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The relationship between receptor for advanced glycation end products expression and the severity of periodontal disease in the gingiva of diabetic and non diabetic periodontitis patients

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ABSTRACT

Objective: Activation of receptor for advanced glycation end products (RAGE) has been implicated in many chronic diseases, including diabetic complications. In this study we examined the relationship between RAGE expression and the morphological changes seen in the gingiva of diabetic and periodontitis patients.

Design: Gingival biopsies from 15 diabetic patients with periodontitis, 25 non diabetic patients with periodontitis and 10 healthy individuals were collected. Sections were stained with haematoxylin and eosin, and immunohistochemically to detect RAGE. Samples were examined in light and fluorescence microscopes and histomorphometric analysis was performed.

Results: Increased number of inflammatory cells and changes in collagen and vasculature were observed in diabetic and non diabetic patients with periodontitis. RAGE was weakly expressed in healthy gingiva. The strongest reaction with anti-RAGE antibody was found in the gingiva of diabetic patients with periodontitis followed by the severe periodontitis patients. RAGE expression in inflammatory cells was stronger than in the epithelium. The number of inflammatory cells in the gingiva was higher in the diabetic periodontitis patients than in the non diabetic severe periodontitis patients.

Conclusions: RAGE is strongly expressed in the gingiva of diabetic patients with periodontitis and with severe periodontitis alone, the latter indicating RAGE activation even in the absence of hyperglycemia. However our findings are based on relatively small sample size. With a larger patient population, some of our other findings may have reached statistical significance.

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Abbreviations: AGEs, advanced glycation end products; CAL, clinical attachment level; DAB, 3,3′-diaminobenzidine; HbA1c, glycated haemoglobin; ICAM-1, intercellular cellular adhesion molecule-1; NF-κβ, nuclear factor-kappa beta; PPD, periodontal pocket depth; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; sRAGE, soluble RAGE; VCAM-1, vascular cell adhesion molecule-1.

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1. Introduction

Periodontitis is considered to be the sixth major complication of diabetes along with retinopathy, nephropathy, neuropathy, microvascular disease and impaired wound healing. The receptor for advanced glycation end products (RAGE) plays an important role in the pathogenesis of diabetic complications.[1] RAGE is a member of the immunoglobulin superfamily of cell-surface receptors that bind to a broad range of ligands. RAGE is an integral membrane protein composed of three extracellular immunoglobulin-like domains, a single transmembrane-spanning region and a short negatively charged cytosolic tail domain responsible for initiating RAGE signalling.[2–4] The first identified ligands of RAGE were advanced glycation end products (AGEs). AGEs are formed due to hyperglycemia and hyperlipidemia associated with diabetes mellitus that promote non-enzymatic glycation and oxidation of tissue proteins or lipids.[5–7] Despite their structural diversity, AGEs bind only to the first 30 amino acids of the V domain of RAGE.[8] Binding triggers an array of signal transduction cascades leading to inflammation. Activation of the full-length RAGE receptor has been implicated in chronic diseases, including diabetic nephropathy, neuropathy, and impaired angiogenesis.[9–13]

In addition to AGEs, other endogenous ligands are implicated in amplifying RAGE-dependent proinflammatory signalling: cytokine-like mediators of the S100 family,[14] amphoterin,[15] amyloid β peptide (Aβ), β-sheet fibrils[16,17] and Mac-1.[18] Thus the structural diversity of RAGE ligands suggests that RAGE is a pattern recognition receptor (PRR)[19] and that RAGE can play a key role in tissue inflammation.

Some of the biological characteristics of RAGE suggest that it may also play a pivotal role in the pathogenesis of periodontal disease by inducing the generation of reactive oxygen species (ROS).[20,21] Infusion of AGEs into normal mice results in generation of thiobarbituric acid reactive substances (TBARS), and other markers of oxidative stress in a variety of tissues including gingiva.[22] One of the key targets of ROS in the cells is the transcription factor, nuclear factor kappa beta (NF-κβ), a critical factor in the transduction of a variety of inflammatory and pro- or anti-apoptotic signals in the cells, depending on the time course, site, and chronicity of the stimulus. An important consequence of RAGE-dependent activation of NF-κβ is the upregulation of RAGE itself[23] and the induction of adhesion molecules like vascular cell adhesion molecules (VCAM-1) and intercellular cellular adhesion molecules (ICAM-1). Furthermore, a possible feedback-loop of increased expression of RAGE arises from AGE/RAGE interaction.[24]

It is well established that periodontitis is a complication of diabetes[25] and diabetic patients are at higher risk for severe and progressive periodontitis,[26–30] but the underlying cellular and molecular mechanisms are not completely understood. We hypothesize that the severity of periodontitis in diabetic patients may be attributed, at least in part, to the different distribution or level of RAGE expression in diabetic gingiva. In this work, we investigate the relationship between the pattern and level of RAGE expression and the morphological changes seen in the gingiva of diabetic and non diabetic patients with periodontitis in order to gain better understanding of cellular mechanisms underlying the increased susceptibility to periodontal disease in diabetics and severity of tissue destruction in diabetic periodontitis.

2. Materials and methods

2.1. Tissue samples

Tissue samples used in this study were obtained from patients of the Faculty of Oral and Dental Medicine, Cairo University, during periodontal surgery, surgical extraction or preprosthetic surgery. All patients gave their university approved informed written consent to participate in the study. Use of human material in this research was in accordance with the Declaration of Helsinki.[31] Only male patients were selected for this study to avoid the possible adverse effects of female ovarian hormones that could increase the gingival inflammation.[32] The age distribution was similar in all groups and ranged from 40 to 60 years (mean age = 48 years). Smokers were excluded from this study. Samples were collected over a six months period, beginning in December 2006 and ending in June 2007. Patient participation was limited to data collection and gingival biopsy. Distribution of all groups is shown in Table 1.

The gingival biopsies (one per person) were collected from 15 diabetic patients (type 2 diabetes mellitus) with periodontitis (diabetic group) and from 25 patients with chronic periodontitis and without diabetes mellitus (periodontitis group). In addition, gingival biopsies were obtained from 10 donors with no known history of diabetes mellitus or periodontitis (control group). The specimens were collected from buccal or lingual free or interdental papilla gingiva of mandibular posterior teeth in case of the diabetic and periodontitis groups, and from maxillary posterior teeth in the control group.

Periodontal evaluation was performed for all the patients using:

1. Gingival index.[33]
2. Periodontal pocket depth (PPD). The site where the biopsy was taken in the periodontitis and diabetic group had a pocket depth of at least 4 mm.
3. Clinical attachment level (CAL).
4. Radiographic examination using panoramic technique. Screening criteria for chronic periodontitis was the presence of at least 5 sites with ≥4 mm horizontal alveolar bone loss on radiographs. Within our clinic setting, these radiographic criteria provided an efficient way to select periodontitis patients with little chance of misdiagnosing gingivitis as periodontitis.

Periodontal pocket depth (PPD) and clinical attachment level (CAL), were used to categorize severity of periodontitis with mild periodontitis defined as mean PPD ≤ 4 mm and CAL ≤ 5 mm; moderate periodontitis as PPD 5–6 mm and CAL 6–7 mm; and severe periodontitis as PPD ≥ 7 mm and CAL ≥8 mm. While these are non-traditional definitions of disease severity, the patient population sampled here had a high prevalence of advanced periodontitis. These criteria