Effect of Ticrynafen on Aqueous Humor Dynamics in Monkeys

Mary Ann Croft, MS; Rong Fang Wang, MD; Steven M. Podos, MD; Arthur H. Neufeld, PhD; Paul L. Kaufman, MD

Objective: To determine the effect of ticrynafen, a non–sulfhydryl-reactive compound similar to ethacrynic acid, on outflow facility in normotensive monkey eyes and on intraocular pressure (IOP) in monkey eyes with laser-induced glaucoma.

Methods: In normotensive eyes, facility (perfusion) was measured shortly before and after bolus or exchange intracameral infusion of ticrynafen or vehicle in opposite eyes, and 3.5 to 4.5 hours after 5 days of twice-daily 2% ticrynafen or vehicle ointment. In glaucomatous eyes, baseline and vehicle diurnal IOP curves were established, 2% ticrynafen ointment was given twice daily for 5 days, and IOP was measured immediately before and 0.5 to 6 hours after each morning treatment.

Results: In normotensive eyes, exchange 2-mL infusion of 0.2-, 1-, or 4-mmol/L ticrynafen increased facility by 33% ± 6% (mean ± SEM), 73% ± 18%, and 60% ± 11%, respectively. Day 5 posttreatment facility was higher in the ticrynafen group than in controls by 28% ± 9%. In glaucomatous eyes, maximum IOP decline, from approximately 35 mm Hg, was 7.5 ± 2.0 mm Hg on day 4 and 9.8 ± 2.4 mm Hg on day 5 of twice-daily ticrynafen treatment.

Conclusion: The facility-increasing, IOP-lowering action of ticrynafen, ethacrynic acid, and derivatives may not depend entirely on sulfhydryl reactivity.

Clinical Relevance: Whether such drugs as ethacrynic acid and ticrynafen prove valuable for glaucoma therapy, at the least they are useful probes to study aqueous outflow mechanisms.


ETHACRYNIC ACID increases outflow facility in living monkey eyes,1,2 enucleated calf eyes,1,3 and organ-cultured, perfused human anterior ocular segments4 and decreases intraocular pressure (IOP) in living normotensive and glaucomatous monkey5-7 and glaucomatous human8 eyes.

Ethacrynic acid is an alkylating agent, giving it sulfhydryl reactivity.9,10 This property may be responsible for both its facility-increasing and toxic corneal effects.5,11 Ticrynafen is a nonalkylating compound,9 structurally similar to ethacrynic acid (Figure 1) but putatively devoid of sulfhydryl reactivity.12 We have determined the effect of ticrynafen on IOP, outflow facility, and anterior segment biomicroscopic appearance in cynomolgus monkey eyes with normal IOP and with laser-induced glaucoma.

MONKEYS WITH BILATERALLY NORMOTENSIVE EYES

Pretreatment baseline facilities in the ticrynafen and contralateral control eyes were similar in all intracameral drug protocols.

Protocol 1

One hour after unilateral bolus intracameral infusion of 10 µL of 2.5-mmol/L ticrynafen (group A, Table), the mean post-drug-baseline facility ratio averaged 1.15 ± 0.05 (n = 6) in the ticrynafen-infused eye and 1.27 ± 0.09 in the vehicle-injected eye. These 15% and 27% increases were both statistically significant (P = .03), were of the magnitude expected for perfusion-induced resistance washout in this system,20 and did not differ significantly (treated-control postdrug facility ratio, 1.03 ± 0.10; treated-control, postdrug-baseline facility ratio, 0.92 ± 0.06; neither differing significantly from 1.0). Thus, there was no apparent ticrynafen-induced facility change. Similar results were seen with the 10-fold higher dose (10 µL of 25-mmol/L ticrynafen; group B, Table); the treated-control, postdrug-baseline facility ratio averaged 1.12 ± 0.11 (n = 6).

Protocol 2

Facility did not increase after 0.04-mmol/L ticrynafen exchange (Table). However, after AC exchange with 0.2-, 1-, and 4-mmol/L ticrynafen, facility relative...
MATERIALS AND METHODS

CYNOMOLGUS MONKEYS

Thirty-nine juvenile and adult cynomolgus monkeys (Macaca fascicularis) of both sexes were studied; 35 were bilaterally ocular normotensive with no biomicroscopically visible anterior chamber cells or flare or other ocular abnormalities, while 4 had stable unilateral ocular hypertension induced by repeated argon laser photocoagulation of the trabecular meshwork. All experiments were conducted in accordance with National Institutes of Health (Bethesda, Md) and institutional guidelines, and with the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research.

ANESTHESIA

For application tonometry and administration of ticrynafen ointment, intramuscular ketamine hydrochloride, 10 to 12 mg/kg, supplemented at approximately 45-minute intervals by 5 mg/kg, was used, supplemented in glaucomatous monkeys by topical 0.3% proparacaine hydrochloride. For anterior chamber perfusion, intramuscular ketamine hydrochloride, 10 mg/kg, was followed by intramuscular pentobarbital sodium, 35 mg/kg.

OUTFLOW FACILITY MEASUREMENT AND INTRACAMERAL DRUG DELIVERY

Total outflow facility was measured by 2-level constant-pressure (approximately 3 and 12 mm Hg above spontaneous IOP) perfusion of the anterior chamber (AC) with Bárány mock aqueous humor. Two variations of the basic method were used. With a 1-needle technique (bolus intracameral infusion of drug and no drug in the reservoir), a second cannulation is avoided; however, the AC contents are not completely mixed or replaced, and drug concentration declines with a half-life of approximately 30 minutes to baseline and adjusted for control eye washout increased significantly, by 33% ± 6% (0.2 mmol/L), 73% ± 18% (1 mmol/L), and 48% ± 11% or 60% ± 11% (4 mmol/L) (Table). In the group treated with 4-mmol/L ticrynafen, there were 2 animals in which the postexchange facilities in the control eyes nearly doubled compared with baseline, so that the facility increase in the control eyes averaged 30% ± 13% for 9 monkeys (Table). When these 2 animals were excluded, the perfusion-induced facility increase in the 7 remaining control eyes was a more typical 14% ± 8% and the facility increase in the contralateral ticrynafen-treated eyes remained at approximately 80%, so that the ticrynafen-vehicle ratios for postdrug facility and postdrug-predrug facility increased to 1.48 and 1.60, respectively (Table).

IOP MEASUREMENT

The IOP was determined with a minified Goldmann application tonometer in monkeys with normotensive eyes, and with a calibrated pneumatic application tonometer (Pneumatonometer model 30, Digilab Inc, Cambridge, Mass) in animals with laser-induced glaucoma.

TICRYNAFEN

Ticrynafen powder and 2% ticrynafen ointment (2% ticrynafen in mineral oil petrolatum base) were obtained (Telor Ophthalmic Pharmaceuticals, Woburn, Mass). The same 2% ticrynafen ointment preparation was used for both the bilaterally normotensive and the unilaterally glaucomatous monkeys. The manufacturer also prepared ethacrynic acid ointment with the use of a similar mineral oil petrolatum base for other studies cited herein. We chose the concentration of ticrynafen based on an IOP-effective concentration of ethacrynic acid.

MONKEYS WITH BILATERALLY NORMOTENSIVE EYES

Protocol 1

Experimental Design. After baseline slitlamp examination, facility was measured simultaneously in both eyes of 11 monkeys for approximately 15 minutes, immediately before and beginning 1 hour after a 10-µL bolus intracameral infusion of 2.5-mmol/L (group A, n = 6), or 25-mmol/L (group B, n = 6) ticrynafen in one eye and vehicle alone in the other (1 monkey was used in both protocols). These doses achieved initial concentrations of 0.25-mmol/L (group A) and 2.5-mmol/L (group B) ticrynafen in the 100-µL cynomolgus anterior chamber. Ticrynafen or vehicle was injected into the inflow tubing of the perfusion apparatus (via a T-piece) of opposite eyes and allowed to wash into the anterior chambers for 5 minutes. The eyes were then exposed to cold air for 3 minutes to enhance convection mixing. The perfusion apparatus was closed to inflow during the interval between baseline and postdrug facility measurements.

Drug Preparation. For 6 animals in group A, the phosphate buffer was adjusted to pH 6.8 with 1.0N hydrochloric acid and filtered through a 0.2-µm acetate filter. Then, 41 mg of ticrynafen was dissolved in 40 mL of phosphate buffer by ultrasonication and further diluted with buffer to 50.0 mL to achieve a final ticrynafen concentration of

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2.5 mmol/L. For 1 monkey in group A and all 5 monkeys in group B, 82.8 mg of ticrynafen and 60.6 mg of TRIS (base) were dissolved in 8.0 mL of purified water, the pH adjusted to 7.0 with 1N hydrochloric acid, and then further diluted with purified water to 10 mL. The solution was then filtered through a 0.2-µm nylon or acetate filter. The vehicle solution was prepared identically, except that 20.0 mg of sodium chloride was substituted for the higher ticrynafen concentration. Solutions were made immediately before the experiment.

Protocol 2

**Experimental Design.** Total outflow facility was measured in both eyes for approximately 45 minutes, immediately before and beginning 0.5 hour after a 10-minute AC exchange with 2 mL of 0.04-, 0.2-, 1-, and 4-mmol/L ticrynafen in one eye and vehicle alone in the other in 4 groups of 4, 5, 7, and 9 monkeys, respectively. The system was closed to inflow during the interval between baseline and postdrug facility measurements. During postexchange facility measurements, the reservoirs contained the respective ticrynafen or vehicle solution.

**Drug Preparation.** TRIS, 242 mg, was dissolved in 7 mL of purified water; ticrynafen, 331 mg, was added and vortexed until dissolved; and water was added to 10 mL. A 1.0-mL aliquot of ticrynafen solution was added to 20 mL of Bärány solution and adjusted to pH 7.4 with 50-µL drops of 1.0N hydrochloric acid while being mixed, and Bärány solution was added to 25 mL. Vehicle solution was prepared identically except for omission of ticrynafen and substitution of 5.0N for 1.0N hydrochloric acid. Both solutions were filtered through a 0.2-µm nylon filter. These solutions are stable for 1 month, but were prepared 1 week before the experiment.

Protocol 3

A 1-cm strip of ointment containing 2% ticrynafen was administered to the cul de sac of one eye and placebo ointment to the opposite eye, randomly selected, of 6 ketamine-anesthetized monkeys with bilaterally normotensive eyes (3 from one of the previous protocols, 5 with 1 previous AC perfusion) twice daily for 5 consecutive days at approximately 9 AM and 3:30 PM. Excess ointment was wiped away 15 minutes after application. On day 1, baseline IOP was measured immediately before treatment. On day 5, IOP was measured immediately before and 1 and 3 hours after morning treatment. Clinical biomicroscopy was performed by a trained ophthalmologist immediately before baseline and final IOP measurements on days 1 and 5. Total outflow facility was determined 3.5 to 4.5 hours after morning treatment on day 5.

All monkeys had biomicroscopically clear ACs bilaterally at the time of the study. One monkey appeared in 4 groups, and 4 others appeared in 2 groups, but no monkey appeared twice in the same group.

**MONKEYS WITH UNILATERAL LASER-INDUCED GLAUCOMA**

The IOP was measured at approximately 9:30 AM and repeated 0.5, 1, 2, 3, 4, 5, and 6 hours later to establish a baseline diurnal curve in both eyes of 4 monkeys with unilateral glaucoma under ketamine anesthesia. The following day, IOP was measured in both eyes starting at approximately 9:30 AM, immediately before and then 0.5, 1, 2, 3, 4, 5, and 6 hours after administration of a 1-cm strip of placebo ointment to the glaucomatous eye only. Beginning 2 days later, 2% ticrynafen ointment was administered twice daily to the glaucomatous eye, at 9:30 AM and 3:30 PM, for 5 days. On each day, IOP was measured in both eyes immediately before the morning 2% ticrynafen dose in the glaucomatous eye and 0.5, 1, 2, 3, 4, 5, and 6 hours thereafter. Immediately after the last IOP measurement, the second dose of 2% ticrynafen was given. Slitlamp examination was performed immediately before the first IOP measurement of the day, and just before IOP measurement every 2 hours thereafter. The contralateral normotensive eyes were untreated throughout the experiment.

**DATA ANALYSIS**

Data are presented as mean ± SEM values. Differences between or ratios of ticrynafen-treated and contralateral vehicle-treated control eyes were tested against 0 or 1, respectively, by the 2-tailed paired t test. The Bonferroni t test was used for the analysis of the multiple-dose study in glaucomatous monkey eyes. The correlation between treated vs control IOP differences and treated/ control facility response was examined by least-squares linear regression, with and without adjusting for baseline IOP.

**MONKEYS WITH UNILATERAL LASER-INDUCED GLAUCOMA**

Six-hour baseline diurnal IOP (day −4) averaged between 34.0 ± 3.1 and 37.5 ± 4.0 mm Hg (Figure 3, A). The IOP immediately before and for 6 hours after ticrynafen-treated eyes averaged 28% ± 9% higher than in the contralateral vehicle-treated controls (Table; n = 6; P = .03). There was no significant correlation between treated vs vehicle eye IOP differences and treated-vehicle eye facility response, with or without adjustment for baseline IOP differences.
vehicle treatment the next day (day −3) averaged between 34.0 ± 2.6 and 37.3 ± 3.3 mm Hg (Figure 3, B). Since there was no apparent effect of the vehicle (Figure 3, H), the vehicle-treated IOP diurnal curve was used for comparison with ticrynafen treatment at the same time of the day in the same eye. The onset of IOP reduction after ticrynafen treatment did not occur until after the seventh dose beginning on day 4. The maximum IOP decline on day 4 averaged 7.5 ± 2.0 mm Hg (P<.05; n = 4) at hour 4, and on day 5 averaged 9.8 ± 2.4 mm Hg (P<.05; n = 4) at hour 2. The IOP in the untreated contralateral normotensive eyes displayed normal diurnal fluctuation during the course of the experiment, averaging between 15.8 ± 1.2 and 18.5 ± 0.7 mm Hg (Figure 4).

Slitlamp examination showed no abnormalities in any vehicle-treated eye or in 3 of 4 ticrynafen-treated eyes. Corneal epithelial edema was noted in 1 ticrynafen-treated eye 2 hours after dosing on days 1 and 4; the edema lessened by 4 hours and disappeared by 6 hours after dosing. However, a confluent epithelial defect occurred in the same eye on day 5 of treatment.

**COMMENT**

In normotensive cynomolgus monkeys, ticrynafen produced an increase in outflow facility when given by AC and reservoir exchange, but not at similar doses given by intracameral bolus injection. Anterior chamber and reservoir exchange, as performed here, allows more rapid administration of drug, more complete mixing of the AC, and better maintenance of the drug concentration during posttreatment facility measurements (as the perfusate in the reservoir contains the desired drug concentration). These pharmacodynamic differences may explain ticrynafen's greater efficacy when given by AC-reservoir exchange.

## Total Outflow Facility After Intracameral or Topical Ticrynafen or Vehicle in Cynomolgus Monkeys*

<table>
<thead>
<tr>
<th>Ticrynafen Concentration and Route</th>
<th>No.</th>
<th>Ticrynafen</th>
<th>Vehicle</th>
<th>Ratio, Ticrynafen/Vehicle</th>
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<tr>
<td>10 µL of 2.5-mmol/L AC bolus</td>
<td></td>
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<td></td>
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<tr>
<td>Preinfusion</td>
<td>6</td>
<td>0.42 ± 0.70</td>
<td>0.37 ± 0.06</td>
<td>1.13 ± 0.11</td>
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<tr>
<td>1 h postinfusion</td>
<td>6</td>
<td>0.49 ± 0.09</td>
<td>0.48 ± 0.08</td>
<td>1.03 ± 0.10</td>
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<tr>
<td>Postinfusion/preinfusion</td>
<td>6</td>
<td>1.15 ± 0.05</td>
<td>1.27 ± 0.09</td>
<td>0.92 ± 9.06</td>
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<td>10 µL of 25-mmol/L AC bolus</td>
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<td></td>
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<tr>
<td>Preinfusion</td>
<td>6</td>
<td>0.32 ± 0.03</td>
<td>0.31 ± 0.04</td>
<td>1.15 ± 0.18</td>
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<tr>
<td>1 h postinfusion</td>
<td>6</td>
<td>0.38 ± 0.07</td>
<td>0.31 ± 0.05</td>
<td>1.26 ± 0.18</td>
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<tr>
<td>Postinfusion/preinfusion</td>
<td>6</td>
<td>1.13 ± 0.12</td>
<td>1.01 ± 0.06</td>
<td>1.12 ± 0.11</td>
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<td>0.04-mmol/L AC exchange</td>
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<td>Preinfusion</td>
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<td>0.26 ± 0.06</td>
<td>0.23 ± 0.07</td>
<td>1.45 ± 0.48</td>
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<tr>
<td>0.5 h postinfusion</td>
<td>4</td>
<td>0.28 ± 0.08</td>
<td>0.23 ± 0.06</td>
<td>1.25 ± 0.32</td>
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<tr>
<td>Postinfusion/preinfusion</td>
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<td>1.13 ± 0.12</td>
<td>1.21 ± 0.34</td>
<td>0.99 ± 0.16</td>
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<td>0.2-mmol/L AC exchange</td>
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<tr>
<td>Preinfusion</td>
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<td>0.35 ± 0.06</td>
<td>0.33 ± 0.04</td>
<td>1.12 ± 0.20</td>
</tr>
<tr>
<td>0.5 h postinfusion</td>
<td>5</td>
<td>0.48 ± 0.11</td>
<td>0.36 ± 0.09</td>
<td>1.29 ± 0.38</td>
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<tr>
<td>Postinfusion/preinfusion</td>
<td>5</td>
<td>1.46 ± 0.14</td>
<td>1.10 ± 0.10</td>
<td>1.33 ± 0.06</td>
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<td>1.0-mmol/L AC exchange</td>
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<tr>
<td>Preinfusion</td>
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<td>0.30 ± 0.03</td>
<td>0.27 ± 0.03</td>
<td>1.17 ± 0.07</td>
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<td>0.5 h postinfusion</td>
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<td>Postinfusion/preinfusion</td>
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<td>2.37 ± 0.27</td>
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<td>4.0-mmol/L AC exchange</td>
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<td>0.29 ± 0.04</td>
<td>0.32 ± 0.04</td>
<td>0.93 ± 0.08</td>
</tr>
<tr>
<td>0.5 h postinfusion</td>
<td>9</td>
<td>0.51 ± 0.05</td>
<td>0.40 ± 0.05</td>
<td>1.36 ± 0.14</td>
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<tr>
<td>Postinfusion/preinfusion</td>
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<td>1.84 ± 0.10</td>
<td>1.30 ± 0.13</td>
<td>1.48 ± 0.11</td>
</tr>
<tr>
<td>4.0-mmol/L AC exchange**</td>
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<td></td>
</tr>
<tr>
<td>Preinfusion</td>
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<td>0.30 ± 0.05</td>
<td>0.33 ± 0.05</td>
<td>0.93 ± 0.10</td>
</tr>
<tr>
<td>0.5 h postinfusion</td>
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<td>0.52 ± 0.07</td>
<td>0.38 ± 0.06</td>
<td>1.47 ± 0.15</td>
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<tr>
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<td>1.80 ± 0.12</td>
<td>1.14 ± 0.08</td>
<td>1.60 ± 0.11</td>
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<tr>
<td>Posttreatment</td>
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<td>0.43 ± 0.03</td>
<td>0.35 ± 0.04</td>
<td>1.28 ± 0.09</td>
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</tbody>
</table>

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*Facility data are mean ± SEM microliters per minute per millimeter of mercury for the number of monkeys indicated, each contributing 1 ticrynafen-treated and 1 vehicle-treated eye. Differences between or ratios of ticrynafen-treated and vehicle-treated eyes were tested against 0 or 1, respectively, by the 2-tailed paired t test. AC indicates anterior chamber.

†P < .05.
‡P < .005.
§P < .10.
¶P < .01.
#Results here are the same as for the section marked with double asterisks, but include 2 animals with high posttreatment facility in the control eye.

**Results here are the same as for the section marked with the number sign, but exclude 2 animals with high posttreatment facility in the control eye.
normalities in 2 of 4 eyes. In monkeys with bilaterally normal eyes (12 of 12 eyes), a 40% ± 15% increase in facility and ocular abnormalities, while 1.5% ethacrynic acid, 0.25 mmol/L, which is also maximal (M.A.C., P.L.K., unpublished data, 1997), produced a nearly identical baseline- and washout-corrected 71% ± 15% (n = 6) facility increase.2

Topical 2% ticrynafen ointment, administered twice daily for 5 days, produced a 28% ± 9% facility increase and no ocular abnormalities, while 1.5% ethacrynic acid ointment, administered once daily for 5 days, produced a 40% ± 15% increase in facility and ocular abnormalities in all eyes (12 of 12 eyes).3 In glaucomatous monkey eyes, topical 2% ticrynafen ointment, administered twice-daily for 5 days, produced an IOP drop similar in magnitude and with fewer corneal abnormalities than once-daily 2.5% or 1.5% ethacrynic acid ointment in a similar base of mineral oil and petrolatum (ticrynafen: 9.8 mm Hg, corneal abnormalities in 1 of 4 eyes; 2.5% ethacrynic acid: 8.5 mm Hg, corneal abnormalities in 3 of 4 eyes; 1.5% ethacrynic acid: 6.5 mm Hg, corneal abnormalities in 2 of 4 eyes). In monkeys with bilaterally normotensive eyes, IOP after unilateral 2% ticrynafen ointment, administered twice daily for 5 days, averaged 1 to 1.5 mm Hg less in the ticrynafen-treated eyes than in the control eyes at the day 3 pretreatment and the 1- and 3-hour posttreatment measurements, but these differences were not statistically significant. Ethacrynic acid, 1.5% ointment, given once daily for 5 days, significantly lowered IOP by 2.8, 1.7, and 3.7 mm Hg before and 1 and 3 hours, respectively, after treatment on day 5 compared with contralateral control eyes.

The small IOP reduction induced by topical ticrynafen in normotensive monkeys is not surprising given the low baseline IOP. When IOP is low, even a substantial effect on inflow or outflow may have little effect on IOP; this is evident from the Goldmann equation.21 In our experiments, IOP of the control eye on day 5, 3 hours after the morning treatment (immediately before facility measurements) was 10.3 mm Hg. Assuming an episcleral venous pressure of 10 mm Hg, and episcleral venous pressure, aqueous formation, and uveoscleral outflow to be the same in both eyes, a 28% higher trabecular facility in the ticrynafen-treated eyes would predict an IOP of 10.2 mm Hg by the Goldmann equation, ie, essentially the same as in the control eyes. In fact, IOP of the ticrynafen-treated eyes on day 5, 3 hours after the morning treatment (immediately before facility measurements) averaged 9.3 mm Hg. As an even more striking example, assume an aqueous formation rate of 1.5 µL/min, IOP of 13 mm Hg, trabecular facility of 0.33 µL·min⁻¹·mm Hg⁻¹, episcleral venous pressure of 10 mm Hg, and uveoscleral outflow of 0.5 µL/min. By the Goldmann equation, even a 52% facility increase to 0.50 µL·min⁻¹·mm Hg⁻¹ would only yield a 1–mm Hg decrease in IOP.

Monkeys with normotensive eyes under ketamine anesthesia and receiving no other drug exhibit a time-dependent decrease in IOP during 6 to 8 hours, perhaps related to ketamine itself, to depth of anesthesia, or to the normal diurnal rhythm for IOP.22 This may partly or completely account for the small but significant contralateral IOP reduction in our ticrynafen-treated animals, rather than a contralateral effect of ticrynafen itself. As we did not measure baseline facility, we cannot say whether facility increased in the control eye. The contralateral (nonglaucomatous) eye in the uniocularly glaucomatous monkeys demonstrated only the normal diurnal IOP decline, with IOP returning to the morning baseline each day, ie, no contralateral effect was evident.

The glaucomatous monkeys had received laser treatment in the midtrabecular meshwork of all 4 quadrants, and we did not measure their perfusion or tonographic

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**Figure 1.** Chemical structures of ethacrynic acid and ticrynafen. Although the right-hand portions of the molecules are identical, ethacrynic acid has substantial sulfhydryl reactivity, whereas ticrynafen does not.

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**Figure 2.** Intraocular pressure (IOP) in 6 cynomolgus monkeys under ketamine hydrochloride anesthesia during twice-daily topical application of ticrynafen in one eye and vehicle in the opposite eye. Data are mean ± SEM IOP (A) in ticrynafen-treated and control eyes, or IOP difference (B) between eyes. BL indicates baseline. Time 0 hours on both days is at 9 AM. Time 0 hours on day 5 is 18 hours after the afternoon treatment on day 4 and immediately precedes the morning treatment on day 5. Asterisk indicates significantly different from ipsilateral pretreatment BL by the 2-tailed paired t test (P<.05).
outflow facility after they received ticrynafen. However, pilocarpine\textsuperscript{23} does produce a substantial IOP decrease and an outflow facility increase in this model, indicating that the meshwork can still respond functionally to mechanical distortion\textsuperscript{24} or a direct drug effect.\textsuperscript{25-27} Epinephrine also lowers IOP in the glaucomatous monkey model,\textsuperscript{23,28} but it is not known whether this effect is caused by enhanced trabecular or uveoscleral outflow.\textsuperscript{29-33}

Some trabecular meshwork between the burns may have been unaffected by the laser treatment or subsequent scarring and remained responsive to the drug, or even damaged trabecular meshwork could be affected functionally by ticrynafen.

Ethacrynic acid produced a small but significant transient IOP rise after the day 5 treatment compared with day 5 baseline values in cynomolgus monkeys with normotensive eyes.\textsuperscript{5} A similar trend was seen with ticrynafen in our normotensive monkeys, but the magnitude was even smaller and not statistically significant.

Some intracamerals doses of some sulfhydryl-reactive compounds may produce an acute facility decrease in enucleated calf and primate eyes,\textsuperscript{34} perhaps because of trabecular cell swelling. No postticrynafen IOP rise was seen on any day in the glaucomatous monkey eyes. In 4 other glaucomatous monkeys, IOP was measured hourly for 6 hours on the third day before, and then daily for 5 days during, once-daily treatment with normal saline. The IOP tended to decline over 5 days, but the change from baseline was not statistically significant at any time point (R.F.W., unpublished data).

Four- or 5-fold excess concentrations of cysteine block the ethacrynic acid–induced facility increase in enucleated calf eyes\textsuperscript{1} and living cynomolgus monkeys (M.A.C., P.L.K., unpublished data, 1997) and the ethacrynic acid–induced shape change in cultured human trabecular meshwork cells.\textsuperscript{35} This suggests that the ethacrynic acid effects might result from sulfhydryl reactivity. However, ticrynafen, which is structurally similar to

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**Figure 3.** Intraocular pressure (IOP) in 4 monkey eyes with laser-induced glaucoma at baseline (BL) before (A) and after topical application of vehicle (B) or 2\% ticrynafen ointment (C-G). All data are mean ± SEM. H, Vehicle-treated minus ipsilateral BL IOP at the corresponding time point. I through M, Ticrynafen-treated minus ipsilateral vehicle-treated IOP at the corresponding time point. Time 0 (always 9:30 AM) is vehicle and all ticrynafen days immediately precedes topical treatment; on treatment days 2 through 5, time is 18 hours after the afternoon ticrynafen treatment on the previous day. Significantly different from 0.0 by the 2-tailed paired t test: asterisk indicates \(P \leq 0.10\); dagger, \(P \leq 0.02\); double dagger, \(P \leq 0.05\).
ethacrynic acid but putatively lacks sulfhydryl reactivity,\textsuperscript{9,12} significantly lowers IOP in glaucomatous and increases outflow facility in normotensive monkey eyes. In addition, a recently published study reported that, in excised bovine eyes, 0.125-mmol/L ticrynafen increases facility by 102\% relative to baseline, significantly more than the 50\% washout-induced facility increase in control eyes, and that, in contrast to ethacrynic acid, the effect is maintained in the presence of a 5-fold excess concentration of cysteine.\textsuperscript{12} Cytoskeletal drugs such as cytochalasins,\textsuperscript{36} latrunculins,\textsuperscript{37} and certain protein kinase inhibitors such as H-738 and staurosporine\textsuperscript{37,39} may exert their facility-increasing effect by altering the shape and adhesion of endothelial cells in the meshwork and along the inner canal wall. Ethacrynic acid and ticrynafen both induced shape changes in cultured calf pulmonary artery endothelial cells and human trabecular meshwork cells,\textsuperscript{12,42} but ethacrynic acid had a greater effect at comparable doses. This might explain why, in our present study, a 4-fold higher concentration of ticrynafen delivered via AC exchange ([ticrynafen]\textsubscript{AC} maintained during facility measurements) was required to produce a facility increase similar to that with ethacrynic acid given via bolus injection.\textsuperscript{2} Nonetheless, given that ticrynafen and ethacrynic acid are of reasonably comparable potency and efficacy, and that the drugs likely lower IOP and increase outflow facility by similar mechanisms (eg, cell shape change; ethacrynic acid,\textsuperscript{35} ticrynafen\textsuperscript{12}), these data suggest that the facility-increasing, IOP-lowering action of ethacrynic acid and derivatives may not depend entirely, if at all, on sulfhydryl reactivity.

Despite evidence of changes in cell shape,\textsuperscript{12,35} volume regulation,\textsuperscript{40} and adhesion consequent to drug effects on cytoskeletal proteins such as actin and tubulin\textsuperscript{12,35} or sodium-potassium-chloride ion cotransport,\textsuperscript{30,41} the cellular biophysical mechanism by which ethacrynic acid and related compounds such as ticrynafen lower IOP and increase outflow facility remains elusive. Whatever the mechanism, it is intriguing to note that ticrynafen increases facility and lowers IOP, apparently in conjunction with a change in cell shape, as does ethacrynic acid, but apparently without microtubule disruption,\textsuperscript{12,42} and does so in the absence of sulfhydryl reactivity. Whether drugs such as ethacrynic acid and ticrynafen prove valuable for glaucoma therapy, they are useful probes for studying aqueous outflow mechanisms.\textsuperscript{43}

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Reprints: Paul L. Kaufman, MD, Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison, WI 53792-7340.
Clinical Sciences Center, 600 Highland Ave, Madison, WI 53792-3220 (e-mail: kaufman@macc.wisc.edu).

REFERENCES


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Galewski has had made an ophthalmomo-thermometer for measuring the temperature of the eye in the living. The bulb is arranged to fit the lower cul-de-sac, where is is allowed to remain three minutes. The normal temperature of the lower cul-de-sac varies from 35.7 to 36.2.


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