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LABORATORY INVESTIGATION

Minimum Endotoxin Concentration Causing Inflammation in the Anterior Segment of Rabbit Eyes

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Abstract

Purpose: This was a quantitative study to investigate the minimum endotoxin concentration causing inflammation in the anterior segment of the eye.

Methods: Endotoxin was injected intracamerally in pigmented rabbits. A quantitative determination of flare and cells in the aqueous was performed using a laser flare-cell photometer, before and until 72 h after the treatment. An area under the curve (AUC) analysis was employed to evaluate the whole inflammatory reaction regarding flare values.

Results: The time course of flare values in each endotoxin group showed a similar pattern with a peak value at 3 h. An AUC corresponding to values for "average +2σ", 19301.8 in control eyes, was considered the cutoff value. Using this cutoff value and the regression curve in endotoxin-treated groups, the minimum endotoxin concentration causing inflammation regarding flare values was determined to be 0.60 endotoxin units (EU). Cell counts (cells/0.5 mm^3·0.5 s) corresponding to the value "average +2σ", 6.07 at 24 h, in control eyes was considered to be the cutoff value. The minimum endotoxin concentration regarding cell counts was determined to be 0.23 EU.

Conclusion: There was a dissociation in response between flare and cells in the aqueous to intracameral endotoxin. The minimum endotoxin concentration causing inflammation ranged between 0.23 and 0.60 EU. **Jpn J Ophthalmol** 2009;53:425–432 © Japanese Ophthalmological Society 2009

Key Words: anterior segment, endotoxin limit, inflammation, laser flare-cell photometer, quantitative evaluation

Introduction

Cataract surgery has advanced greatly, not only with regard to surgical procedures[1] and improved biocompatibility of intraocular lenses[2,3] but also with regard to pharmacological treatment,[4,5] resulting in minimal postoperative inflammation and contributing to an improved quality of life through restoration of good vision. An ophthalmic viscosurgical device (OVD) including hyaluronic acid has played an important role in these developments. Hyaluronic acid is either derived from chicken crest or biosynthesized using bacteria. Not only OVD but also all surgical products should be free from febrile substances, which cause intraocular inflammation. Endotoxins are major toxic substances and candidates for causative substances in early postoperative inflammatory disorders such as toxic anterior segment syndrome.[6–9] In most studies that investigate the effect of endotoxin concentration on inflammation of the anterior segment of the eye, the endotoxin was injected either into the vitreous cavity[10–14] or systemically,[15] and inflammation was evaluated qualitatively either by slit-lamp microscopy or by histopathological methods.[16,17] Therefore, the effect of intracamerally introduced endotoxin on blood–aqueous barrier function could not be evaluated quantitatively. In the present study, intraocular inflammation induced by an intracameral injection of an endotoxin derived from a

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bacterium was quantitatively evaluated to elucidate the minimum concentration causing a breakdown of the blood–aqueous barrier function.[18]

Methods

Preparation of Endotoxin Solution

USP reference standard endotoxin (RSE) was derived from *Escherichia coli* (Sera type O113; H10). Each vial contained 10 000 endotoxin units (EU) of *E. coli* endotoxin in a lyophilized powder form. The RSE in the vial was reconstituted with 5 ml endotoxin-free solution [*Limulus* amebocyte lysate (LAL) reagent water (LRW), Associates of Cape Cod, East Falmouth, MA, USA] following the manufacturer's instructions. Glass tubes and all other implements were certified to be endotoxin-free materials by the manufacturers.

Determination of Endotoxin Solution Concentration

The concentration of endotoxin solutions was determined with the light-scattering method.[19] A newly developed instrument (PA-200C, Kowa, Tokyo, Japan) was used to determine endotoxin concentrations by detection of aggregation reactions with an endotoxin reagent (LAL, ES-II single test, Wako, Osaka, Japan). This instrument is characterized by a wider dynamic range compared with the currently used endpoint chromogenic method (unpublished data).

Experimental Procedures

The study protocol was approved by the Institutional Review Board for Animal Experiment. Pigmented Dutch rabbits weighing around 2.0 kg were used. Seven endotoxin solutions of different concentrations were prepared from one vial of RSE as described above. Preparation of the primary endotoxin solutions was performed in the morning of the experiment day. One-milliliter aliquots of the primary solution were dispensed into five sterile, pyrogen-free borosilicate glass vials. The eyes received doses of 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, or 2.0 EU. Since the doses were delivered in 50-µl volumes, the corresponding concentrations of the endotoxin solution prepared for testing were 0.6, 1.25, 2.5, 5.0, 10.0, 20.0, and 40 EU/ml. Some of the prepared solutions were assayed with the light-scattering method to confirm the endotoxin concentration.

Four Dutch rabbits were randomly assigned to each dose group. Fifty microliters of endotoxin solution was intracamerally injected into one eye of each rabbit with an insulin hypodermic syringe with a 30G needle (Plastipack, Becton Dickinson Japan, Fukushima, Japan), and a sham procedure with 50 µl LRW solution was performed in the fellow eye. In each experiment session, two or three different dose groups were examined. As a preliminary study, 100 µl LRW solution was injected intracamerally ($n = 4$).

Eyes with the following conditions were excluded from the analysis: (1) eyes of rabbits in which the control eye showed extremely high flare values, even with no apparent complications during the treatment; (2) eyes with a marked aqueous loss and shallow anterior chamber after the intracameral injection; and (3) eyes in which flare values markedly increased again in the late phase.

After exclusion of some eyes, an additional experiment was performed so that each endotoxin unit group would contain four eyes, except for the 5.0 EU/ml treated group ($n = 3$).

Intracameral injection was performed under general anesthesia, achieved with an intramuscular injection of xylazine (0.02 mg/kg) (Rompun, Bayer, Leverkusen, Germany) and an intravenous injection of sodium pentobarbital (15 mg/kg) (Nembutal, Abbott Park, IL, USA), and topical anesthesia (benzoyl peroxide, Benoxyl, Santen, Osaka, Japan). The exact amount of endotoxin injected intracamerally was determined by the difference in syringe weight between pre- and post-injection, determined with a B310S balance (Sartorius, Göttingen, Germany). After the intracameral injection, antibiotic eye drops (levofloxacin, Cravit, Santen, Osaka, Japan) were applied.

Clinical Examinations

The treated eyes were evaluated by slit-lamp microscopy following laser flare-cell photometry (Laser Flare-Cell Photometer; FC-2000, Kowa) without anesthesia and in a dark room before and at 3, 6, 9, 24, 48, and 72 h after the treatment. The slit-lamp examination included evaluation of corneal findings, anterior chamber depth, flare and cells in the aqueous, iris involvement, and crystalline lens condition. The observations were graded on a scale of 0 to 4 for each finding according to the modified classification of Hogan et al.[20] Other findings, if noted, were also recorded.

Laser flare-cell photometry was performed under the mydriatic condition achieved by instillation of mydriatic eye drops (Mydrin P, Santen).[21] At least five measurements of flare were performed and their average and standard deviation were calculated.

Cell counts were also performed based on the manufacturer's instructions for the laser flare-cell photometer, and the reliability of the cell count data was confirmed by the following method. In the laser flare-cell photometry, the measurement window of the cell counting unit, 0.5 mm^3/0.5 s, was divided into 128 blocks to exclude noise contamination. Any block contaminated by light noise was automatically discarded, and the number of discarded blocks was displayed. Detected cell counts were converted to apparent cell counts/128 blocks (0.5 mm^3/0.5 s) by using the ratio of discarded blocks to the full 128 blocks. In the present study, if the number of blocks without noise contamination was <64 (= 0.25 mm^3), the result was discarded.

Statistical Analysis

The flare values and cell count data were analyzed by using the Mann-Whitney *U* test or Student *t* test. *P* values less than 0.5 were considered to be significant.

Results

Preliminary Study of Intracameral Injection Volume

Flare values and cell counts in relation to intracameral injection volume were investigated. Figure 1 shows the time course of flare values depending on the intracameral injection volume of LRW, 50 µl or 100 µl. In the eyes undergoing 50-µl injections, flare and cell counts were the same as in the control eyes of the main study. Although the number of eyes in the 100-µl injected group was small, their flare values at 3 h were significantly higher than those in the 50-µl injected group (Mann-Whitney *U* test, $P = 0.0282$). However, with the analysis method of area under the curve (AUC), there was no statistical difference between the 100 and 50 µl groups (Fig. 2), nor was there any significant difference in the cell counts between the 50 and 100 µl groups when the intracameral injection volume was taken into account.

Endotoxin Injection Study

Flare Values

There were four eyes in each endotoxin unit group. The following eyes that met the stated exclusion criteria were excluded from the analysis. Both eyes of rabbits 10 and 27 were excluded because the control eye showed extremely high flare values, although there was no apparent complication during treatment (criterion 1). Endotoxin-treated eyes in rabbits 19 and 24 and control eyes in rabbits 12, 21, and 31 were excluded because they showed a marked aqueous loss and shallow anterior chamber after the intracameral injection (criterion 2). Endotoxin-treated eyes in rabbits 14, 30, and 33, and control eyes in rabbits 15 and 27 were excluded because flare values increased markedly again in the late phase (criterion 3).

Analysis Method 1: Overall Analysis

Figure 3 shows the time course of flare values for 0.6 EU/ml injections. At any given time, there was no significant difference in flare values between endotoxin-treated eyes and their fellow control eyes. Figure 4 summarizes the time course of flare values in each endotoxin unit group. Although

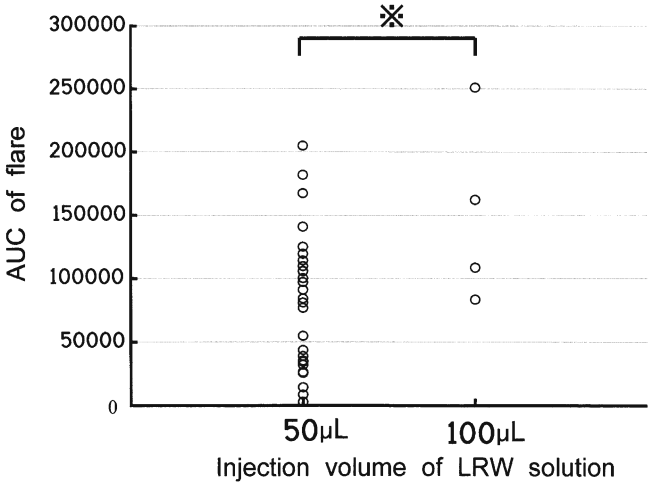

Figure 2. Area under the curve (AUC) analysis of flare values after intracameral LRW injection. There was no significant statistical difference between the 100 µl and 50 µl groups.

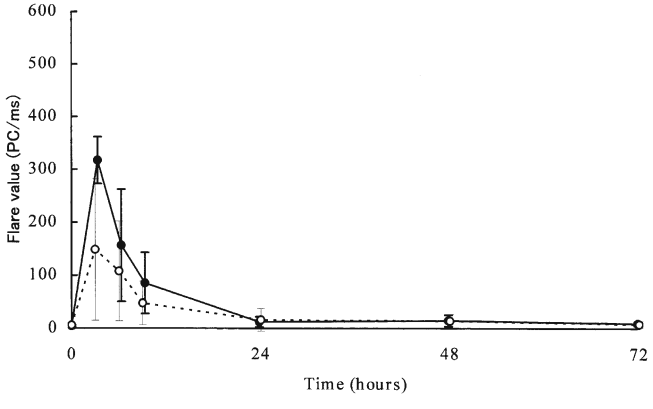

Figure 1. Time course of flare values after intracameral *Limulus* amebocyte lysate reagent water (LRW) injection. The number of eyes in the 100-µl injected group was small but their flare values at 3 h were significantly higher than those in the 50-µl injected group (Mann-Whitney *U* test, $P = 0.0282$). —●—, 100 µl LRW injection; ---○---, 50 µl LRW injection.

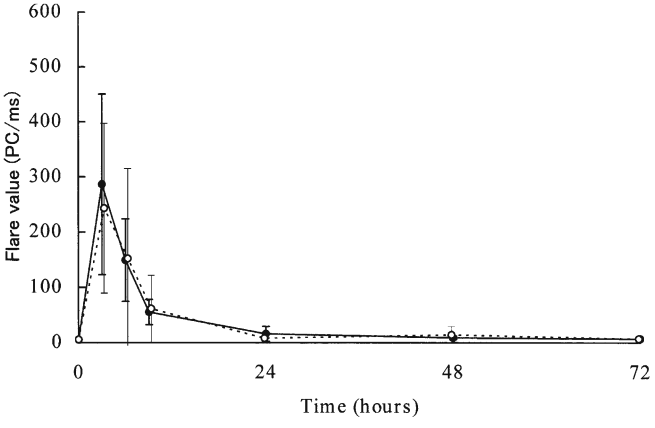

Figure 3. Time course of flare values for 0.6 EU/ml intracameral endotoxin injection. There was no significant difference in flare values between the endotoxin-treated eyes and their fellow control eyes in any of the time periods. —●—, Endotoxin-treated eye; ---○---, fellow control eye.

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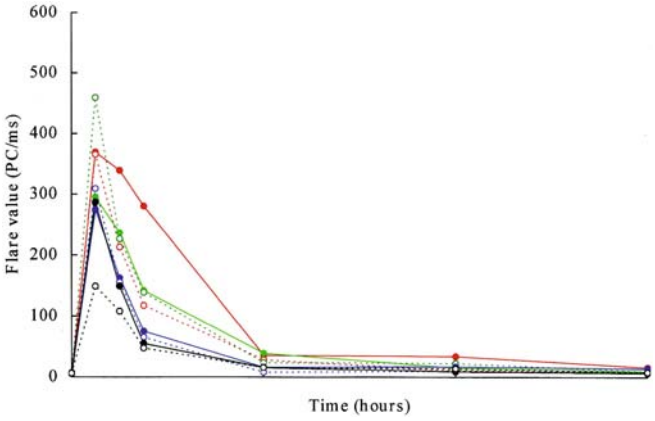


Figure 4. Time course of flare values in each endotoxin unit group. There was a significant statistical difference in flare values at 6 h (P = 0.0014) and 9 h (P = 0.0025) between the 0.6 EU/ml group and the 40 EU/ml group. Endotoxin concentrations: —●—, 40 EU/ml; ---○---, 20 EU/ml; —●—, 10 EU/ml; ---○---, 5 EU/ml; —●—, 2.5 EU/ml; ---○---, 1.25 EU/ml; —●—, 0.6 EU/ml; ---○---, control.

there was no statistical difference in flare values between the 0.6 EU/ml group and the 20 EU/ml group, flare values at 6 h (P = 0.0014) and 9 h (P = 0.0025) differed significantly between the 0.6 EU/ml group and the 40 EU/ml group.

Analysis Method 2: Analysis of Flare Values Resulting from the Actually Injected Endotoxin Units

There was interindividual variation in the number of injected endotoxin units due to surgical manipulations. Therefore, flare values as an index of an inflammatory reaction must be corrected according to the actual intracamerally injected endotoxin units. The actually injected endotoxin units was calculated from the pre- and post-injection insulin syringe weights.

Flare values at each time point for the corrected endotoxin units are shown in Fig. 5a–f. The correlation between flare values and the injected endotoxin concentration was statistically significant at 6 h (r^2 = 0.4199, P = 0.0001), 9 h (r^2 = 0.4893, P = 0.0000), and 48 h (r^2 = 0.2132, P = 0.0117).

Results of the preliminary 50-µl and 100-µl LRW solution injection study showed that in the control eyes, variations of flare values due to differences in the injected amount of endotoxin-free LRW solution could be ignored. Therefore, flare values at each time interval can be considered homogeneous. To determine whether eyes with extremely high flare values could be included as a member of the homogeneous control group, we conducted a Smirnov test. The flare values corresponding to a mean value of +2σ, 296.5 photon counts (PC)/ms at 6 h, 130.5 PC/ms at 9 h, and 29.2 PC/ms at 48 h in the control group were used as the cutoff values to indicate a normal inflammatory response in the eye undergoing paracentesis.

Using the cutoff value in the control group and the regression curve in the endotoxin-treated group, the minimum endotoxin concentration causing inflammation was determined. Table 1 summarizes the minimum endotoxin concentration values at each time point. Analysis method 2 showed that at 9 h, 0.67 EU was the minimum endotoxin concentration causing inflammation in the anterior segment of the eye.

Table 1. Minimum EU causing inflammation

	EU
Flare	
Analysis method 2	
6 h	1.60
9 h	0.67
48 h	1.82
Analysis method 3	
AUC	0.60
Cell count	
9 h[a]	0.01
24 h	0.23

EU, endotoxin unit; AUC, area under the curve.
[a]In eight eyes, cells could not be counted because of intense flare.

Analysis Method 3: Analysis with the Area Under the Curve Method

The time course of flare values showed a single-phase pattern with a peak in the early phase, mostly at 3 h. Therefore, we calculated the AUC using flare values in the pre-injection period and a time course of up to 72 h to evaluate the whole inflammatory pathophysiologic reaction. AUCs for flare values in the control eyes were also calculated. Figure 6 shows the results of the AUC analysis. The same assumption as that in Analysis method 2 was applied. Thus, an AUC corresponding to a mean value of +2σ, 19301.9 in the control group was established as the cutoff value for a nonspecific inflammatory response to intracameral injection of nonactive substances. A statistically significant regression curve based on the AUC for the corrected endotoxin units was obtained (r^2 = 0.4153, P = 0.0002). Based on the cutoff value for the control group and the regression curve in the endotoxin-treated group, the minimum endotoxin concentration causing inflammation was determined to be 0.60 EU by analysis method 3 (Table 1), which agrees well with the minimum concentration determined by analysis method 2.

Cell Counts

Table 2 summarizes the number of eyes in which cell counts were performed with the laser flare-cell photometer. In the early postoperative period (at 3, 6, and 9 h), cells could not be counted by the laser flare-cell photometer in many of the endotoxin-treated eyes, but they could be counted in the fellow control eyes. This difference was due to intense flare in the aqueous in the early period. After 24 h, cells in most eyes could be counted with laser flare-cell photometry.

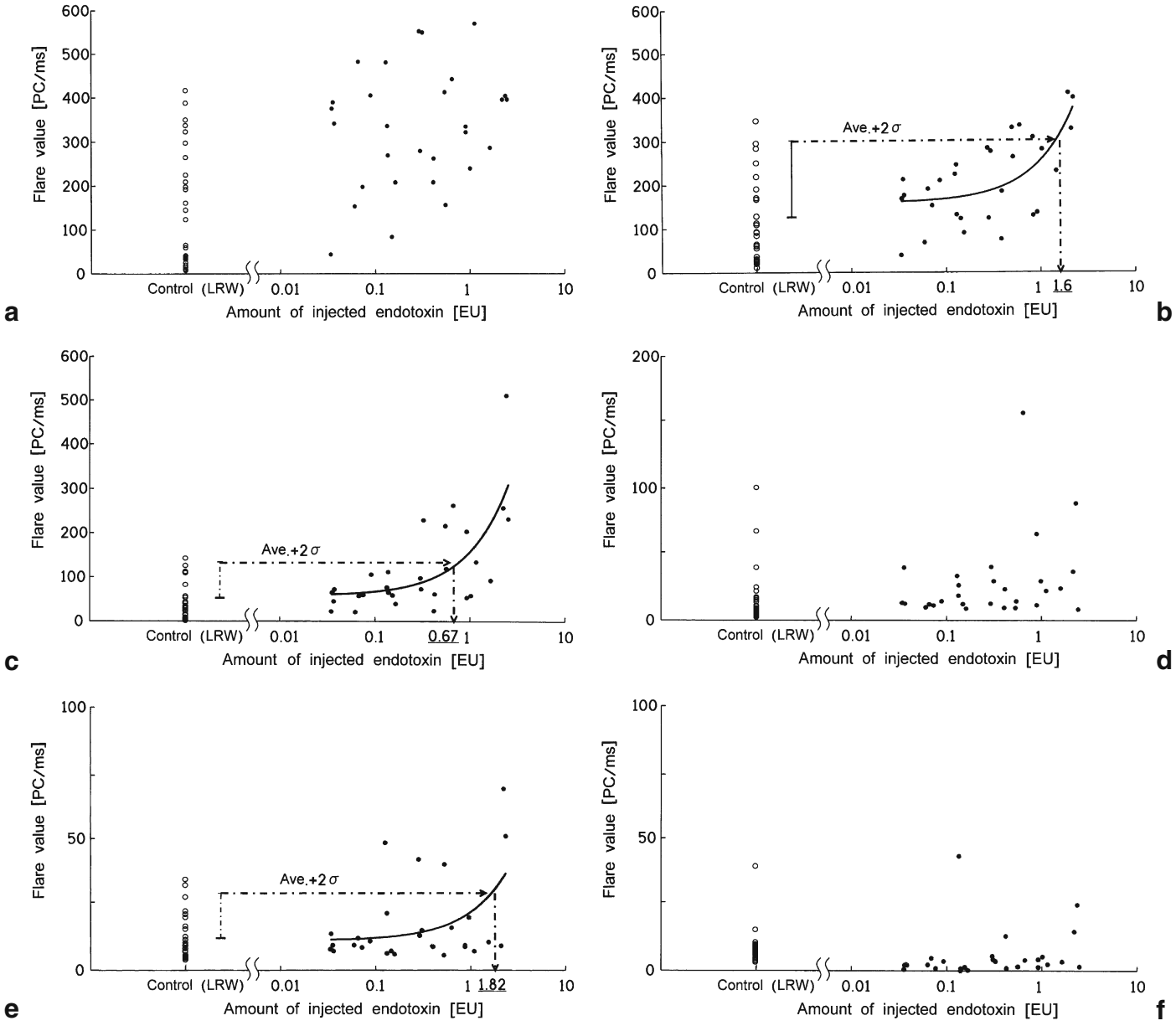


Figure 5a–f. Flare values for actual injected endotoxin concentrations at each time point. There was a significant statistical difference between the flare value and the actual injected endotoxin concentration at 6 h ($r^2 = 0.4199$, $P = 0.0001$), 9 h ($r^2 = 0.4893$, $P = 0.0000$), and 48 h ($r^2 = 0.2132$, $P = 0.0117$). The flare values corresponding to a mean value of +2σ in the control groups were 296.5 PC/ms at 6 h, 130.5 PC/ms at 9 h, and 29.2 PC/ms at 48 h. These flare values are considered to be the cutoff values indicating a normal inflammatory response in an eye undergoing paracentesis. **a** 3 h; **b** 6 h; **c** 9 h; **d** 24 h; **e** 48 h; **f** 72 h.

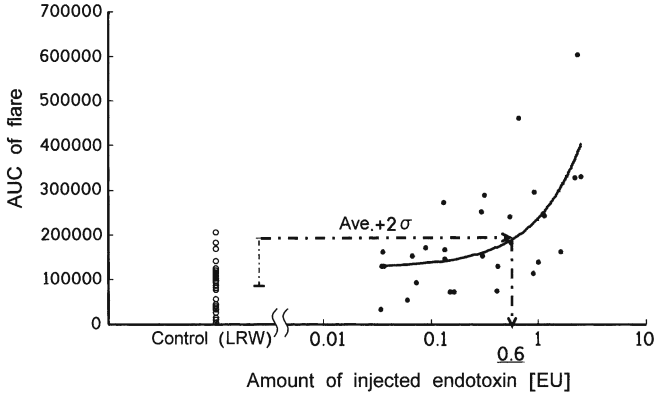


Figure 6. AUC analysis of flare values. AUC corresponding to a mean value of +2σ in the control eyes as a cutoff value is 19301.9 in the control group. The regression curve based on the AUC for the corrected endotoxin units was statistically significant ($r^2 = 0.4153$, $P = 0.0002$). Using the cutoff value for the control group and the regression curve in the endotoxin-treated group, the minimum endotoxin concentration causing inflammation was calculated to be 0.60 EU.

Postoperative results of the slit-lamp examinations are shown in Table 3. There were no statistical differences in cell grading among the different endotoxin unit groups at 3, 6, or 9 h postoperatively. The regression curve between cell counts and actual endotoxin concentrations was not significant at 3 h. At 9 h, a significant regression curve was obtained between cell counts and the actual injected endotoxin concentration ($r^2 = 0.7098$, $P = 0.000$), although eight eyes in which the cells could not be counted by flare-cell photometry were not included (Fig. 7a). These were mostly eyes receiving higher doses of endotoxin. At 24 h, cell count and the actual injected endotoxin concentration were significantly correlated ($r^2 = 0.2277$, $P = 0.0102$) (Fig. 7b). At 48 and 72 h, because of an increase in the number of eyes with a cell count of 0, the correlation between cell count and injected endotoxin units was not significant.

In terms of the minimum endotoxin concentration causing inflammation, cell counts (cells/0.5 mm^3·0.5 s) corresponding to values of "average +2σ", 27.7 at 9 h and 6.07 at 24 h, in the control eyes were considered cutoff values as determined in the flare value study for each time point. Using these cutoff values and the regression curves for the endotoxin-treated eyes, the minimum endotoxin concentration was calculated at each time point. At 9 h, the endotoxin concentration was 0.01 EU. However, as intense flare impeded the laser flare-cell photometer, there were eight missing values in this group, which should be taken into account. The results, therefore, were not reliable. On the other hand, at 24 h, 0.23 EU was obtained as the minimum endotoxin concentration causing inflammation (Table 1).

Table 2. Number of eyes yielding cell photometry

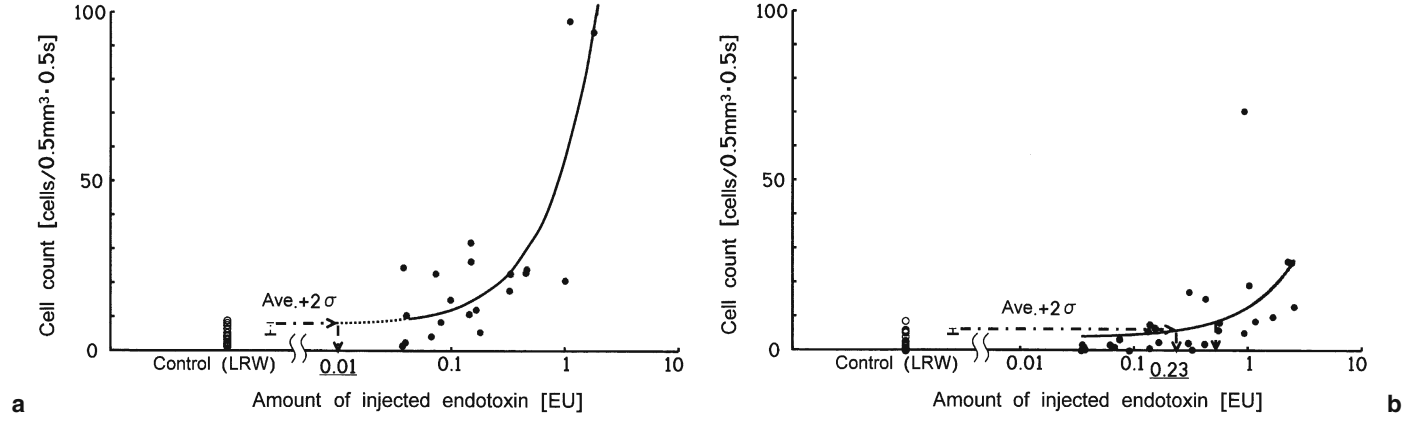
EU/ml	*n*	Pre-op	3 h	6 h	9 h	24 h	48 h	72 h
40	4	4	0	0	1	4	4	4
20	4	4	0	1	2	4	4	4
10	5	5	0	1	3	5	5	5
5	3	3	0	1	2	3	3	3
2.5	5	5	1	3	5	5	5	5
1.25	4	4	1	2	4	4	4	4
0.6	4	4	1	1	4	4	3	4
Control	28	28	14	23	28	28	28	28

Pre-op, preoperative results

Table 3. Cell grading by slit-lamp microscopy

		Cell grade				
EU/ml	Total	0	1	2	3	4
3 h						
40	4	1	0	0	3	0
20	4	1	0	2	1	0
10	5	5	0	0	0	0
5	3	3	0	0	0	0
2.5	5	4	1	0	0	0
1.25	4	4	0	0	0	0
0.6	4	3	1	0	0	0
6 h						
40	4	0	0	0	1	3
20	4	0	0	0	1	3
10	5	0	0	2	2	1
5	3	2	0	0	0	1
2.5	5	1	1	0	2	1
1.25	4	1	0	3	0	0
0.6	4	4	0	0	0	0
9 h						
40	4	0	0	1	3	0
20	4	0	0	1	2	1
10	5	0	0	3	2	0
5	3	1	0	2	0	0
2.5	5	0	2	3	0	0
1.25	4	2	1	1	0	0
0.6	4	4	0	0	0	0

Distribution of cell grades among EU groups shows no significant difference at all time points (χ-squared test).



Figures 7a, b. Cell counts in relation to the actual injected endotoxin concentration at each time point. A significant regression curve between cell counts and endotoxin concentration was obtained at 9 h ($r^2 = 0.7098$, $P = 0.000$) and 24 h ($r^2 = 0.2277$, $P = 0.010$). However, in eight eyes, cells could not be counted at 9 h. Cell counts (cells/0.5 mm^3·0.5 s) corresponding to the average value of +2σ in the control group as a cutoff value were 27.7 at 9 h and 6.07 at 24 h. Using the cutoff value and the regression curve for the endotoxin-treated eyes, the minimum endotoxin concentration was calculated to be 0.01 EU at 9 h and 0.23 EU at 24 h. **a** 9 h; **b** 24 h.

Discussion

In intraocular surgery, it is important to understand the pathophysiology in order to maintain visual function. Postoperative intraocular inflammation is a major problem.[22] Various factors are involved. Prevention of postoperative infection is one important factor, although there are not so many cases.[23] A major complication is postoperative inflammation. The blood–aqueous barrier (BAB) function plays an important role in postoperative inflammation in the anterior segment of the eye. The BAB is located at the nonpigmented epithelium of the ciliary process and the iridial capillary wall.[24] Surgical manipulation, including paracentesis, can cause a breakdown of the BAB,[16,17,24] leading to an increase in protein (flare) and cells in the aqueous humor. Important advances in surgical technique, including closed eye surgery,[1] and the introduction of pharmacological treatments such as nonsteroidal anti-inflammatory drugs[5,25] in addition to steroid drugs, have contributed to the successful suppression of postoperative inflammation in cataract surgery. The development of medical devices such as the intraocular lens[2,26] and the OVD have also supported surgical innovation. In lens implant surgery, OVD plays a key role in securing the space of the anterior segment. Furthermore, new clinical examination methods such as laser flare-cell photometry have played a pivotal role in the evaluation of inflammation in the anterior segment of the eye.[18,27] The laser flare-cell photometer makes noninvasive quantitative assessment of flare and cells in the aqueous possible, although slit-lamp microscopy remains a useful method for examining both flare and cells qualitatively and subjectively.

Endotoxins are a major potent inflammatory substance, and their effects on ocular tissues have been investigated mainly through intravenous injections. Current cataract surgery is carried out using sophisticated phacoemulsification. In this method, the maintained posterior lens capsule separates the intraocular space into anterior and posterior vitreous spaces. Therefore, the endotoxin limits need to be reassessed by using intracameral endotoxin injections.

In this study, an endotoxin solution was introduced intracamerally, and a quantitative analysis was conducted using laser flare-cell photometry. The attempted intraocular injection volume was 50 µl. However, an injection of exactly 50 µl proved technically difficult. Therefore, we determined the actually injected endotoxin units by weighing the syringe before and after the injection. At the same time, measurement of the inflammatory reaction in the fellow control eyes receiving LRW solution showed no significant difference in flare or cells in the aqueous between 50-µl and 100-µl injection groups.

Three flare analysis methods were used. In the first, flare values were analyzed under the assumption that the nominal amount of endotoxin units was intracamerally injected in each group. In this analysis, the time course of flare values in each endotoxin unit group showed a similar monophasic decreasing pattern with a peak at 3 h. Furthermore, this analysis method did not yield any significant difference in flare values among the nominal endotoxin unit groups. In some individuals, however, flare values increased again in the late phase. This phenomenon is similar to the fibrin reaction in postcataract surgery and is due to a secondary deterioration of the BAB and not to the effects of the initial surgical manipulation of the BAB.[27] These individuals were therefore excluded from the assessment of the effects of endotoxin on the BAB. Some individuals had higher flare values in the control eyes, although there was no complication during the endotoxin injection, and some rabbits showed a different reaction to the drugs depending either on individual diathesis or on systemic conditions.[28] The data of these rabbits were excluded as well.

In analysis method 2, flare values were analyzed in relation to the actually injected endotoxin units. In this analysis, the flare value corresponding to the average+2σ in the control group, 0.67 EU, was considered the minimum endotoxin concentration causing inflammation in the anterior segment of the eye.

In analysis method 3, the concept of AUC was introduced. AUC is an index that indicates the overall inflammatory reaction, and it can be used to assess the severity of inflammation. In this analysis, an AUC corresponding to an average of +2σ in the control group was considered the cutoff concentration causing nonspecific responses to paracentesis. Thus, by this method, 0.60 EU was the minimum endotoxin concentration inducing inflammation. The minimum endotoxin concentrations causing inflammation obtained by analysis methods 2 and 3 showed good agreement.

The minimum endotoxin concentration causing inflammation in the anterior segment of the eye was determined to be 0.23 EU according to the cell count analysis. In the cell count analysis, the limitations of laser flare-cell photometry should be taken into account. The laser flare-cell photometer enables quantitative measurement, but the volume is limited to 0.5 mm^3/0.5 s. This means that the observation period is 0.5 s. Furthermore, owing to intense flare, many of the cells in the early posttreatment period, up to 9 h, could not be counted by the laser flare-cell photometer, since an intense flare generates a strong light noise scatter in the anterior segment. At 9 h, the cell count by flare-cell photometry was significantly correlated with actual endotoxin units, but in eight eyes, cell count data were missing. Therefore, 0.23 EU at 24 h was considered to be the correct minimum endotoxin concentration causing an inflammatory response, because at 24 h, the flare-cell photometer could count cells in all of the endotoxin-treated eyes.

Flare and cells in the aqueous humor are important indices of the severity of inflammation in the anterior segment of the eye. However, their pathophysiologic pathways and significance are different. Flares and cells may dissociate occasionally,[18] which can be explained by a difference in the major pathways of the arachidonic acid cascade.[24] Cyclooxygenase plays an important role in the increase in protein in the aqueous, which is observed as flare by slit-lamp microscopy. In the arachidonic acid cascade, cyclogenase biosynthesizes chemical mediators such as

prostaglandins and the platelet-activating factor. Prostaglandins cause dilatation of the blood vessels and increase the leakage of serum protein into the aqueous. Another major enzyme, lipoxygenase, produces leukotrienes from arachidonic acid. Leukotrienes are chemoattractive and cause infiltration of leukocytes into the aqueous.

This study, therefore, has revealed that the minimum endotoxin concentration range causing inflammation in the anterior segment of the eye is 0.23–0.60 EU.

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