Effect of LTD$_4$ on Conjunctival Vasopermeability and Blood–Aqueous Barrier Integrity

David F. Woodward and Sally E. Ledgard

The effect of leukotriene D$_4$ (LTD$_4$) on the microvascular permeability of selected ocular tissues of the guinea pig was evaluated quantitatively by utilizing $^{125}$I-albumin and $^{51}$Cr-erythrocytes. Topically applied LTD$_4$ (1 ng–10 ng) produced a dose-related increase in conjunctival microvascular permeability. However, topical administration of such doses of LTD$_4$ did not promote albumin leakage into the aqueous humor or iris-ciliary body. Intravitreal injection of graded doses of LTD$_4$ also failed to increase the albumin content of the aqueous humor and iris-ciliary body. Thus, although LTD$_4$ increased conjunctival microvascular permeability, it does not appear to disrupt the blood–aqueous barrier of the guinea pig eye. Invest Ophthalmol Vis Sci 26:481–485, 1985

Since its discovery more than 40 years ago, the slow-reacting substance of anaphylaxis (SRS-A) has been suspected to be involved in the pathophysiology of allergic diseases. Unfortunately, the structural identity of SRS-A remained elusive until recently. SRS-A now has been shown to comprise the products of a novel family of 5-lipoxygenase-derived arachidonic acid metabolites. The structure of the major component of immunologically generated guinea pig SRS-A has been defined unequivocally as 5-hydroxy-6-cysteinylglycinyl-7,9,11,14-eicosatetraenoic acid, termed leukotriene D$_4$ (LTD$_4$).

The possibility that leukotrienes are important mediators of allergic inflammation has stimulated considerable interest, and LTD$_4$ has been shown to increase microvascular permeability in a variety of tissues. The purpose of this study was to evaluate quantitatively the effect of LTD$_4$ on the microvascular permeability of selected external and internal ocular tissues. This objective was attained by utilizing radio-labeled serum albumin and erythrocytes and thereby determining extravascular albumin accumulation in the eyelids, bulbar conjunctiva, iris-ciliary body, and aqueous humor.

**Materials and Methods**

**Measurement of Microvascular Permeability**

Albino, male, conscious guinea pigs of the Hartley strain, weighing 350–450 g, were used. $^{125}$I-bovine serum albumin (0.2 ml × 10 $\mu$Ci/ml) and Evans blue (0.2 ml × 2.5%) were injected into the dorsal foot vein 15 min before LTD$_4$ or histamine administration to the eye. Synthetic LTD$_4$ (5S-hydroxy-6R-cysteinylglycinyl-7,9,11,14-eicosatetraenoic acid) was prepared and stored as previously described. LTD$_4$ and histamine solutions (at neutral pH) were administered either topically in a 20-$\mu$l volume or by intravitreal injection in a 2-$\mu$l volume; distilled water was administered to the contralateral eye in an identical manner to provide a control. Intravitreal injection employed a 30-gauge needle, and each eye was pretreated with 0.5% proparacaine (Ophthetic). Animals were killed by intracardiac injection of 0.2 ml T-61 euthanasia solution, which consists of N-[2-(m-methoxy-phenyl)-2-ethylbutyl-(1)-]gamma-hydroxybutyramide (200 mg/ml); 4,4'-methylene-bis (cyclohexyltrimethyl-ammonium iodide) (50 mg/ml); tetracaine hydrochloride (5 mg/ml) with 0.6% v:v dimethylformamide in distilled water. Tissue samples immediately were taken according to the following sequential procedures. Aqueous humor was obtained by means of a 30-gauge needle inserted through the cornea into the anterior chamber. The eyelids then were excised and the eyes carefully enucleated. The bulbar conjunctiva was dissected free of the globe at the limbus and trimmed free of extraocular muscle. The globe then was cut to provide a scleral ring containing the iris and ciliary body; these structures simultaneously were excised with the aid of a microscope. Tissue samples from each individual animal were counted in a $\gamma$ scintillation counter, together with a 1.0-ml blood sample from the same animal. Tissue samples finally were oven-dried at 50–55°C to constant weight.

The extravascular albumin content of the eyelids, conjunctiva, and iris-ciliary body was calculated as...
Fig. 1. Effect of topically administered LTD₄ on the extravascular albumin content of the eyelids. Eyes that received LTD₄ are represented by (●); control eyes are represented by (O). Each point is the mean (±SEM) of five animals.

The investigations described conform to the ARVO Resolution on the Use of Animals in Research.

**Results**

Topically applied LTD₄ over the dose range 1 ng to 10 µg produced a dose-dependent increase in the extravascular albumin content of the eyelids and bulbar conjunctiva (Figs. 1 and 2). A similar increase in microvascular permeability was not apparent in control eyes. The dose-dependent effect of LTD₄ was confirmed for LTD₄-treated eyes by analysis of Spearman rank correlation coefficients; such a relationship was not apparent in the vehicle-treated, control eyes (Table 1). Increased microvascular permeability also

**Corneal Trauma**

Guinea pigs were anesthetized by urethane (2 g/kg ip), and radiolabeled blood elements/Evans blue were injected into the dorsal foot vein as above. Corneal trauma in one eye was caused by introduction of a 30-gauge needle into the anterior chamber with insertion at a slight tangent to the corneal surface. The needle immediately was removed along the same path to form a self-sealing corneal lesion. The contralateral eye served as a nontraumatized control. Aqueous humor samples were withdrawn from both eyes 30 min later.

**⁵¹Erythrocytes and ¹²⁵I-Albumin Preparation**

Ten milliliters of heparinized guinea pig blood were centrifuged three times at 1,000 rpm for 10 min. After each centrifugation, the supernatant and the top layer of cells were discarded, the original volume was restored with normal saline, and the cells were resuspended. The red blood cell suspension then was added to 500 µCi Na₂⁵¹CrO₄ (New England Nuclear) in saline and refrigerated for 18 hr to allow binding of the⁵¹Cr label to the erythrocytes. In order to remove any unbound Na₂⁵¹CrO₄, the red blood cell suspension was recentrifuged three times at 1,000 rpm for 10 min. ¹²⁵I-bovine serum albumin (New England Nuclear) was stored frozen until use, and dilute solutions were prepared in normal saline.

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Table 1. Spearman rank correlation coefficients between tissue albumin content and log_{10} dose LTD₄ for the eyelids, bulbar conjunctiva, aqueous humor, and iris/ciliary body.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Albumin content treated, O.S. vs. log_{10} dose LTD₄</th>
<th>Albumin content control, O.D. vs. log_{10} dose LTD₄</th>
<th>Albumin content O.S.–O.D. vs. log_{10} dose LTD₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyelids</td>
<td>0.806*</td>
<td>0.187</td>
<td>0.798*</td>
</tr>
<tr>
<td>Bulbar conjunctiva</td>
<td>0.857*</td>
<td>0.256</td>
<td>0.830*</td>
</tr>
<tr>
<td>Aqueous humor (topical)</td>
<td>0.285</td>
<td>0.673*</td>
<td>-0.375</td>
</tr>
<tr>
<td>Aqueous humor (intravitreal)</td>
<td>0.316</td>
<td>0.142</td>
<td>0.218</td>
</tr>
<tr>
<td>Iris/ciliary body (topical)</td>
<td>0.060</td>
<td>-0.105</td>
<td>0.089</td>
</tr>
<tr>
<td>Iris/ciliary body (intravitreal)</td>
<td>0.287</td>
<td>0.134</td>
<td>0.451</td>
</tr>
</tbody>
</table>

A significant dose–response relationship is indicated as *P < 0.01.

was visualized as a bluing response in LTD₄-treated eyes.

In contrast to conjunctival tissues, the iris-ciliary body did not exhibit an increase in extravascular albumin content in response to graded doses of LTD₄, applied either topically (Fig. 3a) or injected intravitreally (Fig. 3b). Statistical analysis (Table 1) confirmed this finding, and microscopic examination revealed no evidence of Evans blue-tagged albumin leakage.

The albumin content of the aqueous humor essentially remained unaltered after graded doses of LTD₄ were applied topically (Fig. 4a) or injected into the vitreous humor (Fig. 4b). Furthermore, no bluing of the aqueous humor was observed. According to the correlation coefficient values obtained, an increase in the aqueous humor albumin content of eyes treated with topical vehicle occurred (Table 1). However, the treated (LTD₄) eye–control eye topical comparison and intravitreal studies did not provide confirmation.

The topical 10-μg dose of LTD₄ caused apparent respiratory distress in some animals; a similar phenomenon was not observed as a consequence of intravitreal injection.

In contrast to LTD₄, histamine (1,000 μg), administered topically, produced a significant increase in the albumin content of the aqueous humor; a similar but nonsignificant increase also was associated with intravitreal injection of 100 μg histamine (Table 2). Corneal trauma resulted in a severe response, and albumin leakage into the anterior chamber clearly was visualized as a bluing response (Table 2).

Discussion

 Conjunctival tissues are reported to generate lipooxygenase-derived metabolites from exogenously administered arachidonic acid. However, direct evidence for pathophysiologic changes evoked by lipooxygenase products in the conjunctiva appears to be absent. These studies have demonstrated that the principal component of guinea pig SRS-A, 5S-hydroxy-6R-cysteinylglycinyl-7,9,11,14-eicosatetraenoic acid (LTD₄) is a potent mediator of conjunctival microvascular permeability. The potency of LTD₄ as a permeability factor in the conjunctiva approaches that reported in guinea pig skin, guinea pig trachea, and human skin. Observation of the bluing response revealed that albumin leakage in the eyelids was confined to the inner conjunctival surface, whereas
the bulbar conjunctiva developed a uniform blue coloration. Thus, the numerically smaller response in the eyelids reflects limitation of the microvascular permeability response to the conjunctival region with an absence of cutaneous involvement. The 10-μg dose of LTD₄ applied topically to the conjunctiva also produced signs of significant systemic absorption, observed as respiratory distress, in two instances. This is, perhaps, not surprising, since as little as 0.1 μg LTD₄ administered intravenously, will produce airway constriction in the guinea pig. 19,20 Although 10 μg LTD₄ caused respiratory distress when administered topically, no evidence for a measurable crossover permeability effect in the contralateral, control eye was obtained.

Although a 15-min topical application of LTD₄ potently increased conjunctival microvascular permeability, it did not appear to alter the albumin content of the aqueous humor or the iris-ciliary body. Previous studies in rabbits have shown that microgram doses of other arachidonic acid derivatives elicit measurable changes in anterior segment protein content 21 and intraocular pressure 22 at 15 min posttopical application. Moreover, in the guinea pig, topical histamine caused a significant elevation of aqueous humor albumin content within 15 min. Thus, 15 min might be sufficient for observing effects on the blood–aqueous barrier following ocular surface application. However, it is inappropriate to implicitly assume that the pharmacokinetic and metabolic profile of topically applied LTD₄ would lead to biologically active levels in the anterior segment. Both in vivo and in vitro studies have indicated that the penetration of topically administered prostaglandins may be very restricted 23 and this could, perhaps, equally apply to LTD₄. Therefore, an alternative method of administration was pursued.

LTD₄ effects on blood–aqueous barrier integrity were investigated further by measuring albumin accumulation in the aqueous humor and the iris–ciliary body in response to graded doses of LTD₄ injected intravitreally. Intravitreal injection of LTD₄ failed to disrupt the blood–aqueous barrier of the guinea pig eye. Results obtained with radiolabeled albumin and erythrocytes were confirmed by simple observation of Evans blue leakage; both the iris-ciliary body and the aqueous humor failed to develop a blue coloration. In contrast to LTD₄, intravitreal injection of histamine and corneal trauma appeared to increase the albumin content in the anterior segment over an identical 30-min period. The histamine studies and corneal trauma indicate that albumin leakage into the anterior segment of the guinea pig eye can occur under appropriate conditions, and this provides positive controls for the LTD₄ experiments.

A recent study has demonstrated that intracameral administration of microgram quantities of LTC₄ and LTD₄ does not cause blood–aqueous barrier breakdown in the cat. 24 The rabbit was used to study LTB₄ where maintenance of blood–aqueous barrier integrity was inferred from a lack of effect on intraocular pressure; no direct measurement of aqueous humor protein content was reported. 25 Although the rabbit and the cat frequently are chosen for ocular research, the guinea pig was preferred for peptidoleukotriene studies for the following reasons. Evidence to date suggests that the rabbit is essentially unresponsive to peptidoleukotrienes. Leukotrienes C₄ and D₄ have virtually no effect on rabbit lung preparations 26 and do not increase the microvascular permeability of rabbit skin. 18 In marked contrast, the guinea pig, like humans, responds to LTC₄ and LTD₄ with exquisite sensitivity. Unfortunately, there is little published data on the responsiveness of cats to leukotrienes, 24,27 but the lack of effect of LTD₄ on blood–aqueous barrier integrity in this species is consistent with the guinea pig studies described herein.

The difference between the conjunctiva and the internal blood–aqueous barrier in response to LTD₄ merits further comment. In many tissues, microvascular leakage results from the formation of interendothelial cell gaps in the postcapillary venules. 28 Gap formation appears to permit the unrestricted bulk movement of plasma into the extravascular space. However, studies on the morphology of blood–aqueous barrier breakdown in the rabbit reveal that the ultimate barrier to plasma leakage into the aqueous humor is not the microvasculature, per se, but rather the intercellular clefts associated with the nonpigmented epithelium of the ciliary processes. 29,30 Thus, the difference between the permeability effects of LTD₄ possibly may be explained as gap formation in the conjunctival microvascular bed as opposed to blood–aqueous barrier disruption due to altered patency of tight junctions in the ciliary epithelium. Unfortunately, pertinent anatomic studies of the guinea pig blood–aqueous barrier do not appear to be available to lend further support to this suggestion.

Although exogenously administered LTD₄ does not

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Table 2. Effect of histamine and corneal trauma on the albumin content of the aqueous humor

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Albumin content of aqueous humor (treated eye)</th>
<th>Albumin content of aqueous humor (control eye)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical histamine (1,000 μg) (n = 5)</td>
<td>0.0383 ± 0.0062*</td>
<td>0.0123 ± 0.0027</td>
</tr>
<tr>
<td>Intravitreal histamine (100 μg) (n = 4)</td>
<td>0.0337 ± 0.0092</td>
<td>0.0116 ± 0.0034</td>
</tr>
<tr>
<td>Corneal trauma (n = 8)</td>
<td>0.4999 ± 0.1714*</td>
<td>0.0230 ± 0.0016</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM, and albumin content is expressed as albumin in 1 ml aqueous humor (ml⁻¹ blood).
* P < 0.01 (paired t-test comparison of treated and control eyes).
alter the integrity of the blood–aqueous barrier in the cat and the guinea pig, the effect of endogenously released LTD_{4} on the blood–aqueous barrier and LTD_{4} involvement in allergic uveitis are unlikely to be determined until selective antagonists or synthesis inhibitors become available. In terms of smooth muscle contraction and microvascular permeability, the guinea pig is usually predictive for activity of LTD_{4} in humans.\textsuperscript{10,30} If this trend continues to be valid, these investigations suggest that release of LTD_{4} during immediate hypersensitivity reactions may contribute to the inflammatory response associated with conjunctival allergy.

**Key words:** leukotrienes, conjunctiva, blood–aqueous barrier, inflammation, guinea pig

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**References**