Factors Affecting Therapeutic Concentration of Topical Aminocaproic Acid in Traumatic Hyphema

Epsilon-aminocaproic acid (EACA) decreases rebleeding in traumatic hyphema through antifibrinolytic activity. Therapeutic levels were achieved in aqueous humor of rabbits after topical application. Aqueous humor EACA levels were significantly higher after pretreatment with 0.5% proparacaine. Use of EACA (60%) in a carboxypolymethylene (CPM) vehicle (0.5%, 1%, 2%, 3%, and 4%) was examined. Aqueous humor levels at 4 hours ranged from 6.18-20.42 μg/ml. The 2% and 3% formulas achieved the highest concentrations in aqueous. Use of EACA (15%, 30%, 40%, and 60%) in 4% CPM was also studied. At 2 and 4 hours after treatment, the 30% EACA solution most effectively achieved therapeutic levels. Velcro closure devices were attached to the rabbit's eyelids, and 200 μl of 30% EACA in 2% CPM was administered. After 3 hours the patched eyes had a mean aqueous EACA level of 60.09 μg/ml compared with 8.97 μg/ml in unpatched eyes. When dose size was studied in patched eyes, 200-μl doses achieved aqueous levels of 60.09 μg/ml, and 100-μl doses resulted in levels of 10.40 μg/ml. Since epithelial toxicity was observed in eyes that had been patched, the optimum topical regimen appeared to be 200 μl of 30% EACA in 2% CPM every 6 hours in unpatched eyes. Invest Ophthalmol Vis Sci 31:2389-2394, 1990

Traumatic hyphema is a problem commonly encountered in ophthalmology with potentially sight-threatening complications. Secondary hemorrhage occurs in 18-38% of untreated patients 2-5 days after the original injury. Secondary hemorrhage is usually more severe than the initial hemorrhage and carries a significantly worse prognosis. The incidence of glaucoma, corneal blood staining, and optic atrophy are all higher after secondary hemorrhage.

Epsilon-aminocaproic acid (EACA) inhibits fibrinolysis by inhibiting the activation of plasminogen to plasmin. Previous studies have shown the efficacy of systemically administered EACA (Amicar, Lederle Laboratories, American Cyanamid Company, Pearl River, NY; 50 mg/kg or 100 mg/kg every 4 hr; maximum single dose: 5 g) in significantly reducing the incidence of secondary hemorrhage in masked, controlled laboratory studies using New Zealand white rabbits. The control group and a “placebo” group which received CPM vehicle without EACA had rebleed rates of 33% while the group receiving the EACA preparation had a rebleed rate of 10%. Aqueous humor concentrations of EACA after topical administration were comparable to those achieved with systemic EACA levels. Topically applied EACA in 4% carboxypolymethylene vehicle (CPM) has been shown to be effective in reducing the incidence of secondary hemorrhage in masked, controlled laboratory studies using New Zealand white rabbits. The control group and a “placebo” group which received CPM vehicle without EACA had rebleed rates of 33% while the group receiving the EACA preparation had a rebleed rate of 10%. Aqueous humor concentrations of EACA after topical administration were comparable to those achieved with systemic therapy. With systemic therapy plasma EACA levels were three to 20 times greater than topical EACA. The topical route of administration, which avoids gastric exposure and high plasma EACA levels, should have a lower incidence of systemic side effects.

Our purpose was to optimize the topical EACA formula and dosing schedule using the rabbit model. Both EACA and CPM concentrations were varied. Parameters tested included dose size, frequency, pretreatment with topical anesthetics, and the effect of simulated patching of the eye on aqueous humor EACA concentrations. Traumatized and untraumatized eyes were examined for evidence of toxicity.
Materials and Methods

The EACA preparations were administered topically to female, albino New Zealand rabbits (2-3 kg). All animal protocols were approved by the Eastern Virginia Medical School Animal Care and Utilization Committee and conformed to the ARVO Resolution on the Use of Animals in Research. The preparations were instilled into the inferior fornix. Using the technique described by Loewy and associates,14 blood and aqueous humor samples were obtained at specified times after dosing. Rabbits were anesthetized using 1 mg/kg of acepromazine maleate (Promace; Aveco, Fort Dodge, IA) and 75-100 mg/kg of ketamine (Ketalar; Parke-Davis, Morris Plains, NJ) administered intramuscularly 15-20 min before induction of hyphema. Aqueous humor samples were obtained under anterior chamber paracentesis on anesthetized rabbits using a 27-gauge disposable needle. Blood samples were obtained by venipuncture from marginal ear veins on anesthetized rabbits using standard blood-collecting tubes containing buffered sodium citrate (0.13 mol/l). After clot formation blood samples were centrifuged for 10 min at 7000 rpm (Daman/ICN-S centrifuge, Daman/IEC, Needham Heights, MA), and plasma was collected. All aqueous humor and plasma samples were stored at -10°C until assayed for EACA concentrations.

Protocol for Patching the Eye

To simulate the effect of patching the eye, Velcro (developed in our laboratory out of commercially available Velcro strips) devices were fashioned and attached to the right shaved eyelid of anesthetized rabbits using 27-gauge disposable needle. Blood samples were obtained by venipuncture from marginal ear veins on anesthetized rabbits using standard blood-collecting tubes containing buffered sodium citrate (0.13 mol/l). After clot formation blood samples were centrifuged for 10 min at 7000 rpm (Daman/ICN-S centrifuge, Daman/IEC, Needham Heights, MA), and plasma was collected. All aqueous humor and plasma samples were stored at -10°C until assayed for EACA concentrations.

High-Performance Liquid Chromatography

Analysis of aqueous and plasma samples was done as described by Farid22 and modified by Loewy and associates.14 Plasma or aqueous humor was added to the internal standard (25 μl of W-aminocaproic acid, 80 mg/dl) and 500 μl of alcohol. The sample was centrifuged and evaporated to dryness under vacuum. The residue was resuspended in 750 μl of sodium diphosphate (0.05 mol/l, pH 9.3), and 250 μl of fluorescamine (10⁻³ mol/l in acetonitrile) was added with rapid mixing. Standards were prepared in serum and aqueous humor for each set of analyses. Estimation of EACA content was based on the peak height ratio between E- and W-aminocaproic acid compared with the standards. This method was linear between 0.01 mg/dl (the minimum detectable level) and 30.0 mg/dl. Samples which initially fell outside this range were diluted 1:2 or 1:10 as needed.

Topical Preparations

Preparations were compounded with various concentrations of EACA (Sigma, St. Louis, MO) in CPM vehicle (Carbopol; BF Goodrich, Cleveland, OH) as described in previous reports.20,21 All preparations contained 0.01% ethylenediaminetetraacetic acid (Sigma).

The “control” preparation for the toxicity study consisted of 2% CPM vehicle with 0.01% ethylenediaminetetraacetic acid. Preparations were compounded using sterile technique, titrated to a pH of 7.4, and stored at 4°C.

Hyphema Induction

Unilateral hyphemas were induced in rabbits using the technique devised by Zimmerman et al15 and modified by Allingham and associates.20 Rabbits were anesthetized as described. Before induction of hyphema or anterior chamber paracentesis, proparacaine 0.5% (Ophthetic; Allergan, Irving, CA) was instilled topically. Compressed air was used to propel a 4.5-mm copper-plated steel ball through a 2-m x 1.0-cm aluminum tube. The cornea was positioned perpendicularly 0.5 cm from the tube with eyelids retracted. Nylon mesh (149 mm) (Small Parts, Miami, FL) was placed over the eye to diffuse the force over the corneal surface. This method was successful in approximately 50% of trials. Only one eye was used in each animal. No total hyphemas or perforations occurred. Treatment was initiated 24 hr after hyphema induction in those animals in which a hyphema occurred.

Statistical Analysis of the Data

All data is reported as the mean ± standard error of the mean. One-way analysis of variance and Duncan’s multiple range test were used to determine if differences in aqueous humor EACA concentration among various test groups were significant. Differences were considered statistically significant when $P < 0.05$.

Results

Effects of Proparacaine

To determine the effect of proparacaine pretreatment on aqueous humor levels of EACA, rabbits were divided into two groups. Both groups received 50 μl doses of 60% EACA in 4% CPM vehicle. One group was pretreated with 0.5% proparacaine; the second group received no pretreatment. Plasma and aqueous samples were drawn at 15 and 60 min. Rab-
bits pretreated with proparacaine had mean aqueous humor EACA levels of 39.3 μg/ml and 45.3 μg/ml at 15 and 60 min, respectively, compared with 5.75 μg/ml and 2.8 μg/ml for untreated rabbits (Fig. 1). Therefore, in subsequent experiments all eyes were pretreated with proparacaine.

**Variation of EACA Concentration**

Various concentrations of EACA preparations were studied by dividing rabbits into four groups. All groups received 50 μl of an EACA preparation in 4% CPM vehicle. Concentrations of EACA of 15%, 30%, 40%, and 60% were tested. Aqueous humor and plasma samples were drawn 2 and 4 hr after dosing. Rabbits receiving the 30% EACA formula had the highest aqueous humor levels after 2 hr, while groups receiving 15%, 30%, and 40% formulas had similar levels after 4 hr (Fig. 2).

**Various Concentrations of CPM Vehicle**

In another experiment, rabbits were divided into five groups; all rabbits received a 50-μl dose of 60% EACA formula, but the concentration of the CPM vehicle was varied: 0.5%, 1.0%, 2.0%, 3.0%, and 4.0% vehicles were tested. Aqueous humor and plasma EACA levels were assayed 2 and 4 hr posttreatment. Rabbits receiving EACA in 2.0% CPM gel had the highest aqueous EACA concentrations (65.44 μg/ml) at 2 hr. Four hours posttreatment groups receiving 0.5%, 1.0%, 2.0%, and 3.0% all had similar levels. At both time intervals the groups receiving the 4% formula had the lowest aqueous EACA concentrations. We believed the high viscosity of this formula prevented it from spreading, thus reducing the corneal surface area in contact with the formula (Fig. 3).
Effects of Patching

The effect of patching eyes was tested by attaching Velcro closure devices to the shaved eyelids of rabbits. One group of rabbits had these devices attached; immediately after dosing, the devices were secured while a second group remained unpatched. Both groups received 200 µl of 30% EACA in 2% CPM vehicle. Three hours later aqueous humor samples revealed an EACA level of 60.09 µg/ml in the patched eyes compared with 8.97 µg/ml in unpatched eyes (Fig. 4).

Dose Comparison

The effects of dose were studied using 100-µl and 200-µl doses. After pretreatment with 0.5% proparacaine, one group of rabbits received 100 µl of 30% EACA in 2% CPM. A second group received 200 µl of the same formula. All eyes were patched after dosing. Three hours after treatment the group receiving the 100-µl dose had mean aqueous humor EACA levels of 10.41 µg/ml compared with 60.09 µg/ml after the 200-µl dose (Fig. 5).

Evaluation of EACA or CPM Toxicity

To evaluate possible EACA or CPM toxicity in patched, traumatized rabbit eyes, a masked study was used. A control group of ten rabbits with normal eyes was randomized into a group receiving 30% EACA in 2% CPM vehicle and a group receiving CPM vehicle only (placebo). All groups received 200-µl doses every 12 hr for 5 days and were patched between doses. Fifteen rabbits with traumatized eyes were likewise randomized into groups where one group received 200-µl doses of 30% EACA and the second group received the placebo formula. All rabbits were examined for blepharospasm, photophobia, conjunctival injection, corneal epithelial defects, ulceration, and abnormal red reflex.
Rabbits with traumatized eyes showed blepharospasm, photophobia, and conjunctival injection secondary to the trauma before receiving any topical formula. Conjunctival injection, superficial punctate keratopathy, and epithelial defects developed in all groups during the course of treatment. No rabbit progressed to corneal ulceration (Table 1).

**Discussion**

In 1952 Thygeson and Beard\(^1\) observed a lack of agreement on the management of traumatic-hyphema patients. Despite new therapeutic modalities and many studies in the intervening years, the same statement can still be made today. Problems associated with traumatic hyphema are well known. Many authors have noted the higher rate of complications and worse visual prognosis associated with secondary hemorrhage.\(^1,6,8-12\) For this reason the primary goals in the management of hyphema are the prevention of secondary hemorrhage and control of intraocular pressure.

Hyphemas occur as a result of damage to the vessels of the iris or ciliary body after a blunt, contusive injury to the eye.\(^10-12\) Major vision-threatening complications associated with hyphemas are glaucoma, corneal blood staining, and optic atrophy.

An anti-fibrinolytic agent, EACA has been shown to reduce the incidence of secondary hemorrhage significantly after systemic administration in humans and after topical administration in the rabbit model.\(^9-12,13,15,17,20\) Crouch and associates\(^10,12\) suggested that prevention of clot lysis and retraction allows the injured blood vessels to heal. The lack of widespread acceptance of systemic EACA is related to the incidence of serious adverse reactions including hypotension, dizziness, and nausea and vomiting.

**Table 1. EACA or CPM toxicity**

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<th>Percentage with epithelial defects or SPK</th>
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<tr>
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In this masked study, 15 rabbits with traumatized eyes were randomized into a group receiving 200 \(\mu\)l of 30% EACA in 2% CPM (treatment) and a group receiving 200 \(\mu\)l of CPM vehicle (placebo). Ten rabbits with normal eyes served as a control and were similarly randomized into “treatment” and “placebo” groups. Treatment was repeated every 12 hr for five days. All rabbits were pretreated with proparacaine and patched after treatment. SPK = superficial punctate keratopathy.

Because the drug is cleared by the kidneys, the dose must be adjusted in renal failure. Animal studies have shown possible teratogenic effects; therefore, use in pregnancy is not recommended. The search for an effective topicaly applied agent has been directed at increasing the penetration of EACA into the anterior chamber. In this study we optimized the formula parameters and dosing schedule of EACA in CPM vehicle. We attempted to establish a regimen that would provide therapeutic aqueous humor levels 12 hr after dosing.

Patient compliance improves if dosing intervals can be lengthened. Although patching the eye resulted in therapeutic aqueous humor EACA levels 12 hr after dosing, the development of epithelial defects even in untraumatized, normal eyes precludes the use of patching. We feel that a relative hypoxia is induced by the combination of patching and administration of the EACA preparation. Oxygen tension in the tear film falls from 155 mm Hg when the eye is open to 55 mm Hg when the eye is closed.\(^23\) A shift to anaerobic metabolism in the corneal epithelium may cause the accumulation of lactic acid or a toxic metabolite resulting in epithelial defects. In our previously reported studies using unpatched eyes, neither EACA or CPM produced epithelial damage.\(^20\)

Our study revealed that pretreatment with topical anesthetics results in significantly higher aqueous humor EACA levels compared with administration of EACA formula without pretreatment. In addition to their anesthetic effect, local anesthetics have been shown to disrupt corneal epithelium.\(^24\) In this study, disruption of the epithelium may have facilitated access of EACA to the aqueous humor.

A concentration of 30% EACA in 2% CPM vehicle resulted in the highest aqueous humor levels, and the levels increased as the dose increased. Higher concentrations of EACA, which did not increase aqueous humor levels, markedly changed the physical properties of the preparation resulting in a stiffer gel. The stiffer gel remained in the inferior sulcus and did not spread over the surface of the eye, thus decreasing the surface available for absorption. Similarly, increasing the concentration of the gel above 2% resulted in a stiff gel that did not spread, and penetration of EACA into the aqueous humor was similarly reduced.

In all trials plasma EACA levels were determined concurrently with aqueous humor levels and were nearly undetectable with aqueous humor levels. In addition, plasma EACA levels after topical administration were negligible compared with plasma levels measured after systemic administration.\(^14\)

On the basis of our above results, future studies will employ 30% EACA in 2% CPM applied every 6 hr to...
unpatched eyes. Topical anesthesia will be used to enhance EACA uptake.

Key words: aminocaproic acid (ACA), carboxypolymethylene (CPM), epsilon-aminocaproic acid (EACA), hyphema, secondary hemorrhage

References