Insulin-assisted surgical approach to pre-retinal AAV gene therapy

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Purpose

Current subretinal gene therapy can lead to chronic chorioretinal atrophy in addition to risks of intentionally detaching the macula. Instead, we have developed a gene agnostic pre-retinal surgical approach that utilizes novel insulin-assisted adeno-associated virus (AAV) uptake. We aim to apply AAV pre-retinally in an air-filled eye to transduce the entire retina robustly without risk of inflammation or off-target transduction, as we remove all residual AAV from the eye after 30 minutes.

Methods

24 murine eyes received an intravitreal injection of 7.2E10 vg/eye of AAV9 expressing Green Fluorescent Protein (GFP) in 2 microliters with increasing co-administered intravitreal insulin doses (10-30ng). At one month, *in vivo* fundus imaging, optical coherence tomography (OCT), and electroretinography (ERG) were performed. GFP transduction and expression was evaluated with immunohistochemistry and ELISA. Murine retinal explants were treated with same insulin doses + AAV9 for only 30 minutes. We performed the published “peel and puddle” with vitrectomy and inner limiting membrane (ILM) peel, followed by application of AAV + insulin in 4 minipigs, removing AAV + insulin after 30 minutes and evaluating at 4 weeks.

Results

In the murine intravitreal injections, we observed a *dose response with increasing concentrations of insulin resulting in increasing AAV transduction and GFP expression throughout the retina*. OCT, fundus photography, and ERG did not demonstrate significant inflammation or retinal function degradation. In retinal explants, we found increase in mean GFP intensity and number of transduced cells with addition of insulin to AAV, even with only 30 minutes AAV contact time. In the minipig model, we found again that

insulin + AAV required only 30 minute contact time to confer robust transduction throughout the retina to photoreceptors.

Conclusions

This gene agnostic approach may supplant the current subretinal gene therapy approach by avoiding iatrogenic trauma from retinal detachment, as well as increasing transduction efficiency while avoiding inflammation by removing all residual AAV from the eye after just 30 minutes transduction time. These advantages may also be applied to non-viral techniques, which tend to be challenged by both poor transduction efficiency as well as inflammation.

Layman Abstract (optional): Provide a 50-200 word description of your work that non-scientists can understand. Describe the big picture and the implications of your findings, not the study itself and the associated details.

Current gene therapy in the eye is mostly delivered under the retina. While effective in the first FDA-approved therapy - Luxturna - there are considerable challenges with long term thinning and loss of cells as well as trauma from the injection itself, which causes a temporary retinal detachment. Instead, we have extended upon a previous elegant surgical approach that leaves the retina in place, allowing us to deliver the gene therapy directly to the front surface without lifting it. We do this with the addition of insulin to the gene therapy, which significantly accelerates the gene therapy into retinal cells throughout its thickness. By doing this, we can then remove the excess gene therapy medication before the end of the surgery, avoiding the downstream inflammation problems that have been challenging in the field. We've shown this in three different pre-clinical models, including in pigs with full retinal surgery technique. This approach can be applied to any inherited retinal disease and may be useful for other forms of retinal gene therapy that do not rely on the typical weak viruses that have been used thus far.