be involved in the inactivation of EPI. This hypothesis is supported by enzyme data in vitro, which showed that the inhibitor acts as a competitive substrate with EPI for a partially purified COMT preparation from the rabbit iris–ciliary body (L. P. Bausher, unpublished observations). Iris–ciliary body COMT activity is unaffected by sympathetic denervation, and in most peripheral tissues COMT appears to have a predominantly extraneuronal location.

It is widely accepted that the principal mechanism of inactivation of catecholamines at the neuroeffector junction in most varieties of normal smooth muscle involves reuptake into the presynaptic neuron and binding in intracellular storage granules. Enzymatic inactivation processes are generally found to be unimportant. However, some studies in vitro using tissues which are highly sensitive to catecholamines as a result of prior denervation or treatment with cocaine, suggest a possible role for COMT in the termination of action. Wylie and co-workers have demonstrated potentiation in vivo of the effects of O-methyl transferase inhibitors on blood pressure. Knowledge of the mechanism of the termination of action of EPI in lowering IOP in glaucomatous eyes could be helpful in elucidating the role of the adrenergic system in aqueous humor dynamics. Nerve degeneration in the trabecular region has been reported in open-angle glaucoma and in the human aging process. In the absence of uptake into nerve endings, both denervated and glaucomatous eyes might respond similarly to EPI and drugs which potentiate the effects of EPI. Further clinical and experimental study in this area may be useful.

We are grateful to Mr. Paul O’Connell of The Upjohn Co. for a generous supply of U-0521.

From the Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, Conn. This work was supported in part by United States Public Health Service Grants EY-00785 and EY-00237. Submitted for publication May 25, 1976. Reprint requests: Dr. Larry P. Bausher, Department of Ophthalmology and Visual Science, Yale University School of Medicine, 333 Cedar Street, New Haven, Conn. 06510.

Key words: catechol-O-methyl transferase inhibition, aqueous humor dynamics, intraocular pressure, sympathetically denervated eye, epinephrine, in vivo potentiation, dose-response curves, U-0521.

REFERENCES


The corneal penetration of 6-aminohexanoic acid. ROBERT C. CAMPBELL, ROGER W. NEAULT, AND RICHARD F. BRUBAKER.

6-Aminohexanoic acid is a potent antifibrinolytic agent with the ability to retard dissolution of fibrin clots. The corneal penetration of 6-aminohexanoic acid was measured to determine if therapeutic levels of the drug could be maintained in the anterior chamber by topical administration. The conjunctival sac to anterior chamber transfer coefficient was found to be 1.51 × 10⁻⁴ ± 0.20 × 10⁻⁴ per minute and the corneal permeability found to be 3.46 × 10⁻⁷ ± 0.46 × 10⁻⁷ cm. per second in the rhesus monkey eye. The results indicate that, to establish a therapeutic level in the aqueous humor, a high concentration of drug would have to be used with topical drop administration or a zero order delivery system must be used.

In a prospective, randomized clinical trial, 6-aminohexanoic acid (epsilon-aminocaproic acid)
has been shown to reduce the incidence of secondary hemorrhage in traumatic hyphema from 33 to 3 per cent.\(^1\) The rationale for the use of this drug is that it is thought to retard clot lysis in the injured vessel by inhibiting activators of plasminogen.\(^2\)\(^-\)\(^3\)

The question arises, would it be possible to achieve the same effect by delivering the drug directly to the injured tissue by topical application to the cornea. The pharmacokinetics of topical administration of this lysine-like drug are not known. Its low molecular weight, 131, would favor penetration of the cornea, but its amino acid structure, which results in ionization at all pH's, would slow its penetration. The purpose of this study was to determine if it would be feasible to deliver 6-aminohexanoic acid to the eye by the topical route.

**Materials and methods.**

**Measurement of corneal transfer coefficient.** The technique of Mishima, Hattori, and Yamanouchi\(^4\) was used. Adult rhesus monkeys were anesthetized with intraperitoneal sodium pentobarbital. A reservoir was formed by incising the conjunctiva at the fornix, dissecting it carefully to the limbus and supporting it by sutures to form a cuplike container.

Two 23-gauge needles were inserted at the limbus (posterior to the conjunctival reservoir) into the anterior chamber. The inflow needle was attached to an infusion pump via polyethylene tubing. The anterior chamber was perfused with a mammalian tissue culture medium (Medium 199, Hanks salts with glutamine, without phenol red) at the rate of 20 \(\mu\)l per minute. The outflow needle was attached via polyethylene tubing to a scintillation counter vial. The height of the vial was set 136 mm. above the cornea to produce an intraocular pressure near 10 mm. Hg.

Tritiated 6-aminohexanoic acid (Lederle Laboratories, Pearl River, N. Y.) with specific activity of 42 \(\mu\)Ci per milligram was used. Eleven milligrams of the powder were dissolved in 1 cc. of water to make a stock solution; 50 \(\mu\)l of this stock solution were diluted with 10 cc. of 0.2M phosphate buffer, pH 7.1. An aliquot of the buffered, labeled 6-aminohexanoic acid was dropped directly to the injured tissue by topical application of a single drop. Albino rabbits were anesthetized with intravenous sodium pentobarbital and pentylenetetrazol. The stock solution of labeled 6-aminohexanoic acid referred to above was diluted 1:10 with 0.2M phosphate buffer, pH 7.1; 50 \(\mu\)l of the diluted solution were placed on the surface of the cornea. The lids were “blinking” manually several times, and then left alone. At 15, 30, or 60 min. after application of the drop, the surface of the cornea of each experimental eye was carefully irrigated and dried, and the contents of the anterior chamber aspirated with a 25-gauge needle and a 1 cc. syringe. Approximately 100 \(\mu\)l of aqueous humor were placed into a tared scintillation vial and the radioactivity of the sample determined as described above.

**Determination of the stability of the tritium label on the tracer compound.** A Packard radiochromatogram system was used with thin layer plates of Silica gel G and a solvent system of n-butanol-acetic acid-water, 60:20:20. The chromatographic mobility of the ninhydrin-positive and the radioactive spot was measured with aliquots of the original tracer solution and aliquots of aqueous humor following the single-drop tracer experiment just described.

**Calculation of transfer coefficient.** The following model was used to calculate the transfer coefficient of the drug from the conjunctival reservoir to the anterior chamber, using the data from the first experiment described.

**Definitions.**

\[
\begin{align*}
C_c & = \text{concentration of drug in conjunctival reservoir, \(\mu g/\mu l\).} \\
C_e & = \text{concentration of drug in effluent aqueous humor when steady state has been achieved, \(\mu g/\mu l\).} \\
C_a & = \text{concentration of drug in anterior chamber, \(\mu g/\mu l\).} \\
F & = \text{flow of fluid through the anterior chamber, \(\mu l/min\).} \\
V & = \text{volume of the anterior chamber, \(\mu l\).} \\
A & = \text{area of the cornea.} \\
t & = \text{time, min.} \\
K & = \text{transfer coefficient of drug from reservoir to anterior chamber, referenced to anterior chamber volume, per min.} \\
P & = \text{permeability of the cornea to the drug, cm./sec.} \\
\end{align*}
\]

**Assumptions.**

1. The rate of entry of aqueous humor into the anterior chamber of the rhesus monkey eye is 2.5 \(\mu l/min\), the volume of its anterior chamber is 120 \(\mu l\), and the surface area of its cornea is 87 mm.\(^2\)\(^-\)\(^3\).
2. The anterior chamber is a stirred compartment.
3. \( C_c \gg C_s; C_c \gg C_e \).
4. The entry of drug is a first-order process.
5. The drug is lost exclusively by bulk outflow of aqueous humor.

**Calculations.** The following relation then holds:

\[
\frac{dC_a}{dt} = K(C_c - C_a) - \frac{F_a C_a}{V}
\]  

(1)

Since \( C_c \gg C_a \):

\[
\frac{dC_a}{dt} \approx KC_c - \frac{F_a C_a}{V}
\]  

(2)

In the first experiment, \( C_c \) is constant and samples are taken until \( \frac{dC_a}{dt} = 0 \).

Rearranging,

\[
K = \frac{C_c \cdot F_a}{C_c} \cdot \frac{2.5 + 20}{120}
\]  

(4)

The permeability \( P \) is related to the transfer coefficient \( K \) in the following way

\[
P = \frac{K \cdot V}{A} = \frac{I_e}{I_c} \times 4.3 \times 10^{-4} \text{ cm./sec.}
\]  

(7)

**Results.** The conjunctival sac to anterior chamber transfer coefficient of 6-aminohexanoic acid in the rhesus monkey eye, referenced to the volume of the anterior chamber, was found to be \( 1.51 \times 10^{-4} \pm 0.20 \times 10^{-4} \) per min. (mean ± SE, \( n = 7 \)). (See Table I.) This coefficient represents the fraction of the volume of the anterior chamber that exchanges with the conjunctival reservoir per minute by whatever process brings the drug into the anterior chamber. The corneal permeability can also be calculated from these data and was found to be \( 3.46 \times 10^{-7} \pm 0.46 \times 10^{-7} \) cm./sec. The permeability is a measure of the mass of drug which will pass through a unit area of cornea under a unit concentration gradient per unit time.

Table II summarizes the results of the single-drop experiment carried out in the rabbit eye. The highest concentrations of the drug were found in the earliest samples. Thereafter, the drug concentration fell.

The chromatographic mobility of the ninhydrin-positive spot and the single radioactive peak of the tracer compound on the solvent system described were the same before and after corneal penetration.

**Discussion.** Under the previous assumptions, the concentration and frequency of administration can be estimated from the \( K \) value and from the minimal effective therapeutic level which

<table>
<thead>
<tr>
<th>Eye No.</th>
<th>Radioactivity in conjunctival reservoir, ( I_c ) (counts/min./( \mu )l) ( \times 10^4 )</th>
<th>Radioactivity in anterior chamber at equilibrium, ( I_e ) (counts/min./( \mu )l) ( \times 10^4 )</th>
<th>Transfer coefficient* ( K ) (per min.) ( \times 10^4 )</th>
<th>Permeability, † ( P ) (cm./sec.) ( \times 10^7 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.32</td>
<td>1.91</td>
<td>1.54</td>
<td>3.53</td>
</tr>
<tr>
<td>2</td>
<td>3.28</td>
<td>3.51</td>
<td>2.01</td>
<td>4.61</td>
</tr>
<tr>
<td>3</td>
<td>3.93</td>
<td>4.88</td>
<td>2.32</td>
<td>5.32</td>
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<tr>
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<td>2.76</td>
<td>1.43</td>
<td>3.28</td>
</tr>
<tr>
<td>5</td>
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<td>2.92</td>
<td>1.49</td>
<td>3.42</td>
</tr>
<tr>
<td>6</td>
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<td>3.40</td>
<td>1.06</td>
<td>2.43</td>
</tr>
<tr>
<td>7</td>
<td>5.45</td>
<td>2.13</td>
<td>0.73</td>
<td>1.67</td>
</tr>
</tbody>
</table>

Mean: 1.51 s.D.: 0.54 S.E.: 0.46

*Anterior chamber assumed to have volume of 120 \( \mu \)l.
†Area of cornea assumed to be 87 mm.². See equation 7.

<table>
<thead>
<tr>
<th>Experiment No.:</th>
<th>Anterior chamber concentration (counts/min./100 ( \mu )l)</th>
<th>15 min.</th>
<th>30 min.</th>
<th>60 min.</th>
</tr>
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<tbody>
<tr>
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<td>5.551</td>
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<td>2</td>
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<tr>
<td>3</td>
<td>2,016</td>
<td>1.234</td>
<td>1.433</td>
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<tr>
<td>4</td>
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<td>1.220</td>
<td>5.073</td>
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<td>2.739</td>
<td>5.284</td>
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<tr>
<td>6</td>
<td>3,166</td>
<td>1.910</td>
<td>1.600</td>
<td></td>
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<tr>
<td>Mean</td>
<td>3,170</td>
<td>1,708</td>
<td>1,276</td>
<td></td>
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<tr>
<td>S.D.</td>
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<td>348</td>
<td>521</td>
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</tr>
<tr>
<td>S.E.</td>
<td>775</td>
<td>3,443</td>
<td>1,200</td>
<td></td>
</tr>
</tbody>
</table>

Calculated values for fraction of initial drop concentration in anterior chamber (\( \times 10^6 \)):

Mean: 3.72 S.D.: 2.24 S.E.: 0.90

Each data point is different eye.
†Value > 3 S.D. from mean, discarded.
is known to be approximately 0.05 μg/μl. If the drug is given in drop form (C₀), and the drop disappears from the conjunctival sac as a first-order process, the half-life of which is short (T½₀) compared to the half-life of the drug in the anterior chamber (T½ₐ), the peak concentration (Cₚ) in the anterior chamber will be given approximately by

\[ C_p \approx \frac{T_{½₀}}{K_c \ln 2} = 1.44 \frac{T_{½₀}}{K_c} \]  \hspace{1cm} (8)

If T½₀ for tears is taken to be 1 min, and the mean value of K as measured in rhesus monkey is used, equation 8 becomes

\[ C_p \approx 2 \times 10^{-4} C_d \] \hspace{1cm} (9)

The lowest drop concentration needed to achieve the minimal therapeutic level in aqueous humor would be

\[ C_d = \frac{0.05 \text{ μg/μl}}{2 \times 10^{-4}} = 250 \text{ μg/μl} = 25\% \] \hspace{1cm} (10)

If the drug disappears from the anterior chamber as a first-order process with a half-life of T½ₐ, then the duration of the effective level will increase by T½ₐ every time the drug concentration is doubled. The half-life of many substances in the anterior chamber is approximately 90 min. We would therefore expect that 1 drop of a 50 per cent solution of 6-aminohexanoic acid would achieve a therapeutic level for only 1.5 hr. Single-drop administration of this drug, therefore, does not seem to be a feasible method of maintaining a therapeutic level in the anterior chamber.

The single-drop experiments in rabbit eyes confirm this prediction. For example, the concentration in the anterior chamber at 60 min. following single-drop topical administration to the rabbit eye was 1.8 \times 10^{-4} times the applied concentration (see Table II). This ratio allows us to calculate the drop concentration needed to achieve a therapeutic level and maintain it to 60 min., as follows:

\[ \text{Drop concentration} = \frac{0.05 \text{ μg/μl}}{1.8 \times 10^{-4}} = 28\% \]

It is likely that the corneal epithelium is the greatest barrier to the penetration of 6-aminohexanoic acid into the anterior chamber, although we have not carried out experiments to confirm this assumption. Also, it is possible that the drug might enter the eye via a saturable transport process. Our data neither support nor refute this possibility since all experiments were performed with a single concentration of the drug. If a saturable transport process were present in the cornea for this drug, our calculations would tend to underestimate the concentrations needed to achieve therapeutic levels in the anterior chamber.

These data support the idea that topical delivery of this drug would be difficult to accomplish. Enhancement of penetration might be accomplished by prolonging the contact time of the drug, by altering epithelial permeability with a pH change, or by using a zero-order delivery device. If the last method were used, the rate of delivery of drug would depend primarily on the basal rate of tear flow. If this rate is 35 μl per hour, the delivery device would have to supply at least 240 μg per hour or 5.8 mg per day. This mass of drug is small enough that it is conceivable that several days' supply of the drug could be incorporated into a device which could be worn in the conjunctival cul-de-sac.

From the Department of Ophthalmology, The Mayo Clinic, Rochester, Minn. This study was supported by research grant No. EY00634 of the National Eye Institute, National Institutes of Health, Bethesda, Md. Submitted for publication March 25, 1976. Reprint requests: Richard F. Brubaker, M.D., Department of Ophthalmology, Mayo Medical School, The Mayo Clinic, Rochester, Minn. 55901.

Key words: hyphema, 6-aminohexanoic acid, corneal penetration, monkey, pharmacokinetics, topical drug delivery, antifibrinolysis, eye, anterior chamber.

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