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Transduction of Photoreceptor and Pigmented Epithelial Cells Following a Single Subretinal Injection of AAVHSC17 in Minipigs

Jeff L. Ellsworth, Hillard Rubin, Albert Seymour

Homology Medicines, Inc., Bedford, MA

A novel group of Clade F adeno-associated viruses has been isolated from normal human CD34+ hematopoietic stem cells (AAVHSCs) and has shown high-efficiency nuclease-free gene editing as well as gene transfer capabilities. In non-human primate biodistribution studies, we have observed high-level transduction of retinal cells following a single intravenous delivery of AAVHSC demonstrating that AAVHSCs have tropism for the mammalian retina. The ability of AAVHSCs to also transduce retinal cells following localized delivery to the eye was evaluated. AAVHSC17 packaging a chicken beta actin-promotered self-complementary GFP transgene (AAVHSC17-CBA-scGFP) was prepared by triple transfection in HEK293 cells and purified through two rounds of CsCl density gradient ultracentrifugation. Anesthetized Göttingen minipigs received a single subretinal injection (0.1 mL/eye) in both eyes of either formulation buffer control (n=1) or AAVHSC17-CBA-scGFP, 1.3×10^{12} vg (n=2). Eyes were examined by slit-lamp biomicroscopy and/or indirect ophthalmoscopy following completion of each treatment to confirm location and appearance of the dose. Body weights and food consumption were monitored weekly and twice daily, respectively. Ophthalmic examinations were performed pre-study and on days 3, 8, 15, and at sacrifice on day 28 post-dosing. Spectral domain optical coherence tomography (SD-OCT) for GFP autofluorescence was performed once pre-study and on day 28. At sacrifice, animals were perfused with saline followed by 4% paraformaldehyde, tissues were collected, placed in 4% paraformaldehyde for 24 h, embedded in OCT and frozen. Sections were prepared and analyzed for direct GFP fluorescence and GFP expression by immunohistochemistry (IHC). Animals remained in good health throughout the study. Neither treatment-related ophthalmic changes nor ocular inflammation were observed. SD-OCT images showed regions of GFP autofluorescence that corresponded to the dosing bleb and surrounding tissue in animals treated with AAVHSC17-CBA-scGFP. GFP expression, as determined by direct fluorescence and IHC, was observed in all retinae, optic nerves, optic chiasmata, and optic tracts from animals treated with AAVHSC17-CBA-scGFP. GFP expression was in all retinal layers with greater intensity in cells of retinal pigmented epithelium, photoreceptors, and outer nuclear layer. GFP expression in optic nerves, optic chiasmata and optic tracts was multifocal, less intense and in filament (axon)-shaped structures, while other brain regions examined were negative. No GFP expression was noted in ocular tissues and brains of animals treated with formulation buffer alone. Taken together, these data demonstrate that administration of AAVHSC17-CBA-scGFP by subretinal injection in minipigs was well-tolerated and resulted in GFP expression in all retinal layers. These studies suggest that AAVHSCs may be useful as therapeutic vectors for treating diseases of the eye in humans.