FROM THE COVER

Changing eye color by gene transduction

Intracameral AAV injection lightens iris via melanocyte apoptosis

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Special to *Ophthalmology Times*®

A novel method of changing iris color from brown toward blue was successfully employed in rabbits, with no evidence of resulting inflammation, by injecting into the anterior chamber an adeno-associated virus (AAV) carrying a melanocyte-targeting suicide gene.

Background

With host cell kinases, herpes simplex virus thymidine kinase (HSVtk) phosphorylates ganciclovir (GCV) to GCV triphosphate (GCV-TP), a guanosine analog incorporated into DNA during replication in dividing cells and inducing apoptosis via cell cycle arrest.[1] In most cells, this elicits no inflammatory reaction.[2,3]

Tyrosinase, an enzyme produced only in melanocytes, catalyzes conversion of tyrosine to L-DOPA, the first step in melanin synthesis.

A tyrosinase promoter linked to a HSVtk gene can drive selective expression of the gene in melanocytes.

Procedure

A Dutch Belted rabbit with black and white fur and dark brown eyes received unilateral right eye transcorneal injection of 50 µL AAV6 in phosphate buffered solution into the anterior chamber at a dose of 1E+12 vg, using a 30-gauge needle.

The recombinant AAV vector contained a double-stranded DNA sequence encoding a HSVtk and a mouse tyrosinase promoter.

Pupils were kept miotic with topical 2% pilocarpine during surgery and up

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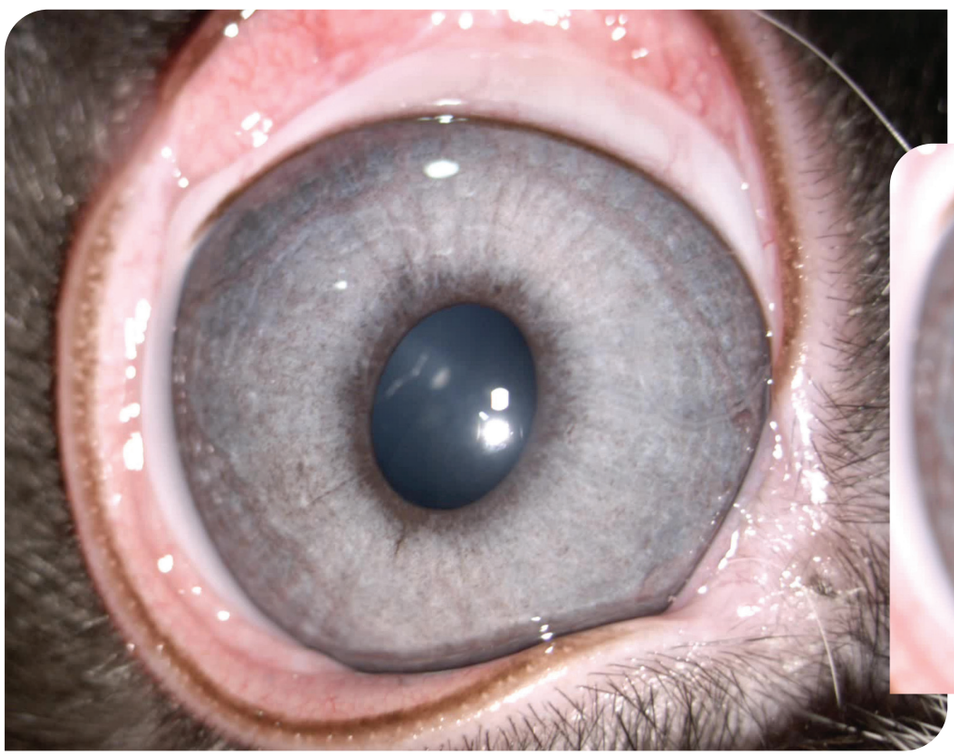

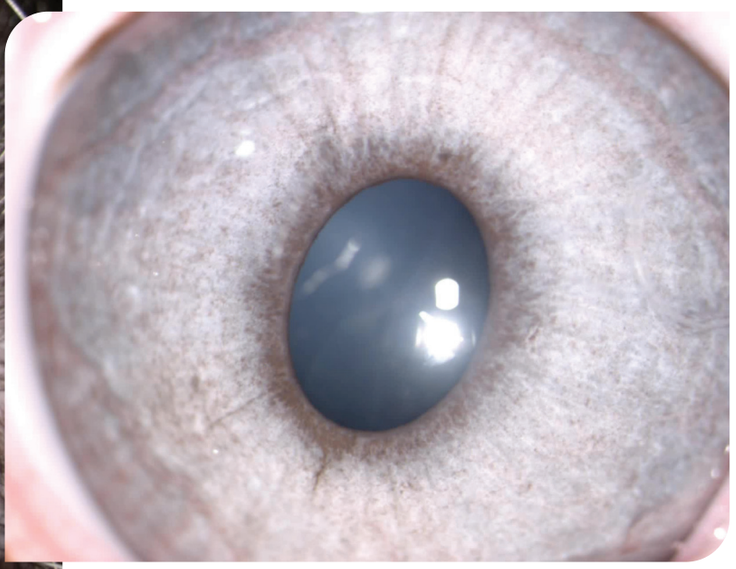

Hematoxylin and eosin staining showed no cellular infiltrate or qualitative difference between treated and contralateral eye in paraffin and cryosections of iris. *(Images courtesy of James Hill, MD, JD)*

COLOR

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to QID for 1 day postoperatively to minimize AAV reflux into the posterior chamber by increasing the velocity of aqueous humor flowing anteriorly through the pupil.

The rabbit then received 50 mg/kg GCV intraperitoneally on days 14 to 18 after AAV injection.

The eyes were examined and photographed by slit lamp on days 0, 1, 3, and 7, then weekly through day 49.

The iris pigment epithelium showed no difference between treated and control eyes, with no observable loss of melanocytes.

Results

The iris of the injected eye (OD) became visibly lighter brown by day 28 after AAV injection, 10 days after GCV administration. By day 35, the iris of the injected eye had developed a bluish gray hue with loss of brown pigmentation, both overall and in a punctate fashion, across the entire anterior surface of the iris in an even distribution.

This color change remained stable, with no further depigmentation observed by day 42 or 49.

The contralateral (control) eye did not change color at any time.

No other changes in ophthalmic examination were observed throughout the 49-day study. IOP remained normal. Cornea and iris looked healthy. No inflammatory cell or flare was observed in the anterior chamber. Lens was transparent. Conjunctiva, sclera, and posterior segment examination remained normal, with normal-appearing vitreous, retina, choroid, and optic nerve, for the entire duration.

We were prepared to treat the injected eye with topical 0.1% dexamethasone at the first sign of anterior chamber inflammation, but this did not occur.

Corneal endothelial cell counts were normal, with no differences between the AAV-treated eye and the contralateral eye.

There was no depigmentation in the rabbit's black fur during the study, indicating no significant loss of melanocytes in hair follicles.

Histology

Hematoxylin and eosin staining showed no cellular infiltrate or qualitative difference between treated and contralateral eye in paraffin and cryosections of iris.

Fontana-Masson staining for melanin revealed loss of anterior iris stromal melanocytes, especially on the anterior surface of the iris, in the treated eye relative to the control eye.

The iris pigment epithelium (IPE) showed no difference between treated and control eyes, with no observable loss of melanocytes. This is especially evident in iris sections perpendicular to ciliary folds, where the IPE runs along edges of the fimbriated regions. The retina and choroid also showed no melanocyte loss.

Immunofluorescent anti-CD68 antibody staining revealed no significant presence of macrophages in the iris and no difference between treated and control eyes.

Other melanocytes protected

This color change technique has 3 requirements to deplete iris melanocytes: (1) a high AAV concentration near target cells for sufficient uptake to express the transgene[4]; 2) tyrosinase expression such that only melanocytes are affected; and 3) DNA replication to incorporate GCV-TP into DNA.[5]

As a result, only iris stromal melanocytes appear to undergo apoptosis with this method because they are capable of mitosis and are exposed to a high AAV concentration in the anterior chamber.

Other melanin-containing ocular cells, including the retinal pigment epithelium,[6] the outer layer of the iris pigment epithelium,[6] and choroidal melanocytes[7] are apparently protected because they are postmitotic and therefore do not replicate their DNA.

Skin and hair melanocytes are unaffected presumably because of massive dilution of AAV in the systemic circulation upon exiting anterior chamber. Moreover, any HSVtk DNA transduced as episomes in proliferating melanocytes would be diluted away with mitosis.[8]

Conclusion

Intracameral AAV delivery of a melanocyte-targeted thymidine kinase gene, followed by nucleoside administration to induce apoptosis, might prove a safe and effective way to change eye color without causing uveitis, glaucoma, or other complications seen with iridial lasers and implants used for this purpose. ▮

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Hill is the founder of Eye Kleur, Inc, and owner of patent applications related to this subject matter.

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