Effect of Paracentesis Upon the Blood-Aqueous Barrier of Cynomolgus Monkeys

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Anterior chamber paracentesis disrupts the blood aqueous barrier (BAB) of rabbits and nonhuman primates, but the magnitude and duration of breakdown in monkeys has not been clarified. We have studied anterior chamber paracentesis in cynomolgus monkeys as a potential model of postoperative BAB breakdown. The effect of a single paracentesis upon fluorescein sodium concentration in the anterior chamber after an intravenous injection was measured in 16 eyes of 8 animals. In an additional 10 eyes of 5 animals, aqueous humor was withdrawn for analysis 24 hours and one week following paracentesis.

Anterior chamber fluorescein concentration was 57 ± 22 ng/ml (mean ± standard deviation) before paracentesis, rose to 81 ± 47 ng/ml 24 hrs after paracentesis, and was 60 ± 36 ng/ml at 72–96 hours. Twenty-four hours after paracentesis, total protein concentration was elevated, but ascorbic acid and transforming growth factor-β levels were not.

Paracentesis in monkeys has only a small and short lasting effect upon BAB integrity and is therefore unlikely to be a good model for assessing the effect of agents designed to stabilize the BAB. However, the short-lived effect of paracentesis may permit the repetitive collection of "primary aqueous" for physiologic and biochemical studies. Invest Ophthalmol Vis Sci 33:165-171, 1992

Breakdown of the blood aqueous barrier (BAB) occurs after intraocular surgery and may play an important role in the postoperative events that determine surgical outcome. Minimization of BAB breakdown after intraocular surgery is generally assumed to be desirable. Steroidal and nonsteroidal agents have been tested for their ability to reduce BAB breakdown after cataract surgery. The success of glaucoma filtration surgery depends upon the nature and magnitude of the postoperative wound healing response. This response may be influenced by the capability of the BAB to prevent blood-borne cells and molecules from gaining access to the interior of the eye.

Paracentesis of the anterior chamber has been used as a method for disrupting the BAB because it is a simple and reproducible technique. Although the effect of paracentesis upon the rabbit eye has been studied extensively, investigations in rabbits may only have limited applicability to the human situation because of the fragility of the BAB in rabbits.

The effect of paracentesis in nonhuman primates has been studied with primary emphasis upon the anatomical site of disruption of the BAB. Much less attention has been paid to quantifying the degree and duration of the disruption of the BAB. We have performed paracentesis in eyes of cynomolgus monkeys. The magnitude and duration of BAB breakdown has been quantified by monitoring the leakage of fluorescein sodium into the anterior chamber and by determining the concentrations of total protein, ascorbic acid, and transforming growth factor-β (TGF-β).

Methods

Animals

Thirteen male cynomolgus (Macaca fascicularis) monkeys, weighing between 5 and 7 kg, were used. Slit-lamp examination of the anterior segment was normal. All experiments were approved by the institutional animal care committee and were performed in accordance with the ARVO Resolution on the Use of Animals in Research. All animals survived these experiments and were used again in unrelated experiments.
Paracentesis

Sixteen eyes of 8 animals underwent a single paracentesis in conjunction with fluorophotometry. The procedure was as follows. After intramuscular ketamine hydrochloride (10 mg/kg) sedation, the animal was placed in a supine position and one drop of proparacaine hydrochloride was instilled in the operative eye. After a lid speculum was placed, a ½ inch 27 gauge needle on a tuberculin syringe was used to enter the temporal peripheral cornea just anterior to the limbus. The globe was stabilized with a forceps grasping the limbus 180° away from the entrance site. Once the upward-facing bevel had entirely entered the anterior chamber, approximately 100 μl of fluid was withdrawn within 2 seconds. The needle had no contact with the iris or lens and contacted the cornea only at the entrance site. The aqueous humor was immediately placed in 1.5 ml Eppendorf tubes and stored in the dark in a -70°C freezer.

Ten eyes of 5 other animals underwent 3 paracenteses to examine changes in aqueous humor composition. Intraocular pressure was first determined in both eyes with a calibrated pneumatonometer (Alcon, Ft. Worth, TX), and the anterior segment was examined with the slit lamp. Paracentesis was always performed in the right eye first. A second paracentesis was performed 24 hours after the first and a third 7 days after the second. After an interval of at least one week, this schedule was repeated in the left eyes. All paracenteses were performed between 8 and 10 a.m. The aqueous humor was immediately placed in 1.5 ml Eppendorf tubes and stored in the dark in a -70°C freezer.

Fluorophotometry

A Fluorotron master (Coherent Medical, Palo Alto, CA) with anterior segment adapter was used to measure anterior chamber fluorescence after the intravenous administration of 0.3 ml of 10% sodium fluorescein (IOLAB, Claremont, CA). This computerized scanning fluorophotometer measures fluorescence continuously at hundreds of depths of the cornea, anterior chamber, and lens. Data are expressed in units of ng/ml fluorescein, although some of the fluorescence may be due to metabolites of fluorescein. The fluorescence in the anterior chamber is readily identifiable and distinguishable from that present in the cornea and lens. A software program provided by Richard Brubaker, MD, allowed us to average the fluorescence measurements in a 1.25 mm thick band positioned in the center of the anterior chamber along the visual axis.

In preliminary experiments, fluorophotometry was performed in normal eyes that had not had surgical manipulation. Under intramuscular ketamine hydrochloride sedation supplemented with intravenous sodium pentobarbital (8 mg/kg), and after endotracheal intubation, scans were performed before the injection of sodium fluorescein and every 15 min for 90 min after injection. Before injection, only a negligible (<5 ng/ml) amount of fluorescein was detected in the anterior chamber. The amount of fluorescence in the anterior chamber increased for the first hour and was stable between 60 and 90 min, as expected.1,10 Thereafter, scans were performed only 90 minutes after fluorescein injection.

Eight animals, not used in the preliminary experiments, underwent anterior chamber paracentesis in the right eye as described above after 2 baseline determinations of the anterior chamber concentration of fluorescein. The 2 baseline determinations were performed at least 2 days apart, with the second done at least 2 days before the paracentesis. Sodium fluorescein was injected intravenously 24 hours and 72–96 hours after paracentesis, and anterior chamber fluorescence was determined. Previous experiments had demonstrated that negligible levels of fluorescence are detectable 48 hours after intravenous injection. At least one week later, the left eyes underwent the identical procedure.

In the first 4 of these 8 animals, intravenous sodium pentobarbital and endotracheal intubation were employed, and the animal remained under pentobarbital anesthesia for the duration of the experiment. We then realized that the same data could be obtained without pentobarbital and endotracheal intubation. Therefore, in the other 4 animals, intravenous fluorescein was administered after intramuscular ketamine injection, and a scan was obtained 90 min after injection with a supplemental dose of ketamine hydrochloride. The amount of fluorescence in the anterior chamber varied with the different anesthetic regimes (Fig. 1), so the effect of anesthesia upon fluorescein concentration and intraocular pressure was examined in one normal eye in each of 5 additional animals.

Protein Determination

Protein concentration was determined using the Bradford11 method adapted for use in 96-well plates. Volumes of aqueous humor analyzed varied from 5–20 μl. Absorbance was measured at 595 nm on a Biotek, Winooski VT plate reader.

Ascorbate Determination

Samples were thawed in the dark and 10 μl were injected directly into a C-8 reverse phase high pressure liquid chromatograph column using a pH 3.5
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Fig. 1. (A) Correlation between the two baseline (pre-paracentesis) measurements (r = 0.888, P = 0.003) from the right eyes. (B) Correlation between baseline measurements in the right and left eyes of the same animal (r = 0.978, P = 0.000). Solid rectangles represent eyes from animals receiving ketamine only; open rectangles represent animals receiving ketamine and pentobarbital.

citric acid buffer. Retention time was 1.8 min and absorbance at 235 nm was monitored.

TGF-β Determination

All samples were acid-activated so the total amount of TGF-β activity (active and latent) in the aqueous humor samples could be determined. TGF-β activity was determined in an assay based on inhibition of the growth of CCL-64 mink lung epithelial cells as described elsewhere. The sensitivity of the assay is 10–20 pg/ml TGF-β, ED₅₀. Although TGF-β3 is 4 times as active as TGF-β1 or TGF-β2 in this assay, we have previously shown that most of the TGF-β in human aqueous humor is TGF-β2.

Statistical Analysis

All data are presented as mean ± standard deviation. Values before and after paracentesis were compared by paired t-test. When there appeared to be no difference between two values, a calculation of the power of the sample size to detect a difference was made. Correlation of the fluorophotometry results between eyes and within the same eye was determined by linear regression analysis.

When both eyes of an individual patient or animal are considered independently, an unappreciated correlation between the 2 eyes may influence the data analysis. Therefore, we analyzed the data for the right and left eyes separately and for both eyes combined. Because the results were the same regardless of how the data were analyzed, we present the data for both eyes combined.

Results

Fluorophotometry in the Unaltered Eye

There was a high degree of correlation between the 2 baseline (pre-paracentesis) measurements (r = 0.888, P = 0.003) from the same eye and between baseline measurements (r = 0.978, P = 0.000) in the 2 eyes of the same animal (Fig. 1).

To determine the effect of 2 different methods of anesthesia upon fluorophotometry, 5 monkeys underwent fluorophotometry and IOP measurement with intramuscular ketamine hydrochloride alone and with intramuscular ketamine followed by intravenous sodium pentobarbital and endotracheal intubation. The fluorescein concentration was higher (86 ± 32 ng/ml) under ketamine and pentobarbital than under ketamine alone (38 ± 13 ng/ml, P = 0.02, t-test), although the IOP was no different (19.4 ± 2.3 vs. 20.2 ± 1.1, P = .338, respectively).

The 4 animals that underwent paracentesis and were anesthetized with sodium pentobarbital accompanied by endotracheal intubation for fluorophotometry also demonstrated a higher pre-paracentesis concentration of fluorescein (71 ± 12 ng/ml) in the anterior chamber than did the other 4 animals that received only ketamine hydrochloride (40 ± 5 ng/ml, P = .003, t-test) (Fig 1). The increase in fluorescein concentration 24 hours after paracentesis was similar for ketamine hydrochloride anesthesia alone (23 ng/ml, 56%) and for ketamine hydrochloride plus sodium pentobarbital (24 ng/ml, 31%).
**Effects of Paracentesis**

**Anterior chamber fluorescein concentration:** The mean anterior chamber fluorescence was higher 24 hours after paracentesis than before paracentesis and had returned to baseline 72–96 hours after paracentesis (Fig. 2). Fluorescein concentration in the contralateral eye was unchanged.

**Protein concentration:** The mean total protein concentration in the aqueous humor obtained from the initial paracentesis, second paracentesis (24 hours later), and third paracentesis (one week later) were $10.2 \pm 4.7$, $14.3 \pm 4.6$, and $10.4 \pm 4.4$ mg/dl, respectively. The increase in protein concentration at 24 hours was statistically significant ($P = 0.01$; Fig. 2), although no flare was detectable on slit-lamp examination.

**TGF-β** Total TGF-β concentration was 1.7 ng/ml before paracentesis and was unaltered by paracentesis (Fig. 3).

**Ascorbic acid:** Ascorbic acid concentration in the aqueous humor obtained during the initial paracentes...
Table 1. Intraocular pressure

<table>
<thead>
<tr>
<th>Eye</th>
<th>Baseline</th>
<th>1 Day after paracentesis</th>
<th>P† value</th>
<th>7 Days after paracentesis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD*</td>
<td>20.2 ± 1.5†</td>
<td>18.6 ± 1.3</td>
<td>0.04</td>
<td>17.4 ± 2.3</td>
<td>0.03</td>
</tr>
<tr>
<td>OS</td>
<td>18.2 ± 2.1</td>
<td>19.4 ± 3.5</td>
<td>0.34</td>
<td>16.0 ± 3.1</td>
<td>0.30</td>
</tr>
<tr>
<td>OU</td>
<td>19.2 ± 2.0</td>
<td>19.0 ± 2.5</td>
<td>0.79§</td>
<td>16.7 ± 2.7</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* N = 5.
† Mean ± SD.
§ Power >95% to detect a 3-mm difference.

Discussion

This study demonstrates that paracentesis in monkeys causes only a mild and short-lived breakdown of the BAB, as measured by slit-lamp examination, fluorophotometry, and aqueous humor composition. Although qualitative data from other studies suggest that the primate BAB is relatively resistant to disruption, only Kronfeld et al quantified the degree or duration of BAB breakdown. Their study involved a protocol that today is neither feasible nor desirable—repeated paracentesis in humans.

The resistance of the monkey BAB to paracentesis can be contrasted with the much greater degree of BAB breakdown that occurs in rabbits and cats. The fragility of the BAB in these species has led some investigators to question their relevance to human physiology. The degree of BAB breakdown in monkeys is similar to that found in humans after paracentesis.

Paracentesis results in a much shorter and milder breakdown of the BAB than cataract surgery with intraocular lens implantation.

We did not measure BAB breakdown earlier than 24 hours after paracentesis. Studies in rhesus monkeys have found 50–100 fold higher aqueous humor protein concentrations 15 minutes and 60 minutes after paracentesis than we found at 24 hours. Thus the marked increase in protein concentration soon after paracentesis appears to resolve with striking rapidity, a decrease that may be associated with normalization of the IOP. We did not observe an increase in fluorescein concentration in the contralateral eye, a finding reported after implant surgery in humans.

The anatomical site of BAB breakdown in nonhuman primates continues to be debated. Raviola concluded that the protein appearing in the anterior chamber after paracentesis was the result of backflow from Schlemm’s canal into the aqueous humor. Other investigators have reported marked anatomic alterations in the ciliary epithelium of the anterior pars plicata. Physiologic studies also implicate the ciliary epithelium as a source of protein and fluorescein leakage.

We chose to study the effect of paracentesis upon ascorbic acid and TGF-β concentrations. Ascorbic acid concentration in the aqueous humor of rabbits decreases following topical application of prostaglandin E2 and after intravitreal injection of bovine serum albumin or bacterial endotoxin. Not only can aqueous humor ascorbic acid concentration be used as a measure of BAB integrity, but it also may play a potential role in wound healing after ocular surgery.

TGF-β is a polypeptide important in wound healing that has been detected in aqueous humor. Altered levels of TGF-β might influence the effect of aqueous humor upon wound healing. Paracentesis had no effect upon aqueous humor levels of ascorbic acid or TGF-β at 24 hours, which correlates with the relatively small changes in fluorophotometry and total protein concentration.

Radius et al performed paracenteses 2 days apart in rhesus monkeys. They found that the aqueous humor from the second, but not the first paracentesis supported the outgrowth of fibroblasts from explants of Tenon’s capsule. In fact, the aqueous humor from the second paracentesis supported outgrowth as well as aqueous humor obtained after a much more invasive procedure—glaucoma filtration surgery. Our data fail to offer an explanation for the growth-enhancing properties of their post-paracentesis aqueous humor. Perhaps paracentesis causes a change in the concentration of a growth factor we have not assayed, such as basic fibroblast growth factor, or perhaps there are species differences.

In experimental situations in which protein concentrations are high, the protein level can influence the measurement of fluorescence emitted from solutions of sodium fluorescein. The small amount of protein in our samples, even after paracentesis, is unlikely to have substantially altered the fluorophotometric data.
Because the BAB differs for each serum component, the study of BAB integrity may involve the examination of many substances. Our study required an intraocular procedure to analyze aqueous humor composition. The advent of the laser flare-cell meter and the measurement of the polarization of fluorescence may permit the noninvasive determination of aqueous humor protein concentration. More sophisticated fluorophotometric techniques may allow the detection of other substances in the aqueous humor. Such technological advances would make studies such as ours easier to perform.

The leakiness of the BAB to fluorescein was greater when the animals received pentobarbital anesthesia. This was not an IOP effect because the IOP was the same regardless of anesthetic. We did not explore whether an alteration in systemic blood pressure or other systemic parameter accounted for this observation.

We had hoped to use anterior chamber paracentesis as a simple and reproducible method of disrupting the BAB with the goal of testing interventions designed to reduce its duration and magnitude. The surprisingly small degree of BAB breakdown leaves little room to demonstrate a decrease in BAB breakdown. A relevant experimental model of BAB breakdown in monkeys will require producing a much larger effect.

The short-lived nature of BAB breakdown after paracentesis raises the possibility that repeated paracenteses could be performed without altering the biochemical composition or physiologic properties of the aqueous humor. Because the scarcity of "primary" aqueous humor from primates is a significant barrier to its study, our results could open the way to obtaining much larger quantities for analysis and experimentation.

Key words: paracentesis, blood-aqueous barrier, nonhuman primates, fluorophotometry, protein determination

Acknowledgments

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References