Suppression of Corticosteroid-Induced Ocular Hypertension in Sheep by Anecortave

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Objective: To confirm the ocular hypotensive effects of anecortave acetate on an ovine model for steroid-induced ocular hypertension. Eyes of normal sheep exhibit a robust steroid-induced ocular hypertensive response. Recent observations in an uncontrolled, interventional case series indicated that anecortave elicited hypotensive effects when administered as a sub-Tenon depot in the eyes of a small sample of patients with glaucoma.

Methods: Intraocular pressure (IOP) was monitored by Perkins applanation tonometry in 16 normal sheep receiving topically administered prednisolone acetate, 0.5%, in both eyes, 3 times daily, a protocol that doubled IOP within 12 days. Half of the sheep had received a unilateral sub-Tenon injection of anecortave in 1 eye prior to the initiation of the bilateral prednisolone instillations, while the 8 remaining sheep received the unilateral anecortave sub-Tenon depot after the IOP was maximally elevated by the prednisolone instillations.

Results: In these 2 sets of experiments, the presence of the anecortave depot suppressed the steroid-induced IOP elevation and reverted the elevated IOP to baseline levels. Measurements of aqueous outflow facility indicated that eyes treated with prednisolone plus anecortave exhibited a 5.8-fold higher outflow facility than the fellow eyes solely exposed to prednisolone, indicating that anecortave prevented the increase in outflow resistance produced by the corticosteroid.

Conclusion: Elucidation of the mechanisms of action of anecortave in animal models may prove relevant to the design of novel interventions for the management of primary open-angle glaucoma.


A Neocortave Acetate (15-mg anecortave acetate suspension; Alcon Laboratories, Fort Worth, Texas) is an angiostatic steroid that is synthesized from cortisol acetate.1 This cortisene is devoid of conventional corticosteroid hormone activity but is an effective inhibitor of neovascularization in several models for experimental angiogenesis.1,2 It has also been administered, with encouraging results, to patients with diabetic retinopathy and age-related macular degeneration in an effort to treat the angiogenic complications of these diseases.2-4

More recently, the therapeutic effects of anecortave were assessed in a diabetic patient with neovascular glaucoma; both regression of anterior segment neovascularization and enduring reduction in intraocular pressure (IOP) were observed.5 In addition, 2 pilot investigations on the effects of anecortave on the IOPs of patients with primary open-angle glaucoma and patients with steroid-related glaucoma were published.5,6 In general, it was observed that a single sub-Tenon injection of anecortave could dramatically lower IOP within 1 week by an average of approximately 30% in these patients, who served as subjects for these uncontrolled, interventional case series.5,6 This IOP reduction was sustained through 12 months of observation.

However, the mechanisms by which anecortave lowers IOP have not been established. Future biochemical and physiological work on the effects of anecortave in an animal model are warranted. The elucidation of the mechanisms of action of anecortave in animal models may prove relevant to the design of novel interventions for the management of primary open-angle glaucoma.

We recently demonstrated the effectiveness of using Corriedale sheep (Ovis aries) as an animal model for glucocorticosteroid-induced ocular hypertension.7 The IOPs of these animals increased ap-
proximately 2.5-fold within 2 weeks of a topical application of prednisolone acetate, 0.5%, 3 times daily. This IOP elevation occurred in 100% of the corticosteroid-treated eyes. After discontinuation of the corticosteroid instillation, the IOPs of the treated eyes declined to baseline values during the course of 1 to 3 weeks. Similar IOP elevations were obtained in all sheep receiving the corticosteroid triamcinolone acetonide, which was applied as a single sub-Tenon injection rather than topically (unpublished observations, O.A.C. et al, 2009).

The 100% incidence of corticosteroid-induced ocular hypertension in *O aries* and the docile nature of the animals, which readily submit to manipulations such as those required for in vivo outflow facility measurements, render this species an ideal model for both examining the mechanisms underlying corticosteroid-induced glaucoma and testing possible IOP-lowering agents. In addition, sheep have eyes with dimensions and volumes similar to humans (e.g., an anterior-posterior axis of approximately 27 mm and an equatorial axis of approximately 30 mm). As in other ruminants, sheep have a trabecular meshwork and an aqueous plexus that is equivalent to the Schlemm canal.

### METHODS

#### ANIMALS

All animal experiments were performed in accordance with the Association for Research in Vision and Ophthalmology guidelines. A total of 16 healthy (female) sheep (Corriedale breed) between 12 and 24 months of age and weighing 35 to 40 kg were selected from a local ranch in Corrientes, Argentina, for this study. The eyes and general health of the animals were considered normal by an ophthalmologist and a veterinarian. The sheep were tagged for individual identification on their ear lobes. Animals were led to a funnel corral and their heads were suitably oriented within a neck yoke. This arrangement allowed movement and one person to hold the sheep’s head while another instilled the drops.

To measure IOP with the Perkins tonometer, the sheep were also guided into the funnel corral and then into the neck yoke. The more elaborate facility measurements entailed anesthetizing the animals and resting them on a table in a nearby shed. For the sub-Tenon injection of anecortave, sheep were anesthetized locally. Between all procedures, the sheep were free to pasture.

#### PREDNISOLONE INSTILLATION PROTOCOL

All eyes of all sheep received prednisolone in this study. After baseline measurements of IOP over the course of 21 days, prednisolone acetate, 0.5%, was instilled in both eyes of 16 sheep. The instillation protocol consisted of 2 drops, 3 times daily at 7 AM, 2 PM, and 7 PM for a 45-day duration. The sheep were divided into 2 sets of 8. In the first set, 1 eye of each sheep received a single sub-Tenon injection of anecortave 45 minutes before the initiation of the bilateral, daily instillations of prednisolone. Eyes receiving anecortave were selected in an alternative manner with an equal number of right and left eyes. In the second set, the bilateral daily instillations of prednisolone were applied for 10 days, which elicited an approximately 2.5-fold increase in IOP, before the anecortave was introduced into the sub-Tenon space of 1 eye. In both sets, the prednisolone administrations were terminated on day 66 of the experiments, while the monitoring of the IOP continued until day 94.

#### GENERAL ANESTHESIA FOR FACILITY DETERMINATION

Food and water were withheld from animals to be anesthetized for 18 and 6 hours, respectively, prior to the administration of anesthesia to minimize ruminal bloating. Because excess salivation is a concern in anesthetized ruminants, immediately prior to anesthesia atropine (0.5 mg/kg) was administered intramuscularly (IM). Additional doses (0.5 mg/kg IM) were given at 30-minute intervals as necessary. For anesthesia, a combination of ketamine (5 mg/kg IM) and xylazine (0.1 mg/kg IM) was administered. Maintenance doses (one-fourth the induction dose) were applied intravenously as required to maintain the necessary depth of anesthesia, which was determined by a lack of flexor withdrawal response to stimulation of a hind limb and the loss of blink reflex. Before inserting needles through the cornea (for outflow facility measurements, as described below), a local anesthetic was applied to the ocular surface (1-2 drops of proparacaine, 0.5%).

#### SUB-TENON INJECTION OF ANECORTAVE

Two drops of proparacaine, 0.5%, were applied to the ocular surface. Then, a single 1-mL injection of sterile anecortave (75 mg/mL, or 7.5%) was administered via sub-Tenon injection using a 30-gauge needle inserted 5 mm from the limbus in 1 eye while the contralateral eye received an injection of vehicle. Alcon Research chose the dose and volume of anecortave to align with that used in an earlier (unrelated) study the company conducted.

For the injections, the distance from the limbus was determined from the width of a 5 mm × 30 mm Schirmer strip that was held between the limbus and the injection site. The conjunctiva at the injection site was grasped with fine forceps, and an initial oblique conjunctival puncture was made with the needle bevel facing up. The needle was then pushed deeper (continuing at an oblique angle) to create another puncture in the Tenon capsule, in such a manner that the Tenon puncture did not underlie the conjunctival puncture. The needle was pushed through the Tenon capsule until the tip reached the sclera (as determined by feel). Care was taken to avoid puncturing the sclera itself. Also, because the Tenon capsule consists of several layers, care was also taken to administer a sub-Tenon (to create a juxtascleral depot) and not an intra-Tenon injection. Immediately following the injection, the needle was rapidly withdrawn. The volume injected (1 mL) yielded a characteristic 180° to 240° quasi-doughnut-shaped bolus of fluid around the limbus. Each sheep treated with anecortave received the drug in 1 eye, while the contralateral eye received a sub-Tenon injection of the vehicle. The injected eyes then received 2 drops of tobramycin ophthalmic solution, 0.3%.

#### MEASUREMENT OF IOP

Animals were led to a funnel corral and their heads were suitably oriented within a neck yoke to enable an ophthalmologist to measure IOP with the Perkins tonometer. Before the IOP measurement, 2 drops of topical proparacaine, 0.5%, followed by 2 drops of fluorescein, 0.25%, were instilled. Two sets of measurements were taken on each eye, alternating between eyes. All IOP measurements were taken between 2 and 4 PM every 2 or 3 days. The Perkins tonometry readings were converted to millimeters of mercury as described in detail previously.
Prednisolone and anecortave acetate. Contralateral eyes of each sheep received single sub-Tenon injections of either anecortave or vehicle 45 minutes before the initiation of bilateral, daily instillations of prednisolone on day 21 of the experiments (first arrow). On day 66, the daily topical instillations of prednisolone were terminated (second arrow). Error bars indicate standard error of the mean.

AQUEOUS OUTFLOW FACILITY MEASUREMENTS ON ANESTHETIZED SHEEP

Following attainment of a surgical plane of anesthesia (as described previously), the anterior chamber was cannulated with a 30-gauge steel needle and subjected to a constant flow infusion of a balanced salt solution at rates of 1, 3, and 5 µL/min while simultaneously monitoring IOP. Each flow rate was maintained for 5 minutes or until the IOP stabilized. Thereafter, the process was repeated for a total of 3 IOP measurements at each flow rate. The pressure developed by the infusion (change in IOP) was measured with either a calibrated inline pressure transducer or with a second 30-gauge needle inserted into the anterior chamber connected to a calibrated pressure transducer. Outflow facility, or outflow conductance, was determined from the inverse of the regression line slope drawn through a mean pressure–flow rate curve. This approach was our implementation of methods described earlier to measure outflow facility in vivo.6,10

The same sheep that were used to measure the effects of prednisolone and anecortave on IOP were also used for the outflow facility measurements. As reported earlier,7 we found that termination of the corticosteroid instillation resulted in the sheep’s IOPs declining to baseline values during 1 to 3 weeks. In the present study, 1 month was allowed to elapse after discontinuation of prednisolone instillations, at which point IOP was normal. Then, of the 16 original sheep, 9 were randomly selected for the outflow conductance measurements following a second exposure to the drugs. Because these animals had been treated earlier, with only 1 eye having received anecortave (both eyes had received prednisolone), in this phase of the experiments, the drug administrations were reinitiated with the contralateral eye from 10.1 mm Hg (0.7 mm Hg) to 27.5 mm Hg (0.5 mm Hg) (n=8), a value approximately 2.4-fold higher than the mean IOP of 11.0 mm Hg (0.6 mm Hg) (n=8) in the fellow eye, which had been administered anecortave (P<.001, as either paired or unpaired data).

Intraocular pressure in both eyes of the 16 normal sheep was measured prior to any treatment to establish baseline values. The measured Perkins tonometry readings and the equivalent IOP as determined from a calibration curve indicated baseline pressures between 9.3 and 11.2 mm Hg and a rounded mean of 10.1 mm Hg, values similar to those obtained earlier.7

The present experiments were designed to determine if the administration of anecortave would both (1) prevent the IOP elevation in a sheep model for glucocorticosteroid-induced ocular hypertension and (2) reduce the elevation in IOP after its establishment by daily topical instillations of corticosteroid. Prednisolone was administered as the IOP-elevating agent as used previously.7

The first set of experiments (8 sheep) tested if anecortave prevented the IOP elevation elicited by prednisolone (Figure 1). After recording the control IOP of both eyes during a period of 21 days, the contralateral eyes of each sheep received a single 1-mL sub-Tenon injection of either the vehicle or 7.5% anecortave acetate solution (75 mg) under topical anesthesia. Forty-five minutes later, the daily topical instillations of prednisolone, 0.5%, were initiated. Twelve days later (day 33 of the protocol), the eyes injected with the vehicle reached a mean IOP of 26.4 mm Hg (0.7 mm Hg) (n=8), a value approximately 2.4-fold higher than the mean IOP of 11.0 mm Hg (0.6 mm Hg) (n=8) in the fellow eye, which had been administered anecortave (P<.001, as either paired or unpaired data).

The IOP of the anecortave-treated eyes remained indistinguishable from that of the control level until day 42, when the pressure increased slightly to 12.1 mm Hg (0.3 mm Hg) (Figure 1). This IOP reading was significantly higher than the previously measured value of 11.1 mm Hg (0.2 mm Hg) on day 38 (P<.005, as paired data). Thereafter, further gradual increases occurred until the mean IOP reached 26.0 mm Hg (0.4 mm Hg), a level virtually identical to that of the vehicle-treated fellow eye on day 66 (P>7, as unpaired, 2-tailed data; P>3, as paired data). The daily prednisolone instillations were then terminated, which resulted in a gradual bilateral return of IOP to the baseline level over the course of 2 to 3 weeks.

The second set of experiments (Figure 2) aimed to determine whether or not the administration of anecortave would reverse the elevated IOP produced by the daily prednisolone instillations in the sheep model. The prednisolone dosing was initiated bilaterally on day 21 in 8 normal sheep. Mean IOP increased in 1 eye from 10.4 mm Hg (0.8 mm Hg) to 27.5 mm Hg (0.5 mm Hg) (n=8), and in the other eye from 10.1 mm Hg (0.7 mm Hg) to

Figure 1. Intraocular pressures of 8 sheep treated with prednisolone and anecortave acetate. Contralateral eyes of each sheep received single sub-Tenon injections of either anecortave or vehicle 45 minutes before the initiation of bilateral, daily instillations of prednisolone on day 21 of the experiments (first arrow). On day 66, the daily topical instillations of prednisolone were terminated (second arrow). Error bars indicate standard error of the mean.

Figure 2. Aqueous outflow facility measurements on anesthetized sheep. The first set of experiments (8 sheep) tested if anecortave prevented the IOP elevation elicited by prednisolone (Figure 1). After recording the control IOP of both eyes during a period of 21 days, the contralateral eyes of each sheep received a single 1-mL sub-Tenon injection of either the vehicle or 7.5% anecortave acetate solution (75 mg) under topical anesthesia. Forty-five minutes later, the daily topical instillations of prednisolone, 0.5%, were initiated. Twelve days later (day 33 of the protocol), the eyes injected with the vehicle reached a mean IOP of 26.4 mm Hg (0.7 mm Hg) (n=8), a value approximately 2.4-fold higher than the mean IOP of 11.0 mm Hg (0.6 mm Hg) (n=8) in the fellow eye, which had been administered anecortave (P<.001, as either paired or unpaired data).

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STATISTICAL ANALYSIS

The significance of experimentally elicited changes in IOP and facility measurements were analyzed using the t test as either paired or unpaired data, with α = .05 chosen as the level of significance. There are instances in which the 2 eyes from the same animal can react equally to a treatment, in which case paired analysis can be used. On the other hand, there is evidence that fellow eyes are not identical, in which case unpaired tests should be used. To avoid uncertainties, we used both tests. Data are presented in mean (standard error of the mean) unless otherwise indicated.
26.5 mm Hg (0.4 mm Hg) (n=8) between days 21 and 31. After recording the pressure readings on day 31, the contralateral eyes of each sheep received a single sub-Tenon injection of either anecortave or vehicle, and the thrice-daily instillations of prednisolone were continued until day 66. Between days 31 and 66, the IOPs of the eyes receiving anecortave reverted to baseline levels, reaching a value of 11.6 mm Hg (0.7 mm Hg) on day 49 (Figure 2). The IOP of the eyes receiving the vehicle was 25.3 mm Hg (1.5 mm Hg) on day 49. Thereafter, the IOPs of the eyes that had received the anecortave depot gradually increased, resulting in the IOPs of the contralateral eyes of each sheep becoming identical between days 59 and 66. After the daily prednisolone instillations were ended, the IOPs declined bilaterally to the baseline level in 2 weeks.

To determine if the IOP-lowering effect of anecortave could be attributed to an improvement in aqueous outflow facility, outflow conductance was measured bilaterally in eyes of 9 sheep, with 1 eye solely exposed to prednisolone and the fellow eye treated with prednisolone plus anecortave. After 15 days of bilateral prednisolone instillations, the IOP was 11.8 mm Hg (0.5 mm Hg) in eyes pretreated with a sub-Tenon injection of anecortave and 26.7 mm Hg (0.4 mm Hg) in the fellow eyes that received a sub-Tenon injection of vehicle. The 2 outflow conductance values from the contralateral eyes were then determined within a half hour of each other (obtained values compiled in the Table). The mean outflow conductance value for the eyes receiving prednisolone plus anecortave were consistently higher in each animal. On average, the eyes administered anecortave exhibited a 5.8-fold higher outflow conductance value of 0.46 µL/(min × mm Hg) (0.15 µL/[min × mm Hg]).

Glucocorticosteroids, such as prednisolone, exhibit therapeutic versatility given their common usage as anti-inflammatory, immunosuppressive, and anti-angiogenic agents. However, glucocorticosteroids also elicit adverse ocular effects, such as causing cataracts and increasing IOP. Individuals susceptible to the latter adverse effect may require treatment for glaucoma.

The phenomenon of glucocorticosteroid-induced ocular hypertension has been recognized for decades, and a number of predisposing risk factors have been identified among patients who received various corticosteroid treatments; yet the mechanisms by which glucocorticosteroids induce IOP elevation have not been determined. It is recognized that this adverse effect is due to a reduced trabecular aqueous humor outflow associated with morphological and biochemical changes in the trabecular meshwork. As such, a thorough understanding of the cellular processes eliciting corticosteroid-induced ocular hypertension may shed light on the cause of primary open-angle glaucoma.

An elucidation of the mechanisms by which anecortave both suppressed and reduced the IOP elevation produced by prednisolone in the present study could be equally important in understanding primary open-angle glaucoma. Anecortave was designed to retain the anti-angiogenic (or angiostatic) activity of the glucocorticoids, but to be devoid of conventional steroid hormone activity. Its ocular hypotensive effect was apparently discovered by serendipity in patients, an effect in accord with unpublished observations that anecortave was able to suppress dexamethasone-induced ocular hypertension in rabbits. These observations led to the design of the present study on sheep.

We preferred the sheep model for corticosteroid-induced ocular hypertension than the rabbit model because only about 50% of rabbits treated chronically with glucocorticoids such as dexamethasone develop ocular hypertension, and responders to dexamethasone are com-

![Figure 2. Intraocular pressures of 8 sheep treated with prednisolone and anecortave acetate. Contralateral eyes of each sheep received daily instillations of prednisolone beginning on day 21 of the experiments (first arrow). On day 31, the contralateral eyes of each sheep received single sub-Tenon injections of either anecortave or of vehicle (second arrow). On day 66, the daily topical instillations of prednisolone were terminated (third arrow). Error bars indicate standard error of the mean.](https://archopht.jamanetwork.com/)

### Table. Effect of Anecortave on Aqueous Outflow Facility in the Presence of Prednisolone

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>Prednisolone (n=9)</th>
<th>Prednisolone and Anecortave (n=9)</th>
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*Facility measurements from fellow eyes of each animal were determined within a half hour of each other at the point in the experiments (see “Results” section) at which the effect of anecortave acetate on intraocular pressure was near maximal.

*Mean (standard error of the mean), 0.08 (0.02) µL/[min × mm Hg] significantly larger by approximately 5.8-fold, with P < .02 as 2-tailed paired data; P < .01 as paired data.
monly defined as those exhibiting IOP elevations of at least 5 mm Hg. In contrast, all treated sheep responded to prednisolone with 2.5-fold increases in IOP as reported previously. Moreover, we did not observe cataract formation in any of the animals used in this or the previous study, possibly because of the limited time (45 days) of corticosteroid treatment as well as the low dose and frequency of instillation.

Although prednisolone and anecortave share angiostatic activities, these compounds appear to elicit antagonistic effects on the aqueous outflow facility of the sheep in which this parameter was measured. The average outflow conductance value in eyes treated with prednisolone was 0.08 μL/(min × mm Hg), and was approximately 5.8-fold higher, or 0.46 μL/(min × mm Hg) in eyes simultaneously exposed to prednisolone plus anecortave (Table). To the best of our knowledge, values for outflow conductance in normal sheep have not been previously reported. We did not determine the effects of anecortave on the outflow conductance values of control eyes because we had access to a limited supply of the compound, which was provided by Alcon. Thus, anecortave could be a corticosteroid blocker alone or act in another fashion to reduce IOP elevation due to other causes. In addition, we did not measure the outflow conductance values in normal sheep because we chose to limit the number of sheep that we submitted to this invasive protocol. However, we expect the 0.46 μL/(min × mm Hg) value to approximate the control outflow conductance of the normal sheep eye. This value is similar to that obtained in eyes of other species, eg, 0.2 to 0.4 μL/(min × mm Hg) in rabbits and 0.3 μL/(min × mm Hg) in humans. As such, it is possible that anecortave reversed the increase in aqueous outflow resistance produced by prednisolone so that outflow conductance in the combined presence of the 2 agents was similar to that in the normal eye.

The higher aqueous outflow facilities of eyes simultaneously exposed to prednisolone and anecortave (Table) may explain both the initial prevention of corticosteroid-induced IOP elevation by anecortave (Figure 1) and the IOP reduction elicited by this cortisone when administered after the induction of ocular hypertension (Figure 2). The favorable IOP management elicited by anecortave was transient in the face of continual thrice daily prednisolone instillations (Figure 1 and Figure 2). Our study was not designed to directly determine an explanation for the transient effect. However, the anecortave depot could be seen to disappear from the eye in approximately 30 days, suggesting that the drug was being depleted, and insufficient quantities remained to antagonize the IOP-elevating effects of prednisolone.

The fact that remnants of the anecortave depot could be observed in the eye for such a prolonged period indicated that the injections were confined to the sub-Tenon space. The volume of fluid injected yielded a 180° to 240° doughnut-shaped bolus around the limbus which persisted as a continuous arch for more than 10 days. Thereafter, evidence of the anecortave depot could be seen as speckled coloration on the eye for about 30 days. If the injected fluid had been placed into the subconjunctival space, the fluid would have more rapidly spread and disappeared within a few days.
REFERENCES