Measurement of Blood–Retinal Barrier Breakdown in Endotoxin-Induced Endophthalmitis

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Purpose. Endophthalmitis is a severe inflammatory disorder with profound visual consequences. Treatment of this disorder has been limited by the lack of quantitative information regarding retinal responses to severe inflammation. The purpose of this study was to measure the effect of endotoxin-induced endophthalmitis on blood–retinal barrier (BRB) function in vivo using contrast-enhanced magnetic resonance imaging (MRI).

Methods. Endophthalmitis was produced by injecting Escherichia coli endotoxin into the mid-vitreous of pigmented rabbits. Contrast-enhanced MRI was performed at selected intervals thereafter. In all cases, a clinical grading system was used to assess the severity of inflammation before imaging. In a dose-response experiment, total vitreous protein was measured from vitreous specimens obtained 1 day after endotoxin injection and immediately after the imaging procedure.

Results. At 1 day after injection, endotoxin produced a selective breakdown of the inner BRB at all doses evaluated (0.01 μg to 500 μg). Permeability–surface area product normalized to the area of leaky retina (PS') increased from 1.35 ± 0.78 × 10⁻⁴ cm/minute (mean ± SEM, n = 4 eyes) at a dose of 0.01 μg to 8.15 ± 2.22 × 10⁻⁴ cm/minute (n = 4 eyes) at a dose of 10 μg. Inner BRB integrity was restored by day 28 after injection. In general, changes in PS', blood–aqueous barrier leakage, mean clinical score, and vitreous protein concentration were found, but the correlation between any two of these parameters was poor.

Conclusion. Leakage of contrast appears early in the course of endotoxin-induced endophthalmitis and is a self-limited process. In future studies, these quantifiable changes in BRB permeability should prove useful in the assessment of various therapeutic interventions.
One promising approach to the measurement of BRB permeability in endophthalmitis is contrast-enhanced magnetic resonance imaging (MRI). This non-invasive technique can be used to measure directly and accurately the permeability surface area product (PS), spatial extent, and location of the disrupted BRB even in the presence of media opacities.\textsuperscript{12,13} The MRI technique measures the change in the water signal intensity produced as a contrast agent (for example, gadolinium diethylenetriaminepentaacetic acid [Gd-DTPA]) enters the vitreous space.\textsuperscript{14} In the rabbit model, it is also possible to distinguish inner from outer BRB breakdown in vivo.\textsuperscript{15}

In this study, BRB breakdown was measured using contrast-enhanced MRI in an established model of ocular inflammation produced by a single intravitreal injection of bacterial endotoxin in the rabbit eye. The purpose of these studies was to establish the relationship between endotoxin dose and BRB permeability and to examine the time course of BRB breakdown. Because the preretal blood vessels in the rabbit eye are confined to a thin strip of superior retina and the remainder is avascular, the site of BRB breakdown (i.e., inner versus outer BRB) could also be identified. To search for possible correlations with BRB permeability measurements, blood-aqueous barrier leakage (an MRI-based parameter) and vitreous protein concentrations were measured. In addition, clinical observations of tissue responses to inflammation were measured using an established grading system that was developed for a rabbit model of bacterial endophthalmitis.

MATERIALS AND METHODS

Animal Preparation

Fourteen Dutch-belt pigmented rabbits weighing 2 to 2.5 kg each were used in the study, which was conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. For all procedures, anesthesia was induced with intramuscular ketamine HCl (35 mg/kg) and xylazine HCl (2 to 4 mg/kg). Pupils were dilated with one drop each of 2.5% neosynephrine and 1% tropicamide. During prolonged procedures (see Magnetic Resonance Imaging), anesthesia was maintained with continuous intravenous infusions of ketamine HCl (20 to 40 mg/kg per hour) and xylazine HCl (2 to 4 mg/kg per hour) through an auricular vein.\textsuperscript{16} Systemic blood pressure and heart rate were monitored through an auricular arterial catheter.\textsuperscript{13} Each animal was intubated and ventilated with a Harvard (South Natick, MA) ventilator. Blood gas monitoring was performed periodically using an AVL 995 blood-gas machine (AVL, Roswell, GA), and the core body temperature of the animal was maintained at 37°C to 38°C through a circulating water blanket connected to a constant temperature bath. When required, euthanasia was performed under deep anesthesia with an intravenous injection of saturated potassium chloride solution.

Endotoxin solutions were prepared by dissolving lyophilized lipopolysaccharide (Escherichia coli, serotype 055:B5) (Sigma, St. Louis, MO) in Balanced Salt Solution (BSS; Alcon, Fort Worth, TX), which was then filter-sterilized by passage through a 0.22-μm filter unit. The volume of injection was kept constant (50 μl). The mid-vitreous of one eye of each animal was injected as follows: 0.01 μg (n = 4 eyes); 0.1 μg (n = 9 eyes); 1 μg (n = 12 eyes); 10 μg (n = 4 eyes); 500 μg (n = 1 eye); balanced salt solution alone (n = 4 eyes). Endotoxin injection was performed by trans-scleral passage of a sterile 30-gauge needle and observation of the needle tip in the mid-vitreous through the dilated pupil. Eyes were examined immediately after injection by indirect ophthalmoscopy to ensure that increased intraocular pressure had not interrupted the blood supply to the retina and choroid.

Experimental Groups

Dose–Response Group. Twenty-one eyes in this group were imaged at 24 hours after intravitreal injection of endotoxin. Thereafter, these animals were killed. For each dose, n = 4 (except the 500 μg dose, where n = 1). The vitreous of all eyes other than the eye that received 500 μg of endotoxin was salvaged for protein determination (see Vitreous Protein Determinations).

Time-Course Group. Eyes in this group received either 0.1 μg (five eyes) or 1 μg (eight eyes) of endotoxin. These animals were imaged at 24 hours and on days 3, 7, 14, and 28 after endotoxin injection.

Magnetic Resonance Imaging

Magnetic resonance imaging was performed on an 0.5T Super Toshiba machine (Toshiba, San Francisco, CA) using a 10-cm surface coil positioned over both orbits. The field of view was 10 cm, and slice thickness measured 3.5 mm with a 0.7-mm gap; Echo Time (TE) = 15 msec, and Repetition Time (TR) = 400 msec. Each image required 6.9 minutes to acquire. The animal was positioned so that a single coronal slice could be chosen through the center of both eyes oriented perpendicularly to the medullary ray. The imaging procedure involved acquiring a control image before the intravenous injection of 1.0 mmol/kg of Gd-DTPA (Magnevist; Berlex Labs, Wayne, NJ) which was injected over 5 minutes. Three serial images were then acquired after injection.

Image Analysis

Images collected on the Toshiba magnet were transferred to a data station and analyzed on a Macintosh
Ilci (Apple, Elk Grove, CA) using Image 1.50 application software (NIH, Baltimore, MD). The program contained user-modified subroutines to expedite image registration and analysis of permeabilities. For each image, a region of interest in the vitreous that completely surrounded the area of enhancement was selected, and the mean signal intensity was measured. The permeability surface area normalized to the area of leaky retina (PS') was then calculated as described.12,14 Time between injection and imaging was calculated from the center of injection to the center of image acquisition. PS' values derived from enhancements of 30% or less were not used because they are not as reliable as those derived from greater enhancements.14 These eyes, as well as eyes with no measurable leakage, were analyzed using the temporal correlation method, VISIBLE, of Wood et al,17 which was developed to detect subtle intensity changes if leakage is not readily apparent on an image after injection.

Integrity of the blood–aqueous barrier was also assessed from the magnetic resonance images. Leakage was compared between eyes by measuring the rate of anterior chamber enhancement during the MRI experiment (a time interval of approximately 25 minutes). The slope of enhancement was then normalized to the initial signal intensity on the precontrast image. This provided a measure of the relative rate of Gd–DTPA leakage, λ, which is expressed in the units of minutes-1.18

Changes in protein concentration that accompany inflammation might influence the relaxivity (R1) of Gd–DTPA in water, thus causing error in PS' and λ determinations. However, the R1 used in this study has an estimated error of 10% to 20%.12 To determine how protein concentrations changes might affect the measurements, the relaxivity of albumin in cerebrospinal fluid at 100 MHz and 37°C (0.0015 s⁻¹/g·l) was considered. Based on this information, a maximum

Table 1. Clinical Grading System*5

<table>
<thead>
<tr>
<th>Tissue Response</th>
<th>Points</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea Transparency</td>
<td>Clear</td>
<td>Mild</td>
<td>Moderate (iris visible)</td>
<td>Severe (bare iris detail)</td>
<td>Opaque (no iris view)</td>
<td></td>
</tr>
<tr>
<td>Vessels</td>
<td>None</td>
<td>&lt;1 mm</td>
<td>1 to 3 mm from limbus</td>
<td>3 to 4 mm from limbus</td>
<td>≥5 mm</td>
<td></td>
</tr>
<tr>
<td>Abcess</td>
<td>None</td>
<td>&lt;1 mm</td>
<td>1 to 2 mm</td>
<td>3 to 4 mm</td>
<td>≥5 mm</td>
<td></td>
</tr>
<tr>
<td>Anterior Chamber</td>
<td>Protein flare</td>
<td>None</td>
<td>Trace</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>None</td>
<td>Trace</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td></td>
</tr>
<tr>
<td>Fibrin/hypopyon</td>
<td>None</td>
<td>Mild strands</td>
<td>Moderate strands</td>
<td>Severe strands &gt;15%</td>
<td>No iris detail</td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>None</td>
<td>Mild</td>
<td>Moderate</td>
<td>≥50%</td>
<td>8-ball</td>
<td></td>
</tr>
<tr>
<td>Iris</td>
<td>Blood vessels</td>
<td>None</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
<td>Neovascular</td>
</tr>
<tr>
<td>Vitreous</td>
<td>Protein flare</td>
<td>None</td>
<td>Trace</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Opacities</td>
<td>None</td>
<td>Mild</td>
<td>Multiple clumps</td>
<td>Red reflex</td>
<td>Totally opaque</td>
<td></td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>None</td>
<td>25%</td>
<td>50%</td>
<td>75%</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Optical media</td>
<td>Clear</td>
<td>View of vessel outline only</td>
<td>Sharp red reflex</td>
<td>Dull red reflex</td>
<td>Totally opaque</td>
<td></td>
</tr>
</tbody>
</table>

* Clinical score is sum of points in each category. Note: If cornea is opaque, add 20 points; if anterior chamber is opaque, add 15 points. Adapted from Meredith TA, Trabelsi A, Miller MJ, Aguilar E, Wilson LA. Spontaneous sterilization in experimental Staphylococcus epidermidis endophthalmitis. Invest Ophthalmol Vis Sci. 1990;31:181–186.
FIGURE 2. Representative magnetic resonance images of dose-response eyes acquired 1 day after intravitreal injection and at approximately 25 minutes after intravenous injection of Gd-DTPA (1.0 mmol/kg). A control eye (A) and eyes that received endotoxin doses of 0.01 µg (B), 0.1 µg (C), 1 µg (D), and 10 µg (E) are shown. Note the absence of vitreous enhancement in the control eye and increasing vitreous enhancement in endotoxin-injected eyes as a function of dose. The location of leakage in these images is restricted to the region of vitreous overlying the medullary ray (arrow, C), thus representing inner BRB breakdown. No evidence of outer BRB breakdown is observed. In addition, the anterior chamber and vitreous adjacent to the ciliary body in endotoxin-injected eyes show greater enhancements than in the control eye, indicating the presence of blood-aqueous barrier breakdown.
Clinical Grading

Before each imaging procedure, eyes were examined using a portable slit lamp. Indirect ophthalmoscopy was then performed. A clinical grading score (Table 1) was assigned to each eye using the system described by Meredith et al. The observer was masked to the dose of endotoxin in the dose-response group during clinical grading.

Vitreous Protein Determinations

Eyes were enucleated immediately after death. The sclera was quickly incised 3 mm posterior to the limbus, and the vitreous was aspirated as completely as possible into a plastic syringe. The vitreous was immediately frozen and stored at −20°C. Excised frozen vitreous was later thawed, to which 0.4 ml of cold, distilled water was added and gently vortexed. Triplicate 100-μl samples were taken for protein assay. Protein determinations were carried out using the Bio-Rad (Hercules, CA) protein assay method with bovine serum albumin as a standard.

Fundus Photography and Fluorescein Angiography

Parallel studies were performed in which two additional eyes were injected with 0.1 μg of intravitreal endotoxin. This dose of endotoxin was thought to be low enough to allow fundus photography based on experience with the DR group. Photography and angiography were performed before injection of endotoxin and on days 3 and 7 after injection. Red-free photographs and angiograms were obtained using a Topcon TRC-50X 35 mm fundus camera (Topcon, Paramus, NJ). Angiograms were performed after injection of 0.2 ml of sodium fluorescein (Fluorescite; Alcon Laboratories, Fort Worth, TX) into an auricular vein.

Statistical Analyses

Statistical analyses were performed using either paired or unpaired Student’s t-tests. Analysis of variance was not performed.

TABLE 2. Total Vitreous Protein on Day 1 After Intravitreal Injection of Endotoxin

<table>
<thead>
<tr>
<th>Dose (μg)</th>
<th>Protein (mg/ml) (mean ± SEM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0†</td>
<td>0.735 ± 0.057§</td>
</tr>
<tr>
<td>0.01</td>
<td>1.45 ± 0.25</td>
</tr>
<tr>
<td>0.1</td>
<td>1.09 ± 0.031§</td>
</tr>
<tr>
<td>1</td>
<td>1.56 ± 0.176§</td>
</tr>
<tr>
<td>10</td>
<td>1.78 ± 0.20§</td>
</tr>
</tbody>
</table>

* n = 4 eyes in each group.
† Balanced Salt Solution-injected eyes.
§ Significantly different from all other groups, unpaired t-test.
§ Significantly different from each other, unpaired t-test.
TABLE 3. Anterior Chamber Gd-DTPA Leakage After Endotoxin Injection

I. Dose-Response Group*

<table>
<thead>
<tr>
<th>Endotoxin Dose (µg)</th>
<th>0†</th>
<th>0.01</th>
<th>0.1</th>
<th>1</th>
<th>10</th>
<th>All doses ≥ 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ × 10⁻² min⁻¹ (mean ± SEM)</td>
<td>5.19 ± 1.25</td>
<td>8.50 ± 0.99</td>
<td>7.69 ± 0.49</td>
<td>10.0 ± 1.09‡</td>
<td>8.22 ± 1.26</td>
<td>8.61 ± 0.50§</td>
</tr>
<tr>
<td>Number of eyes</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>16</td>
</tr>
</tbody>
</table>

* Values obtained 1 day after endotoxin injection.
† BSS-injected eyes.
‡ P = 0.026; §P = 0.009.

II. Time-Course Group

A. High-Dose (1 µg) Group

<table>
<thead>
<tr>
<th>Time After Endotoxin Injection (days)</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ × 10⁻² min⁻¹ (mean ± SEM)</td>
<td>9.67 ± 0.86</td>
<td>9.42 ± 0.90</td>
<td>9.04 ± 0.88</td>
<td>6.67 ± 1.85</td>
<td>3.07 ± 0.45*</td>
</tr>
<tr>
<td>Number of eyes</td>
<td>7†</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

*P < 0.05 compared to all other days.
† n = 8 for PS' and clinical scores; λ could not be analyzed in 1 eye.

B. Low-Dose (0.1 µg) Group

<table>
<thead>
<tr>
<th>Time After Endotoxin Injection (days)</th>
<th>1</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ × 10⁻² min⁻¹ (mean ± SEM)</td>
<td>8.22 ± 1.07</td>
<td>6.73 ± 0.91</td>
<td>6.91 ± 1.27</td>
<td>5.84 ± 1.67</td>
<td>5.72 ± 0.93</td>
</tr>
<tr>
<td>Number of eyes</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

used to assess correlation, and the Newman–Keuls test was used for multiple comparison. In all tests, P < 0.05 signified statistical significance.

RESULTS

Dose–Response Group

Clinical Findings. Figure 1 shows the clinical grading for animals in the dose–response group. All doses of endotoxin produced a clinical score that was significantly greater than zero (P < 0.05). In contrast, clinical grading scores for control eyes were zero in all instances. Differences in clinical grading scores between the three lowest dose groups were not statistically significant; however, a significant difference was noted between the score obtained for the highest dose group (10 µg) and the three lower dose groups.

Blood–Retinal Barrier Integrity. In all eyes that received endotoxin, Gd–DTPA leakage into the vitreous was confined to the inner BRB irrespective of the dose administered. No leakage was found over the inferior avascular retina, which indicated that the outer BRB remained intact. This was confirmed using VISIBLE.17

Figure 2 illustrates representative images obtained for each dose of endotoxin administered.

Figure 1 shows that PS' increased with dose of endotoxin in dose–response eyes. The mean PS' at the lowest dose (0.01 µg) was 1.35 ± 0.78 × 10⁻⁴ cm/minute, which was not significantly different from zero (P = 0.18). The only significant increase in mean PS' occurred between the lowest dose (0.01 µg) and the next higher dose (0.1 µg), at which the PS' (5.07 ± 0.90 × 10⁻⁴ cm/minute) became significantly different from zero (P = 0.011). The mean PS' in the lowest dose group was significantly lower (P < 0.05) than in all other dose groups. No significant differences were found between the mean PS' of any two groups that received an endotoxin dose of 0.1 µg or higher.

Although PS' was positively correlated with the clinical scores (P = 0.009; r² = 0.47), the shape of the two dose–response curves appeared different. As shown in Figure 1, the clinical scores appeared most sensitive to an increase in dose from 1.0 to 10 µg, whereas the PS' appeared most sensitive to a change in dose from 0.01 µg to 0.1 µg. In the eye that received 500 µg of endotoxin (not shown in Fig. 1), the PS'
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FIGURE 4. Clinical scores (mean ± SEM) for eyes in the time-course experiment. The number of eyes averaged per data point is given in Table 3-II. Eyes injected with 1 μg (dashed line) or 0.1 μg (solid line) of endotoxin showed the predicted course of inflammatory response and resolution. At all times after injection, the higher dose yielded mean clinical scores that were significantly larger (P < 0.05) than those produced by the lower dose. At 28 days after injection, the mean clinical scores of the high-dose group remained significantly greater than zero (P < 0.001). Scores obtained in 1-μg eyes were significantly different on any two days (P < 0.05). *Significantly different from day 3 (P < 0.05).

was comparable (9.6 × 10⁻⁴ cm/minute) to values obtained in the 10-μg group, suggesting a saturation phenomenon.

Vitreous Protein Concentrations. Table 2 shows the mean vitreous protein concentrations for endotoxin-injected dose–response eyes. All doses of endotoxin produced vitreous protein concentrations that were significantly greater (P < 0.05) than the control group. A significant correlation (P = 0.0007; r² = 0.48) was demonstrated between vitreous protein concentration and clinical scoring. In contrast, no significant correlation (P = 0.1325; r² = 0.12) was noted between PS' and vitreous protein levels.

Blood–Aqueous Barrier. In the dose–response group, the relative rates of Gd–DTPA leakage into the anterior chambers are summarized in Table 3-I. The mean leakage parameter (λ) pooled from all endotoxin-injected eyes was increased (8.6 ± 0.5 × 10⁻² minutes⁻¹; mean ± SEM; n = 16) compared to control eyes (5.2 ± 1.2 × 10⁻² minutes⁻¹; n = 4; P = 0.009). However, there were no significant differences between any two doses of endotoxin used. There was no significant correlation between λ and PS', clinical score, or vitreous protein concentration.

Time-Course Group

Clinical Findings. Figure 4 illustrates the mean clinical grading scores for time-course eyes. Peak clinical scores (maximum score, 30 points) occurred on days 1 (1.0 μg dose) and 3 (0.1 μg dose) and averaged 17.7 ± 1.95 (mean ± SEM, n = 8) and 8.5 ± 1.32 (n = 5), respectively. A subsequent rapid decrease in score was observed over the first 8 days after injection, followed by a more gradual decline. The mean clinical scores remained significantly greater than zero (P < 0.05) on all days of observation in both dose groups, except for day 28 in the lower dose (0.1 μg) group.

Blood–Retinal Barrier Integrity. Figure 5 illustrates the change in PS' over time in time-course eyes at the two doses studied. Peak PS' was found on day 1 and averaged 4.86 ± 0.61 × 10⁻⁴ cm/minute (mean ± SEM; n = 5) for the 0.1 μg-dose, and 7.0 ± 0.80 × 10⁻⁴ cm/minute (n = 8) for the 1 μg-dose. These values were not significantly different from comparable values obtained in the dose–response experiments. The PS' progressively declined over time for both high and low doses of endotoxin. Using analysis of variance tables, the change in PS' over time in the 1-μg eyes was statistically significant (P = 0.0073, r² = 0.954). The time course of change in PS' was more variable in the 0.1-μg group; therefore, no similar correlation was found. However, both groups decreased to a PS' of zero on day 28 after injection.

Fluorescein Angiographic Findings. A representative fluorescein angiogram performed on day 3 after the administration of 0.1 μg of intravitreal endotoxin is shown in Figure 3. Progressive hyperfluorescence was noted over the medullary ray in all cases. No hyperfluorescent areas were seen in other portions of the fundus, but not all inferior areas were visible through the vitreous exudate.

Blood–Aqueous Barrier. In the time-course study, there was a gradual decrease in λ over 28 days of observation for both doses studied (Table 3-II). There was no significant correlation found between λ and PS' or clinical score within any group.

DISCUSSION

This study describes the dynamic behavior of the BRB in a well-established model of endotoxin-induced endophthalmitis, as revealed by contrast-enhanced MRI. Retinal leakage of contrast was limited to the inner BRB in all eyes. It was greatest on day 1 after endotoxin injection (the first time the animals were imaged) and then decreased, but it persisted through day 14 after injection. By day 28, there was no detectable leakage. The severity of leakage was dose dependent, but the largest increase in permeability occurred between doses of 0.01 μg and 1.0 μg. Further increases in dose produced little or no increase in permeability.

The time course of blood–aqueous barrier breakdown was comparable to the findings of other investigators who examined markers of acute inflammation in the aqueous in this or similar animal mod-
FIGURE 5. PS' (mean ± SEM) for eyes in the time-course experiment. In general, PS' decreased with time after injection of 0.1 µg (solid line) or 1.0 µg (dashed line) of endotoxin. The number of eyes averaged per data point is given in Table 3.II. In general, PS' decreased in both groups over time after endotoxin injection. The two groups were significantly different (P = 0.048) on day 3 only. The variability seen in the lower dose group may have resulted from the low number of eyes studied on day 7 and later. Note that unlike the clinical scores, PS' in both groups reached a mean of zero on day 28. *Significantly (P < 0.05) greater than zero. †Significantly greater than zero (day 28).

Table 3-H. In general, PS' decreased in both groups over time after endotoxin injection. The number of eyes averaged per data point is given in Table 3.II. In general, PS' decreased in both groups over time after endotoxin injection. The two groups were significantly different (P = 0.048) on day 3 only. The variability seen in the lower dose group may have resulted from the low number of eyes studied on day 7 and later. Note that unlike the clinical scores, PS' in both groups reached a mean of zero on day 28. *Significantly (P < 0.05) greater than zero. †Significantly greater than zero (day 28).

In general, PC decreased with time after injection of 0.1 µg (solid line) or 1.0 µg (dashed line) of endotoxin. The number of eyes averaged per data point is given in Table 3.II. In general, PC decreased in both groups over time after endotoxin injection. The two groups were significantly different (P = 0.048) on day 3 only. The variability seen in the lower dose group may have resulted from the low number of eyes studied on day 7 and later. Note that unlike the clinical scores, PC in both groups reached a mean of zero on day 28. *Significantly (P < 0.05) greater than zero. †Significantly greater than zero (day 28).

For example, McGahan and Fleisher 25 found that the level of aqueous inflammatory infiltrates and myeloperoxidase activity varied in a dose-dependent fashion in the endotoxin-injected rabbit eye. Aqueous protein concentration behaved in a similar manner. They did not examine the effect of endotoxin on BRB integrity. In the present study, permeability increased by a factor of 3.8 between the lowest two doses used: 0.01 µg and 0.1 µg. An increase in dose from 0.1 µg and 10 µg produced only a 1.5-fold rise in PS', which suggests a saturation effect at the higher doses. Blood–aqueous barrier leakage increased with dose, but a dose–response relationship was not observed as clearly as BRB permeability.

Clinical assessment demonstrated poorer dose dependency than did PS' in this study. Clinical scores were significantly, though weakly, correlated (r² = 0.47) with PS' values in the dose–response group. One reason for the weakness in the correlation of these two variables is apparent in Figure 1. Mean clinical scores remained relatively unchanged over a dose range that produced the largest change in PS' (0.01 µg to 1.0 µg). Between the higher doses of endotoxin (1.0 µg and 10 µg), there was a disproportionate increase in mean clinical score. This phenomenon is probably caused by a systemic error in the grading system that requires the addition of a modifier of the full quota of points (+15) to compensate for heavy fibrin and inflammatory exudate in the anterior chamber (Table 1). Because this occurred mainly at the highest endotoxin dose, the mean score of the group was significantly higher than it was in the other groups that received lower doses.

In the time-course study, mean clinical scores decreased with time as did the mean PS'. However, unlike PS', the clinical scores never reached zero by day 28. It is likely that long-term changes in the appearance of ocular tissues account for this finding. The vitreous continued to have decreased clarity at the end of the time course, but this was not necessarily representative of ongoing inflammatory activity. Therefore, in a self-limited inflammatory process, we would predict decreases in the correlation between PS' and clinical scores as the period of observation increases, as long as structural changes interpreted as signs of inflammatory activity persist.

Unlike clinical assessment, no correlation was found between vitreous protein concentration and PS', although protein concentrations were similar to those measured by other investigators in endotoxin-injected rabbit eyes. For example, McGahan 20 showed a mean vitreous protein concentration of 1.66 mg/ml at 24 hours after intravitreal injection of 0.1 µg of E. coli endotoxin, compared to 0.33 ± 0.063 mg/ml in control eyes. In her study, she followed vitreous protein concentrations over time and found that peak concentrations occurred on the 7th day after injection, at which time a 3.3-fold increase over control values was observed. The low concentration of protein on day 1, combined with inherent variability of all protein assays, could have resulted in the poor correlation between protein concentration and permeability in the present study.

The time course of protein accumulation in the endotoxin-injected rabbit eye 20 points to another difficulty in comparing vitreous protein concentrations with real-time permeability values. Mean PS' in the present study decreased between days 1 and 7. Therefore, it appears likely that the cumulative measure of protein concentration cannot be quantitatively related to PS' at 1 day or longer after endotoxin injection.
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without a knowledge of PS' at all preceding time points. Other factors that relate to vitreous protein concentration also could impact on the comparison with PS'. First, total vitreous protein concentrations may be affected by factors other than BRB breakdown, such as cellular synthesis or release of intracellular proteins from damaged cells. Second, there may be a differential effect of endotoxin on BRB permeability to molecules of dissimilar sizes. The difference in molecular weight between Gd-DTPA (MWt 550) and proteins normally excluded from the vitreous by an intact BRB (such as albumin, MWt 66,290) is considerable. This problem could potentially be overcome by using high molecular weight contrast agents. Third, because BRB breakdown is a focal process in this model, protein accumulation is also likely to occur focally in the vitreous. The relationship between whole vitreous protein concentration and the concentration of protein overlying the site of leakage is not known.

To our knowledge, the present study is the first to report direct, noninvasive, in vivo measurement of BRB breakdown in a model of endophthalmitis. Cheng et al. performed proton magnetic resonance spectroscopy on vitreous supernatants of rabbit eyes with Streptococcus pneumoniae endophthalmitis and demonstrated an increased methyl resonance signal intensity that they suggested was an indirect marker of BRB breakdown caused by entry of lipoproteins into the vitreous. However, it appeared late in the time course (day 2 after introduction of the bacteria). In addition, several of the problems associated with changes in vitreous protein concentration (vide supra) apply to indirect measurement of lipoproteins in the vitreous as well.

In summary, contrast-enhanced MRI was used in this study to provide specific information regarding the location and extent of the BRB involved at the time of examination. We speculate that with this type of information, it may be possible to predict the severity of other aspects of retinal involvement (that is, visual loss) or the likelihood of response to drugs that poorly penetrate the intact BRB. In the latter case, the temporal profile of changes in BRB permeability in this model should prove useful as a baseline for the evaluation of various therapeutic interventions. Such studies in this model and other models of bacterial endophthalmitis are under way in our laboratory.

Key Words: blood–retinal barrier, blood–aqueous barrier, endophthalmitis, magnetic resonance imaging, endotoxin, rabbit eye

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