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PLASMA AND URINE PC-1 ACTIVITY IN TYPE 2 DIABETICS TREATED WITH METFORMIN

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Summary. *The plasma cell differentiation antigen (PC-1) is an inhibitor of insulin receptor tyrosine kinase activity and is a major factor of insulin resistance in patients with type 2 diabetes mellitus. We have studied lymphocyte PC-1 (ecto-alkaline phosphodiesterase I, APD I) in obese type 2 diabetics, as well as plasma level and urinary excretion of PC-1. Fifteen patients (8 female, 7 male) with a BMI 34.11±3.37 kg/m^2, were studied before and after 3 months of metformin (1000 mg b.i.d.) treatment. Lymphocyte PC-1 in patients with type 2 diabetes was markedly increased, and metformin treatment brought it to the level slightly over control. Plasma PC-1 was significantly reduced after metformin treatment, from 37 to 14 mU/L. However, urinary excretion of PC-1 was not changed by metformin treatment. A highly significant correlation between lymphocyte and plasma PC-1 was obtained (r = 0.909 and 0.529, before and after metformin treatment, respectively). Glucoregulation was found improved by metformin treatment as the mean glycosylated hemoglobin was found decreased from 8.15 before to 7.07% after therapy with metformin. In conclusion, an increased lymphocyte PC-1 activity and its reversal by metformin treatment were demonstrated in obese type 2 diabetics. Plasma PC-1 activity was found also decreased after metformin treatment and correlated significantly with lymphocyte PC-1 activity. Urine PC-1 was not found to correlate with lymphocyte PC-1. A high correlation of plasma and lymphocyte PC-1 before metformin treatment should be evaluated for using plasma PC-1 in demonstration of insulin resistance in type 2 diabetics. Plasma but not urine PC-1 correlates with insulin resistance in obese type 2 diabetes mellitus.*

Key words: *PC-1, Type 2 diabetes, insulin resistance, metformin*

Introduction

Insulin resistance, manifesting as impaired insulin-stimulated glucose uptake in skeletal muscle and other key insulin target tissues, is a feature of type 2 diabetes mellitus, which has a dominant role in its pathogenesis (1). Recently some advances have been made in identification of transmembrane glycoprotein PC-1 as a potential factor of insulin resistance in both type 2 diabetics and obese non-diabetic subjects. Elevated PC-1 content is associated with defective insulin signaling, and furthermore, it was suggested that PC-1 inhibits a key-step in insulin signaling, the insulin receptor tyrosine-kinase activity (2). Overexpression of PC-1 was found to attenuate the insulin action in obese, insulin-resistant type 2 diabetics (3). Soluble forms of PC-1 have been identified in serum of experimental animals and humans (4,5). It is proposed that soluble PC-1 originates from proteolytic cleavage of membrane-integrated molecules (2). Some studies have been performed in effort to improve the understanding of role of soluble PC-1 in insulin resistance. Frittitta et al. have found that decreased level of soluble PC-1 could be a predictor of future insulin resistance development (4).

Metformin (dimethylbiguanide) is an oral antihyperglycemic agent used worldwide in therapy of type 2 diabetes (6). It primarily reduces the rate of glucose production through a reduction in gluconeogenesis (7). One of the proposed actions of metformin is increasing insulin sensitivity of the target tissues, possibly through the reversal of the PC-1 activity (8). Three-month metformin treatment of obese type 2 diabetics resulted in significant decrease of previously markedly increased lymphocyte PC-1 values (8).

The aim of this study was to investigate correlation of PC-1 level in plasma and urine with lymphocyte PC-1 in obese type 2 diabetics and changes in PC-1 level before and after metformin therapy. Evidence were obtained that plasma but not urine PC-1 correlates with insulin resistance in type 2 diabetes mellitus.

Patients and Methods

Fifteen patients (8 females, 7 males) with type 2 diabetes mellitus, as diagnosed by the National Diabetes Data Group (9), who had a glycosylated hemoglobin

value above the upper limit of normal, and plasma C-peptide concentration of at least 1.5 ng/mL, were studied. Patients with type 2 diabetes receiving dietary and/or sulphonylurea therapy were included. Patients were excluded if they had abnormal hepatic or renal function or had a recent atherosclerotic event. The patients received 1000 mg of metformin twice daily orally for three months.

Isolation and culture of human PBMC

Peripheral blood mononuclear cells (PBMC) were isolated from 10 ml of freshly drown heparinized (50 IU/ml) blood, layered over Ficoll-Hypaque (Lymphoprep, Nyegard, Oslo, Norway), washed twice in RPMI 1640 (Flow Laboratories, Irvine, UK) culture medium containing 25 mM HEPES, 2 mM glutamine, penicillin (100 U/ml) and streptomycin (100 mg/ml), and resuspended at a concentration of 2×10^6 /ml in the same medium supplemented with 10% fetal calf serum (FCS). PMBC were incubated for 48h at 37°C in an atmosphere of 95% air and 5% CO_2.

Lymphocyte PC-1 activity

Basal values of PC-1 in lymphocyte culture were determined, as well as enzymatic activity of PC-1 in plasma and urine.

Non-adhering cells from the culture plates were transferred to centrifuge tubes after appropriate washing with saline. PC-1 (alkaline phosphodiesterase I, APD) activity was determined in 50 mM Tris-HCl buffer, pH 8.0, 130 mM NaCl, 1 mM $MgCl_2$, with 1.5 ml p-nitrophenyl thymidine 5'-phosphate as a substrate. Incubation was carried out at 37°C for 3-10 min with gentle agitation, under zero-order kinetic conditions. The enzyme reaction was stopped with 0.1 ml of 1M sodium hydroxide. The p-nitrophenol formed was measured at 405 nm.

Plasma and urine PC-1 activity

Enzymatic activity of PC-1 in plasma and urine was determined in identically prepared incubation medium, except for the different incubation time, under zero-order kinetic conditions.

Results

Baseline characteristics of patients with type 2 diabetes are presented in Table 1. These were obese diabetics with a body mass index (BMI) of 34.11±3.37 kg/m^2. Both fasting plasma glucose and glycosylated hemoglobin were over the normal values prior to metformin therapy. After three months of metformin therapy, the mean fasting plasma glucose value was significantly lower than before the treatment. The glycosylated hemoglobin also changed significantly ($p<0.001$) in comparison to the pre-treatment values.

Lymphocyte, plasma and urine PC-1 activity

Lymphocyte PC-1 activity in patients with type 2 diabetes was markedly ($p<0.001$) increased, and it was over the activity previously found in healthy controls (12) (Table 2). A three month treatment with metformin significantly reduced lymphocyte PC-1 from 22.5 to 9.4 nmol/min per 10^6 Ly (Table 2). Basal plasma level of PC-1 was 37 mU/L, and was significantly reduced by metformin treatment to 14 mU/L ($p<0.001$). Urine PC-1 was 2.69, and was not significantly changed by metformin therapy (Table 2).

Table 1. Baseline characteristics of type 2 diabetes mellitus patients treated with metformin

Characteristic	*Before metformin*	*After metformin*
Age (years)	51.12±6.85	
Duration of diabetes (years)	3.20±0.40	
Body mass index (kg/m^2)	34.11±3.37	33.35±3.75*
Fasting plasma glucose (mmol)	10.05±1.75	7.20±1.36**
Glycosylated hemoglobin (%)	8.15±1.72	7.07±1.49**
Fasting plasma insulin (mU/ml)	29.47±14.16	24.47+7.52*

Values are means ±SD
* $p<0.003$ vs pre-treatment value
**$p<0.001$ vs pre-treatment value

Table 2. Lymphocyte, plasma and PC-1 activity in type 2 diabetes mellitus

	Lymphocyte PC-1 (nmol/min per 10^6 Ly)	*Plasma PC-1 (mU/L)*	*Urine PC-1 (U/L)*
Before metformin	22.50 (18.8-88.2)	37 (22-220)	2.69 (1.1-4.79)
After metformin	9.40* (4.5-21.9)	14* (3-40)	2.04 (1.45-3.93)

Values are given as median, with range in parenthesis
* $p<0.001$ vs before metformin

Plasma PC-1 significantly correlated to lymphocyte PC-1 before ($r = 0.909$, $p<0.01$) and after metformin treatment ($r = 0.529$, $p<0.01$). Value of urine PC-1 did not correlate to lymphocyte PC-1 neither prior to nor after the metformin treatment (Table 3).

Table 3. Correlation of plasma and urine PC-1 with lymphocyte PC-1

	Plasma PC-1	*Urine PC-1*
Before metformin	0.909*	0.257
After metformin	0.529*	0.258

* $p<0.01$

Plasma and urine PC-1 correlated significantly before metformin treatment ($r = 0.504$, $p<0.01$). However, after treatment no correlation was obtained ($r = 0.101$).

Discussion

Insulin resistance of target tissues is an important factor of type 2 diabetes mellitus development and progression. Inhibition of tyrosine kinase activity could be a key-step in impaired insulin signaling (10,11). It is suggested that PC-1 can directly bind to the alpha-subunit of insulin receptor, which inhibits insulin signal transduction (11). Our previous studies have confirmed

the elevated content of PC-1 in lymphocytes of type 2 diabetics compared to the control subjects (8), implicating the importance of PC-1 for pathogenesis of type 2 diabetes.

Various methods have been tried for identification of insulin-resistant subject in clinical practice, since the hyperinsulinemic-euglycemic clamp can be used only for the scientific purposes, but none of them has shown sufficient accuracy. In the present study, we have found an increased enzymatic activity of PC-1 in plasma of patients with type 2 diabetes, which highly correlated to the lymphocyte PC-1 content in these patients ($r = 0.909$). Plasma PC-1 activity was significantly decreased after three-month treatment with metformin, again with high correlation to the lymphocyte PC-1 ($r = 0.529$). Since it was already documented that increased value of PC-1 implicates the presence of insulin resistance in both diabetics and non-diabetic subjects (12,13), our findings suggest that the level of circulating PC-1 could be used in clinical practice for easier identification of insulin-resistant subjects. The elevated plasma PC-1 in diabetics could be explained by the proteolytic cleavage of surface molecules, shed to the plasma by lymphocytes (5). Urine PC-1 did not correlate to the plasma and lymphocyte PC-1 activity, which could be explained by the fact that urine values mainly depend on the renal function. A correlation of plasma and urine PC-1 levels before metformin treatment suggests that a part of serum PC-1 is filtered by the kidney.

In the study of Frittitta et al. (4), PC-1 was found to circulate in human plasma, and its concentration was related to insulin sensitivity. However, in their study, the plasma PC-1 was decreased in insulin-resistant compared to insulin-sensitive nondiabetic subjects.

In conclusion, an increased level of lymphocyte and plasma PC-1 was found in obese type 2 diabetics, which reversed to the normal values after three-month metformin treatment. Plasma PC-1 highly correlated to the lymphocyte PC-1 activity and should be considered as a clinical indicator of insulin resistant state. Plasma, but not urine PC-1, was found to correlate with insulin resistance in obese patients with type 2 diabetes mellitus.

References

1. Reaven GM: Pathophysiology of insulin resistance in human disease. Physiol Rev 1995; 75: 473-486
2. Goldfine ID, Maddux BA, Youngren JF, Trischitta V, Frittitta L. Role of PC-1 in the etiology of insulin resistance. Ann NY Acad Sci 1999; 892: 204-22.
3. Maddux BA, Sbraccia P, Kumakura S, Sasson S, Youngren J, Fisher A, Spencer S, Grupe A, Henzel W, Stewart TA et al. Membrane glycoprotein PC-1 and insulin resistance in non-insulin-dependent diabetes mellitus. Nature 1995; 373: 448-451.
4. Frittitta L, Camastra S, Baratta R, Costanzo BV, D'Adamo M, Graci S, Spampinato D, Maddux BA, Vigneri R, Ferrannini E, Trischitta V. A soluble PC-1 circulates in plasma: relationship with insulin resistance and associated abnormalities. J Clin Endocrinol Metabol 1999; 84: 3620-3625.
5. Bollen M, Gijsbers R, Ceulemans H, Stalmans W, Stefan C. Nucleotide pyrophosphatases/phosphodiesterases on the move. Crit Rev Biochem Mol Biol 2000; 35: 393-432.
6. Bailey CJ. Metformin-an update. Gen Pharmacol 1993 Nov; 24: 1299-1309.
7. Hundal RS, Krssak M, Dufuor S, Laurent D, Lebon V, Chandramouli V, Inzucchi SE, Schumann WC, Petersen KF, Landau BR, Shulman GI. Mechanism by which metformin reduces glucose production in type 2 diabetes. Diabetes 2000; 49: 2063-2069.
8. Stefanović V, Antić S, Mitić-Zlatković M, Vlahović P. Reversal of increased lymphocyte PC-1 activity in patients with type 2 diabetes treated with metformin. Diabetes Metab Res Rev 1999; 15: 400-404.
9. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979; 28: 1039-1057.
10. Kumakura S, Maddux BA, Sung CK. Overexpression of membrane glycoprotein PC-1 can influence insulin action at a post-receptor site. J Cell Biochem 1998; 68: 366-77.
11. Maddux BA, Goldfine ID. Membrane glycoprotein PC-1 inhibition of insulin receptor function occurs via direct interaction with the receptor alpha-subunit. Diabetes 2000; 49: 13-19.
12. Frittitta L, Spampinato D, Solini A, Nosadini R, Goldfine ID, Vigneri R, Trischitta V. Elevated PC-1 contents in cultured skin fibroblasts correlates with decreased in vivo and in vitro insulin action in nondiabetic subjects. Diabetes 1998; 47: 1095-1100.
13. Gu HF, Almgren P, Lindholm E, Frittitta L, Pizzuti A, Trischitta V, Groop LC. Association between the human glycoprotein PC-1 gene and elevated glucose and insulin levels in a paired-sibling analysis. Diabetes 2000 Sep; 49: 1601-1603.

AKTIVNOST PC-1 U PLAZMI I URINU PACIJENATA SA TIPOM 2 DIJABETES MELITUSA TRETIRANIH METFORMINOM

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Kratak sadržaj: *Plazma-ćelijski antigen (PC-1) je inhibitor tirozin-kinazne aktivnosti insulinskog receptora i jedan od važnih faktora u nastanku insulinske rezistencije u pacijenata sa tipom 2 dijabetes melitusa. Predmet ovog istraživanja bilo je određivanje aktivnosti limfocitnog PC-1, kao i nivoa PC-1 u plazmi i urinu gojaznih tip 2 dijabetičara. Ispitivano je 15 pacijenata (8 žena i 7 muškaraca) pre i posle tromesečne terapije metforminom (1000 mg dnevno podeljeno u dve doze per os). U studiju su uključeni gojazni tip 2 dijabetičari čiji je indeks mase tela na početku ispitivanja bio 34,11±3,37 kg/m^2. Limfocitni PC-1 u pacijenata sa tipom 2 dijabetesa bio je značajno povišen, da bi nakon terapije metfominom došlo do njegovog smanjenja na vrednosti bliske normalnim. Tretman metforminom signifikantno je smanjio nivo PC-1 u plazmi, sa 37 na 14 mU/L. Vrednosti PC-1 u urinu, međutim, nisu se značajno promenile tokom terapije. Utvrđena je visoko signifikantna korelacija između vrednosti PC-1 u plazmi i limfocitima (r = 0,909 pre terapije, a r = 0,529 posle terapije metforminom). Efekat metformina na stanje glikoregulacije bio je povoljan (srednja vrednost glikoziliranog hemoglobina smanjila se sa 8,15 pre na 7,07% posle terapije). Rezultati dobijeni ispitivanjem gojaznih tip 2 dijabetičara uključenih u studiju pokazuju značajno povišene početne vrednosti limfocitnog PC-1, kao i njihovo smanjenje na vrednosti bliske normalnim nakon terapije metforminom. Tromesečna terapija metforminom dovela je, takođe, do sniženja vrednosti PC-1 u plazmi, dok promene PC-1 u urinu nisu bile značajne. Nađena je visoko signifikantna korelacija vrednosti PC-1 u plazmi i limfocitima, a nema značajne korelacije između urinarnog i limfocitnog PC-1. Visoku korelaciju vrednosti PC-1 u plazmi i limfocitima pre lečenja metforminom treba dalje ispitivati da bi se PC-1 u plazmi mogao koristiti pri identifikaciji insulin-rezistentnih tip 2 dijabetičara. Zaključeno je da u gojaznih bolesnika sa tipom 2 dijabetesa nivo PC-1 u plazmi a ne PC-1 u urinu korelira sa prisustvom insulinske rezistencije.*

Ključne reči: *PC-1, tip 2 dijabetes melitusa, insulinska rezistencija, metformin*