Trabecular Meshwork and Lens Partitioning of Corticosteroids

Implications for Elevated Intraocular Pressure and Cataracts

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Objective: To determine whether adverse effects such as elevated intraocular pressure and cataracts, which are lower with dexamethasone when compared with fluocinolone acetonide or triamcinolone acetonide, may be explained in part by the differences in drug lipophilicity and partitioning of these drugs into the trabecular meshwork and lens.

Methods: The n-octanol/phosphate-buffered saline (pH 7.4) partition coefficient (log distribution coefficient [D]) and bovine/human ocular tissue partition coefficients were determined for triamcinolone, prednisolone, dexamethasone, fluocinolone acetonide, triamcinolone acetonide, and budesonide at 37°C.

Results: The log D of the corticosteroids ranged from 0.712 to 2.970. The ranges of tissue:PBS partition coefficients following drug incubation at 0.4, 2.0, and 10.0 µg/mL were 0.35 to 1.56, 0.30 to 2.12, and 0.30 to 1.95, respectively, for the bovine lens, 0.87 to 4.18, 0.71 to 4.40, and 0.69 to 5.86, respectively, for the human lens, and 2.98 to 9.48, 2.41 to 9.16, and 1.71 to 9.96, respectively, for the bovine trabecular meshwork. In general, tissue partitioning showed a positive correlation with log D. Dexamethasone, with lipophilicity less than triamcinolone acetonide and fluocinolone acetonide, exhibited the least amount of partitioning in the trabecular meshwork and lens among these 3 corticosteroids commonly used for treating diseases at the back of the eye.

Conclusion: Binding of corticosteroids to the trabecular meshwork and lens increases as drug lipophilicity increases.

Clinical Relevance: Less lipophilic corticosteroids with limited partitioning to the trabecular meshwork and lens may result in reduced incidence of elevated intraocular pressure and cataracts.

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Dexamethasone, fluocinolone, prednisolone, and triamcinolone or their derivatives have been shown to be beneficial in treating several disorders of the eye including uveitis, macular edema, and choroidal neovascularization. However, corticosteroid use is associated with risks including ocular hypertension, or elevated intraocular pressure (IOP), and lens opacification (cataracts). Several pathological changes have been reported in the trabecular meshwork (TM) and lens as a consequence of corticosteroid-induced ocular hypertension and cataract, respectively.

Even though it is well established that corticosteroids induce pathological changes in the TM and lens, it is currently not possible to rank the adverse effects of various corticosteroids based solely on their glucocorticoid activity. This is particularly relevant for corticosteroids administered intravitrally. Currently, US Food and Drug Administration–approved corticosteroid products for the back of the eye include Ozurdex (biodegradable dexamethasone implant; for macular edema; 0.7 mg), Retisert (non-biodegradable fluocinolone acetonide implant; for uveitis; 0.59 mg), and Trivaris/Triesence (triamcinolone acetonide suspension; uveitis and topical corticosteroid responsive inflammatory conditions; 4 mg). Of these products, the incidence of adverse effects is the highest for fluocinolone acetonide and the least for dexamethasone. To better understand these differences, which might in part depend on the affinity of the drug for the TM and lens, the purposes of this study were to determine the lipophilicity of corticosteroids and to further determine their partition coefficients in the TM and lens.

In this study, we used bovine lens, human lens, and bovine TM for partition...
An Expression of Concern about this article has been published.

Table 1. Optimized Mass Spectrometry Instrument Parameters for the Corticosteroid Analysis

<table>
<thead>
<tr>
<th>Corticosteroid</th>
<th>MRM</th>
<th>DP, V</th>
<th>FP, V</th>
<th>EP, V</th>
<th>CE, V</th>
<th>CXP, V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triamcinolone</td>
<td>395/343</td>
<td>65</td>
<td>200</td>
<td>10</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>361/343</td>
<td>65</td>
<td>200</td>
<td>10</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>393/373</td>
<td>60</td>
<td>200</td>
<td>10</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Fluocinolone acetonide</td>
<td>453/413</td>
<td>60</td>
<td>200</td>
<td>10</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Triamcinolone acetonide</td>
<td>435/415</td>
<td>30</td>
<td>200</td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Budesonide</td>
<td>431/413</td>
<td>55</td>
<td>200</td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Corticosteronea</td>
<td>347/329</td>
<td>40</td>
<td>200</td>
<td>10</td>
<td>23</td>
<td>15</td>
</tr>
</tbody>
</table>

Abbreviations: CE, collision energy; CXP, collision cell exit potential; DP, declustering potential; EP, entrance potential; FP, focusing potential; MRM, multiple reaction monitoring.

aCorticosterone served as the internal standard.

MATERIALS AND PROCEDURES

Triamcinolone, triamcinolone acetonide, fluocinolone acetonide, and budesonide were purchased from Spectrum Chemical and Laboratory Products, New Brunswick, New Jersey. Prednisolone, dexamethasone, and n-octanol were purchased from Sigma-Aldrich, St Louis, Missouri. High-performance liquid chromatography-grade acetonitrile and methanol were purchased from Fisher Scientific, Philadelphia, Pennsylvania. Freshly excised bovine eyes were purchased from G and C Packaging Co, Colorado Springs, Colorado.

Human cadaver eyes from anonymous donors were obtained from the San Diego Eye Bank, San Diego, California, in accordance with a protocol approved by the institutional review board. All procedures were in compliance with the Declaration of Helsinki for research involving human tissue. The age of the donors ranged from 55 to 60 years.

METHODS

Tissue Isolation

Enucleated eyes from 3-year-old cows were obtained on ice and used within 2 hours after the cows were killed. Human eyes maintained at 4°C were received from the eye bank within 24 hours after death. The experiment was initiated within an hour after receiving the human eyes.

During the entire process of tissue isolation, eyes and tissues were maintained at 4°C. For isolating the TM from bovine eyes, previously described methods were followed. Briefly, the cornea was removed carefully by cutting it away from the limbus region. Beginning at the limbus, approximately 3- to 10-mm-deep circular tissue was isolated. The remaining optic cup with the intact lens was kept on ice until further use. The circular piece of tissue was cut into quadrants and each quadrant was processed as follows. With the corneoscleral surface facing downward, the iris and pectinate ligament layer were removed carefully, exposing the velcrolike structure of the TM. The ciliary body posterior to the TM band was separated by an incision, ensuring that it does not contaminate the TM. The TM and ciliary body could be clearly distinguished by their color. The TM band appeared gray, while the ciliary body was black. Finally, the TM band was detached from the sclera. The optic cup that was kept aside on ice was used for isolating the intact lens. Both tissues were gently rinsed with PBS (pH 7.4).
CORTICOSTEROID EXTRACTION RECOVERY

For calculating the percentage of extraction recovery, the following formula was used: % recovery = [analyte peak area of standard with spiking before extraction × 100] / [analyte peak area of corresponding standard with spiking after extraction procedure]. For prespiking or spiking before extraction, 100 mg of tissue with a known concentration of analyte mixture (10.0 µL of 40.0-µg/mL solution of 6 steroids) and internal standard (10.0 µL of 50.0-µg/mL corticosterone) was homogenized in 300.0 µL of PBS (pH 7.4). After homogenization, extraction with 2.0 mL of ethyl acetate was done by vortexing for 15 minutes using a multilute vortexer (VX-2500; VWR International, Marlborough, Massachusetts). Organic solvent was separated after centrifugation for 15 minutes at 3000 rpm. After removing the organic solvent under nitrogen, final reconstitution was achieved with 1.0 mL of acetonitrile. In postspiking or spiking after extraction, tissues were first homogenized in PBS followed by extraction with organic solvent, which was separated after centrifugation. The organic extract was spiked with a known concentration of analyte mixture and internal standard. After mixing, the organic solvent was removed under nitrogen and final reconstitution was done in 1.0 mL of acetonitrile. Quality control samples were prepared by direct dilution of the analyte and the internal standard in 1.0 mL of acetonitrile. The matrix effect was calculated using the following formula: % matrix effect = [analyte peak area of standard with spiking after extraction procedures × 100] / [analyte peak area of corresponding unextracted standard]. After reconstitution, samples were analyzed using liquid chromatography tandem mass spectrometry.

IN VITRO TISSUE PARTITIONING STUDIES

These studies were performed to measure the relative affinity of each corticosteroid toward different ocular tissues and PBS (pH 7.4). All of the corticosteroids were used as a cocktail mixture for partitioning studies to minimize any experimental variation. Three concentrations of corticosteroids (0.2, 4.0, and 10.0 µg/mL) in PBS were selected for the partitioning study. Portions of 100 mg of TM or lens (n = 5) were incubated with 0.5 mL of corticosteroid solution in PBS for 6 hours at 37°C. At the end of the incubation period, samples were centrifuged for 15 minutes at 10,000 rpm. The PBS supernatant was removed and tissues were washed with 0.5 mL of fresh PBS. Samples were again centrifuged for 15 minutes at 10,000 rpm, and the wash buffer was separated. During the sample processing for drug content estimation in tissue and buffer, corticosterone was added as the internal standard to all tissue samples before extraction, similar to our extraction recovery study. Similarly, internal standard was
added to buffer samples prior to analysis. Tissue partitioning was estimated as the tissue:buffer ratio of drug concentration.

STATISTICAL ANALYSES
Comparison of in vitro tissue partitioning between tissues and 6 different corticosteroids was performed using 1-way analysis of variance followed by Tukey post hoc analysis. Statistical significance was set at $P < .05$.

RESULTS
ESTIMATION OF EXTRACTION RECOVERY
In preparation for our partitioning studies, we initially determined the efficiency of extraction of our corticosteroids from various bovine ocular tissues using ethyl acetate and dichloromethane and determined that ethyl acetate is superior for corticosteroid extraction. Table 2 represents the percentage of extraction recovery of each corticosteroid in the TM and lens using ethyl acetate. All percentages of extraction recovery fell in the range of approximately 80.7% to 108.9%.

PHYSICOCHEMICAL PROPERTIES OF CORTICOSTEROIDS
Mean n-octanol/PBS (pH 7.4) partition coefficients (log D) of corticosteroids at 37°C are summarized in Table 3. The log D of corticosteroids ranged from 0.712 to 2.970 with the following rank order: triamcinolone < predni-
solone = dexamethasone < fluocinolone acetonide = triamcinolone acetonide < budesonide (P < .05).

**TISSUE PARTITION COEFFICIENTS**

Corticosteroid tissue partition coefficients for TM and lens are depicted in Figure 2. In general, the tissue partitioning followed the trend, with coefficients being higher in the TM than in the lens. It is noteworthy that for several corticosteroids in each tissue, partition coefficient values were the highest at the lowest concentration used for the partitioning study. For example, triamcinolone showed the highest TM:PBS and lens:PBS partitioning ratios at 0.4 µg/mL compared with 2.0 and 10.0 µg/mL. The TM and lens tissue partition coefficients of corticosteroids at all concentrations showed positive correlation with drug lipophilicity (Figure 3).

To our knowledge, we are the first to report that corticosteroid lipophilicity and relative partitioning into the TM meshwork and lens, respectively, may explain elevated IOP and cataract. We observed that an increase in lipophilicity, corticosteroids exhibit greater ocular tissue partition coefficients in the TM and lens.

*n*-Octanol:buffer (pH 7.4) partition studies at 37°C indicated that all corticosteroids assessed are lipophilic with log D values ranging from 0.712 to 2.970. The relative D of the corticosteroids between lipophilic *n*-octanol and aqueous buffer at physiologic pH ranged from 2.25-fold to 921.86-fold. For dexamethasone, fluocinolone acetonide, and triamcinolone acetonide, the mean measured log D values were 1.955, 2.560, and 2.585, respectively. The mean D values for fluocinolone acetonide and triamcinolone acetonide were 4.02- and 4.26-fold higher compared with dexamethasone, respectively. Typically, the literature reports for partition coefficients are determinations at room temperature. Also, on several occasions, predicted log D values as opposed to actual measures have been reported. The *n*-octanol:buffer partition coefficients measured in this study are expected to be more relevant to interpret physiological differences in drug distributions. Based on these measurements, it is likely that tissue entry of fluocinolone acetonide and triamcinolone acetonide will be greater than that of dexamethasone. In addition to those 3 US Food and Drug Administration–approved corticosteroids for diseases at the back of the eye, we investigated prednisolone, triamcinolone, and budesonide, spanning a broader spectrum of lipophilicity, to determine whether corticosteroid lipophilicity correlates with TM and lens partition coefficients. Among the various corticosteroids assessed, triamcinolone was the least lipophilic molecule (mean log D of 0.712) and budesonide was the most lipophilic molecule (mean log D of 2.970), with the rank order being triamcinolone < prednisolone = dexamethasone < fluocinolone acetonide = triamcinolone acetonide < budesonide (Table 3).

Although the mechanism of corticosteroid-induced IOP elevation is much debated, there is general agreement that corticosteroids induce IOP elevation through their action in the TM. At physiologically relevant pH and temperature, all corticosteroids assessed in this study exhibited preferential accumulation in the TM compared with PBS (partition coefficients >1.00, with the range being 1.71-9.97). At a 0.4-µg/mL buffer concentration, the TM partition coefficients of fluocinolone acetonide and triamcinolone acetonide were 1.85- and 1.56-fold higher when compared with dexamethasone. Similar trends were also evident when the corticosteroids were incubated at...
2.0 and 10.0 µg/mL. As the drug concentrations increased from 0.4 to 10.0 µg/mL, the TM partition coefficients for all corticosteroids except budesonide decreased, suggesting that the binding sites in the TM for several corticosteroids could become saturated by an increase in drug concentration. The rank order for trabecular meshwork partition coefficients at 0.4-µg/mL drug concentrations was triamcinolone < prednisolone < dexamethasone < triamcinolone acetonide < fluocinolone acetonide < budesonide. At 10.0 µg/mL, there was no difference between the partition coefficients of fluocinolone acetonide and triamcinolone acetonide; the remaining trend mirrored the observations made for 0.4 µg/mL. Thus, the TM preferentially accumulates all corticosteroids compared with the aqueous medium, with the drug accumulation increasing with an increase in drug lipophilicity.

Probably due to its protein-rich and hydrophilic nature, bovine lens accumulation of all corticosteroids was lower (4.90- to 8.43-fold) compared with the TM. While triamcinolone, prednisolone, and dexamethasone preferentially remained in the aqueous medium (tissue:buffer partition coefficient <1.00), triamcinolone acetonide, fluocinolone acetonide, and budesonide remained preferentially in the bovine lens (partition coefficient >1.00). The rank order for the lens:buffer partition coefficient at a drug concentration of 0.4 µg/mL was triamcinolone < prednisolone < dexamethasone < triamcinolone acetonide ≈ fluocinolone acetonide < budesonide. At 10.0 µg/mL, the partition coefficient of dexamethasone was less than that of prednisolone, while the rest of the trend remained the same. A reduction in partition coefficients with an increase in drug concentration was evident for triamcinolone, dexamethasone, and triamcinolone acetonide. At 0.4 µg/mL, the lens:buffer partition coefficients of fluocinolone acetonide and triamcinolone acetonide were 1.53- and 1.44-fold higher compared with dexamethasone. Thus, although the bovine lens has a lower affinity for corticosteroids compared with the TM, the tissue partition coefficient increases with an increase in drug lipophilicity. Compounds with a log D of 1.995 (dexamethasone) or less exhibited preferential retention in the aqueous buffer compared with the lens tissue. Similar to bovine lens, human lens partition coefficients also increased with an increase in drug lipophilicity. The rank order of corticosteroid partition coefficients in the human lens was triamcinolone < prednisolone < dexamethasone < triamcinolone acetonide < fluocinolone acetonide < budesonide. In the human lens, all corticosteroids except triamcinolone accumulated preferentially in the tissue compared with the aqueous medium. With an increase in drug concentration, there was a decrease in the partition coefficient for all corticosteroids except budesonide.

Compared with the bovine lens, the human lens partition coefficients of corticosteroids were 1.53- to 3.00-fold higher. This may be explained by age- and species-related differences in the lens tissue. It is known that with aging, the proportion of insoluble proteins inside the lens increases, whereas the proportion of soluble α-crystallin decreases.11,12 In the bovine lens, for instance, the percentage of insoluble protein increases from about 5% at birth to 20% to 30% at 3 years and 80% to 90% at 5 to 6 years.11,12 Using a dynamic light-scattering technique, it has been shown that the α-crystallin index, a measure of soluble α-crystallin in human eyes, decreases significantly (P < .001) with aging in patients aged 7 to 86 years.13 The α-crystallin index decreased from 32% in patients aged 7 to 25 years at 15% in patients aged 56 to 65 years and further to less than 5% in patients older than 75 years.13 The human eyes used in our study were obtained from donors aged 55 to 60 years, whereas the bovine eyes were obtained from 3-year-old cows.

To determine whether clinical adverse effects of corticosteroids correlate with their tissue partitioning data, we collected clinical data (Table 4) from the literature on percentages of incidences of IOP elevation and cataract after intravitreous treatment with dexamethasone, fluocinolone acetonide, and triamcinolone acetonide. It is noteworthy that the rank order for ocular adverse effects parallels the corticosteroid partition coefficients in the tissues. However, because dose, duration of assessment and exposure, and patient disease states were not similar in the clinical reports for dexamethasone, fluocinolone acetonide, and triamcinolone acetonide, some caution should be exercised in final interpretations.

In conclusion, corticosteroid partitioning into the TM and the lens increases with an increase in lipophilicity. Accumulation in the lens is lower than in the TM for all corticosteroids. All corticosteroids preferentially accumulate in the TM rather than the buffer. All corticosteroids except triamcinolone, prednisolone, and dexamethasone accumulate preferentially in the lens compared with the buffer in the case of bovine tissue. Dexamethasone exhibits a lower n-octanol:water partition coefficient as well as lower lens and TM partition coefficients com-

Table 4. Occurrence of Cataracts and Intraocular Pressure Elevation Following Intravitreous Administration of Corticosteroids

<table>
<thead>
<tr>
<th>Corticosteroid</th>
<th>Total Dose, mg</th>
<th>Indication</th>
<th>Patients, No.</th>
<th>Subjects With IOP Elevation, No. (%)</th>
<th>Duration of Treatment and Effect Assessment, mo</th>
<th>Subjects With Cataract, No. (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td>0.7</td>
<td>ME</td>
<td>421</td>
<td>106 (25.2)</td>
<td>6</td>
<td>15 (3.8)</td>
<td>Allergan Inc.14 2009</td>
</tr>
<tr>
<td>Fluocinolone acetonide</td>
<td>0.59 or 2.1</td>
<td>NIPU</td>
<td>278</td>
<td>163 (58.6)</td>
<td>7.8</td>
<td>37 (13.3)</td>
<td>Gillies et al.15 2006</td>
</tr>
<tr>
<td>Triamcinolone acetonide</td>
<td>4</td>
<td>CNV and AMD</td>
<td>75</td>
<td>31 (41.3)</td>
<td>12</td>
<td>4 (5.3)</td>
<td>Girgis et al.16 2003</td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; CNV, choroidal neovascularization; IOP, intraocular pressure; ME, macular edema; NIPT, noninfectious posterior uveitis.
pared with fluorocinolone acetonide and triamcinolone acetonide. These differences offer an additional explanation for the clinically observed lower incidence of ocular adverse effects with dexamethasone compared with fluorocinolone acetonide and triamcinolone acetonide.

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REFERENCES


Correction

Error in Box. In the Lessons in Leadership article titled “Interview With Alfred Sommer, MD, MHS” by Sommer and Bartley, published in the May 2011 issue of the Archives (2011;129[5]:653-654), a typographical error occurred in the “Introduction to ‘Lessons in Leadership’” box. The author’s affiliation should have appeared as follows: “George B. Bartley, MD, Chair Emeritus, Department of Ophthalmology, Mayo Clinic, Rochester, Minnesota, and Chief Executive Officer Emeritus, Mayo Clinic, Jacksonville, Florida.” This article was corrected online.