Blood Serum Content of PAMG-1 Protein Binding Insulinlike Growth Factor 1 (Somatomedin C) in Patients with Diabetes Mellitus

S. V. Nazimova, S. S. Obernikhin, M. N. Boltovskaya, N. A. Starosvetskaya, E. I. Zaraiskii, and B. B. Fuks

UDC 616.379-008.64-07:616.153.96]-092

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 116, № 9, pp. 302-304, September, 1993
Original article submitted April 29, 1993

Key Words: *PAMG-1; PP12; IGF-BP-1; diabetes*

Many reports showing an important role of insulinlike growth foactor (IGF-1) in pancreatic secretory function reguation have appeared of late. In physiological concentrations this factor has been found to suppress insulin secretion. Administration of recombinant IGF-1 to humans and animals drastically reduced plasma insulin content [7]. In contrast to many peptide hormones, insulin included, IGF-1 is found in native plasma not in a free form but in a complex with binding proteins (BP) biffering in their functions, relation to growth hormone and insulin, as well as in molecular weight. These complexes determine IGF-1 stability, activity, and availability for target cells [4]. IGF-BP-1 os one of the IGF-BP family members; previously it was identified and investigated as placental protein 12, (PP12) [6], immunochemically identical to placenta-specific α_1-microglobulin (PAMG-1) [3]. IGF-BP-1 (PP12, PAMG-1) is produced mainly by the liver but also by ovarian granulous cells [5], endometrium in the secretory phase, minor IGF-BP of the amniotic fluid, in which its content can constitute as much as 10% of the total protein [6,12]. In contrast to other binding protein IGFs, PP12 (PAMG-1) inhibits IGF-1 binding to receptors and mitogenic activity [14]. Insulin is the principal regulator of IGF-BP-1 production in the liver [4,15]. Insulin has been shown to reduce the synthesis of this factor by suppressing its gene transcription [12]. Hence, three proteins, insulin, IGF-1, and IGF-BP-1, are closely related through mutual reguation of synthesis and functional activity. At present the role of IGF-BP-1 in regulating of carbohydrate metabolism in the body is still unknown. Some authorities report an increase of the IGF-BP-1 blood serum level in diabetes [16], diabetic nephropathy [8], and pregnancy complicated by diabetes [17]. The IGF-BP-1 level in these investigations was assessed either qualitatively (by the ligand blooting method) or quantitatively, using polyclonal antibodies, and once, rather than over time.

The present study involved repeated measurements of PAMG-1 (IGF-BP) in diabetics using monoclonal antibodies to two different PAMG epitopes.

MATERIALS AND METHODS

At first PAMG-1 concentrations were measured once in 11 diabetics whose case histories had not been thoroughly analysed. The blood serun PAMG-1 levels were then measured over time (at least three times at 2-week intervals) in 31 patients with

Research Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow . (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0009-1143$12.50

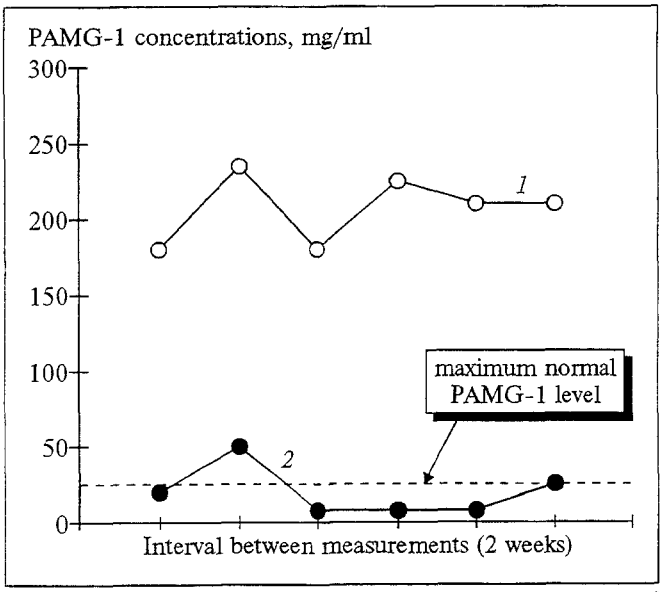

Fig. 1. Individual curves characterizing time course of PAMG-1 in insulin-dependent (*1*) and non-insulin-dependent (*2*) diabetes. Abscissa: interval between measurements (2 weeks); ordinate: PAMG-1 concentrations, mg/ml; horizontal broken line: maximum normal PAMG-1 level.

diabetes mellitus. Eleven of these patients (9 men and 2 women aged 18 to 42) presented with type I diabetes, or insulin-dependent mellitus (IDDM), and 20 (6 men and 14 women 32 to 67) with type II, or non-insulin-dependent diabetes mellitus (NIDDM). The disease duration was five to fifteen years. The control group consisted of 48 healthy nonpregnant women and 56 healthy men. In addition, 85 patients with various somatic diseases were examined, 4 with essential hypertension, 21 coronary parients, 10 patients with acute and chronic cholecystitis, 3 with acute and chronic pneumonia, 8 with urolithiasis, 4 with hepatitis, 2 with pyelonephritis, 7 with chronioc renal insufficiency, and 26 with other disorders such as reumatic fever, asthma, or injuries. Blood serum aliquots were stored at -20°C and tested three times. No repeated freezing of the samples was permitted.

Serum PAMG-1 levels were measured by sadnwich enzyme immunoassay, as described previously [1], using monoclonal antibodies to two different epitopes of PAMG-1 molecule obtained earlier. Test system sensitivity for PAMG-1 measurements was 3 ng/ml, the variability coefficient in a series of samples being 3.2%. Enzume immunoassay results were assessed using Multiscan Titertek (Flow, GB). Calibration curves for estimation of the PAMG-1 concentration were plotted of PLM paper.

Hybridomata producing monoclonal antibodies to PAMG-1 were Preparated by a standard method by fusing splenocytes of BALB/C mice immunized by 3-4-fold intraperitoneal injections of 100 μg purified PAMG-1 in normal saline and myeloma SP 2/0 [1].

RESULTS

The serum PAMG-1 level of healthy nonpregnant women varied from 0 to 20 ng/ml, that of healthy men from 0 to 25 ng/ml. The serum PAMG-1 concentrations in all somatic disease patients did not surpass the levels in normal subjects exept for two patients, one with chronic hepatitis, in whom the protein level was 100 ng/ml and the oher with cholecystitis, with protein level of 36 ng/ml. Preliminary single measurements in 11 diabetics revealed a noticeable increase of serum PAMG-1 in 83.3% of patients. The results of repeated measurements of serum PAMG-1 levels in the 31 diabetics are presented in Table 1. One can see that the protein level surpassed the normal values in all patients with insulin-dependent diabetes, being ten times higher or more in 54% of cases. In patients with non-insulin-dependent diabetes the PAMG-1 level surpassed the normal value in 80% of cases, in 63% the concentration of this protein surpassing the norm by only 1.5-2 times. Note that in the insulin-dependent condition the {AMG-1 concentration showed a stable tendency to increase during all measurements without noticeable fluctuations in protein level, whereas in non-insulin-dependent disease the PAMG-1 levels varied within a wide range and sometimes dropped to normal values. Figure 1 shows the most characteristic curves reflecting the individual time course of serum PAMG-1 levels in insulin-dependent and non-insulin-dependent diabetes, the intervals between measurements being rwo weeks. No relation-

TABLE 1. Serum PAMG-1 Levels in Diabetes Mellitus

Type of diabetes	Number of patients	Increased PAMG level			Normal PAMG-1 level, 0-25 ng/ml	Increased PAMG-1 level in group (% of patients)
		30-50 ng/ml	50-100 ng/ml	100 ng/ml		
Insulin-dependent (I)	11	3 (27%)	2 (19%)	6 (54%)	0	100
Non-insulin-dependent (II)	20	10 (63%)	3 (18.5%)	3 (18.5%)	4 (20%)	80

ship between the PAMG-1 level and patient sex or age was detected in patients with either types of diabetes. Blood serum PAMG-1 levels varied withing a wide range in diabetics with both types of the disease: 10-230 ng/ml in type I and 0-360 mg/ml in type II.

Suikkari *et al.* [16 used polyclonal antibodies in PAMG-1 (PP12) testing. Their results were similar in many respocts to our findings: a marked rise of the PP12 level in insulin-dependent diabetes and a less noticeable but reliable increase on this protein level in non-insulin-dependent diabetes. We used a test system with monoclonal antibodies, which may be responsible for the differences in the absolute values characterizing serum PAMG-1 in our research and Suikkari's study. The significant increase of the serum PAMG-1 concentration in insulin-dependent diabetes may be due to the fact that it is insulin which regulates PAMG-1 (IGF-BP-1) production and serum content [4]. Insulin has been shown to suppress IGF-BP-1 gene transctiption in HEPG 2 cells [12] acting on the promoter of this gene. Ooi et al. [10] revealed an increased IGF-BP-1 mRNA content in the liver of rats with experimental diabetes and showed that an insulin injection reduced its level. The less pronounced increase of the IGF-BP-1 content in non-insulin-dependent diabetes may be due to a not so marked insulin deficiency and to the fact that the inhibitory effect of insulin on IGF-BP-1 production in cells (e.g., in hepatocytes) is not realized in full measure because of defective reception of insulin by the cell [9].

We observed a serum PAMG-1 increase in 80% of patients with type II diabetes. The diagnosis, particularly an early one, of this form of diebetes is rather dificult [13]. The usefulness of the enzyme immunoasssay of PAMG-1 for the early diagnosis of non-insulin-dependent diabetes melitus is still being debated.

The poblem of the pathogenetic role of the PAMG-1 concentration increase in diabetes, particularly so in pregnant patients, is an important one, for this protein is an inhibitor of the mitogenetic and growth function of IGF-1. This problem lies outside the scope of the present study; we would only mention that some authorities have demonstrated a potent effect of IGF-BP-1 inhibitors on embryonal growth and nervous system development [2].

REFERENCES

1. M. N. Boltovskaya, E. I. Zaraiskii, B. B. Fuks, *et al., Byull. Eksp. Biol. Med.*, № 10, 397-400 (1991).
2. W. Balkan, R. P. Rooman, A. Hurst-Evans, *et al., Teratology*, **38**, 79-86 (1988).
3. H. Bohm and W. Kraus, *Arch. Gynec.*, **229**, 279-291 (1980).
4. C. A. Conover, P. D. K. Lee, J. A. Kanalev, *et al., J. Clin. Endocr.*, **74**, № 6, 1355-1360 (1992).
5. M. Julkunen, R. Koistinen, K. Aalto-Setala, *et al., FEBS Lett.*, **236**, 295-302 (1988).
6. R. Koistinen, N. Kalkkinen, M. L. Huhtala, *et al., Endocrinology*, **118**, № 4, 1375-1378 (1986).
7. J. L. Leahy and K. M. Vandekerhove, *Ibid*, **126**, № 3, 1593-1598 (1990).
8. P. D. K. Lee, R. L. Hintz, J. B. Sperry, *et al., Pediat. Res.*, № 4, 309-315 (1989).
9. D. Muller-Wieland and W. Krone, *Diabet. Croat.*, **20**, № 1, 7-13 (1991).
10. G. T. Ooi, C. G. Orlowski, A. L. Brown, *et al., Molec. Endocri.*, **4**, 321-328 (1990).
11. G. Povoa, G. Enberg, H. Jornvall, *et al., Europ. J. Biochem.*, **144**, 199-204 (1984).
12. D. R. Powell, A. Suwanichkul, M. Z. Cubbage, *et al., J. Clin. Biol. Chem.*, **266**, № 28, 18868-18-876 (1991).
13. K. R. Ratzmann, *Med. Aktuelle*, **18**, № 2, 87-88 (1992).
14. O. Ritvos, T. Ranta, J. Jalkanen, *et al., Endocrinology*, **122**, № 5, 2150-2157 (1988).
15. D. K. Snyder and D. R. Clemmons, *J. Clin, Endocrin.*, **71**, 1632-1636 (1990).
16. A. M. Suikkari, U. A. Koivisto, E. M. Rutanen, *et al., Ibid.*, **66**, № 2, 266-272 (1988).
17. G. N. Than, J. E. Csaba, G. D. Szabo, *et al., Arch. Gynec.*, **236**, № 1, 41-46 (1984).