Details Methods

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

Ocular Pharmacokinetics

Both eyes of adult New Zealand albino rabbits (n=40) received a topical application of 50 µL of ophthalmic emulsion, composed of carboxylic acyl caprylic acid triglycerides, caprylic 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. Rabbits were euthanized 15-120 minutes after dosing by intravenous injection of pentobarbital, 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 the retina concentrations of aganirsen 90 minutes and 8 hours after topical delivery. Animals were sedated with ketamine (8 mg/kg, I.M.) for all procedures unless stated otherwise. 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 under sedation with ketamine (10 mg/kg) were euthanized by intravenous sodium pentobarbital overdose. Eyes were enucleated and flash frozen before analysis using the same methods employed for rabbit studies.

Laser-induced Choroidal Neovascularisation

Twenty-six adult African green monkeys with normal ocular health received 16 twice-daily topical doses of aganirsen, beginning 2 days prior to the 14 days after laser photocoagulation. Dosing comprised 54 µL of aganirsen (21.5, 43 or 86 µg) on to each eye. Monkeys were randomly assigned to treatment groups (n=8 for aganirsen groups, n=8 for vehicle-treated group) 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. Fluorescein angiography, fundus Imaging and optical coherence tomography (OCT) was performed 4 weeks after laser photocoagulation. To assess anti-angiogenic activity of aganirsen, angiographic activity of (OCT) was performed 4 weeks after laser photocoagulation. To assess anti-


dose-dependent inhibition of corneal angiogenesis in both animals and humans. Prior studies have confirmed aganirsen exopods are safe for human use and in rabbit models penetration of the posterior chamber of the eye has been demonstrated following topical delivery. Insulin receptor subtype-1 has been shown to play a key role in retinal angiogenesis. Together, these findings highlight the potential merits of employing aganirsen for the treatment of retinal neovascular diseases via topical application. Taking advantage of the genetic and anatomic homology between humans and Old world primates, the present study was conducted in African green monkeys to assess the ocular pharmacokinetics and anti-angiogenic efficacy of aganirsen in a laser-induced model of choroidal neovascularisation (CNV) following topical administration.

Results

Peak quantities of aganirsen in the iris and ciliary body and retina were observed 90 minutes after topical delivery of a single 86 µg dose (1.72 mg/mL) on the cornea. Animals were euthanized 15, 30, 60, 90 or 120 minutes after administration. Results shown are mean ± SEM. * P≤0.05 compared with 30 and 60 minutes. Corneal application of aganirsen was estimated to result in delivery of 0.82% of active compound to retina in rabbits, achieving levels previously shown to exhibit anti-angiogenic activity in vitro.

Retinal aganirsen levels in African green monkeys were significantly higher following administration of a single topical dose of the 86 µg formulation compared with aganirsen levels following instillation of a 21.5 µg dose (Fig. 2).

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 once-daily 86 µg dosing regimen conferred significant attenuation of laser-induced CNV in a non-human primate model.

Retinal delivery is likely to occur via the trans-scleral route although further studies are required to assess ocular pharmacokinetics.

References