Receptors for advanced glycation end-products (AGE)—expression by endothelial cells in non-diabetic uraemic patients


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Abstract

Background. Cellular actions of advanced glycation end-products (AGE) are mediated by a receptor for AGE (RAGE), a novel integral membrane protein. Immunohistochemical studies show only low-level RAGE antigen expression in endothelial cells.

Design. It was the purpose of the study to compare expression of RAGE antigen by endothelial cells in non-diabetic uraemic patients (n = 8) with non-uraemic controls (n = 11). Samples of arterial tissue were obtained at the time of renal transplantation (in uraemic patients) and abdominal surgery (in controls). RAGE antigen was visualized using guinea-pig anti-RAGE IgG and PAP technique.

Results. Marked staining for RAGE was noted in endothelial cells, both arterial endothelium and endothelium of vasa vasorum of normoglycaemic uraemic patients, but was not demonstrable in endothelial cells of large arteries and only faintly expressed in vasa vasorum of non-uraemic individuals.

Conclusion. Normal endothelial cells do not constitutively express RAGE antigen; in contrast it is expressed by arterial and capillary endothelial cells of uraemic patients. The observation is of note in view of the putative role of AGE of causing atherosclerotic and non-atherosclerotic vascular lesions.

Introduction

Accumulation of advanced glycation end-products (AGE) from the products of non-enzymatic glycation proteins [1-3] has been related to late vascular and non-vascular complications of diabetes mellitus [1,4-8]. AGE interact with cells of the vessel wall via specific receptors which had been identified on endothelial cells, but are also present on mononuclear phagocytes and other cells [9,10]. The best characterized receptor has been termed receptor for AGE or RAGE. RAGE is a new member of the immunoglobulin superfamily of cell-surface molecules. It consists of an extracellular domain of three immunoglobulin-like domains, of a single transmembrane spanning domain, and a highly charged cytosolic tail [11]. Intergenic studies showed that RAGE is responsible for clearance of AGE from the intravascular space. RAGE mediates interaction of AGE with the vessel wall and AGE-induced gene expression [12]. Engagement of these receptors results in increased endothelial cell monolayer permeability [9], oxidant stress [13,14] and adhesivity for mononuclear phagocytes [13] as well as induction of mononuclear phagocyte chemotaxis and cytokine/growth factor generation [15-20].

In normal bovine tissue a recent survey showed expression of RAGE in smooth-muscle cells, nervous tissue, and vessel wall, excluding, however, vascular endothelial cells [21]. In contrast, RAGE is expressed by endothelial cells of vasa vasorum in patients, both diabetic and non-diabetic, with arterio-occlusive disease [22].

Atherosclerosis is a common feature of uraemia. Furthermore, several authors showed accumulation of AGE in the circulation of uraemic patients [23,24]. This led to the question of whether RAGE antigen is expressed by endothelial cells of uraemic patients [25]. This issue was addressed in the present study, where we compared samples of the iliac artery of normoglycaemic uraemic patients (obtained at the time of renal transplantation) and of mesenteric arteries in non-diabetic non-uraemic individuals (obtained at the time of abdominal surgery).

Subjects

Description of patients

We obtained samples of the iliac artery from six patients of both sexes ranging from 28 to 59 years, who had been on dialysis for a median period of 32,333 months (18-73 months) and who had documented fasting normoglycaemia. Underlying renal diseases were chronic, membranoproliferative, or perimembranous glomerulonephritis.
As controls we used 11 patients of both sexes, ranging from 1 to 79 years who had had abdominal surgery for the following reasons: resection of carcinoma of the large intestine (n = 7), liver transplantation (n = 3), and removal of a toe in a polydactylic patient (n = 1). The vessels were mesenteric arteries (taken from the site of branching) and a terminal branch of A. dorsalis pedis respectively. The protocol was approved by the local ethical committee and informed consent was obtained.

Harvesting and preparation of tissues

The samples were immediately snap-frozen in isopentane—liquid nitrogen and then stored at −80°C.

Immunological techniques

For immunostaining we used the IgG of a polyclonal antiserum which had been raised in guinea-pigs against a single band of bovine RAGE (=35 kDa). The latter had been purified from lung tissue. As described elsewhere [21,26]. The antibody has been well characterized, including Western blotting, ELISA and immunostaining. Staining was specific since it was blocked by preincubation of antibody with purified bovine RAGE.

For immunostaining cryostat sections (4–12 μm) were cut and fixed in ice-cold acetone for 10 min, incubated with anti-RAGE IgG raised against bovine RAGE in a humidified atmosphere overnight at 4°C. Second antibody (rabbit anti-guinea-pig immunoglobulin, Dakopatts, Glostrup, Denmark) was added for 30 min at room temperature. Subsequently the third antibody (swine anti-rabbit immunoglobulin, Dakopatts, Glostrup, Denmark) was added for 30 min. This was followed by incubation with Dakopatts rabbit PAP (soluble complexes consisting of rabbit antibody to horseradish peroxidase and horseradish peroxidase) for 30 min. Between each step of this procedure sections were washed for 10 min in phosphate-buffered saline (PBS) at room temperature. Peroxidase activity was detected using aminothiocarbazole as chromogen. In control sections, primary antibody was replaced by PBS or normal serum. Immunohistochemical staining was evaluated without knowledge of the diagnosis by three independent investigators (JG, IK, and RW). The results were expressed as a score, as is more fully described in the Results section.

Statistical analysis

Statistical analysis was performed using the Mann–Whitney–Wilcoxon test.

Results

Microvascular endothelium

The control group consisted of 11 patients as described above. Endothelium from vasa vasorum of normal vessels demonstrated variable, low-intensity staining for RAGE antigen as shown in Figure 1 (A). Although staining for RAGE was not intense, it was the result of specific binding of the antibody to the antigen, as it was not observed with non-immune IgG (not shown) and preadsorption of the antibody with purified bovine RAGE abrogated the staining (data not shown, see Subjects and methods).

The staining was quantified as described above, showing that eight of the 11 controls were immunohistochemically negative and three had little staining (one+) as shown in Figure 2 (A).

This is consistent with the results of the bovine survey [21] and with previous results of our group [22].

In uraemic, non-diabetic patients (n = 8) half of the sections (4) showed marked immunostaining (+ +) as shown in Figure 1 (B). The other half (4) was also positive (+). This is shown in Figure 2 (B). There were no negative sections in regard to vasa vasorum in this group. This difference is described by P < 0.05 in the Mann–Whitney–Wilcoxon test.

Macrovascular endothelium

Macrovascular endothelium was negative for RAGE in eight of the 11 non-diabetic, non-uraemic control individuals as shown in Figure 1 (C) and moderately positive (+) in three of the controls.

The distribution of anti-RAGE positivity (see Figure 2 (C)), therefore, was comparable to microvessels of the control group. This result confirmed previous work in bovine and human tissue [21,22].

The macrovascular endothelium of renal arteries in uraemic, non-diabetic, patients was positive for RAGE in all patients evaluable (n = 6). A typical immunohistochemical picture of this is given in Figure 1 (D). Five of six patients in this group showed strong positivity (+ +) and one patient was moderately but clearly positive (+). This is shown in Figure 2 (D). The difference in immunopositivity is described by P < 0.05 in the Mann–Whitney Wilcoxon test.

Smooth-muscle cells

RAGE appeared to be unchanged in vascular smooth-muscle cells (data not shown), a cell type which constitutively expresses considerable amounts of RAGE according to the bovine survey [21] and our previous work [22].

Discussion

The above results clearly document expression of the RAGE antigen by endothelial cells of large arteries and their vasa vasorum in non-diabetic uraemic patients. This finding contrasts with the near absence of RAGE antigen in endothelial cells of non-uraemic patients. The latter finding is in agreement with previous observations in bovine tissue [21] and human tissue [22].

Expression of RAGE has also been shown in endothelial cells of vascular tissue obtained from diabetic patients [22] or patients with ischaemia secondary to arterio-occlusive disease [22].

It is conceivable, though not directly proven, that AGE interact with RAGE on endothelial cells. As this ligand–receptor system may play a role in arterio-occlusive disease [22] and diabetes without uraemia,
the presence and enhancement of RAGE in uraemia implies that vascular complications in uraemia might, at least in part, be due to a similar mechanism. This thesis is supported by the fact that AGEs are increased in non-diabetic patients on dialysis [27], correlate with serum creatinine, and decrease after kidney transplantation. Furthermore, an AGE-modified form of beta-2-microglobulin is a major component of the amyloid associated with haemodialysis [23,28,29].

The mechanism by which RAGE is enhanced, however, has still to be elucidated. A possible reason for enhanced RAGE in uraemia may be cytokines like IL1 and TNF that are known to be increased in uraemia. We have previously observed that TNF enhances RAGE expression in cultured endothelial cells (unpublished observation). Such cytokine regulation of RAGE expression is also supported by previous work in which TNF increased the binding of AGE albumin to mononuclear phagocytes [15]. A cytokine-mediated enhancement of RAGE expression in uraemia may therefore be possible.

AGEs, enhanced in uraemia, might also induce RAGE. In this context, AGEs may represent a modern-day version of ‘middle-molecules’. If they were pathophysiological relevant, their presence would provide a strong rational for the use of high-flux membranes, as these membranes reduce AGE levels in uraemia [24]. One might speculate if this would in return reduce RAGE levels and therefore provide a significant therapeutic tool in preventing vascular and non-vascular [30] complications in uraemia.

Non-AGE ligands of RAGE could potentially also modulate RAGE expression. One of such candidates is amphoterin [31]. It has been described so far in connection with nerve outgrowth and development. As its relation to vascular complications remains unclear, we wish to refrain from further speculation at this point. The proinflammatory, proproliferative, and

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**Fig. 1.** Immunohistochemistry (magnification x 340).
A. Vasa vasorum of normal vessels exhibit little or no RAGE-like immunoreactivity in endothelial cells.
B. Immunoreactivity for RAGE in uraemic individuals is strongly positive in vasa vasorum.
C. Macrovascular endothelium is largely negative for RAGE in normal individuals.
D. Uraemic patients show strong immunopositivity for RAGE in macrovascular endothelial cells.
repair-mechanism-like effects of AGE and RAGE might play an important role in the development of vascular and proliferative complications in kidney disease. This, however, requires the occurrence of RAGE. We claim that RAGE is abundantly present in endothelial cells of vessels in uraemia.

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