Uveoscleral Outflow following Cyclodialysis in the Monkey Eye Using a Fluorescent Tracer

Keiko Suguro, Carol B. Toris, and Jonathan E. Pederson

Cyclodialysis was performed in one eye of each of eight cynomolgus monkeys. Two days later, the intraocular pressure was 1.6 ± 0.7 mmHg in eyes with cyclodialysis and 12.0 ± 0.7 mmHg in fellow control eyes. 10^4 M fluorescein-isothiocyanate dextran (70,000 molecular weight) was perfused into the anterior chamber of each eye for 30 min. The eyes were enucleated and dissected into sclera, choroid, retina, iris, and ocular fluid. Samples were homogenized and centrifuged, and the fluorescence of the supernatant was measured. Expressed as equivalent volumes of aqueous, the rate of anterior chamber movement of tracer via uveoscleral pathways was 1.40 ± 0.17 μl/min in cyclodialysis eyes and 0.34 ± 0.10 μl/min in control eyes. Cyclodialysis results in a fourfold increase in uveoscleral outflow, contributing to the observed hypotony. Invest Ophthalmol Vis Sci 26:810-813, 1985

The mechanism of hypotony in cyclodialysis is commonly thought to be reduced aqueous humor formation (hyposecretion), presumably due to shallow detachment of the ciliary body. However, the rapid pressure rise following closure of a cyclodialysis cleft suggests that fluid may be passing through the cyclodialysis cleft into the suprachoroidal space. Experimental cyclodialysis in the rabbit has shown that uveoscleral outflow is greatly enhanced. Furthermore, recent fluorophotometric studies on four patients with cyclodialysis clefts have revealed nearly normal aqueous flow rates (Brubaker RF, personal communication; Pederson JE, unpublished data). This would suggest that an increase in aqueous humor outflow is the cause of hypotony. Since the intraocular pressure is far below the episcleral venous pressure, conventional outflow is precluded and unconventional (uveoscleral) outflow alone must predominate.

In the present study, the rate of uveoscleral outflow following cyclodialysis in the monkey eye is measured using fluorescein-isothiocyanate (FITC) dextran as the tracer. This method is advantageous since it obviates the need for radioisotopic analysis.

Materials and Methods

Ten cynomolgus monkeys weighing 2.2–3.8 kg were used in the study. Animal usage conformed to the ARVO Resolution on the Use of Animals in Research. In eight animals, the intraocular pressure (IOP) was measured with a Perkins applanation tonometer (Clemente Clarke; London, England) under ketamine hydrochloride anesthesia (20 mg/kg im). Sodium pentobarbital (10 mg/kg) was then administered iv, with supplemental doses as needed. Cyclodialysis was performed in one eye as follows. A small limbal conjunctival peritomy was made superotemporal. A small sclerotomy was then made 2-3 mm posterior to the limbus. A cyclodialysis spatula was inserted through the sclerotomy and was swept into the anterior chamber, disinserting the ciliary body for about 2-3 mm. Occasionally, a small iridodialysis was inadvertently created. The sclerotomy was closed with 8-0 silk sutures.

Two days later, the IOP was measured and slit-lamp exam was performed under ketamine anesthesia. A needle-knife track was made in the peripheral cornea of each eye with a Ziegler knife. A 23-gauge needle attached to a polyethylene tube was inserted through the Ziegler track into the anterior chamber. Aqueous was allowed to drain into the tubing and was analyzed for protein content. The needle was removed and a second needle was inserted. This needle was attached to a polyethylene tube and reservoir filled with 10^4 M FITC-dextran (70,000 MW) in phosphate buffer, and secured at a height of 20 cm above the anterior chamber. The fluorescent solution was perfused through the anterior chamber for 30 min. The animal was then killed by an iv overdose of sodium pentobarbital. The eyes were enucleated, extraocular tissue was excised, and the eyes were rinsed externally. The corneas were removed and all FITC-dextran was flushed from the anterior surface of the iris. The eyes were trans-
ferred to a clean Petri dish for dissection and no further washing was done. Eyes were divided at the equator and separated into iris, retina, anterior uvea, posterior uvea, anterior sclera, posterior sclera, and all remaining fluid, including vitreous. After weighing, iris, retina, fluids, and uvea were homogenized in 1.0 ml of 0.1 M phosphate buffer. Sclera was minced, divided into three separate samples, then homogenized in 0.5 ml of buffer for 3–5 minutes. The three samples were then combined. All samples were centrifuged for 15 min, and the fluorescence of the supernatant determined with a xenon flash fluorophotometer. Since the fluorescence of FITC-dextran may be quenched by the tissues, one animal without FITC-dextran perfusion was dissected as above. The fluorescence was measured for background values. Furthermore, a known concentration of FITC-dextran was added to the scleral and uveal supernatant to construct separate standard curves.

In another animal, the eyes were removed 2 days after cyclodialysis for histopathologic examination.

### Results

The mean IOP before and after surgery is given in Table 1. Cyclodialysis resulted in dramatic fall in IOP by the second postoperative day. Compared to control eyes on the same day, this fall is highly significant (*P* < 0.001). The control eyes also had a significant drop in intraocular pressure before vs after cyclodialysis in the opposite eye (*P* < 0.005). The aqueous protein concentrations 2 days after surgery were 2.17 ± 0.45 mg/ml in cyclodialysis eyes and 0.27 ± 0.02 mg/ml in control eyes (*P* < 0.005).

The amount of FITC-dextran recovered in the various tissues of the eye is given in Table 2, expressed as equivalent volumes of anterior chamber fluid (mass of FITC-dextran recovered/anterior chamber concentration). The anterior chamber concentration was assumed to be the concentration of the perfusate solution. Since some aqueous humor from the posterior chamber would dilute this slightly during the perfusion, the true value for equivalent volumes of anterior chamber fluid would be slightly underestimated. The amount of FITC-dextran recovered in cyclodialized eyes was significantly higher than control eyes for anterior and posterior uvea, anterior and posterior sclera, and fluid. Retinal and iris tissue values were not significantly different. The rate of uveoscleral outflow (sum of all tissue values/30 min) was four times greater in cyclodialized eyes than control eyes and was highly significant.

The values listed for fluid include vitreous, posterior chamber aqueous (minimal), and suprachoroidal fluid. In eyes with cyclodialysis and inadvertent iridodialysis, the posterior chamber region was slightly fluorescent. This may have increased these values somewhat. During dissection, the suprachoroidal space was noticeably filled with fluorescent fluid in eyes with cyclodialysis. In control eyes, this was much less obvious.

Eyes studied histopathologically revealed a normally attached ciliary body in the control eye and a completely disinserted ciliary body following cyclodialysis (Figs. 1, 2).

### Discussion

The finding of increased uveoscleral outflow in the presence of cyclodialysis-induced hypotony in the monkey eye is consistent with previous rabbit studies. This pathogenetic mechanism has also been assumed from clinical observations. From purely physiologic considerations, if the intraocular pressure is below the episcleral venous pressure, uveoscleral outflow must be the sole source of aqueous outflow. Conventional outflow depends on the presence of an intraocular pressure higher than the episcleral venous pressure. This conclusion does not eliminate the possibility that reduced aqueous humor formation may also contribute to the hypotony. However, this cannot be the sole mechanism.

The finding of reduced aqueous humor flow (hypersecretion) in the presence of cyclodialysis-induced hypotony in humans may be due to coexistent

### Table 1. Intraocular pressure (mmHg)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cyclodialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>14.8 ± 0.5</td>
<td>14.4 ± 0.5</td>
</tr>
<tr>
<td>After</td>
<td>12.0 ± 0.6*</td>
<td>1.6 ± 0.7*</td>
</tr>
</tbody>
</table>

Data are mean ± SE.

* *P* < 0.001.

### Table 2. Anterior chamber fluid recovered (µl)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cyclodialysis</th>
<th>Control</th>
<th><em>P</em></th>
</tr>
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<tbody>
<tr>
<td>Iris</td>
<td>0.66 ± 0.30</td>
<td>0.36 ± 0.13</td>
<td>0.22</td>
</tr>
<tr>
<td>Anterior uvea</td>
<td>8.37 ± 1.89</td>
<td>2.53 ± 0.79</td>
<td>0.004</td>
</tr>
<tr>
<td>Posterior uvea</td>
<td>5.06 ± 1.05</td>
<td>0.25 ± 0.13</td>
<td>0.003</td>
</tr>
<tr>
<td>Anterior sclera</td>
<td>10.62 ± 1.63</td>
<td>5.09 ± 1.43</td>
<td>0.002</td>
</tr>
<tr>
<td>Posterior sclera</td>
<td>9.54 ± 0.87</td>
<td>1.37 ± 0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Retina</td>
<td>0.58 ± 0.22</td>
<td>0.03 ± 0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Fluid</td>
<td>7.27 ± 1.95</td>
<td>0.68 ± 0.08</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>42.10 ± 5.06</td>
<td>10.32 ± 3.09</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Rate of uveoscleral outflow (µl/min) 1.40 ± 0.17 0.34 ± 0.10 <0.001

Data are mean ± SE.

* Paired t-test analysis.
Iridocyclitis. In support of this, aqueous secretion tends to return to normal in the late postoperative period following cyclodialysis, as the inflammation abates. In four patients with cyclodialysis clefts, without anterior chamber inflammation, quantitative fluorophotometry revealed aqueous flow rates between 1.2 and 2.2 μl/min (Brubaker RF, personal communication; Pederson JE, unpublished data). The eyes in the present study had an eightfold increase in aqueous protein following cyclodialysis. This may have reduced the aqueous humor formation rate, adding to the hypotony. It was not possible to delay the perfusion for several weeks to allow the inflammation to subside since the cyclodialysis cleft spontaneously closes.

The method used for studying uveoscleral outflow in the present study is a modification of a previously described technique. The advantage of a fluorescent tracer over radioisotopic tracer is in convenience of disposal of tissues. However, quenching of fluorescence is a theoretic problem, which was overcome by creating separate fluorescence standard curves for each tissue studied. The osmotic effect of the FITC-dextran would be about 2 mmHg since a 10^{-4} M solution was used. This would have a negligible effect on the results.

The IOP during the experiment was held at 20 cm H_{2}O (about 15 mmHg). This was chosen so as to maximize the differences between the eyes. It is technically difficult to maintain the IOP at less than 2 mmHg, and it is difficult to ensure adequate filling of the anterior chamber with tracer. Undoubtedly, the rate of uveoscleral outflow would have been lower if perfusion had been done at 2 mmHg. Since the anterior chamber was evacuated and refilled during the perfusion, it is possible that the blood-aqueous barrier was disturbed, particularly in the control eyes. However, the results were similar to those of previous studies in cynomolgus monkeys, indicating that this is not a serious drawback.

In a comparable study using radioisotopic tracers, the rate of uveoscleral outflow in normal eyes was 0.42 μl/min, compared with 0.34 μl/min in the present study. However, in the previous study, extraocular tissues were also included, which if subtracted, would yield 0.38 μl/min. Extraocular tissues were not
Fig. 2. Light micrograph of ciliary body 2 days following cyclodialysis. Ciliary body is entirely detached from scleral spur (hematoxylin and eosin, ×32).

included in the present study since that would include some fluid exiting the eye by conventional outflow pathways. Recent evidence indicates that the conjunctival and episcleral blood vessels are permeable to horseradish peroxidase and thus, possibly, to FITC-dextran as well. Therefore, tracer may leak into the extraocular tissues from those vessels during the perfusion, causing an overestimate of uveoscleral outflow.

In summary, the bulk of evidence at the present time suggests that cyclodialysis-induced hypotony is caused primarily by increased uveoscleral outflow. If iridocyclitis is also present, reduced aqueous humor formation may contribute to the low intraocular pressure but cannot be the major determinant.

Key words: uveoscleral outflow, cyclodialysis, fluorescein-isothiocyanate dextran, hypotony, cynomolgus monkey eye, aqueous humor dynamics

References