# Peptain-1 blocks ischemia/reperfusion-induced capillary degeneration in mice

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## Background

- Global prevalence of diabetes mellitus – 463 million adults globally, 31 million Americans (IDF 2019).
- Diabetic retinopathy is the leading cause of visual impairment for working-class adults worldwide.

**Role of Inflammation**

- Pro-inflammatory cytokines IFN-γ, TNF-α, MCP-1, IL-1β, and IL-6 are upregulated in the diabetic retina.
- VEGF is the current target of most therapeutics; 30% of patients progress despite anti-VEGF therapy.

**Crystallins**

- Crystallins are the major structural proteins expressed in ocular tissues
  - αA and αB crystallin also function as small heat shock proteins, found particularly in RGCs and Müller glial cells.
- Peptain-1 is the core 20 amino acid peptide of αB crystallin
  - Previously shown to block apoptosis of LECs, cataract formation, and protect against RGC degeneration

*We show that peptain-1 protects against apoptosis of retinal cells and may have potential as a therapeutic for diabetic retinopathy.*

## Methods

**Conjugation of peptain-1 with Cy5 and testing the entry of intravitreally injected peptain-1-Cy5 into the retina**

- Peptain-1 was conjugated to Cy5 at the C-terminus
- Mice (C57BL6/J) were injected intravitreally with 1µg of peptain-1-Cy5 in 2µL PBS. Contralateral eyes were used as control.
- After 4h, retinas were harvested for flatmounting. Cy5 fluorescence intensity was measured.

**Determining the effect of peptain-1 on pro-inflammatory cytokine-mediated apoptosis in HRECs**

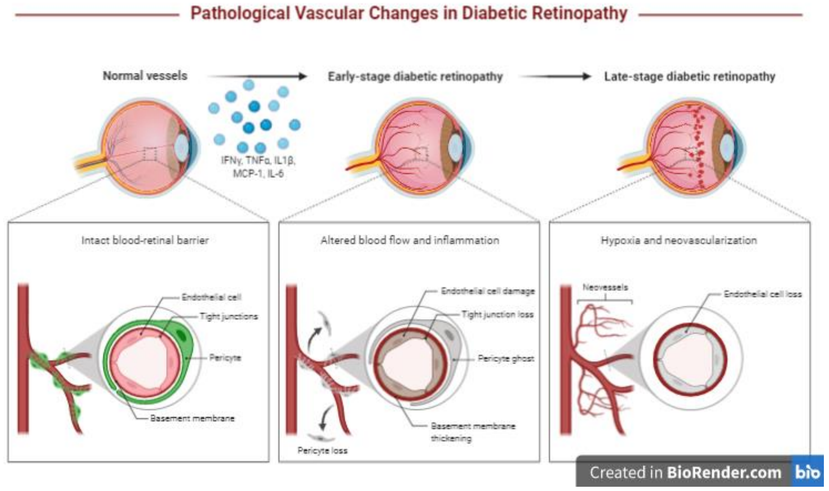
- HRECs were isolated and cultured on 0.2% gelatin-coated plates in DMEM, 10% fetal bovine serum, penicillin/streptomycin, and incubated at 37C.
- HRECs were treated with peptain-1 or scrambled peptide for 3h in serum-free medium and then a combination of inflammatory cytokines for 48h
- Scrambled sequences: scrambled 1 (Scr-1 : SLKEKRNFDVSEVKHVLFVDP) and scrambled 2 (Scr-2 : FEPSVRFSKVDHLVKENDLVK
- Cell lysates were prepared and protein concentration measured. Immunoblotting was performed for cleaved-caspase-3.
- Densitometry was performed with ImageJ software.

**Intravitreal injection of peptain-1 and ischemia/reperfusion injury in mice to induce acellular capillaries in retina**

- Mice were randomly allocated to treatment with peptain-1 or scrambled proteins.
- They were anesthetized and IOP increase induced by intravitreal normal saline drip for 1h. Ischemia injury was stopped and reperfusion allowed to occur. At this time, mice received treatment with either peptain-1 or scrambled peptide by intravitreal injection.
- The contralateral eye served as a control.

**Capillary bed preparation and analysis of acellular capillaries**

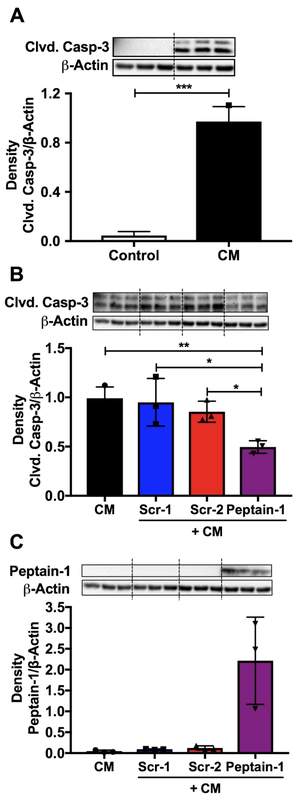
- 14d after initial injection of peptide, mice were sacrificed and enucleated. Retinas were isolated by dissection under light microscopy and treated with elastase (40U/mL) for 35 min at 37C with gentle agitation.
- After removal of the ILM and overnight incubation in Tris-HCl, pH 7.8, the remaining neuronal tissues were carefully dislodged. PAS stain was used to visualize the isolated capillary layer. Acellular capillaries per visualized field at 40X magnification were counted and analyzed.

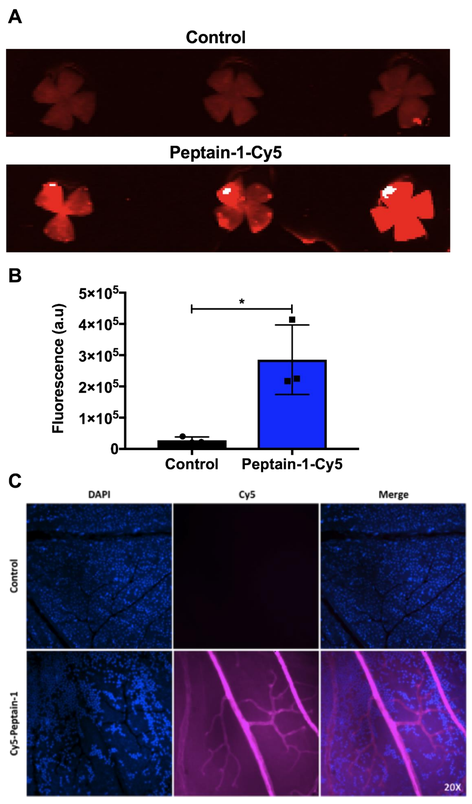



## Results

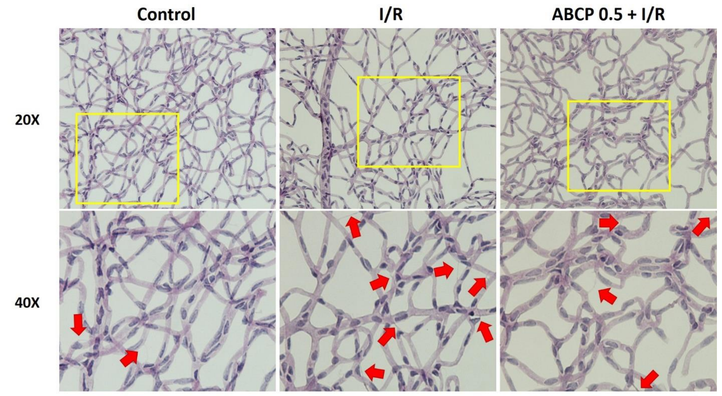
**1. Peptain-1 protects HRECs against pro-inflammatory cytokine-mediated apoptosis**

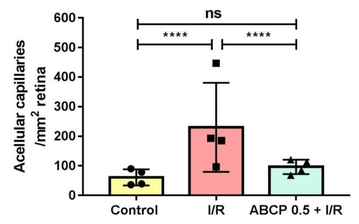
**2. Intravitreally injected peptain-1 reaches the retina**



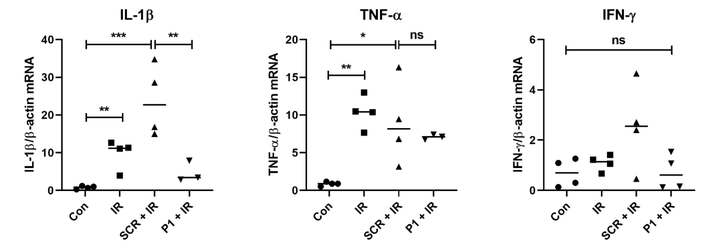




**3. Intravitreally administered peptain-1 inhibits acellular capillary formation caused by I/R injury**




**4. Intravitreally injected peptain-1 prevents pro-inflammatory cytokine upregulation due to I/R injury**




## Discussion

- Diabetic retinopathy is the most common cause of visual impairment in industrialized countries.
- Ischemic stress is directly tied to the intracellular inflammatory response, but current therapies primarily target later stages of disease.
- This study indicates that peptain-1 enters cells and blocks caspase-3-mediated apoptosis in HRECs. Previously shown to have similar effect on RGCs and rat retinal explants.
- Pericyte apoptosis is an early event in DR and is followed by apoptosis of endothelial cells, leading to increased vascular permeability, macular edema, and angiogenesis.
- Peptain-1 blocked capillary cell death in both the *in vitro* and *in vivo* experimental models, making it a potential therapeutic approach for DR.

To view references: