Leukotriene 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 eliminated the constriction and 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 nitroprusside⁹ 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 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.
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 separated 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 metallurgical microscope. Postinjury testing of the microvascular response began 15 minutes after endothelial injury.

During the laser/Evans blue studies the sulfusate 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 sulfusate at 24°C to duplicate the conditions under which endothelium dependence was demonstrated.

The blocker wasICI 198615 ([1-[2-methoxy-4-[[phenylsulfonyl]-amino carbonyl[phenyl[methyl]-IH-indazol-6-yl]-carboxyl]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 ammonium 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 diluted in mock CSF. 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. 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 injury, and a 4±1% constriction at a site 100 μm from the injury. Thus, the responses to LTC₄ were the same and the effect of laser/Evans blue injury on the response was the same irrespective of the source of LTC₄.

The LTC₄-D₄ receptor blocker was then tested against LTC₄. This experiment was designed to test the effect of ICI 198615 against both the constricting action of LTC₄ and against the slight relaxing action that was unmasked by endothelial injury. Fifteen mice were tested over 5 days. Five mice were tested before and after laser/Evans blue injury. LTC₄ (10⁻⁶ M) caused a 9±1% (mean±SEM) constriction before injury, a 3±1% relaxation after injury, and 8±1% constriction 15 minutes later at a site 100 μm from the injury. Thus, we see again the endothelium-dependence of constriction by LTC₄ and the unmasking by endothelial injury of a slight dilator response. The responses before injury and 100 μm away were obtained in the presence of DMSO, the vehicle for ICI 198615. The response at the injured site was obtained in the presence of 10⁻⁶ M ICI 198615. This response, a 3±1% relaxation, was the same as that obtained at the injured site in the absence of ICI 198615, as shown in the previous experiments summarized in Table 1. Thus, the dilator response unmasked by endothelial injury was not inhibited by the LTC₄-D₄ receptor blocker.

Over the same days that ICI 198615 was tested against the postinjury response, we also assessed the effect of the blocker on the constriction produced in uninjured vessels by LTC₄ (Table 2). LTC₄ (10⁻⁶ M) caused a 9±1% (mean±SEM) constriction on its first application in the presence of DMSO, the vehicle for ICI 198615. Fifteen minutes later, the same dose of LTC₄ was applied together with 10⁻⁶ M ICI 198615. Three mice failed to respond to LTC₄, and the

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LTC₄, leukotriene C₄. All values are mean±SEM for n mice.

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<th>TABLE 2. 10⁻⁴ M ICI 198615 Inhibits Response to 10⁻⁴ M LTC₄</th>
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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).13,14 As yet, no one has developed a blocker that will distinguish endothelium-dependent constriction from endothelium-independent relaxation.

The slight relaxation produced by LTC₄ after endothelial injury, though trivial in size, was consistent. It was not affected by the LTC₄-D₄ receptor blocker, so it may not have been mediated by these receptors. More likely, the LTC₄-D₄ receptors responsible had a lower affinity for the blocker than did the endothelial receptor that mediated constriction. The consistent unmasking of dilation represents a principle we have previously elaborated upon—namely, that a single agonist may have multiple, simultaneous, opposite actions on vascular tone. One action is endothelium-dependent and the other is a result of direct action on the vascular smooth muscle.6-8 Under the conditions of our experiments, the endothelium-dependent constriction 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.6,7 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,1-3 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 vasoconstriction in these situations.

References


Key Words • leukotrienes • microcirculation • mice • endothelium
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