Severity of diabetic microvascular complications is associated with a low soluble RAGE level

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Abstract

Aims. – The receptor for advanced glycation end-products (RAGE) has been implicated in diabetic microvascular complications, but several lines of evidence suggest that the soluble isoform of RAGE (sRAGE) may protect against AGE-mediated vessel damage. The characterized AGE N\textsubscript{e}-carboxymethyllysine (CML) is associated with diabetic microvascular complications. In the present study, we measured blood levels of sRAGE and CML-protein in diabetic patients with and without microvascular complications.

Methods. – Thirty patients with type-2 diabetes were recruited into the study, comprising 20 who had no microvascular complications, and 10 who had both retinal and renal complications. sRAGE was measured in serum by ELISA, and CML by competitive ELISA.

Results. – sRAGE blood levels were similar in both the controls and diabetic patients without microvascular complications. In patients with complications, the mean sRAGE blood level was significantly decreased (1068 ± 231 pg/mL) compared with diabetic patients without complications (P = 0.028). CML-protein was increased in all diabetic patients, but to a higher extent in those who had microvascular complications.

Conclusion. – The association of low sRAGE with high CML-protein levels in diabetic patients who developed severe diabetic complications supports the hypothesis that sRAGE protects vessels against AGE-mediated diabetic microvascular damage.

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Résumé

Association entre des concentrations faibles de RAGE soluble et complications microvasculaires du diabète.

Objectifs. – Le récepteur des produits de la glycation avancée (RAGE) est connu pour son rôle dans la survenue des complications microvasculaires du diabète. La forme soluble du RAGE (sRAGE) neutraliserait les AGE et leur toxicité vasculaire. La N\textsubscript{e}-carboxymethyllysine (CML) est un AGE trouvé en excès chez les patients diabétiques souffrant de complications microvasculaires. Au cours de ce travail, nous avons dosé les concentrations sériques de sRAGE et de CML chez des patients diabétiques avec ou sans complications microvasculaires.

Méthodes. – Le sRAGE et la CML ont été dosés par des techniques immunologiques dans le sérum de 30 diabétiques, dont 20 indemnes de rétinopathie et de néphropathie, et dix atteints de celles-ci.

Résultats. – Les concentrations de sRAGE étaient similaires chez les témoins non diabétiques et les diabétiques indemnes de complications microvasculaires. Les patients atteints de complications avaient des concentrations significativement inférieures (1068 ± 231 pg/mL) comparés aux patients sans complications (P = 0,028). Les concentrations de CML-protéine étaient augmentées chez les diabétiques et de manière plus importante chez les patients atteints de complications microvasculaires.

Abbreviations: AGE, advanced glycation end-product; RAGE, receptor for AGE; sRAGE, soluble RAGE; CML, N\textsubscript{e}-carboxymethyllysine.

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Conclusion. – L’association de concentrations faibles de sRAGE et de concentrations élevées de CML chez les patients qui ont développé des complications microvasculaires sévères est cohérente avec l’hypothèse selon laquelle le sRAGE pourrait protéger les vaisseaux de la toxicité des AGE.

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Keywords: Type-2 diabetes; Advanced glycation end-products receptor (RAGE); Soluble RAGE; Microvascular complications

Mots clés : Diabète de type 2 ; Produits de la glycation avancée (AGE)

1. Introduction

Retinopathy and glomerulosclerosis are common complications of diabetes mellitus. There is substantial evidence to support the involvement of advanced glycation end-products (AGE) binding to its receptor (RAGE) in the development of diabetic microvascular complications [1]. Activation of RAGE induces oxidative stress, increased permeability and an inflammatory response in the vessel wall [2]. RAGE is a member of the immunoglobulin superfamily, and was originally described as a transmembrane multiligand receptor. Recently, a soluble isoform of RAGE (sRAGE), generated by splicing the RAGE gene transcript, has been described [3]. In diabetic patients with end-stage renal disease, low circulating sRAGE is a predictor of cardiovascular mortality [4], suggesting that sRAGE may protect against AGE-mediated vessel damage. We previously observed that Nε-carboxymethyllysine (CML)-protein was increased in diabetic patients with microvascular complications [5]. In the present study, we measured blood levels of sRAGE and CML-protein in diabetic patients with and without microvascular complications.

2. Material and methods

2.1. Patients

Our study was approved by the internal review boards of the participating institutions, and the informed consent of all patients was obtained. Patients were recruited from our diabetes departments between November 1999 and December 2000. A total of 30 patients (eight women and 22 men) with type-2 diabetes were included in the study. Urinary albumin excretion (UAE) was routinely determined [6]. Patients with persistent microalbuminuria (30–300 mg/24 h), a clinical symptom of glomerulosclerosis, were considered to have nephropathy. Retinopathy was defined as the presence of microaneurysms, haemorrhage, hard and soft exudates, intraretinal abnormalities and/or clearly evident venous beading [7]. Glycated haemoglobin (HbA1c) was measured using variant II (Biorad-France, Marnes la Coquette, France), and CML-protein was measured by competitive ELISA, using purified antibodies specific for CML [5]. Twenty patients had no microvascular complications, and 10 had both retinal and renal complications. Patients’ characteristics are presented in Table 1. Patients treated with angiotensin–converting enzyme (ACE) inhibitors or angiotensin-receptor blockers (ARB) were excluded from the study as these agents were reported to be capable of modifying sRAGE blood levels. Non-diabetic gender-matched subjects (n = 29) were enrolled as controls. These subjects were younger (mean age: 52.1 ± 1.0 years, range: 39–62) than the diabetic patients (mean age: 59.7 ± 1.0 years, range: 50–70) (P < 0.001). However, in this study, neither sRAGE nor CML blood levels were correlated with age.

2.2. sRAGE measurement

sRAGE protein was measured in the serum of both diabetic patients and control subjects using human Quantikine

Table 1

<table>
<thead>
<tr>
<th>Study participants’ characteristics</th>
<th>Control subjects (n = 29)</th>
<th>Diabetic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without complications (n = 20)</td>
<td>With complications (n = 10)</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>28</td>
<td>35</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.1 ± 1.0</td>
<td>59.7 ± 1.2</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>0</td>
<td>7.1 ± 2.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22 ± 2.1</td>
<td>26.2 ± 0.9</td>
</tr>
<tr>
<td>Glycaemia (g/L)</td>
<td>0.9 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.0 ± 1.0</td>
<td>7.7 ± 0.6</td>
</tr>
<tr>
<td>UAE (mg/24 h)</td>
<td>&lt; 30</td>
<td>9.2 ± 1.4</td>
</tr>
<tr>
<td>CML-protein (pmol/mg prot)</td>
<td>8.7 ± 1.4</td>
<td>31.0 ± 5.1</td>
</tr>
<tr>
<td>Treatment (% of patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>0</td>
<td>6.2</td>
</tr>
<tr>
<td>Oral agent</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>Insulin</td>
<td>0</td>
<td>12.5</td>
</tr>
<tr>
<td>Oral agent + Insulin</td>
<td>0</td>
<td>6.2</td>
</tr>
</tbody>
</table>
| Complications: retinopathy and urinary albumin excretion (UAE; 30–300 mg/24 h); CML, Nε-carboxymethyllysine.

*P < 0.05, **P < 0.01 versus diabetics without complications.
RAGE (R&D Systems, Abingdon, Berks, UK) according to the manufacturer’s instructions. Briefly, 50 µL of serum was incubated on a monoclonal anti-RAGE antibody precoated plate. After washing, enzyme-linked polyclonal antibody specific for the extracellular domain of RAGE was added. The reaction was revealed by substrate solution and optical density was measured.

2.3. Statistical analysis

Serum sRAGE levels were presented as follows (Fig. 1):

- the solid line within the box represents the median;
- the dotted line represents the mean;
- the vertical lines extending beyond the boxes indicate the 25% and 75% percentiles;
- the horizontal bars outside the boxes represent the 10% and 90% percentiles;
- the dots indicate values outside of this range.

Statistical significance was determined using one-way analysis of variance (ANOVA) followed by the parametric Dunnett’s test. A P value less than 0.05 was considered statistically significant.  

3. Results

Patients with microvascular complications had significantly higher glycaemia (P < 0.05) and higher levels of HbA1c (P < 0.01) compared with patients without microvascular complications. CML-protein was increased in diabetic patients compared with controls (36.3 ± 5.0 versus 8.7 ± 1.4 pmol/mg protein, respectively; P < 0.001). CML-protein blood levels were higher in patients who had microvascular complications compared with those who did not (46.4 ± 12 versus 31.0 ± 5.1 pmol/mg protein, respectively), as has been previously reported [5] although, in the present group of patients, the difference was not statistically significant.

Serum sRAGE levels in both the diabetic patients (with and without complications) and healthy controls were similar (1472 ± 121 versus 1400 ± 111 pg/mL, respectively). No statistical correlation was found between sRAGE blood levels and other parameters such as age, body mass index (BMI), CML-protein in serum, HbA1c or diabetes duration. However, patients with renal and retinal complications had significantly (P < 0.05) lower blood levels of sRAGE (1068 ± 136 pg/mL) compared with patients without complications (1575 ± 139 pg/mL) (Fig. 1).

4. Discussion

In the present study, lower levels of sRAGE were found in patients who developed both diabetic retinopathy and nephropathy. CML-protein was increased in all diabetic patients, but was highest in those with microvascular complications [5,8]. The association between low levels of sRAGE and high levels of CML-protein in patients with microvascular complications suggests that the production of sRAGE was probably insufficient to clear the excess CML-protein. This unbalanced ratio allowed circulating AGE to bind to cell RAGE, leading to endothelial dysfunction, an early step in diabetic microvascular complications. On the other hand, patients with higher sRAGE and lower CML-protein levels did not exhibit vascular complications. sRAGE may act as a decoy of AGE to maintain vascular homeostasis. This hypothesis is consistent with the results showing that, in type-2 diabetes, a decrease of sRAGE is associated to a high incidence of cardiovascular mortality [4]. In type-1 diabetes, a low sRAGE blood level is related to the severity of retinopathy and to vessel-wall thickness [9]. In type-2 diabetes, sRAGE is correlated with serum levels of macrophage colony-stimulating protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α). sRAGE has been proposed as a possible marker of vascular inflammation [10]. Blocking RAGE activation with the use of recombinant sRAGE limited the development of atherosclerotic lesions in ApoE-null mice, in which RAGE ligands mediate vascular and inflammatory stress [11–14]. sRAGE expression is affected by drugs such as ACE inhibitors and rosiglitazone, and patients treated with these drugs have significantly higher levels of circulating sRAGE [15]. Thus, stimulation of sRAGE production should be considered as a potential therapeutic target in diabetes and AGE-related vascular disease.

In conclusion, we have found that sRAGE blood levels are lower in diabetic patients who have both retinal and renal complications, supporting the hypothesis that sRAGE, by limiting the interaction of AGE with cell membrane RAGE, can protect vessels against AGE toxicity.
References