L-649,923: An Antagonist of Cardiac and Vascular Leukotriene D₄ Receptors

A.-L. Sirén, J. Eimerl, and G. Feuerstein

Uniformed Services University of Health Sciences, Bethesda, Maryland, U.S.A.

Summary: The capacity of L-649,923—sodium (ßS,γR*)-4-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)-propylthio)-γ-hydroxy-ß-methylbenzene butanoate—to block vascular receptors of leukotriene D₄ (LTD₄) was examined in the conscious rat. Hindquarter (HQ), renal, and mesenteric blood flow and vascular resistance were evaluated in the conscious rat chronically equipped with miniaturized Doppler probes for organ blood flow measurement by directional pulsed Doppler technique. In addition, cardiac output was measured by thermodilution technique in conscious rats equipped with minithermists in the ascending aorta. Systemic hemodynamic variables, mean arterial pressure, and heart rate were monitored through femoral catheters. LTD₄ (1 or 10 μg/kg) produced a marked dose dependent increase in the mesenteric vascular resistance associated with a marked decrease in blood flow whereas no consistent effects were demonstrated in the renal circulation. LTD₄, at 1 μg/kg, increased the HQ blood flow whereas the higher dose of LTD₄ produced a biphasic response: an early increase followed by a decrease in blood flow. Infusion of LTD₄, 3 μg/kg per min over 10 min decreased cardiac output and increased total peripheral resistance. L-649,923 (10 or 30 mg/kg, i.v.) effectively blocked the LTD₄-induced mesenteric constriction and the second phase of HQ vasoconstriction but did not modify the LTD₄ induced HQ vasodilation. L-649,923 also effectively attenuated the cardiac effects of LTD₄ infusion. These studies suggest that L-649,923 could preserve cardiac and vascular functions in pathologic states mediated by cysteinyl leukotrienes, such as traumatic or endotoxin shock. Key Words: Leukotriene D₄—Cardiovascular system—Leukotriene antagonist—Mesenteric blood flow—Renal blood flow—Hindquarter blood flow—Anaphylaxis.

Cysteinyl leukotrienes (LT) are potent mediators produced by the 5-lipoxygenase pathway of arachidonic metabolism (1). The LTs have been shown to be extremely potent substances in producing pulmonary derangements (2,3) similar to the human disorder asthma (4), and to produce typical inflammatory reactions including wheal and flare (5,6). Furthermore, LTs have been implicated in several trauma situations such as burns, bone fracture, and surgical wounds (7–10) as well as endotoxic shock (11,12). Cardiac and vascular derangements seem to be of primary importance in leukotriene-induced shock states (for review see ref. 13).

The potential involvement of LTs in multiple pathophysiologic processes stimulated substantial efforts in designing and testing compounds that might antagonize LT actions in various organs. Several LT antagonists have already been tested and described in detail (for review see ref. 14) with the primary view of prophylactic or therapeutic capacity in asthma. However, all the LT antagonists described so far demonstrated only limited potency and partial specificity. Furthermore, only a limited number of studies attempted to examine the capacity of LT antagonists to block the vascular receptors of LTs. Vascular actions of such antagonists might be an essential part of a protective effect in situations where systemic cardiovascular derangements (e.g., cardiac anaphylaxis, shock) rather than pulmonary are the primary pathophysiologic processes.

Our laboratory has previously described the capacity of two known LT antagonists, FPL-55712 and LY-171883, to block vascular responses to...
LTD₄ and LTE₄ (15). Whereas both compounds have demonstrated partial blocking capacity of the constrictive effect of LTD₄ on specific vascular beds, the doses that were necessary to produce the protective action were quite high and elicited intrinsic systemic and regional hemodynamic responses (15).

More recently, a new LT antagonist, L-649,923, has been described (16). This compound has been shown to be a competitive antagonist of the pulmonary effects of LTD₄ in vitro and in vivo (16). However, the capacity of L-649,923 to block cardiac and vascular LTD₄ receptors has not been studied as yet.

In this paper we have examined the capacity of L-649,923 to block LTD₄ actions on selected vascular beds and the cardiac output of the conscious rat. This species has been selected for these studies because LTs produce no consistent pulmonary effects and have no effect on platelet aggregation (17). Furthermore, by utilizing the directional pulsed Doppler velocimetry for blood flow measurements it is possible to examine several discrete vascular responses instantaneously in the conscious state that cannot be accomplished by other methods.

**MATERIALS AND METHODS**

Male Sprague-Dawley rats (Taconic Farms) weighing 250–350 g were used in all studies (n = 26). Surgical procedures were performed under anesthesia after intramuscular injection of ketamine (130 mg/kg) and acepromazine (1.3 mg/kg) solution. Experiments were carried out on the conscious unrestrained rats 1–3 days after operation. All rats were housed individually in standard plastic cages with food and water ad libitum.

**Regional blood flow measurements with directional pulsed Doppler technique**

The directional pulsed Doppler method was selected to measure organ blood flow in the hindquarter, renal, and mesenteric vessels. Although it does not allow quantitative blood flow monitoring, this method is superior compared with other available techniques because: (a) it can be used chronically in conscious animals; (b) it allows continuous on-line recording of blood flow; and (c) it can detect instantaneous and transient changes in blood flow within seconds after the drug administration. Haywood et al. (18) have shown that the velocity signals recorded from the Doppler flow probes are directly and reliably proportional to changes in true volume flow measured by electromagnetic flowmetry.

Miniaturized Doppler flow probes were implanted around the lower abdominal aorta for hindquarter (HQ) blood flow measurement. The superior mesenteric (M) and the left renal (R) arteries were also implanted with such probes by the method previously described by Haywood et al. (18). Briefly, a midline laparotomy was performed, and the M, R, and lower abdominal aorta above its bifurcation were carefully isolated under a dissecting microscope. Miniaturized Doppler flow probes (Valpey-Fisher) were then loosely sutured (6-0 silk) around each vessel. The insulated wire leads of the probes were tunneled beneath the skin, exteriorized at the nape of the neck, and soldered to a receptacle that was then attached to the skull with small screws and dental acrylic. The femoral artery and vein were cannulated with polyethylene catheters (PE-50) that were also tunneled beneath the skin to exit at the neck. Catheters were regularly flushed (0.2 ml) with heparinized saline (100 U/ml) and secured by a soft spring wire attached to the animal's neck with an adhesive collar.

On the day of the experiment the arterial line was connected to a pressure transducer (Narco Bio-Systems model RP 1500i) coupled to a strain gauge coupler (Narco Bio-Systems type 7179) and a biotachometer coupler (Narco Bio-Systems type 7032). Mean arterial blood pressure (MAP) and heart rate (HR) were continuously recorded on a Narcotrace 80 computerized dynograph and sampled (automatically or manually) at 15–60 s intervals by a Northstar-Hazeltine computer. A connector line was attached between the blood flow receptacle and a directional pulsed Doppler flowmeter (University of Iowa, Bioelectrical Engineering). Output signals of Doppler shifts >4 kHz were simultaneously transformed to mean flow velocity (kHz) and recorded continuously on the Narcotrace 80 through universal couplers (type 7178). Local vascular resistance was calculated by dividing the MAP by the Doppler shift in kilohertz, as previously described (15). It is noteworthy that measurements of MAP from the femoral artery cannula in the rat are virtually the same as the MAP in other arteries (e.g., brachial, mesenteric) in normal, hypotensive, or hypertensive states (19). Changes in regional blood flow and local vascular resistance were further expressed as percent changes from control values. No differences in the basal signal level were found between the various experimental groups.

**Experimental protocols**

On the day of experiment each animal was allowed to stabilize for at least 30 min while basal (control) levels of the hemodynamic variables were recorded. Vehicle injections (0.9% NaCl, 300 μL i.v.) were used as controls for injection effects. To assess the consistency of the various vascular responses, a bolus injection of norepinephrine (0.3–1 μg/kg i.v.) was used; this test dose of norepinephrine caused a prompt increase in MAP, a decrease in HR, and a decreased blood flow in HQ, R, and M. After the stabilization period each animal was scheduled to one of the following protocols: (a) administration of LTD₄ (1 or 10 μg/kg, i.v.) as bolus injections in ascending order; or (b) administration of LTD₄ (1 or 10 μg/kg, i.v.) 15 min after treatment with the LT antagonist L-649,923 (10 or 30 mg/kg, i.v., given over 0.5–1 min).

**Measurement of cardiac output**

In eight additional rats, the effect of L-649,923 on LTD₄ actions on cardiac output and total peripheral resistance (TPR) was investigated. Because bolus injections of LTD₄ induced only transient changes in hemodynamic variables that are not possible to detect by using the thermodilution method, a continuous infusion of LTD₄ was used in these experiments. The rats were anesthetized with pentobarbital (40 mg/kg i.p.) and PE-50 tubing was inserted into the femoral artery and vein. These catheters were tunneled beneath the back skin and exited at the back of the neck as previously described. An incision was made
TABLE 1. Hemodynamic effects of L-649,923 in the conscious rat

<table>
<thead>
<tr>
<th></th>
<th>ΔMAP (mm Hg)</th>
<th>ΔHR (beats/min)</th>
<th>Δ Blood flow (%)</th>
<th>Δ Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HQ</td>
<td>R</td>
</tr>
<tr>
<td>Phase 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>-5 ± 1</td>
<td>3 ± 12</td>
<td>+20 ± 6a</td>
<td>-28 ± 18</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>-38 ± 5b</td>
<td>-135 ± 33</td>
<td>-90 ± 5b</td>
<td>-92 ± 4a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+111 ± 39a</td>
<td>-75 ± 16b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-10 ± 5</td>
<td>+173 ± 108b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+807 ± 275a</td>
<td>+693 ± 250a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+1565 ± 756a</td>
<td></td>
</tr>
<tr>
<td>Phase 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>+13 ± 2a</td>
<td>-46 ± 8b</td>
<td>+6 ± 5</td>
<td>+41 ± 14a</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>+24 ± 4b</td>
<td>-51 ± 11b</td>
<td>+15 ± 6a</td>
<td>+19 ± 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-13 ± 4</td>
<td>+72 ± 42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+4 ± 3</td>
<td>+15 ± 4a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-19 ± 8</td>
<td>+8 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-49 ± 8b</td>
<td>-28 ± 15</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; HQ, hindquarter; R, renal; M, mesenteric; n, 6–10. Maximal changes recorded 5–10 min after drug administration. L-649,923 was injected intravenously. Values indicate mean ± SEM.

Significance from baseline: *p < 0.05, **p < 0.01 (Student-Newman-Keul test).

at the midline of the neck from the cricoid to the clavicle, and PE-50 tubing was inserted into the right atrium through the external jugular vein. The left common carotid artery was exposed and ligated, and a thermistor (MX2-780-33 model THMP f 1.5, Teflon reusable, Columbus Instruments) was advanced through the carotid into the ascending aorta (placement above the aortic valve was confirmed in each animal at the end of the experiment and by the shape of the dilution curve). All lines were secured by a soft spring wire through the cage as described earlier. Twenty-four hours after surgery, the arterial line was connected to a blood pressure transducer for hemodynamic recordings.

The cardiac output was measured by thermodilution technique as previously described (20). In brief, the thermistor was attached to the computerized Cardiomax II (CMX2-780-k with the microprobe option R, Columbus Instruments). The dead space of the venous line was first flushed with 0.05 ml of 0.9% (wt/vol) NaCl (saline) at room temperature (22°C); after a brief stabilization period (10 s to ensure normal core temperature) an additional injection of 0.2 ml normal saline (22°C) was rapidly injected using a 1 ml syringe. Cardiac output was recorded in the following way: a control period of 15 min included two to three cardiac output measurements to test for consistency and placement of the probe and also to get control values for MAP and HR. Continuous infusion of LTD4 (3 μg/kg-1 per min-1) over 10 min (total LTD4 dose 30 μg/kg) into the jugular vein (27 μL/min) was done by means of a Harvard infusion pump (Harvard Instruments) before and 15 min after treatment with L-649,923 (30 mg/kg). The timer on the automatic data collection system was started at the beginning of the LTD4 infusion, and data points were taken at time (t) 1, 5, 10, 15, and 30 min. The animal was allowed to completely recover from the first LTD4 infusion (at least 1.5 h) before the adminis-

FIG. 1. Original tracing of the hemodynamic responses to L-649,923 in the conscious rat. AP, arterial pressure; HQ, hindquarter; R, renal; M, mesenteric. Values above the pressure tracing denote levels of mean arterial pressure and heart rate at the baseline 30 s and 3 min after the injection of L-649,923 intravenously.

Dissolved in saline before its use. Each aliquot was thawed only once and diluted with distilled water and kept frozen (−70°C) until used. LTD₄ was aliquoted in saline before its use. Each aliquot was thawed only once and diluted with distilled water and kept frozen (−70°C) until used. LTD₄ was dissolved in saline before its use. Each aliquot was thawed only once and diluted with distilled water and kept frozen (−70°C) until used. LTD₄ was dissolved in saline before its use. Each aliquot was thawed only once and diluted with distilled water and kept frozen (−70°C) until used. LTD₄ was provided by Merck Frosst. LTD₄ was aliquoted in distilled water and kept frozen (−70°C) until used. LTD₄ was dissolved in saline before its use. Each aliquot was thawed only once and diluted with distilled water and kept frozen (−70°C) until used. LTD₄ was thawed only once and diluted with 0.9% NaCl to a final volume of 200–300 µl for injection into the animal.

**Drugs**

Pure synthetic LTD₄ and L-649,923 were kindly provided by Merck Frosst. LTD₄ was aliquoted in distilled water and kept frozen (−70°C) until used. LTD₄ was dissolved in saline before its use. Each aliquot was thawed only once and diluted with 0.9% NaCl to a final volume of 200–300 µl for injection into the animal.

**Statistics**

Data are presented in text and figures as mean ± SEM for the indicated number of rats. Analysis of variance (ANOVA) with repeated measures and ANOVA followed by Student-Newman-Keuls test, Dunnett’s test, or Kruskal-Wallis test was used for statistical evaluation of the data. Where appropriate, a two-tailed paired Student’s t-test was also used. Differences were considered significant when the p-value was <0.05.

**RESULTS**

**Influence of L-649,923 on blood pressure and heart rate responses to LTD₄**

L-649,923 at the higher dose (30 mg/kg i.v.) induced initial ("phase 1") hypotensive (−37 ± 5 mm Hg, p < 0.01) and strong bradycardic (−135 ± 33 beats/min, p < 0.01) responses. These effects became apparent 30 s after the drug administration and were followed by the pressor effect ("phase 2"), which reached its maximum 5 min after the drug injection and subsided in 15 min (Table 1, Fig. 1). The baseline values of hemodynamic parameters immediately before LTD₄ administration were not different in L-649,923-treated rats compared with saline-treated rats (Table 2). Intravenous injection of LTD₄ induced moderate increases in blood pressure and heart rate (Fig. 1).

**L-649,923 blocked the pressor response to the low dose of LTD₄ (1 µg/kg) but only partially attenuated the effect of the high dose (Fig. 2).**

**Influence of L-649,923 on blood vessel responses to LTD₄**

L-649,923 induced transient dilator/constrictor responses in HQ, R, and M blood vessels (Fig. 1, Table 1). At the low dose (10 mg/kg) an initial renal vasoconstriction followed by a vasodilation in all three vascular beds was observed whereas the higher dose (30 mg/kg) induced vasoconstriction in all three vascular beds. However, all these responses subsided within 5–10 min. The blood flow values before LTD₄ administration in L-649,923-treated animals did not significantly differ from levels after saline (Table 2).

**FIG. 2. Maximal changes in mean arterial pressure and heart rate after LTD₄ administration in L-649,923-treated rats. LTD₄ (1–10 µg/kg) was injected intravenously before (open bars) and 15 min after intravenous injection of L-649,923 at doses (10 mg/kg (hatched bars) and 30 mg/kg (cross-hatched bars)). Vertical bars denote SEM. Asterisk indicates statistical significance by Student-Newman-Keuls test (*p < 0.05).**

**TABLE 2. Baseline values of mean arterial pressure (MAP), heart rate (HR), regional blood flow, and vascular resistance before LTD₄ administration in the conscious rat**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>S</th>
<th>L 10</th>
<th>L 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>11</td>
<td>114 ± 4</td>
<td>121 ± 4</td>
<td>126 ± 4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>11</td>
<td>394 ± 14</td>
<td>371 ± 11</td>
<td>359 ± 15</td>
</tr>
<tr>
<td>Blood flow (kHz)</td>
<td>11</td>
<td>3.7 ± 0.5</td>
<td>3.4 ± 0.6</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>HQ</td>
<td>11</td>
<td>3.2 ± 0.6</td>
<td>3.4 ± 0.6</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>R</td>
<td>10</td>
<td>5.1 ± 0.7</td>
<td>5.5 ± 0.6</td>
<td>5.8 ± 0.7</td>
</tr>
<tr>
<td>M</td>
<td>10</td>
<td>36 ± 4</td>
<td>42 ± 5</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>Resistance (mm Hg/kHz)</td>
<td>11</td>
<td>44 ± 5</td>
<td>45 ± 6</td>
<td>47 ± 7</td>
</tr>
<tr>
<td>HQ</td>
<td>10</td>
<td>23 ± 2</td>
<td>27 ± 5</td>
<td>27 ± 5</td>
</tr>
</tbody>
</table>

Values (mean ± SEM) indicate levels immediately before the commencement of LTD₄ administration after pretreatments with saline (s). 10 mg/kg L-649,923 (L 10), or 30 mg/kg (L 30). HQ, hindquarter; R, renal; M, mesenteric; n, number of rats; LTD₄, leukotriene D₄.
Effect of L-649,923 on cardiac output response to LTD₄ infusion

Infusion of LTD₄ to the conscious rat produced a slight increase in mean arterial pressure and heart rate (Fig. 6). These changes were not significantly affected by L-649,923. LTD₄ infusion produced a marked decrease in cardiac output that was accompanied by an increase in TPR (Fig. 7); both variables slowly returned to basal levels upon cessation of the infusion. L-649,923, at a dose of 30 mg/kg, did not significantly attenuate the initial decrease in cardiac output and the increase in TPR produced by LTD₄ infusion but totally blocked the later reduction in cardiac output and increase in TPR (Fig. 7).

DISCUSSION

The cardiac and vascular responses produced by LTD₄ in this study are consistent with previous reports in the anesthetized and conscious rat (15,17, 21,22). The profound decrease in cardiac output is most probably the result of reduction in O₂ delivery to the heart due to decrease in coronary blood flow because LTD₄ has been shown to produce profound coronary constriction in the rat coronary...
L-649,923 as a Leukotriene Antagonist

The vascular responses to LTD4 in the rat are most probably mediated by LTD4 receptors on the vascular smooth muscle because they are not blocked or modified by any antagonist of the known autacoids (24) nor are they mediated by cyclooxygenase metabolites of arachidonic acid (15).

The nature of the vascular receptors of LTs has been only sparsely explored. Most of the pharmacologic studies using LT antagonists have focused on bronchopulmonary preparations or other nonvascular smooth muscles (e.g., ileal). However, blockade of the coronary and other vascular receptors to LTs might be of critical importance in view of protecting the heart and other essential organs from the consequences of systemic anaphylaxis or other forms of tissue injury where large amounts of LTs are produced.

The two LT antagonists, FPL-55712 and LY-171883, have been shown to block the effect of LTD4 at doses that already produce intrinsic cardiovascular effects (15) and even these doses blocked only part of the vascular response. Thus, FPL-55712 (10 mg/kg) or LY-171883 (30 mg/kg) blocked approximately 50% of the vasoconstriction produced in the HQ, R, and M vessels in this same experimental model.

Interestingly, LY-171883 but not FPL-55712 also blocked the vasodilation produced by LTD4 or LTE4 in the HQ (15).

The newer LT antagonist tested in this study, L-649,923, produced clear dose dependent pressor effects in the conscious rat that followed a brief initial hypotension and bradycardia. Furthermore, L-649,923 produced biphasic effects in the blood vessels tested. The low dose increased the blood flow in the HQ, R, and M circulation, in part because of direct vasodilation; the higher dose produced a secondary constrictor response. The nature of the vasodilation and the secondary vasoconstriction produced by this drug is still obscure. Some intrinsic agonistic activity of L-649,923 might be preserved in this preferential antagonist of leukotrienes. Release of pressor mediators (e.g., catecholamines) is contradicted by the finding that a profound hypotensive response accompanied the vasoconstriction. In any case, the hemodynamic responses provoked by L-649,923 were short lasting and all the hemodynamic indexes were normal at the time of the LTD4 administration.

L-649,923, at a dose of 30 mg/kg, completely blocked the increase in the mesenteric vascular resistance after 1 μg/kg LTD4 and preserved the flow to the mesenteric region; this dose of L-649,923 still...
efficacy of L-649,923 to block vascular receptors of LTD₄. Therefore, it is suggested that such a compound could exert a protective role in situations where coronary or other peripheral arterioles are exposed to constrictor levels of LTs, as anticipated in systemic anaphylaxis (25). However, further studies are necessary to fully explore the intrinsic activity of this compound and especially its potential acute constrictive effect on the coronary vessels.

Acknowledgment: This work was supported by Uniformed Services University of Health Sciences protocol No. G19218 and G19214. The opinions or assertions contained herein are the private ones of the author(s) and are not to be construed as official or as necessarily reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. There is no objection to its presentation and/or publication. The experiments reported herein were conducted according to the principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Medicine, National Research Council, DHEW Publication No. (NIH) 80-23, 1980. We thank Mrs. Rhoda Press for excellent technical assistance and Mrs. Laura Garza for preparing the manuscript.

REFERENCES


