POSTERIOR CHAMBER PHARMACOKINETICS AND ANTI-ANGIOGENIC EFFICACY OF AN ANTISENSE OLIGONUCLEOTIDE TARGETING INSULIN RECEPTOR SUBSTRATE-1

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Objectives

To evaluate the ocular pharmacokinetics and anti-angiogenic efficacy of aganirsen, an antisense oligonucleotide that inhibits insulin receptor substrate-1 (IRS-1) expression, in African green monkeys following topical delivery.

Background

Aganirsen is an inhibitor of IRS-1 previously shown to dose-dependently inhibit corneal angiogenesis in animals and humans [1-3]. Prior studies have confirmed aganirsen eyedrops are safe for human use [4] and penetrate the posterior chamber of the eye in rabbits following topical delivery [1]. A key role for IRS-1 in retinal angiogenesis has been confirmed [5]. Together, these findings suggest aganirsen may be an effective treatment for retinal neovascular diseases via topical delivery.

Detailed Methods

All work was conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Ocular Pharmacokinetics

Adult New Zealand albino rabbits (n=60) received a topical application of 50 µL of ophthalmic emulsion, composed of carbomer, caprylic/capric acid triglycerides, cetyl alcohol, glycerol and polyethylene glycol stearates, sodium hydroxide, and water) containing aganirsen (1.72 mg/mL), giving a local delivery of 86 µg of active compound to each eye. Rabbits were euthanized 15-120 minutes after dosing, eyes enucleated and snap frozen and ocular tissues sub-dissected and homogenized for analysis by ion-exchange chromatography.

Twelve adult African green monkeys (*Chlorocebus sabaeus)* were recruited to evaluate retinal concentration of aganirsen following topical delivery. Animals were sedated with ketamine (8 mg/kg, I.M.) and xylazine (1.6 mg/kg, I.M.) and a single topical dose (54 µL) of aganirsen emulsion was administered on to each eye at a dose of either 21.5, 43 or 86 µg. Animals were euthanized, eyes enucleated, flash frozen and analyzed as listed above.

Laser-Induced Choroidal Neovascularization (CNV)

Twenty-six adult African green monkeys received 16 twice-daily topical doses of aganirsen, beginning 2 days prior (day -2) to laser photocoagulation and continuing for 14 days after lasering. Dosing comprised 54 µL of aganirsen (21.5, 43 or 86 µg) or vehicle (ophthalmic emulsion) on to each eye. Monkeys were randomly assigned to treatment groups (n=6-8) and all dosing, laser photocoagulation and image analysis conducted with observers masked to treatment. Six laser spots were concentrically spaced approximately 1.5 disc diameters from the fovea as described previously [6]. Fluorescein angiography (FA), fundus imaging and optical coherence tomography (OCT) was performed 4 weeks after laser photocoagulation. Late phase fluorescein leakage was graded as described previously [6], whereby I=no hyperfluorescence; II=hyperfluorescence without leakage and no significant residual staining in late-phase angiograms; III=hyperfluorescence early or mid-transit with late leakage and significant residual staining; IV=hyperfluorescence early or mid-transit with late leakage extending beyond borders of the treated area. Maximal CNV complex area was assessed using ImageJ (National Institute of Health, Bethesda, MD).

Results

Peak aganirsen levels in the iris and ciliary body and retina were observed 90 minutes after topical delivery of a single dose in rabbits (Fig. 1). Aganirsen was not detected in the untreated contralateral eye of unilaterally dosed rabbits (n=3, data not shown).

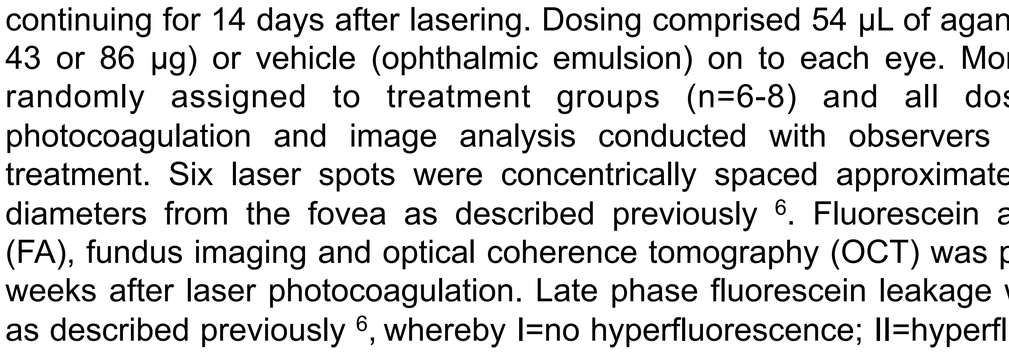

Fig. 1. Aganirsen concentration in the iris and ciliary body (A) and retina (B) of rabbits (n=12 per group) after topical administration of an 86 µg dose (1.72 mg/g). Results shown are mean ± SEM. * P<0.05 compared with 30 and 60 minutes. Corneal aganirsen dosing was estimated to result in delivery of 0.83% of active compound to retina in rabbits, achieving levels previously shown to elicit anti-angiogenic activity *in vitro* [1].

A single 86 µg aganirsen instillation produced significantly higher retinal tissue concentrations in African green monkeys compared with a 21.5 µg dose (Fig. 2).

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Results (continued)

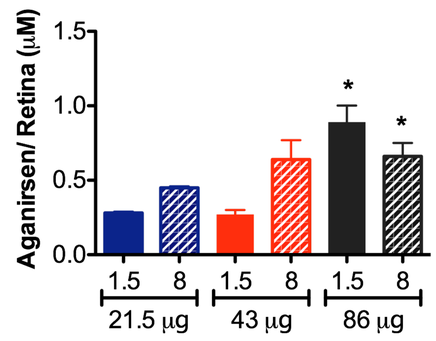

Fig. 2. Aganirsen concentration in the retina of African green monkeys after topical administration of a single dose (21.5, 43 or 86 µg). Animals were euthanized 1.5 or 8 hours after dosing. Results shown are mean ± SEM. * P<0.05 compared with equivalent time point at 21.5 µg dose.

A single topical dose of 43 or 86 µg of aganirsen was sufficient to elicit significant reduction in retinal IRS-1 expression in African green monkeys (Fig. 3).

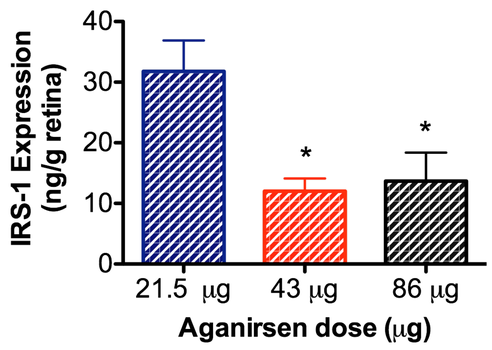

Fig. 3. Effect of aganirsen on IRS-1 expression in retina of African green monkeys. Expression of IRS-1 was assessed in retinal tissue 8 hours after dosing. Significant reduction in IRS-1 expression was observed at 43 µg (P=0.0039) and 86 µg (P=0.031) doses compared with eyes receiving a 21.5 µg dose. Results shown are mean ± SEM.

Significant and near complete inhibition of grade IV CNV lesion development and significantly reduced CNV complex size was observed at the highest dose explored (86 µg; Fig. 4 & 5) in an African green monkey laser-induced model.

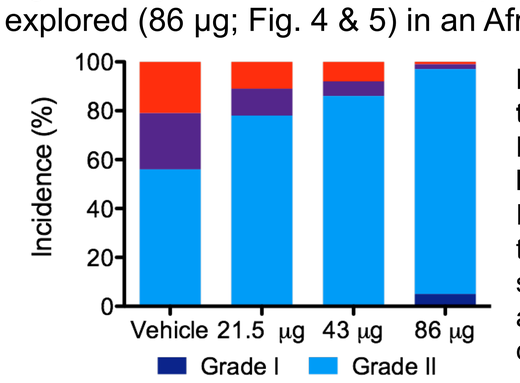

Fig. 4. Graded scoring of week 4 FA images after topical aganirsen or vehicle administration. Percent incidence of graded fluorescein staining and leakage (n=36–78 lesions graded per group). Incidence of grade IV lesions, those representing the most severe neovascularization, was significantly lower (P=0.0005) in twice-daily 86 µg aganirsen-treated eyes than in vehicle-treated controls.

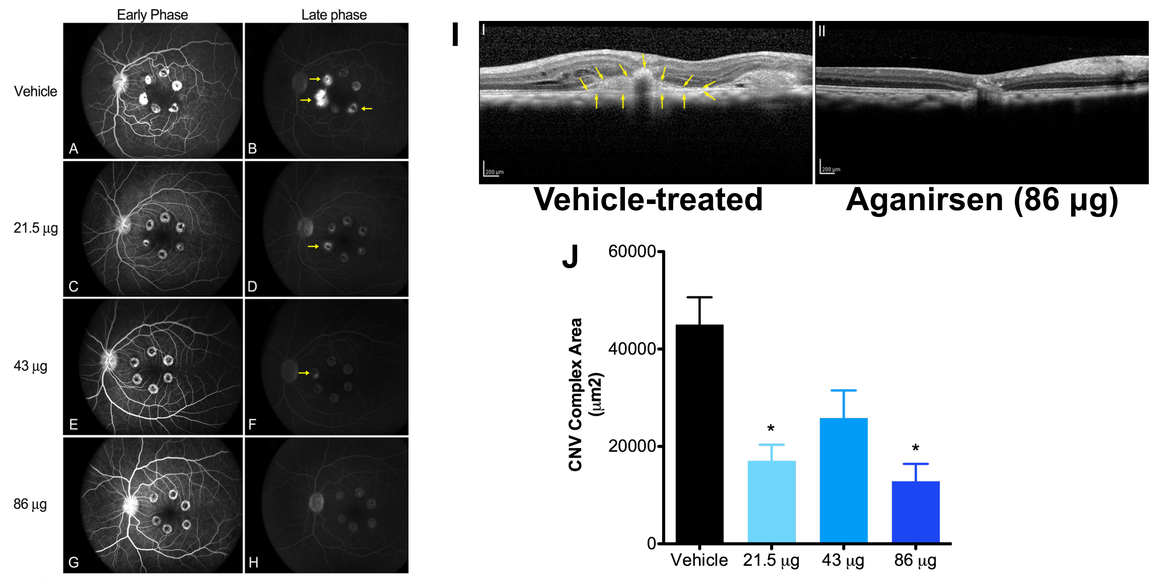

Fig. 5. Fluorescein angiography, OCT imaging and CNV area analysis of OCT images after laser photocoagulation and topical aganirsen administration. Representative early (30s) and late (6 minute) angiograms collected 4 weeks post-laser from vehicle or aganirsen-treated animals (A-H). Late phase fluorescein leakage was almost completely inhibited at the 86 µg dose (H). Representative OCT images highlight reduced CNV complex formation in aganirsen-treated eyes (I). Maximal CNV complex area was significantly lower in eyes receiving 21.5 or 86 µg doses of aganirsen than vehicle-treated control eyes (J), p<0.0001).

Conclusions

1. Significant quantities of aganirsen are delivered to the retina following topical instillation of a single 86 µg dose to the cornea.
2. Retinal levels obtained following single dose administration are dose-dependent.
3. Following topical delivery of a single 86 µg dose, bioactive levels of aganirsen are achieved in the retina.
4. A twice-daily 86 µg dosing regimen conferred significant attenuation of laser-induced CNV in a nonhuman primate model.
5. Retinal delivery is likely to occur via the trans-scleral route although further studies are required to assess ocular pharmacokinetics.

References

1. Al-Mahmood S, Colin S, Farhat N, et al. *The Journal of pharmacology and experimental therapeutics.* May 2009;329(2):496-504.
2. Cursiefen C, Bock F, Horn FK, et al. *Ophthalmology.* Sep 2009;116(9):1630-1637.
3. Hos D, Regenfuss B, Bock F, et al. *Investigative ophthalmology & visual science.* Jul 2011;52(8):5778-5785.
4. Kain H, Goldblum D, Geudelin B, et al. *British journal of clinical pharmacology.* Aug 2009;68(2):169-173.
5. Jiang ZY, He Z, King BL, et al. *Journal of biological chemistry.* Aug 22 2003;278(34):31964-31971.
6. Goody RJ, Hu W, Shafiee A, et al. *Experimental eye research.* Jun 2011;92(6):464-472.