation of the complement system can

---

Abbreviations: AAT: alpha 1-antitrypsin; PE: plasma exchange; PVC: polyvinylchloride; C3, C3d, C4: complement proteins; CH_{50}, AP_{50}: 50% haemolytic titers in classical and alternative complement pathways; EDTA: ethylenediaminetetraacetic acid.

Offprint requests should be sent to I. Csípő, H-4004 Debrecen, Móricz Zs. u. 22., Hungary

Akadémiai Kiadó, Budapest

take place /5/. The polyvinylchloride (PVC) membranes were found to be weaker activators than the polysulfon and cellulose acetate membranes /11/.

The purpose of this study was to investigate whether an activation of the complement system takes place when PVC tubes are used in the blood cell separator. We examined the changes in both the classical and alternative complement pathways by an analysis of functional haemolytic titers. Besides, the serum levels of C3, C3d, C4, albumin and alpha 1-antitrypsin were determined.

Materials and methods

22 PE-s were carried out in five patients, in three with myasthenia gravis, one with systemic lupus erythematosus, and a pregnant woman with Rh incompatibility. PE was performed with Haemonetics Modell 30S Cell Separator. About one litre of plasma was removed during each treatment. The first blood sample was taken 10 min before, and the second, 10 min after the PE. Sera dispensed in small volumes were stored at minus 30 ^{o}C. (Repeated freezings and thawings were avoided.)

Functional haemolytic titrations for total classical and alternative complement pathway components were performed as described elsewhere /10, 12/.

Levels of C3, C3d, C4, albumin and alpha 1-antitrypsin were assayed by radial immunodiffusion. The slides were prepared with 1% agar in 0.02 M barbital buffer with 0.01 M EDTA at pH 8.4 /8, 9/.

Statistical analysis: We regarded every treatment as a separate case, so we compared the pre- and postcentrifugation results using the paired "t" test and calculating the correlation coefficients (r).

Results

Changes in serum albumin concentration during PE

About one litre of plasma (about 30% of the original total plasma volume) was taken from each patient during each PE. In order to control the real value of haemodilution, we chose the serum albumin levels as a marker. We found, on the average, 28% decrease in the level of albumin at the end of 22 PE-s (Table I).

Table I

Serum albumin levels before and after plasma exchange (g/l)

No. of samples	Before treatment Mean ± S.D.	After treatment Mean ± S.D.
22	39.1 ± 10.93	28.47 ± 8.42

Classical (CH_{50}) and alternative pathway (AP_{50}) titers, C3, C3d and C4 levels before and after PE

We found significant (about 30%) decreases in the titers of both classical ($P < 0.001$) and alternative ($P < 0.01$) complement pathways during PE (Fig. 1).

The decrease in the level of the early component of the classical pathway, C4, was 36%, and in the level of central component of both activation pathways, C3, was found to be 33% during PE (Fig. 2).

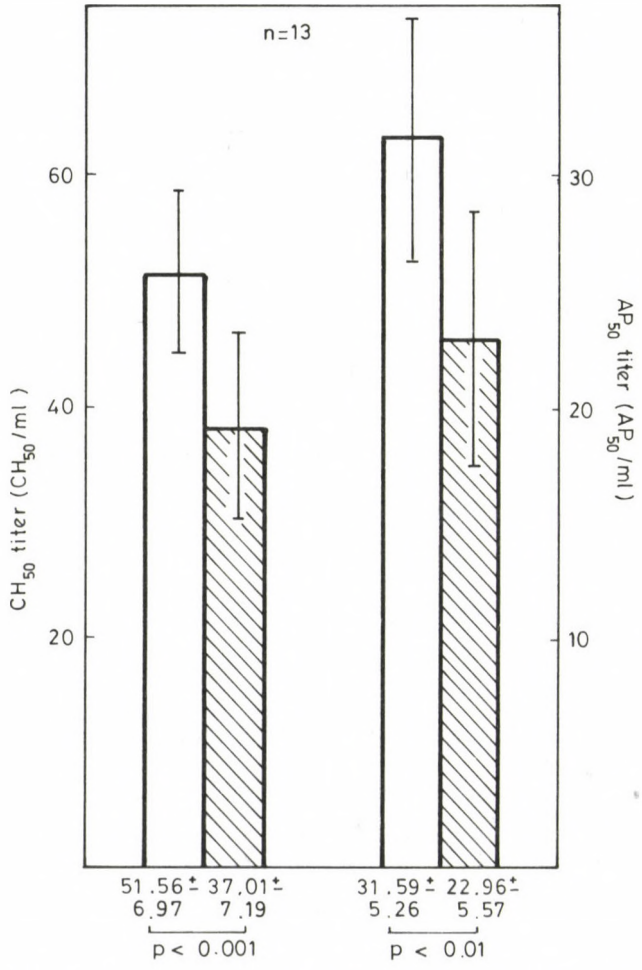


Fig. 1. Classical (CH_{50}) and alternative pathway (AP_{50}) complement titers (n = 13) before (□) and after (▨) PE (means ± S.D.)

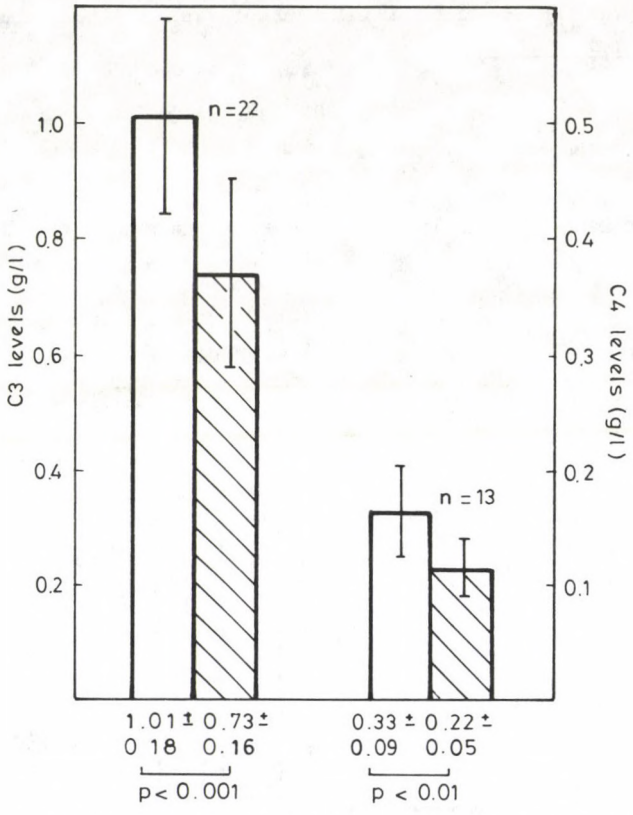


Fig. 2. C3 (n = 22) and C4 (n = 13) levels before (□) and after (▨) PE (means + S.D.)

The levels of C3d were also examined in 13 cases, and the decrease measured was about 29%. The values of C3d before and after PE were characterized by the diameter (d^2) of immunodiffusion precipitation rings (Table II).

In these 13 cases we also compared the CH_{50}, AP_{50}, C3 and C4 levels to the values of C3d by calculating the correlation coefficients (Table III).

Table II

Serum C3d levels before and after plasma exchange (mm^2)

No. of samples	Before treatment Mean ± S.D.	After treatment Mean ± S.D.
13	63.37 ± 21.37	45.05 ± 18.03

Table III

Comparison of correlation coefficients between C3d and CH_{50}, AP_{50}, C3, C4 levels before and after plasma exchange

	Correlation coefficient before PE	after PE
	C3d	C3d
C3	0.58	0.57
C4	0.70	0.56
CH_{50}	0.87	0.87
AP_{50}	0.59	0.87

Good correlations were found between the values of CH_{50}, AP_{50} and C3d before and after PE, suggesting that the applied therapeutical method did not result in detectable differences in the activation state of the complement system, because the decrease of CH_{50}, AP_{50} and C3d are about the same as the decrease of the albumin concentration (Table I).

Changes in alpha 1-antitrypsin levels during PE

The levels of acute-phase reactant globulin, alpha 1-antitrypsin (AAT), were measured before and after PE in 22 cases. The decrease in serum concentration of AAT was 36% ($P < 0.01$) at the end of PE (Table IV). This rate of decrease was in accordance with the value of haemodilution, suggesting that no acute phase-reaction had occurred during PE.

Table IV

Serum alpha 1-antitrypsin content before and after plasma exchange (g/l)

No. of events	Before treatment Mean ± S.D.	After treatment Mean ± S.D.
22	2.67 ± 0.84	1.69 ± 0.64

Discussion

The methods of extracorporeal circulation (haemodialysis, cardiopulmonary bypass, leukapheresis, plasmapheresis) were reviewed by Gardinali et al. /5/. They found some complement activation in all methods using artificial membranes. McLeod et al. /13/ compared the complement activating properties of some membranes. Polysulfon and cellulose acetate membranes activated complement more markedly than PVC did.

In this study our aim was to investigate the effects of PVC tubes used during the centrifugation PE method on the complement system and acute phase-reaction.

Blood samples were taken before and immediately after PE. During PE we did not take blood samples, for the plasma expander Gelifundol (gelatine) might have an effect on the complement system itself /4/.

According to Derksen et al. after an exchange of 1-2 litres of plasma, the serum concentration of individual plasma components (e.g. albumin) can show a reduction of 35% to 65% /3/. In our practice the exchange of about one litre of plasma caused a 28% decrease in serum albumin level (besides the original plasma volume corrected by Gelifundol).

We found that each of CH_{50}, AP_{50}, C4, C3, C3d and AAT decreased by the same rates (28 to 36%) during PE, suggesting that neither Gelifundol nor the PVC tubes can result in an effective and significant activation of the complement system or acute-phase reactions, for these rates of decrease do not an excess the value of haemodilution (28%). Namely, the decreases significantly greater than 28 to 36% would mean the signs of complement consumption, derived from the activation of the complement system.

Hypersensitivity reaction (dyspnoe, hypotension, sweating) was observed but rarely in our patients, whose symptoms could rather be attributed to the effect of ethylene-oxide used for sterilization of sets /2/ than to the PVC tubes.

In conclusion, our data support the view that the application of PVC tubes in blood processors is a rather safe and useful technique, causing no activation of the complement system, acute-phase reactions and/or the granulocytes /1/.

REFERENCES

1. Bedő, Z., Sipka, S., Csípő, I., Kormos, T., Lebedin, J. S., Shmuskovich, B. J., Chuchalin, A. G., Szegedi, Gy.: Leukocytosis induced by plasma exchange. Acta Med. Hung. 48, 219—224 (1991)

2. Bommer, J., Barth, H. P., Wilhelms, O. H., Schindele, H., Ritz, E.: Anaphylactoid reactions in dialysis patients: role of ethylene-oxide. Lancet 2, 1382—1385 (1983)

3. Derksen, R. H. W. M., Schuurman, H. J., Gmelig Meyling, F. H. J., Struyvenberg, A., Kater, L.: The efficacy of plasma exchange in the removal of plasma components. J. Lab. Clin. Med. 104, 346—354 (1984)

4. Füst, Gy., Medgyesi, G. A., Rajnavölgyi, É., Csécsi-Nagy, M., Czikora, K., Gergely, J.: Possible mechanism of the first step of the classical complement activation pathway: Binding and activation of C1. Immunology 35, 873—879 (1978)

5. Gardinali, M., Circardi, M., Agostoni, A., Hugli, T. E.: Complement activation in extracorporeal circulation: Physiological and pathological implications. Pathol. Immunopathol. Res. 5, 352—370 (1986)

6. Hambling, T. J.: An apheresis register. Lancet 2, 550—552 (1982)

7. Heal, J. M., Bailey, G., Helpingstine, C., Thiem, P. A., Leddy, J. P., Bucholcz, D. H., Nusbacher, J.: Non-centrifugal plasma collection using cross-flow membrane plasmapheresis. Vox. Sang. 44, 156—166 (1983)

8. Johnson, F. R., Agnello, V., Williams, R. C.: Opsonic activity in human serum deficient in C2. J. Immunol. 109, 141—145 (1972)

9. Mancini, G., Carbonara, A. O., Heremans, J. F.: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2, 235—239 (1965)

10. Mayer, M. M.: Complement and complement fixation. In: Kabat, E. A., Mayer, M. M. (Eds): Experimental Immunochemistry. Charles C. Thomas Publisher Springfield, 1961, pp. 133—240.

11. McLeod, B. C., Viernes, A., Sasetti, R. J.: Complement activation by plasma separation membrane. Transfusion 23, 143—147 (1983)

12. Platts-Mills, T. A. E., Ishizaka, K.: Activation of the alternate pathway of human complement by rabbit cells. J. Immunol. 113, 348—358 (1974)

Acta Medica Hungarica, Vol. 48/3—4, pp. 219—224 (1991)

LEUKOCYTOSIS INDUCED BY PLASMA EXCHANGE

Z. BEDŐ[1], S. SIPKA[1], I. CSÍPŐ[1], T. KORMOS[2], J. S. LEBEDIN[2], B. J. SHMUSHKOVICH[2], A. G. CHUCHALIN[2], GY. SZEGEDI[1]

[1]3rd Department of Internal Medicine, University Medical School of Debrecen, Hungary, and [2]2nd Institute of Medicine, Moscow, USSR

(Received: January 16, 1991)

A significant increase was found in the number of white blood cells, including neutrophils, in the peripheral blood of patients during plasma exchange. However, the spontaneous chemiluminescence of peripheral white blood cells (basically characterized the activated state of neutrophils) was not elevated. This finding suggests a relative deficiency in the metabolism of neutrophils during plasma exchange. This idea is supported by the observation that the levels of malonyldialdehyde in plasmas, after plasma exchange, do not increase, indicating that the activation dependent lipidperoxidation in neutrophils did not take place.

Keywords: plasma exchange, side effects, neutrophils, chemiluminescence.

Introduction

Therapeutic plasma exchange (PE) has become a routinely used procedure in the treatment of diseases caused by autoantibodies and/or immunocomplexes /10, 3/.

Beside its apparent advantages, little is known about side effects of PE. It is obvious that verification any of its side effects has great theoretical and practical importance because the patients suffering from autoimmune disease, for whom PE is indicated, are usually rather sensitive to infections.

Abbreviations: PE: plasma exchange, MDA: malonyldialdehyde, PBS: phosphate-buffered saline, TBA: thio-barbituric acid, WBC: white blood cell, CL: chemiluminescence.

Offprint requests should be sent to Z. Bedő, H-4004 Debrecen, Móricz Zs. u. 22., Hungary

Akadémiai Kiadó, Budapest

Therefore, in this study we compared the numbers and activation states of peripheral neutrophil granulocytes — playing crucial roles in the defence against infections — before and after PE. We found that PE was followed by leukocytosis, basically, an elevation in the number of neutrophils. At the same time, the spontaneous chemiluminescence (CL) of neutrophils did not increase in proportion with leukocytosis, suggesting a relative damage to these cells. The same tendency was observed by the measurement of the levels of malonyldialdehyde (MDA) in the plasmas of patients before and after PE.

Materials and methods

Patients: 25 PE-s were carried out in six patients (3 patients with systemic lupus erythematosus and 3 others with myasthenia gravis).

For the PE the Haemonetics 30 S blood cell processor was used. The approximately 30-40% of the original plasma volume was taken out during each treatment. Citrate was used as anticoagulant. Venous blood samples (heparinized, citrated and not anticoagulated) were taken 10 min before PE and 10 min after finishing PE. The whole PE procedure lasted 120 minutes in general. In each case both the quantitative and qualitative WBC count was determined.

Measurement of spontaneous chemiluminescence of peripheral white blood cells in whole blood: 1.0 ml of heparinized human whole blood was diluted to 3 times its original volume with phosphate-buffered saline (PBS). The CL intensity was measured in the presence of 10^{-6} mol/l of luminol (final concentration) by a Nuclear Chicago Isocap/300 liquid scintillation counter (Searl, Ind. USA), in the off coincidence mode. The total number of photons measured 3 times at 5-min intervals was used as a characteristic value for a given sample /12/. The value of the spontaneous CL of a single WBC was calculated by the index: CL cpm/number of WBC in 1 µl.

Determination of MDA levels in citrated plasma samples: lipid peroxidation was assayed by Ohkawa's method (10) with slight modifications. 0.20 ml citrated plasma was diluted by addition of 0.20 ml 8.1% SDS in distilled water. This solution was added to 1.50 ml of 20% acetic acid (pH 3.5) together with 1.50 ml 0.8% thiobarbituric acid (TBA). After incubation (95 °C, 60 min), the TBA reactive substance (MDA) was extracted by addition of 4.00 ml of n-butanol-pyridine (15:1) and measured in a fluorescence spectrophotometer (excitation: 532 nm; emission: 570 nm). The content of MDA was calculated from a standard curve obtained with MDA, and was expressed as nmoles MDA/ml serum.

Albumin levels were determined by radial immunodiffusion /9/.

The 25 PE-s in 6 patients were carried out at various periods of time and not at regular intervals. Because of the relatively long intervals (3-4 days) between PE-s, we thought it would be acceptable, from a statistical point of view, to regard every treatment as a separate case. Therefore, in the statistical analysis (using Student's *t*-test), we compared the laboratory values before and after PE.

Results

Changes in numbers of white blood cells, neutrophils and in chemiluminescence before and after PE

Figure 1 shows that there are significant elevations in numbers of both total WBC-s and neutrophils of peripheral blood, measured before and after PE in our cases (qualitative and quantitative cell counts) (Fig. 1).

At the same time, the spontaneous CL intensity of these cells (characterizing basically the metabolic activity of neutrophils in whole peripheral blood) (8) does not increase in parallel with the number of cells. The non-significant elevation shows or suggests a relative metabolic damage of neutrophils during PE (Fig. 2).

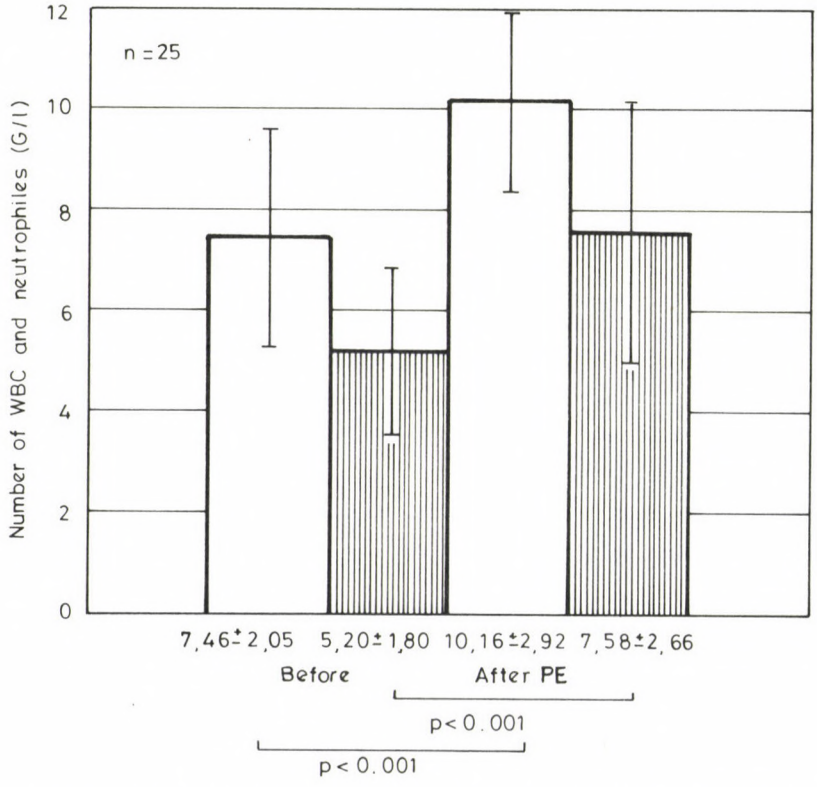


Fig. 1. Total WBC count (empty bars) and number of neutrophils (dotted bars) before and after PE (means ± S.D.)

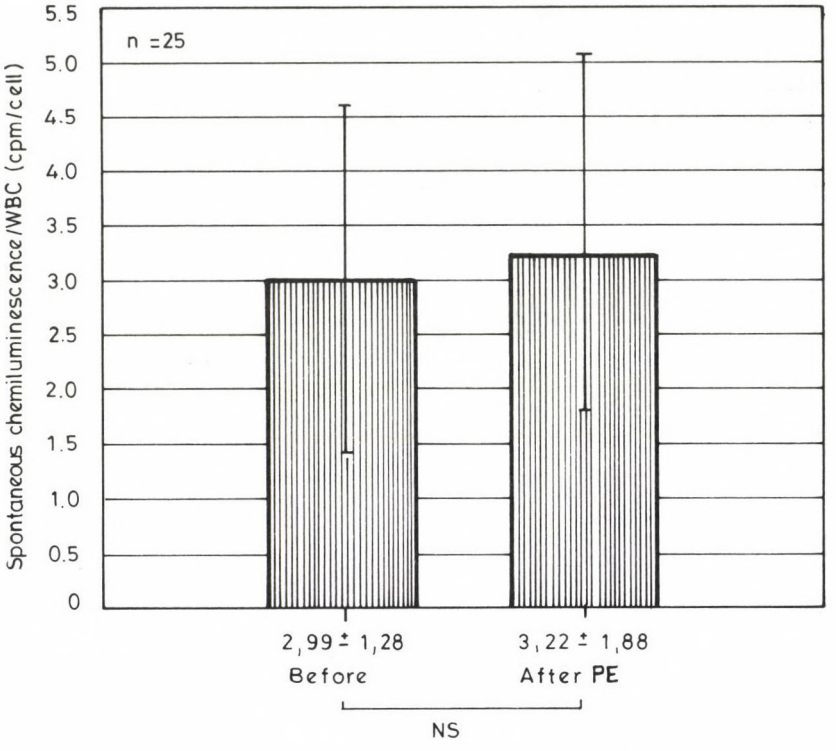


Fig. 2. Change of whole blood chemiluminescence before and after PE (means ± S.D.)

Change in MDA levels during plasma exchange

MDA is an endproduct of lipidperoxidation /1, 5/. Its extracellular quantity can also be an indicator of the intensity of energy dependent metabolic processes taking place in the cells /14/. In our cases, the plasma

Table I

Plasma malonyldialdehyde (nmol/ml) and albumin (g/l) levels before and after PE

Plasma components	Number of samples	Pre-PE values mean ± S.D.	Post-PE values mean ± S.D.
MDA (nmol/ml)	14	2.32	1.70
		0.95	0.79
Albumin (g/l)	22	39.11	28.47
		10.93	8.42

levels of MDA decreased after PE only as much as the dilution of plasma components took place. Namely, the lower MDA values after PE compared to the values before PE are in accordance with the changes in the plasma albumin concentrations, suggesting that an artificial dilution of proteins and other plasma components (like MDA) is taking place during PE (Table I). Our results show that PE does not cause a significant increase in the lipidperoxidation of blood cells (Table I).

Discussion

We tested anticoagulated blood samples (plasma and WBC) taken before and after PE. During the procedures we did not measure the samples because the gelatine molecules used in plasma expander solution Gelifundol influence the function of leukocytes. Our aim was to compare the metabolic state of WBC at the beginning and at the end of the procedures. Therefore, we measured the spontaneous CL of peripheral WBC /4/, in which more than 90% of the photons were emitted by the neutrophils /8/.

Since, the number of neutrophils in WBC (qualitative cell counts) changed in parallel with the total number of WBC (quantitative cell counts), and because 90% of the CL in a sample could come from the neutrophils, we regarded the values of CL as data representing quantitatively the metabolic activity of neutrophils in a sample.

The significant elevation in number of WBC (including neutrophils) can be explained in our results, supposedly, by the elevation of interleukin-1 levels, produced during a slight acute phase reaction induced PE /6/. However, we have to mention that clinical symptoms of a real acute phase reaction could be rarely observed. That means that the PE procedure — based upon Dideco sets — is clinically a well-tolerated medical treatment for patients.

In our system we have found a decreased spontaneous CL of peripheral neurophils calculated upon the increased number of WBC after PE. These phenomena can possibly be explained by the mechanical and osmotic stresses caused by the PE procedure /7/. At the same time, the lack of increase in plasma MDA levels also seems to exclude the possibility of a significant increase in the lipidperoxidation (elevated metabolism of peripheral WBC). Thus, our data do not support the idea that — like during hemodialysis /2, 13/ — a prolonged activation of neutrophils occurs.

REFERENCES

1. Babior, B. M., Kipnes, R. S., Curnutte, J. T.: Biological defence mechanisms: the production by leukocytes of superoxide, a potential bactericidal agent. J. Clin. Invest. 52, 741—744 (1973)

2. Bauer, H., Brunner, H., Franz, H. E., Bultmann, B.: Leukocyte function test during hemodialysis with different dialysis membrane. Contr. Nephrol. 36, Kargel, Basel, 1983, pp. 9—14.

3. Council Report: Current status of therapeutic plasmapheresis and related techniques. JAMA 253, 819—825 (1985)

4. Czirják, L., Dankó, K., Sipka, S., Zeher, M., Szegedi, Gy.: Polymorphonuclear neutrophil function in systemic sclerosis. Ann. Rheum. Dis. 46, 302—306 (1987)

5. Dahle, L. K., Hill, E. G., Hollmann, R. T.: The thiobarbituric acid reaction autooxidation of polyunsaturated fatty acid methyl esters. Arch. Biochem. Biophys. 98, 253—261 (1962)

6. Docci, D., Bilancioni, R., Baldrati, L., Capponcini, C., Tirci, F., Feletti, C.: Elevated acute phase reactants in hemodialysis patients. Clin. Nephrol. 34, 88—91 (1990)

7. Huestis, D. W.: Risk and safety practices in hemapheresis procedures. Arch. Pathol. Lab. Med. 113, 273—278 (1989)

8. Johnston, R. B., Lehmeyer, J. E., Guthrie, L. A.: Generation of superoxide anion on chemiluminescence by human monocytes during phagocytosis and on contact with surface-bound immunoglobulin G. J. Exp. Med. 143, 1551—1556 (1976)

9. Mancini, G., Carbonara, A. O., Heremans, J. F.: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2, 235—239 (1965)

10. Ohkawa, H., Ohishi, N., Yagi, K.: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351—358 (1979)

11. Shumak, K. H., Rock, G. A.: Therapeutic plasma exchange. N. Engl. J. Med. 310, 762—771 (1984)

12. Sipka, S., Szentmiklósi, A. J., Nagy, A., Taskov, V., Szegedi, Gy.: Inhibition of the zymosan-induced chemiluminescence of human phagocytes by adenosin, polyadenylic acid and agents influencing adenosin metabolism. Allergol. et Immunopathol. 17, 209—212 (1989)

13. Skubitz, K. M., Craddock, P. R.: Reversal of hemodialysis granulocytopenia and pulmonary leukostasis. J. Clin. Invest. 67, 1383—1391 (1981)

14. Stossel, T. P., Mason, R. J., Smith, A. L.: Lipid peroxidation by human blood phagocytes. J. Clin. Invest. 52, 638—645 (1974)

Acta Medica Hungarica, Vol. 48/3—4, pp. 225—229 (1991)

PROSTAGLANDIN E_2 IN RENAL TRANSPLANT RECIPIENTS

M. M. H. EL-SHARABASY, M. N. EL-NAGGAR

Chemistry Department, Faculty of Science, Mansoura University, Egypt

(Received: August 7, 1991)

Prostaglandin E_2 (PGE_2), sodium, potassium and creatinine were determined in the blood and urine of 50 renal transplant recipients treated for at least one year post transplantation with cyclosporine A or azathioprine as immunosuppressive agent. Fourteen healthy subjects were used as a control group. The urinary PGE_2 excretion was significantly decreased in the renal transplant recipients on azathioprine therapy while it was unchanged in the patients treated with cyclosporine A. At the same time, a significant decrease in urinary excretion of sodium and potassium was found. On the other hand, a high elevation of blood PGE_2 concentration was observed while no significant changes were seen in sodium and potassium in the blood of these renal transplant recipients. It is suggested that an association exists between urinary PGE_2 reduction and immunosuppressive treatments in renal transplant recipients and that PGE_2 may regulate intrarenal haemodynamics and influence renal tubular electrolyte excretion. Finally, urinary PGE_2 can be used as an indicator of successful renal transplantation.

Keywords: prostaglandin E_2, sodium, potassium, creatinine, renal transplant recipients, cyclosporine A and azathrioprine.

Introduction

Prostaglandin are synthesized in many tissues in minute amounts. They are highly active pharmacologically causing, e.g., contraction of smooth muscle — of the uterus among them — vasodilation and platelets aggregation /2/. In addition, prostaglandins are important intrarenal hormone involved in the regulation of renal blood flow, renal haemodynamics and water diuresis /3/. Since urinary prostaglandins E_2 (PGE_2) concentration was found in-

Offprint requests should be sent to M. M. H. El-Sharabasy, Chem. Dept., Faculty of Science, Mansoura University, Egypt

Akadémiai Kiadó, Budapest

creased 1 to 7 days before any changes in the conventional biochemical indicators of acute rejection, measurement of urinary PGE_2 may be a useful and early indicator of acute renal transplant rejection /9/. Furthermore, a high increase in urinary PGE_2 excretion, but no or minimal increase in the total excretion rates was observed in patients with acute oliguria renal failure immediately after the transplantation. In this respect, rejection crises were characterized by a two-fold increase in PGE_2 excretion with a subsequent fall induced by steroid treatment, and the PGE_2 excretion correlated closer with urinary sodium excretion than diuresis /6/. This prompted us to study the urinary excretion of PGE_2, sodium and potassium, as well as the concentration of these agents in the blood of renal transplant recipients under immunosuppressive therapy. Cyclosporine A or other conventional therapy, e.g. azathioprine was applied for at least one year post renal transplantation.

Table I

Mean values of PGE_2, sodium and potassium in urine of controls and of renal transplant recipients treated with immunosuppressive agents

Group	PGE_2 ng/g. creatinine	Sodium mEq/g. creatinine	Potassium mEq/g. creatinine
Healthy subjects			
Mean ± S.D.	6327 ± 2823	129.0 ± 60.2	32.0 ± 16.40
n =	13	14	14
Renal transplant recipients			
Mean ± S.D.	5294 ± 2324	90.5 ± 42.6*	18.0 ± 8.73**
n =	40	42	41
Cyclosporine A recipients			
Mean ± S.D.	6229 ± 3460	99.4 ± 38.9*	29.4 ± 17.50
n =	25	27	25
Conventional therapy recipients			
Mean ± S.D.	4720 ± 2163	95.2 ± 33.3*	21.4 ± 8.67*
n =	15	15	16

* = $P < 0.05$

** = $P < 0.001$

Patients and methods

This study was carried out on 50 adult patients, 32 males and 18 females, at least one year after successful renal transplantation at the Urology and Nephrology Center, Mansoura University, Egypt. Patients were divided into two groups according to the treatments immunosuppressive agent: 30 patients were treated with cyclosporine A and 20 patients with azathioprine. All patients received long-term immunosuppressive therapy. Fourteen healthy persons (8 males and 6 females) were used as a group for comparison. Blood and urine samples were taken from both patients and controls in a fasting state.

The radioimmunoassay method of Jaffa and Parker /5/, the atomic absorption spectrophotometric method of Willis /11/ and the method of Van Pilsum and Bovis /10/ was used for determination of PGE_2, sodium and potassium, and creatinine, respectively. Serum and urine samples were tested. The statistical analyses were performed according to Snedecor's method /8/.

Results

The mean value of the urinary PGE_2 excretion was significantly decreased ($P < 0.05$) in the renal transplant recipients as compared to the mean control value (Table I). This reduction was accompanied by a highly

Table II

Mean values of PGE_2, sodium, potassium and creatinine in the blood serum of healthy subjects and renal transplant recipients

Group	PGE_2 (ng%)	Sodium (mEq%)	Potassium (mEq%)	Creatinine (mg%)
Healthy subjects				
Mean ± S. D.	3150 ± 1767.6	146.9 ± 14.36	4.67 ± 0.71	0.52 ± 0.18
n =	12	14	14	13
Renal transplant recipients				
Mean ± S.D.	5808 ± 2446.7**	145.0 ± 10.78	4.27 ± 0.83	1.03 ± 0.65**
n =	25	28	28	29
Recipients on cyclosporine A therapy				
Mean ± S.D.	5489 ± 2914.0**	147.4 ± 11.94	4.32 ± 0.94	1.07 ± 0.58**
n =	14	16	15	15
Recipients on conventional therapy				
Mean ± S.D.	6327 ± 2363.7**	144.0 ± 8.08	4.42 ± 0.86	1.22 ± 0.61**
n =	11	17	17	15

* = $P < 0.05$

** = $P < 0.001$

significant decrease ($P < 0.001$) in the concentrations of sodium, potassium and creatinine in the urine of the same patients. At the same time, the concentration of PGE_2 was significantly increased ($P < 0.05$) in the blood of renal transplant recipients, rather than the corresponding mean value of controls. Blood creatinine was also increased, but within the normal range (Table II). Moreover, no significant changes were observed in the blood concentrations of sodium and potassium (Table II).

The results obtained from the two groups of renal transplant recipients were similar to each other (Tables I and II).

Discussion

The renal prostaglandin system has proved to participate in some renal disorders, we investigated the behaviour of PGE_2 and its influence on sodium and potassium levels in the urine and blood of renal transplant recipients treated with immunosuppressive agents. The urinary PGE_2 excretion was decreased in all of our renal transplant recipients and its decrease was accompanied by a marked decrease in urinary excretion of sodium and potassium. These findings can be explained by assuming that prostaglandins are important intrarenal hormones concerned with the regulation of renal blood flow and electrolyte excretion and that this is so not only in some kidney disorders but also in renal transplant recipients treated with cyclosporine A or being on some other conventional therapy /2/.These assumptions are in agreement with other previous findings, viz., that in patients with hypercalciuria, prostaglandin synthetase inhibition with indomethacin is followed by a marked reduction of calcium and sodium. These observations are related to an evidence that renal prostaglandin activity is an important factor in the mechanism of hypercalciuria /1/.

Haylor and Lote /4/ provided an evidence for a direct tubular action of endogenous prostaglandins on distal-nephron sodium excretion. There is also good circumstantial evidence to suggest that prostaglandins may influence calcium reabsorption by the kidney and prostaglandins have been shown to alter water, sodium and calcium transport across frog skin /7/. In our immunosuppressived recipients the reduction of the urinary PGE_2 excretion followed a marked decrease in urinary sodium and potassium excretion may determine the renal handling of sodium and potassium by controlling the tubular function; furthermore, the reduction in PGE_2 excretion was attribut-

ed to an impaired kidney function. This is supported by the significant elevation of blood creatinine concentration. Besides, the elevation of PGE_2 in the blood of renal transplant recipients can be attributed to the kidney dysfunction which enables secretion of this prostaglandin in the urine; moreover, this elevation of PGE_2 mainly due to the possible activation of monocytes by immunosuppressive agents used as treatment for renal transplant rejection.

REFERENCES

1. Buck, A. C., Sampson, W. F., Lote, C. J., Blacklock, N. J.: The influence of renal prostaglandins on glomerular filtration rate (GFR) and calcium excretion in urolithiasis. J. Urology, 53, 485—491 (1981)
2. Campbell, P. N., Smith, A. D.: "Biochemistry illustrated". Churchill, Livingstone, New York, 1982, pp. 206—208
3. Frolich, J. C., Rosenkranz, B., Kitajima, W., Fisher, C., Walker, L.: Prostaglandin as a renal hormone. In: Clinical Pharmacology of prostacyclin, ed. Lewis, P. J., O'Grady, J., Ch. 8, 1981, pp. 53—58.
4. Haylor, J., Lote, C. J.: Renal function in conscions rats after indomethacin. Evidence for a tubular action of endogenous prostaglandin. J. Physiology, 298, 371—381 (1980)
5. Jaffa, B. M., Parker, C. W.: Radioimmunoassay for prostaglandins. Radioassay Clin. Med., 33—48 (1974)
6. Ladefoged, J.: Urinary prostaglandin E_2 excretion in renal allotransplantation in man. Prostaglandins, 34, 611—618 (1987)
7. Lote, C. J., Rider, J. B., Thomas, S.: The effect of prostaglandin E_2 on the short circuit current and sodium, potassium, chloride and calcium movements across isolated frog (Rana temporaria) skin. Pflüger Archiv für die gesamte Physiologie des Menschen und der Tier, 352, 145—153 (1974)
8. Snedecor, G. W.: Statistical Methods applied to Experiments in Agriculture and Biology. 5th ed., Ames. College Press, 1956, p. 132.
9. Thompson, D., Rowe, D. J., Briggs, J. D.: Measurement of urine prostaglandin E_2 as a predictor of acute renal transplant rejection: Preliminary findings. Ann. Clin. biochem., 22, 161—165 (1985)
10. Van Pilsum, J. F., Bovis, M.: Effects of various protein precipitants on recoveries of creatinine added to plasma. Clin. Chem., 3, 90—95 (1957)
11. Willis, J. B.: Analysis of biochemical material by atomic absorption spectrophotometry. In: Methods of Biochemical Analysis (ed. Glick, D.), Interscience Publishers, New York, 1963, pp. 1—67.

Acta Medica Hungarica, Vol. 48/3—4, pp. 231—235 (1991)

BOOK REVIEWS

ACTIVATION TO ACQUISITION: Functional Aspects of the Basal Forebrain Cholinergic System

Edited by Russell T. Richardson

(Birkhäuser, Boston 1991) Price: 168 Swiss Francs

The chapters of this book — written by leading investigators of the field — provide an excellent review of the current knowledge and hypotheses about the role of the basal forebrain cholinergic system (BFCS). Contributions from most disciplines of the neurosciences can be found in the book, which allow the reader to gain a broad understanding of the anatomy, physiology and behavioural implications of this system.

The functional importance of the cholinergic basal forebrain neurons began to be recognized in the mid-1970s, when these neurons were discovered to provide a diffuse innervation of the entire cortical mantle. Later they were shown i) to represent the last link in the ascending reticular activating system of Moruzzi and Magoun (1949), ii) to have a powerful influence on the plastic properties of cortical neurons during development as well as in the adult (learning and memory), and iii) to play a crucial role in the pathogenesis of Alzheimers' disease. Due to these fundamental functional and pathophysiological implications, the BFCS has become a focus of research in many laboratories over the past decade. This book represents the most complete account to date of the huge body of information accumulated during this period about the BFCS.

In the first chapter the editor summarizes the aims of the book, and presents the question which had to be addressed by all contributors: "Based on your critical evaluation of relevant studies, what is the most likely function of the basal forebrain cholinergic system?". A brief but comprehensive summary is given about the several functional processes suggested to involve the BFCS. The proposed hypotheses are not mutually exclusive, in almost all interpretations acetylcholine (ACh) is considered a neuromodulator. The view which appears to emerge from all chapters is that the cholinergic system is altering the functional properties of other brain structures over time (from transient to permanent effects), which may involve selected target neurons, selected target areas, or even the entire cerebral cortex.

The following three chapters describe the classical and chemical neuroanatomy of the basal forebrain with particular emphasis on the functional implications of characteristic structural features. A chapter by Koliatsos and Price gives a historical overview, and summarizes our present knowledge of the afferent and efferent connections, local circuits, neurotransmitters, and nerve growth factor (NGF) receptor content of the BFCS. In the next, rather short chapter Mesulam reviews recent work on the topographical organization of the cholinergic basal forebrain-neocortex projection in primates, and describes the specific behavioural and neurophysiological aspects of this organization. More detailed anatomy, based largely on tracing studies, is provided by Price and Carnes, who conclude the chapter by speculating about the functional implications of the specific input-output relationships of BFCS neurons.

The next four chapters are based primarily on electrophysiological studies of the role of the BFCS in cortical activation, learning and memory. The activity state of the cerebral cortex is either aroused (open-loop, or

Akadémiai Kiadó, Budapest

interactive state), or non-activated (closed-loop, or non-interactive state). What is the biological significance of this dichotomy of states? Why must the cereoral cortex be aroused to interact with the environment? "Why is the brain not hard-wired exclusively for the information-processing mode as seen in computers? What are the neurophysiological mechanisms involved in the switching between the diametrically opposite functional states?" In their chapter Buzsáki and Gage attempt to answer the last question, and at least to discuss the other basic neurobiological questions through their own studies on the BFCS. They hypothesize that the basal forebrain cholinergic neurons play a crucial role in cortical arousal via a dual mechanism: i) they enhance the efficacy of specific thalamic afferents to drive their cortical target neurons (direct cortical effect), and ii) they suppress the rhythmic oscillations of the thalamic "pacemaker" neurons by their axons innervating the reticular nucleus of the thalamus, thus, allowing the relay of specific information to the neocortex. The possible role of BFCS neurons in learning and memory is analysed in a chapter by Richardson and DeLong, who review their studies on the firing properties of single basal forebrain neurons during different behavioural paradigms, and describe a model how cholinergic neurons may facilitate associative learning. The relations of conditioning, different forms of long-term potentiation (LTP), and cholinergic facilitation of glutamatergic transmission are summarized in a chapter by Gorman and Woody. The cholinergic modification of intracellular responses and excitability, and its ultimate effect on receptive field plasticity in a behavioural state-dependent manner is the subject of a chapter by Ashe and Weinberger, who present a hypothetical model of "adaptive information processing" for the auditory cortex.

Learning and memory are the focus of the two subsequent chapters (by Olton et al. and Kesner and Johnson), but from more of a behavioural viewpoint in which the relations of the BFCS to other brain structures and their functions is analysed.

In the subsequent, most fascinating, two chapters the role of the cholinergic afferents in the regulation of sensory receptive fields is reviewed. Kasamatsu and Imamura emphasise the necessity of an integrated cholinergic noradrenergic action in the regulation of visual cortical plasticity during development (e.g. the formation of ocular dominance columns). In their chapter Dykes et al. summarize their work on long lasting enhancement of cortical responses evoked by sensory stimuli. They conclude that cortically released ACh facilitates synaptic modification, and that the mechanisms of cortical plasticity are fundamentally similar in all areas of sensory neocortex at all postnatal ages.

In the last chapter by Woolf and Butcher a highly speculative model is presented addressing "the basic computation style that cholinergic basal forebrain cells could use in order to perform cognitive operations". The cholinergic neurons are suggested to be involved in cognition by altering the anatomical connections among different groups of neurons representing specific bits of information.

This book consists of thorough reviews of anatomical, electrophysiological and behavioural data on the BFCS, and, in addition, the author of all chapters share their personal views and hypotheses about the possible functions of this system. According to John Platt, strong inference is the most effective method for making progress in science, since it results in the formulation of multiple, testable hypotheses. The book contains a most fascinating collection of such hypotheses. It is highly recommended to neuroscientists involved in studies of the BFCS, the cerebral cortex, learning and memory, and cognitive processes.

TAMÁS FREUND

Adrenoreceptors: structure, mechanisms, function

(Edited by E. Szabadi and C. M. Bradshaw,

Birkhäuser Verlag, Basel--Boston--Berlin)

408 pages. Price: 98 Swiss Francs

The material of this volume was presented at the Symposium on the pharmacology of adrenoreceptors (June 1990, University of Manchester), the third of the symposia organized as a satellite of a Word Congress of Pharmacology (IUPHAR Congress in Amsterdam, 1990.). It reflects the immense development in this field of science during the four-year period that has elapsed since the preceding symposium.

The structure of adrenoreceptors has been revealed in the last 5 to 6 years, and our view has been greatly modified due to the new knowledge. The existence of further subtypes of the $alpha_1$, $alpha_2$, $beta_1$ and $beta_2$ adrenoreceptors, a question much debated before, has been proved. The diversity of the receptors has been suggested by new data obtained with the classical techniques on the one hand, and numerous subtypes were identified by means of methods of molecular biology on the other. Newly-discovered agonists and antagonists have contributed to the recognition of the exact /physiological role of the receptor types (and subtypes)/.

The newly-described subtypes of receptors suggest that soon drugs were selective than those available today will be developed for use in therapy.

Much has been recognized of the molecular mechanism activated by adrenergic receptors. For beta and $alpha_2$ receptors various elements of the G protein family (G_s and G_i, respectively) play the mediator role, whereas for $alpha_1$ receptors the phosphoinozitol pathway which is activated via phospholipase C is involved in the usual biochemical mechanism.

As to the adrenergic receptors in the cardiovascular system, it should be taken into account that the receptors of various vascular areas may behave differently, and that their diversity is a general rule.

Changes in adrenergic receptors may play an important role in some clinical conditions. It has been shown that a lasting rise in the plasma catecholamine level is associated with a decrease in number of beta adrenoreceptors in the cardiovascular system. In contrast, an increase in the sensitivity of alpha and beta receptors should be reckoned with when the autonomous regulation involves a lasting decrease in the plasma catecholamine level.

This book should be read by those who are dealing with the pharmacology and/or physiology of adrenoreceptors or meet these receptors in the course of their research work.

G. B. MAKARA

New Perspectives in Histamine Research

(Ed. H. Timmerman, H. van der Groot).

Agents and Actions Supplements, vol. 33. A satellite symposium of the XIth International Congress of Pharmacology of IUPHAR, July 6–8, 1990, Noordwijkerhout, the Netherlands. Birkhäuser Verlag, Basel, Boston, Berlin, 1991. 434 pages. Price: 128 Swiss Francs

There is a long history of histamine research, still this field of research is always full of exciting new findings. Histamine has been known as a neurotransmitter, inflammatory mediator and a factor in anaphylaxis, as well as in cardiac and gastrointestinal functions. In addition to these, some new roles of histamine were presented at this Symposium, e.g. its role as an immune modulatory autacoid or as a mediator of the mitogenic response. The book contains 34 papers presented at the Symposium dealing with nearly all aspects of the role of histamine in the regulation of physiological processes.

Japanese authors (Wada et al., Osaka, Japan) have identified the histaminergic neuron system in rat brain by immunocytochemical techniques using antibodies against histidine decarboxylase or histamine itself. The histaminergic neurons are located exclusively in the tuberomamillary nucleus in the posterior basal hypothalamus and send their varicose fibres to almost all regions of the brain.

Histamine has a stimulatory, but indirect, effect on the release of pituitary derived pro-opiomelanocortin peptides: ACTH, β-endorphin, α-melanocyte stimulating hormone (Knigge et al., Copenhagen, Denmark) that occurs by stimulation of H_1 and H_2 receptors and seems to be mediated via release of corticotropin-releasing hormone.

One of the newest findings was presented by the researchers of the Unité de Neurobiologie et Pharmacologie, INSERM, Paris (Arrang et al.), who identifying among a series of methylated histamine derivatives (R)α, (S)β,-dimethyl histamine as a novel potent and selective H_3 receptor agonist. The drug seems to display a very low animal toxicity, it might find various clinical applications. The same group (Ruat et al.) designed the first ^{125}I-labelled probes specific for the H_1 and H_2 receptors. The reversible ^{125}I-probes allowed them to extend the pharmacology of these receptors in several biological preparations and to establish their interaction with G protein.

Barnes P. (London, U.K.) summarized the role of different histamine receptors in mediating allergic airway disease.

The association of histamine receptors with the intracellular second messenger systems is discussed by S. J. Hill (Nottingham, U.K.). H_2 receptors are coupled via a G_S regulatory protein to adenylate cyclase and stimulate cyclic AMP formation, while H_1 receptors mediate many of their effects via the products of inositol phospholipid hydrolysis. It is becoming clear that there is a substantial "cross-talk" between these intracellular second messenger systems in a number of tissues.

The problem of a preoperative histamine H_1 + H_2 prophylaxis was tackled by Lorenz et al. (Marburg, Germany) including randomized controlled clinical trials and cross-sectioned studies with plasma histamine measurements and administration of H_1 + H_2 antagonist.

The use of histamine H_2-agonists seems to be a new promising approach to the treatment of congestive heart failure. Buschauer and Baumann (Berlin and München, Germany) developed a potent H_2 agonist, a guanidine derivative having a potent positive inotrope effect.

Brandes et al. (Winnipeg, Canada) provided evidence that the histamine mobilized from cytoplasmic stores is a mediator of the mitogenic response to concavalin A in mouse spleen cells.

Khan and Melmon (Stanford, CA, USA) summarized our knowledge of the function of histamine and its congener derivatives as immune modulators. The receptors for histamine are non-randomly distributed on lymphocytes. Suppressor T cells can control the expression of histamine receptors on helper and cytolytic T cells. Histamine regulates lymphokine release and vice versa histamine release is influenced by lymphokines.

The book is highly recommended for pharmacologists and biochemists involved in monoamine studies.

KATALIN SZ. SZALAY

CONGRESSES

THE ELEVENTH ANNUAL SCIENTIFIC MEETING AND EXHIBITION of the SOCIETY OF MAGNETIC RESONANCE IN MEDICINE
will be held August 8—14, 1992, in Berlin, Germany.

For more information, contact SMRM, 1918 University Avenue, Suite 3C, Berkeley, CA 94704, USA.
Telephone: (510) 841-1899. FAX: (510) 841-2340

WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER
PATHOLOGY OF CANCER FOR NON-PATHOLOGISTS
— AN INTERNATIONAL COURSE—
Oslo, 15—19 June 1992

Further information and application forms may be obtained from:
The Unit of Education and Training
International Agency for Research on Cancer
150, cours Albert Thomas
F-69372 Lyon Cedex 08

PRACTICAL BODY IMAGING:
US, CT and MRI in the 1990's
Marriott's Tenaya Lodge at Yosemite, CA
May 18—22, 1992

For further information, please contact:
Radiology Postgraduate Education
Room C-324
University of California School of Medicine
San Francisco, California 94143-0628

INTERVENTIONAL HEPATOBILIARY RADIOLOGY:
PERCUTANEOUS PORTACAVAL SHUNTS, BILIARY STENTS, TUMOR CHEMOEMBOLIZATION, LITHOTRIPSY
Ritz-Carlton, San Francisco, California
June 4—6, 1992

For further information, please contact:
Radiology Postgraduate Education
Room C-324
University of California School of Medicine
San Francisco, California 94143-0628

Akadémiai Kiadó, Budapest

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat

INFORMATION FOR AUTHORS

Acta Medica Hungarica is published under the auspices of the Hungarian Academy of Sciences. Manuscripts and editorial correspondence should be sent to the editorial office: H-1450 Budapest 9, P.O. Box 67.

Original articles dealing with clinical and experimental medicine will be accepted with the understanding that they have not been and will not be published elsewhere and are subject to editorial revision.

Form of manuscripts

Two copies of the manuscript typewritten double-spaced with margins at least 4 cm wide should be submitted. Pages should be numbered consecutively. The first page should contain (1) the title of the paper (2) the initials and first name(s) of the author(s), (3) name of the institution where the work was done, (4) name and address of the author to whom correspondence and offprint requests should be addressed — this will appear as a footnote; (5) an abstract not exceeding 250 words which states the purposes of the study, the main findings and principal conclusions. Below the abstract provide 3 to 10 keywords that will assist indexers in cross-indexing the article.

The text of the paper should be divided into sections with the headings: Introduction, Materials (Patients) and Methods, Results, Discussion, References.

Unusual abbreviations should be identified in an alphabetical list typed after the abstract and keywords.

Drugs must be referred to by their WHO code designation (Recommended International Nonproprietary Names); use of proprietary names is unacceptable.

The international system of units (SI) should be used for all measurements.

References

These should be cited in the text as numbers in square brackets. The list of references should contain in alphabetical order of the first authors' names the following: authors' last names with initials; for journal articles the title of the paper (lower case), journal title abbreviated according to the style used in Index Medicus, volume number, inclusive page numbers, year of publication in parentheses; for books the title (upper and lower case), publisher, place and date of publication. Only manuscripts accepted for publication may be included in the reference list.

Examples:

1. Stagg, B. H., Temperly, J. M., Wyllie, J. H.: The fate of pentagastrin. Gut **12**, 825—829 (1971)
2. Falkner, F.: Prevention in Childhood of Health Problems and Adult Life. WHO, Geneva 1980
3. Fishman, A. P.: Dynamics of pulmonary circulation. In: Hamilton, W. F., Dow, P. (eds): Handbook of Physiology. American Physiological Society, Washington 1963, pp. 65—79

Tables

Each table should be typed on a separate sheet. They should be numbered consecutively with Roman numerals and have a brief specific title. The data presented in the table must be logically and clearly organized and should be self-explanatory. Omit internal horizontal and vertical rules. Cite each table in the text and indicate its approximate place on the margin.

Illustrations

Figures should be submitted in duplicate. They must be numbered consecutively with arabic numerals. All figures should bear the name of the first author, the figure number and an arrow indicating the top. Cite each figure in the text and indicate its approximate place on the margin. If a figure has been published, acknowledge the original source and submit written permission from the copyright holder to reproduce the material. Figure captions should be submitted typed double-spaced on a separate sheet.

Proofs and reprints

The first authors will receive (1) comments and suggestions of the Editorial Board for improving their paper; (2) a set of proofs for correction; corrected proofs should be returned without delay to the editorial office; (3) 50 reprints free of charge.

Index: 26.015