

Urokinase and Its Receptor:

Novel Therapeutic Target in Diabetic Retinopathy

Proteinases may be a promising target for early therapeutic intervention in diabetic retinopathy.

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Recent observations in our laboratory suggest a novel role for proteinases in alteration of the blood-retinal barrier (BRB) in diabetes.^{1,2} Increased microvascular permeability in the retina is a central event in the progression of diabetic retinopathy (DR), and a focus of our laboratory is to evaluate the contribution of extracellular proteases, mainly urokinase plasminogen activator (uPA), its receptor (uPAR), and matrix metalloproteinases (MMPs) to endothelial barrier function.¹

The BRB is largely maintained by endothelial cell junctions that are composed of several families of specialized cell junctional proteins, such as occludin in tight junctions and vascular endothelial (VE)-cadherin, the primary component of adherens junctions. Cell junctions regulate tissue homeostasis and the flow of solutes from the circulatory system to the tissue. Increased retinal microvascular permeability is a consequence of altered endothelial barrier function, and our studies point to a central role played by proteinases in this compromised barrier function. This role suggests the use of proteinase inhibitors as a promising target for early therapeutic intervention in DR.

CELLULAR EXPRESSION, DISTRIBUTION OF VE-CADHERIN

In an earlier study, we sought to determine if diabetes

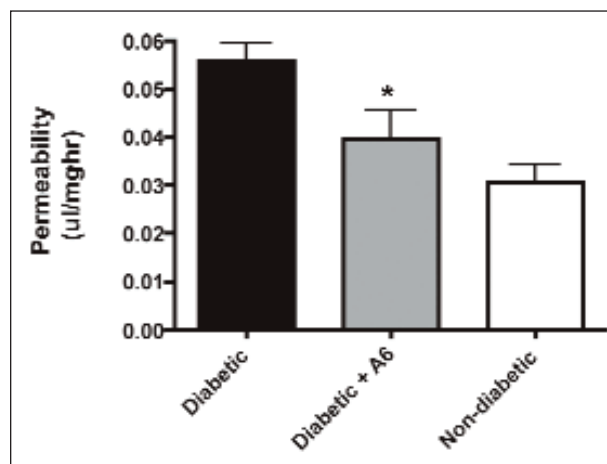


Figure 1. A6 prevents an increase in retinal permeability in diabetic rats.

alters the cellular expression and distribution of VE-cadherin in retinal endothelial cells, and if this alteration is mediated by proteinase activity. In streptozotocin-induced diabetic rats, an increase in retinal permeability was seen as early as 2 weeks of diabetes with a coincident loss of VE-cadherin protein in the diabetic vasculature. Real-time polymerase chain reaction analysis of isolated retinal vasculature showed an upregulation of uPA, uPAR, MMP-2 and MMP-9 mRNA levels in the diabetic retina.

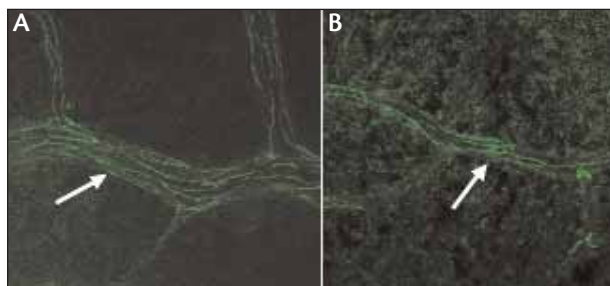


Figure 2. A nondiabetic rat (A). The loss of VE-cadherin correlates with altered retinal vascular permeability in the diabetic rat (B).

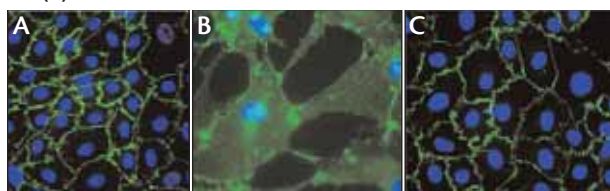


Figure 3. Retinal endothelial cells untreated (green staining at cell borders for VE-cadherin) (A). Cells treated with AGE—loss of cell-cell contact (B). Cells treated with AGE plus urokinase inhibitor Å6—normalized cell-cell junctions are seen (C).

Systemic administration of an MMP inhibitor for 2 weeks after induction of diabetes was able to block both increases in retinal permeability and loss of VE-cadherin, suggesting that mechanisms of permeability in diabetes were proteinase-dependent. Stimulation of bovine microvascular retinal endothelial cells with advanced glycation endproducts (AGE)-bovine serum albumin (BSA), a potent inducer of permeability, caused a reduction of VE-cadherin on the cell surface, which was again rescued by a MMP-inhibitor.

In a separate experiment, we demonstrated that MMP-9 is able to release an approximately 75-KDa fragment of the VE-cadherin ectodomain, large amounts of which was detected in the conditioned media of AGE-BSA-treated cells. This proteolytic degradation of the VE-cadherin molecule from the cell surface was abolished in the presence of a MMP inhibitor. These observations led us to conclude that a possible mechanism by which diabetes contributes to the BRB is through proteolytic degradation of VE-cadherin.

UROKINASE SYSTEM AND THE BRB

A peptide inhibitor that blocks the interaction of uPA with uPAR was used to study the direct contribution of uPA/uPAR to retinal vascular permeability. The Å6 peptide has also been shown to have antiangiogenic activity, and has been tested in a phase 2 clinical trial in patients with gynecological cancer. More than 40% of patients dosed continuously with Å6 experienced disease stabi-

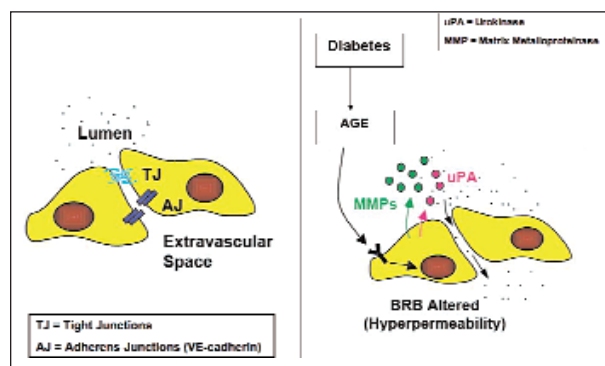


Figure 4. Protease-induced alteration of the BRB.

lization. We have previously showed that Å6 can suppress both retinal and choroidal neovascularization in animal models of retinal neovascularization. Diabetic rats received intraperitoneal injections of Å6 100 mg/kg daily for 2 weeks after induction of diabetes, and retinal vascular permeability was assayed using the Evans Blue dye technique. Å6 treatment significantly reduced retinal vascular permeability levels as compared to untreated diabetic rats.

To elucidate how inhibition by Å6 affects cell junctions, bovine retinal microvascular endothelial cells were treated with AGE-BSA in the presence or absence of Å6. Under these conditions, VE-cadherin surface expression and induction of MMPs were examined. AGE-BSA treatment increased monolayer permeability, loss of cell surface VE-cadherin expression, decreased cell-cell contact, and a drastic upregulation of MMP-2 and 9. All of these effects were effectively reversed by the simultaneous treatment with Å6.

These results indicate that the uPA/uPAR axis, through their ability to regulate induction of MMP activity in endothelial cells, plays a crucial role in breakdown of the BRB during diabetes. Thus, the urokinase system might be a formidable target for early therapeutic intervention in DR. ■

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1. Navaratna D, Meniciccu G, McGuire P, Das A. Urokinase and its receptor—A novel therapeutic target in diabetic retinopathy. #0099-OR. Presented at the American Diabetes Association 67th Scientific Sessions, June 22-26, 2007, Chicago.
2. Navaratna D, McGuire P, Meniciccu G, Das A. Proteolytic degradation of VE-cadherin alters the blood-retinal barrier in diabetes. *Diabetes*. Published online May 29, 2007.