tween 20 and 30 mmHg as in most open-angle glaucoma patients. This can best be obtained by delivering about 50 joules of energy with 0.5 to 1.0 second duration exposures. Our method is simply a refinement of the original recommendations of Gaasterland and Kupfer. Using our parameters, sustained elevations have been achieved in the majority of recently treated primate eyes with one treatment session. We continue to try to improve the method to achieve more moderate IOP levels.

One can compare this treatment to therapeutic human laser angle treatment with some reservations. The cynomolgus monkey cornea is 10 mm in diameter, hence its trabecular area is about 85% of human trabecular area. The energy levels per unit area of meshwork to achieve the same result would differ by this factor. In addition, there are minor differences in meshwork structure that might be important in ways not yet understood. Taking the size difference into account, we estimate that the energy exposure for IOP elevation in the normal human eye would be 60 joules. In the present method of argon laser trabeculoplasty, 100 spots of energy at about 1.0 watt are placed with a 50-µm spot size and 0.1-sec duration around the entire angle. Calculated energy with this treatment is, therefore, 10 joules. In a normal eye this energy level should be below that associated with IOP rise (in fact by a factor of 6). However, the human eyes being treated are, of course, not normal. In addition to being the eyes of older individuals, they have compromised outflow capacity. It is not surprising, then, that occasional eyes have temporary or prolonged IOP increases after trabeculoplasty, especially with repeated treatment.

One feature of our data relative to trabeculoplasty deserves further comment. Using 0.1-sec deliveries, we have not caused a sustained IOP elevation, even with repeated treatment. In part, this may result from the low total energy used in 0.1-sec delivery sessions. It is possible that short duration laser exposure does not lead to detrimental effects upon trabecular function, even when the total energy is the same as that in longer duration exposures. For example, in one eye, 208 deliveries of 0.2 sec, 1.5 watt power did not cause IOP elevation despite a total energy of 62.4 joules. This same energy with a 0.5-sec duration has caused IOP rise. Further study of laser effects is merited to delineate the relationship between exposure duration, toxicity, and therapeutic effect. Exposures greater than 0.1 sec should be avoided in human trabecular treatment because of the potential for angle damage and IOP elevation.

Key words: glaucoma, argon laser, trabecular meshwork, intraocular pressure, trabecuoplasty, surgery

From the Glaucoma Service, Wilmer Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland. Supported in part by PHS Research Grants EY02120 (Dr. Quigley) and EY01765 (Core Facility Grant, Wilmer Institute), both awarded by the National Eye Institute, Bethesda, Maryland, and by an unrestricted award from the Alcon Research Institute, Fort Worth, Texas. Submitted for publication: February 4, 1983. Reprint requests: Harry A. Quigley, MD, Maumenee B-110, Wilmer Institute, Johns Hopkins Hospital, Baltimore, MD 21205.

References


Absence of an Effect of Topical Dexamethasone on Endothelial Permeability and Flow of Aqueous Humor
Steven W. Rice, William M. Bourne, and Richard F. Brubaker

Twenty-four normal human subjects were studied before and after one week of treatment with 0.1% topical dexamethasone. Intraocular pressure, corneal thickness and endothelial cell size were measured. The flow of aqueous humor and the endothelial permeability to fluorescein were determined using fluorophotometry. In addition, the relationship between the initial location of an iontophoretic depot of fluorescein and its kinetics was studied. There was a small and significant increase in intraocular pressure in the eyes treated with dexamethasone but no significant change in corneal thickness, endothelial permeability or the rate of aqueous humor flow. The elimination of fluorescein from the eye was slightly higher when the fluorescein depot was placed adjacent to the superior limbus than when the depot was placed in the central cornea. Smaller right to left differences were observed when fluorescein was placed peripherally than when it was placed cen-

0146-0404/83/0900/1307/$1.05 © Association for Research in Vision and Ophthalmology
The increased precision with peripheral placement is probably due to the improved signal-to-noise ratio for fluorescence measurements of the anterior chamber through the unstained cornea. Invest Ophthalmol Vis Sci 24:1307–1311, 1983

Topical steroids have been demonstrated to exert a number of effects on the cornea. It has been shown that topical dexamethasone causes a small but significant increase in corneal thickness in approximately 35% of normal eyes. The increase is especially marked when accompanied by an increase in intraocular pressure. Conversely, topical corticosteroids may decrease corneal thickness when corneal edema is present, as in the case of endothelial cell damage. Hara has indicated that thinning of the cornea may occur after topical hydrocortisone treatment. He hypothesized that these changes may be due to a reduced passive imbibition rate, increased evaporation or increased endothelial pump activity.

It is important to know the mechanisms responsible for these observed changes in corneal thickness. Alteration of endothelial permeability by steroids could produce such changes.

Anselmi et al. studied the effect of topical dexamethasone on endothelial permeability in five steroid responsive subjects and found no significant change in permeability or aqueous flow rate after one month of treatment despite a rise in intraocular pressure. In order to increase the statistical power and to eliminate the possible secondary effects of the intraocular pressure rise per se, we have tested a larger number of normal subjects with similar fluorescent tracer techniques to look for steroid effects on the corneal endothelium and aqueous humor flow. At the same time, we studied the relation between fluorescein kinetics in the eye and the initial location of the corneal depot.

Materials and Methods. The sample consisted of 24 normal human subjects between 18 and 39 years of age. Each subject had an eye examination prior to the experiment. Individuals with evidence of ocular disease or history of previous ocular disorders were excluded. Each subject underwent fluorophotometry for determination of aqueous flow rate and endothelial permeability, tonometry, and specular microscopy before and after one week of steroid treatment in one eye.

Fluorescein was administered to both eyes between 7:30 and 8:30 am by the iontophoretic method using an electrode of 2% agar with 10% fluorescein. This procedure produced a round, discrete 5-mm depot of fluorescein beneath the corneal epithelium. Intraocular pressures were measured by applanation tonometry. The excess fluorescein was removed from the conjunctival sac by irrigation. In 12 of the 24 subjects the depot was placed in the central cornea, while in the remainder the depot was placed with its upper edge tangent to the superior limbus. Following iontophoresis the anterior chamber volume was measured photogrammetrically. Measurements of the applied mass of fluorescein and the concentrations in the cornea and anterior chamber were recorded at 30 min, 1 hr, and hourly for a total of 8 hrs using a modified Zeiss photoslit-lamp camera system. In eyes where the depot was placed peripherally, measurements of fluorescent intensity were taken with the eye fixating 30° above the horizon. In these eyes neither the excitation beam nor the fluorescent signal passed through the depot. Mass measurements in these eyes were taken with the upper portion of the excitation field where the fluorophotometer is less sensitive. Thus, it was necessary to make a separate calibration factor for this measurement. The central and peripheral mass calibration factors differed by 10%. After the 8-hr measurement, intraocular pressure was measured again, and contact specular microscopy was performed to determine the thickness of the central cornea.

One eye was selected at random to receive one drop of 0.1% dexamethasone phosphate (Decadron Ophthalmic Solution, Merck) four times daily for 1 week. The Decadron vehicle with preservative served as a placebo and was administered to the fellow eye in the same manner as the steroid preparation. Each subject was asked to chart each dose to document compliance. Neither the investigator nor the subject knew the identities of the solutions until the data had been collected.

After 1 week of treatment each subject returned and fluorophotometry, tonometry, anterior chamber photography and specular microscopy were performed again. In addition, the corneal endothelium was photographed for determination of cell size and standard deviation of cell size.

The rate of aqueous flow and the endothelial permeability to fluorescein were calculated using the least squares method (Method #2) described by Nagataki and Brubaker. The anterior chamber volumes for these calculations were determined photogrammetrically. The outlines of 50 contiguous endothelial cells or more were traced by hand with an electronic digitizer (Numonics, Inc.) from the projected images of endothelial photographs. Individual cell areas were calculated.

The precision of the measurements of aqueous flow, cornea to anterior chamber transfer coefficient (Kc), and anterior chamber elimination coefficient (Ko) were determined from right to left differences in
pretreated eyes and from before and after differences in placebo-treated eyes. Stratification according to location of the iontophoretic depot permitted comparison of the effect of this location on the precision of the determination. For the purposes of comparison, precision was calculated as the mean of the absolute differences between each of the eye pairs of each test group. Statistical analyses were performed using Student’s t-test and a Wilcoxon test for paired or unpaired samples where appropriate.

Results. The results of the measured and calculated variables are summarized in Table 1. The intraocular pressures were slightly and significantly higher in the eyes treated with dexamethasone than in those treated with placebo \((P < 0.05)\) and higher than pretreatment values. There was no corresponding increase in the rate of aqueous flow in the dexamethasone treated eyes. Dexamethasone was not found to affect corneal thickness, \(K_{ca}\), \(K_o\), mean endothelial cell size, or anterior chamber volume, all of which were within normal limits.

When the data were stratified according to the location of the iontophoretic depot, the values for \(K_{ca}\) in the two strata were not significantly different. However, the elimination coefficient \((K_o)\) calculated for the eyes that received the fluorescein via peripheral depot was slightly and significantly higher than that calculated from a central depot \((P < 0.001)\) (Table 2). The flow of aqueous humor was calculated to be higher in the subjects who were tested with a peripheral depot, but the difference was not statistically significant \((P < 0.1)\) (Table 3).

The precision, as determined on the basis of pretreated right to left differences, was significantly better in the group tested with the peripheral depot for \(K_{ca}\), \(K_o\), and flow. Precision was not as good in either stratum when comparing pre to post placebo treated eyes. Greater precision was observed in the group

<table>
<thead>
<tr>
<th>Table 1. Summary of results*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>(K_{ca} (\text{min}^{-1} \times 10^3))</td>
</tr>
<tr>
<td>(K_o (\text{min}^{-2} \times 10^2))</td>
</tr>
<tr>
<td>Aqueous flow rate ((\mu)l/min)</td>
</tr>
<tr>
<td>IOP: initial</td>
</tr>
<tr>
<td>(mmHg) final</td>
</tr>
<tr>
<td>Corneal thickness (mm)</td>
</tr>
<tr>
<td>Anterior chamber volume ((\mu)l)</td>
</tr>
<tr>
<td>Mean endothelial cell size ((M^2))</td>
</tr>
</tbody>
</table>

* Mean ± 1 standard deviation.

<table>
<thead>
<tr>
<th>Table 2. Summary of results (central vs. peripheral depot)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
</tr>
<tr>
<td>Post-treatment</td>
</tr>
<tr>
<td>(Dexamethasone)</td>
</tr>
<tr>
<td>(Placebo)</td>
</tr>
</tbody>
</table>

* Mean ± 1 standard deviation.
\(\dagger 0.05 < p < 0.1\).
\(\dagger\dagger p < 0.001\).
tested with the peripheral depot but statistical significance could not be demonstrated in this comparison (Table 3).

**Discussion.** From this study we conclude that dexamethasone in this dosage and for this duration does not exert a clinically significant effect on endothelial permeability to fluorescein or on the rate of flow of aqueous humor through the anterior chamber. This study confirms the study of Anselmi, Jones, and Maurice.4 Their subjects were steroid responders and had a mean increase in intraocular pressure of 19.2 mmHg. Our subjects were not selected for steroid responsiveness nor did they use steroids long enough for this characteristic to become manifest.12 It is clear from the study of Anselmi et al. that the elevation of intraocular pressure did not occur primarily as a result of increased production of aqueous humor. However, a small effect of corticosteroids on aqueous formation could have been masked by indirect effects of the large change in intraocular pressure. In our study, the elevation of intraocular pressure was too small to mask a clinically significant effect of corticosteroids on flow.

Endothelial permeability is directly related to \( K_{ca} \) and corneal thickness by the following equation:

\[
\text{permeability} = \frac{K_{ca} \cdot \text{stromal thickness}}{0.64}
\]

where 0.64 is the anterior chamber-cornea equilibrium distribution ratio for fluorescein as determined by Ota et al.13 Neither \( K_{ca} \) nor corneal thickness were changed by the present treatment. Thus, it appears that endothelial permeability to fluorescein is not altered by dexamethasone.

It should be noted that both the steroid and placebo preparations contained benzalkonium chloride in a concentration (0.02%) sufficient to produce an increase in epithelial permeability.14 This effect is negligible in the present study since it is known that the epithelium plays a minor role in corneal hydration15 and since this effect would have been present in both eye pairs.

It appears that the location of the initial depot of fluorescein in the cornea is not critical to the results. Brubaker16 predicted from theoretical considerations that limbal placement would yield slightly higher values of aqueous humor flow but was unable to demonstrate a difference in five normal subjects tested with a central depot in one eye and a peripheral depot in the other eye. Also, Jones and Maurice could demonstrate no difference.6 In the present study, 12 subjects received a central depot in both eyes on both test days and 12 subjects received a peripheral depot. The \( K_{o} \) and flow values in the group with peripheral placement of the iontophoretic depot were slightly higher, as predicted theoretically.16 In the case of \( K_{o} \) values, the difference was statistically significant. This difference may also have been due to a real difference between the two groups of normal subjects rather than to the location of the depot; in any case, it is too small to affect, by itself, clinical decisions. We concluded as did the previous two investigators that no clinically significant systematic error is introduced by varying the site of the fluorescein depot in the normal cornea.

We believe, however, that peripheral placement of the depot results in more precise measurements, particularly of \( K_{ca} \) (and endothelial permeability). Peripheral placement of the depot permits an unobstructed view of the fluorescence in the anterior chamber whereas central placement makes it necessary that the weak fluorescence of the anterior chamber be viewed through an intensely fluorescent cornea. Corrections for the boundary function between the cornea and the anterior chamber can be applied16 but the signal to noise ratio is more favorable when the depot is not in the direct line of view of the anterior chamber. The favorable signal to noise ratio for anterior chamber measurements is partly offset by the relatively greater ease of measuring the initial fluorescence of a central depot and the greater subject convenience of fixing a target on the horizon rather than looking upward 30°. The net effect, however,
as shown in Table 3 is that right to left differences are smaller when the depot is placed in the upper cornea.

Key words: Endothelial permeability, aqueous flow rate, fluorophotometry, corticosteroids

Acknowledgment

The authors thank Merck, Sharp & Dohme Research Laboratories, West Point, Pennsylvania, for providing the dexamethasone and placebo solutions.


References


An Improved Fixation Technique for Maintaining the Fine Structure of the Nuclear Zone of Neonatal Mouse Lens

Yosumichi Yajima

The fine structure of the nuclear zone of neonatal mouse lenses can vary considerably according to the fixation used. When normal neonatal mouse lenses are fixed in a commonly used chilled glutaraldehyde solution, the nuclear zone develops a grossly visible opacity, and irregular sized protein granules appear in the subsequent sections. Similar artifacts of aggregated irregular sized protein granules appear when cataractous mouse lenses are conventionally processed. These artifacts can be avoided by soaking the lens in 0.15 M reduced glutathione solution for 10–15 min before fixation in a phosphate buffered 2% glutaraldehyde solution (pH 7.4) at 27–35 C. Normal lenses treated in this manner maintain trans- lucency in the nuclear zone throughout the fixation-embedding procedure, and the resulting sections show finely uniform granularity with the cell membrane well preserved. Similarly processed nuclear portions of cataractous lenses of Nakano mice show uniformly aggregated protein granules, measuring about 350Å in diameter. The cell membranes in the cataractous zone are also not interrupted. Invest Ophthalmol Vis Sci 24:1311–1316, 1983

An Improved Fixation Technique for Maintaining the Fine Structure of the Nuclear Zone of Neonatal Mouse Lens

Yosumichi Yajima

The fine structure of the nuclear zone of neonatal mouse lenses can vary considerably according to the fixation used. When normal neonatal mouse lenses are fixed in a commonly used chilled glutaraldehyde solution, the nuclear zone develops a grossly visible opacity, and irregular sized protein granules appear in the subsequent sections. Similar artifacts of aggregated irregular sized protein granules appear when cataractous mouse lenses are conventionally processed. These artifacts can be avoided by soaking the lens in 0.15 M reduced glutathione solution for 10–15 min before fixation in a phosphate buffered 2% glutaraldehyde solution (pH 7.4) at 27–35 C. Normal lenses treated in this manner maintain translucency in the nuclear zone throughout the fixation-embedding procedure, and the resulting sections show finely uniform granularity with the cell membrane well preserved. Similarly processed nuclear portions of cataractous lenses of Nakano mice show uniformly aggregated protein granules, measuring about 350Å in diameter. The cell membranes in the cataractous zone are also not interrupted. Invest Ophthalmol Vis Sci 24:1311–1316, 1983

The nuclear zone of normal neonatal mouse lenses invariably become opaque after cooling or fixation with glutaraldehyde-containing fixatives. This lens opacity formation due to cooling has been termed cold cataract. Although physiologic and physical studies on this phenomenon have been extensively reported,1,2 the mechanism for opacity formation upon glutaraldehyde fixation has not been described. Upon comparing various fixation techniques we have found that the fine structure of the nuclear zone of neonatal mouse lens can considerably vary depending upon the technique used. It is particularly difficult to maintain the fine homogeneous granular appearance of crystallin substance in the cytoplasm.

Here we present an improved fixation method for the preservation of lens cell fine structure. This method is of particular interest for the adult cataractous mouse lens.

0146-0404/83/0900/1311/$1.10 © Association for Research in Vision and Ophthalmology