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Role of advanced glycation end product receptors in the pathogenesis of diabetic retinopathy

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Abstract

Problem: Advanced glycation end products (AGEs) and the interaction with their receptors (RAGE) play an important role in the pathogenesis of diabetic retinopathy (DR). Our study investigated whether serum soluble (s) RAGE (sRAGE) could serve as a prognostic tool for identifying the susceptibility to DR. Moreover, we examined the association between soluble forms of vascular cell adhesion molecules (sVCAM-1), nitric oxide (NO) and sRAGE levels in serum and the severity of DR. **Methods:** Circulating levels of sRAGE, sVCAM-1, and NO were examined in 37 type 2 diabetic patient and 20 age-matched healthy nondiabetic subjects using ELISA. The diabetic subjects were categorized as patients without retinopathy, patients with nonproliferative DR (NPDR), and patients with proliferative DR (PDR). **Results:** Serum sRAGE levels were significantly lower in patients with NPDR and PDR than in healthy controls and in those without retinopathy (1331.13±126.13, 934.87±66.27 vs. 1712.69±167.3, 1833.1±153.06 pg/ml, respectively, $P<.05$). Serum sVCAM-1 and NO were significantly higher in diabetic patients (1310.215±54.712 vs. 616.55±12.9 ng/ml and 96.432±0.864 vs. 28.78±5.88 μmol/l, respectively, $P<.05$) and were positively associated with the severity of DR. **Conclusions:** The results indicate that sRAGE is an endogenous protection factor against the occurrence of accelerated DR.

Keywords: AGEs; sRAGE; sVCAM-1; NO; DR

1. Introduction

Diabetes mellitus (DM), ranked as the fourth leading cause of death by disease globally (Kowluru & Chan, 2007), has become one of the most challenging problems of the 21st century. The disease affects more than 230 million people worldwide, and this number is expected to reach 350 in 2025. Diabetic retinopathy (DR) is one of the most significant complications of DM, it occurs in 90% of patients after 20–30 years from the disease diagnosis, and its advanced form, proliferative diabetic retinopathy (PDR), affects over 60% of diabetic patients (Fong, Aiello, & Gardner, 2003). Studies proposed that DR signs may reflect generalized microangiopathic processes that affect not only the eyes but also organs elsewhere in the body (Mohamed, Gillies, & Wong, 2007). Therefore, apart from being a manifestation of microvascular damage in the retina, DR should be viewed as a biomarker of underlying widespread deleterious effects of abnormal glucose metabolism on the systemic microcirculation (Cheung & Wong, 2008).

A hallmark of early DR is the change in the structure and cellular composition of the microvasculature (Ciulla, Amador, & Zinman, 2003). The degree of retinopathy was classified into two stages: NPDR and PDR (Miura et al., 2004). The diabetic milieu causes pericyte dropout from retinal blood vessels leading to dysfunction of endothelial cells (ECs) which is responsible for maintaining the blood–

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retinal barrier. The latter translates to an array of vascular abnormalities typical of NPDR, basement membrane thickening, microaneurysms, hemorrhages, and, eventually, capillary occlusion. Blood vessels with reduced pericyte coverage are more susceptible to angiogenic factors and neovascularization, which marks the beginning of PDR (Motiejunaite and Kazlauskas, 2008). The incidence of PDR was found to be associated with longer duration of diabetes and higher blood glucose levels compared with NPDR (Park et al., 2008).

The duration of diabetes and severity of hyperglycemia are the major risk factors for developing retinopathy. Once present, duration of diabetes appears to be a less important factor than hyperglycemia for progression from earlier to later stages of retinopathy (Snow, Weiss, & Mottur-Pilson, 2003). Pathogenic mechanisms linking hyperglycemia with DR include increased polyol pathway flux, protein kinase C activation, increased advanced glycated end product (AGE) formation, increased hexosamine pathway flux, enhanced formation of reactive oxygen species, increased production of vascular endothelial growth factor, and altered generation of endothelial NO (Yokoi et al., 2007).

The hypothesis, receiving considerable interest, is the role of protein glycation. The result of nonenzymatic glycoxidation of macromolecules is the formation of AGEs. AGEs are complex, heterogeneous molecules that cause protein cross-linking, formed by the Maillard process (Nessar, 2005). Receptors of AGEs (RAGE) are members of the immunoglobulin superfamily of cell surface molecules. The AGE–RAGE system appears to play a central role in the development/progression of diabetic micro- and macrovascular complications (Gohda et al., 2008). Engagement of RAGE by AGEs activates its downstream signaling and subsequently evokes oxidative stress and inflammatory responses in vascular wall cells, thus contributing to the development and progression of DR (Yamagishi et al., 2008). RAGE is a multiligand receptor composed of three extracellular domains including a V-type domain that possesses ligand binding properties and two C-type immunoglobulin domains. A fourth transmembrane domain anchors RAGE in the membrane and is connected to a highly charged fifth intracellular domain that mediates interaction with cytosolic transduction molecules (Lindsey, Cipollone, Abdullah, & McGuire, 2009).

Three major RAGE mRNA variants are expressed. They encode the full-length RAGE (full-length type), a variant protein lacking the NH2-terminal region (N-truncated type), and another variant lacking the COOH-terminal region (C-truncated type). Because the V domain of RAGE has been shown to be the binding site for AGE ligands, the N-truncated RAGE does not bind AGE (Yonekura et al., 2003). The C-truncated type lacks the transmembrane domain and is secreted extracellularly and detected in serum as endogenous secretory (es) RAGE (Katakami et al., 2009). The ratio of the expression of the three variants is different between ECs and pericytes. This difference contributes to our understanding of the molecular basis for the diversity of cellular responses to AGEs and for individual variations in the susceptibility to diabetic vascular complications (Yonekura et al., 2003).

There are other forms of soluble RAGE (sRAGE) that are proteolytically cleaved from cellular surface by action of matrix metalloproteinases (MMPs) and shed into the bloodstream. So, total circulating sRAGE is a sum of esRAGE and RAGE which is shed by MMP action (Katakami et al., 2009). Unlike cell-surface RAGE, sRAGE contributes to the removal/detoxification of AGEs by acting as a decoy. Although it retains the capacity to bind ligands, it blocks RAGE-dependent cellular responses (Basta, 2008).

Cell adhesion molecules, such as soluble vascular cell adhesion molecule-1 (sVCAM-1), may take part in the process of neovascularization, involved in the development of retinopathy. Its elevated plasma level reflects a deterioration of the endothelium function (Siemianowicz, Francuz, Gminski, Telega, & Syzd, 2005).

Retinopathy could serve as a useful biomarker to improve risk stratification in people with diabetes as it is a common, uniquely specific, and noninvasively assessable measure of diabetic microangiopathic burden (Cheung & Wong, 2008). The current study showed that RAGE is related to clinical factors indicating deterioration of DR and that its soluble forms may act as a naturally occurring inhibitor of the signaling induced by the interaction of AGEs with its cellular receptor. Thus, sRAGE may have a protective role against the progression of DR.

2. Subjects and methods

We recruited 57 participants (age range, 50–72 years). All patients were diagnosed by ophthalmoscopy and fundus stereophotography after pupil dilation by a specialized ophthalmologist. Determination of the presence or absence of DR was conducted in a masked fashion. For each participant, a data sheet was completed with the patient's identification code, age, sex, duration of diabetes, and the antidiabetic agent used, if any. Exclusion criteria included ischemic cerebrovascular and cardiovascular disorders, hepatic and renal disorders, presence of hematological diseases, and history of malignancy. The study protocol was approved by the Department of Biochemistry Council, Faculty of Pharmacy, Ain Shams University. This council has an ethics authority. Informed consent was obtained from all participants.

Participants were divided into four groups. Their descriptive data are shown in Table 1. Group I is composed of healthy volunteers (n=20) with no DM or systemic or local eye lesion. Group II is composed of type 2 diabetic patients (n=14) who did not develop DR. Absence of DR was defined as complete absence of macular edema, hard exudates, blot hemorrhage, microaneurysms, cotton wool spots, or neovascularization. Group III is composed of type 2