pH and Drug Ionization Affects Ocular Pressure Lowering of Topical Carbonic Anhydrase Inhibitors

William F. Brechue and Thomas H. Maren

**Purpose.** To evaluate the effect of drug ionization on the ocular hypotensive activity of topical carbonic anhydrase inhibitors.

**Methods.** Ocular normotensive New Zealand albino and ocular hypertensive Dutch Belted pigmented rabbits were used. Tonometric intraocular pressure levels were taken after topical application of 50 nl of drug (at various concentrations and pH values) to one eye with the contralateral eye used as an untreated control. The drugs tested were MK-927, L-662,583, and AHR-16329. Eye tissues were analyzed for drug by our enzymatic methods.

**Results.** In all cases, the more ionized the applied drug the greater the ocular hypotensive activity. Tissue distribution studies showed that there was more drug found in the eye after the ionized form of a drug was applied than that found after application of the less ionized forms.

**Conclusions.** Increasing the ionization of three ampholyte topical carbonic anhydrase inhibitors increases their ocular hypotensive activity. These data taken with ocular disposition data suggest that ionized compounds of this type are more readily sequestered in the cornea, which serves as a drug depot for prolonged drug delivery and activity. Invest Ophthalmol Vis Sci. 1993;34:2581-2587.

Recently, several carbonic anhydrase (CA) inhibitors were introduced that, when applied topically to the cornea, significantly reduce intraocular pressure (IOP) in normotensive and hypertensive animals and humans. In general, these compounds are ampholytes with a basic amine (pK1 between 5.5 and 6.0) and an acidic sulfonamide group (pK2 of 8.3–8.9). Delivered as their hydrochloride salts, these drugs have relatively good water and lipid solubility in the pH range of 4.5–6; such solutions elicit a significant reduction in IOP. In this pH range these compounds are 50–95% ionized. Similarly, ionization of the sulfonamide at the pH 9 level yields soluble solutions. We originally supposed that at neutral pH, where ionization was about 10% and lipid solubility was increased, corneal permeability would increase and result in greater IOP reduction. However, we were surprised to find that decreasing drug ionization and increasing lipid solubility actually reduced ocular hypotensive activity. This article explores and extends these observations with several structurally different compounds. Theoretical and practical implications of these findings are discussed. Preliminary accounts of this work were presented in abstract form at ARVO meetings along with attempts to rationalize the findings in terms of ionic trapping mechanisms.

**METHODS**

Adult male rabbits weighing 2–3 kg, either ocular normotensive New Zealand albino (18.6 ± 2.1 mmHg) or ocular hypertensive Dutch Belted pigmented (30.5 ± 2.5 mm Hg) were used. The experimental procedures conform to the Association for Research in Vision and Ophthalmology...
sion and Ophthalmology Resolution on the use of animals. The rabbits were kept in individual cages with food and water provided ad libitum. The animals were maintained on a 12-hr:12-hr light/dark cycle in a temperature controlled room (21–26°C). More details on the handling, care, and housing of animals have been given previously.11

**Drug Administration**

All drugs were topically applied. MK-927 was administered as a 2% (53 mM) or 0.5% (13.3 mM) solution. The nascent pH level of these solutions was 4.5–4.8. L662,583 was always used as a 2% solution (48 mM). Its nascent pH was 4.4. Both of these compounds were obtained from Merck Sharp & Dohme Research Laboratories (West Point, PA). AHR16329 was given as a 2% (63 mM) or 0.5% (9 mM) solution. Its nascent pH level was 4.6–4.8. This compound was obtained from the A. H. Robins Company. All solutions were prepared and applied in 0.5% hydroxyethylcellulose. To alter ionization of the drug, solutions were titrated with 1 N sodium hydroxide to the desired pH. Solutions of 0.5% MK-927 and AHR-16329 were used to allow study over the entire pH profile of the drug. The upper limit of water solubility for a 2% solution is about pH 5.5; lower limit for 2% solution is about pH 9.

Using a syringe, a 50-μl drop was applied to the upper quadrant of the eye and allowed to flow over the cornea. The contralateral eye served as a control during pressure studies. We have determined that hydroxyethylcellulose vehicle alone does not affect IOP.2

**Physiologic Measures**

**Tonometric IOP.** IOP was measured using a Digilab model 30R pneumatonometer (Bio-Rad, Cambridge, MA).212 The pressure readings were matched with two-point standard pressure measurements at least twice each day using a Digilab Calibration Verifier. All IOP measurements were made by the same investigator with the same tonometer.

One drop of 0.5% proparacaine (Alcaine, Alcon Inc., Humaco, PR) diluted 1:2 with saline, was instilled in each eye immediately before each set of pressure determinations. The animals were familiarized with the experimental procedure by handling and routine pressure measurements taken before the experiments. Rabbits were not sedated or otherwise restrained during IOP measurements except for the hand of the investigator placed lightly on the back and shoulders of the rabbit. IOP was measured three times at each time interval and the means reported. IOP measurements were taken immediately before drug administration, after which IOP was determined at 30 min and then hourly until the drug effect had dissipated. In all IOP studies, rabbits that showed a consistent difference in IOP between eyes during baseline measurements or any sign of eye irritation were excluded from the study, ≥ 3%.

**Manometric IOP.** To determine onset of pressure reduction during the first hour after treatment, IOP was measured continuously using manometric techniques.15 Rabbits were anesthetized with sodium

### Table 1. Physicochemical Properties of Topical and Systemic Sulfonamides

<table>
<thead>
<tr>
<th></th>
<th>$K_1$ vs. CA II (nM)</th>
<th>$NH^+$</th>
<th>$pK_{SO_4NH^-}$</th>
<th>$OH^-$</th>
<th>CHCl$_3$ Buffer pH 7.4</th>
<th>Solubility (mM)</th>
<th>$In$ vitro $(\times 10^3 \cdot hr^{-1})$</th>
<th>$In$ vivo $(\times 10^3 \cdot hr^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK-927*</td>
<td>4</td>
<td>5.8</td>
<td>8.3</td>
<td>—</td>
<td>pH 5</td>
<td>0.3</td>
<td>60</td>
<td>0.4</td>
</tr>
<tr>
<td>L-662,583</td>
<td>0.3</td>
<td>5.7</td>
<td>8.4</td>
<td>10.8</td>
<td>pH 6</td>
<td>0.8</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>AHR16329†</td>
<td>7</td>
<td>6.0</td>
<td>8.9</td>
<td>—</td>
<td>pH 4.6</td>
<td>0.01</td>
<td>&gt;50</td>
<td>—</td>
</tr>
<tr>
<td>Methazolamide‡</td>
<td>10</td>
<td>—</td>
<td>7.4</td>
<td>—</td>
<td>pH 7.4</td>
<td>0.60</td>
<td>3</td>
<td>3.2</td>
</tr>
<tr>
<td>Ethoxzolamide‡</td>
<td>1</td>
<td>—</td>
<td>8.0</td>
<td>—</td>
<td>pH 7.2</td>
<td>0.06</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

* From reference 2.
† From reference 5.
‡ From reference 1.
Data on L-662,583 are new.
Drug Ionization Alters CAI Pressure Lowering

![Chemical Structures]

**Figure 1.** Structures of the three compounds investigated in this study.

pentobarbital (30 mg/kg intravenous) with additional 30–60-mg doses titrated as needed. Animals were intubated by tracheotomy and ventilated on a positive displacement ventilator. End tidal carbon dioxide levels were maintained at 4.2 ± 0.4% throughout the experiment. Body temperature was maintained at 39 ± 0.5°C with a heating pad. The right femoral artery and vein were isolated and cannulated. The arterial line was used to monitor systemic arterial pressure and to sample blood. The venous line was used to inject anesthesia.

Animals were prone with their heads supported upright by a stereotaxic device such that the head was at about the normal standing height, approximating the position during tonometric IOP studies. Needles (25-gauge) were introduced into the anterior chamber of the eye through the cornea. A 4 cm, PE 50 polyethylene catheter connected the needle to an RP-1500 pressure transducer (Narco-Bio Systems, Houston, TX). Output was recorded on a DMP-4B Narco physiograph (Narco-Bio Systems, Houston, TX). Calibrations were performed daily with a mercury manometer. Animals were killed with a pentobarbital overdose followed by supersaturated potassium chloride solution.

Baseline arterial pressure, IOP, and arterial blood gases were monitored for 30–45 min after surgical preparation and insertion of eye needles and before starting experiments to ensure steady-state levels in the animals.

**Drug Distribution in Eye**

The distribution of MK-927 was studied 30 min and 2 hr after topical administration of 1 drop 0.5% MK-927 in 1% hydroxyethylcellulose at pH 4.7, 7.0, and 8.9. Rabbits were killed by intraperitoneal injection of 50 mg/kg pentobarbital followed by centripetal pooling of blood away from the head. Each eye was washed for 5 sec under a stream of water. The aqueous humor was sampled, and the corneas were excised and then briefly rinsed again before removal of the ciliary process. These were nearly blood-free and were excised from well-blotted uveas by scraping with a flat scalpel blade. The tissue was carefully blotted to remove excess liquid before weighing. Tissue samples (5–60 mg) were homogenized in 0.2 ml distilled water and briefly boiled before assay for drug concentration. These experiments were carried out by Dr. Curtis Conroy.

**Calculations**

For all IOP experiments drug was administered to only one eye leaving the contralateral eye as an untreated control. The ocular hypotensive activity is expressed as the average difference in IOP between the treated (T) and control (C) eyes (IOP^T-C, mmHg), thus minimizing the diurnal, seasonal, and interindividual variations commonly observed in the rabbit. Change in IOP^T-C versus time curves were plotted for each animal after tonometric studies. The area between the IOP = 0 line and the IOP/time curve was integrated to yield the area under the line (AUL) or the integrated time effect.

**Statistics.** All data are expressed as mean ± SE. Data were analyzed using a one-tailed t test. Original alpha levels were set at 0.05 with the per comparison error rate corrected by the modified Bonferroni technique.

**RESULTS**

Figure 2 shows the effect of ionization on the ocular hypotensive activity of 2% MK-927. At the low and high pH, the most ionized forms of the drug (≈ 90%), pressure lowering is the same both in peak IOP reduction (3.5 mm Hg) and area under the no-effect line (AUL, 11 mm Hg/hr). When the drug is applied in the less ionized form (72%, pH 5.4), peak IOP reduction is 3.1 mmHg, which is not statistically different from the other pH solutions. However, by 2 hr the pressure lowering is significantly less than after application of the more ionized forms resulting in an attenuated duration of activity, seen as a significantly lower AUL, 6.1 mmHg/hr. To study this phenomenon more completely, we used a 0.5% solution that would confer water solubility over a wide pH range. The results are shown in Figure 3. Again, at the ionization extremes,
FIGURE 2. Effect of ionization on the ocular hypotensive activity of 2% MK-927 in albino rabbits. Values are mean ± SE. Mean starting pressures were 18.6 ± 2.4 mmHg. --- pH 4.6, 94% ionized; ▲ pH 5.4, 72% ionized; ■ pH 9.1, 86% ionized. Area under the no effect line (AUL) is given. n = 8 for each pH experiment.

91% (pH 4.8) and 84% (pH 9), the IOP lowering is the same with respect to peak reduction (2.5 and 2.7 mm Hg, respectively) and AUL (6.7 and 6.6 mm Hg/hr, respectively). Intermediate levels of ionization (56% at pH 5.7 and 33% at pH 8) resulted in slightly less peak IOP reduction and AUL. The most significant finding was that at pH 7, the least ionized (11%) and most lipid soluble form of the drug, peak IOP reduction (0.8 mm Hg) and AUL (1.2 mm Hg/hr) were far lower than those that occurred with the more ionized drug. Ocular pressure activity was essentially and surprisingly eliminated. Figure 4 shows the same general phenomenon for MK-927 in Dutch Belted pigmented rabbits.

FIGURE 3. Effect of ionization on the ocular hypotensive activity of 0.5% MK-927 in albino rabbits across the pH range. Values are mean ± SE. Mean starting pressures were 18.5 ± 1.6 mm Hg. Solution pH, percentage of ionization and AUL are given, n = 8 for each pH experiment.

FIGURE 4. Effect of ionization on the ocular hypotensive activity of MK-927 in ocular hypertensive pigmented rabbits. (A) 2% solution. Mean starting pressures were 31 ± 3.4 mmHg. ■ pH 4.5, 95% ionized; pH 5.4, 72% ionized. (B) 0.5% solution. Mean starting pressures were 30 ± 2.1 mmHg. ■ pH 4.8, 91% ionized; pH 7.0, 11% ionized; ▲ pH 9.1, 86% ionized. AUL is given, n = 8 for each individual pH experiment. Values are mean ± SE.

The upper curve shows the 2% experiment and the lower curve the 0.5% experiment. These animals were naturally ocular hypertensive, and therefore pressure lowering and AUL were significantly greater than in the albino rabbits. However, the same effect of ionization is seen, decreasing ionization reduces the pressure lowering ability of MK-927.

Figure 5 shows the results of manometric pressure studies performed in the albino rabbit. These experiments show clearly that the initial pressure lowering (1.4 mm Hg at 20 min) is independent of ionization, but between 30 and 60 min the ocular hypotensive activity is significantly greater with the low pH, high ionization drug.

Figures 6 and 7 show the same ionization phenomenon with other topical CA inhibitors with ampholyte characteristics. L-662,583 has a very different ring system from MK-927 (Fig. 1). AHR-16329 is an organic sulfamate and is also structurally quite different from both other compounds (Fig. 1).

Table 2 gives tissue distribution data of MK927.
Drug Ionization Alters CAI Pressure Lowering

The more ionized form of the applied drug (pH 4.7 and 8.9) results in significantly higher drug levels in the cornea, anterior aqueous humor, and ciliary process at 30 min compared to those after application of the less ionized form. These differences in cornea and ciliary process persisted through 2 hr. Differences in the aqueous humor were small throughout. Expanded data along with their theoretical basis are being prepared for publication.

DISCUSSION

Our main finding is that in this series of drugs the ionized forms are more active than unionized. The three compounds used are structurally different, but all contain a sulfonamide group that confers inhibitory activity against CA. Another major similarity is that each compound is an ampholyte consisting of an ionizing group with pK of 5.5–6 and an acidic sulfonamide group with pK of 8.3–8.9. Their ampholytic characteristic confers relatively good water and lipid solubility. Each compound has a constellation of physico-chemical properties that allows good corneal and tissue permeability and significant reduction of IOP.

### TABLE 2. Effect of Ionization on Distribution of MK-927 in Ocular Tissues and Fluids (μmol/kg)

<table>
<thead>
<tr>
<th>Tissue/Treatment</th>
<th>30 min</th>
<th>2 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cornea</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4.7</td>
<td>10.4 ± 1.3</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>2.5 ± 0.4</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>pH 8.9</td>
<td>9.9 ± 1.1</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td><strong>Anterior Aqueous</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4.7</td>
<td>2.5 ± 0.4</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>1.5 ± 0.3</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>pH 8.9</td>
<td>3.8 ± 0.4</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td><strong>Ciliary Process</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4.7</td>
<td>7.2 ± 0.8</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>3.0 ± 0.8</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>pH 8.9</td>
<td>6.5 ± 1.4</td>
<td>2.3 ± 0.2</td>
</tr>
</tbody>
</table>

From reference 10 and amended by Dr. Curtis Conroy. (To be published.)
We have shown that these ampholytes have another property that, in addition to good corneal and non-corneal permeability, appears to be a necessity for effective long-term lowering of IOP: namely the ability to sequester in the cornea and form a drug delivery depot.\(^2,13\) Analysis of corneal drug levels and decay from the cornea and other ocular tissue and fluids suggests that the cornea controls the process. The cornea continually supplies drug to the ciliary process, replacing delivery from plasma, as for systemic CA inhibitors.\(^5\)

Each of the three compounds have similar in vivo corneal permeability comparable to that of methazolamide but significantly less than for ethoxzolamide (see Table 1). Methazolamide and analogs and ethoxzolamide in solution do not lower IOP by the topical route; it is significant, however, that there is activity with some of the homologs and ethoxzolamide when administered as a suspension in which case there must be some depot effect.\(^12,16\) After topical application of methazolamide or ethoxzolamide, in solution, high corneal and aqueous humor concentrations are reached early but decline quickly.\(^1,17\) Giving ethoxzolamide as a 2% suspension, where modest ocular hypertensive activity is seen, increases the cornea/aqueous humor ratio from 1.1 to 4.\(^12\) Trifluormethazolamide also penetrates the cornea and reduces IOP.\(^1\) This fluorination of methazolamide increased the cornea/aqueous humor ratio of drug from 0.67 to 1.2\(^11,17\) as a result of increased ionization and corneal sequestration.

Each of the compounds studied here result in cornea/aqueous humor ratios of 2.5–5 when the drug is administered in the ionized form\(^6\) (and unpublished data). Other ocular hypertensive agents have similar ratios, epinephrine, \(^4,18\) and timolol, \(^10,19\) notably, these are also bases.

The theoretical effect of increasing ionization of CA inhibitors would be to greatly decrease in vivo permeability. However, previous studies have shown that acidic ionized compounds adequately penetrate the cornea at about 1/4 the rate of the unionized species.\(^9\) We now show that in the case of MK-927 corneal permeability is independent of ionization of the base (Table 1).\(^2\) The same pattern is seen for AHR16329 whereas for L-662,583 it appears that the unionized species is somewhat more permeable (Table 1).

It is emphasized that formation of the anionic species does not in itself confer activity in all structural series. We have shown clearly that methazolamide and its more active analogs do not lower pressure when administered as their anionic solutions at pH 8–9.\(^1,16\) The same is true of acetazolamide and ethoxzolamide.\(^1\) What appears necessary is the anionic species (at high pH) in an ampholyte setting, in the current case an unionized base. Conversely, at low pH levels corneal sequestration and IOP lowering may be dictated also by ionization of the base while the acid appears neutral.

The effects of ionization on IOP lowering suggests for several reasons that it affects the ability of the drugs to form a corneal depot, rather than affecting permeability. First, in vivo corneal permeability is independent of ionization (Table 1). Second, the similarity in IOP reduction at the different pH levels before 30 min (Fig. 5) is probably related to noncorneal absorption of drug to the ciliary processes. We have shown that MK-927 appears in the anterior uvea before detection in aqueous humor and is associated with a significant decrease in IOP.\(^13\) The differences in IOP response after 30 min reflects the rapid decline of drug from the eye because of rapid corneal clearance. From Table 2 it can be seen that decreasing ionization results in significantly lower corneal, aqueous humor, and ciliary process drug levels at all times; the data show that the cornea/aqueous humor ratio decreases twofold to threefold with decreasing ionization. The corneal depot effect results in a higher concentration of drug in the ciliary processes with the more ionized drug (pH 4.7; 8.9) compared to the unionized drug (pH 7).

The significance of these findings may relate to clinical practice. Significant problems encountered when placing many drugs on the cornea involve discomfort, irritability, tearing, and redness. Therefore, a goal in developing topical drugs relates to corneal comfort and the attempt to apply drugs as close to physiologic pH as possible. Most drugs are bases and given in acid solution; epinephrine, dipivefrine, pilocarpine, and MK-927 sting at pH < 6. Because the topical CA inhibitors are relatively inactive at neutral pH and are maximal at pH 9, usage at this pH level may eliminate many of the corneal discomfort problems without sacrificing pressure lowering activity.

In conclusion, ampholyte carbonic anhydrase inhibitors when applied topically to the eye, significantly reduce IOP because of a series of physicochemical properties that result in good corneal and noncorneal permeability and the ability to sequester in the cornea. Decreasing the percentage of ionization of the applied drug does not alter in vivo corneal or noncorneal permeability or early IOP reduction (< 30 min). Decreasing ionization attenuates the ability of these drugs to sequester in the cornea resulting in lower drug levels in the ciliary process after 30 min. Thus neutral pH greatly limits their effectiveness in lowering IOP. This is analogous to methazolamide, which has good corneal permeability and solubility but does not significantly reduce IOP after topical application because of
lack of corneal sequestration. The mechanisms or anatomic location of this corneal depot remain to be investigated.

**Key Words**

ampholytes, intraocular pressure, ionization, AHR-16329, L662,583, MK-927

**Acknowledgment**

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**References**