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Efficacy assessment and pre-clinical toxicology of AAV2/8-hCARp.hCNGB3, a *CNGB3* gene therapy vector

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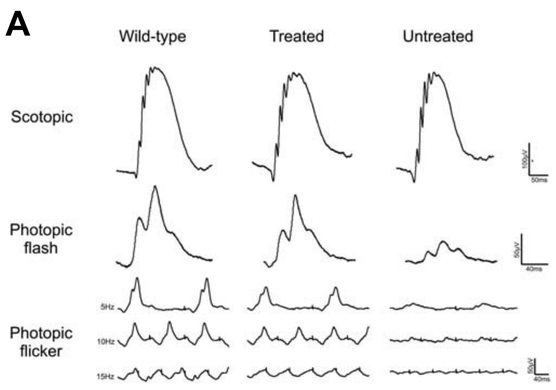
Introduction

Achromatopsia is an inherited autosomal-recessive condition characterised by intact rod function and absent cone function. The cone cyclic nucleotide-gated (CNG) channel is the final critical effector in the phototransduction cascade (the biological conversion of light energy to electrical signalling) and mutations in the β subunit (CNGB3) are the leading cause of achromatopsia. We developed AAV2/8-hCARp.hCNGB3, an AAV2/8-based vector, carrying a human CNGB3 gene driven by a fragment of human cone arrestin promoter that leads to rescue of cone function in Cngb3-deficient mice at wildtype levels [(1)].

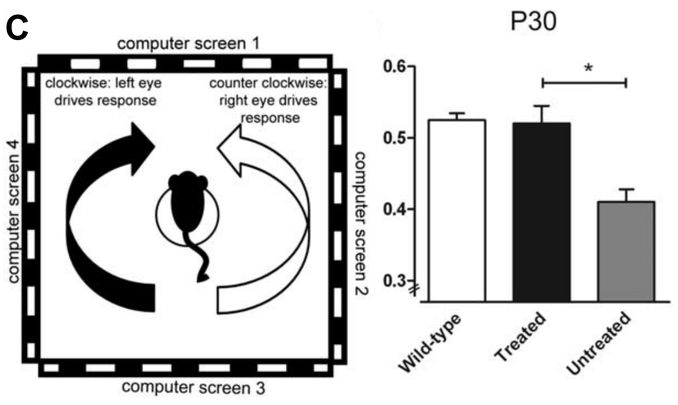
Efficacy in mice

The efficacy of AAV2/8-hCARp.hCNGB3 in *Cngb3*$^{-/-}$ mice was assessed following subretinal injection.

Delivery of AAV2/8-hCARp.hCNGB3 resulted in long-term restoration of cone function, as determined by electroretinography (ERG). There was a three-fold increase in photopic b-wave amplitudes (to 85% of WT levels; A) in animals treated at 1 month, and this was maintained for at least 9 months post-injection (B).

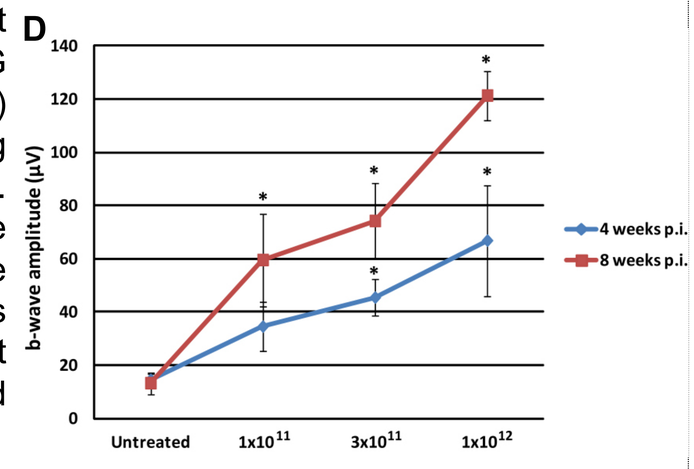


Visual acuity (assessed by optomotor responses) was restored to WT levels in mice treated at 1 month, but not in mice treated at 6 months (C).



As the Carvalho *et al.* study used a single dose of high titre AAV vector (2x10^{12} vg/mL), we titrated the efficacy of AAV2/8-hCARp.hCNGB3 in the same model using lower titres that corresponded to those planned for a proposed Phase I/II clinical trial (1x10^{11}, 3x10^{11} and 1x10^{12} vg/mL) .

There was a clear and significant dose response in ERG amplitudes from treated eyes (D) indicating that increasing amounts of AAV2/8-hCARp.hCNGB3 led to a greater rescue of photopic responses. These data indicate that all tested titres are able to provide significant improvement in cone-mediated responses (n=3. *; P<0.05).

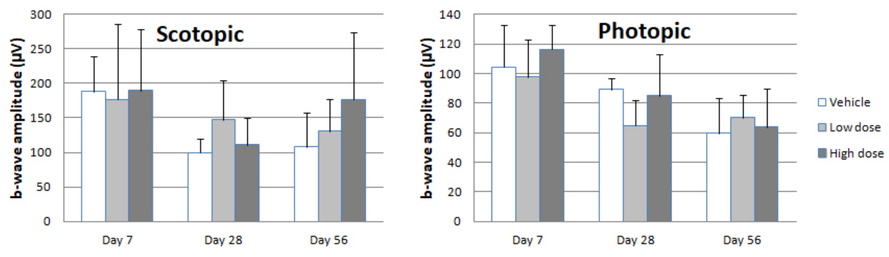


Toxicology – Retinal function & structure

Retinal function and structure were assessed in wild-type mice (C57Bl/6J) and wild-type rabbits (New Zealand White) to determine ocular toxicity associated with high levels of CNGB3 protein in the wild-type retina.

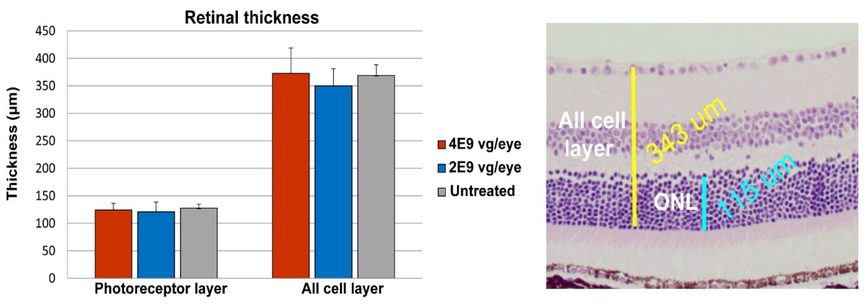
Wild-type mice and rabbits were assessed in an acute toxicological study probing for toxicity at 7, 28 and 56 days. Mice were also used in a longer study with a 9 month endpoint. The acute toxicological studies assessed the effect of a Low vector dose (1.3x10^{9} vg/eye in mice, 0.6x10^{11} vg/eye in rabbits), a High vector dose (4x10^{9} vg/eye in mice, 2x10^{11} vg/eye in rabbits) and Vehicle. The long-term study assessed a High dose (4x10^{9} vg/eye).

In all the studies and all assessed time-points there were no significant decreases in scotopic or photopic electroretinography (ERG) responses.



Rod-mediated (scotopic) and cone-mediate (photopic) ERG responses in wild-type mice at 7, 28 and 56 days after subretinal delivery.

Retinal thickness measurements were obtained from all eyes and no significant decreases were observed between cohorts.



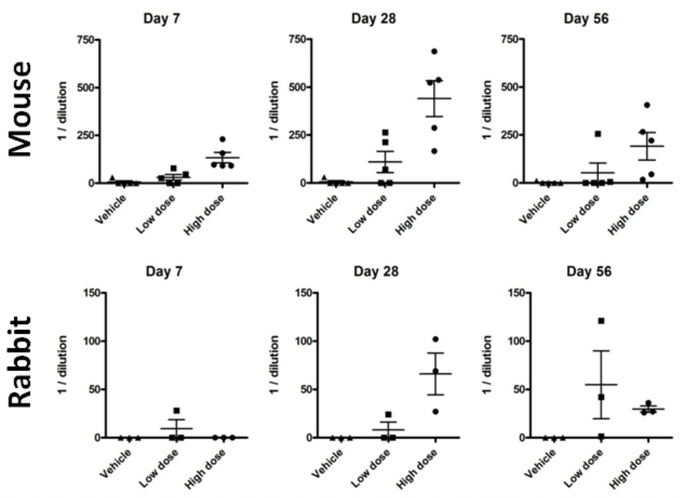
Retinal thickness measurements of treated versus untreated retinas in wild-type mice at 9 months after subretinal delivery. Representative image of a High dose treated retina section is also shown.

Biodistribution and immune responses

Real-time PCR for vector genome sequences was quantified in absolute numbers against a standard curve of amplicon DNA.

In both mice and rabbits major organs were sampled for the analyses with gonads and tissues of the contralateral uninjected eye included in the rabbit studies. Some dissemination of vector was observed for both species leading to detection of vector genomes mainly in the liver, as would be expected.

Anti-AAV5 capsid antibodies and anti-AAV5 neutralising antibody (NA) titres were established. Anti-AAV5 IgG responses were marginal in both species for treated animals. Neutralising antibodies against the AAV5 capsid were consistently found in vector injected animals after Day 7, as expected.



Previously, subretinal administration of AAV2/2-hRPE gave rise to neutralising antibody titres up to 1 in 1000 in trial participants without safety concerns, suggesting that the neutralising antibody titres detected here should not pose a risk to patient health[(2)].

Conclusions

Administration of AAV2/8-hCARp.hCNGB3 resulted in a positive dose response at all titres planned for the clinical trial.
In toxicology studies, AAV2/8.hCARp.hCNGB3 did not adversely affect retinal structure or function. Some vector dissemination was detected as well as low level immune responses against the vector capsid, without concomitant pathology. We conclude that subretinal administration of AAV2/8.hCARp.hCNGB3 is safe for use in clinical trials.

References:
1. Carvalho *et al.*, Hum Mol Genet (2011) 23(16): 3161-3175.
2. Bainbridge *et al.*, N Engl J Med (2015) 14;372(20): 1887-1897.

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