PEPTAIN-1 BLOCKS ISCHEMIA/REPERFUSION-INDUCED RETINAL CAPILLARY DEGENERATION IN MICE

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ABSTRACT

Purpose: To evaluate the ability of peptain-1, a 20 amino acid peptide derived from the αB-crystallin core domain, to block apoptosis of human retinal endothelial cells (HRECs) in vitro and retinal capillary degeneration in mice subjected to retinal ischemia/reperfusion (I/R) injury.

Methods: HRECs were treated with either peptain-1 or scrambled peptides (200 μg/mL) for 3 h and a combination of proinflammatory cytokines (IFN-γ 20 ng/mL + TNF-α 20 ng/mL+ IL-1β 20 ng/mL) for an additional 48 h. Apoptosis was measured with cleaved caspase-3 formation via western blot, and by TUNEL assay. C57BL/6J mice (12 weeks old) were subjected to I/R injury by elevating the intraocular pressure to 120 mmHg for 60 min, followed by reperfusion. Peptain-1 or scrambled peptide (0.5 μg) was intravitreally injected immediately after I/R injury and 7 days later. One microliter of PBS was injected as vehicle control, and animals were euthanized on day 14 post-I/R injury. Retinal capillary degeneration was assessed after enzyme digestion followed by periodic acid–Schiff staining.

Results: Our data showed that peptain-1 entered HRECs and blocked proinflammatory cytokine-mediated apoptosis. Intravitreally administered peptain-1 was distributed throughout the retinal vessels after 4 h. I/R injury caused retinal capillary degeneration. Compared to the scrambled peptide, peptain-1 protected capillaries against I/R injury. Additionally, peptain-1 inhibited microglial activation and reduced proinflammatory cytokine levels in the retina following I/R injury.

Discussion: Our study suggests that peptain-1 could be used as a therapeutic agent to prevent capillary degeneration and neuroinflammation in retinal ischemia.

Peptain-1 inhibits proinflammatory cytokine-induced apoptosis in HRECs

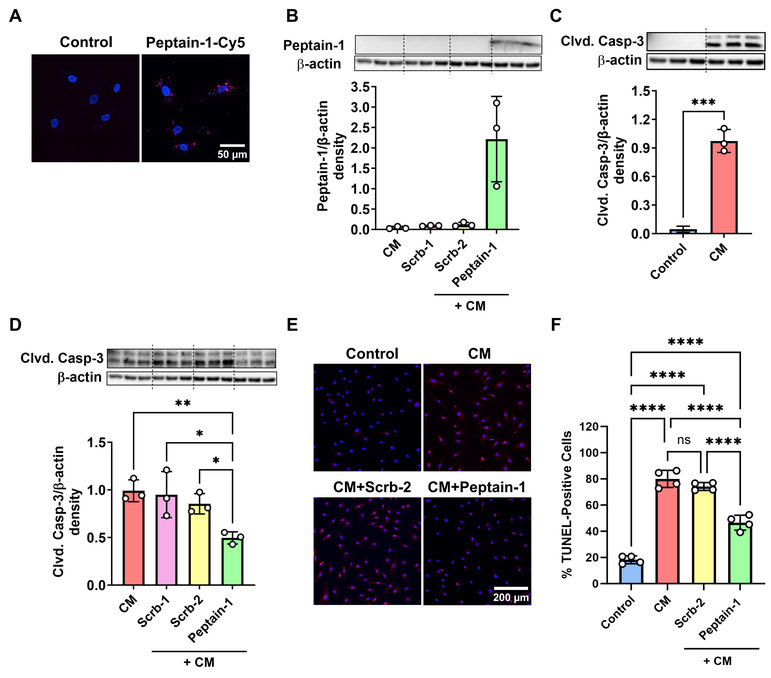

Figure 2. Cy5-conjugated peptain-1 was treated (10 μg/mL) for 24 h and visualized by confocal microscopy, demonstrating that peptain-1 is cell permeable **(A)**. Cells were treated with or without peptide (200 μg/mL) for 3 h and then with a mixture of IFN-γ, TNF-α and IL-1β (20 ng/mL) to induce apoptosis for an additional 48 h. Western blotting demonstrated the presence of peptain-1 in the cell lysates **(B)**. Western blotting showed that cleaved caspase-3 was increased after 48 h of cytokine stimulation, while peptain-1 effectively inhibited this increase **(C-D)**. The graph represents the mean ± SD of triplicate measurements. The TUNEL assay was used to assess apoptosis, and representative confocal microscopy images are shown in **(E)**. TUNEL-positive cells were labeled in red, and cell nuclei were labeled with DAPI (blue). The percentage of TUNEL-positive cells in each treatment group is presented in **(F)**. The graph represents the average of TUNEL-positive cells from each well in each treatment group ± SD. Peptain-1 significantly reduced the number of apoptotic cells under inflammatory stress. CM = cytokine mixture and Scrb-1 and Scrb-2 = scrambled peptide 1 and 2. *p<0.05, **p< 0.01, ***p< 0.001, and ****p< 0.0001.

Peptain-1 inhibits retinal capillary degeneration following I/R injury

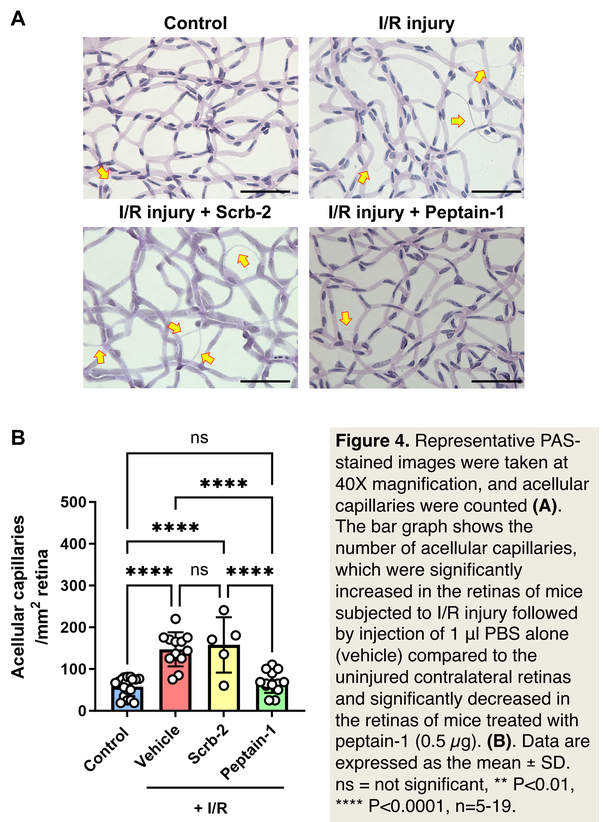

Figure 4. Representative PAS-stained images were taken at 40X magnification, and acellular capillaries were counted **(A)**. The bar graph shows the number of acellular capillaries, which were significantly increased in the retinas of mice subjected to I/R injury followed by injection of 1 μl PBS alone (vehicle) compared to the uninjured contralateral retinas and significantly decreased in the retinas of mice treated with peptain-1 (0.5 μg). **(B)**. Data are expressed as the mean ± SD. ns = not significant, ** P<0.01, **** P<0.0001, n=5-19.

Peptain-1 is permeable to the retinal capillaries

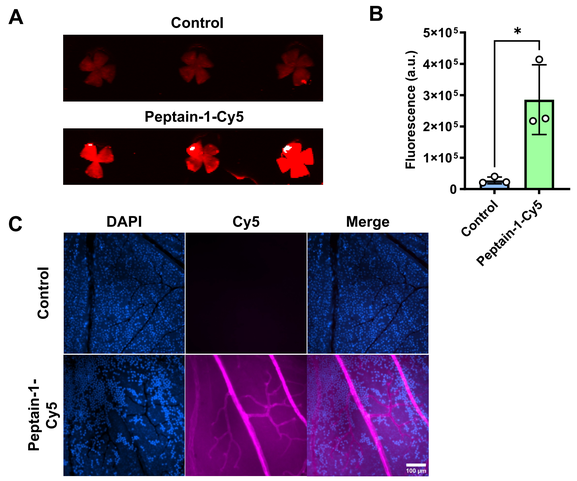

Figure 1. Cy5-conjugated peptain-1 is intravitreally injected into the mouse retinas. Mice were sacrificed 4 h **(A-B)** and 24 h **(C)** after injection. Fluorescence intensity was detected in the retinal flat mount **(A)**, homogenate **(B)** and blood vessels **(C)** of the injected eye. Uninjected contralateral eyes were used as a control. The nuclei were stained with DAPI (blue). *p<0.05. a.u.= arbitrary units.

Peptain-1 inhibits inflammatory cytokine upregulation and microglial activation in I/R-injured retinas

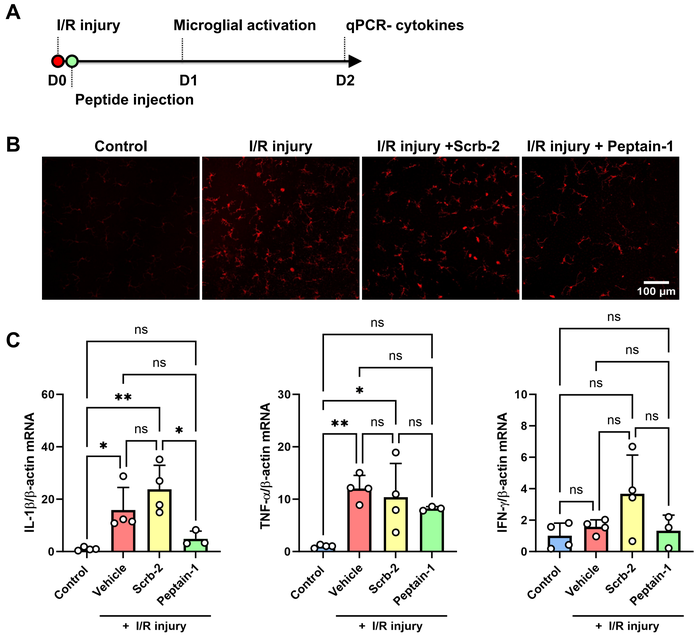

Figure 3. The timeline for peptide injections and I/R injury is shown in **(A)**. Mice were subjected to I/R injury and injected with 1 μl of PBS alone (vehicle), 0.5 μg peptain-1, or scrambled peptide was intravitreally injected immediately after I/R injury. **(B)** One day after retinal I/R injury, retinas were isolated, whole retinal flatmounts were immunostained for Iba1 (red) to identify activated microglia. The results showed that I/R injury induces microglial activation, but treatment with peptain-1 reduced this activation. **(C)** Two days after I/R injury, mice were euthanized, retinas were dissected out, and total RNA was lysed from the retinas. The mRNA levels of proinflammatory cytokines, IL-1β, TNF-α, and IFN-γ were measured by qPCR. The injection of peptain-1 significantly reduced IL-1β mRNA levels that were induced by I/R injury. Data are expressed as the mean ± SD. ns = not significant, * P<0.05, ** P<0.01, *** P<0.001, n=3-4.

SUMMARY

- Peptain-1 attenuates cytokine mediated apoptosis in primary HREC.
- After intravitreal injection, peptain-1 is able to enter the retina and is found in retinal blood vessels.
- *In vivo* experiments suggest peptain-1 attenuates neuroinflammation and retinal capillary degeneration after I/R injury.

CONCLUSION

- Peptain-1 is protective against capillary degeneration and neuroinflammation during retinal ischemia.
- Peptain-1 has potential as a therapeutic agent in cases such as early diabetic retinopathy where there is known ischemic insult.

ACKNOWLEDGEMENT

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