Steroid-Induced Ocular Hypertension in Normal Sheep

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Purpose. To determine whether the ovine eye develops elevation of intraocular pressure (IOP) in response to corticosteroid applied topically.

Methods. IOP was monitored by Perkins applanation tonometry in a group of 18 sheep receiving topically administered 0.5% prednisolone acetate in one eye (experimental), three times daily, for a period of 3 or four 4 weeks after the establishment of baseline IOP values. Perkins readings were converted to actual mm Hg using a calibration curve derived from in vivo manometric measurements. IOP was monitored for an additional 1 to 3 weeks after discontinuation of corticosteroid treatment.

Results. Baseline IOP in normal sheep was 10.6 ± 1.4 mm Hg (mean ± SD; $n = 36$ eyes). The IOP of the experimental eyes began to increase after 1 week of prednisolone treatment in all sheep and reached a peak 1 week later (27.5 mm Hg experimental vs. 11.7 mm Hg fellow, control eye; $P < 0.001$). After the discontinuation of corticosteroid instillation, the IOP of the treated eyes declined to the baseline values over the course of 1 to 3 weeks.

Conclusions. Ovine eyes exhibit a robust steroid-induced ocular hypertensive response, with 100% occurrence in this trial. The mechanisms of steroid-induced glaucoma may be related to those involved in primary open-angle glaucoma and could provide insight into primary open-angle and clues to its treatment. (*Invest Ophthalmol Vis Sci.* 2009;50:669–673) DOI:10.1167/iovs.08-2410

The administration of glucocorticosteroids by a variety of routes can lead (in susceptible persons) to the development of ocular hypertension and open-angle glaucoma. This occurs because of a reduction in aqueous humor outflow that is associated with morphologic and biochemical changes in the trabecular meshwork.[1,2] 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. However, although steroid-induced ocular hypertension has been recognized for more than 50 years,[3] and a number of predisposing risk factors have been identified among patients receiving various corticosteroid treatments,[1,2] the biological processes contributing to the increased intraocular pressure (IOP) are largely unknown and are the subject of contemporary investigations. Moreover, basic trabecular meshwork physiology is not yet fully characterized.

We have previously reported that the bovine eye is a reliable and reproducible in vivo model for steroid-induced glaucoma.[4] In our earlier report, topically applied prednisolone acetate induced IOP elevation in 100% of the animals, and the IOP returned to normal values when treatment was discontinued. We report here that the IOP response to topical prednisolone treatment in normal sheep resembles that observed earlier with cows, thereby identifying a second mammalian species with an apparent 100% susceptibility to corticosteroids that may also serve as a model for steroid-induced ocular hypertension. These experiments have been conducted to determine whether another mammal exhibiting a higher-than-plasma aqueous humor concentration of chloride, such as sheep,[5] responds to corticosteroids with elevations in IOP. In addition, in vivo experiments are simpler to perform in sheep than in the cows.

Materials and Methods

Animals

All animal experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Eighteen healthy sheep (female; Corriedale breed) between 18 and 36 months of age and weighing 35 to 40 kg selected from a local ranch in Corrientes, Argentina, were used for this study. They were tagged on their ear lobes for individual identification and were herded from pasture whenever it was necessary to have drops instilled or IOP measured. The sheep were guided into a funnel corral ending in a loose-fitting yoke (*cepo*; Fig. 1). This arrangement allowed for free movement of the head, which could be held by one person while another instilled the drops. To measure IOP, the sheep were guided into the funnel corral and then into the neck yoke. This procedure took approximately 4 minutes per animal; otherwise, the sheep were free to pasture.

Drugs and Protocol

After baseline measurements of IOP over the course of 1 week, 0.5% prednisolone acetate (Ultracortenol; Novartis Ophthalmics, Hettlingen, Switzerland) was instilled in one eye each of five sheep. As a control, an artificial tear preparation (Alcon Lagrimas II; Alcon Argentina, Tortuguitas, Argentina) was instilled in the contralateral eye. For the first experiment, control and experimental instillations consisted of 2 drops, 3 times daily at 7 AM, 2 PM, and 7 PM for a 3-week duration of intervention in five sheep. In four other sheep, the 3-week intervention protocol was extended for an additional week, during which the frequency of instillation was reduced to twice daily. In another group of nine animals, drops were instilled 3 times a day for a period of 4 weeks and then reduced to twice daily for 2 weeks before the drops were discontinued. Animals were monitored for an additional 1 to 3 weeks after the discontinuation of the corticosteroid treatment.

Plastic bottles containing the drops were covered with a tape, either red (artificial tears) for the control eyes or green (prednisolone) for the drug-treated eyes, thus masking the identity of the agent administered to the examiner.

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FIGURE 1. Sheep in the funnel corral and yoke (cepo), which allowed for free movement of the head during the instillation of eye drops.

Instillation of Drops

One of the authors (RG) taught the field hands who cared for the sheep the procedure for instilling the drops. They were given both types of drops, as described, from an investigator (OAC) who was not directly involved with the IOP measurements, and they were provided sealed instructions on what "color drop" to instill to right or left eyes, how many times, and when. The field hands did not know the contents of the bottles.

Measurement of IOP with the Handheld Perkins Applanation Tonometer

Once the animal was held in the yoke and its head was properly oriented, an ophthalmologist measured the IOP with the Perkins tonometer. Before the IOP measurement, 2 drops of topical 0.5% proparacaine (Alcon Argentina) followed by 2 drops of 0.25% fluorescein were instilled. Two sets of measurements were taken on each eye, alternating first one eye and then the other (Fig. 2). The ophthalmologist measuring the IOP was unaware of the treatment of each eye. All IOP measurements were taken between 2 PM and 4 PM at least once a week.

FIGURE 2. IOP measurement with a Perkins applanation tonometer on the left eye of a sheep held by two field hands. The photograph illustrates the docile nature of the sheep during the IOP measurement, though such measurements were commonly performed with the animals in the cepo during the course of this study.

Calibration of the Perkins Tonometer in Sheep Eyes by Cannulation In Vivo

A manometric measurement by cannulation was performed in three eyes of three additional sheep. The purpose was to determine the actual IOP in these animals and to calibrate the Perkins tonometer in vivo. With the sheep in the yoke, before cannulation, 3 drops of 0.5% proparacaine were instilled in the eye. Two minutes later, a 25-gauge butterfly needle was introduced into the anterior chamber. The butterfly's tubing was connected to a custom-made pressure-recording instrument. This instrument consisted of three parts: an inline pressure transducer (Ohmeda model TNF-R; Ohmeda Ltd, Singapore), a custom-made amplifier, and a high-impedance custom-made millivolt meter. The pressure transducer was connected to the butterfly tubing through a valve. The signal of the transducer was fed into the amplifier. The amplifier was calibrated against a column of water connected to the transducer so that its output in millivolts corresponded to pressure in millimeters of mercury. The amplifier output was read on the liquid crystal display screen of the voltmeter.

Intraocular pressure was continuously recorded by the pressure transducer (model TNF-R; Ohmeda) and was altered by adjusting the height of a variable column of balanced salt solution attached to a second cannulation needle inserted in the anterior chamber, 180° from the previous one. The IOP was then sequentially fixed to 10-, 20-, 30-, and 40-cm water pressure (10 mm Hg = 13.6 cm H_2O pressure). A handheld Perkins applanation tonometer with a clinically used Goldmann applanation tip was used. The instrument was powered off after each measurement. Readings were recorded for each pressure level, and the values obtained are compiled in Table 1. The manometric (true) IOP (after converting the water column levels to millimeters of mercury), were plotted against the Perkins readings (Fig. 3), and the equation for the line obtained by regression analysis was used to calculate the IOP of the 18 sheep treated with prednisolone. R^2 was 0.94, indicating a very good correlation.

Data Analysis

IOP differences between experimental and control eyes were analyzed using analysis of variance, with time and group as independent variables. Tukey-Kramer post hoc testing was performed to determine which time points had significantly elevated IOP.

RESULTS

The IOP in both eyes of the 18 normal sheep was measured before any treatment to establish the baseline values in these animals (Table 2). The measured Perkins tonometer reading and the equivalent IOP, as determined from the Perkins cali-

TABLE 1. In Vivo Calibration of Perkins Tonometry Readings

Sheep*	Water Column (cm H_2O)	Water Column (mm Hg)	Digital Meter (mm Hg)	Perkins Units
1	10	7.4	6.9	1
	20	14.7	12.5	1.5
	30	22.1	18.8	3.5
	40	29.4	23.7	5.5
2	10	7.4	7.1	1
	20	14.7	13.1	2
	30	22.1	19.2	4
	40	29.4	22.6	5
3	10	7.4	6.5	1
	20	14.7	13.7	2
	30	22.1	19.4	4
	40	29.4	23.9	6.5
Mean ± SD of 3 eyes		7.4		1.0 ± 0.0
		14.7		1.8 ± 0.3
		22.1		3.8 ± 0.3
		29.4		5.7 ± 0.8

Manometric calibration of the Perkins reading was performed in vivo by cannulation of three eyes of three sheep. Perkins values were plotted against water pressure values (in mm Hg), as shown in Figure 4.

* All readings were taken in the left eye.

bration curve (Fig. 3), indicated a normal IOP of between 9 and 13 mm Hg and a mean value of approximately 10.6 mm Hg. These values were lower than the 16 to 17 mm Hg levels seen earlier in the bovine.[4]

IOP values obtained on a weekly basis during the course of the experiments indicated substantial elevation in the eyes treated with corticosteroid ($P < 0.001$ ANOVA). The protocols in the three sets of experiments depicted in Figures 4, 5, and 6 were identical during the initial 4 weeks so that the IOP of the experimental eye at week 2 (23.2 ± 1.1 mm Hg [mean ± SE]; $n = 18$), representing 7 days of prednisolone administration, was significantly higher than that of the contralateral control eye (11.2 ± 0.4 mm Hg; $n = 18$; $P < 0.001$, paired *t*-test) and was significantly higher than baseline ($P < 0.05$, post hoc testing).

At the third week, representing 14 days of steroid treatment, the IOP values for the respective control ($n = 18$) and experimental ($n = 18$) eyes were 11.7 ± 0.3 and 27.5 ± 0.8 mm Hg ($P < 0.001$, paired *t*-test). IOP elevation persisted for 1 to 2 weeks after treatment discontinuation (Figs. 4–6).

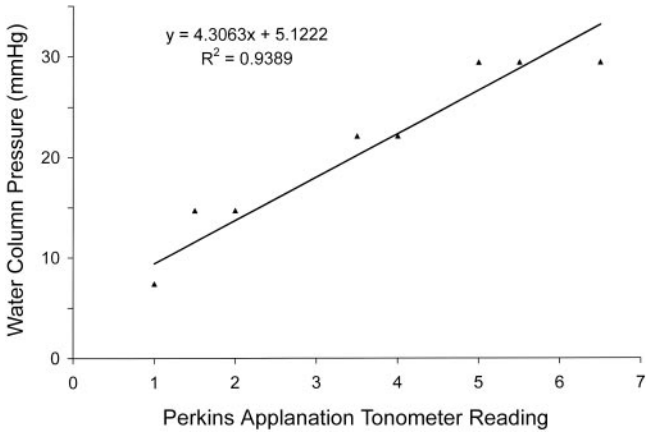

FIGURE 3. Manometric (true) IOP in cannulated sheep eyes in vivo compared with Perkins applanation tonometer reading. Plot of data shown in Table 1; the 12 Perkins values in the table are shown as eight points because four overlap. The equation for the line obtained by regression analysis was used to calculate the IOP of normal sheep exposed to topical prednisolone during the course of this study.

In the second set of experiments (Fig. 5), the frequency of the prednisolone instillation was reduced to twice daily for 1 week (weeks 4–5 of the experiment), and in the third set of experiments (Fig. 6), it was reduced to the same frequency for 2 weeks. During this period the IOP remained elevated. IOP differences between contralateral eyes in the three groups of sheep were not significantly different at any time point for the first 5 weeks ($P > 0.2$, ANOVA).

DISCUSSION

Corticosteroid administrations evoke ocular hypertension because of increased aqueous outflow resistance.[1,2] Studies on cultured trabecular meshwork cells and on organ cultured eyes and ultrastructural studies on human specimens suggest that the following general mechanisms are involved: (1) mechanical changes in the microstructure of the trabecular meshwork resulting from reorganization of actin stress fibers and formation of reversible actin networks mediated by trabecular meshwork glucocorticoid receptors[6,7]; (2) increased deposition of extracellular matrix material, altering the ultrastructure of the outflow pathway (this fine fibrillar material accumulating in the juxtacanalicular region can be related to increases in glycosaminoglycans, elastin, and fibronectin production caused by steroids)[8–10]; (3) reduction in the protease activities and phagocytic properties of the cells, leading to decreases in the breakdown of substances in the trabecular meshwork[11–13]; (4) transcellular or paracellular reduction of water flow in the trabecular meshwork.[14,15]

Gene expression profile analysis of human trabecular meshwork cells exposed to prolonged dexamethasone treatment found that some actins and actin-associated proteins are involved in the development of cross-linked actin networks in the treated cells.[16] In addition, a trend toward decreased expression of protease genes and increased expression of protease inhibitors in the dexamethasone-exposed cells was identified.[16]

We propose that cultured cell studies, such as those described, be supplemented by work on trabecular meshwork material procured from live animal models (e.g., cows and sheep). Previously, it had been shown that corticosteroids induce ocular hypertension in rabbits, but the results were variable and the amount of corticosteroid necessary was often

TABLE 2. Intraocular Pressure of Normal Sheep

	Perkins Tonometer Reading		IOP as Determined by Perkins Tonometry (mm Hg)*	
Sheep	Right Eye	Left Eye	Right Eye	Left Eye
1	1.75	2.0	12.4	13.5
2	1.5	1.73	11.3	12.3
3	1.5	1.5	11.3	11.3
4	1.5	1.5	11.3	11.3
5	1.75	1.75	12.4	12.4
6	2.0	1.5	13.5	11.3
7	1.5	1.0	11.3	9.1
8	1.0	1.0	9.1	9.1
9	1.5	1.5	11.3	11.3
10	1.0	1.13	9.1	9.6
11	1.5	1.5	11.3	11.3
12	1.0	1.38	9.1	10.7
13	1.0	1.25	9.1	10.2
14	1.0	1.0	9.1	9.1
15	1.0	1.38	9.1	10.7
16	1.0	1.0	9.1	9.1
17	1.25	1.75	10.2	12.4
18	1.0	1.0	9.1	9.1
Mean ± SD	1.32 ± 0.33	1.38 ± 0.31	10.5 ± 1.5	10.8 ± 1.4

* IOPs were obtained from the Perkins tonometer readings with the calibration curve shown in Figure 3.

lethal.[17] Moderate elevations of IOP can be produced in some cats by topical administration of corticosteroids,[18] whereas corticosteroid-induced ocular hypertension is inducible in cynomolgus monkeys; in one series, 5 of 11 monkeys exhibited increased IOP on topical dexamethasone treatment.[19] (For a more extensive review of other open-angle glaucoma animal models, see Simoens et al.[20] and Guyomard et al.[21].) In contrast, cows and sheep exhibit 100% incidences of elevated IOP in response to prednisolone administration and are thus potentially suitable for characterizations of the biochemical and molecular factors involved in the increase in aqueous outflow resistance. In this regard, the ovine species may be a superior model given the shorter amount of time (1 week) needed to evoke a significant increase in IOP, whereas 3 weeks of steroid instillation were required in the case of the bovine.[4]

Sheep are docile animals and are particularly well suited for in vivo experiments (e.g., outflow studies), which are extremely difficult to perform in cows. This advantage outweighs the current lack of extensive genetic information on these animals. On the other hand, in terms of aqueous secretion, ovine physiology appears to be similar to human physiology,[5] and trabecular meshwork anatomy appears to be similar to that of the primates.[20,21] The major advantages of using an ovine steroid-induced model of IOP elevation are the consistency and

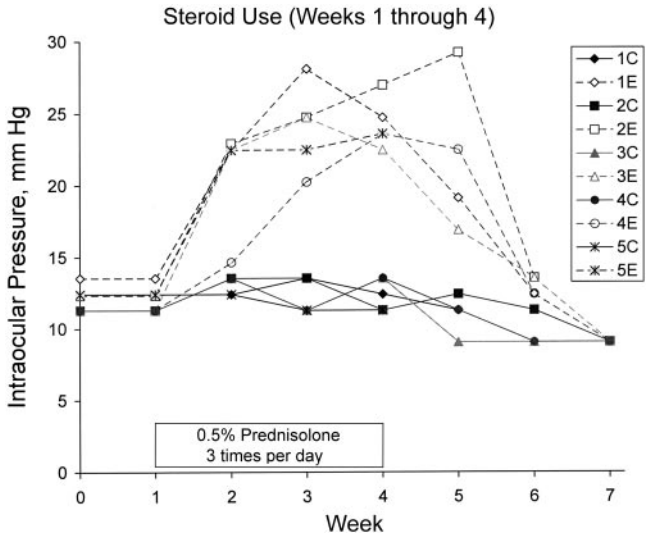

FIGURE 4. Weekly plot of the IOP from five sheep treated unilaterally with topical corticosteroid. The three times daily instillation of drops began on the seventh day of the experiment. Control eyes (C) received artificial tears, and the contralateral experimental eyes (E) received 0.5% prednisolone acetate (*open symbols*). An effect by the steroid persisted after discontinuation of the treatment. Animal 5 was killed at week 4.

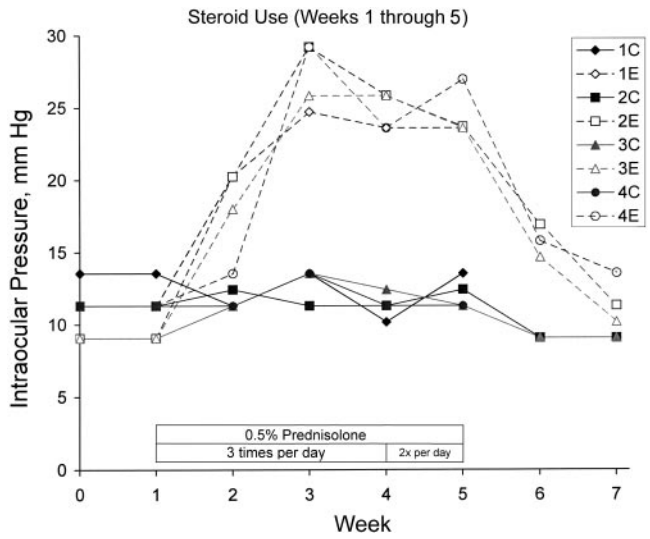

FIGURE 5. Weekly plot of the IOP from four sheep treated unilaterally with topical corticosteroid. Values from eyes treated with prednisolone are plotted with *open symbols* connected by *dashed lines*. Between the fourth and fifth weeks of the experiment, the frequency of prednisolone instillation was reduced from three times daily to twice daily. Animal 1 was killed at week 5; data collection from eye 4C was discontinued after week 5 because of a corneal ulcer.

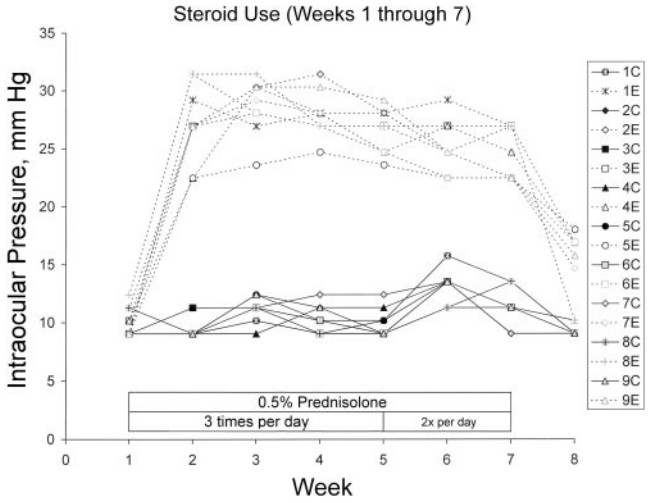

FIGURE 6. Weekly plot of IOP of nine sheep treated unilaterally with topical corticosteroid. In this set of experiments, prednisolone administration began on the same day immediately after the first IOP measurement. Values from eyes treated with prednisolone are plotted with *open symbols* or *faint symbols* connected by *dashed lines*. After 4 weeks of treatment, the frequency of the prednisolone instillation was reduced from three times daily to twice daily. IOP was monitored for an additional week after treatment discontinuation.

robustness of the IOP response and the relatively low cost compared with studies in primates.

Prolonged topical corticosteroid treatment can cause significant adverse effects, including cataracts and corneal ulcers. We did not observe cataract formation in any of the animals used in this study, but a corneal ulcer did develop in one eye. Perhaps the limited time (maximum 6 weeks) of steroid treatment and the low dose and frequency of instillation are factors related to the low frequency of adverse effects that were observed.

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