Pronase E improves gene transduction of retinal ganglion cells

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Purpose: Intravitreal injection is used for gene delivery to retinal ganglion cells, but has low efficiencies of transduc- tion. The aim of the present study was to optimise recombi- nant adenovirus-associated vector (rAAV)-mediated gene transduction of retinal ganglion cells by co-administration with proteolytic enzymes.

Method: Female Sprague-Dawley rats (n = 4) received a 5 µL intravitreal injection containing 5e+9 genome copies (gc) rAAV. The rAAV contained a bistronic cassette expressing green fluorescent protein (rAAV2/2.CAG.NGB. HA.IRES.GFP.pA). Further groups received co-administration of Pronase E (n = 6) or Heparinase III + Hyaluronidase (n = 8). The fellow eye received a vehicle injection. Retinal function was analysed at 2 weeks by scotopic electroretinogra- phy (ERG). At 3 weeks, GFP expression was assessed in vivo using a confocal scanning laser ophthalmoscope (cSLO). Ani- mals were then euthanized for whole mount retinas.

Results: The mean in vivo GFP expression on cSLO fundal images in rAAV + Pronase E eyes (360 of fundus ±0) was significantly greater than rAAV + Hep/Hyal (70 ± 35;P < 0.001) or rAAV alone (200 ± 47;P < 0.001) eyes. Analysis of retinal whole mounts supported this pattern of increased transduction with Pronase E. The ERG b-wave of PBS-injected eyes (1195 ± 59 µV) was not different from rAAV-only injected eyes (1246 ± 101 µV; P = 0.46), rAAV + Pronase E eyes (1004 ± 105 µV; P = 0.18) or rAAV + Hep/Hyal eyes (1150 ± 64 µV; P = 0.33).

Conclusion: Retinal ganglion cell transduction by rAAV was enhanced by co-administration of Pronase E. Importantly, this effect was not associated with increased retinal toxicity, as assessed by electroretinography. The use of pronase E should be considered for delivery of candidate neuro-protective genes to retinal ganglion cells.