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LEUKOTRIENES IN MUCOSAL DAMAGE AND PROTECTION

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Exposure of the rat gastric mucosa to ethanol stimulates the generation of leukotriene (LTC_4) and 15-hydroxyeicosatetraenoic acid, but not of thromboxanes and prostaglandins. Lipoxygenase activation is not found with other topical irritants or nonsteroidal anti-inflammatory drugs. A number of gastroprotective drugs dose-dependently inhibit the stimulatory action of ethanol on mucosal LTC_4 formation closely parallel to their protective activity suggesting that ethanol-induced damage and activation of lipoxygenases may involve common targets which are simultaneously counteracted by certain types of protective agents. Selective inhibition of 5-lipoxygenase, however, does not confer protection against gastric mucosal damage caused by topical irritants or non-steroidal anti-inflammatory drugs. Thus, although leukotrienes may mediate certain reactions elicited by gastric ulcerogens such as submucosal venular constriction and mucosal microvascular engorgement, they do not appear to be major mediators of ulcerogen-induced tissue necrosis. The contribution of other products of the various pathways of arachidonic acid metabolism to gastric mucosal injury and the mechanism underlying the close interrelationship between protection and inhibiton of LTC_4 formation observed with certain compounds remains to be investigated.

Key words: Experimental ulcer, gastric mucosal damage, gastric mucosal protection, leukotrienes, lipoxygenase inhibitors, indomethacin, non-steroidal anti-inflammatory drugs

INTRODUCTION

Leukotrienes (LT) are products of arachidonic acid generated via the 5-lipoxygenase pathway. While LTB_4 is a potent chemotactic and chemokinetic agent, the cysteinyl leukotrienes C_4, D_4, and E_4 primarily have spasmogenic activity. Thus, cysteinyl leukotrienes are potent bronchoconstrictors, contract gastrointestinal smooth muscle and are constrictors in most vascular beds (1). When LTC_4 and LTD_4 are infused into the arterial supply of the isolated in situ perfused rat stomach they cause a rapid and marked decrease of vascular flow (2, 3). Furthermore, LTC_4,

but not LTD_4 was reported to produce venular constriction and vascular stasis in the rat gastric submucosa (4). Finally, LTC_4 and LTD_4 increase microvascular permeability thus promoting plasma exudation (5). Thus, Pihan et al. (6) reported that in the rat stomach intraarterial administration of LTC_4 or LTD_4 caused vascular injury as revealed by increased monastral blue deposition in mucosal collecting venules and submucosal vessels. In rats LTC_4 and LTD_4 administered alone do not cause visible damage to the gastric mucosa. Gastric mucosal injury induced by various noxious agents is, however, markedly aggravated in the presence of LTC_4 or LTD_4 (6, 7), an effect probably resulting from the impairment of mucosal blood supply elicited by the leukotrienes.

Gastric mucosal damage induced by topical irritants such as ethanol, strong acid or strong base are accompanied by constriction of submucosal venules and microcirculatory changes in the mucosa leading to a decrease in mucosal blood flow, vascular stasis, engorgement of microvessels and leakage of plasma proteins and blood cells (8, 9, 10). Whether local synthesis of chemical mediators is involved in eliciting such changes is not clear so far. The actions of exogenously administered cysteinyl leukotrienes resemble the vascular changes observed after exposure of the gastric mucosa to topical irritants (4). We, therefore, investigated whether endogenous leukotrienes are involved in mucosal injury caused by chemical agents. Since in a number of organ systems inhibition of the cyclooxygenase pathway of arachidonic acid metabolism can enhance formation of leukotrienes, we also studied whether increased synthesis of leukotrienes contributes to gastric mucosal damage caused by non-steroidal anti-inflammatory drugs.

METHODS

Experiments were performed in male Wistar rats, deprived of food, but not water for 24 hrs before the experiments. Rats were treated orally with 1.5 ml of absolute ethano or 1 ml of 25% NaCl, 0.2 N NaOH, 0.6 N HCl or acidified taurocholate (100 mM tauro cholate in 0.2 N HCl) or water. Five min after instillation of the irritant the stomachl were removed in ether anesthesia and mucosal damage was assessed using a scoring system based on the number and length of hemorrhagic lesions as described previously (11). Then fragments of the glandular mucosa between the necrotic bands were excised. Tissue aliquots of 40 mg were incubated in 0.5 ml of oxygenated Tyrode solution at 37°C for 10 min. Release of eicosanoids into the incubation media was measured radioimmunologically (11).

In additional experiments groups of 6—8 rats received graded doses of protective drugs or 5-lipoxygenase inhibitors by oral intubation 30 min prior to intragastric instillation of the necrotizing agent. Indomethacin-induced gastric lesions were produced by oral administration of 20 mg/kg of the drug. In these experiments lesion formation and ex vivo gastric mucosal eicosanoid generation was assessed 5 hrs after dosing of

indomethacin. In further experiments rats were pretreated orally 30 min prior to indomethacin (20 mg/kg) with the 5-lipoxygenase inhibitor A-63162 (12) and mucosal damage and ex vivo mucosal eicosanoid generation was assessed 5 hrs after indomethacin.

RESULTS

Effect of necrotizing agents on gastric mucosal leukotriene formation

Intragastric instillation of ethanol dose-dependently stimulates the release of LTC_4 from the gastric mucosa during a subsequent in vitro incubation (*Fig. 1*). High pressure liquid chromatography analysis shows that the rat gastric mucosa (under basal conditions as well as after ethanol challenge) releases practically exclusively LTC_4, while only trace amounts of LTD_4 and LTE_4 can be detected (11). Release of the cyclooxygenase-derived vasoconstricting and pro-ulcerogenic thromboxane is not enhanced by intragastric ethanol (11). Likewise, no significant increase in mucosal formation of prostaglandins is elicited by topical ethanol (*Fig. 1*). On the other hand, formation of 15-hydroxyeicosatetraenoic acid in the gastric mucosa is significantly increased by ethanol challenge suggesting activation of other lipoxygenases in addition to 5-lipoxygenase (13).

Hypertonic solution (25% NaCl), strong base (0.2 N NaOH), strong acid (0.6 N HCl) or acidified taurocholate introduced intragastrically cause severe mucosal damage. Only 0.2 N NaOH slightly increases mucosal LTC_4 formation. This effect is, however, inconsistent and reaches statistical

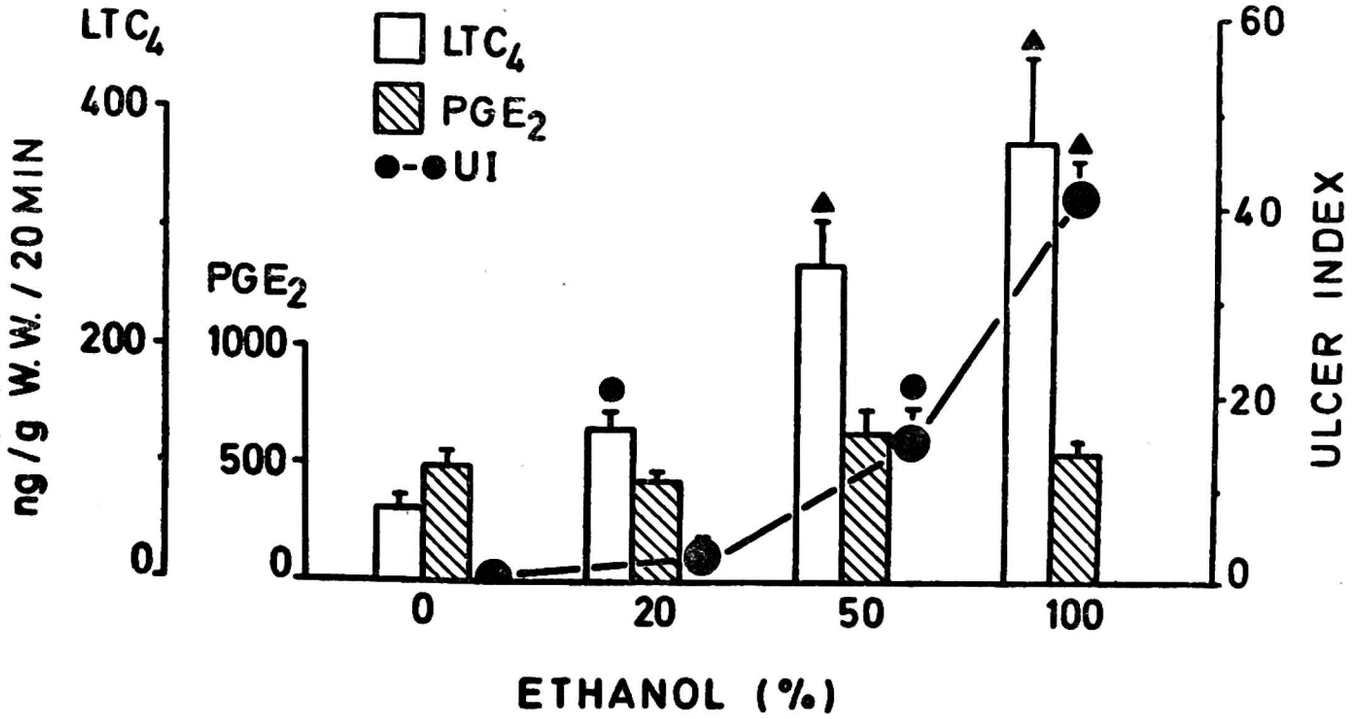

Fig. 1. Effect of intragastric instillation of graded doses of ethanol on ex vivo mucosal formation of LTC_4 and PGE_2 in rats in comparison with the production of hemorrhagic mucosal lesions (UI). ● $p < 0.01$; ▲$p < 0.005$ compared to water-treated controls. Data are partially derived from Peskar et al. (11).

Table 1. Effect of topical irritants on lesion production and LTC_4 formation in the rat gastric mucosa

	Control	NaOH (0.2 N)	HCl (0.6 N)	NaCl (25%)	TC (100 mM)
Lesion Index	1±1	44±5*	36±4*	46±6*	39±3*
LTC_4 (ng/g/10 min)	27±5	48±6*	14±3*	35±7	38±9

Gross mucosal damage and ex vivo LTC_4 formation was assessed 5 min after oral instillation of the irritants. Results represent the means±SEM of 6 rats each group. *p at least < 0.05 compared to controls. Data are derived from Peskar (13).

significance only in part of the experiments. Hypertonic solution and acidified taurocholate does not increase mucosal LTC_4 formation and HCl even decreases the release of measurable LTC_4 (*Table 1*). These findings indicate that the increased LTC_4 formation observed after ethanol exposure of the gastric mucosa is not just the consequence of tissue damage.

Effect of gastroprotective agents

To investigate if the increased formation of LTC_4 has a mediatory function in ethanol-induced mucosal injury we studied the effect of various gastroprotective agents on mucosal LTC_4 release. These experiments

Table 2. Effect of sulfhydryl-containing and -blocking agents, metals and non-steroidal anti-inflammatory drugs on lesion production and formation of LTC_4 and PGE_2 in the rat gastric mucosa after ethanol challenge

Compound	Dose mg/kg	Lesion Index	LTC_4	PGE_2
		% Inhibition		
Dimercaprol	30	86±1*	99±1*	92±2*
Cysteamine	250	88±4*	95±3*	67±4*
Diethylmaleate	250	91±5*	95±2*	71±5*
Iodoacetamide	50	85±5*	68±9*	50±3*
LiCl	20	96±1*	80±3*	51±6*
$ZnCl_2$	40	88±1*	81±4*	37±11
Aspirin	400	62±9*	89±1*	99±1*
Proquazone	45	86±4*	78±5*	91±6*
Diflunisal	400	74±5*	88±4*	60±2*

Rats were challenged with intragastric ethanol 30 min after oral drug treatment. Five min later gross mucosal injury and ex vivo mucosal eicosanoid formation was assessed. Values were calculated as % inhibition compared to the corresponding vehicle-treated groups and represent the means±SEM of 6 rats. * p at least < 0.01 compared to the corresponding control group. Data are derived from Peskar (13).

showed that certain protective compounds substantially suppress the stimulatory action of ethanol on gastric mucosal LTC_4, while others do not inhibit leukotriene formation even in the presence of near-maximal protection. Compounds with inhibitory action on leukotriene formation include certain non-selective lipoxygenase inhibitors such as nordihydroguaiaretic acid (11) or BW755C (14), sulfhydryl-containing or -blocking agents and metals (15) and certain non-acidic as well as salicylate-type non-steroidal antiinflammatory drugs (16, 17). The inhibitory effects of these agents on gastric mucosal LTC_4 formation are dose-dependent and closely parallel their protective activity. The protection afforded by compounds inhibiting LTC_4 formation cannot be explained by increased formation of protective prostaglandins. As shown in *Table 2*, most agents inhibit formation of PGE_2 (and also of 6-keto-$PGF_{1\alpha}$, data not presented) in addition to LTC_4 and some agents virtually abolish mucosal prostaglandin formation in doses conferring near-maximal protection (15). This indicates that in rat gastric mucosa inhibition of the 5-lipoxygenase pathway does not increase metabolism of arachidonic acid via the cyclooxygenase pathway. This may be due to the fact that cysteinyl leukotrienes and prostaglandins are generated in different types of cells. Alternatively, arachidonic acid which is not utilized by 5-lipoxygenase may rapidly be reacylated into phospholipid stores before reaching the site of cyclooxygenase. Extensive and rapid reacylation of liberated arachidonic acid

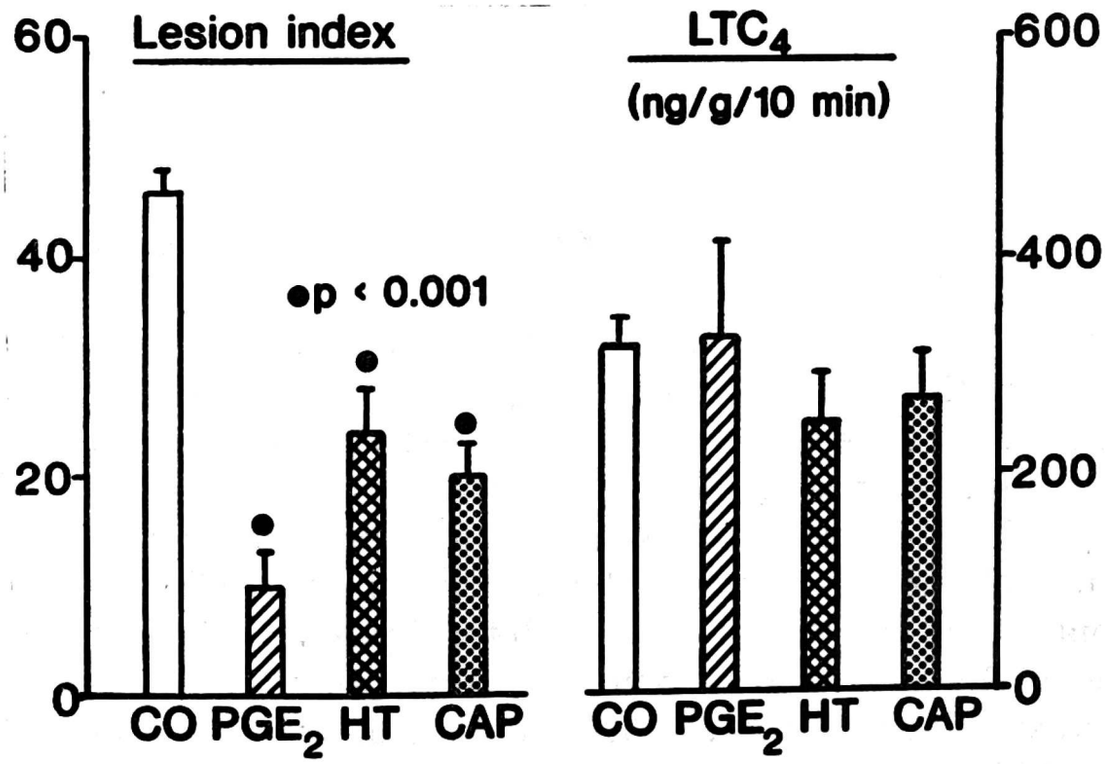

Fig. 2. Effect of oral treatment with PGE_2 (200 μg/kg), hydrotalcit (HT, 750 mg/kg) or capsaicin (CAP, 500 μg/kg) on gastric lesion production and ex vivo mucosal LTC_4 formation caused by intragastric ethanol. Values represent the means ± SEM of 6—8 rats.

controlling the levels of free fatty acid has been demonstrated to occur in certain cellular systems such as bone marrow-derived macrophages (18).

Sufficient generation of prostaglandins in the gastric mucosa is an important factor in the maintenance of mucosal integrity. Inhibition of prostaglandin formation by non-steroidal anti-inflammatory drugs may render the gastric mucosa more vulnerable against the effect of certain noxious agents and leads to mucosal ulceration. In contrast, drug-induced protection against an ulcerogen such as ethanol is completely independent of the endogenous prostaglandin system and can occur even when prostaglandin formation is fully suppressed (15). This indicates that the role of endogenous gastric prostaglandins differs in physiological mucosal defence mechanisms and pharmacologically induced protection against a noxious agent.

Not all protective compounds inhibit ethanol-stimulated gastric mucosal leukotriene formation. Thus, PGE_2 (19), the antacid hydrotalcit (20) and capsaicin (21) all protect against ethanol-induced gastric mucosal damage in the presence of full stimulation of LTC_4 formation (*Fig. 2*). This further supports the view that the mechanism underyling the increase in LTC_4 generation elicited by ethanol is not necrosis of mucosal cells. The results also indicate that the inhibition of leukotriene formation observed with certain gastroprotective agents is not due to lack of damage to the mucosa, but a phenomenon occuring parallel to protection.

Effect of selective 5-lipoxygenase inhibition

The marked stimulation of gastric mucosal LTC_4 formation after ethanol challenge and the close interrelationship between gastroprotection and inhibition of LTC_4 generation observed with a number of protective drugs initiated the hypothesis that LTC_4 may act as a mediator of mucosal damage. Indeed, in the rat stomach treatment with lipoxygenase inhibitors was shown to prevent the characteristic microcirculatory changes preceding ethanol-induced mucosal damage. Thus, in indomethacin-pretreated rats the constriction of arterioles and venules elicited by submucosal application of ethanol was inhibited by BW755C (22). Likewise, the mucosal hyperemia and venoconstriction induced by intraluminal ethanol was prevented by BW755C or nordihydroguaiaretic acid (10, 23). In the rabbit small intestine the selcective 5-lipoxygenase inhibitor L-651, 392 caused a significant reduction of intraluminal protein loss indicating protection against ethanol-induced microvascular damage

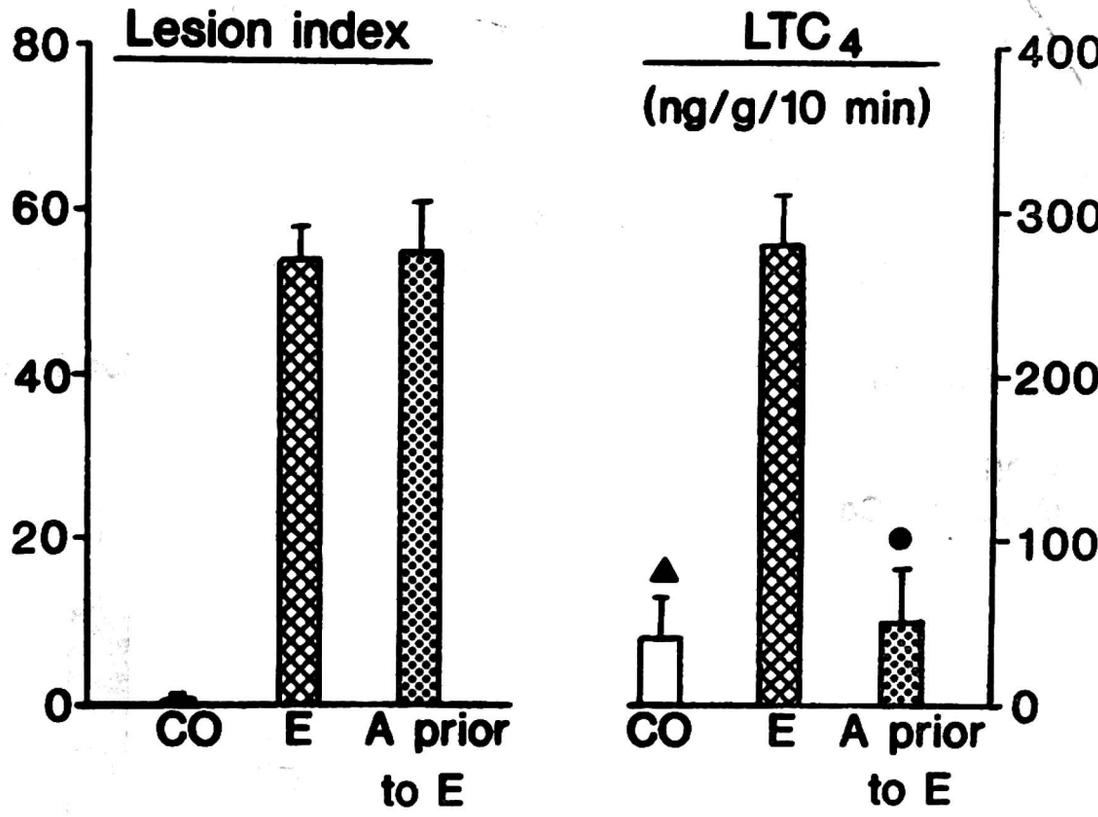

Fig. 3. EEffect of the 5-lipoxygenase inhibitor A-63162 on gastric damage and ex vivo mucosal LTC_4 release in ethanol-treated rats. A-63162 (A, 30 mg/kg) was administered orally 30 min prior to intragastric instillation of ethanol (E) and rats were sacrificed 5 min later. Controls (CO) received the corresponding vehicle. Values represent the means ±SEM of 6—8 rats.

(24). These findings suggest that certain reactions elicited in the microcirculation by ethanol may be leukotriene-mediated. However, as shown in *Fig.* 3, pretreatment of rats with the selective 5-lipoxygenase inhibitor A-63162 does not prevent the necrotizing effect of ethanol, although it fully counteracts its stimulatory action on mucosal LTC_4 formation. Similar findings have been observed with other selective 5-lipoxygenase enzyme inhibitors (25, 26) or the 5-lipoxygenase activation inhibitor MK-886 (15). Leukotriene-mediated vascular reactions may thus not be crucial for the development of tissue necrosis. The protection against ethanol-induced gastric damage observed with non-selective lipoxygenase inhibitors and certain cysteinyl-leukotriene receptor antagonist (11, 25, 27, 28, 29, 30) are obviously not related to their effects on the leukotriene system. They may be due to additional properties such as radical scavenging or other not yet identified actions. As shown by the increased formation of 15-hydroxyeicosatetraenoic acid, topical ethanol activates several enzymes of the arachidonic acid cascade. It remains te be investigated whether products of additional metabolic pathways may be involved in ethanol-induced gastric mucosal damage which may be spared by selective 5-lipoxygenase inhibitors.

Gastric mucosal LTC_4 and non-steroidal anti-inflammtory drugs

Studies in non gastric tissues have shown that inhibition of cyclooxygenase can enhance formation of leukotrienes. This effect has been attributed either to a shift of the common substrate arachidonic acid away from cyclooxygenase to the 5-lipoxygenase pathway (31) or to the suppression

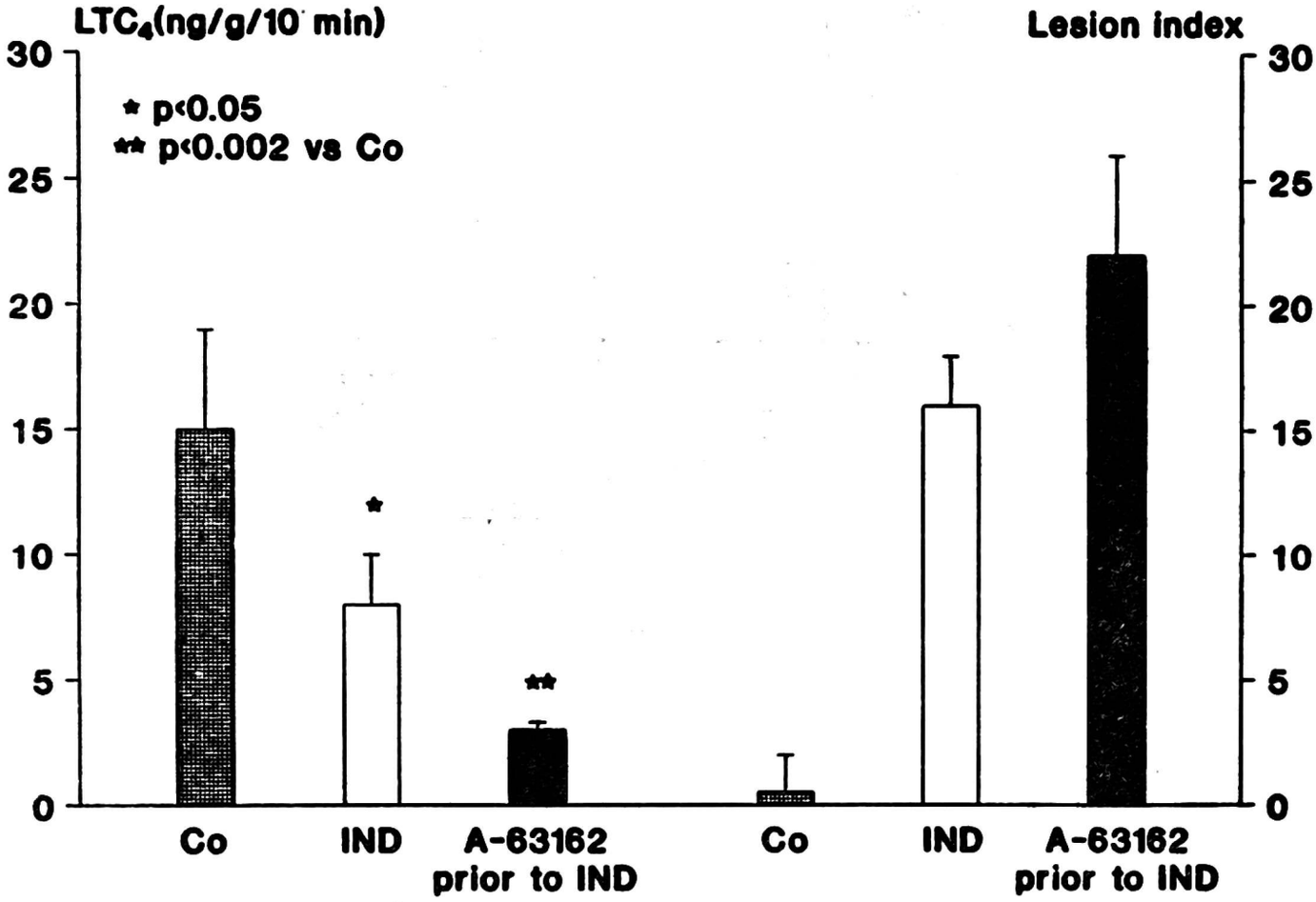

Fig. 4. Effect of A-63162 on gastric lesions and mucosal LTC_4 formation caused by indomethacin. Groups of 6 rats received A-63162 (45 mg/kg) 30 min prior to oral administration of indomethacin (20 mg/kg). Gastric damage and ex vivo release of LTC_4 from mucosal fragments was assessed 5 hrs after dosing of indomethacin. Mucosal LTC_4 formation was reduced in indomethacin-treated compared to vehicle-treated rats ($p < 0.05$) and was further reduced by pretreatment with A-63162 ($p < 0.002$ vs. controls and $p < 0.01$ vs. rats treated with indomethacin only, respectively). Pretreatment with A-63162 did not diminish mucosal damage caused by indomethacin. Data are derived from Peskar (16).

of prostaglandin formation which inhibit leukotriene biosynthesis by increasing intracellular cAMP (32). It has been speculated that the gastrotoxic effect of non-steroidal anti-inflammatory drugs is not only related to reduced synthesis of protective prostaglandins, but also to an increased formation of pro-ulcerogenic leukotrienes (33). We have investigated in rats whether inhibition of cyclooxygenase by non-steroidal anti-inflammatory drugs increases gastric mucosal release of LTC_4. The drugs

studied include indomethacin (15), sodium salicylate and aspirin (17), proquazone, 4-aminosalicylic acid, 2,4-dihydroxybenzoic acid, diflunisal, methyl salicylate, ibuprofen, lonazolac, naproxen and bezydamine (16). Although drug effects range from no or moderate to near-maximal inhibition of prostaglandin formation, gastric mucosal LTC_4 generation is either not affected or inhibited (15, 16, 17). None of the drugs increases formation of LTC_4 even in the presence of severe lesion formation. Furthermore, as shown in (*Fig. 4*), pretreatment of rats with the 5-lipoxygenase inhibitor A-63162 in a dose which further reduces mucosal LTC_4 formation does not prevent gastric lesions caused by indomethacin. Similarly, suppression of gastric mucosal LTC_4 formation by MK-886 does not protect against indomethacin-induced gastric lesions (15). These findings suggest tissue selective effects of non-steroidal anti-inflammatory drugs on the various pathways of arachidonic acid metabolism and do not support the hypothesis that the gastric mucosal leukotriene system is involved in the gastrotoxic properties of non-steroidal anti-inflammatory drugs.

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