Gliclazide treatment lowers serum ICAM-1 levels in poorly controlled type 2 diabetic patients

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SUMMARY

Objective: To investigate the potential effect of gliclazide on serum ICAM-1 (intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1) levels in poorly controlled type 2 diabetic patients.

Patients and methods: The study included 104 patients, randomly divided into two groups. Group A comprised 53 patients (26 men) treated with gliclazide with a mean age of 67.5±9.9 years, a mean diabetes duration of 13.4±5.4 years and a mean HbA1c of 8.6±1.1 %. Group B comprised 51 patients (25 men) treated with glibenclamide with a mean age of 66.4±10.9 years, a mean diabetes duration of 13.2±6.1 years and a mean HbA1c of 8.4±1.3 %. A third group of 30 healthy controls (15 men) with a mean age of 63.3±10.4 years was also included. Serum levels of ICAM-1 and VCAM-1 were measured at the beginning of the study and after six months of treatment.

Results: Pretreatment serum ICAM-1 and VCAM-1 levels did not differ between groups A and B, while they were significantly higher (P=0.0001) than in healthy controls. No significant difference in HbA1c body mass index, blood pressure control and lipid profile between the two groups was observed after the sixth month of treatment. In group A, serum ICAM-1 levels after six months of treatment were significantly reduced from 623.12±61.17 ng/ml to 370.14±49.92 ng/ml (P=0.01), while no reduction was found in VCAM-1 levels. In group B, no reduction was found in serum ICAM-1 and VCAM-1 levels after the end of the study.

Conclusions: Our results suggest that gliclazide treatment reduces serum ICAM-1 levels in poorly controlled type 2 diabetic patients. This reduction is independent of the hypoglycaemic action of gliclazide.

Key-words: Adhesion molecules · Atherosclerosis · Diabetes mellitus type 2 · Gliclazide.

RéSUMé

Le traitement par gliclazide diminue les concentrations sériques d'ICAM-1 chez les diabétiques de type 2 mal équilibrés.

Objectif : L'objectif de ce travail consistait à chercher un effet du gliclazide sur les concentrations sériques d'ICAM-1 et de VCAM-1 chez des patients atteints de diabète de type 2.

Patients et méthodes : Dans cette étude, ont été inclus 104 patients, repartis en deux groupes par randomisation. Le groupe A, traité par gliclazide, comportait 53 patients (26 hommes), d'âge moyen 67,5 ± 9,9 ans, durée du diabète 13,4 ± 5,4 ans et HbA1c 8,6 ± 1,1 %. Le groupe B, traité par glibenclamide, comportait 51 patients (25 hommes), d'âge moyen 66,4 ± 10,9 ans, durée du diabète 13,2 ± 6,1 ans et HbA1c 8,4 ± 1,3 %. Un troisième groupe comportait 30 témoins (15 hommes) âgés en moyenne de 63,3 ± 10,4 ans. Les concentrations sériques d'ICAM-1 et de VCAM-1 ont été mesurées avant puis après six mois du traitement.

Résultats : Avant traitement, les concentrations sériques d'ICAM-1 et de VCAM-1 étaient semblables entre les deux groupes, mais significativement plus élevées que chez les témoins (P = 0,0001). Après 6 mois de traitement, l'HbA1c, l'indice de masse corporelle, la pression artérielle et les paramètres lipidiques du sérum restèrent identiques dans les deux groupes. Dans le groupe A traité par gliclazide, les concentrations sériques d'ICAM-1 diminuèrent significativement après six mois de traitement, passant de 623,12 ± 61,17 ng/ml à 370,14 ± 49,92 ng/ml (P = 0,01). Les concentrations sériques de VCAM-1 restèrent stables. Dans le groupe B, traité par glibenclamide, aucune modification des concentrations sériques d'ICAM-1 et ni de VCAM-1 ne fut observée.

Conclusions : Nos résultats suggèrent que le gliclazide réduit les concentrations sériques d'ICAM-1 chez les patients atteints de diabète de type 2 mal équilibrés. Cette réduction est indépendante de l'action hypoglycémiant de gliclazide.

Mots-clés : Athérosclérose · Diabète sucré · Gliclazide · Molécules d'adhésion.

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Introduction

One of the earliest steps in the process of atherosclerosis is increased binding of neutrophils to endothelial cells [1-6]. Experimental studies have shown that hyperglycaemia per se and through increased glycation of albumin stimulates human monocyte adhesion to endothelial cells by inducing intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) expression [6-9]. The deleterious effect of hyperglycaemia is of particular importance in patients with diabetes mellitus [2,6-8,10-12]. Antidiabetic treatment should, therefore, ideally not only lower blood glucose, but also contribute to reduced expression of adhesion molecules [2,6-8].

Gliclazide, a second-generation sulphonylurea, is a drug with a beneficial action beyond glycaemic control [13]. Both in vitro [14-18] and in vivo [19-21] studies have shown that it is a free radical scavenger, which reduces oxidative stress. This antioxidant activity has been found to be independent of glycaemic control [16,19,20] and is attributed to the unique presence of an amino azabicyclo-octane ring grafted on to the sulphonylurea [19,20]. In vitro investigations have shown that gliclazide scavenges free radicals not only in the large vessels, but also in the pancreas [22] and in the microvasculature of the retina [23]. Furthermore, gliclazide, in vitro as well as in vivo, reduces platelet adhesion and aggregation and increases fibrinolysis by acting on tissue plasminogen activator [10,13]. It also lowers smooth muscle cell dysfunction in vitro [18]. Moreover, gliclazide reduces monocyte adhesion to endothelial cells in vitro [6,7,17] and, according to two studies, in patients with type 2 diabetes mellitus [24,25]. Finally, recent studies have shown that gliclazide reduces the expression of adhesion molecules (ICAM-1, E-selectin, P-selectin) in vitro [6,8,9].

However, reduction in serum adhesion molecules due to gliclazide in vivo has not been demonstrated. There is one study in 8 patients in whom administration of gliclazide was found to reduce monocyte adhesion to endothelial cells without any effect on serum ICAM-1 and VCAM-1 levels [24]. Thus, the aim of the present study was to investigate the potential effect of gliclazide on serum adhesion molecule levels in type 2 diabetic patients with poor glycaemic control.

Patients and methods

This study initially recruited 106 patients, who were randomised 1:1 to one of two treatment groups (gliclazide vs glibenclamide). We used a stratified randomisation method to achieve approximate balance of important characteristics, such as age, gender, diabetes duration and other parameters [26]. Previous antidiabetic treatment was interrupted. After a wash-out period of three weeks, patients in group A started treatment with gliclazide, while patients in group B started treatment with glibenclamide. Two patients from group B were finally excluded from the study and were considered drop-outs because of poor compliance. All patients were recruited from the Diabetic Department of “O Agios Dimitrios” General Hospital of Thessaloniki. A third group of 30 healthy controls was also included. Characteristics of the three groups are summarized in table I. The study was conducted in accordance with the Helsinki Declaration of Human Rights and all patients gave their informed consent.

Diabetes mellitus was diagnosed according to the American Diabetes Association criteria [27]. All patients had poor metabolic control. Poor metabolic control was defined as HbA1c exceeding 7% [28]. Blood pressure, low-density lipoprotein (LDL)–cholesterol, high-density lipoprotein (HDL)–cholesterol, triglycerides, and body mass index (BMI = weight/height squared) were measured. Smoking habits during the last ten years (smokers vs non-smokers) were also recorded.

Diabetic patients were evaluated for the presence of macro and microvascular complications. Diagnosis of coronary artery disease was based on a history of myocardial infarction, revascularisation procedure and/or findings in the ECG suggestive of coronary ischaemia (Minnesota coded) [29,30]. Peripheral arterial occlusive disease was evaluated by means of ankle-brachial-index (ABI) measurement with a Doppler apparatus. Peripheral arterial occlusive disease was diagnosed in patients with ABI<0.9 [31]. No patient had evidence of cerebrovascular disease, defined as history of stroke or focal neurological deficits on clinical examination. Retinopathy was defined as at least two microaneurysms and/or retinal haemorrhage and/or other signs of retinal damage [32]. Microalbuminuria was defined as an albumin excretion rate ≥20μg/min in the absence of uncontrolled hypertension and/or urinary tract infection [33]. Neuropathy was diagnosed by a standardised clinical examination using the Neuropathy Disability Score (NDS) and defined as an NDS≥34,35).

Exclusion criteria were: a) concurrent or recent treatment with statins, angiotensin-converting enzyme (ACE) inhibitors, metformin, glitazones, non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, cortisone, immunosuppressive agents or cytotoxic drugs; b) cancer, coagulation disorders, disorders of the immune system, chronic renal insufficiency, chronic obstructive pulmonary disease or other serious systemic disease; c) recent surgery.

Both in the diabetic patients and in the healthy controls, serum levels of ICAM-1 and VCAM-1 were measured by sandwich enzyme-linked immunoassay (ImmuKontakt ELISA assay, AMS Biotechnology Ltd, Abingdon, UK). For ICAM-1, the sensitivity of the assay was 3.3 ng/ml, the intra-assay coefficient of variance was 4.1% and the inter-assay coefficient of variance was 7.6%. For VCAM-1, the sensitivity of the assay was 0.9 ng/ml, the intra-assay coefficient of variance was 3.1% and the inter-assay coefficient of
variance was 5.2%. Blood samples were collected under fasting conditions. In the diabetic patients, measurements were made at the beginning of the study and after a treatment period of six months.

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences) 11.0. Normally distributed quantitative variables were analysed by an unpaired t-test. Significance of qualitative variables was assessed by the chi-square test (with Yates’ correction for 2x2 contingency tables). Data were expressed as mean ± Standard Deviation. Statistical significance was defined at a level of 5% (P<0.05).

Results

There was no significant difference between the groups in age, gender, diabetes duration, HbA1c, blood pressure, BMI and serum lipids. Similarly, no difference was observed between the groups in frequency of microvascular and macrovascular complications (table I).

Pre-treatment serum ICAM-1 and VCAM-1 levels did not differ significantly between the two groups. However, they were significantly higher in both groups, as compared with controls (table II).

Post-treatment serum ICAM-1 but not VCAM-1 levels were significantly (p=0.04) lower in group A than in group B. Serum ICAM-1 and VCAM-1 levels were significantly higher in both groups, as compared with controls (table II).

Treatment with gliclazide resulted in a significant (P=0.01) reduction in serum ICAM-1 but not VCAM-1 levels in group A. No difference in serum adhesion molecules was demonstrated after treatment with glibenclamide in group B (table II).

No significant reduction in HbA1c was observed after completion of treatment with gliclazide or glibenclamide. Finally, no significant change in blood pressure, BMI, and serum lipids was noted at the end of the study.

Discussion

The present study showed that treatment with gliclazide, but not with glibenclamide, resulted in a significant reduction in serum ICAM-1 levels in poorly controlled type 2 diabetic patients. It should be noted that this reduc-

Table I

| Characteristics of patients and healthy controls included in the study. |
|-----------------|-----------------|-----------------|
|                  | Group A         | Group B         | Control         |
| Number of patients | 53 (26 men)     | 51 (25 men)     | 30 (15 men)     |
| Age (years, mean±SD)* | 67.5±9.9        | 66.6±10.9       | 63.3±10.4      |
| Duration of diabetes (years, mean±SD)* | 13.4±5.4        | 13.2±6.1        |               |
| Body mass index (kg/m², mean±SD)* | 28.5±1.8        | 28.1±2.1        | 26.9±2.4      |
| Smoking (n, %)* | 30 (56.60%)     | 28 (54.90%)     |               |
| Coronary artery disease (n, %)* | 5 (19.2%)       | 5 (23.8%)       |               |
| Peripheral arterial occlusive disease (n, %)* | 4 (7.5%)        | 3 (5.9%)        |               |
| Retinopathy (n, %)* | 13 (24.5%)      | 12 (23.5%)      |               |
| Microalbuminuria (n, %)* | 2 (3.8%)        | 2 (3.9%)        |               |
| Neuropathy (n, %)* | 12 (22.6%)      | 11 (21.6%)      |               |
| Pretreatment BP (mm Hg, mean±SD)* | 145.4±4.5       | 148.5±5.2       | 110.5±3.5     |
| Post-treatment BP (mm Hg, mean±SD)* | 141.5±6.5**     | 145.5±4.8**     |               |
| Pretreatment LDL (mg/dl, mean±SD)* | 142.6±16.5      | 150±17.8        | 121.1±19.1    |
| Post-treatment LDL (mg/dl, mean±SD)* | 140±17.5**      | 146.2±18.3**    |               |
| Pretreatment HDL (mg/dl, mean±SD)* | 34.1±2.9        | 32.4±3.1        | 39.8±3.3      |
| Post-treatment HDL (mg/dl, mean±SD)* | 33.2±2.2**      | 35±2.8**        |               |
| Pretreatment TG (mg/dl, mean±SD)* | 199.4±20.5      | 187.6±22.4      | 152.4±28.1    |
| Post-treatment TG (mg/dl, mean±SD)* | 181.7±21.4**    | 183.1±25.6**    |               |
| Pretreatment HbA1c (%)* | 8.6±1.1%        | 8.4±1.3%        | 5.3±0.4%      |
| Post-treatment HbA1c (%)* | 7.9±1.4%**      | 7.8±1.7%**      |               |
| Sulphonylurea dose (mg, mean±SD) | 196.5±47.1      | 6.4±2.1         |               |

BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: Triglycerides
*No significant difference between the two treatment groups.
**No significant difference between pre- and post-treatment values in each of the two treatment groups.
tion in ICAM-1 levels was independent of metabolic control, since no significant reduction in HbA1c was achieved after treatment with gliclazide. Reduction in ICAM-1 was also not attributable to any effect on serum lipids, BMI or blood pressure, since these parameters were not affected by the hypoglycaemic treatment. In addition, no reduction in ICAM-1 levels was found after treatment with glibenclamide. Hence, reduction in ICAM-1 levels was shown to be a specific benefit of gliclazide treatment. Our findings are in accordance with in vitro studies, which have shown that gliclazide reduces expression of ICAM-1 in human endothelial cell cultures [6,8,9]. Reduction in ICAM-1 levels is independent of the hypoglycaemic action of gliclazide [16,19,20] and has been attributed to inhibition of a protein kinase C pathway and to inhibition of the oxidative stress-sensitive transcription factor nuclear factor–kappa B due to the antioxidant properties of gliclazide [6,8,9]. In vitro research has also shown that reduced expression of ICAM-1 is only achieved by gliclazide and not by glibenclamide or glimepiride [6,9,17]. Reduced expression of adhesion molecules contributes to the action of gliclazide in reducing monocyte adhesion to endothelial cells of diabetic patients both in vitro [6,7,17] and in vivo [24,25].

In contrast to our findings, Desfaits et al. reported that administration of gliclazide reduced monocyte adhesion to endothelial cells in vivo [24]. In the same study, there was no difference at baseline between diabetic patients and control subjects in serum adhesion molecule levels [24]. Nonetheless, this study included a very small number of patients and this may be one reason why no difference in serum adhesion levels could be demonstrated. Moreover, as has already been observed by Elhadd et al. [36], 50% of the patients were on ACE inhibitors, which may contribute to reduction in endothelial dysfunction, and all patients were on metformin, which may contribute to reduction in lipid peroxidation. Treatment with these drugs may have interfered with monocyte adhesion [20]. In contrast to the study by Desfaits et al. [24], we included a selected sample of patients with long-standing diabetes and poor metabolic control, who had increased levels of serum adhesion molecules. Moreover, no patient was on ACE inhibitors, glitazones or metformin, and so the potential action of these drugs on endothelial function was avoided.

Pre-treatment serum VCAM-1 levels in our study were significantly higher in both groups of diabetic patients than in healthy controls. This is in accordance with previous findings [6,7,12,37-39]. Although treatment with gliclazide resulted in a significant reduction in ICAM-1 levels, it did not lead to reduction in VCAM-1 levels. The difference between the effect of gliclazide on serum ICAM-1 and VCAM-1 levels in our study may be due to a difference in

### Table II

<table>
<thead>
<tr>
<th>Adhesion molecules</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>Statistical evaluation</th>
<th>Comparison between the groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A: ICAM-1 (ng/ml, mean±SD)</td>
<td>623.12±61.17</td>
<td>370.14±49.92</td>
<td>P=0.01</td>
<td>Pre-treatment ICAM-1</td>
</tr>
<tr>
<td>A vs B: P=0.91, A vs Controls: P=0.0001, B vs Controls: P=0.0001</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Group A: VCAM-1 (ng/ml, mean±SD)</td>
<td>1381.24±228.51</td>
<td>1172.97±248.66</td>
<td>P=0.67</td>
<td>Pre-treatment VCAM-1</td>
</tr>
<tr>
<td>Group B: ICAM-1 (ng/ml, mean±SD)</td>
<td>581.27±56.91</td>
<td>564.46±50.17</td>
<td>P=0.8</td>
<td></td>
</tr>
<tr>
<td>A vs B: P=0.9, A vs Controls: P=0.0001, B vs Controls: P=0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B: VCAM-1 (ng/ml, mean±SD)</td>
<td>1276.15±201.44</td>
<td>1269.51±210.68</td>
<td>P=0.99</td>
<td></td>
</tr>
<tr>
<td>Controls: ICAM-1 (ng/ml, mean±SD)</td>
<td>192.51±35.12</td>
<td>_</td>
<td>_</td>
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</tr>
<tr>
<td>A vs B: P=0.04, A vs Controls: P=0.007, B vs Controls: P=0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls: VCAM-1 (ng/ml, mean±SD)</td>
<td>413.12±101.87</td>
<td>_</td>
<td>_</td>
<td></td>
</tr>
<tr>
<td>A vs B: P=0.9, A vs Controls: P=0.0001, B vs Controls: P=0.0001</td>
<td></td>
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</tr>
</tbody>
</table>

ICAM-1: intercellular adhesion molecule 1; VCAM-1: vascular cell adhesion molecule 1.
mode of action on ICAM-1 and VCAM-1. Nevertheless, there is no data to support this explanation. There is little evidence about the action of gliclazide on serum VCAM-1 levels. Of the three studies performed in vitro [6,8,9], VCAM-1 levels were measured only in one and were found to be reduced by gliclazide administration [8]. Desfaits et al. were the only ones who studied gliclazide in vivo and found, in agreement with our results, that it had no effect on VCAM-1 levels [24]. The difference between ICAM-1 and VCAM-1 levels may be related to some other difference in mode of induction between the two adhesion molecules. Walpola et al. for example have found that shear stress has a different influence on ICAM-1 and on VCAM-1 levels [40]. Furthermore, levels of circulating ICAM-1 may be particularly important in migration of increased numbers of T-lymphocytes into active lesions and more closely related to the activity of atherosclerosis [41]. VCAM-1 may play an important role in early atherogenesis but a less important role in advanced, complex lesions [42]. Although correlation between serum VCAM-1 levels and degree of atherosclerosis has been reported [43,44], increase in serum ICAM-1 levels has been more extensively documented as a very sensitive marker of the activity of atherosclerosis in patients with coronary artery disease (acute coronary syndrome [45-50], chronic ischaemic heart disease [48,51] and risk of future myocardial infarction [52]). It seems reasonable, therefore, that reduction in serum ICAM-1 levels in our patients may be more sensitive than VCAM-1 in detecting the reduction in the activity of atherosclerosis due to gliclazide-induced reduction of monocyte adhesion. However, further research is needed to clarify this issue.

In conclusion, this study suggests that treatment with gliclazide lowers serum ICAM-1 but not VCAM-1 levels in type 2 diabetic patients with poor metabolic control. This reduction is independent of the hypoglycaemic action of gliclazide. Our findings confirm the beneficial action of gliclazide on serum adhesion molecules that was shown by in vitro studies [6,8,9]. Since monocyte-endothelium interaction is of crucial importance in the pathogenesis of atherosclerosis [2-5], it seems reasonable that gliclazide treatment in patients with type 2 diabetes mellitus may prevent the development and the progression of macrovascular disease. However, further studies are needed, so that the long-term benefit from the action of gliclazide on serum adhesion molecules in type 2 diabetic patients with or without manifest atherosclerosis might be fully elucidated.

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