Nurture vs. nature in diabetic vasculopathy: roles of advanced glycation endproducts and the receptor for them

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Nurture *vs* Nature in Diabetic Vasculopathy: Roles of Advanced Glycation Endproducts and the Receptor for Them

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Abstract.

As is diabetes itself, diabetic vasculopathy is a multi-factor disease. Studies conducted in this lab revealed advanced glycation endproducts (AGE) as the major environmental account for vascular cell derangement characteristic of diabetes, and the receptor for AGE (RAGE) as the major genetic factor that responds to them. AGE fractions that caused the vascular derangement were proven to be RAGE ligands. When made diabetic, RAGE-overexpressing transgenic mice exhibited the exacerbation of the indices of nephropathy, and this was prevented by the inhibition of AGE formation. We also created RAGE-deficient mice. They showed marked amelioration of diabetic nephropathy. Extracellular signals and nuclear factors that induce the transcription of human RAGE gene were also identified, which would be regarded as risk factors of diabetic complications. Through an analysis of vascular polysomal poly(A)$^+$RNA, we came across a novel splice variant coding for a soluble RAGE protein, and named it endogenous secretory RAGE (esRAGE). esRAGE was able to capture AGE ligands and to neutralize the AGE action on endothelial cells, suggesting that this variant has a potential to protect blood vessels from diabetes-induced injury. The AGE-RAGE system should thus be regarded as a candidate molecular target for overcoming this life- and QOL-threatening disease.

Introduction

The number of peoples who are suffering from diabetes mellitus is steadily increasing. Before being lethal, quality of life (QOL) is greatly impaired in the patients with diabetes due to vascular complications like retinopathy and nephropathy. To understand how blood vessels are impaired in the diabetic state is, therefore, crucially important. As is diabetes *per se*, diabetic complications are caused by multiple environmental and genetic factors, and we should identify such accounts one by one. Those efforts should reveal effective molecular targets for our overcoming this life- and QOL-threatening disease.

1. **Advanced glycation endproducts (AGE) and the receptor for AGE (RAGE)**

In 1912, Maillard described that reducing sugars like glucose can react non-enzymatically with the amino groups of proteins to form Schiff bases and then Amadori compounds. These early glycation products undergo further reactions, including dehydration, condensation and crosslinking, yielding irreversible protein derivatives termed AGE. The AGE formation and accumulation are most accelerated under diabetes. Non-enzymatic pathways that are distinct from the classical Maillard reaction but can also yield AGE have recently been known (1). They use short-chain carbonyl compounds, such as glyceraldehyde, glyoxal, glycolaldehyde, methylglyoxal and 3-deoxyglucosone, as intermediates. These short-chain aldehydes are more reactive than glucose, taking shorter time period to accomplish the AGE formation, and do occur in human bodies. Most AGE structures derived from the short-chain carbonyl remain to be defined.

Cellular receptors to which AGE can bind have been described. Among them is RAGE, which was initially isolated from bovine lung (2). It belongs to the immunoglobulin superfamily, having three immunoglobulin-like domains in the N-terminal extracellular segment, one transmembrane region and a short C-terminal intracytoplasmic stretch. RAGE is also known as a multi-ligand receptor not only for AGE but also for amphoterin, S100 proteins, amyloid-β proteins and transthyretin.

We prepared AGE by incubating BSA with glyceraldehyde, glyoxal, glycolaldehyde, methylglyoxal and 3-deoxyglucosone, and tested their abilities to bind purified human RAGE protein using a BIACORE surface plasmon resonance system.

Glyceraldehydes- and glycolaldehyde-derived AGE were found to be associated with the sensor chip, as the control glucose-derived AGE was. Apparent dissociation constant (Kd) values of RAGE interaction with glyceraldehyde-derived AGE and glycolaldehyde-derived AGE were estimated to be ~300 nM and ~1.4 μM, respectively, when assessed either by the BIACORE assay or by Scatchard analysis with intact cells and ^{125}I-labeled AGE. The results indicated that glyceraldehydes- and glycolaldehyde-derived AGE are new RAGE ligands.

We thus next examined whether glyceraldehydes- and glycolaldehyde-derived AGE inhibit the growth of human pericytes; pericyte loss is a hallmark of diabetic retinopathy. As a result, glyceraldehydes- and glycolaldehyde-derived AGE were found to significantly decrease human brain pericyte synthesis of DNA at lower concentrations than did glucose-derived AGE. On the contrary, when administered to EC, glucose-, glyceraldehydes- and glycolaldehyde-derived AGE significantly increased viable cell numbers and upregulated the levels of VEGF mRNA. The results indicate that the RAGE-engaging AGE fractions occurring in our internal milieu should be regarded as an environmental account for vascular injury in diabetes.

2. **RAGE transgenic mice**

To evaluate *in vivo* the hypothesis that the AGE-RAGE system may participate in the development of diabetic vascular complications, We created transgenic mice that overexpress human RAGE proteins in vascular cells, and crossbred them with another transgenic mouse line that develops insulin-dependent diabetes early after birth (3). The resultant double transgenic mice showed statistically significant increases in kidney weight, albuminuria, glomerulosclerosis index and serum creatinine compared with the diabetic control, while blood glucose, hemoglobin A_{1c} and serum AGE levels were essentially invariant between the two groups. The increases in serum creatinine and sclerosis index were effectively prevented with (±)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-ylacetanilide, an inhibitor of AGE formation. Indices diagnostic of diabetic retinopathy were also most prominent in the double transgenic. Thus, this transgenic approach has supported the concept that AGE-RAGE system plays an active role in the development of diabetic complications, and has developed a useful animal model for testing remedies.

Further, mice deficient for RAGE showed amelioration of diabetic nephropathy, as

evidenced by loss of nephromegaly, significant decrease in albuminuria, improved glomerulosclerosis and no elevation of serum creatinine. This again emphasizes that RAGE is functionally involved in the development of diabetic nephropathy.

3. **Regulation of human RAGE gene**

In light of the findings with RAGE transgenic mice, it is reasonable to assume that upregulation of the endogenous RAGE gene would aggravate diabetic vascular derangement. Accordingly, we screened factors that can influence the EC level of RAGE mRNA. We identified three inducers of RAGE gene transcription: AGE ligands themselves, tumor necrosis factor-α (TNF-α) and 17β-estradiol (4). TNF-α has been known to be responsible for insulin resistance. The action on RAGE gene unveiled another side of this cytokine; that is, an increased TNF-α level in diabetic patients may worsen diabetic complications through RAGE induction. Between the AGE and RAGE a positive feedback loop may exacerbate diabetic vasculopathy. The RAGE gene activation by estradiol may provide a biochemical basis for the well-known fact that pregnancy worsens diabetic complications.

4. **esRAGE**

We analyzed poly(A)$^+$RNA isolated from polysomes of human EC and pericytes, and isolated previously undescribed splice variants of RAGE mRNA (5). Three major variants were identified: the known full-length membrane-bound form, a novel N-terminally truncated membrane-bound form and a novel C-terminally truncated soluble form. The ratio of expression of these variants differed from one cell type to another; C-truncated > full-length = N-truncated in EC; full-length > N-truncated > C-truncated in pericytes. We named the C-truncated form “esRAGE (endogenous secretory RAGE)”. esRAGE would be cytoprotective, because it is able to capture AGE outside cells. In effect, this variant was found to effectively neutralize the AGE action on EC and does exist in human circulation. An enzyme-linked immunosorbent assay system for esRAGE has been developed, and, with it, diabetic subjects with or without complications are now being screened.

Conclusion

AGE are senescent proteins that accumulate during prolonged diabetic exposure, and can also engage cell surface RAGE, this resulting in the deterioration of vascular functions. The AGE-RAGE system thus should be a candidate target for overcoming this life- and QOL-threatening disease.

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