Leukotrienne Constriction of Mouse Pial Arterioles in Vivo Is Endothelium-Dependent and Receptor-Mediated

William I. Rosenblum, MD, Guy H. Nelson, MS, and Hiroyuki Nishimura, MD

The diameters of pial arterioles of mice were monitored in vivo with an image-splitting technique and television microscopy. Concentrations of leukotriene C₄ as low as 10⁻⁷ M constricted the arterioles. The leukotriene C₄-D₄ receptor blocker ICI 198615 (10⁻⁸ M) inhibited the response. Endothelial injury by a helium-neon laser/Evans blue technique unmasked a slight but consistent relaxation that was not inhibited by 10⁻⁸ M ICI 198615. Since leukotrienes are produced by the brain and enter the cerebrospinal fluid in ischemia, head trauma, and subarachnoid hemorrhage, the possibility that leukotrienes C₄ and D₄ contribute to decreases in cerebral blood flow during these conditions should be considered. However, the present data makes such a possibility far less likely because the endothelium is frequently injured in these conditions, and therefore the ability of leukotrienes to constrict vessels would be severely curtailed. (Stroke 1990;21:1618-1620)

Leukotrienes are among the many candidates for significant vasoconstrictor activity in diseased brain. They are released during ischemia-reperfusion, trauma, and/or subarachnoid hemorrhage.¹⁻³ We and others have demonstrated the vasoconstrictor action of leukotriene C₄ (LTC₄) and leukotriene D₄ (LTD₄) on pial arterioles of several species in vivo.⁴

Recently, the existence of endothelium-dependent constriction has been revealed.⁵⁻⁷ Therefore, each vasoconstrictor must now be re-examined to determine whether it has an endothelium-dependent mode of action. Such a finding should cause re-evaluation of the potential pathogenetic significance of the leukotriene constrictors since there is frequently endothelial damage in the settings where leukotrienes are released.

In pial arterioles of mice we demonstrated that serotonin, sodium arachidonate, and high-dose histamine produced constrictions that were abolished by minor endothelial injury. The injurious agent was light from a helium-neon laser in the presence of intravascular Evans blue.⁶⁻⁸ This injury does not affect the underlying smooth muscle, as shown by retention of normal responses to well-known endothelium-independent dilators like sodium nitroprusside⁸ and 8-bromoguanosine 3':5'-cyclic monophosphate⁹ and by normal constriction to uridine 5'-triphosphate, a known endothelium-independent constrictor. In the following study we used the same technique to test the endothelium-dependence of LTC₄ constriction. We also used a potent inhibitor of LTC₄ receptors to demonstrate the receptor-dependence of the LTC₄ action.

Materials and Methods

The materials and methods have been described in detail⁶⁻⁸ and will therefore be abbreviated here. Male mice, ICR strain (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) were anesthetized with urethane. Tracheotomy and craniotomy were performed, the dura was stripped, and the pial vessels on the brain surface under the transparent arachnoid were exposed. Experiments were performed as follows. The brain surface was continuously irrigated with mock cerebrospinal fluid (CSF) (Elliott's solution¹⁰), mean±SEM pH 7.35±0.01, flowing at 0.8 ml/min. All drug solutions were diluted in this mock CSF at this pH and applied at this flow rate unless noted otherwise. The mice were kept at 37° C with a heating mattress.

In each mouse, a single arteriole 30–40 μm in internal diameter was arbitrarily selected for study. Its diameter was monitored with a television microscope and image-splitter and was continuously recorded on a strip chart¹¹,¹² before and after drug...
application. One milliliter of LTC₄ was applied. The
maximum change in diameter expressed as a percentage
of the diameter during the minute preceding
drug application (baseline diameter) was used as the
response. Successive applications of LTC₄ were sepa-
rated by 15-minute intervals.

A 0.5% solution of Evans blue was injected at 0.5
ml/100 g body wt via the tail vein. Thirty minutes
later, the endothelium was damaged at a spot 36 μm
in diameter by directing the beam of a 6-mW helium-
noon laser through the optics of a metallurgic micro-
scope. Postinjury testing of the microvascular re-
sponse began 15 minutes after endothelial injury.

During the laser/Evans blue studies the suffusate
of mock CSF and all drug solutions were kept at
24°C to minimize laser damage. The studies of the
LTC₄-D₄ receptor blocking agent were therefore
conducted with the suffusate at 24°C to duplicate the
conditions under which endothelium dependence was
demonstrated.

The blocker²⁵,²⁶ was ICI 198615 ([1-[[2-methoxy-
4-[[phenylsulfonyl]-amino carbonyl]phenyl[methyl]-
1H-indazol-6-yl]-carboxylic acid cyclopentyl ester]. The
drug was supplied by ICI Pharmaceuticals,
Wilmington, Del. LTC₄ was obtained as the methyl
ester (Sigma Chemical Co., St. Louis, Mo.) or as a
concentrated solution in methyl alcohol and ammo-
nium acetate (BIOMOL Research Laboratories,
Inc., Plymouth Meeting, Pa.). In either case the
concentrate was diluted in mock CSF immediately
before use. The ICI 198615 was dissolved in dimethyl
sulfoxide (DMSO), and this stock solution was di-
centrated solution in methyl alcohol and ammo-
nium acetate. The final concentration of
DMSO was never more than 0.001%, and control
experiments always included equivalent amounts of
DMSO together with LTC₄ but without ICI 198615.

At the end of each experiment, 100 μl blood was
obtained from the carotid artery and PaO₂, PaCO₂,
and pH were measured as guides to the general
condition of the mice. These values were similar from
study to study and will not be referred to again. The
mean±SD values were PaO₂=109±8 mm Hg,
PaCO₂=33±2 mm Hg, and pH=7.37±0.04.

Results

Both sources of LTC₄ caused constriction of pial
arterioles, in agreement with our prior report.⁴ In
each case the laser/Evans blue injury eliminated the
constriction and instead a slight relaxation appeared.
This is shown in Table 1. When 10⁻⁷ M LTC₄ methyl
ester was used (experiment 1), arterioles constricted
7±1% (mean±SEM) before endothelial injury and
relaxed 3±1% after (p<0.01 by Wilcoxon's test).
Fifteen minutes later, at an uninjured site 100 μm
away, LTC₄ constricted the arterioles 5±1%, so loss
of constriction at the injured site could not have been
caused by fatigue.

When LTC₄ was prepared from a concentrate in
methyl alcohol and ammonium acetate (experiment 2),
10⁻⁷ M caused a 7±3% constriction (mean±SEM)
before endothelial injury, a 2±1% relaxation after

<table>
<thead>
<tr>
<th>Change in diameter (%)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
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<tbody>
<tr>
<td>Before laser</td>
<td>-7±1</td>
<td>-7±3</td>
</tr>
<tr>
<td>After laser</td>
<td>3±1*</td>
<td>2±1*</td>
</tr>
<tr>
<td>100 μm away</td>
<td>-5±1</td>
<td>-4±2</td>
</tr>
</tbody>
</table>

LTC₄, leukotriene C₄. All values are mean±SEM for n mice.
Original diameter was unchanged throughout each experiment.
*p<0.01 different from response before laser or 100 μm away by
Wilcoxon's test in experiment 1, paired t test after logarithmic
transformation of data in experiment 2 because n was too small for
Wilcoxon's test.


Leukotriene Constriction

TABLE 2. 10⁻⁴ M ICI 198615 Inhibits Response to 10⁻⁸ M LTC₄

<table>
<thead>
<tr>
<th>n</th>
<th>Diameter (μm)</th>
<th>Change in diameter (% of baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before ICI</td>
<td>With ICI</td>
</tr>
<tr>
<td>10</td>
<td>34</td>
<td>-9±1*</td>
</tr>
</tbody>
</table>

LTC₄, leukotriene C₄. All values are mean±SEM for n mice.
*p<0.01 different from constriction with ICI 198615 by Wilcoxon's test.
mean±SEM response of all 10 mice to LTC4 was a constriction of only 2±1%. After washout of LTC4 and ICI 198615 and 15 minutes of suffusion with mock CSF, the LTC4 was applied again in the presence of DMSO. A constriction of 7±1% was obtained. This was essentially the same as that seen before the blocker. Both the pre- and post-ICI 198615 responses to LTC4 were significantly larger than the response in the presence of ICI 198615 (p<0.01 for each comparison by Wilcoxon’s test).

**Discussion**

The new findings are that constriction of pial arterioles by LTC4 is endothelium-dependent, that the response to LTC4 changes from constriction to a slight relaxation after endothelial injury, and that the constriction but not the relaxation is inhibited by very low doses of the LTC4-D4 receptor blocker ICI 198615.

The endothelium-dependence of the response to LTC4 was established by showing that endothelial injury abolished the response. The receptor-dependence of the contractile response was shown by its almost-total abolition by very low doses of a very potent and selective blocker of the LTC4-D4 receptor(s). As yet, no one has developed a blocker that will distinguish receptor-dependence constricting action of LTQ outweighed the receptor-dependence and the other is a result of direct action on vascular tone. One action is endothelium-dependent and the other is a result of direct action on the vascular smooth muscle. Under the conditions of our experiments, the endothelium-dependent constricting action of LTC4 outweighed the endothelium-independent relaxation.

Previously, we had demonstrated endothelium-dependent constriction in vivo in mouse pial arterioles for serotonin, sodium arachidonate, and high-dose histamine. Each of these agents also appeared to depend on a product of cyclooxygenase action in the endothelium, suggesting that thromboxane or a constricting prostaglandin might be the endothelium-derived mediator released by each agonist. We have not attempted to define the mediator for LTC4 in the present studies. We will do so when we obtain a selective inhibitor of prostaglandin receptors.

Meanwhile, the importance of the present findings resides in their implication for the potential action of LTC4 or LTD4 in disease. Since these agents may be present in high local concentrations in ischemia-reperfusion, trauma, or subarachnoid hemorrhage, they could be considered potential constrictors that lead, perhaps in an additive or synergistic fashion with other constrictors, to significant reductions in cerebral blood flow. However, the present data makes this scenario far less likely. Ischemia, subarachnoid hemorrhage, and head trauma can injure endothelium. Since this would abolish the endothelium-dependent constriction produced by LTC4 and/or LTD4, it is far less likely that they contribute to vasocostriction in these situations.

**References**


**Key Words** • leukotrienes • microcirculation • mice • endothelium
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