Preventing vision loss in murine diabetes by vascular stabilization

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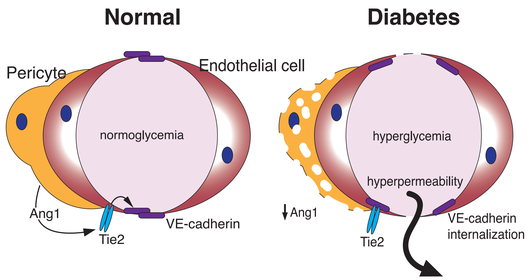
Introduction

Diabetic retinopathy is the leading cause of blindness among the working age population and a 50% increase in its prevalence is expected by 2030[1]. Pericyte loss, vascular hyperpermeability, and increased vascular endothelial growth factor-A (VEGF-A) production are critical to its pathogenesis[2,3]. Angiopoietin 1 (Ang1), secreted by pericytes, maintains a stable and mature vasculature by preventing VEGF-A-induced vascular hyperpermeability and promoting vascular endothelial (VE)-cadherin stabilization[4].



Hypothesis

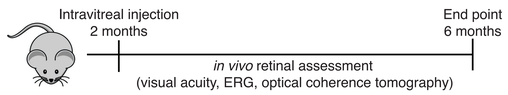
Stabilizing the vasculature with COMP-Ang1 will prevent retinal structural and functional deficits in diabetic retinopathy

Methods

Mice. Mice heterozygous for the Ins2 mutation (C57BL/6-Ins2Akita/J (Ins2Akita)) experience hypoinsulinemia and hyperglycemia by 4 weeks of age. We used only male Ins2Akita with blood sugar levels greater than 280 mg/dL or age-matched controls (background strain C57BL/6J).

COMP-Ang1. Cartilage oligo matrix protein (COMP)-Ang1 is an Ang1 variant with enhanced solubility and stability.

AAV2. Consitutive expression of COMP-Ang1 (or control) was accomplished with adeno-associated viral vector serotype 2 (AAV2.COMP-Ang1 and AAV2.AcGFP).



Retinal vasopermeability. Vascular hyperpermeability was established with Evans Blue method.

Visual Acuity. Optomotor head tracking response was used to determine visual acuity.

Retinal electrical function. Electroretinography (ERG) was performed on anesthetized mice under scotopic conditions.

Vasculature

Structure

Figure 1

COMP-Ang1 prevents endothelial loss in diabetes

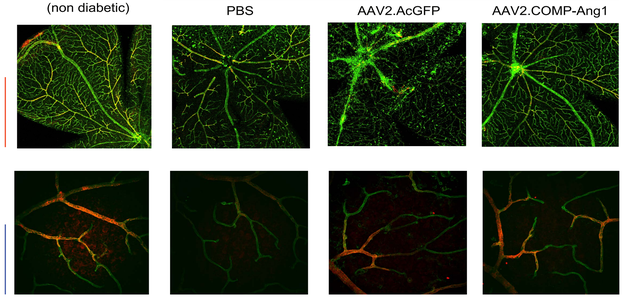


Figure 1 AAV2.COMP-Ang1 mitigates diabetic retinal capillary dropout
(a) Retinal flatmounts of 6 month-old mice and stained for isolectin (endothelial cell marker, green) and α-SMA (pericyte marker, red). Ins2Akita mice experienced endothelial and pericyte dropout compared to C57BL/6J mice. Endothelial cell loss was prevented by AAV2.COMP-Ang1.

Function

Figure 2

COMP-Ang1 increases endothelial resistance and decreases hyperpermeabilty

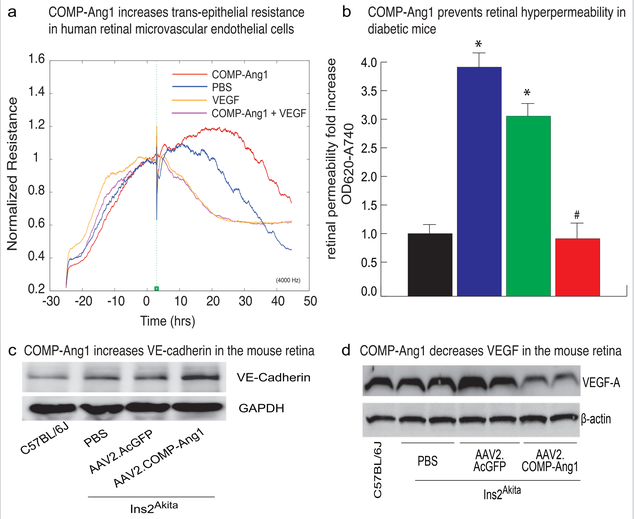


Figure 2 COMP-Ang1 increases vascular barrier function
(a) COMP-Ang1 (100 ng/mL) increased resistance of HrMVECs (n=3) but did not overcome VEGF (50 ng/mL)-induced decreases in resistance. (b) AAV2.COMP-Ang1 returned vascular hyperpermeabilty to control levels. (c) AAV2.COMP-Ang1 increased VE-cadherin expression in Ins2Akita mouse retinas. (d) AAV2.COMP-Ang1 decreased VEGF-A in Ins2Akita mouse retinas. n=3 mice/group, data are mean ± s.e.m. *P<0.001 vs.C57BL/6-J, #P=0.02 vs. AAV2.AcGFP.

Retina

Figure 3

COMP-Ang1 prevents ganglion cell layer loss and retinal thinning in diabetes

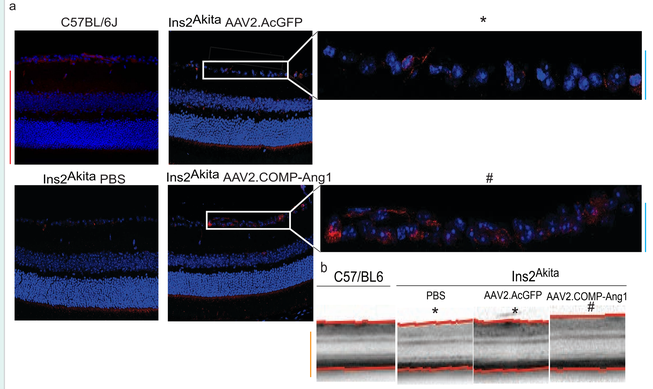


Figure 3 AAV2.COMP-Ang1 prevents diabetes-induced retinal ganglion cell layer degeneration
(a) Cross sections of 6 month old mouse retina stained for VE-cadherin (red) or nuclei (DAPI, blue). Right, magnified view of the ganglion cell layer from AAV2.COMP-Ang1 and AAV2.AcGFP treated mice demonstrating increased VE-cadherin staining. (b) Optical coherence tomography (OCT) measuring retinal thickness. AAV2.COMP-Ang1 prevented diabetes-induced retinal thinning, n=5 mice/group, *P=0.03 vs. C57BL/6J, #P=0.03 vs. AAV2.AcGFP

Figure 4

COMP-Ang1 preserves visual acuity and retinal function in diabetes

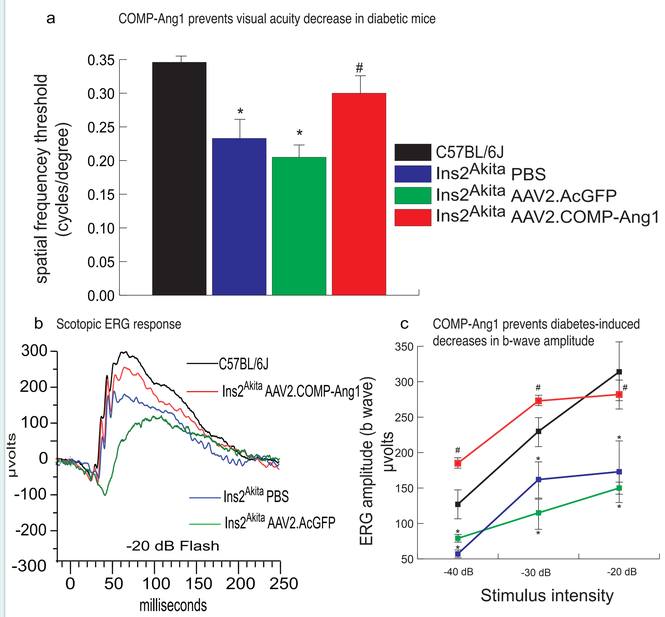


Figure 4 AAV2.COMP-Ang1 prevents visual function loss in diabetic retinopathy
Visual acuity was determined by testing optomotor responsiveness. (a) Ins2Akita mice exhibited decreased tracking response and AAV2.COMP-Ang1 prevented the decrease in visual acuity; n=6 mice/group. (b) Representative ERG. (c) Decreased b-wave amplitudes in Ins2Akita mice treated with PBS or AAV2.AcGFP compared to C57BL/6JL6 mice; AAV2.COMP-Ang1 prevented the decrease in amplitude. n=5 mice/group, data are mean ± s.e.m. *P=0.0001 vs. C57BL/6J. #P=0.01 vs. AAV2.AcGFP.

Discussion

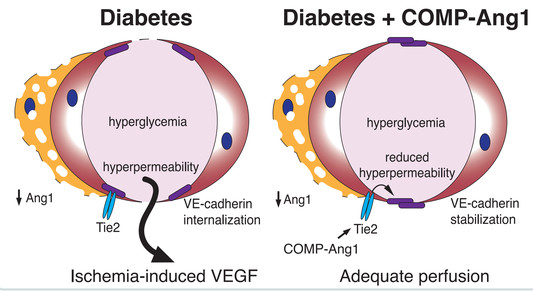
The Ins2Akita mouse exhibits several hallmarks of non-proliferative diabetic retinopathy including increased VEGF-A protein expression, vascular hyperpermeability and hypoperfusion.

Current therapy for diabetic retinopathy focuses on suppressing VEGF either through ablating large portions of the retina with laser photocoagulation or administering anti-VEGF antibodies, neither of which addresses the underlying cause.

We hypothesized that stabilizing the vasculature would promote proper perfusion and prevent the retinal ischemia responsible for increased VEGF production.

AAV2.COMP-Ang1 *prevents* diabetes-induced:
- **endothelial dropout**
- **vascular hyperpermeability (despite persistent pericyte dropout)**
- **retinal thinning and ganglion cell loss**
- **visual acuity and retinal function deficits**

We propose that AAV2.COMP-Ang1 normalizes the vasculature by increasing VE-cadherin stability and preventing the endothelial cell loss seen in Ins2Akita mice. The normalized vasculature results in enhanced perfusion, reduced hypoxia-driven VEGF production (further contributing to vascular stability) and reduced ganglion cell layer loss. This is turn prevents decreases in visual acuity and ERG responsiveness, which decrease in diabetic patients due to ischemia.



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