Lymphocyte ecto-5’-nucleotidase in obese type 2 diabetic patients treated with gliclazide

V Stefanovic¹, S Antic², M Milojkovic³, G Lazarević², P Vlahovic¹

SUMMARY

Objective: Lymphocyte 5’-nucleotidase is sensitive to superoxide anion, and is an indicator of oxidative stress in humans. The aim of this study was to assess the effect of the sulfonylurea drugs gliclazide and glibenclamide on lymphocyte ecto-5’-nucleotidase of type 2 diabetic patients.

Methods: Thirty obese type 2 diabetic patients were treated for three months after randomisation either with gliclazide or glibenclamide. Basic laboratory parameters (glycemia, fructosamine, C-peptide), plasma malondialdehyde levels (MDA) as well as lymphocyte 5’-nucleotidase activity were determined, for all patients and 16 healthy controls, before and after the treatment.

Results: 5’-nucleotidase activity in diabetic patients before treatment with gliclazide was 1.61 ± 0.16 nmol/min/10⁶ lymphocytes, and was significantly (P< 0.01) increased compared with the level in healthy controls. After three months of gliclazide treatment, ecto-5’-nucleotidase activity fell significantly by 47.39% and 36% in unstimulated Con A- and PMA-stimulated lymphocytes, respectively. Glibenclamide treatment had no effect on ecto-5’-nucleotidase of type 2 diabetic patients. Glyceregulation was improved, as plasma fructosamine decreased from 53.4 to 42.1 and from 50.5 to 43.4 U/g proteins after gliclazide and glibenclamide treatment, respectively. Plasma MDA levels markedly decreased after gliclazide treatment but not glibenclamide treatment.

Conclusion: These results show that gliclazide treatment inhibits the activity of lymphocyte ecto-5’-nucleotidase and presumably decreases the concentration of adenosine at the cell surface. A decrease in 5’-nucleotidase activity and attenuation of adenosine production may be a factor in the protection of tissue injury in type 2 diabetic patients.

Key-words: Diabetes mellitus type 2 · Ecto-5’-nucleotidase · Adenosine · Lymphocyte · Gliclazide · Glibenclamide.

REZUMÉ

Effets du traitement par gliclazide sur l’ecto-5’-nucleotidase lymphocytaire des diabétiques de type 2 obèses

Objectif : La 5’-nucleotidase lymphocytaire est sensible à l’anion superoxyde et est un indicateur du stress oxydant chez l’homme. Le but de ce travail était d’examiner l’effet de sulfonylurées, comme le gliclazide et le glibenclamide, sur l’ecto-5’-nucleotidase des diabétiques de type 2.

Méthodes : Trente diabétiques obèses de type 2 ont été traités après randomisation pendant trois mois par gliclazide ou glibenclamide. La glycémie, la fructosamine et le peptide C, les concentrations plasmatiques de malondialdéhyde (MDA) ainsi que l’activité de la 5’-nucleotidase lymphocytaire ont été mesurés chez les malades et chez 16 témoins avant et après traitement.

Résultats : L’activité de la 5’-nucleotidase des malades diabétiques avant traitement par gliclazide était 1,61 ± 0,16 nmol/min/10⁶ lymphocytes, significativement plus élevée (P < 0,01) que celle des témoins. Après trois mois de traitement par gliclazide, l’activité de l’ecto-5’-nucleotidase des lymphocytes non stimulés et stimulés par la concanavaline ou le PMA, diminua respectivement de 47,39 % et de 36 %. Le traitement par glibenclamide n’eut aucun effet sur l’ecto-5’-nucleotidase des diabétiques de type 2. La glycorégulation s’améliora comme en témoigne la diminution de la fructosamine plasmatique de 53,4 à 42,1 et de 50,5 à 43,4 U/g de protéines après traitement par gliclazide et glibenclamide, respectivement. Une diminution nette des concentrations plasmatiques de MDA fut observée après traitement par gliclazide, mais non après traitement par glibenclamide.

Conclusion : Ces résultats indiquent que le traitement par gliclazide inhibe l’activité de la 5’-nucleotidase lymphocytaire et diminue vraisemblablement la concentration d’adénosine à la surface cellulaire. La diminution de l’activité de la 5’-nucleotidase et la réduction de production d’adénosine pourraient être impliquées dans la protection vis-à-vis des lésions cellulaires chez les diabétiques de type 2.

Mots-clés : Diabète de type 2 · Ecto-5’-nucleotidase · Adénosine · Lymphocyte · Gliclazide · Glibenclamide.

Address correspondence and reprint requests to:
V Stefanovic. Institut za nefrologiju i hemodijalizu, Medicinski fakultet, Bul. Zorana Djindjica B1, 18000 Nis, Serbia.
stefan@ni.ac.yu

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Introduction

Conflicting results exist on the causative role of adenosine in insulin resistance. 5'-nucleotidase is likely to play a key role in the regulation of the local concentration of adenosine [1,2]. Lymphocyte 5'-nucleotidase is sensitive to superoxide anion, and is an indicator of oxidative stress in humans [3]. The increased 5'-nucleotidase activity could be related to the increased oxidative stress [4].

The role of reactive oxygen species in diabetes and its complications are well known. Two therapeutic agents commonly used in the treatment of diabetes are the sulfonylureas gliclazide and glibenclamide. These drugs effectively reduce blood sugar in type 2 diabetes mellitus by augmenting insulin release. Over the past 15 years, studies have shown that gliclazide not only lowers blood glucose but also has free radical-scavenging ability that relates to the unique aminoazabicyclo-octane ring grafted onto the sulfonylurea [5]. Gliclazide scavenged O$_2^-$, OH$^-$ and nitric oxide (NO) in a dose-dependent manner whereas glibenclamide was without effect. These findings suggest that gliclazide is not only effective in reducing blood sugar but may also be beneficial by inhibition of lipid and protein denaturation, which leads to the development of diabetic complications [6].

The aim of this work was to comparatively study the effect of the two sulfonylureas gliclazide and glibenclamide on lymphocyte ecto-5'-nucleotidase, an indicator of oxidative stress, in type 2 diabetic patients, as well as the plasma malondialdehyde (MDA) level.

Subjects and methods

Patients

The investigation included 30 newly diagnosed (3-12 months), obese (body mass index [BMI] ≥ 30 kg/m$^2$) type 2 diabetic patients, as defined by revised National Diabetes Data Group (NDDG)/World Health Organization (WHO) criteria. Subjects were selected if they failed to achieve satisfactory glucose control with dietary treatment alone, for three months. Five patients in the gliclazide group and six in the glibenclamide group had grade 1 hypertension, and were treated with angiotensin-converting enzyme inhibitors. This was a randomised, open, parallel study. Patients were randomised to treatment with gliclazide 80 mg orally daily, or glibenclamide 10 mg orally daily, for three months. Basic laboratory parameters (glycaemia, fructosamine, C-peptide) as well as lymphocyte 5'-nucleotidase activity were determined for all patients before and after the treatment.

Statistical analysis

Data were expressed as means ± SE, or medians. The Student t-test and non-parametric analysis (Mann-Whitney) were used when appropriate. Differences were considered significant at P < 0.05.

Results

The baseline characteristics of the type 2 diabetes are shown in table I. All subjects were obese (BMI 31 ± 3.4 and 33.5 ± 5.0 kg/m$^2$) before treatment with gliclazide and gli-
Treatment with gliclazide and glibenclamide did not produce significant changes in body mass. Both gliclazide and glibenclamide treatments produced a statistically significant reduction in blood glucose (P < 0.001). The same effect was noted with a statistically significant decrease in fructosamine (P < 0.001) after the three-month period of treatment. The C-peptide level was not significantly changed by sulfonylurea treatment. Total cholesterol but not triglyceride levels decreased significantly after the three-month period of treatment. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were not significantly changed.

The pretreatment plasma MDA level in type 2 diabetic patients was increased, being 143.7% and 146.0% of the control group MDA level in the gliclazide and glibenclamide groups, respectively. Gliclazide treatment significantly decreased plasma MDA to 81.4% of the pretreatment level. Glibenclamide treatment of diabetic patients had no effect on plasma MDA (table II).

### Discussion

This study has shown increased 5'-nucleotidase activity in type 2 diabetic patients, related to the increased oxidative stress in diabetes. Gliclazide, but not glibenclamide, reduced lymphocyte 5'-nucleotidase activity to the level of healthy controls. The plasma MDA level was also increased in diabetic patients but partially reduced by gliclazide treatment of type 2 diabetic patients. Glibenclamide was without effect on the antioxidant activity.

In the study by Fava et al. [10], type 2 diabetic patients received glibenclamide or gliclazide in a 12-week, randomized, observer-blinded, parallel study. At 12 weeks, gliclazide patients had lower plasma lipid peroxides (13.3 ± 3.8 micromol/L vs. 19.2 ± 4.3 micromol/L, P = 0.0001) than the glibenclamide patients. Gliclazide was found to reduce oxidative stress in type 2 diabetic patients by improving plasma antioxidant status [10]. There is increased oxidative stress in elderly diabetics, however, this is not due to a reduced erythrocyte antioxidant defense potential but, rather, increased free radical production possibly due to hyperglycaemia [11].

Oxidative stress plays a role in the pathological processes that occur in the diabetic patient. Excessive oxidative stress...
has adverse effects on islet cell survival and function and accelerates complications in target organs and tissues [12,13,14]. Advanced glycation end products and the free radicals generated in this process can both be implicated in the accelerated atherosclerosis and vascular and prothrombotic microangiopathic changes of diabetes [5]. The rate of formation of free radicals is dependent on the rate of protein glycation and therefore the level and duration of hyperglycaemia. In diabetes, where increased glycation and oxidation are fundamental to the pathogenesis of diabetic vascular disease, agents such as gliclazide, with its antioxidant activities [10,11], may have an enhanced therapeutic role.

Antioxidant therapy may potentially play a critical role in reducing morbidity and mortality in diabetes. However, at the current time, studies have not allowed us to quantitatively determine to what extent oxidative stress plays a role in deterioration of islet function and worsening of the complications. Understanding the molecular mechanisms of oxidative stress and damage will provide insight into the role of specific antioxidants in different disease processes. Progress may come as specific therapies are developed to correct components of oxidant stress.

The results suggest that gliclazide treatment inhibits the activity of lymphocyte ecto-5'-nucleotidase and presumably decreases the concentration of adenosine at the cell surface. Decrease in ecto-5'-nucleotidase activity and attenuation of adenosine production by gliclazide may be a factor in the protection of tissue injury in type 2 diabetic patients.

Further studies need to be performed on the evolution of oxidative stress over time, on which complications are affected, when and to what extent, and on whether intervention with antioxidants slows or reverses the process, and, if so, at what stage of the disease. Studies will have to be placebo-controlled, prospective, and powered with a large number of patients to detect significant differences in antioxidant effects.

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References


Table III
Lymphocyte 5'-nucleotidase activity in type 2 diabetes patients treated with gliclazide or glibenclamide.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>5'-nucleotidase activity (nmol/min/10⁶ Ly)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unstimulated</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>0.95 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.94 (0.78-1.20)</td>
</tr>
<tr>
<td>DM-Before gliclazide</td>
<td>15</td>
<td>1.61 ± 0.16a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.49 (1.04-2.68)</td>
</tr>
<tr>
<td>DM-After gliclazide</td>
<td>15</td>
<td>0.82 ± 0.06a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.79 (0.65-1.11)</td>
</tr>
<tr>
<td>DM-Before glibenclamide</td>
<td>15</td>
<td>1.57 ± 0.12b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.38 (0.95-2.27)</td>
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<tr>
<td>DM-After glibenclamide</td>
<td>15</td>
<td>1.66 ± 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.44 (1.04-2.68)</td>
</tr>
</tbody>
</table>

Values are means ± SE, medians, range in parentheses.
a vs. DM-Before gliclazide P< 0.001.
b vs. Control P< 0.01.
c vs. Control P< 0.05.


