

COMPARISON OF THE DAMAGE-PROMOTING EFFECTS OF  
LEUKOTRIENES DERIVED FROM EICOSAPENTAENOIC ACID  
AND ARACHIDONIC ACID ON THE RAT STOMACH

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While leukotrienes are classically considered to be mediators of inflammation, their biological actions and the observation of increased production in some experimental models have led to the suggestion that they play a role in the pathogenesis of gastric ulceration (for a recent review, see reference 1). In recent years, there has been considerable interest in the possibility of dietary manipulation of inflammatory diseases, especially through increasing the dietary intake of fish oils. The rationale for these studies is that leukotrienes derived from the  $\omega$ -3 fatty acid eicosapentaenoic acid (EPA), which is a major constituent of marine oils, are generally less potent, in terms of their proinflammatory actions, than those derived from arachidonic acid (AA). For example, in one study, leukotriene B<sub>5</sub> was reported to be 10–30-fold less potent as a chemotactic factor for human neutrophils *in vitro* than leukotriene B<sub>4</sub> (LTB<sub>4</sub>) (2). There have been a number of recent reports of beneficial effects of fish oil diets in experimental models of gastric and duodenal ulcer (3–7). Whether or not these beneficial effects can be attributed to shifts in the production of leukotrienes by the stomach (e.g., from LTB<sub>4</sub> to LTB<sub>5</sub>) is not clear. This is in part because the damage-promoting effects of the leukotrienes derived from EPA are not known. The present study was therefore performed to characterize the effects of the EPA-derived peptido-leukotrienes, LTC<sub>5</sub> and LTD<sub>5</sub>, on the rat stomach, and to compare these effects with the arachidonate-derived LTC<sub>4</sub> and LTD<sub>4</sub>.

#### Materials and Methods

Male Wistar rats with weights in the 180–220 g range were obtained from Charles River Breeding Laboratories, Inc. (Montreal, Canada) and were fed standard chow pellets and tap water *ad libitum*. For 18–22 h before an experiment, the rats were deprived of food, but not water.

The rats were anesthetized with sodium pentobarbitone (60 mg/kg, *i.p.*). An *ex vivo* gastric chamber preparation was used, as described in detail previously (8). This preparation allows for direct viewing of the gastric mucosa throughout the experiment. Solutions can be directly applied to the mucosa and subsequently withdrawn from the chamber. Transmucosal potential difference was recorded continuously during the experiment using apparatus

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described previously (8). A carotid artery was cannulated for intra-arterial administration of the leukotrienes. The leukotrienes were administered via this route so that first-pass clearance by the liver and lung could be avoided.

*Effects on Susceptibility to Injury.* At the beginning of the experiment, a blood sample (75  $\mu$ l) was taken from the carotid cannula for determination of resting hematocrit. Each experiment consisted of six 10-min periods, with the solution bathing the mucosa (5.0 ml) being changed at the beginning of each period. During all periods except the third, the mucosa was bathed with 50 mM hydrochloric acid made isosmotic with mannitol. During the third period, the mucosa was "challenged" with 20% ethanol (vol/vol). All luminal bathing solutions were warmed to 37°C before being added to the chamber. At the end of each period, the luminal bathing solution was removed by syringe, weighed, then frozen for subsequent determination of protein concentration (9). An intra-arterial infusion of one of the leukotrienes or the vehicle was started at minute 17 (i.e., 3 min before the challenge with 20% ethanol) and was continued for 5 min. The following leukotrienes were tested: leukotrienes C<sub>4</sub>, D<sub>4</sub>, C<sub>5</sub>, and D<sub>5</sub>. Leukotrienes C<sub>4</sub> and D<sub>4</sub> were tested at doses of 0.1, 1, and 3  $\mu$ g/kg/min, while leukotrienes C<sub>5</sub> and D<sub>5</sub> were tested at doses of 1, 3, and 5  $\mu$ g/kg/min. At the end of the experiment, a second blood sample was taken from the carotid cannula for determination of hematocrit. The mucosa was photographed using color transparency film for subsequent planimetric determination of the surface area of the mucosa that exhibited hemorrhage (8).

*Effects on Gastric Blood Flow.* The rats were anesthetized and an ex vivo chamber was prepared, as described above. Gastric blood flow was measured using a laser-Doppler flowmeter, as has been described in detail elsewhere (10). A hard-tip pencil probe (P-431; TSI Inc., St. Paul, MN) was mounted in a micromanipulator over the chamber preparation. The probe was connected to a flowmeter (BPM 403A; TSI Inc.) for continuous recording of gastric blood flow. The probe was lowered onto the mucosal surface, and basal recordings were made for at least 20 min. The site of placement of the probe was random, except that it was always placed within the corpus region. Blood flow was recorded continuously, and basal readings were taken for at least 15 min before any leukotriene was tested. Each of the four leukotrienes used in the studies described above were infused intra-arterially for a total of 3 min. For LTC<sub>4</sub> and LTD<sub>4</sub>, the doses tested were 0.05, 0.1, and 1  $\mu$ g/kg/min. For LTC<sub>5</sub> and LTD<sub>5</sub>, the doses tested were 0.1, 1, 2, and 5  $\mu$ g/kg/min. After infusing one of the leukotrienes, gastric blood flow was allowed to return to basal levels before another test was started. The order of testing of the different leukotrienes and doses within a preparation were randomized. Each dose of each leukotriene was tested in at least four separate preparations.

*Statistical Analysis.* All data are expressed as the mean  $\pm$  SEM. Comparisons between groups of data were made using the student's *t* test, with the exception of the changes in hematocrit, where a paired *t* test was used.

*Materials.* All leukotrienes were kindly provided by Dr. A. W. Ford-Hutchinson of Merck-Frosst Research Laboratories. The leukotrienes were stored in aliquots at -70°C. On the day of an experiment, an aliquot was thawed and then diluted appropriately in PBS (pH 7.4).

## Results

*Effects on Susceptibility to Injury.* The resting transmucosal potential difference (PD) ranged between 45 and 55 mV (mucosa negative to serosa) in all experiments. In control rats receiving intra-arterial PBS, the topical application of ethanol caused a decrease in the PD of  $\sim$ 30 mV, followed by a gradual return to basal levels. By the end of the experiment, 30 min after removal of 20% ethanol from the chamber, the PD in the control group had recovered to  $96 \pm 6\%$  of basal levels. Hemorrhagic erosions formed during the 30-min period after the challenge with 20% ethanol, but were limited to the periphery of the chamber, and involved  $<1\%$  of the total glandular mucosa. The application of ethanol resulted in a marked increase in the concentration of protein in the luminal bathing fluid, but in subsequent periods the amount of protein efflux returned towards basal levels.

Intra-arterial infusion of LTC<sub>4</sub> or LTD<sub>4</sub> at a dose of 0.1 μg/kg/min did not significantly affect any of the three parameters of mucosal damage, when compared with the control group. However, when infused at doses of 1 or 3 μg/kg/min, both LTC<sub>4</sub> and LTD<sub>4</sub> produced significant increases in hemorrhagic damage area (Fig. 1) and protein efflux into the luminal solution (Fig. 2). Furthermore, the transmucosal PD in rats receiving intra-arterial LTC<sub>4</sub> or LTD<sub>4</sub> at these doses did not return to basal levels by the end of the experiment (Fig. 3). Significant differences between

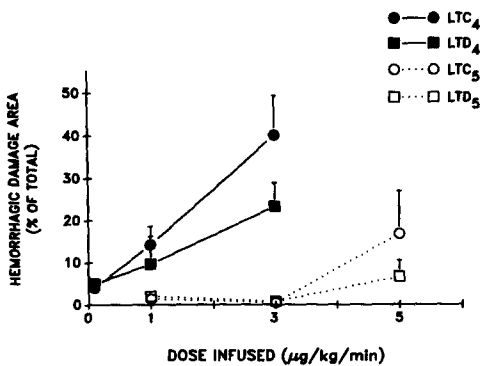


FIGURE 1. Extent of hemorrhagic damage to the gastric mucosa induced by topically applied 20% ethanol and intra-arterially infused leukotrienes. Each leukotriene was infused, at the doses shown, for 5 min. In the control group receiving the vehicle intra-arterially, the damage area involved <1% of the total glandular mucosa. Each point represents the mean ± SEM of at least four experiments.

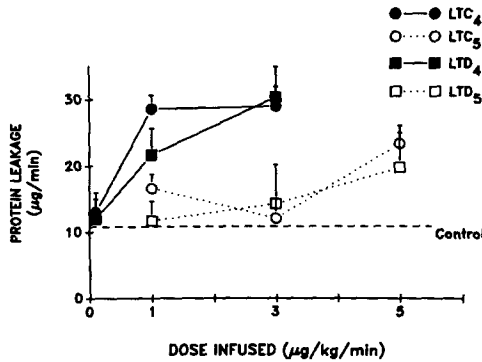


FIGURE 2. Effects of intra-arterial infusion of various leukotrienes on the leakage of protein into the gastric lumen during the 30-min period after topical application of 20% ethanol. The mean protein leakage for the control group, which received the vehicle intra-arterially, is indicated by the dotted line. LTC<sub>4</sub> and LTD<sub>4</sub> induced significantly ( $p < 0.05$ ) more protein leakage than the control group at doses of 1 and 3 μg/kg/min, while LTC<sub>5</sub> and LTD<sub>5</sub> significantly increased protein leakage only at the 5 μg/kg/min dose. Each point represents the mean ± SEM of at least four experiments.

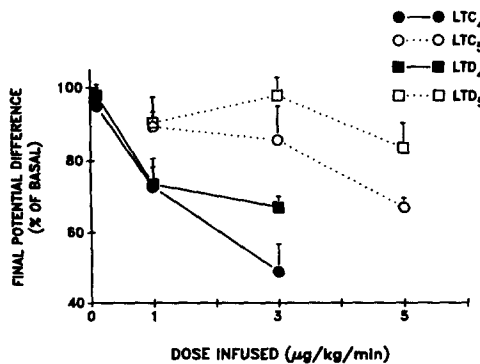


FIGURE 3. Final transmucosal potential difference values for groups of rats that received a leukotriene intra-arterially and topical application of 20% ethanol. These potential difference values were taken 30 min after the removal of ethanol from the gastric chamber. In the control group, the potential difference at the end of the experiment was 96 ± 6% of the basal level. LTC<sub>4</sub> and LTD<sub>4</sub> caused significant ( $p < 0.05$ ) reductions of the final potential difference when infused at doses of 1 or 3 μg/kg/min, whereas LTC<sub>5</sub> and LTD<sub>5</sub> only caused a significant depression of potential difference when infused at the 5 μg/kg/min dose. Each point represents the mean ± SEM of at least four experiments.

the effects of similar doses of LTC<sub>4</sub> and LTD<sub>4</sub> were not observed with any of the three parameters of damage.

Intra-arterial infusion of LTC<sub>5</sub> or LTD<sub>5</sub> at doses of 1 or 3  $\mu\text{g}/\text{kg}/\text{min}$  did not significantly affect hemorrhagic damage area, the efflux of protein into the lumen, or the recovery of transmucosal PD after topical application of 20% ethanol (Figs. 1-3). At a dose of 5  $\mu\text{g}/\text{kg}/\text{min}$ , both of the EPA-derived leukotrienes significantly increased hemorrhagic damage area and protein efflux, while only LTC<sub>5</sub> significantly affected the recovery of PD towards basal levels.

Marked differences between the AA- and EPA-derived leukotrienes were also observed when their effects on hematocrit were examined. For example, LTC<sub>4</sub> and LTD<sub>4</sub> produced small, but significant ( $p < 0.05$ ), increases in hematocrit at a dose of 3  $\mu\text{g}/\text{kg}/\text{min}$  ( $9.6 \pm 1.5\%$  and  $12.3 \pm 3.2\%$ , respectively). On the other hand, LTC<sub>5</sub> and LTD<sub>5</sub> did not produce significant changes in hematocrit at any of the doses tested.

*Effects on Gastric Blood Flow.* Intra-arterial infusions of LTC<sub>4</sub> or LTD<sub>4</sub> for 3 min produced marked reductions in gastric blood flow at doses of 0.1 or 1  $\mu\text{g}/\text{kg}/\text{min}$  (Fig. 4). While there were not significant differences between the magnitude of the reduction in blood flow with the two AA-derived leukotrienes, only LTD<sub>4</sub> produced a statistically significant reduction of flow at the dose of 0.1  $\mu\text{g}/\text{kg}/\text{min}$ . At the dose of 1  $\mu\text{g}/\text{kg}/\text{min}$ , the two AA-derived leukotrienes reduced gastric blood flow by 40-60%.

Infusion of the two leukotrienes derived from EPA, at doses of 0.1, 1, or 2  $\mu\text{g}/\text{kg}/\text{min}$ , did not significantly alter gastric blood flow (Fig. 4). At a dose of 5  $\mu\text{g}/\text{kg}/\text{min}$ , both LTC<sub>5</sub> and LTD<sub>5</sub> produced significant decreases in flow, and there was no significant difference between the two leukotrienes in terms of the magnitude of the reduction of flow.

## Discussion

Numerous studies have implicated a role for peptido-leukotrienes in the pathogenesis of experimental gastric ulceration (1). While a link has never been conclusively made, it is presumed that the vasoconstrictor actions of these leukotrienes in the gastric submucosa are responsible for the observed ability of these compounds to increase the susceptibility of the gastric mucosa to injury. It has further been suggested that effects of peptido-leukotrienes on vascular permeability may contribute to their "pro-ulcerogenic" actions (3).

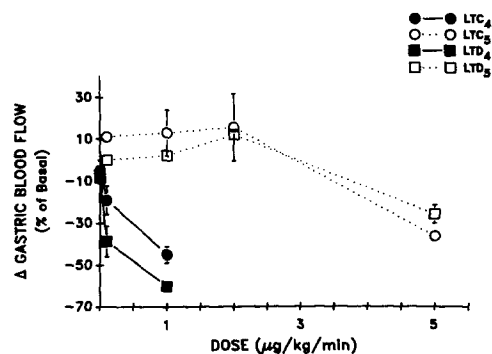


FIGURE 4. Effects of intra-arterial infusion of various leukotrienes (for 3 min) on gastric blood flow. Blood flow was measured by laser-Doppler flowmetry, and the changes in blood flow as shown represent the difference (percentage) between pre-infusion and post-infusion levels. LTC<sub>4</sub> significantly ( $p < 0.05$ ) reduced gastric blood flow at doses of 0.1 and 1  $\mu\text{g}/\text{kg}/\text{min}$ ; LTD<sub>4</sub> significantly reduced flow at a dose of 1  $\mu\text{g}/\text{kg}/\text{min}$ . LTC<sub>5</sub> and LTD<sub>5</sub> only produced a significant reduction of gastric blood flow when infused at the 5  $\mu\text{g}/\text{kg}/\text{min}$  dose. Each point represents the mean  $\pm$  SEM of at least four experiments.

Very little is known of the actions of the peptido-leukotrienes derived from EPA *in vivo*, in particular, their actions on the stomach. Most previous studies on EPA have focused on LTB<sub>4</sub> vs. LTB<sub>5</sub> (2, 11, 12), although there are also data from *in vitro* studies of smooth muscle contractility to suggest that LTC<sub>5</sub> is less potent as an agonist than LTC<sub>4</sub> (13). A number of recent studies have been performed in which the effects of fish oil diets on the resistance of the gastric mucosa to injury have been assessed (3-7). An underlying assumption of these studies is that the fish oil diet modifies eicosanoid production such that prostaglandins with more potent protective actions and/or leukotrienes with less potent pro-ulcerogenic actions are produced. The results of the present study show that there is indeed a clear difference in the potency of leukotrienes derived from AA vs. those from EPA. Regardless of which index of mucosal damage is used (i.e., damage area, protein efflux, PD), a similar pattern was observed. There were also marked differences in potency between the AA- and EPA-derived leukotrienes in terms of their effects on gastric blood flow. LTC<sub>4</sub> and LTD<sub>4</sub> were at least five times more potent than LTC<sub>5</sub> and LTD<sub>5</sub>. It seems likely that the changes in blood flow induced by the leukotrienes are an important component of the mechanism of action of increasing susceptibility to injury by 20% ethanol. It is possible, however, that other actions of these leukotrienes contributed to the increased susceptibility to damage observed after their administration.

It should be noted that the addition of significant quantities of fish oil or pure EPA to a diet will undoubtedly affect the synthesis of many other products besides leukotrienes. Whether or not changes in the synthesis of prostaglandins may explain the observations of increased mucosal resistance to injury has not yet been determined, although one group has reported that an EPA-derived prostaglandin (PGF<sub>3α</sub>) is, in fact, less potent as a protective agent than its AA-derived counterpart (PGF<sub>2α</sub>) (14). Clearly, further studies are required not only to clarify the role of leukotrienes in experimental and human gastrointestinal ulceration, but also to clarify the mechanism through which the addition of EPA or fish oil to the diet results in increased resistance of the gastroduodenal mucosa to injury.

### Summary

The ability of leukotrienes derived from eicosapentaenoic acid were compared with counterpart leukotrienes derived from arachidonic acid in terms of their ability to affect susceptibility of the stomach to injury induced by a topical irritant and their ability to alter gastric blood flow. Intra-arterial infusion of leukotriene C<sub>4</sub> (LTC<sub>4</sub>) and LTD<sub>4</sub> (0.1-3 μg/kg/min for 5 min) produced dose-dependent increases in gastric mucosal damage induced by topically applied 20% ethanol, as assessed macroscopically, by changes in transmucosal potential difference and by measurement of efflux of protein into the gastric lumen. Similar doses of LTC<sub>5</sub> or LTD<sub>5</sub> did not produce significant changes in any of these three parameters, when compared with control rats receiving the vehicle. With a higher dose of LTC<sub>5</sub> or LTD<sub>5</sub> (5 μg/kg/min), significant damage was observed. LTC<sub>4</sub> and LTD<sub>4</sub> were also found to be more potent at reducing gastric blood flow than LTC<sub>5</sub> and LTD<sub>5</sub>. These results demonstrate that the peptido-leukotrienes derived from eicosapentaenoic acid (LTC<sub>5</sub> and LTD<sub>5</sub>) are on the order of five times less potent than the leukotrienes derived from arachidonic acid (LTC<sub>4</sub> and LTD<sub>4</sub>), in terms of increasing the susceptibility of the gastric mucosa to damage and reducing gastric blood flow. These

results may have important implications in terms of the hypothesis that fish oil diets may be protective or may accelerate healing in ulcerative diseases of the gastrointestinal tract.

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