Leukotriene B₄ promotes reactive oxidant generation and leukocyte adherence during acute hypoxia

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We have recently shown that an acute reduction of the inspired Po₂ initiates a rapid microvascular response characterized by enhanced leukocyte-endothelial interactions (39), followed by leukocyte emigration into the perivascular space, as well as increased vascular permeability (38). Evidence to date implicates an alteration in the balance between reactive oxygen species (ROS) and nitric oxide (NO) as a key event in the microvascular response to hypoxia. In support of this view, ROS formation (measured using fluorescent probes) was increased within the mesenteric microcirculation during hypoxia (37). Pretreatment with antioxidants attenuated the hypoxia-induced increase in ROS formation (37) as well as leukocyte adherence and emigration (39). Increasing tissue levels of NO, on the other hand, also attenuated leukocyte-endothelial interactions during hypoxia (39).

One potential consequence of oxidant stress due to an altered ROS/NO balance is local formation of lipid inflammatory mediators. These substances can be quickly produced on release of arachidonic acid from the cell membrane (22, 35), which is consistent with their potential role as mediators of the rapid microvascular response to hypoxia. In fact, hypoxia is known to increase circulating levels of one type of these mediators, leukotriene B₄ (LTB₄) (31), although no previous studies have examined its actions on the microcirculation in vivo under these conditions.

LTB₄ is a potent proinflammatory mediator that promotes leukocyte adherence and emigration and increases vascular protein leakage (14, 21). These responses, which are characteristic of acute inflammation, are the same as those noted in the mesenteric microcirculation during acute hypoxia. In addition, LTB₄ has been shown to increase ROS generation in polymorphonuclear (PMN) leukocytes (4, 34), which again is consistent with the responses to hypoxia. However, conflicting results have been reported showing no effect of LTB₄ on ROS generation in leukocytes (27).

The overall goal of this study was to evaluate the hypothesis that LTB₄ mediates microvascular responses to acute systemic hypoxia. If this were the case, we anticipated that 1) exogenous LTB₄ would produce similar effects on the microcirculation as observed during hypoxia and 2) LTB₄ receptor blockade should attenuate hypoxia-induced microvascular responses. Accordingly, experiments were designed to 1) examine the effects of exogenous LTB₄ on the mesenteric microcirculation, 2) verify the effectiveness of LTB₄-dimethylamide (LTB₄-DMA), an LTB₄ receptor antagonist (26), to prevent responses to exogenous LTB₄, and 3) evaluate the ability of LTB₄-DMA to attenuate hypoxia-induced microvascular alterations. Intravital microscopy was used to examine leukocyte-endothelial adhesive interactions and ROS generation in mesenteric venules of anesthetized rats.

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METHODS

All surgical and experimental procedures were approved by the Animal Care and Use Committee of the University of Kansas Medical Center. The University of Kansas is accredited by the American Association for the Accreditation of Laboratory Animal Care. Guidelines set by the National Institutes of Health and the Public Health Service Policy on the humane use and care of laboratory animals were followed at all times.

Surgical Preparation

Male Sprague-Dawley rats (200–300 g) were fasted overnight and anesthetized with an injection of urethane (1.5 g/kg im). Polyethylene catheters (PE-50) were placed into the right jugular vein for fluid administration and into the right common carotid artery for systemic arterial pressure measurement and blood sampling. A tracheotomy was performed using polyethylene tubing (PE-240).

Adhesive Interactions of Circulating Leukocytes with Mesenteric Venules

During all procedures, the animal's temperature was maintained at 36–37°C by using a homeothermic blanket system connected to an intrarectal temperature probe (Harvard Apparatus, Natick, MA). Lactated Ringer solution was infused via the jugular vein (2 ml/h) while blood pressure was continuously measured via the carotid artery cannula connected to a digital blood pressure monitor (Micro-Med, Louisville, KY). A radiocautery (Harvard Apparatus) was used to open the abdomen along the midline. The animal was then positioned on a Plexiglas sheet over the stage of a Zeiss Axiovert inverted microscope. A section of the small intestine was carefully exteriorized and positioned over a glass coverslip on the Plexiglas sheet so that the mesenteric venules could be viewed. The mesentery was then covered with a piece of Saran wrap to prevent drying of the tissue and to be viewed. The mesentery was then covered with a section of the small intestine with mesenteric venules. Venules were selected for experiments using the following criteria: 1) straight, unbranched vessels at least 100 μm in length, 2) diameters of 20–40 μm, 3) no adjacent vessels within 100 μm of the venule, and 4) two or fewer adherent leukocytes within a 100-μm segment of the venule during the control period. The mesentery was superfused (2 ml/min) with saline (37°C) to keep the tissue moist and warm.

Images of the mesenteric venules (×40 objective) were recorded on a video cassette recorder with a time-date generator (Panasonic) by using a video camera (Panasonic). Venular diameter was measured with a video caliper (Microcirculation Research Institute, College Station, TX). An optical Doppler velocimeter (Microcirculation Research Institute) was used to measure centerline red blood cell velocity within venules. Average red blood cell velocity was calculated as centerline velocity/1.6 (10). Venular wall shear rate, which represents the physical force generated at the vessel wall due to movement of blood, was calculated as 8 × (average red blood cell velocity/venular diameter) (17).

Adhesive interactions of leukocytes with mesenteric venules were assessed off-line during playback of the videotapes as follows: rolling leukocytes were defined as those leukocytes moving along the venular endothelium at a rate lower than red blood cell velocity. The velocity of rolling leukocytes was estimated by measuring the time it takes for a leukocyte to move between two points 100 μm apart along the vessel (39). Rolling velocity was measured for five leukocytes during each minute, and these values were then averaged to obtain a single estimate for this minute. The total number of rolling leukocytes passing a given point in the vessel was expressed as the number of leukocytes rolling per minute (rolling leukocyte flux). The total number of adherent leukocytes was determined in each minute by counting the number of leukocytes that remained stationary for more than 30 s (39).

Measurement of ROS Generation by Use of Dihydrorhodamine 123

Dihydrorhodamine 123 (DHR), an oxidant-sensitive probe, was used to assess ROS generation in the mesenteric microcirculation (37). Reactive oxidants, primarily hydroperoxides and hydroxyl radical, oxidize DHR to rhodamine 123, a fluorescent molecule. After the animal was allowed to stabilize after surgery, DHR (10 μg/kg iv) was injected into the animal. A period of 20 min was allowed for the probe to equilibrate within the microcirculation before the experiments. Fluorescence was recorded on videotape by using an intensified charge-coupled device camera (Hamamatsu Photonics, Shizouka, Japan) and later measured by image analysis (NIH Image, 1.61). The fluorescent signal was measured in five adjacent circles of 5-μm diameter along the vessel and averaged to obtain a single estimate of fluorescence intensity. The same field of view was maintained throughout the experiment to ensure that measurements of DHR fluorescence were obtained in the same section of the venule. Values for fluorescence were expressed as a percentage of the values observed during the normoxic control period.

Drugs and Chemicals

Solutions of LTβ4, LTβ4-DMA, anti-rat PMN antibody, bicarbonate buffer (pH 7.4), DHR, and t-butyl hydroperoxide were prepared on the day of the experiment. LTβ4 solutions were prepared in bicarbonate buffer. Potassium superoxide (KO2) was made immediately before superfusion onto the mesentery. The commercial sources for drugs used in this study are: LTβ4, Cayman Chemical (Ann Arbor, MI); LTβ4-DMA, Biomol (Plymouth Meeting, PA); anti-rat PMN antibody (AI-A51140), Accurate Chemical (Westbury, NY); urethane, t-butyl hydroperoxide, and KO2, Sigma Chemical (St. Louis, MO); and DHR, Molecular Probes (Portland, OR).

Experimental Protocols

Experiments began after a stabilization period of 30 min after surgery. Animals spontaneously breathed room air or hypoxic gas mixtures through a two-way valve (2384 series, Hans Rudolph, Kansas City, MO) attached to the tracheal tube. Arterial blood samples were collected at the end of each period and were analyzed for pH, PO2, and PCO2 with appropriate electrodes at 38°C and then adjusted to the rat's rectal temperature by use of temperature-correction factors for rat blood (13).

Series 1: LTβ4-induced leukocyte-endothelial adhesive interactions in normoxia. Animals were randomly assigned to a control group in which the mesentery was superfused with saline for 40 min or a treatment group in which the mesentery was superfused with saline and with 1, 5, and 20 nM LTβ4 for 10-min periods, with 10-min recovery periods between each dose. During the recovery periods, superfusates were carefully removed from the mesentery, which was then rinsed with saline.

The ability of the LTβ4 receptor antagonist, LTβ4-DMA, to attenuate LTβ4-induced responses was also examined.
protocol of these experiments was the same as described above except that a 5 mM concentration of LTB\textsubscript{4}–DMA was added to the superfusates.

**Series 2: LTB\textsubscript{4}–induced ROS generation in normoxia.** The protocol for these experiments consisted of a 10-min control period, a 20-min equilibration period after injection of DHR, and a 20-min treatment period with 20 nM LTB\textsubscript{4}. The ability of LTB\textsubscript{4}–DMA to attenuate LTB\textsubscript{4}–induced ROS generation was determined by superfusing the mesentery with 20 nM LTB\textsubscript{4} plus 5 mM LTB\textsubscript{4}–DMA. Recordings of DHR fluorescence were made for ~15 s at the end of each period.

To assess the contribution of adherent leukocytes to LTB\textsubscript{4}–induced ROS generation, leukopenic animals were also studied. Animals were given injections of anti-rat PMN antibody (0.5 ml in 1.5 ml saline ip) at 24 and 4 h before surgery. The protocol described above to measure LTB\textsubscript{4}–induced DHR fluorescence was used.

**Series 3: Effect of LTB\textsubscript{4} receptor blockade on hypoxia-induced leukocyte-endothelial adhesive interactions.** The protocol consisted of a 10-min control period, after which the mesentery was superfused with either saline or LTB\textsubscript{4}–DMA for the remainder of the experiment. Twenty minutes later, the animals were made hypoxic for 10 min; this was followed by a 10-min normoxic recovery period.

**Series 4: Effect of LTB\textsubscript{4} receptor blockade on hypoxia-induced ROS generation.** Animals were randomly assigned to groups in which the mesentery was superfused with either saline or LTB\textsubscript{4}–DMA (5 mM). The protocol consisted of a 10-min control period, a 20-min equilibration period after intravenous injection of DHR, superfusion of saline or LTB\textsubscript{4}–DMA, a 10-min hypoxic period, and a 10-min normoxic recovery period. Recordings of DHR fluorescence were made for ~15 s at the end of each period.

**Series 5: Effect of LTB\textsubscript{4}–DMA on oxidant-induced DHR fluorescence.** The goal of these experiments was to determine whether LTB\textsubscript{4}–DMA interferes with oxidant-induced DHR fluorescence in vivo or in vitro. In the first in vivo experiments, the protocol consisted of a 10-min control period, then a 20-min equilibration period after the injection of the fluorescent probe, followed by topical application of either 1 M KO\textsubscript{2} or 1 M KO\textsubscript{2} plus 5 mM LTB\textsubscript{4}–DMA to the mesentry. Recordings of DHR fluorescence were made during ~15 s at the end of the 20-min equilibration period and when KO\textsubscript{2} solutions were applied to the mesentry. The protocol for the next in vivo experiments was the same except that solutions of 10\textsuperscript{-8}, 10\textsuperscript{-6}, and 10\textsuperscript{-3} M t-butyl hydroperoxide with and without LTB\textsubscript{4}–DMA (5 mM) were applied to the mesentry for 1-min periods. The solutions were carefully removed, and the mesentery was rinsed with saline after each period. Recordings of DHR fluorescence were made for ~15 s at the end of each period.

In the in vitro experiments, 1 ml of either KO\textsubscript{2} (1 M) or t-butyl hydroperoxide (10\textsuperscript{-8}, 10\textsuperscript{-6}, or 10\textsuperscript{-3} M) was added to a Petri dish containing 1 ml of the DHR solution with or without LTB\textsubscript{4}–DMA (5 mM). Fluorescence intensity was measured during ~15 s after the DHR solution was placed in the dish and immediately after addition of the oxidants.

**Statistical Analysis**

Data are presented as means ± SE. Analysis of variance with Bonferroni’s pairwise comparison of means, Student’s t-test, and paired t-test were used to compare groups (Statistix 4.0 Analytical Software, St. Paul, MN). Values of P < 0.05 were considered to be statistically significant.

**RESULTS**

**Series 1: LTB\textsubscript{4}–Induced Leukocyte-Endothelial Adhesive Interactions in Normoxia**

Table 1 shows results from the experiments carried out to determine the dose-related effects of exogenous LTB\textsubscript{4} on leukocyte adherence, rolling flux, and venular shear rate, as well as the effectiveness of the LTB\textsubscript{4} receptor antagonist in blocking the effects of LTB\textsubscript{4}. Leukocyte-endothelial adherence progressively increased in response to higher doses of LTB\textsubscript{4} super- fused over the mesentery. Leukocyte rolling flux also increased with progressively greater doses of LTB\textsubscript{4}, whereas shear rate did not change significantly. The addition of LTB\textsubscript{4}–DMA to the superfusate completely blocked LTB\textsubscript{4}–induced leukocyte adherence and the increase in rolling flux. Shear rate was not significantly changed when LTB\textsubscript{4}–DMA was given in combination with LTB\textsubscript{4}.

**Series 2: LTB\textsubscript{4}–Induced ROS Generation in Normoxia**

The addition of exogenous LTB\textsubscript{4} to the mesenteric microcirculation significantly increased DHR fluorescence intensity compared with control levels (Fig. 1). Also, LTB\textsubscript{4}–DMA completely prevented LTB\textsubscript{4}–induced ROS generation. Finally, leukopenia did not significantly attenuate LTB\textsubscript{4}–induced DHR fluorescence in mesenteric venules (Fig. 1).

**Series 3: Effect of LTB\textsubscript{4} Receptor Blockade on Hypoxia-Induced Leukocyte-Endothelial Adhesive Interactions**

Figure 2 shows the effect of hypoxia on leukocyte adherence (top) and venular shear rate (bottom) in saline- and LTB\textsubscript{4}–DMA-treated animals. During the control period, the number of adherent leukocytes was

| Table 1. Effect of LTB\textsubscript{4} on leukocyte adherence, rolling flux, and venular shear rate during normoxia |
|-------------------------------------------------|------------------|------------------|------------------|
|                                                  | LTB\textsubscript{4} (n = 5) | LTB\textsubscript{4} + LTB\textsubscript{4}–DMA (n = 5) |
| Leukocyte adherence, leukocytes/100 μm           | Control 1 nM | 5 nM | 20 nM | Control 1 nM | 5 nM | 20 nM |
|                                                  | 0.8 ± 0.3 | 6.3 ± 1.8* | 9.3 ± 2.0† | 14.7 ± 2.2† | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.4 ± 0.2 | 0.0 ± 0.0 |
| Leukocyte rolling flux, leukocytes/min           | Control 1 nM | 5 nM | 20 nM | Control 1 nM | 5 nM | 20 nM |
|                                                  | 36.7 ± 3.6 | 49.0 ± 5.3† | 60.2 ± 10.7† | 69.0 ± 6.0† | 54.4 ± 7.3 | 55.8 ± 6.6 | 50.4 ± 7.1 | 50.8 ± 8.7 |
| Shear rate, s\textsuperscript{-1}                | Control 1 nM | 5 nM | 20 nM | Control 1 nM | 5 nM | 20 nM |
|                                                  | 35 ± 50 | 376 ± 47 | 434 ± 69 | 414 ± 92 | 571 ± 71 | 548 ± 71 | 517 ± 65 | 493 ± 74 |

Values are means ± SE obtained during the 10th minute of each period. LTB\textsubscript{4}, leukotriene B\textsubscript{4}; LTB\textsubscript{4}–DMA, LTB\textsubscript{4}–dimethylamide. *P < 0.05 and †P < 0.01 vs. control values.
not significantly different from zero in either the saline- or the LTB4-DMA-treated rats. Hypoxia caused a rapid, progressive increase in the number of adherent leukocytes in saline-treated animals. During the normoxic recovery period, no further increase in leukocyte adherence occurred. Although a slight decrease was observed, the number of adherent leukocytes remained significantly greater than control values throughout the recovery period. In contrast, there was no increase in leukocyte adherence in the LTB4-DMA-treated animals during both hypoxia and the recovery period.

Hypoxia resulted in a significant decrease in shear rate in both the saline- and LTB4-DMA-treated rats (Fig. 2, bottom). Shear rate decreased to ~32% of the control values in both groups of animals. Because venular diameter did not change during hypoxia, the reduction in shear rate was entirely due to decreased blood velocity. Shear rate rapidly returned to baseline values in both groups during the normoxic recovery period.

Leukocyte rolling velocity significantly decreased during hypoxia in both the saline- and LTB4-DMA-treated groups. During recovery, rolling velocity increased compared with values during hypoxia. Leukocyte rolling velocity in the tenth minute of each period in saline-treated rats (n = 6) was: control, 33.6 ± 2.7 μm/s; hypoxia, 17.0 ± 1.6* μm/s; and recovery, 45.7 ± 7.5 μm/s; leukocyte rolling velocity in LTB4-DMA-treated rats (n = 5) was: control, 34.6 ± 7.2 μm/s; normoxia plus LTB4-DMA, 78.4 ± 10.2 μm/s; hypoxia plus LTB4-DMA, 41.4 ± 4.9* μm/s; and recovery plus LTB4-DMA, 102 ± 10.9 μm/s (*P < 0.01 vs. control in saline- and LTB4-DMA-treated rats). Leukocyte rolling flux did not significantly change during hypoxia in either group of animals: control, 41.8 ± 4.6 leukocytes/min; hypoxia plus saline, 35.3 ± 5.3 leukocytes/min; and recovery plus saline, 54.2 ± 6.9* leukocytes/min; control, 48.6 ± 6.7 leukocytes/min; normoxia plus LTB4-DMA, 48.2 ± 7.6; hypoxia plus LTB4-DMA, 48.0 ± 5.4 leukocytes/min; and recovery plus LTB4-

Fig. 1. Effect of leukotriene B4 (LTB4; 20 nM) on reactive oxygen species (ROS) generation in the mesenteric microcirculation. *P < 0.05 vs. control; †P < 0.01 vs. LTB4.

Fig. 2. Cumulative results for changes in leukocyte adherence (top) and shear rate (bottom) during the normoxic control, hypoxia, and normoxic recovery period. Saline, n = 6; LTB4-dimethylamide (DMA), n = 5.
After a 10-min hypoxic period, fluorescence intensity in the saline-treated animals increased over 1.6 times above control values ($P < 0.05$). Conversely, in the LTB4-DMA-treated animals, DHR fluorescence was increased by only 860.2% after 10 min of hypoxia, which was significantly less than that observed in the hypoxia plus saline group ($P < 0.01$ between groups).

Although the increase in ROS generation during hypoxia was reduced by LTB4-DMA-treatment, it remained significantly greater than control values ($P < 0.05$). In both groups, DHR fluorescence during normoxic recovery was significantly less than that observed during hypoxia.

Series 5: Effect of LTB4-DMA on Oxidant-Induced DHR Fluorescence

KO2 and $t$-butyl hydroperoxide significantly increased DHR fluorescence in mesenteric venules in vivo as well as in vitro (Table 2). LTB4-DMA did not diminish the oxidant-induced increase in ROS generation under either condition.

DISCUSSION

The results from this study demonstrate that LTB4 plays a major role in the mechanism of hypoxia-induced microvascular responses. This contention is based on the following: 1) exogenous LTB4 produced rapid increases in ROS levels and leukocyte adherence to mesenteric venules during normoxia, and 2) LTB4-DMA, a receptor antagonist to LTB4, prevented hypoxia-induced increases in ROS generation and leukocyte-endothelial adherence. In addition, LTB4-DMA also blocked the responses to exogenous LTB4 and did not attenuate DHR fluorescence produced by oxidants (KO2 and $t$-butyl hydroperoxide) either in vitro or in vivo. In this regard, the increased fluorescence intensity produced by KO2 administration should not be interpreted as showing that KO2 directly oxidizes DHR. KO2 rapidly degrades in solution to form hydrogen peroxide, which has been reported to be a potent stimulant of DHR oxidation (14). These results suggest that the effects observed with LTB4-DMA were related to its actions as a competitive antagonist of the LTB4 receptor rather than nonspecific actions, i.e., acting as an oxidant quencher or interfering with DHR cleavage or fluorescence.

Results from our previous studies pointed toward ROS generation as a key event in hypoxia-induced microvascular injury because interventions that blocked ROS formation also reduced leukocyte adherence and emigration as well as increased vascular permeability (37–39). An initiating role of ROS in hypoxia-induced leukocyte-endothelial adhesive interactions is consistent with recent studies implicating ROS as intracellular signals in various physiological processes (1, 7–9). According to this view, a possible sequence of events leading to microvascular alterations is the formation of lipid inflammatory mediators secondary to ROS generation. The present study clearly shows that the underlying mechanism is more complex than this, given that the LTB4 antagonist substantially reduced ROS generation, suggesting that the major proportion of ROS is formed after LTB4 production.

These results do not provide information regarding the source of LTB4 in hypoxia. It is unlikely that this inflammatory mediator originates from endothelial cells because they have been reported either to lack or to have extremely low levels of the enzyme 5-lipoxygenase, which converts LTA4 to LTB4 (16, 22, 23). On the other hand, mast cells and leukocytes are capable...
of producing significant amounts of LTB4 under some conditions. Mast cells are abundant within the mesentery adjacent to the microvasculature, where release of inflammatory mediators could readily alter endothelial function. Degranulation of mast cells and release of LTB4 are important events in the microvascular inflammatory response associated with ischemia/reperfusion and sepsis (12, 19, 25). In vitro studies have demonstrated that various agonists can stimulate LTB4 from leukocytes, in addition to mast cells (30). At the present time, however, it is not known whether hypoxia can directly cause LTB4 formation from either mast cells or leukocytes.

Our results suggest that LTB4 formation during hypoxia results in ROS generation. It is known that ROS may be generated in many different cell types. Our laboratory’s studies (37), including the present one, show that the venular endothelial layer as well as adherent leukocytes are important sites of ROS generation during hypoxia. In the present study, we found that leukopenia did not influence the increase in ROS in response to exogenous LTB4. On the other hand, we have observed that hypoxia in leukopenic rats is accompanied by an increase in ROS generation, although to a significantly lower level than that observed in rats with normal levels of circulating leukocytes (28). These results suggest that leukocytes are an important, but not the sole, source of ROS during hypoxia. The fact that administration of LTB4-DMA reduced the level of hypoxia-induced ROS generation in normal rats below that observed in leukopenic rats suggests that LTB4 stimulates both leukocyte-dependent and leukocyte-independent ROS formation.

The mechanism by which LTB4 is generated in hypoxia is not clear at the present time. Elevated circulating LTB4 levels have been observed in humans exposed to altitude, suggesting that this mediator could play a role in responses to hypoxia in intact organisms (31). LTB4 formation can be induced in several cell types by various stimuli, including increased intracellular calcium (24), cytokines, and other inflammatory mediators (36). Prominent among these is oxidative stress, which has been shown to be a potent stimulant of LTB4 production (15, 32). Thus, although our results suggest that LTB4 formation precedes most of the ROS generation during hypoxia, a role for ROS as an initiating event in hypoxia-induced microvascular responses cannot be ruled out, as evidenced by the fact that there is still some ROS generation in LTB4-DMA-treated animals. It has been clearly demonstrated that ROS may act as initiators of cellular responses in hypoxia in several systems, including endothelial cells (18), cardiomyocytes (11), and carotid body chemoreceptors (29). It is possible that ROS generated in leukocytes or mast cells may stimulate the formation of LTB4 and other lipid inflammatory mediators, which could eventually promote further ROS production in endothelial cells and leukocytes.

Although the mechanism of LTB4 generation in hypoxia is not yet clear, it is apparent that this mediator plays an important role in the vascular endothelial response to hypoxia. Our results, however, do not exclude the involvement of other lipid inflammatory mediators in this phenomenon. In fact, we recently observed that a receptor antagonist to platelet activating factor (PAF), a lipid inflammatory mediator formed from membrane phospholipids, also attenuated leukocyte adherence to mesenteric venules during hypoxia (6). In that study, pretreatment with WEB2086 (a PAF receptor antagonist) attenuated leukocyte adherence to mesenteric venules in rats breathing 10% O2. Taken together, the results from the previous as well as our present study implicate a central role for both LTB4 and PAF in the promotion of leukocyte-endothelial adhesive interactions during hypoxia. Several potential explanations could account for our observations that pretreatment with either the LTB4 or PAF receptor antagonist nearly completely prevented hypoxia-induced leukocyte adherence. First, hypoxia may result in subthreshold increases in tissue levels of each mediator, such that either alone is insufficient to promote leukocyte-endothelial interactions. However, if their actions were potentiated when acting in concert, microvascular responses could occur during hypoxia whereas an antagonist to either mediator would prevent these responses. In support of this possibility, several examples of potentiating interactions between LTB4 and PAF have been reported (20, 21, 33). A second possibility consistent with our data involves sequential actions of these inflammatory mediators, i.e., one mediator stimulates generation of the other. For example, PAF has been shown to stimulate LTB4 release from mast cells and leukocytes (2, 3, 5). In addition, antagonists to either of these inflammatory mediators have also been shown to nearly completely

**Table 2. Effect of LTB4-DMA on oxidant-induced DHR fluorescence**

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<th>KO2</th>
<th>ROS-Dependent Fluorescence, % of control</th>
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<td></td>
<td>In vitro</td>
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<tr>
<td></td>
<td>10^{-6} M</td>
</tr>
<tr>
<td>Saline</td>
<td>149 ± 13%*</td>
</tr>
<tr>
<td>LTB4-DMA</td>
<td>191 ± 17%†</td>
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Values are means ± SE. In vitro KO2 groups, n = 6; in vivo KO2 groups, n = 5; in vitro and in vivo t-butyl hydroperoxide groups, n = 6. DHR, dihydrorhodamine 123. *P < 0.05; †P < 0.01 vs. control.
prevent leukocyte adherence after ischemia and reperfusion (21).

In summary, this study demonstrates that the lipid inflammatory mediator LTB4 participates in the mechanism of microvascular responses to hypoxia. Local administration of LTB4 to the mesenteric microcirculation produced effects that were similar to those observed during systemic hypoxia, namely leukocyte-endothelial adherence and ROS generation. Blockade of LTB4 receptors prevented these microvascular alterations produced by exogenous LTB4 and by hypoxia. These results add a new dimension to our understanding of the mechanism of hypoxia-induced microvascular changes by showing that the actions of LTB4 are critical for increased ROS generation during hypoxia. Further studies are needed to define the cellular sources of inflammatory mediators and sites of ROS production, as well as their potential interactions, during hypoxia.

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