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PROLONGED SULFONYLUREA TREATMENT HAS NO EFFECT ON LYMPHOCYTE PC-1 EXPRESSION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Summary. *Plasma cell antigen (PC-1) is an inhibitor of insulin receptor tyrosine kinase, which plays an important role in insulin resistance pathogenesis in type 2 diabetes mellitus, obesity and some other insulin resistant states. In a recent study we have demonstrated an increased lymphocyte PC-1 activity in patients with type 2 diabetes and its reversal by 3-month metformin treatment, corresponding to the improvement of insulin sensitivity. The aim of this study was to investigate the effect of prolonged treatment with two commonly used sulfonylurea agents, gliclazide and glibenclamide, on lymphocyte PC-1 expression.*
Twenty-six newly diagnosed, obese type 2 diabetes patients with a body mass index $\geq$30, and 14 healthy controls were enrolled in the study. Basal, concanavalin A (Con A)-stimulated, and phorbol-12-myristate-13-acetate (PMA)-stimulated lymphocyte PC-1 was determined in 26 diabetic patients before and after 3-month of gliclazide (12 patients) and glibenclamide (14 patients) treatment.
Fasting plasma glucose and blood fructosamine decreased significantly after treatment. Pre-treatment lymphocyte PC-1 activity in diabetic patients was significantly higher than in controls. Treatment with gliclazide or glibenclamide did not produced any significant effect in unstimulated, Con A-stimulated or PMA-stimulated PC-1 activity.
In conclusion, this study has confirmed an increased lymphocyte PC-1 activity in type 2 diabetes. However, in contrast to the metformin action, prolonged treatment with two commonly used sulfonylurea agents, gliclazide and glibenclamide, did not produce any significant effect on lymphocyte PC-1. This study has established that sulfonylurea drugs have no effect on PC-1.

Key words: *Diabetes mellitus, insulin resistance, PC-1, sufonylurea treatment, gliclazide, glibenclamide*

Introduction

Insulin resistance is a complex disorder which is central to the pathophysiology of type 2 diabetes and a number of other disease states (1). In obese humans with type 2 diabetes, insulin resistance is accompanied by elevated levels of plasma cell membrane glycoprotein (PC-1) and decreased insulin receptor (IR) tyrosine kinase activity in peripheral tissues (2). PC-1 inhibits IR tyrosine kinase by direct binding to the insulin receptor alpha subunit (3) and affects insulin action at a post-receptor site, by decreasing p70 ribosomal S6 kinase stimulation (4). Current investigations implicate that therapeutic modification of PC-1 expression would be of great benefit for insulin-resistant type 2 diabetics.

Treatment of obese type 2 diabetics includes several groups of antihyperglycemic agents. Metformin, a biguanide oral antidiabetic agent, was shown to affect insulin resistance by decreasing enzymatic activity of overexpressed PC-1 molecules after a 3-month treatment in obese type 2 diabetics (5). Other, frequently used oral antidiabetics, include the second generation sulphonylurea agents gliclazide and glibenclamide (glyburide). They act primarily through sulphonylurea receptors by triggering insulin release and improving beta cell function (6). Gliclazide expresses an insulin-sensitizing effect on a postreceptor level, apparently independent of the insulin receptor tyrosine kinase activity (7). One of suggested gliclazide actions is TNF-alpha production inhibition, which is supposed to delay type 2 diabetes complications (8). It was shown that gliclazide treatment normalized pyruvate dehydrogenase activity in lymphocyte of type 2 diabetics, which could also contribute to the improved insulin sensitivity after gliclazide treatment (9). So far, no effect of sulphonylurea treatment on PC-1 expression has been established. Therefore, the aim of this sudy was to investigate the effect of sulphonylurea agents glibenclamide and gliclazide on PC-1 expression in obese type 2 diabetics during a prolonged, 3-month treatment.

Subjects and Methods

The investigation included 26 newly diagnosed (3-12 months), obese (BMI≥30 kg/m^2) type 2 diabetics, as defined by revised NDDG/WHO criteria. The subjects were selected if failed to achieve satisfactory glycoregulation with dietary treatment only. Two groups were formed, one (12 subjects) recieving gliclazide, 80 mg per os daily, and the other (14 subjects) treated with glibenclamide, 10 mg per os/d. The treatment lasted for three months for both groups. Basic laboratory parameters (glycemia, fructosamine, C-peptide) as well as lymphocyte PC-1 activity were determined for all patients before and after the treatment.

A control group was formed of 14 healthy subjects, matched to the clinical group. Control subjects included had no personal history of, or first degree relatives with diabetes type 1 or type 2, hypertension or dyslipidemia. None of them was receiving any medications.

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^{o}C in an atmosphere of 95% air and 5% CO_2.

PC-1 of lymphocytes

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^{o}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.

Results

Baseline characteristics of type 2 diabetics are given in Table 1. All subjects were obese (BMI for gliclazide group 31±3.42 prior to treatment, 31.53±3.48 kg/m^2 after the treatment; glibenclamide group 33.53±4.94 before the treatment, 33.36±4.79 kg/m^2 after the treatment). Treatment with neither gliclazide nor glibenclamide caused significant changes in body mass. Both gliclazide and glibenclamide produced satisfactory blood glucose reduction, which was statistically significant (p<0,001). The same effect was noted in statistically significant fructosamine decrease (p<0,001) after three-month sulphonylurea treatment. Level of C-peptide was not signficantly changed by sulphonylurea treatment.

Lymphocyte PC-1 activity

Lymphocyte PC-1 activity before and after gliclazide and glibenclamide treatment are given in Fig. 1-3. In non-stimulated cultured lymphocytes, enzymatic activity of PC-1 was statistically over control values both before and after the sulphonylurea treatment (gliclazide 294%, glibenclamide 313% expressed as percentage of control value, p<0.001). A statistically increased activity of PC-1 was found in ConA (p<0.01) and PMA stimulated lymphocytes (p<0.001) before treatment compared to the control group. These, statistically increased levels, were maintained after the three-month treatment with gliclazide and glibenclamide. After gliclazide treatment, in non-stimulated lymphocytes, PC-1 activity was 275% of control value (p<0.001), in ConA stimulated 150% (p<0.01), and in PMA stimulated 475% of control value (p<0.001). After glibenclamide treatment, these values were 289%, 153%, and 544% of control value, respectively, with identical statistical significance as in gliclazide group.

Disscusion

Overexpression of PC-1 membrane glycoprotein in peripheral tissues is considered as an important pathogenetic factor in insulin-resistant states, including obese type 2 diabetes mellitus (2). A several-fold increase in PC-1 activity was documented in sceletal muscled, dermal fibroblasts, and adipose tissue of insulin resistant subjects (11), as well as in cultured lymphocytes of obese type 2 diabetics (5). PC-1 inhibits insulin signalling via direct interaction with insulin receptor alpha subunit (3),

Table 1. Baseline characteristics of type 2 diabetics before and after treatment with sulphonylurea agents gliclazide and glibenclamide

Subjects (n=26)	Gliclazide (n=12)		Glibneclamide (n=14)	
	Before treatment	After treatment	Before treatment	After treatment
Age (years)	59.88±10.57 (45-76)		53±4.88 (43-61)	
BMI (kg/m^2)	31±3.42	31.53±3.48	33.53±4.94	33,36±4,79
Fasting glycemia (mmol/l)	10.11±2.08	7.67±1.89*	10.87±1.28	7.22±1.50*
Fructosamine (U/gr protein)	53.41±5.51	42.12±3.18*	50.51±6.46	43.46±5.57*
C-peptide (pmol/l)	1.40±0.84	1.07±0.29	0.96±0.37	0.88±0.18

Values are means ± S.E.M.

***p<0.001 in comparison to value before treatment**

and affects other downstream signalling events at a post-receptor site (4). Previously, a beneficial effect of a three-month metformin treatment on PC-1 activity was reported, suggesting the mechanism by which metformin reduces insulin resistance in obese type 2 diabetics (5).

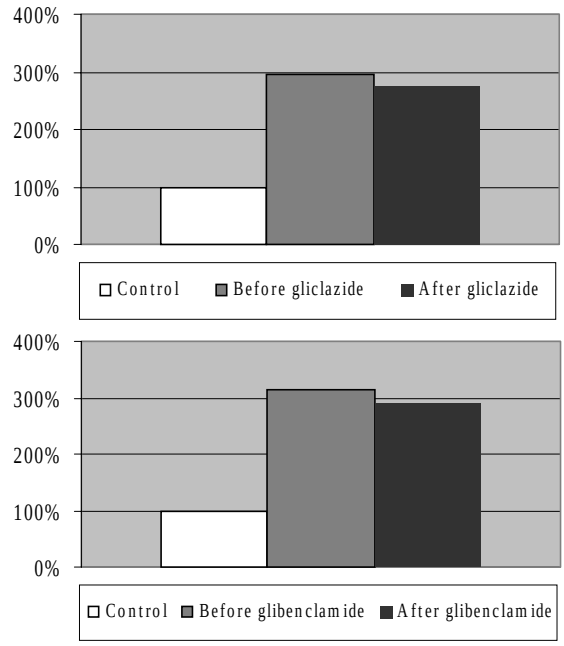

Fig. 1. PC-1 acitivity of non-stimulated lymphocytes before and after 3-month treatment with gliclazide and glibenclamide. Values given are percentage of control value ($p<0.001$ before and after treatment in comparison to control value)

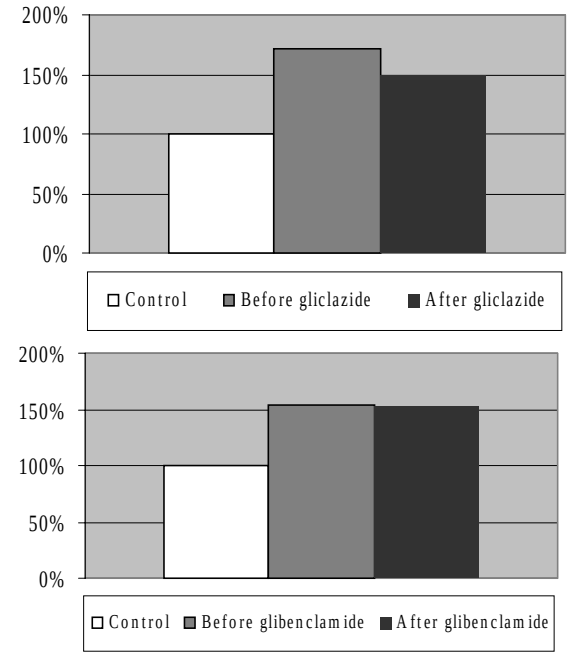

Fig. 2. PC-1 acitivity of ConA stimulated lymphocytes before and after 3-month treatment with gliclazide and glibenclamide. Values given are percentage of control value ($p<0.01$ before and after treatment in comparison to control value)

Sulphonylurea treatment, which has been the backbone of oral antidiabetic treatment for decades, predominantly acts through sulphonylurea receptors, which are found in various tissues (6). Sulphonylureas trigger insulin release from pancreatic beta cells, thereby lowering the blood glucose level. The limiting factor in sulfonylurea therapy is beta-cell exhaustion, when the treatment becomes ineffective (10). Improvement in insulin sensitivity was reported after treatment with sulphonylurea agent gliclazide (9). It inhibits the production of TNF-alpha, and brings lymphocyte pyruvate dehydrogenase to the normal values, thereby increasing insulin sensitivity (8,9). Proposed mechanisms of that gliclazide action are insulin receptor tyrosine kinase-independent (9).

Our results have shown an increased PC-1 activity in cultured lymphocytes of obese type 2 diabetics. These findings confirm our previous observations obtained by investigation of insulin-resistant obese subjects with type 2 diabetes (5). In contrast to the reported metformin action (5), prolonged sulphonylurea treatment with gliclazide and glibenclamide did not affect PC-1 activity, which maintained increased on pretreatment level, in both non-stimulated and ConA and PMA stimulated lymphocytes. Although glycoregulation was obtained, no beneficial effect on PC-1 activity was noted.

In conclusion, this investigation confirms increased PC-1 activity in cultured lymphocytes of obese type 2 diabetics. A prolonged, 3-month sulphonylurea treatment with gliclazide and glibenclamide had no significant effect on PC-1 activity.

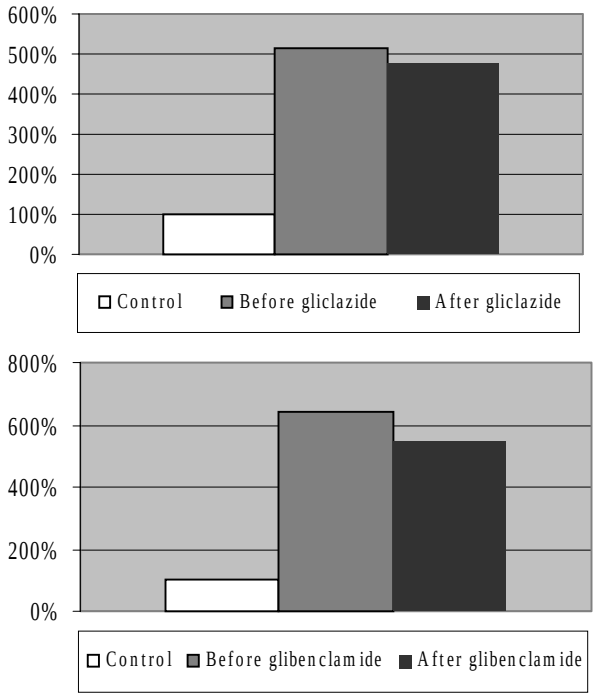

Fig. 3. PC-1 acitivity of PMA stimulated lymphocytes before and after 3-month treatment with gliclazide and glibenclamide. Values given are percentage of control value ($p<0.001$ before and after treatment in comparison to control value)

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TERAPIJA PREPARATIMA SULFONILUREJE NEMA EFEKTA NA EKSPRESIJU PC-1 U KULTURI LIMFOCITA PACIJENATA SA TIPOM 2 DIJABETES MELITUSA

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Kratak sadržaj: *Plazma-ćelijski antigen (PC-1) je inhibitor tirozinkinazne aktivnosti insulinskog receptora i igra značajnu ulogu u nastanku insulinske rezistencije u tipu 2 dijabetes melitusa, gojaznosti i u drugim stanjima koja se karakterišu smanjenjem insulinske senzitivnosti. Saopšteno je da je tromesečni tretman metforminom doveo do smanjenja aktivnosti PC-1 u kulturi limfocita gojaznih tip 2 dijabetičara, sa odgovarajućim poboljšanjem insulinske senzitivnosti. Cilj ovog istraživanja bio je ispitivanje efekta tromesečnog tretmana često korišćenim preparatima sulfonilureje gliklazidom i glibenklamidom na limfocitnu ekspresiju PC-1.*

Ispitivanjem je obuhvaćeno 26 novootkrivenih, gojaznih (BMI≥30 kg/m^2) tip 2 dijabetičara i 14 zdravih osoba (kontrolna grupa). Pre i posle tromesečne terapije gliklazidom (12 pacijenata) i glibenklamidom (14 pacijenata), određene su bazalne, ConA (konkavalin A)-stimulisane i forbol-12-miristat-13-acetat (PMA)-stimulisane vrednosti limfocitnog PC-1.

Nivo glikoze našte kao i nivo fruktozamina statistički su se značajno smanjili nakon tretmana preparatima sulfonilureje. Vrednost PC-1 pre terapije kod gojaznih tip 2 dijabetičara bila je statistički značajno povišena u odnosu na kontrolnu grupu. Tretman gliklazidom ni glibenklamidom nije doveo do statistički značajnog smanjenja aktivnosti limfocitnog PC-1 kako u nestimulisanim, tako i u ConA i PMA stimulisanim limfocitima.

U zaključku, ovo istraživanje potvrđuje prisustvo povišene limfocitne aktivnosti PC-1 u tipu 2 dijabetesa. Za razliku od metformina, dva često korišćena preparata sulfonilureje, gliklazid i glibenklamid, u toku tromesečne terapije nisu dovela do značajnog smanjenja limfocitne aktivnosti PC-1. Ovim istraživanjem utvrđeno je da preparati sulfonilureje nemaju efekta na aktivnost PC-1.

Ključne reči: *Dijabetes melitus, insulinska rezistencija, PC-1, preparati sulfonilureje, gliklazid, glibenklamid*