Nonsulfhydryl-reactive Phenoxyacetic Acids Increase Aqueous Humor Outflow Facility

David L. Epstein, Bruce C. Roberts, and Laura L. Skinner

Purpose. The phenoxyacetic acid, ethacrynic acid (ECA), has potential use in glaucoma therapy because it acts to increase aqueous outflow in vivo and in vitro. In human trabecular meshwork (HTM) cell culture, ECA acts to change cell shape and attachment, effects that have been correlated with microtubule (MT) alterations and chemical sulfhydryl (SH) reactivity. To further explore these actions, we evaluated two non-SH reactive phenoxyacetic acids, indacrinone and ticrynafen, and the MT-disrupting drug vinblastine.

Methods. Excised bovine and porcine eyes were perfused and outflow facility measured. Calf pulmonary artery endothelial and HTM cells were grown in culture and cytoskeletal effects evaluated after drug treatment.

Results. Indacrinone, ticrynafen, and vinblastine all caused an increase in outflow facility. In contrast with ECA, the outflow effects of indacrinone and ticrynafen were not blocked by excess cysteine. Although indacrinone and ticrynafen produced changes in cell shape in vitro, the β-tubulin staining pattern of treated cells was not altered. Vinblastine caused cell shape change and the expected MT disruption.

Conclusions. Phenoxyacetic acids can increase aqueous outflow facility and alter HTM cell shape and attachment in vitro by a non-SH, non-MT mechanism (which is probably shared also by ECA). These findings suggest the possibility of a broader class of glaucoma drugs that may be directed at the HTM. An understanding of the cellular target for these drugs has implications both for potential glaucoma therapy and for the cytoskeletal mechanisms involved in normal outflow function. Invest Ophthalmol Vis Sci. 1997; 38:1526–1534.

We have been investigating the sulfhydryl (SH) reactive phenoxyacetic acid (PXA), ethacrynic acid (ECA), (Fig. 1) as a potential glaucoma drug1-10 and as a probe of outflow pathway cell function.11-13 Ethacrynic acid has been shown to increase aqueous humor outflow facility in vitro1,2,4 and in vivo1,8,10. These effects have been related to observed induced changes in the cytoskeleton, particularly in the microtubules (MT), in cultured trabecular and endothelial cells.11-13 We have hypothesized that the observed cytoskeleton-related cell shape and attachment alterations observed in vitro11-13 have direct correlations to the changes observed in vivo in the dimensions of the outflow pathway (through the juxtacanalicular tissue and inner wall of Schlemm’s canal).1,2

A diuretic14 still in use in humans,15 ECA was initially chosen for study because of its sulfhydryl (SH) reactivity1 and systemic safety profile. Findings in previous physiological and biochemical investigations had indicated that outflow pathway function was extremely reactive to SH modification.16-21 In further evidence of this, simultaneous perfusion of excess cysteine, an SH-containing amino acid, with ECA was observed to block ECA’s induced increase in outflow facility both in vitro and in vivo.1

To test further this “sulfhydryl hypothesis,” that is, that SH reactivity was required for such drug action, we wished to evaluate under similar conditions two other PXAs, indacrinone and ticrynafen, (Fig. 1) which were previously evaluated as diuretics in humans22 but which did not have SH reactivity. In addi-
Phenoxyacetic Acids Increase Outflow

![Chemical structure of the three phenoxyacetic acids: ethacrynic acid, indacrinone, and ticrynafen.](image)

Ethacrynic Acid

Indacrinone

Ticrynafen

FIGURE 1. Chemical structure of the three phenoxyacetic acids: ethacrynic acid, indacrinone, and ticrynafen.

tion, to explore further the role of cellular MTs in outflow pathway function, we have evaluated the outflow and cell culture effects of vinblastine, a known MT-disrupting drug.23

We observed that such an MT-disrupting drug as well as non-MT acting (non-SH acting) PXAs can increase aqueous humor outflow facility and alter cell shape in the human trabecular meshwork (HTM) in vitro, suggesting the presence of multiple cytoskeletal targets in the outflow pathway for potential glaucoma drug therapy.

METHODS

Outflow Facility Measurements

Bovine and porcine eyes were obtained, freshly enucleated and chilled, from a commercial abattoir and were perfused within 4 hours. Our standard constant-pressure perfusion technique was followed using a Grant stainless steel corneal fitting.1'4'16'17 Radial iridotomies were performed to prevent artificial deepening of the anterior chamber. The medium for perfusion was Dulbecco's phosphate-buffered salt solution (DPBS; Gibco, Gaithersburg, MD) with 5.5 mM D-glucose (Sigma, St. Louis, MO) added. All solutions were filtered through a 0.2-μm Nuclepore filter (Nuclepore, Pleasanton, CA).

The baseline facility was determined after the eye had been perfused at 15 mm Hg and 25°C for 1 hour to achieve a steady state flow value. The corneal fitting was removed, and the anterior chamber was then gently emptied with a cellulose sponge (Weck-Cel, Research Triangle Park, NC) and refilled with the same kind of perfusion medium as that containing the drug under study. Fellow eyes used in control experiments received only the medium for perfusion and drug vehicle. Sham manipulations were performed in fellow control eyes. Drug and control solutions were then perfused for the remainder of the experiment. Outflow facilities (C) of experimental and control eyes were measured every hour for 5 hours. Drug effects are expressed as the percentage of change in outflow facility in a 5-hour period from the baseline value of the experimental eye, minus the percentage change in the control eye.

Values are expressed as means ± standard errors. A paired two tailed student’s t-test analysis was performed.

Drugs

Indacrinone was kindly provided by DA Koechel (Toledo, OH), Alcon Laboratories (Ft. Worth, TX), and Santen Pharmaceuticals (Osaka, Japan). Ticrynafen was provided by DA Koechel and Santen Pharmaceuticals. Vinblastine and taxol were obtained from Sigma Chemical Company. Indacrinone and ticrynafen were initially dissolved in the perfusion medium at a concentration of 10 mM and further diluted in the perfusion medium for perfusion experiments. For cell culture experiments indacrinone and ticrynafen were dissolved in Hanks Balanced Salt Solution (Gibco) with 50 mM Bis Tris Propane (Sigma) as a buffer and diluted in the cell culture medium. Vinblastine was dissolved directly in the perfusion medium (perfusion experiments) or growth medium (cell culture experiments) at the desired concentration. Taxol was dissolved in 100% dimethyl sulfoxide (DMSO; Sigma) and then diluted in cell culture medium for cell culture experiments (final DMSO concentration was 1%). During cell culture drug exposures, the cells were exposed to the drugs continuously for the entire incubation period. In taxol experiments, taxol was present for the pretreatment and for the subsequent drug exposure.

Cell Culture

Both HTM and calf pulmonary artery endothelial cells (CPAE) (obtained from the American Type Culture Collection [CCL 209; Rockville, MD]), which we have used as a possible surrogate11 for Schlemm's canal endothelium, were grown in culture, treated with indacrinone, ticrynafen, or vinblastine, and stained with antibodies to β-tubulin (Sigma; clone Tub 2.1), Rhodamine-conjugated Phalloidin (Sigma), and phosphotyrosine antibodies or paxillin antibodies (Transduction Laboratories, Lexington, KY). The primary antibodies were labeled with fluorescently conjugated goat antimouse antibodies (Bio Source International, Camarillo, CA).

Human cadaver eyes were obtained from the National Disease Research Interchange (NDRI; Philadelphia, PA) within 48 hours of the donor's death. The whole globes were soaked in complete growth medium (Dulbecco’s modified Eagle’s medium [DMEM]) with
TABLE 1. Effect of 0.125 mM Indacrinone on Outflow Facility in Excised Cow Eyes

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Indacrinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.35 ± 0.20</td>
<td>2.21 ± 0.19</td>
</tr>
<tr>
<td>5 hours</td>
<td>3.36 ± 0.29</td>
<td>4.64 ± 0.56</td>
</tr>
</tbody>
</table>

(+43%)(+113%)

* n = 10.
† P < 0.01.

20% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml amphotericin B (Gibco, Grand Island, NY) for 15 minutes at room temperature. The globes were bifurcated at the equator with a scalpel blade. The ciliary body, iris, and lens were gently teased out of the anterior segment. The trabecular meshwork was isolated from surrounding tissues with incisions both anterior and posterior to the meshwork. The trabecular meshwork was grasped with forceps and removed. This dissection and explant technique are similar to that described previously. The HTM was then grown in complete DMEM. Passages 1 through 8 were used for experiments.

The CPAE cells were grown in minimum essential medium (MEM) that contained 20% fetal bovine serum, 100 U/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml amphotericin B (Gibco). Passages 1 through 12 were used in experiments.

All cells were maintained at 37°C with 7% CO2, passaged at confluence using a solution of 0.05% trypsin and 0.53 mM EDTA (Gibco, Grand Island, NY) and split at a ratio of 1:4. For experiments, cells were plated onto glass coverslips that were coated with gelatin (Sigma). On reaching confluence the cells were washed three times in serum-free growth medium and treated with serum-free growth medium that contained study drug. At the end of a drug exposure, the medium and drug were removed, cells were rinsed in 37°C 0.5% Triton-X and 0.25% glutaraldehyde in PEM for 2 minutes. Cells were fixed in 37°C 0.5% Triton-X and 0.25% glutaraldehyde in PEM for 2 minutes. Cells were fixed in 37°C 50 mM PIPES (ICN Biochemicals, Inc., Cleveland, OH), 10 mM EGTA, 6 mM MgSO4 (Sigma) buffer (PEM) and permeabilized in 37°C 0.5% Triton-X and 0.25% glutaraldehyde in PEM for 2 minutes. Cells were fixed in 37°C 50 mM PIPES (ICN Biochemicals, Inc., Cleveland, OH), 10 mM EGTA, 6 mM MgSO4 (Sigma) buffer (PEM) and permeabilized in 37°C 0.5% Triton-X and 0.25% glutaraldehyde in PEM for 2 minutes. Cells were fixed in 37°C 50 mM PIPES (ICN Biochemicals, Inc., Cleveland, OH), 10 mM EGTA, 6 mM MgSO4 (Sigma) buffer (PEM) and permeabilized in 37°C 0.5% Triton-X and 0.25% glutaraldehyde in PEM for 2 minutes.

RESULTS

Outflow Effects

In bovine eyes 0.125 mM indacrinone increased outflow facility 113% ± 24% compared with a 43% ± 6% increase in outflow in control eyes (washout effect25; n = 10; P < 0.01; Table 1). Two additional pairs of eyes were perfused with 0.03 mM indacrinone and demonstrated, after 5 hours, an 89% increase in outflow compared with a 54% increase in outflow in control eyes. Ticrynafen (0.125 mM) increased outflow 102% ± 25% versus a 50% ± 20% increase in outflow in control eyes (n = 6; P < 0.025; Table 2).

TABLE 2. Effect of 0.125 mM Ticrynafen on Outflow Facility in Excised Cow Eyes

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Indacrinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.38 ± 0.24</td>
<td>1.87 ± 0.41</td>
</tr>
<tr>
<td>5 hours</td>
<td>3.26 ± 0.35</td>
<td>3.51 ± 0.56</td>
</tr>
</tbody>
</table>

(+50%)(+102%)

* n = 6.
† P < 0.025.
Phenoxyacetic Acids Increase Outflow

TABLE 3. Effect of 0.125 mM Indacrinone/0.625 mM Cysteine on Outflow Facility in Excised Cow Eyes*

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Indacrinone/Cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.25 ± 0.09</td>
<td>2.42 ± 0.23</td>
</tr>
<tr>
<td>5 hours</td>
<td>3.39 ± 0.09</td>
<td>4.54 ± 0.27</td>
</tr>
</tbody>
</table>

(+52%) (+91%)†

* n = 6.
† P < 0.005.

In bovine eyes 10 μM vinblastine caused a 70% ± 8% increase in outflow compared with a 45% ± 6% increase in outflow in control eyes (n = 8; P < 0.01) at 5 hours. In porcine eyes 10 μM vinblastine increased outflow 43% ± 13% compared with a 26% ± 4% increase in outflow in control eyes after 5 hours (n = 11; P = 0.29). Vinblastine (100 μM) after 4 hours increased outflow 36% ± 10% compared with a 21% ± 5% increase in outflow in control eyes. (n = 23; P = 0.001).

Cell Culture Experiments

Confluent CPAE and HTM cells that were incubated with 0.1 to 0.5 mM indacrinone or ticrynafen demonstrated a dose- and time-dependent change in cell shape beginning at approximately 60 minutes and progressing through 150 minutes. The treated cells showed a pronounced change in cell shape. The changes began at 60 minutes, appearing as small gaps between cells where cells had retracted from their neighboring cells. The retraction continued for the remainder of the time course. The progressive change in cell shape resulted in the observation that at 150 minutes the treated cells appeared to occupy less than half of their beginning surface area. In addition to this retraction, beginning at 60 minutes the cells lost cell-to-cell attachments, and displayed significant disruption of the actin staining pattern (Fig. 3). This loss of actin stress fiber-staining pattern appeared to coincide with the observed changes in cell shape. In general, the cells did not round up completely and did not lose their attachments to the substratum. Similar actin and cell shape effects have been reported for EGA, which we have confirmed (data not shown). Although EGA, indacrinone, and ticrynafen produced comparable disruption in actin staining, the observed changes in cell shape appear greater with EGA at comparable doses.

In contrast with EGA (data not shown) β-tubulin staining was not degraded by indacrinone or ticrynafen at any dose or time point studied. Early time points (up to 60 minutes) showed no alteration of β-tubulin staining compared with that shown in controls. As the cells changed shape (retracted) the β-tubulin stain demonstrated intact MTs that were pushed in toward the nucleus of the cell by the retracting cell membrane (Fig. 4). The paxillin staining pattern of the treated cells was identical to that in controls until the cell shape and actin changes were observed (Fig. 5). When the cells retracted, the peripheral plaques of paxillin staining were lost. Responses to phosphotyrosine (data not shown) and vimentin (data not shown) staining of the treated cells were likewise identical to those in controls at time points preceding observed cell shape changes.

Cells pretreated with 10 to 20 μM taxol for 1 hour demonstrated an increased staining pattern of β-tu-
FIGURE 3. Human trabecular meshwork (HTM) cells stained for filamentous actin after treatment with (A) sham manipulations for 150 minutes (control); (B) 0.5 mM indacrinone for 150 minutes; (C) 0.5 mM ticrynafen for 150 minutes; (D) 50 μM vinblastine for 150 minutes; (E) phase photograph of HTM cells sham treated for 150 minutes; (F) phase photograph of HTM cells treated with 0.5 mM indacrinone for 150 minutes. B and C demonstrate disruption of actin staining and alteration of cell shape and attachment. D demonstrates the cell shape change (and lack of actin disruption) induced by vinblastine. Note the change in cell shape and retraction in F. All drug effects were fully reversible. Original magnification for A to D, ×1000; E and F, ×100.

bulin (data not shown). However, taxol did not inhibit the indacrinone- or ticrynafen-induced cell shape changes. As seen in non-taxol-treated cells, the β-tubulin staining pattern suggested that the MTs in the indacrinone- and ticrynafen-treated cells were being pushed in toward the cell nucleus by the retracting cell membrane.

The CPAE and HTM cells treated with 1 μM, 10 μM, and 50 μM vinblastine demonstrated a rapid and complete loss of β-tubulin staining with paracrystal formation (Fig. 4). Vinblastine at 10 μM and 50 μM produced a total loss of the MT pattern by 5 minutes of treatment. No change in the actin stress fiber-staining pattern was observed with vinblastine treatment (Fig. 3). Paxillin and phosphotyrosine staining were also unaffected by vinblastine in these experiments (data not shown). Incubation with 10 μM and 50 μM vinblastine produced cell shape changes that began between 30 and 60 minutes and progressed with time. These cell shape changes were of lesser magnitude than those produced by indacrinone, ticrynafen and ECA. The vinblastine-treated cells appeared to have been “pulled” apart from their neighboring cells. Some cell-to-cell attachments were maintained (Fig. 3) and no loss of cell substrate attachment was observed. The cells were retracted to approximately two thirds of their initial surface area. When the cells were preincubated with 10 μM or 20 μM taxol for 1 hour and exposed to vinblastine, the loss of β-tubulin staining pattern and the resulting cell shape changes were attenuated when compared with that in non-taxol-pre-treated cells (data not shown).

All cytoskeletal and cell shape changes induced by exposure to indacrinone, ticrynafen, and vinblastine were completely reversible within 24 hours with reintroduction of the control medium. Sham-treated control cells and those treated with indacrinone, ticrynafen, and vinblastine showed positive staining with fluorescein diacetate and were negative for staining when incubated with propidium iodide. As a positive control, cells treated with 0.8 mM ECA for 2 hours, a concentration known to be cytotoxic,11 showed no fluorescein diacetate staining and were positive for propidium iodide staining.

DISCUSSION

Our results suggest that PXAs (Fig. 1), which as a drug class seem to have the potential to increase outflow facility and treat glaucoma, must have at least two distinct cellular targets, only one of which directly involves MTs. In results of comparable studies and doses of ECA, indacrinone and ticrynafen are shown to increase outflow facility (Tables 1, 2) and to alter trabecular cell shape in vitro (Fig. 3) but without altering MT (β-tubulin) staining (Fig. 4). Our recent results26 have indicated that, in addition to its early MT effect, ECA also causes early loss of paxillin and phosphotyrosine staining, implying an interaction with focal contacts and possibly their phosphorylation reactions: This effect could represent an important mechanism of action.27'28 However, in the current results the PXAs indacrinone and ticrynafen were observed not to cause a loss of paxillin (Fig. 5) or phosphotyrosine staining before the observed cell shape changes. This would suggest that in addition to MT and focal contact actions, PXAs must have at least one other cytoskeletonally related cellular target.

It is remarkable that changes in one chemical side-group of PXAs (Fig. 1) can be seemingly responsible for these dramatic differences in cytoskeleton targeting. The SH reactivity of ECA is believed to be caused by the CH2 = C double bond in the side-group, which adds to SH groups by a Michael-type reaction.45 Tubulin has reactive SH groups and ECA has been shown to bind directly to them.29'30 Indacrinone and ticrynafen lack this ethylene double-bond side-group and would therefore be expected to lack SH reactivity.
Phenoxyacetic Acids Increase Outflow

FIGURE 4. Human trabecular meshwork cells stained for β-tubulin after treatment with (A) sham manipulations for 150 minutes (control); (B) 0.5 mM indacrinone for 150 minutes; (C) 0.5 mM ticrynafen for 150 minutes; (D) 50 µM vinblastine for 150 minutes. Note that whereas vinblastine (D) produces a loss of β-tubulin staining, with indacrinone and ticrynafen (B and C, respectively) intact microtubules are observed. Original magnification, ×1000.

In confirmation of this, unlike ECA whose outflow effect is blocked by simultaneous excess cysteine,1 in similar experiments with excess cysteine, indacrinone (Table 3) and ticrynafen (Table 4) maintained their outflow-enhancing actions. It is further noteworthy that despite producing pronounced changes in cell shape and attachment in vitro similar to, but perhaps not as great, as the that caused by the related PXA, ECA, indacrinone and ticrynafen did not alter the β-tubulin staining pattern of HTM and CPAE cells. It seems likely that the lack of SH reactivity of indacrinone and ticrynafen might explain this finding.

These new observations raise the question of whether, in fact, the MT action of ECA is involved in the observed cell shape and outflow effects, as has been proposed.11,12 In contrast, we observed that vinblastine, a known MT-disrupting drug,23 caused an increase in outflow facility in bovine and porcine eyes (Fig. 2) that was correlated with cell shape (Fig. 3) and MT effects (Fig. 4) in cell culture. Taxol, a drug that stabilizes MTs,29,31 attenuated these observed cell shape changes, produced by vinblastine in vitro, as it also did in those produced by ECA.11 In contrast, taxol did not interfere with the cell shape effects produced by indacrinone and ticrynafen. We therefore conclude that MT depolymerization can produce both an in

FIGURE 5. Human trabecular meshwork cells stained for paxillin after treatment with (A) sham manipulations for 150 minutes (control); (B) 0.5 mM indacrinone for 150 minutes. No change in paxillin staining related to indacrinone was observed. Original magnification, ×1000.
vitro change in HTM cell shape (Fig. 3) and an increase in outflow facility (Fig. 2), and that probably part, but not all, of ECA’s action may involve its MT effect. It is noteworthy that taxol has been observed to interfere with ECA’s effect of increasing outflow facility.12

Nevertheless, the magnitude of both the cell shape change observed in vitro and the increase in outflow facility seems less with vinblastine than with ECA (or indacrinone or ticrynafen). However, it should be remembered that MT-depolymerizing drugs (for example, colchicine) may require an unexpected latency for lowering of intraocular pressure to be obtained.22,33 This latency may be responsible for the observed lack of outflow effect of nocodazole, another MT-depolymerizing drug, in similar short-term perfusions in excised eyes.12 In addition, porcine eyes, more plentiful in North Carolina than bovine eyes, seem to demonstrate a lessened washout effect25—a progressive decrease in outflow resistance with prolonged perfusion in control eyes (Fig. 2)—that may also act to diminish the magnitude of the increase in outflow facility in drug-treated eyes observed in vitro. It is interesting that taxol may act to diminish the magnitude of the washout effect.12

We did not directly compare the outflow facility-increasing effects of indacrinone and ticrynafen with that of ECA1 in this study, but the magnitude of the changes seem similar at comparable doses. Because of the limited supply of indacrinone and ticrynafen, we were not able to conduct a full dose-response perfusion study, but chose comparable doses to ECA, with which 0.1 mM seems to be the top of the outflow-enhancing, dose-response curve in excised eyes.41 In contrast, the magnitude of the observed cell shape changes with indacrinone and ticrynafen (Fig. 3) seemed less than with ECA1; and even increasing the concentration of drug fivefold (0.1 to 0.5 mM) did not reproduce the full magnitude of the ECA-induced cell shape change, even though the actin-disruption effect (which is difficult to quantitate) may have been comparable in this dose range with all three drugs. We therefore hypothesize that all three PXAs share a common mechanism for altering cell shape that is not related to MTs or to focal contacts but that the greater cell shape effect observed with ECA is probably related to this added MT-depolymerizing mechanism (and possibly also the focal contact action26). Thus, MT actions may well be desirable in pharmacologic targeting of the HTM in potential glaucoma therapy and, by implication, may be involved in cellular homeostatic mechanisms in outflow pathway function.12 Another important conclusion is that for drug screening the magnitude of the cell shape change observed in HTM cells in vitro may relate quantitatively, but not strictly qualitatively, to the observed outflow effect in a living eye, where the morphologic correlate of a very dramatic increase in outflow may be a very small change in the dimensions of the flow pathway between cells.1,34

An explanation for the likely role of MTs in the induced changes in cell shape and attachment that have now been observed with ECA11 and vinblastine (Fig. 3) cannot be definitively stated. There are no unequivocal direct attachments of MTs to the cell membrane in higher species, although the MT motor protein, dynein, has been indirectly implicated in membrane attachment.35 One hypothesis relates to the concept of tensegrity,36,37 in which cell shape is determined by a balance of outwardly directed MT forces and inwardly directed actin forces. With loss of MTs, unopposed actin forces lead to cell shape changes and loss of cell-to-cell attachments. In fact, in separate studies we have observed that MT-depolymerizing drugs can cause trabecular cell contraction, as assayed by wrinkle production on silicone membranes.38 Cellular contraction in the HTM, possibly by a tensegrity mechanism, may be important both pharmacologically and physiologically. Another possibility involves the dynamic nature of MTs, which are constantly undergoing polymerization (lengthening) and depolymerization (shortening)—reactions termed dynamic instability39,40 that might be involved in some type of cellular signaling. Microtubules also act as “railroad tracks,” conveying substances to and from the cell membrane,41 and it is conceivable that outflow facility effects may relate to such a function. Motor molecules35,42 involved in these processes (e.g., kinesin and dynein) may be directly affected by ECA.43

A very important practical conclusion from our results is that SH reactivity is not required for the potential therapeutic outflow effects of PXAs to occur. Sulphydryl reactivity has severely limited the potential use of ECA itself for topical administration, except perhaps in a prodrug form,5 because of topical irritation. The results of this study therefore offer the potential of a broader range44 of topical antiglaucomatous agents than previously thought with ECA alone. Such non-SH reactive PXAs44 as indacrinone and ticrynafen also have the advantage of previous systemic use.22 Obviously, to design potentially even more effective outflow agents, it is very important to perform three-dimensional structural analysis to learn what reactive chemical groups might be shared by ECA, indacrinone, and ticrynafen. It seems very important to determine the mechanism of action for the outflow and the in vitro cell shape changes induced by these non-SH-reactive PXAs that presumably does not involve MT (or focal contact) actions. This information could provide important new insight into future outflow facility drug therapies and could have potential
Phenoxyacetic Acids Increase Outflow

relevance to normal and abnormal cytoskeleton function in the outflow pathway.\textsuperscript{15,46}

In addition, that an MT-disrupting drug like vinblastine can cause an increase in measured aqueous humor outflow and in vitro cell shape changes in the HTM, suggests that other MT drugs should be studied for potential outflow efficacy and for further insights into HTM cell function.

**Key Words**
ethacrynic acid, indacrinone, microtubules, outflow facility, ticrynafen

**References**


