Pharmacological Prevention of RAGE-Mediated RPE Damage: Implications for Age-Related Macular Degeneration

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Purpose: Oxidative damage appears to play an important role in the pathogenesis of AMD. Advanced glycation end-products (AGEs) and other oxidatively damaged molecules accumulate in the retina with advancing age, increasing oxidative stress and forming a significant component of drusen. The receptor for advanced glycation end-products (RAGE), which recognizes many of these molecules and initiates inflammatory responses, is associated with AMD and localizes to the RPE and Bruch’s membrane. Furthermore, RAGE activation has been shown to induce cell death in cultured ARPE-19 cells. We hypothesize that RAGE-mediated damage to the RPE contributes to the pathogenesis of AMD and that RAGE-inhibiting compounds may hold promise as therapeutic agents.

Methods: Cultured HEK293 and ARPE-19 cells were exposed to AGE products and various RAGE inhibitors including semi-synthetic glycosaminoglycan ethers (SAGEs), a novel class of RAGE antagonists (GlycoMira, Inc). Cells were assayed for response to these compounds by western blot, MTS cell viability assays, and immunocytochemical examination of cell death and cell stress. In vitro experiments were used to examine the concentrations at which SAGEs would effectively inhibit the interaction of RAGE with both physiological RAGE ligands and AGE products.

Results: HEK293 and ARPE-19 cells treated with AGE products exhibited a significant degree of cell death as well as upregulation of RAGE (data not shown). Cell death was dose-dependent and proceeded in a progressive cascading fashion. Effects were also noted in cell adhesion, an area in which the RAGE receptor has been implicated in other organ systems. In vitro studies showed that SAGEs inhibited RAGE interaction with S100b and the AGE product CML-BSA at ng/ml concentrations. Similarly, the measured effects of RAGE ligand administration in cultured cells were mitigated by co-administration of SAGES in the ng/ml range.

Conclusions: SAGES show significant promise as an anti-RAGE therapy and warrant further investigation in vivo. The cascading pattern of cell death observed in cultured ARPE-19 cells implies that RAGE access on non-apical surfaces may be required for the observed response, and suggests a potential role for mechanical disruption of the RPE and Bruch’s membrane in the pathological response to oxidative injury. The successful rescue of the induced disease phenotype by a RAGE antagonist implies that RAGE antagonism may hold promise as a treatment to prevent death of the RPE in AMD and other oxidative damage-related retinal diseases.


Figure 1. In Vitro RAGE inhibition with SAGE compounds

Figure 2. Cell death in AGE-exposed ARPE-19 cells

Figure 3. Dose response curves in HEK293 cells

Light microscopy was performed on tissue sections of normal and pathological retinae as follows: A. H&E stain B. Oligonucleotide staining by in situ hybridization with digoxigenin-labeled probes for the gene encoding the choriocapillaris-specific transcript C. DAPI (nuclear stain) D. Green: Molecular Probes Fixable Live/Dead Cell Stain Kit (L23101, Eugene, OR). This fluorescent dye binds to DNA and RNA only when the cellular membrane is highly permeable.

Serum starvation induced cell loss near the edges of the coverslip, in the BSA control (A), and this cell death was exacerbated by the presence of AGE (B). ODSSH, an earlier, weaker RAGE-antagonist, failed to rescue cell death at the concentrations used (C). SAGES virtually eliminated the effects of AGE exposure. However, the presence of SAGES appears to inhibit cell adhesion, consistent with the known physiological roles of the RAGE receptor in epithelial adherence.

Confluent ARPE-19 cells matured in 1% FBS for 21 days were exposed in serum-free media for 40 hours to ribose-modified AGE-BSA, O-desulfated heparin (ODSSH), and semi-synthetic glycosaminoglycan ethers (SAGEs) then processed for histology as follows: A. AGE + 200 µM AGE, B. 25 µM AGE, C. 25 µM AGE + 200 µM ODSSH, D. 25 µM AGE + 200 µM SAGE.