Steroid-Induced Ocular Hypertension in Normal Cattle

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Objectives: To determine whether the bovine eye develops elevation of intraocular pressure (IOP) in response to topical corticosteroid use and to develop a reliable model of steroid-induced elevation of IOP in an animal.

Methods: Intraocular pressure was monitored by Perkins applanation tonometry in a group of 12 cows receiving topically administered prednisolone acetate in 1 eye 3 times a day for a period of 49 days after the establishment of baseline IOP values. Perkins readings were converted to IOP in mm Hg using calibration curves derived from in vitro cannulation manometric experiments and validated with in vivo manometric measurements. Intraocular pressure was also monitored for 50 days after the discontinuation of corticosteroid therapy.

Results: Intraocular pressure began to increase after 3 weeks of treatment in 100% of the cow eyes receiving corticosteroid and reached a peak 1 week later. Peak intraocular IOP differences between the corticosteroid-treated eye and the fellow control eye reached up to 15 mm Hg and began to decline after the discontinuation of treatment but remained significantly elevated for a period of 3 more weeks.

Conclusions: Bovine eyes exhibit a robust steroid-induced ocular hypertensive response, with 100% occurrence in this trial. The IOP elevation caused by corticosteroid slowly subsides after discontinuation of treatment.

Clinical Relevance: The mechanisms of steroid-induced glaucoma may be related to those involved in primary open-angle glaucoma and could provide the clues to elucidate the pathogenesis of the latter. The high prevalence of corticosteroid-induced elevation of IOP in the cow and the large amount of tissue available will permit studies on the mechanism of this phenomenon not previously possible.

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LUCOCORTICOSTEROIDS, administered by a variety of routes, may elevate intraocular pressure (IOP). This induced ocular hypertension, which generally occurs within weeks in susceptible individuals, is usually reversible. However, it may produce glaucomatous optic neuropathy if the duration of corticosteroid therapy is lengthy. The open-angle glaucoma so produced resembles primary open-angle glaucoma, including the mechanism by which IOP is elevated, a decrease of trabecular meshwork outflow facility. It has been suggested that there is a distinct and common relationship between this ocular response to corticosteroids and primary open-angle glaucoma. Therefore, understanding the cellular processes that lead to corticosteroid-induced ocular hypertension may illuminate the cause of primary open-angle glaucoma. Unfortunately, these processes remain elusive.

In humans, the elevation of IOP induced by corticosteroids appears to occur in only about one third of those tested, with an even smaller percent exhibiting marked elevations. The search for other animal species in which corticosteroids elevate IOP underscores the need to find animal models in which the investigation of pathogenesis can be carried out. Corticosteroids will induce ocular hypertension in rabbits, but the results are quite variable, and the amount of corticosteroid necessary is often lethal. Moderate elevations of IOP may be produced in cats by topical administration of corticosteroids. Corticosteroid-induced ocular hypertension may be induced in cynomolgus monkeys as well; in one series, 5 of 11 monkeys treated with topical dexamethasone showed increased IOP.

Many investigations have been reported that study the effects of glucocorticoids on the trabecular meshwork in an attempt to unravel the family of diseases...
called glaucoma. These include explorations in cultured trabecular meshwork cells, organ-cultured eyes, and in vivo models, investigating morphology, gene expression, extracellular matrix, cytoskeleton, and cell adhesion molecules, to name a few. Most often, human trabecular meshwork cells are utilized; sometimes other species’ cells are used, rarely the whole eyes such as those of the rabbit. The cow eye may be ideal for these studies because of similarities with the human eye. For example, recent investigations report that the concentration of chloride in the bovine aqueous humor is higher than that in plasma and that isolated bovine ciliary epithelium transports chloride and is inhibited by carbonic anhydrase inhibitors, as in humans. Dexamethasone treatment of cultured bovine trabecular meshwork cells does produce alterations in extracellular matrix proteins and cell contacts. However, although IOP of normal cattle has been reported, it is unknown whether cow eyes are corticosteroid responsive, although they would provide large amounts of tissue material for further studies. Thus, a study was carried out to explore the IOP response of the bovine eye to the administration of a topical corticosteroid.

METHODS

ANIMALS

All animal experiments were performed according to the Association for Research in Vision and Ophthalmology (ARVO) guidelines. Twelve healthy (female) cows between 3 and 5 years of age and weighing 350 to 420 kg were selected from a local ranch in Corrientes, Argentina, for the corticosteroid study. They were of a common type in Argentina named Braford, a cross between Brahman and Hereford. Two of the cows were pregnant, and 1 lost the fetus during the study because of unrelated reasons, without affecting the health of the mother. The cows were tagged for individual identification on their ear lobes. They were herded from pasture whenever it was necessary to instill the drops or to measure IOP. They were guided into a funnel corral and yoke (Figure 1). With time, the cows became accustomed to the routine, and drops could be instilled while they were in the open field. To take the IOP, the cows were guided into the funnel corral and then into the neck yoke. This procedure took about 4 minutes per cow. Otherwise, the cows were free to pasture. The other set of 8 untreated cows of various breeds that underwent IOP measurement were selected at random, and IOPs were measured just prior to sacrifice at the local slaughterhouse.

DRUGS AND PROTOCOL

After 1 baseline measurement of IOP (cows 1-6) or 2 such measurements 1 week apart (cows 7-12), 1% prednisolone acetate (Falcon Pharmaceuticals, Fort Worth, Tex) or 0.5% prednisolone acetate (Ultracortenol; Novartis Ophthalmics, Hettlingen, Switzerland) was instilled in 1 eye. (The study was initiated with the 1% prednisolone solution but was changed to the 0.5% solution for reasons of availability after 1 week of treatment.) As a control, an artificial tear preparation (Alcon Lagrimas II; Alcon Argentina, Tortuguitas, Argentina) was instilled in the contralateral eye. Both control and experimental instillations consisted of 2 drops, 3 times daily at 8 AM, 2 PM, and 6:30 PM for the duration of intervention. The 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. In addition, the IOP in both eyes of another group of 8 cows was measured to determine the normal cow IOP.

INSTILLATION OF DROPS

One of the authors taught the cowboys who were in charge of the cows how to instill the drops, which was done mostly in the field. They were given the 2 types of drops as described above from a different investigator with sealed instructions on what “color drop” to instill to right or left eyes, how many times, and when. The cowboys did not know the contents of the bottles.

MEASUREMENT OF IOP WITH THE HANDHELD PERKINS APPLANATION TONOMETER

Once the cow was held in the yoke and a cowboy moved the cow’s head to a proper orientation, the 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 (Figure 2). The ophthalmologist measuring the IOP was unaware of the treatment of each eye. All IOP measurements were taken between 8:30 AM and 10 AM at least once a week. Although IOPs were taken at various intervals, the drugs were applied 3 times daily during the 49-day treatment period.

MEASUREMENT OF IOP IN COW EYES BY CANNULATION IN VIVO

A manometric measurement by cannulation was performed in 4 eyes of the 12 cows while in the study. The purpose was to determine the actual IOP in these cows and to use these values to compare with those obtained with the Perkins tonometer in vivo as well as to confirm the calibration of the Perkins tonometer done in isolated cow eyes. With the cows in the yoke, prior to cannulation, 3 drops of 0.5% proparacaine were instilled in the eye. About 2 minutes later, with the head held by hand, a 25-gauge butterfly needle was introduced into the anterior chamber (Figure 3). The butterfly’s tubing was con-
connected to a custom-made pressure-recording instrument. This instrument consisted of 3 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 mm Hg. The amplifier output was read on the liquid crystal display screen of the voltmeter.

**CALIBRATION OF THE PERKINS TONOMETER IN ENUCLEATED COW EYES**

Four cow eyes were obtained from the slaughterhouse. Eyes were transported to the laboratory on ice immediately after enucleation. All globes were verified to be intact with visually clear corneas. The eyes were cannulated with a 26-gauge needle at 90° to the visual axis through clear cornea 1 to 2 mm anterior to the limbus with the aid of an operating microscope. The absence of leaks was verified microsco-

cally throughout the experiment. Intraocular pressure was controlled by adjusting the height of a variable column of balanced salt solution attached to the needle (open stopcock method). Intraocular pressure was verified and continuously recorded by a pressure transducer (Ohmeda model TNF-R) connected to a second cannulation needle inserted in the anterior chamber in a similar fashion, 180° away from the previous one. Intraocular pressure was sequentially adjusted to 15-, 25-, 35-, 45-, and 55-cm water pressures (10 mm Hg = 13.6 cm H₂O pressure). The eyes were supported in a small cup, and the cornea was applanated after application of fluorescein solution. A Perkins handheld applanation tonometer with a clinically used Goldmann applanation tip was used. The instrument was powered off after each measurement. Five measurements were made at each pressure level, and the mean was calculated. The readings obtained were plotted against the manometric (true) IOP (after converting to millimeters of mercury values), as shown in **Figure 4**.

**RESULTS**

The IOP measurements in both eyes of 8 normal cows were used to determine the baseline values of IOP in these animals (Table 1). The corresponding Perkins tonometer reading and the equivalent IOP as determined from the Perkins calibration curve (Figure 4) indicated a normal IOP of between 16 and 17 mm Hg.

The Perkins IOP measurements during the course of the experiment (corrected from the calibration curve in Figure 4), as depicted in **Figure 5** in mean absolute values and in **Figure 6** as IOP differences (ΔIOP) between the corticosteroid-treated eye and the contralateral eye of each animal, showed substantial elevations of IOP in the eyes treated with corticosteroid. The ΔIOP was significant between days 28 and 77 (21 to 70 days after the onset of steroid administration) (P < .001, analysis of variance; P < .05, Bonferroni test). The IOP remained elevated at the same level between days 35 and 56 (days 28-49 on steroid) (P > .05, Bonferroni test).

To confirm the accuracy of the elevated IOP measurements obtained with the Perkins tonometer in the experimental cows, IOP was determined manometri-
In vivo in 4 eyes (the eyes of cows 4 and 7 receiving corticosteroid drops and cows 1 and 8 receiving artificial tear drops) on 2 occasions (occasion 1, day 67 for cows 1 and 4 and day 60 for cows 7 and 8; occasion 2, day 84 for cows 1 and 4 and day 77 for cows 7 and 8) immediately after measurement with the Perkins tonometer (Table 2). The IOP measured by cannulation manometrically was not significantly different from the IOP determined by calibrated Perkins applanation tonometry (Table 2). The IOP measured by cannulation manometrically was not significantly different from the IOP determined by calibrated Perkins applanation tonometry (Table 2). The IOP measured by cannulation manometrically was not significantly different from the IOP determined by calibrated Perkins applanation tonometry (Table 2). The IOP measured by cannulation manometrically was not significantly different from the IOP determined by calibrated Perkins applanation tonometry (Table 2). The IOP measured by cannulation manometrically was not significantly different from the IOP determined by calibrated Perkins applanation tonometry (Table 2). The IOP measured by cannulation manometrically was not significantly different from the IOP determined by calibrated Perkins applanation tonometry (Table 2).

Table 1. Intraocular Pressure of Normal Cows

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Perkins Tonometer Reading</th>
<th>Intraocular Pressure as Determined by Perkins Tonometry, mm Hg*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right Eye</td>
<td>Left Eye</td>
</tr>
<tr>
<td>1</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>2.25</td>
</tr>
<tr>
<td>3</td>
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<tr>
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<td>2.5</td>
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<tr>
<td>6</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>7</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>8</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.94 ± 0.50</td>
<td>2.09 ± 0.63</td>
</tr>
</tbody>
</table>

*As determined from calibration curve (see Figure 4).

Intraocular pressure in the cow has been previously reported. In one study, using the MacKay-Marg tonometer, mean IOP values of 27.5 and 28.2 mm Hg were obtained. Mean IOP with the TonoPen was 26.9 mm Hg; however, with both instruments, there was a large range of values (12-42 mm Hg). Another study reported a mean IOP of 23.4 mm Hg with the TonoPen. However, both studies did not correlate IOP measurements with these instruments with actual IOP as measured by cannulation. Using Perkins handheld applanation tonometry, the readings in the normal cow eye are numerically very low.
Using a calibration curve derived from in vitro manometric measurements, the control, presumably normal, mean of IOP in the cow is 16 to 17 mm Hg. The reliability of these calibration curves is confirmed by the in vivo cannulations performed on some of the experimental eyes. Corticosteroid drops produce a substantial elevation of IOP in cow eyes. This occurs rapidly over a few days during the third to fourth weeks of treatment and is subsequently maintained during corticosteroid administration. Peak IOPs of 30 to 35 mm Hg are typical in all cow eyes so treated. The IOP slowly reverts to normal over 4 to 5 weeks after discontinuation of corticosteroid administration. A small rise of IOP occurs in the fellow eyes of these cows, the cause of which will require additional study. The intensity and maintenance of this steroid response in all the cows treated with corticosteroid drops are unlike those in any other species previously tested. Moreover, the large volume of ocular tissue that can be made available from the cow represents a remarkable resource for further studies of this phenomenon.

Further studies of cytoskeleton, extracellular matrix, receptors, integrins, and growth factors certainly can be carried out by comparing the corticosteroid-treated and untreated fellow eyes. The availability of this model indicates that tissue-cultured cells may no longer be necessary for these investigations. Limitations on prior studies using whole tissue, such as those indicating that there are changes in the aqueous pathway glycosaminoglycans in rabbit eyes treated with dexamethasone, will be obviated by this model.

This study establishes the bovine eye as a reliable and reproducible in vivo model for steroid-induced glaucoma. We have defined a dosage level, dosage frequency, and rate of onset to reach a doubling of IOP, as well as the rate of return to normal IOP when treatment is discontinued. This will enable other investigators to use this model...
for further studies. Such studies could include experiments in bovine eyes with increased IOP to determine the dynamics of aqueous humor flow. In addition, the large amounts of tissue available can allow investigations of gene and protein expression\(^\text{17,18}\) that may shed light on the mechanisms involved in steroid-induced glaucoma and, potentially, primary open-angle glaucoma.

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REFERENCES