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Editorial

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ROLE OF LEUKOTRIENES AND PLATELET ACTIVATING FACTOR IN GASTRIC MUCOSAL DAMAGE AND REPAIR

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Gastric mucosal integrity depends upon the balance between „aggressive" factors and „defensive" mechanisms. The formation of mucosal lesions results from the disruption of defense lines, including the breaking of unstirred mucus layer, the reduction of surface hydrophobicity, extensive exfoliation of surface epithelium, penetration of offending agents deeply into the mucosa and damage to the microvessels. The release of proinflammatory and vasoactive mediators such as leukotrienes (LT), thromboxanes, platelet activating factor (PAF), endothelins and others has been thought to be involved in the pathomechanism of mucosal injury, especially damage to the microvascular endothelium, increased vascular permeability, reduction in mucosal blood flow, vascular stasis, tissue ischemia and glandular cell necrosis. This paper reviews the mechanisms and possible pathogenetic implication of two related compounds, LT and PAF in acute mucosal injury by topical irritants such as ethanol, aspirin, bile salts and by stress. LT and PAF arise from similar membrane phospholipids and may regulate the biosynthesis of one another in the damaged mucosa. Although pharmacological studies have clearly demonstrated the noxious effects of cysteinyl LT and PAF on the mucosa, especially when exposed to topical irritants, recent publications have challenged the primary role of these mediators in the pathogenesis of mucosal lesions and ulcerations because the treatment with agents that selectively antagonize their biosynthesis or the receptor sites at the target cells did not always interrupt the chain of events leading to mucosal injury. The role of these mediators in the mucosal repair processes has been little studied but both cysteinyl LT and PAF seem to delay the restitution and healing of the mucosa. Further studies are necessary to clarify to what extent the biosynthesis of LT and PAF and the pharmacological inhibition of their action on the target tissues is related to noxious, protective and reparative events in the mucosa exposed to mild irritants and ulcerogens.

Key words: *cytoprotection, microvasculature, phospholipids, leukotrienes, platelet activating factor.*

INTRODUCTION

Previous research related to acute gastric mucosal lesions has been largely centered on the aggressive environment of the gastric lumen, particularly gastric acid, pepsin and bile salts. Over the last 15 years, a great many studies have been focused on the protective actions of prostaglandins (PG) and cytoprotection (1). The recent surge of interest in mucosal protection has shown that gastric mucosal integrity is dependent upon the balance between the „aggressive" factors and „defensive" properties of the mucosa.

Gastric mucosa is exposed to the constant action of a variety of irritants of exogenous or endogenous origin which may induce mucosal damage (1, 2). The maintenance of mucosal integrity involves several lines of defense such as the mucus-alkaline secretion, an unstirred layer at the mucosal surface, mucosal barrier, surface hydrophobicity, rich blood flow and rapid mucosal cell restitution and replication, all working in concert to withstand continual aggressive assault (1—4).

The exposure of the stomach to topical irritants and ulcerogenic agents results in characteristic morphological, ultrastructural and functional changes which reflect an imbalance between aggressive factors and defensive mechanisms. Such an imbalance is accompanied by the disruption of the unstirred layer, the reduction of surface hydrophobicity and excessive exfoliation of the surface epithelial cells. These changes permit offending agents and aggressive factors to penetrate the mucosa, to injure mucosal microvessels and glandular cells, and to release proinflammatory factors and vasoactive substances such as leukotrienes (LT), platelet activating factor (PAF), thromboxanes (TX), endothelins and others. Injury to the microvascular endothelium leads to microvascular stasis and cessation of oxygen and nutrient delivery, thus adding an ischemic component to the direct toxic injury of the mucosal cells (4). Within a few minutes after the exposure of the mucosa to topical irritants, an excessive exfoliation of surface epithelium occurs and concomitantly rapid restitution or migration of mucosal cells from the preserved area of gastric pits to the denuded basement membrane takes place. This is followed by a more prolonged renewal of gastric epithelium due to the proliferation of the progenitor „neck" cells, which normally lasts up to several days (4—6). The process of the re-epitheliazation of the damaged mucosa involving both rapid restitution and cell renewal is helped by the microclimates created by a gelatinous layer composed of mucus, desquamated epithelial cells and mucosal exsudate on the mucosal surface. Such a protective cover or „mucoid cap" enables also local disposal of irritants, especially hydrogen ions, by the release of bicarbonate ions (7, 8).

More aggressive agents such as high concentration of acid, bile salts, aspirin, stress or necrotizing substances result in more extensive damage including deeper mucosal vasculature (9). Stasis, vasocongestion and thrombus formation lead to macroscopic hemorrhagic erosions and necrosis that are a characteristic picture of acute mucosal damage (9—12). The mucosal damage is followed by rapid repair and healing that involve a variety of locally generated humorals (13). The aim of this paper is to review the role of leukotrienes and platelet activating factor in the mucosal damage and repair.

Role of LT in acute gastric mucosal damage

Arachidonic acid (AA), that is liberated from membrane phospholipid stores by the action of phospolipases, may be metabolized via several pathways. In the cyclooxygenase pathway, AA is transformed to unstable endoproxidase intermediates PGG_2 and PGH_2 ,which are subsequently metabolized to prostaglandin PGE_2 PGF_{2alfa}, PGI_2, PGD_2 and TXA_2. PG have been shown to possesse a wide range of gastrointestinal effects among which the most remarkable is their ability to protect the gastrointestinal mucosa against the damaging effect of various noxious agents (1, 2, 4, 11—13).

In 1974, an alternative pathway of arachidonate metabolism was described (14) and the biosynthesis of a family of highly biologically active compounds, named leukotrienes (LT) was identified (14—16). This pathway is catalyzed by various lipoxygenase enzymes and results in the formation of noncyclized monohydroxy acids and hydroperoxy acids (HETEs and HPETEs) and LT. 5-HPETE, which is formed from AA by the action of 5-lipoxygenase, is either enzymatically or nonenzymatically processed to 5-HETE or acts as precursor in the LT biosynthesis. Further conversion of 5-HPETE by 5-lipoxygenase yields the unstable intermediary 5,6-epoxide LTA, which can be enzymatically hydrated to form biologically active LTB_4 or can be spontaneously nonenzymatically hydrolyzed to several distinct 5,12-dihydroxyeicosatetraenoic acids (5,12,diHHETE) showing only minor biological activity (*Fig. 1*).

An alternate pathway of LTA_4 metabolism is mediated by enzyme glutathione-S-transferase and leads to the formation of LTC_4. Successive shortening of the peptide side-chain results in the conversion of LTC_4 to LTD_4 and further to LTF_4. These compounds are named cysteinyl or sulfidopeptide leukotrienes.

The initial step in the metabolism of LTC_4 involves the removal of

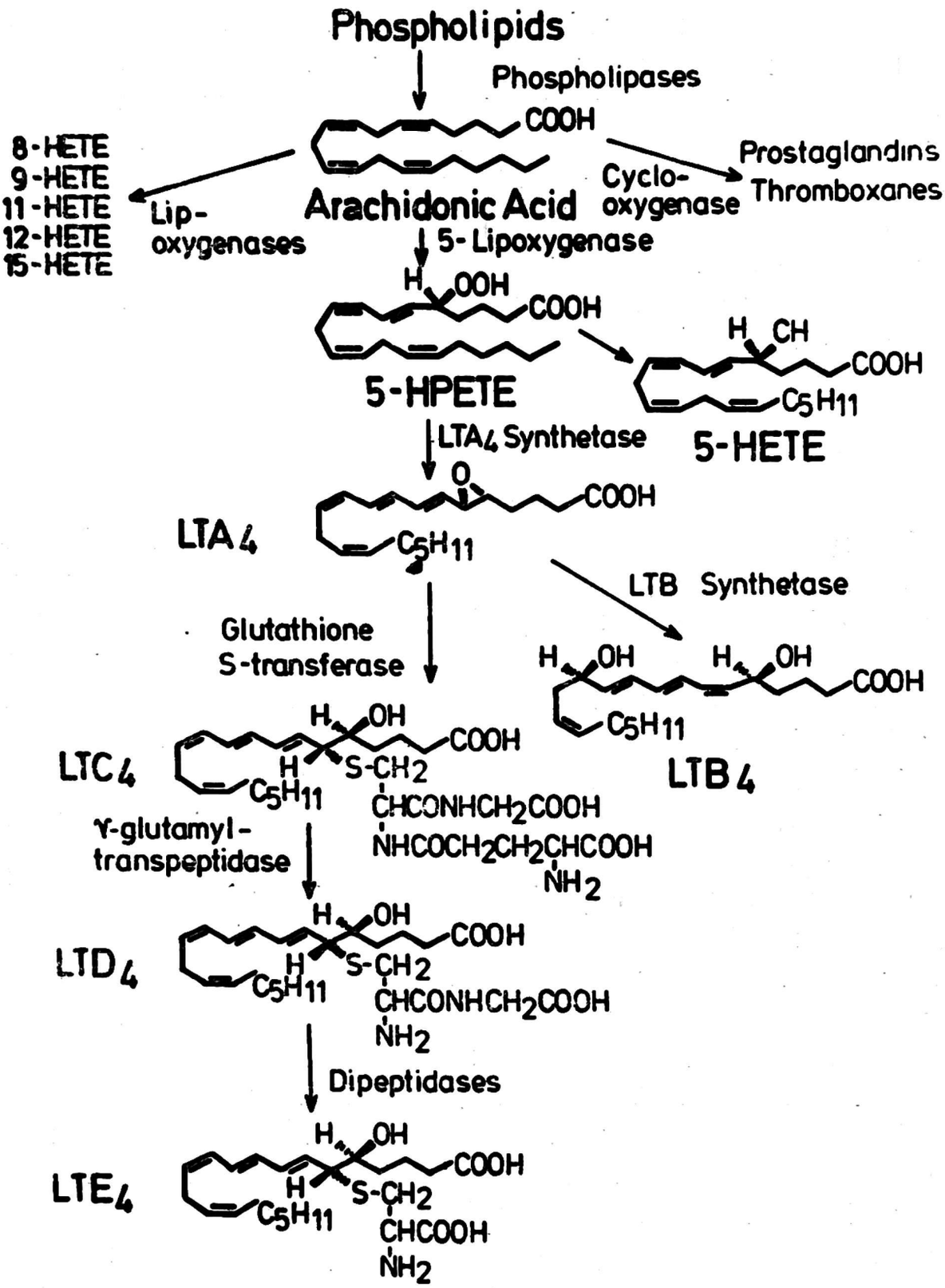

Fig. 1. Metabolism of arachidonic acid via the 5-lipoxygenase pathway.

the gamma-glutamyl group by the membrane-bound enzyme gamma-glutamyl transferase leading to the formation of LTD_4. Removal of the glycine residue of LTD_4 by dipeptidases results in the formation of LTE_4. The latter can be further metabolized to LTF_4 by addition of glutaminic acid to cysteine residue under the influence of gamma-glutamyl transferase. The structure of LT metabolites in vivo and their routes of excretion is species specific. In rodents, cysteinyl LT are excreted mainly

in bile as N-acetyl LTE_4 while in humans they are excreted in urine in the form of LTE_4.

LTC_4, LTD_4 and LTE_4 exhibit some differences in their biological activity but they convert from one active form to another by simple peptide cleavage. LTB_4 has very potent chemokinetic and aggregating activity on human and rodent polymorphonuclear cells. It is a potent chemotactic agent for leukocytes, eosinophils, monocytes and fibroblasts. In addition, LTB_4 causes adherence of leukocytes to the vascular endothelium followed by diapedesis and stimulates the release of lysosomal enzymes. LTB_4 induces plasma exsudation in various experimental models by a leukocyte-dependent mechanism and enhances the vascular permeability (15, 16).

The primary actions of the cysteinyl leukotrienes are directed to smooth muscle, blood vessels and secretory cells (15—17). The spasmogenic activity of these LT was recognized long ago as the smooth muscle--contracting activity of the slow-reacting substance of anaphylaxis (SRS-A) released by the anaphylactic reaction. LT contract the smooth muscle cells of the blood vessels, pulmonary airways, gastrointestinal tract and the myocardial and endothelial cells. These contractions are characterized by a slow onset and prolonged course. Regional differences have been found regarding the effects of LT on the rat gastrointestinal tract. Both LTC_4 and LTD_4 elicited concentration dependent contractions of the isolated rat stomach and colon and these effects were inhibited by LT receptor antagonist FPL 55712 (17). In contrast, the duodenum and ileum failed to respond to LT possibly due to the lack of LT-specific receptors in these regions of the gut.

Effects of LT on gastric blood flow were studied in rats and in dogs. The first observation that some LT exert a vasoconstrictive effect on gastric vasculature was made by Whittle et al. in 1985 (18) using direct microscopy of the gastric submucosal vessels. They showed that LTC_4 caused almost immediate and pronounced dose-dependent contraction of the arterioles and venules characterized by a segmental and focal nature. LTB_4 and LTD_4 had no significant vasoactive effect on the rat submucosal microcirculation. The vasoconstriction by LTC_4 was not due to the release of an AA cyclooxygenase product such as TXA_2 because pretreatment with indomethacin did not affect the vascular changes induced by LTC_4. Venular vasoconstriction caused by LTC_4 led to extensive stasis and sluggish blood flow resembling the vascular changes induced by necrotizing substances such as ethanol (9). Using canine *ex vivo* stomach preparation in a lucite chamber, we confirmed that, indeed, LTC_4 infused i.a. resulted in a dose-dependent reduction in total and mucosal blood flow to the stomach accompanied by the inhibition of gastric acid res-

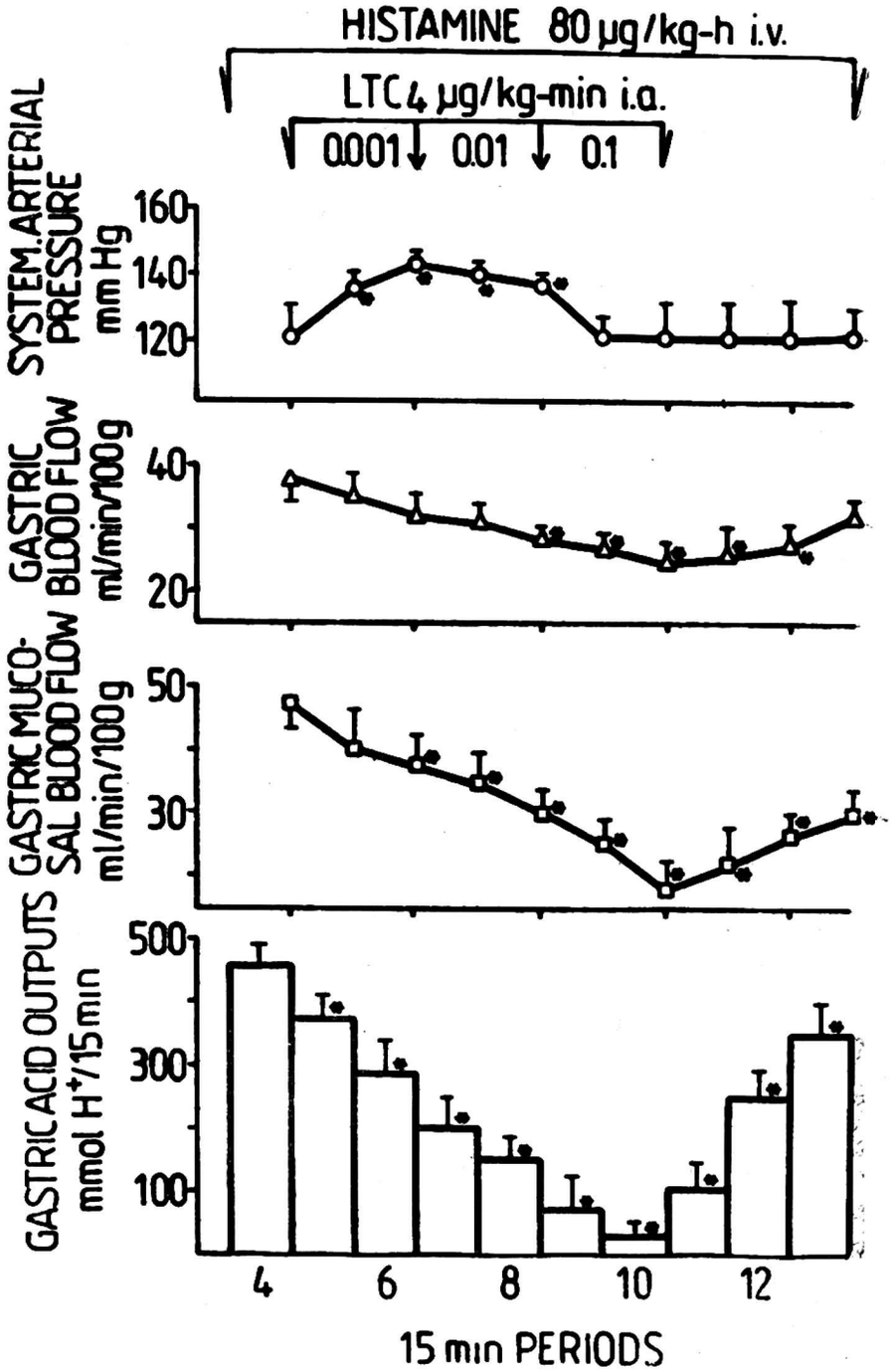

Fig. 2. Effects of gradually increasing doses of LTC_4 infused i.a. on systemic blood pressure, gastric blood flow, gastric mucosal blood flow (measured by H_2-clearance) and histamine-induced gastric acid secretion from the fundic portion of the stomach in lucite chamber of anesthetized dogs. Mean ± SEM of 5 experiments on 5 dogs. Asterisk indicates significant change as compared to the control value obtained with histamine alone.

ponse to histamine (19). Systemic blood pressure tended to increase (*Fig. 2*). Arteriovenous oxygen difference was unaffected but the calculated oxygen consumption declined in these experiments mainly because of mucosal vasoconstriction and increased gastric vascular resitance. It should be emphasized that only LTC_4 and LTD_4 showed a characteristic reduction in gastric circulation, oxygen consumption and acid secretion, whereas LTB_4 was completely ineffective (19).

The gastric inhibitory effect of LTC_4 was also observed in conscious dogs with Heidenhain pouch and gastric fistula stimulated by histamine pentagastrin and other stimuli (20). This inhibitory effect was probably secondary to the diminution of the blood flow to the stomach because *in vitro* isolated gastric glands showed no change when LTC_4 was added to the incubation medium (20) nor did the inhibition when stimulated by histamine, carbachol and DBcAMP. In other studies (21), LTC_4, LTD_4 and LTE_4 also did not affect basal acid production from the enriched rat isolated parietal cells but potentiated prestimulated secretion. Only high concentrations of LTC_4 and LTD_4 were found to inhibit acid secretion but at lower concentrations a typical augmentation of the secretion prestimulated by histamine, forskolin or DBcAMP was observed. LTB_4 had no effect on basal or prestimulated acid production. It was concluded that in rats, cysteinyl LT have a direct effect on the partietal cells by interacting with intracellular mechanisms which are activated by membrane histamine-H_2 or muscarinic receptors. The difference in the action of LT on acid secretion may represent the species specific effect of these agents on the parietal cells or might be explained by the existence of functionally different LT receptor subtypes in the gastric mucosa. It is of interest that LTC_4 in the canine stomach induced the stimulation of alkaline secretion that was partly mediated by endogenous PG because pretreatment with indomethacin reduced this effect. This increase in alkaline secretion was not caused by mucosal damage and passive bicarbonate diffusion from the plasma because the mucosal integrity was not affected as determined by measurement of the transmucosal potential difference (PD) in the stomach in these experiments. It should be mentioned that despite these remarkable alterations in the gastric circulation no visible mucosal damage was observed in animals receiving LT.

Our *in vitro* studies with isolated gastric glands have also demonstrated that LTC_4, which showed only a slight effect on acid production, did not affect the viability of the glands exposed to indomethacin, their survival time or the release of the cytoplasmic enzyme LDH. Pretreatment with PGE_2 prolonged the bioviability of the glands and markedly inhibited the leakage of LDH from these glands, while LTC_4 was ineffective in this respect (*Fig. 3*). This indicates that LTC_4 has no direct damaging action on the mucosal cells.

The question remains whether LT are generated in the gastric mucosa in sufficient amounts to contribute to the damage observed after topical irritants and whether the alterations in the microvasculature are involved in this injury. Numerous studies have demonstrated that tissues of the gastrointenstinal system have a capacity of generating

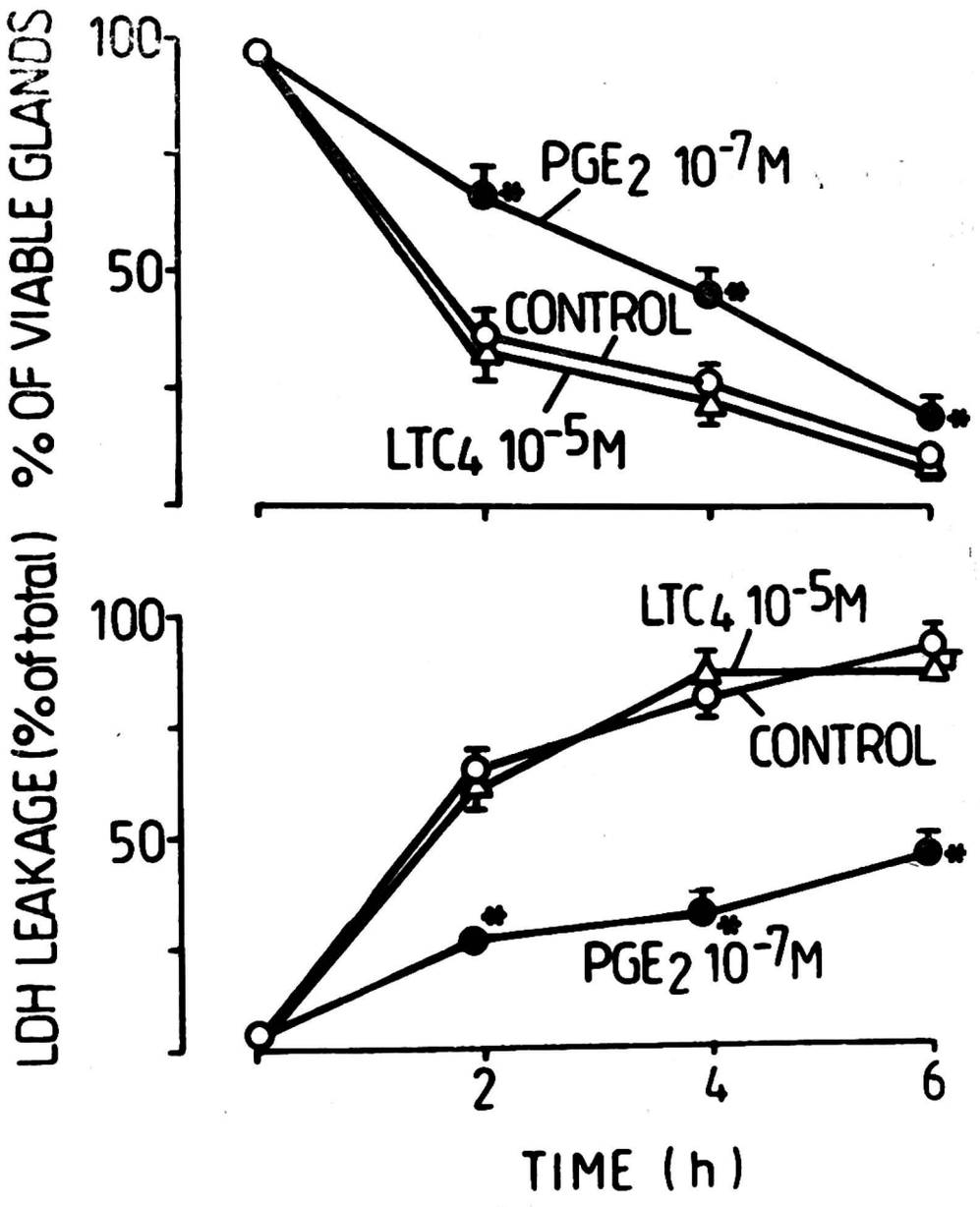

Fig. 3. Viability of isolated rat gastric glands as measured by the Fast Green exclusion assay and the release of LDH from these glands after their exposure to 1.5 mM indomethacin alone (control) or indomethacin plus PGE_2 (10^{-7}M) or LTC_4(10^{-5}M). Means ± SEM of 6 separate preparations of gastric glands. Asterisk indicates significant change as compared to the control value.

not only cyclooxygenase but also lipoxygenase products of arachidonate metabolism (13, 25). As shown by Peskar and her colleagues (13, 25) LT release from the human and guinea pig gastric mucosa is greatly enhanced after antigen challenge and this effect can be prevented by pretreatment with FPL55712. Mucosa of the glandular portion of the rat stomach has a particularly high capacity of generating spontaneously LT, and this generation is not affected by ionophore A23187 indicating that phospholipase and 5-lipoxygenase are not affected by an influx of calcium ions (*Fig. 4*). LT generated by the fragments of the gastrointestinal mucosa under basal or stimulated conditions consisted mainly of a mixture of LTC_4, LTD_4 and LTE_4. Fragments of the mucosa in the incubation medium rapidly converted exogenous LTC_4 to LTD_4 and

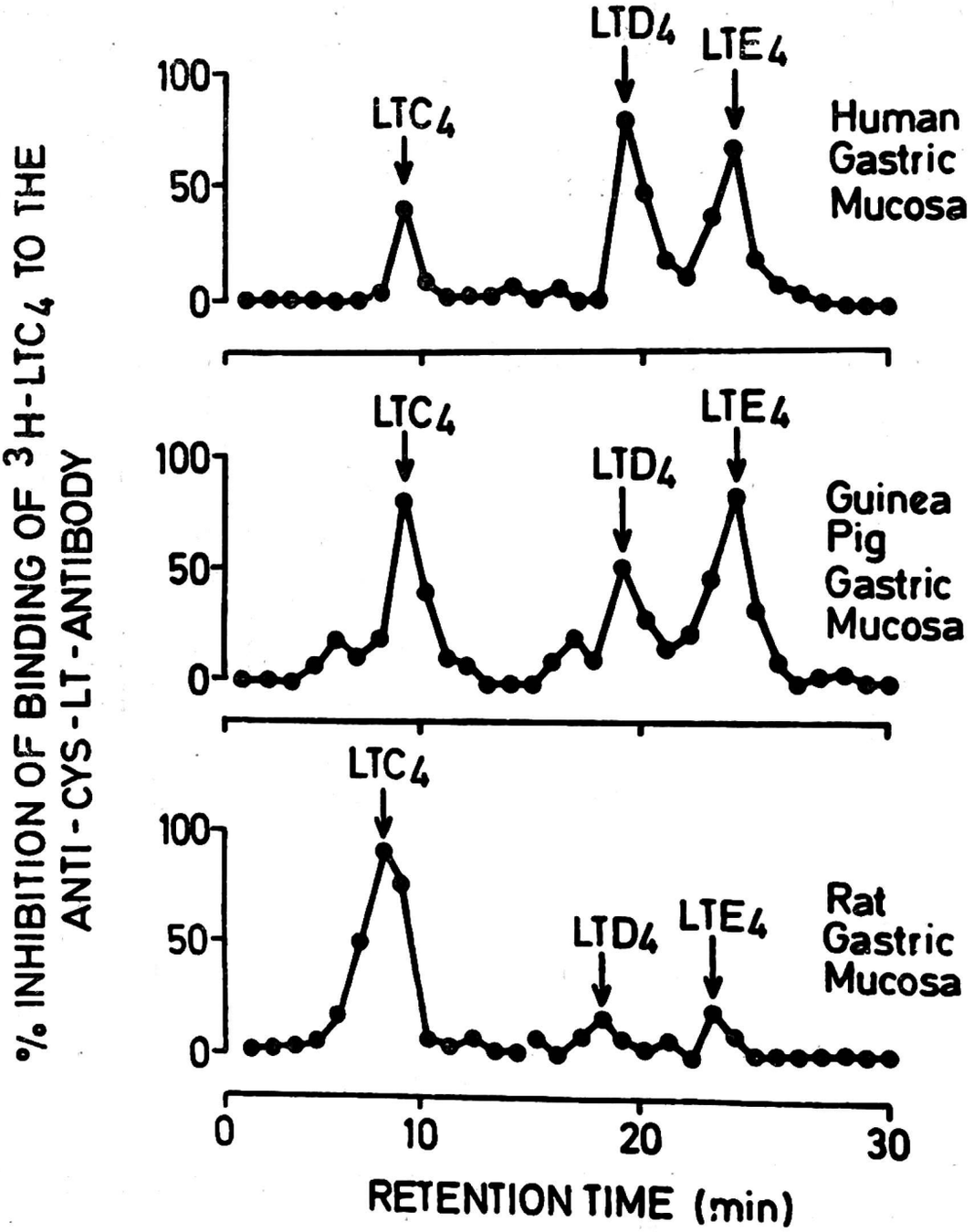

Fig. 4. Profile of cysteinyl LT released by human guinea pig and rat gastric mucosa. LT formation was stimulated by ionophore A2187 (human and guinea pig) or in vivo exposure to ethanol (rat). LT were determined by specific RIA using antiserum that recognizes LTC_4, LTD_4 and LTE_4 [from Peskar (13)].

LTE_4. These findings indicate that the mucosa contains the enzymatic machinery to produce and metabolize cysteinyl leukotrienes.

The role of LT in the vascular changes observed during acute gastric mucosal damage has been the subject of extensive investigations. Pihan et al (22) reported that close i.a. infusion of LTC_4 or LTD_4 caused widespread vascular damage as revealed by monastral blue labelling of gastric microvessels. We used protein leakage into the chamber and Evens blue leakage into the gastric mucosa as the indicators of vascular permeability and PD as an overall index of mucosal integrity. As shown

in (*Fig. 5*), LTC_4 used in a dose of 1 μg/kg/min infused i.a. did not affect vascular permeability or mucosal integrity but the exposure to ethanol resulted in marked changes in both these parameters.

Our findings that LTC_4 and LTD_4 do not affect the permeability of mucosal capillaries in the canine stomach are largely consistent with

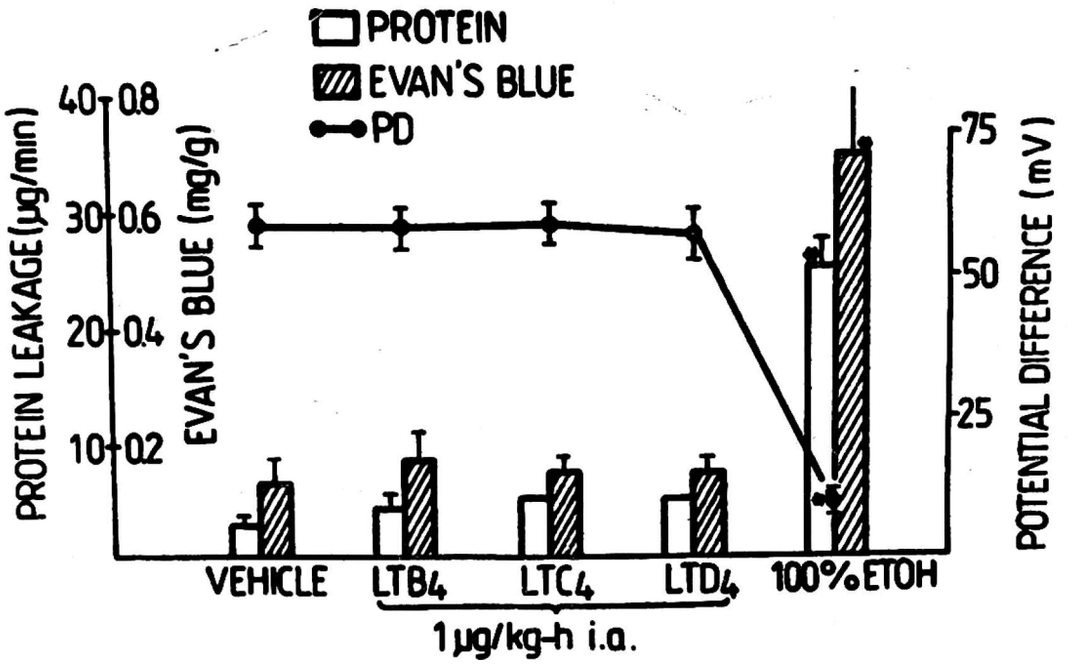

Fig. 5. Protein leakage and Evans blue leakage from the canine gastric mucosa and transmucosal potential difference (PD) of the mucosa in dogs with close i. a. infusion of various LT or topical application of 100% ethanol in anesthetized dogs. Mean ± SEM of 5 dogs. Asterisk indicates significant ($P < 0.05$) change as compared to the values obtained with vehicle control.

the hypothesis that LT cause an extensive vasoconstriction and this may result in tissue anoxia with increased susceptibility of the mucosa to damage rather than cause direct injury to the endothelial or mucosal cells (18, 19). Thus, while cysteinyl LT do not seem to be ulcerogenic by themselves, they may potentiate the noxious effects of damaging agents.

This notion is supported by our finding (23) that LTC_4 given to rats did not cause any substantial damage to the intact mucosa but only augmented the mucosal injury induced by absolute ethanol, bile salts, aspirin or stress (*Fig. 6*). Although this increase in the mucosal susceptibility to damage was dose-dependent and started with a dosage as low as 10 μg/kg of LTC_4, it is not clear whether this LT is generated in sufficient amounts in the mucosa to induce alterations in the gastric microvasculature and to predispose the surrounding mucosa to lesions by various irritants. Since an intact microcirculation is fundamental to the functional and structural integrity of the mucosa (9—11), the actions

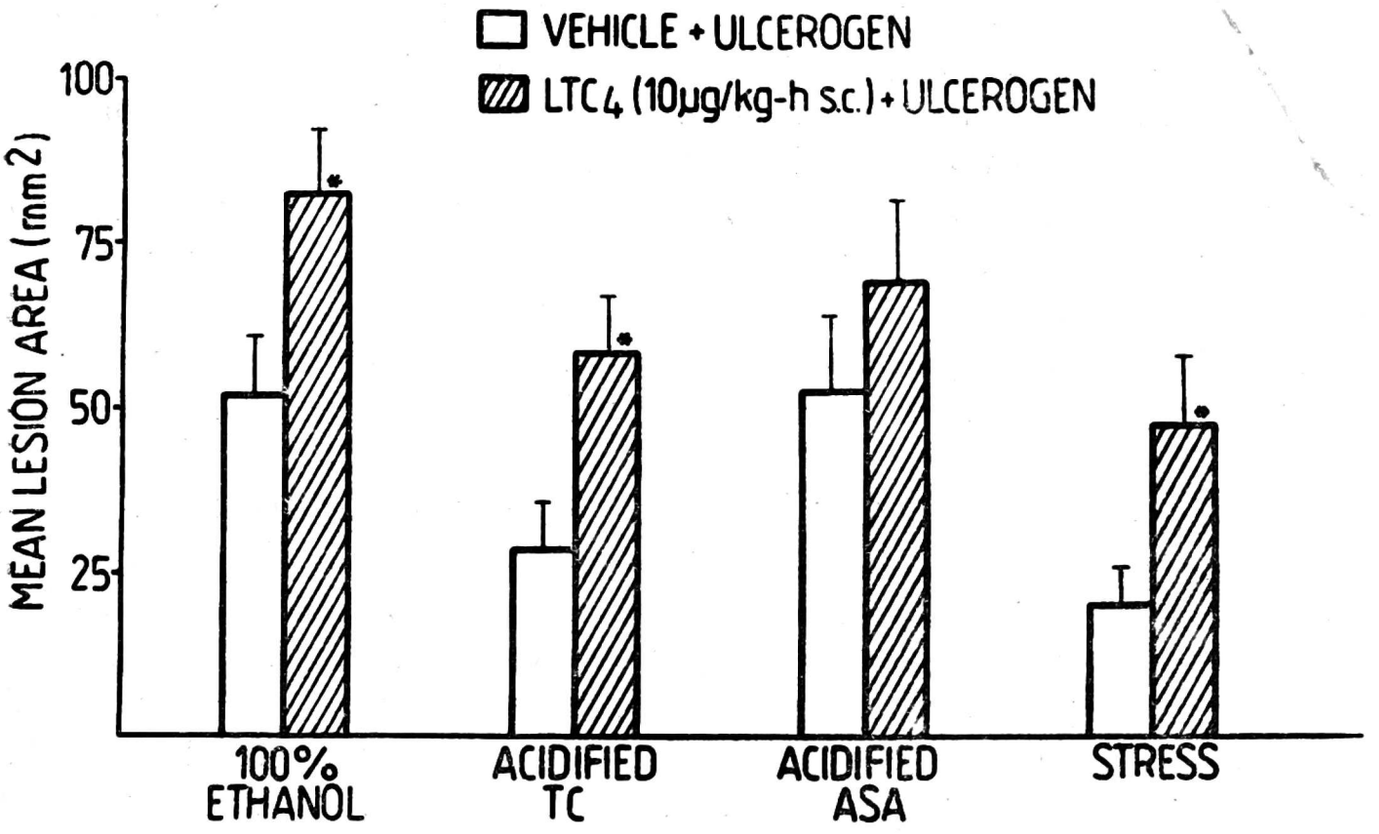

Fig. 6. The effects of s. c. administration of LTC_4 (10 µg/kg-h) on the mean area of gastric lesions induced by 100% ethanol, acidified taurocholate (TC), acidified aspirin (ASA) or water immersion and restraint stress in rats. Mean ± SEM of 8—10 experiments on 8—10 rats. Asterisk indicates significant ($P < 0.05$) increase above the vehicle control value ([from Konturek et al. (29)].

of leukotrienes on the vasculature may be of importance in mediating or promoting gastric injury or ulcerations. It is well established that various models of acute gastric mucosal damage such as that induced by ethanol, bile salts, aspirin or stress conditions are accompanied by marked vascular damage (9—14). As shown by Guth (9) and Szabo and his colleagues (10—12) or Oates (24), the alternations in mucosal microvasculature in ethanol-induced damage of mucosa closely resemble those observed after direct application of LTC_4 to the rat gastric mucosa suggesting a possible involvement of this vasoactive LT in the vascular effects of ethanol in the mucosa.

Peskar et al (25) provided a major contribution to the concept of LT being involved in the pathophysiology of gastric ulcerations by showing for the first time that gastric mucosa produces large quantities of immunoreactive LTC_4. They also demonstrated that LT generation increased dose-dependently upon the exposure of the mucosa to increasing concentrations of ethanol. The increase in the mucosal biosynthesis of LTC_4 is closely correlated with the degree of hemorrhagic damage to the mucosa produced by ethanol, whereas the mucosal formation of cycloxygenase products such as TXB_2 or PGE_2 was unchanged (25). Certain

gastroprotective agents such as carbenoxolon or nordihydroguiaretic acid (NDGA) dose dependently reduced the area of mucosal damage produced by ethanol and the release of LTC_4 by the mucosa. The maximal increase in mucosal release of LTC_4 was observed as early as 1 min after exposure to ethanol (25). It is, therefore, reasonable to assume that the microcirculatory changes caused by ethanol may be at least in part mediated by cysteinyl LT. This effect may represent a selective activation of the 5-lipoxygenase pathway of arachidonate metabolism because other products of arachidonate metabolism such as TXB_2 or PGE_2 were not affected by ethanol.

The finding that the exposure of the gastric mucosa to ethanol increases mucosal production of LTC_4 while causing mucosal damage has been amply confirmed by various investigators (13, 23, 26—30). We found (23, 29) that such ethanol-induced mucosal damage with accompanying increase in LTC_4 release may be prevented by pretreatment of the agent FPL 55712, which antagonizes the cysteinyl receptors (*Fig.* 7). This prevention by FPL 55712 occurred without alteration in the generation of LTC but with

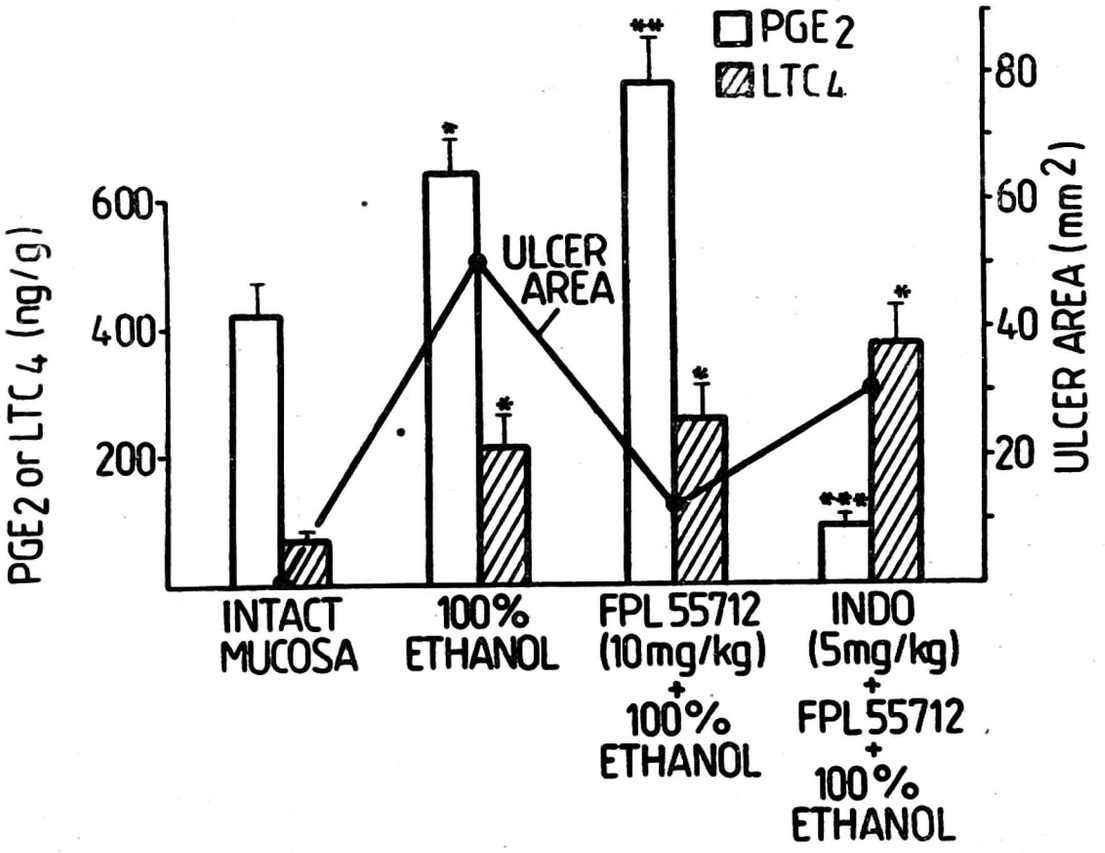

Fig. 7. PGE_2 and LTC_4 generation and mean lesion area in rat stomach exposed to 100% ethanol alone, FPL 55712 (10 mg/kg) combined with ethanol or indomethacin (5 mg/kg) combined with FPL 55712 plus ethanol. Mucosal generation of PGE_2 and LTC_4 in the intact mucosa is also shown. Mean ± SEM of 8—10 experiments on 8—10 rats. Single asterisk indicates significant increase above the value in the intact mucosa. Double asterisks indicate significant increase above the value obtained with 100% ethanol alone and triple asterisk indicate significant decrease below the value obtained in intact mucosa.

significant increase in the biosynthesis of PGE_2. Indomethacin, which almost completely suppressed mucosal PGE_2 production, partly reversed the protective effects of FPL 55712 but again did not affect the enhanced production of LT. It is of interest that FPL 55712 reduced not only ethanol-induced gastric damage but also that produced by other ulcerogens and was the most effective against stress-ulcerations (23). These effects might be attributed to the enhancement of PGE_2 formation by FPL 55712 rather than to the antagonism of LTC receptors by this agent. In the aspirin-treated stomach, the increase in LT generation was relatively small, but unlike other types of mucosal injury aspirin caused almost complete suppression of mucosal biosynthesis of PGE_2 so that the ratio of LTC_4/PGE_2 was greatly increased. This increase might be responsible for the proulcerogenic effect of aspirin.

Important evidence supporting the involvement of LTC_4 in the pathogenesis of acute gastric mucosal lesions derived from studies with gastroprotective agents affecting the formation of LT in ethanol-treated mucosa. There is a close relationship between the gastroprotection obtained with a number of drugs and the inhibition of LT formation (13, 23, 25, 26, 29). There is a long list of gastroprotective agents including carbenoxolone, NDGA, sodium salicylate, sulfhydryl containing substances such as cysteamine, colloidal bismuth (30). However, other gastroprotective durgs including Al/Mg-containing antacids or sucralfate, which are known to stimulate mucosal PGE formation, fail to affect enhancement in the production (by ethanol) of LTC_4 generation in the gastric mucosa (13). These findings indicate that ethanol-induced stimulation of gastric mucosal LTC_4 synthesis may occur despite pronounced protection, implying that the increased biosynthesis of LTC_4 may not be a secondary phenomenon resulting from gastric mucosal injury.

Proofs of LT involvement as a mediator of acute mucosal damage should be obtained from experiments using highly selective inhibitors of the 5-lipoxygenase pathway. Although it is the most informative of all the approaches undertaken in such studies, it has proved to be the most difficult and the results so far obtained are very divergent. A number of inhibitors of lipoxygenase have been shown to prevent gastric mucosal damage. We showed that NDGA reduced the ethanol-induced mucosal damage and increased the LTC_4 release by ethanol (29), but this inhibitor is not specific and its protective effects may possibly be related to the enhancement of mucosal PG generation or to some other actions such as anti-oxidant or free radical scavenging (25).

Whittle and his associates (27) used acetohydroxamic acids such as BWA4C, which was found to be a highly effective inhibitor of both LTB_4 and LTC_4 formation, indicating inhibition of the common 5-lipoxygenase

enzyme pathway. This inhibitor is specific for 5-lipoxygenase as it does not affect the biosynthesis of PG cycloxygenase. BWA4C was completely ineffective as a protective agent against ethanol damage (27). Another 5-lipoxygenase inhibitor, BW755C, also suppressed the LTC_4 formation and did not affect PG biosynthesis, but was highly protective against ethanol damage (28). However, this drug caused gastroprotection at a dose much lower than that required for the inhibition of LT formation. Again, gastroprotection by BW755C may occur through a mechanism that appears to be unrelated to the inhibition of LT synthesis and possibly results from the scavenging of oxygen free radicals or from the inhibition of 12- or 15-lipoxygenase which are only weekly inhibited by BWA4C (27). It is likely that the products of these enzymes such as hydroperoxy metabolites or free radicals, are involved in ethanol-induced damage. For comparison, indomethacin was used in another series of experiments and caused only suppression of PG formation without major changes in the LT formation and of course, without gastroprotection (39). At a high dosage there was significant inhibition of LTC_4. This could be ascribed to the inhibition by indomethacin of phospholipase A_2 with subsequent reduction in the availability of arachidonic acid for both cyclo- and lipo-oxygenase enzymes (31).

Wallace et al (26) compared the gastroprotective and LTC suppressing effects of numerous agents and confirmed that following exposure to ethanol there is an increase in LTC formation. Certain agents such as BW755C reduced in parallel fashion the formation of gastric ulcerations and of LTC biosynthesis. Other substances such as L651392 (LT biosynthesis inhibitor) or dexamethasone strongly suppressed LTC biosynthesis but failed to show any gastroprotective activity. Rioprostil, PGE_1 analog, and FPL 52694, mast cell stabilizer, were protective without inhibiting LTC formation (26). Thus, there was no significant correlation between mucosal protection and the inhibition of LT formation, so the inhibition of 5-lipoxygenase does not necessarily confer protection on the gastric mucosa. These studies led to the conclusion that LTC_4 is unlikely to play an important role as mediator of ethanol induced gastric mucosal damage.

In this issue of the Journal, the paper of Peskar (30) demonstrates that the selective inhibition of 5-lipoxygenase does not confer protection against gastric mucosal damage induced by non-anti-inflammatory drugs such as indomethacin or topical irritants other than ethanol. It seems unlikely that the mucosal LT system is involved in the injurious action of non-steroidal anti-inflammatory compounds (30) since the pronunced reduction of LTC_4 generation caused by 5-lipoxygenase blocker, A-63162 did not result in any significant mucosal protection against indomethacin damage. Furthermore, hypertonic solutions such as 25% NaCl or chemical noxious agents such as 0.2 N NaOH, 0.6 N HCl or acidified taurocholate

did not cause any consistent increase in mucosal LTC_4 generation despite the extensive mucosal damage. This suggests that the increased release of LTC_4 from the gastric mucosa exposed to ethanol is not just a consequence of cell necrosis and damage (30). Interestingly, PGE_2 (31, 32). or capsicin (33), which exhibit gastroprotective activity, failed to affect the increased LT formation induced by absolute ethanol, indicating that the protection may be gained even in the presence of full stimulation of LT biosynthesis. This is almost in agreement with a previous report (28) showing that oral treatment with PGE_2 prevented hemorrhagic mucosal damage caused by ethanol without reducing LTC biosynthesis while 16, 16 dimethyl-PGE_2 prevented both the gastric mucosal lesions and the increase in mucosal LT formation. The difference in the effects of these PG may be related to the nature and dose of PGE_2 used in this study (28). Recently Osada et al (34), using the HPLC system demonstrated that a measurable amount of LT could be detected after 1 h of 0.6 N HCl administration, whereas mucosal lesions appeared only within 15 min after exposure to the damaging agent. In our hands (Fig. 8) the introduction

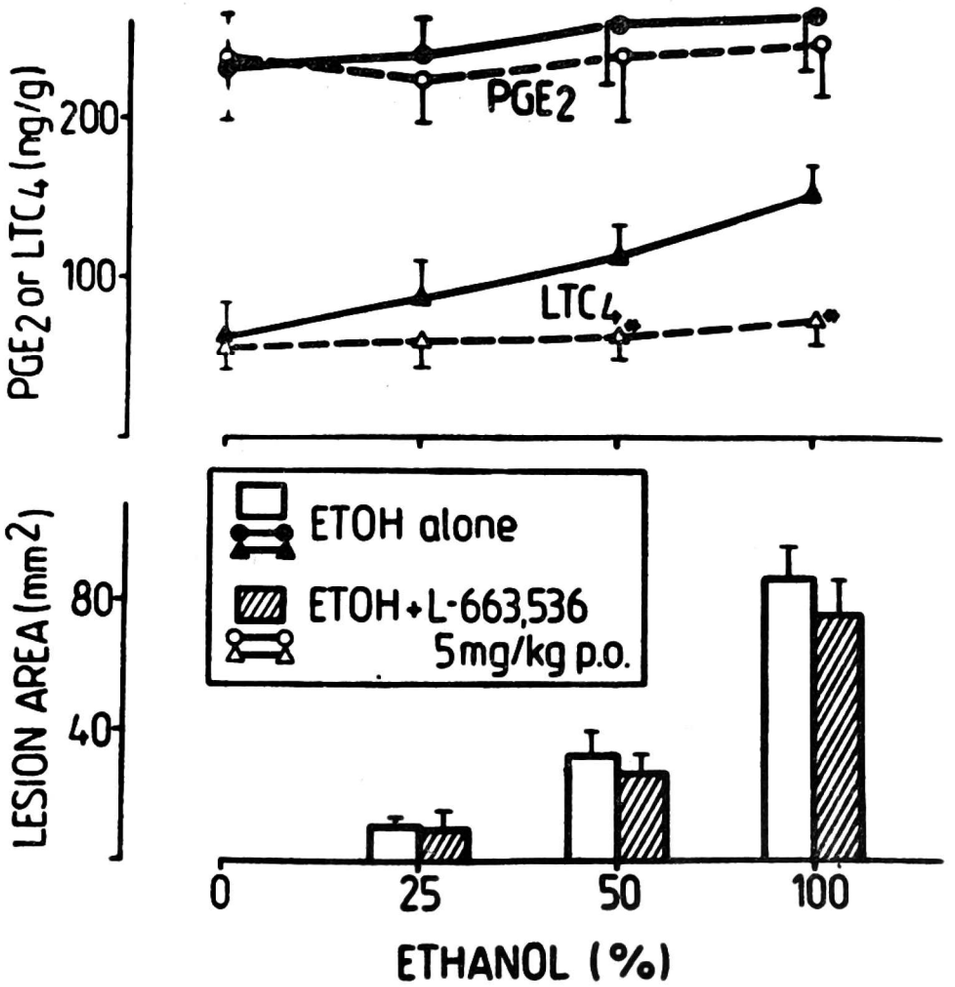

Fig. 8. Meaan are of gastric lesions and mucosal generation of PGE_2 and LTC_4 following intragastric administration of 1 ml of ethanol alone in gradually increasing concentrations without and with pretreatment (30 min before ethanol) with L-663, 536, a potent 5-lipoxygenase inhibitor. Mean ± SEM of 8—10 experiments on 8—10 rats. Asterisk indicates significant decrease below the control value obtained with ethanol alone.

of ethanol into the stomach resulted in a dose-dependent increase in mucosal damage and concomitant increments in the mucosal generation of LTC_4 (but not PGE_2). Pretreatment with a potent and selective 5-lipoxygenase inhibitor (L-663,536) almost completely abolished increments in the mucosal formation of LTC_4 but failed to affect mucosal damage by exposure to ethanol. Several studies support the hypothesis that LT are not the primary mediators of the gastric ulcerogens but that extensive mucosal injury may result in the increased formation of LT (35, 36).

Although endogenous LT are unlikely to play a key role in the pathogenesis of acute gastric lesions, little is known about the effects of LT on the repair and healing of acute gastric lesions and chronic ulcerations. As mentioned in the introduction, the processes of mucosal repair and healing following acute gastric damage dependent on the protection of denuded gastric mucosa from the damaging effect of the gastric environment, such al luminal acid and other noxious agents (6, 37, 38). Kuroiwa et al (39) reported recently that the maintenance of the mucosal blood flow by PG greatly contributes to mucosal healing of stress lesions. It is expected that LT may markedly impair the restitution process by reducing

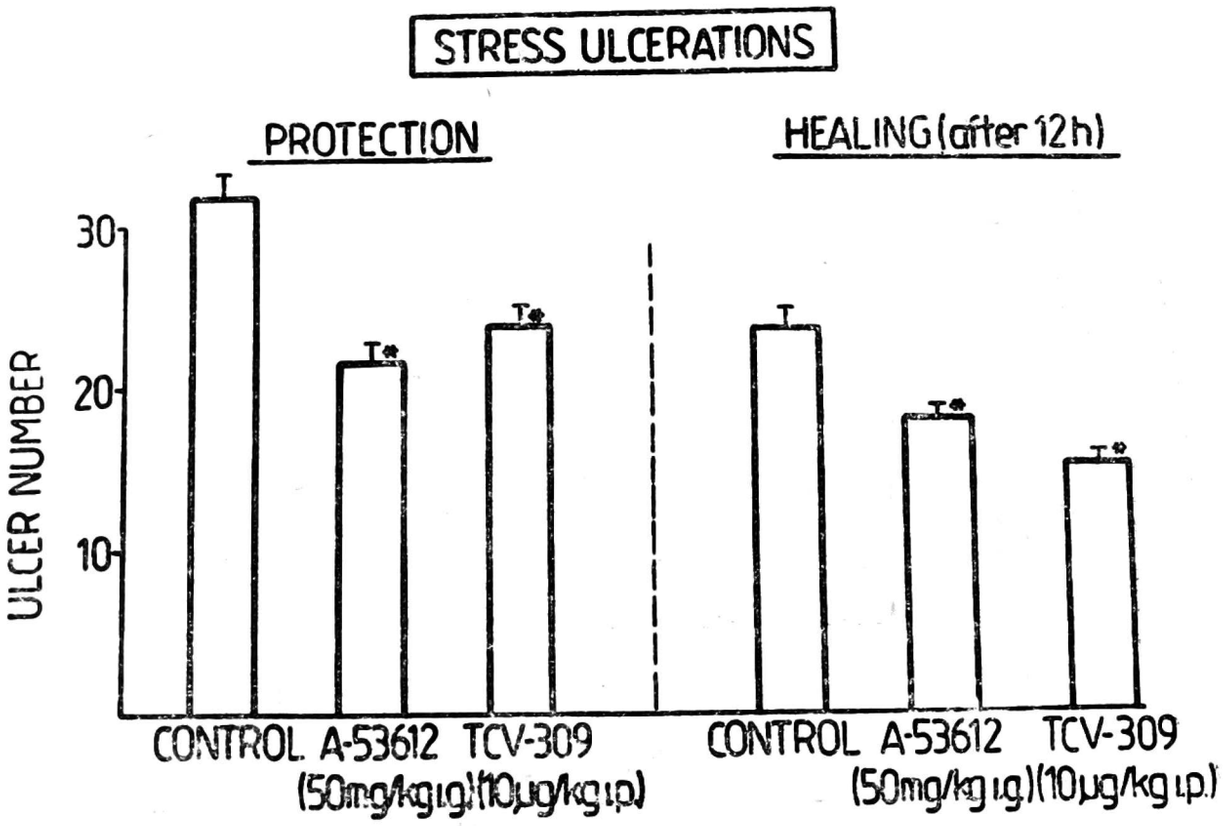

Fig. 9. The effect of intragastric administration of A-53612, a potent 5-lipoxygenase inhibitor, or intraperitoneal administration of TCV-309 a potent antagonist of PAF receptors, on the formation (protection) or healing of acute gastric mucosal lesions induced by water immersion and restraint stress. Both agents prevented in part, the formation of lesions and significantly enhanced the repair of damage as observed 12 h after withdrawal of stress. Means ± SEM of 8—10 tests on 8—10 rats. Asterisk indicates significant decrease of ulcer number as compared to control rats treated with vehicle.

blood flow and the delivery of oxygen and nutrients to the gastric tissue, However such a study has not yet been undertaken and even the finding that PG promote the restitution of surface epithelium is difficult to interpret, since these compounds may also reduce the severity and depth of the cell damage in the regeneration zone rather than to have a direct effect on the restitution process (37, 38). Recently, we (39) found that pretreatment with a potent inhibitor of 5-lipoxygenase, A 53612, markedly reduced the formation of stress lesions and improved the healing of these lesions (*Fig. 9*). Further studies are needed to determine the mechanism of action of LT and their role in the repair and healing processes of the mucosa damaged by various irritants and ulcerogens. It is possible that the balance of protective PG and proulcerogenic LT is a relevant factor in the mucosal protection and repair.

PAF in acute gastric mucosal damage

PAF (1-0-alkyl-2-0-acetyl-sn-glycero-3-phosphocholine) is an endogenous low molecular weight phospholipid which is formed by a variety of cell types including macrophages, monocytes, neutrophiles, eosinophils and endothelial cells on activation of phospholipase A_2. The same enzyme also liberates the eicosanoid precursor, arachidonic acid (41—43). PAF is not a metabolite of arachidonic acid and its immediate precursor, lyso-PAF is released from membrane-bound alkyl-phospholipids by the action of phospolipase A_2 (*Fig. 10*). Lyso-PAF is metabolized by acetylo-CoA transferase into PAF acether and this is finally broken down back to lyso-PAF by acyl hydrolase (42). The induction of the PAF biosynthesis is thus controlled by the activation of phospholipase A_2 (41-44). Since this enzyme is inhibited by glicocorticoids (via polypeptide lipocortin) hence the glucocortycoids such as dexamethasone greatly reduces the biosynthesis of PAF and septic shock-induced gastrointestinal lesions as well hemoconcentration and hypotention (45—48). The catabolism of PAF is catalyzed by two enzymes; the plasma PAF is rapidly hydrolyzed by the acid labile enzyme, acetyl hydrolase, giving rise to lyso-PAF. Phospholipase A_2 can also convert PAF back to lyso-PAF.

PAF has been found to be released systemically to act as putative mediator of the circulatory changes observed e. g. during endotoxin shock (septic shock) and inflammation (49). There is ample evidence supporting the important role of PAF as mediator of many symptoms of septic shock (49—51). Injection of PAF into laboratory animals produced various symptoms of septic shock, including systemic hypotention due to peripheral vasodilation, pulmonary hypertension, bronchoconstriction,

increased vascular permeability, stimulation of platelet aggregation, enhancement of blood coagulation and stimulation of neutrophil aggregation and degranulation, and stimulation of lysosomal enzyme release into the circulation (49—54). The fact that only the naturally occuring stereoisomer (R) is biologically active suggests that PAF acts via specific receptors. High affinity binding sites for PAF have been recently identified on platelets, neutrophiles, lung membranes, liver and endothelial cells (47).

A feature of septic shock is widespread gastrointestinal hemorrhages and lesions (49—54, 51). All these effects may be ameliorated by pretreatment with dexamethasone which inhibits PAF formation (54). A second line of evidence supporting the role for PAF in septic shock is that the release of PAF increased after the administration of endotoxin to animals and endotoxin produced similar gastric damage to the of PAF (52). Furthermore, several groups (44, 45) have reported that a PAF receptor

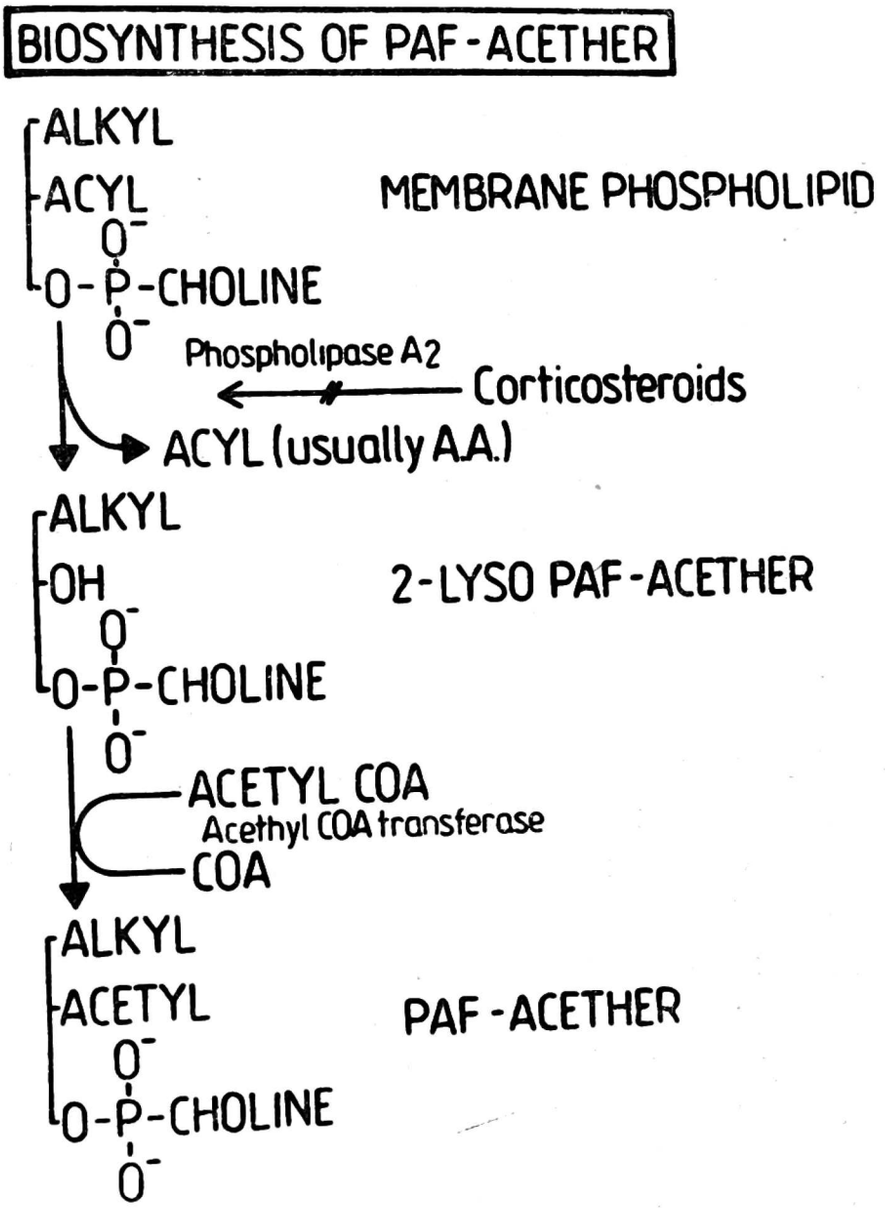

Fig. 10. Schematic diagram of biosynthetic pathway for PAF. The action of phospholipase A_2 can be inhibited by corticosteroids leading to the decrease in PAF synthesis. Lyso-PAF is the intermediate in both the anabolism and catabolism of PAF.

antagonist such as extract of Ginko biloba leaves may block the hypotension and other systemic effects of endotoxin and reduce the gastrointestinal damage associated with endotoxin shock. We found that PAF infused into the artery supplying the fundic portion of the canine stomach in a lucite chamber resulted in a dose-dependent reduction in the mucosal blood

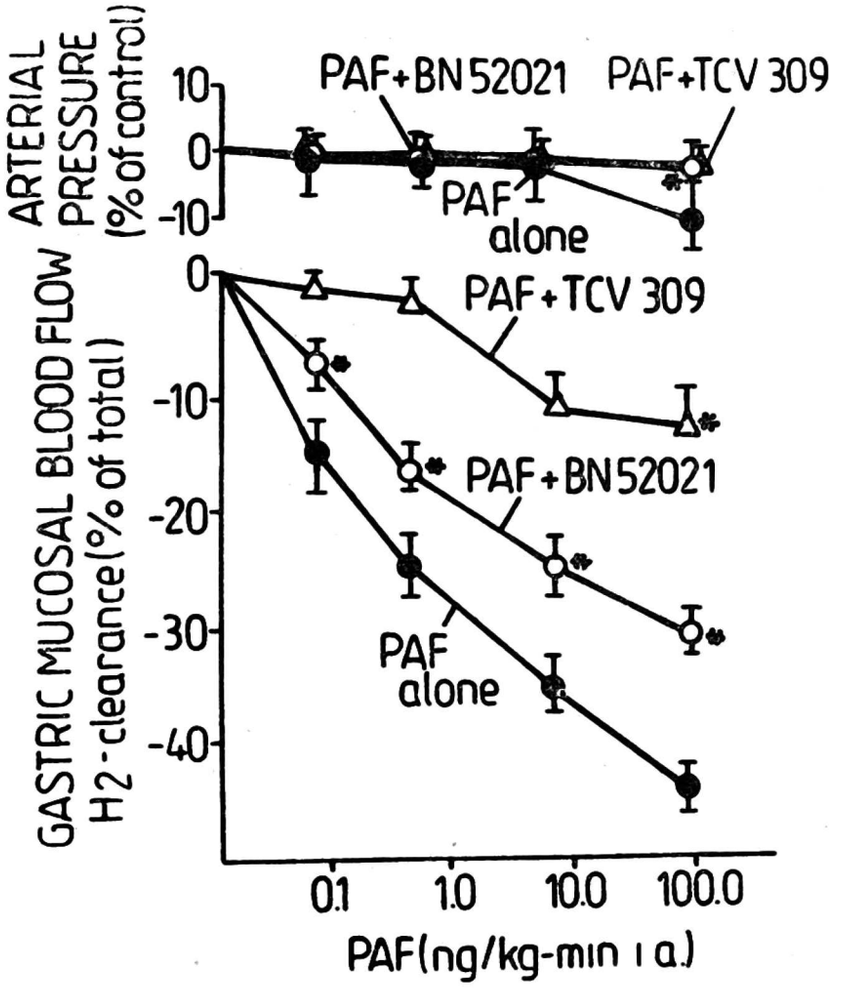

Fig. 11. Arterial blood pressure and mucosal blood flow of fundic portion of the stomach in the lucite chamber after close i. a. infusion of PAF in gradually increasing doses of anesthetized dogs without and with treatment with potent PAF receptor antagonist BN 52021 (10 mg/kg-h) or TCV 309 (20 μg/kg-h). Mean ± SEM of 5 tests on 5 dogs. Asterisk indicates significant change as compared to the value obtained with PAF alone

flow (as measured by H_2-hydrogen gas clearance) and in a fall in arterial pressure. These effects were prevented, in part, by the pretreatment with PAF receptor antagonists such as BN 52021 or TCV-309 suggesting that they are mediated by specific receptors in the gastric vasculature (*Fig. 11*).

Rosam et al (57) were the first to report that close intra-arterial infusion results in a dose-dependent increase in macroscopic and histological damage to the gastric mucosa; a dose of about 10 ng/kg-min is the threshold dose for significant damage compared with a control. In a dose of 5 ng/kg-min histological damage was observed in the form of vascular congestion in the mucosa, subepithelial edema, glandular disruption, vasocongestion

and hemorrhagic damage in the upper part of the mucosa. Systemic blood pressure started to decline at a dose of 25 ng/kg-min. Since the structurally related breakdown product lyso-PAF did not produce gastric damage (54), the effect of PAF cannot be considered as merely an effect of the lytic action of a small molecular phospholipid.

In our hands, PAF alone injected i.p. a dose of 10 ng/kg caused only small gastric mucosal lesions but when combined with topical irritants such as ethanol, taurocholate or aspirin, it greatly potentiated mucosal lesions. This effect could be almost completely prevented by pretreatment with a potent antagonist of PAF receptors such as BN 52021 (Fig. 12).

The characteristic feature of mucosal damage by PAF is vasocongestion accompanied by extensive damage to the vascular wall. Effects similar to these of PAF (100 ng/kg-min) can ben obtained by administration of E coli endotoxin (25 mg/kg) (51—53). Owing to vascular wall damage there is extensive leakage of plasma protein into the gastrointestinal lumen (51). These effects can be reduced by pretreatment with a PAF antagonist such as CV-3988 (53) or BN52021 (58). PAF or endotoxin-induced plasma leakage in the gastrointestinal tract (except the colon) is consistent with the macroscopic and histological observation of extensive necrosis in these tissues (51).

The result of plasma exsudation was profound hemoconcentration with increased hematocrit. Leukopenia preceded hemocontraction and this might probably be explained by neurotrophil aggregation and degranulation leading to the release of LTC_4 (44, 59). Significant hemoconcentration, a consequence of increased vascular permeability coupled with prolonged hypotention probably accounted for the sluggishness of the mucosal blood flow caused by PAF (59, 60).

The mechanism by which PAF produces gastrointestinal damage is still unknown, nor is it clear whether PAF mainly originates from the circulating blood or locally in the gastric mucosa. It appears that PAF might originate from the mucosa itself but under certain conditions such as septic shock additional amounts could be formed in the body and released into the circulation.

One convenient approach to test whether or not PAF contributes to acute gastric mucosal damage by various ulcerogens is the use of a PAF-receptor antagonist such as BN 52021. PAF given alone i. v. in a dose (10 μg/kg) that caused only small mucosal damage, greatly enhanced the ethanol- taurocholate or aspirin-induced mucosal lesions (*Fig. 12*). This antagonist had, however, no influence on the extent of mucosal damage by any of these irritants alone (29). This indicates that PAF is unlikely to play a major role in the pathogenesis of acute mucosal damage induced by topical irritants. Braquet et al (61) reported that stress-induced damage

could be reduced by a PAF receptor antagonist but this required a dose several times higher (61). We found that a potent PAF receptor antagonist, TCV-309, prevented in part the acute mucosal lesions provoked by water immersion and stress (see *Fig. 9*).

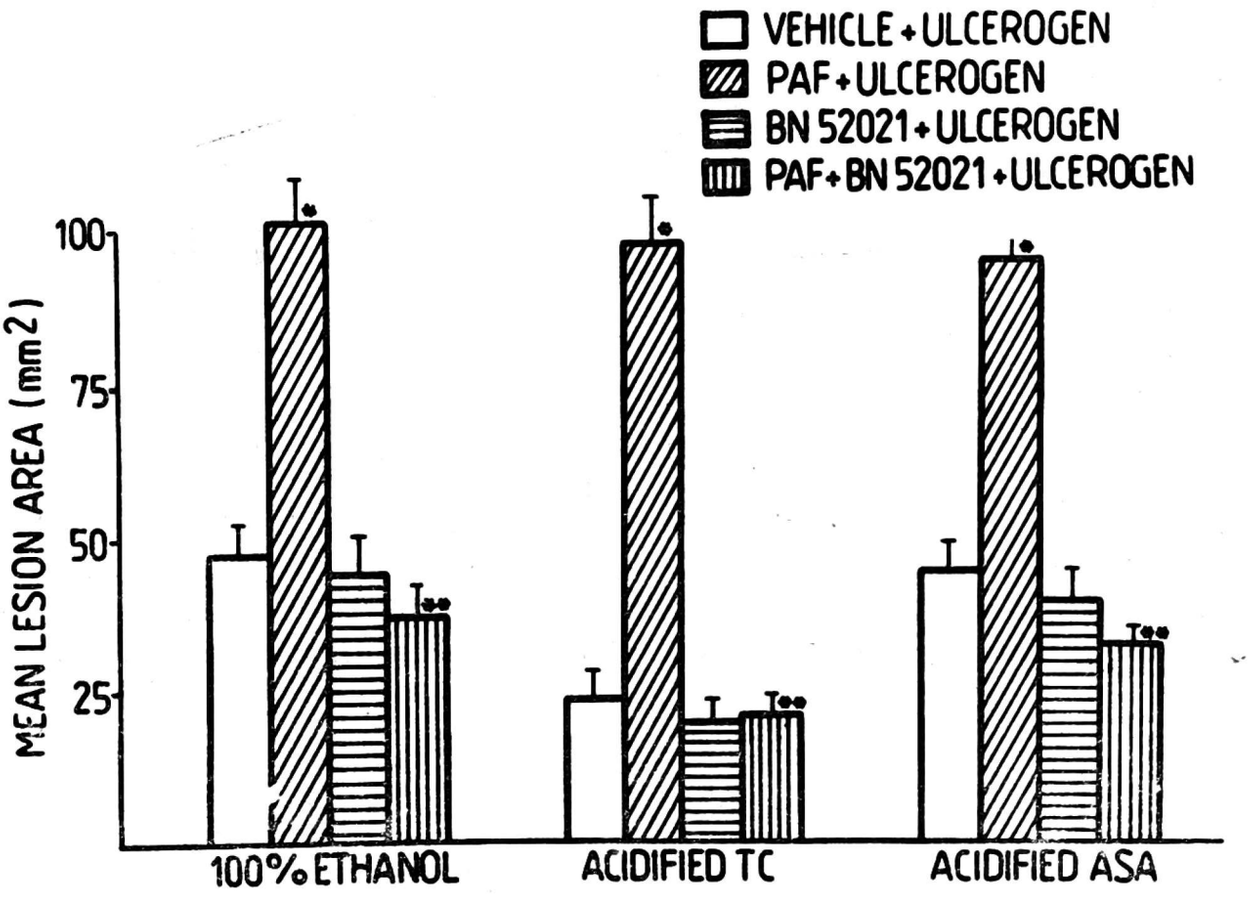

Fig. 12. Mean area of gastric lesions induced by 100% ethanol, acidified taurocholate (TC) acidified aspirin (ASA) and stress applied alone (vehicle) or in combination with PAF (10 μg/kg), BN 52021 (20 mg/kg) or PAF+BN 52021. Mean ± SEM of 8—10 rats. Single asterisk indicate significant increase above control (vehicle) value obtained with ulcerogen alone. Double asterisks indicate significant decrease below the value obtained with the combination of ulcerogen plus PAF [From Konturek et al (20)].

Another condition where a PAF antagonist was found to be effective was endotoxin-induced gastric damage. This was originally described by Braquet at al (61) but according to our experience (*Fig. 13*) not only the PAF antagonist but also the thromboxane synthesis inhibitor. OKY-1581, and the LTC_4 receptor antagonist, FPL-55712, were effective, suggesting that endotoxin-induced damage is mediated by TX and LT. It is of interest that PAF-induced mucosal damage in intact or ethanol-treated mucosa can be suppressed by LT synthesis inhibitor or LT receptor antagonist (29) indicating that PAF, like endotoxin, damages gastric mucosa via LTC_4. Indeed, the more detailed studies of Peskar (62) on vascularly perfused rat stomach demonstrated that PAF releases dose-dependently LTC_4 and this can be partly blocked by NDGA which in our studies prev-

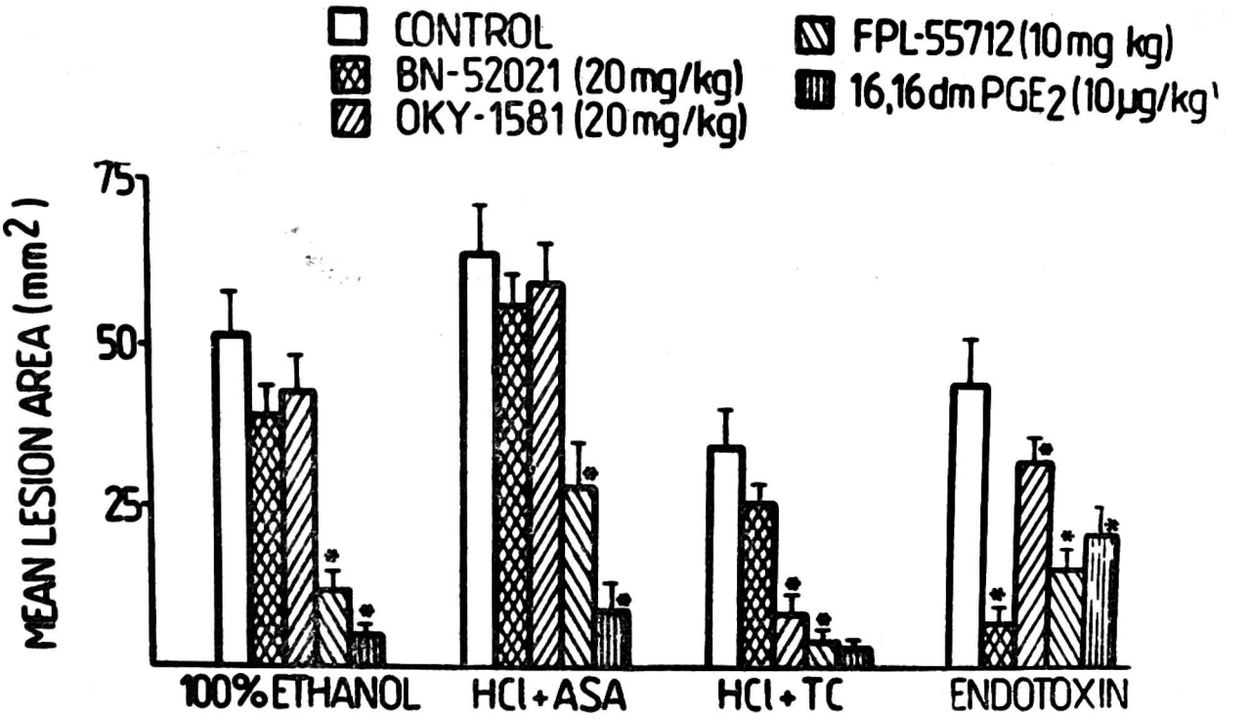

Fig. 13. Mean area of gastric lesions induced by 100% ethanol, acidified aspirin (ASA), acidified taurocholate (TC) or endotoxin alone or in combination with BN 52021, OKY 1581, FPL 55712 or 16,16 dimethyl PGE_2. Means $\pm$SEM of 8—10 rats. Asterisk indicates significant decrease below the value obtained with ulcerogen alone.

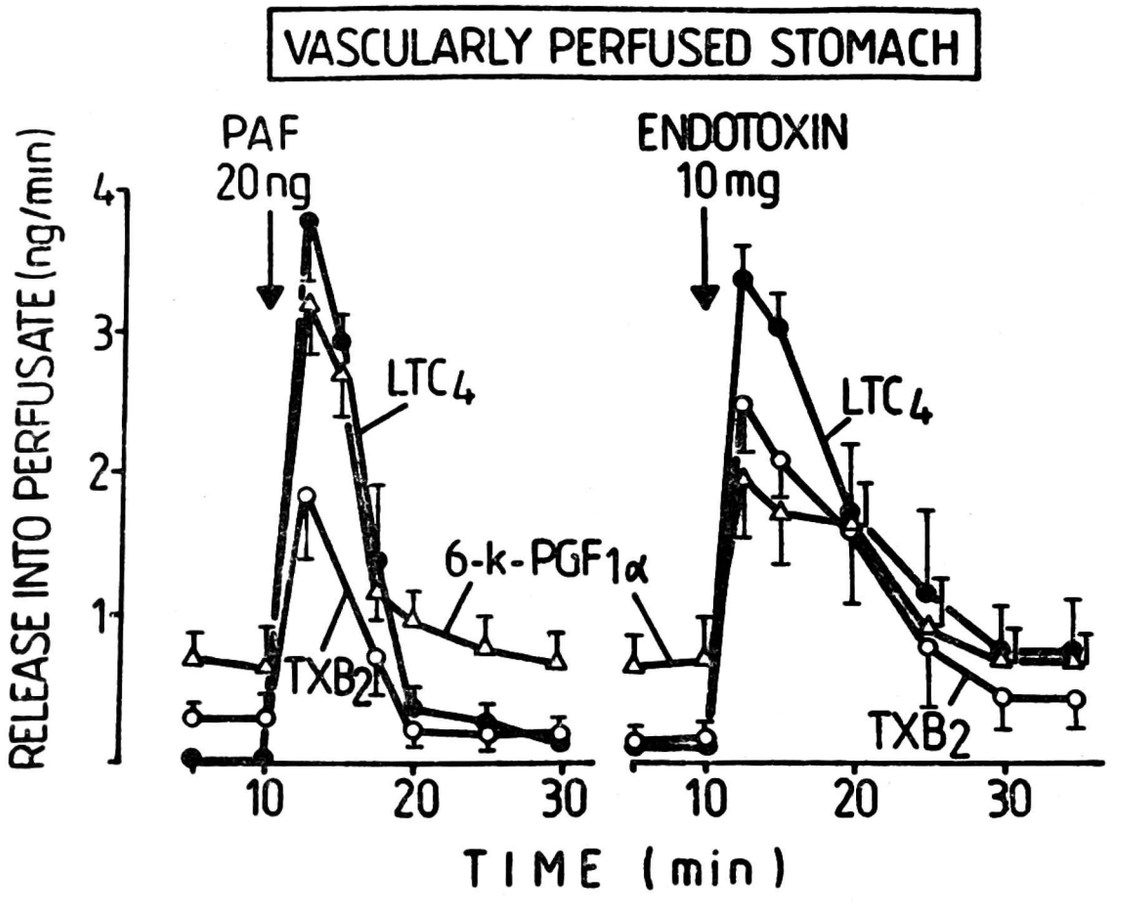

Fig. 14. The release of immunoreactive PAF, LTC_4, TXB_2 and 6-keto-PGE_{2k} from the vascularly perfused isolated rat stomach before and after administration of PAF (20 µg) or endotoxin (10 mg). Mean $\pm$SEM of 5 tests on 5 perfused stomach.

ented PAF-induced mucosal damage (29). PAF also released TX and PG from the stomach and this, in turn, may explain the protective effect of TX inhibitor and the augmenting action of indomethacin on PAF-induced gastric damage. Similar increase in the release of LTC_4, TX and PG was observed following the administration of endotoxin, suggesting that both PAF and endotoxin act via the liberation of various products of arachidonate metabolism (*Fig. 14*).

The important role of LT in the gastric mucosal damage induced by PAF and endotoxin is supported by our finding that both these compounds greatly increased in vivo the generation of LTC_4 while increasing gastric damage. The combination of PAF plus ethanol or PAF plus endotoxin resulted in the augmentation of gastric lesions and further increments in mucosal biosynthesis of LTC_4 (*Fig. 15*). Other effects of PAF, not only gastric, but also intestinal lesions (63) and hemoconcentration, can be reproduced by LTC_4 and LTD_4, thus reinforcing the notion that these LT mediate PAF-induced mucosal damage (54).

Recent study by Cucala and Wallace (65) has demonstrated that intracerebroventricular administration of PAF resulted in potent inhibition of acid secretion without causing gastric mucosal damage. These

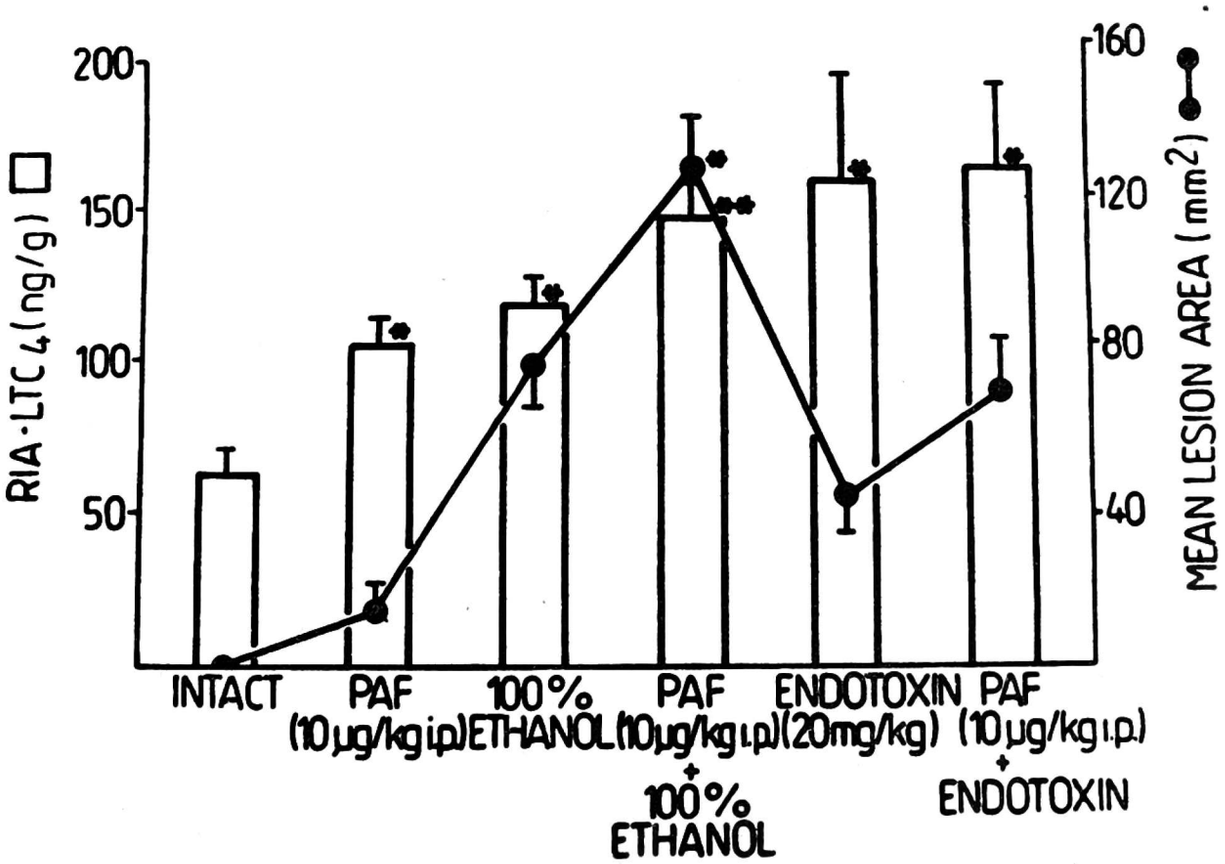

Fig. 15. Mucosal generation of LTC_4 and mean lesion area in rat stomach after adminin tration of PAF (10 µg/kg i. p.), 100% ethanol (1 ml i. g.), PAF plus ethanol, endotoxis (20 mg/kg) and endotoxin plus PAF. Mean ± SEM of 8—10 rats. Single asterisk indicate significant increase above the value obtained in intact stomach. Double asterisks indicate significant increase above the value obtained with PAF or ethanol alone.

effects of PAF were opposite to those of TRH which increased acid secretion and induced gastric damage. Furthermore, PAF applied centrally did not affect the blood pressure, suggesting that this membrane-active phospholipid may play a different role when administered either peripherally or centrally. These data also suggest the participation of membrane phospholipids as PAF in gastric modulatory system at the central level.

REFERENCES

1. Robert A. Cytoprotection by prostaglandins. Gastroenterology 1979; 77: 761—767.
2. Robert A, Nezamis BE, Lancaster C, Hanchar A. Cytoprotection by prostaglandins in rats: prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. Gastroenterology 1979; 77: 433—443.
3. Davenport H. The gastric mucosal barrier. Past, present and future. Mayo Cain Proc 1975; 50: 507—514.
4. Hollander D, Tarnawski AS (eds). Gastric Cytoprotection. New York; Plenum 1989.
5. Lacy DR, Ito S. Microscopic analysis of ethanol damage to rat gastric mucosa after treatment with a prostaglandins. Gastroenterology 1982; 83: 619—625.
6. Lacy DR, Ito S. Rapid epithelium restitution on the rat gastric mucosa after ethanol injury. Lab Invest 1984; 51: 573—583.
7. Wallace J. Increased resistance of the rat gastric mucosa to hemorrhagic damage after exposure to an irritant. Role of the „mucoid cap" and prostaglandins synthesis. Gastroenterology 1988; 94: 22—32.
8. Wallace J. McKnight G. The mucoid cap over superficial gastric damage in the rat. A high-pH microenviroment dissipated by nonmaterial anti-inflammatory drugs and endothelin. Gastroenterology 1990; 99: 295—304.
9. Guth PH, Paulsen G, Nagata H. Histology and microcirculatory changes in alcohol--induced gastric lesions in the rat: Effect of prostaglandins cytoprotection. Gastroenterology 1984; 87: 1083—1090.
10. Szabo S, Trier AS, Brown A, Schnoor J. Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in rat. Gastroenterology 1985; 88: 1948—1953.
11. Szabo S, Phial G, Trier AS. Alterations in blood vessels during gastric injury and protection. Scand J Gastroenterology 1986; 21: S92—96.
12. Pihan G, Majzoubi D, Handenschild C, Trier AS, Szabo S. Early microcirculatory stasis in acute gastric mucosal injury in the rat and prevention by 16, 16-dimethyl prostaglandin E2 or sodium thiosulfate. Gastroenterology 1986; 91: 1415—1426.
13. Wallace JR. Endogenous Mediators of Gastrointestinal Diseases. Boca Raton; CRC 1989.
14. Hamberg M, Samuelsson B. Prostaglandin endoperoxides; Novel transformation of arachidonic acid in human platelets. Proc Natl Acad Sci USA 1974; 71: 3400—3408.
15. Samuelsson B. The Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammations. Science 1983; 220: 568—575.
16. Lewis RA, Austen KF. The biologically active leukotrienes. Biosynthesis metabolism, receptors, functions and pharmacology. J Clin Invest 1984; 73: 889—910.

17. Goldenberg MM, Subers EM. The reactivity of rat isolated gastrointestinal tissues to leukotrienes. Eur J Pharmacol 1982; 78: 463—470.
18. Whittle BJR, Or en-Woman, Guth PH. Gastric vasoconstrictor actions of leukotriene C_4, PG_2 and thromboxane mimetic V-46619 on rat submucosal microcirculation un vivo. Am J Physiol 1985; 248: G580—586.
19. Pawlik W, Konturek S, Gustaw P, Sendur R, Czarnobilski K, Beck G, Jendralla M. Gastric vasoconstrictive and secretory effects of leukotrienes C_4 and D_4 in canine stomach. In Samuelsson B, Paoletti R, Ramwell (eds) Advances in Prostaglandin, Thromboxane and Leukotriene Research, Vol. 17, New York, Raven 1987, pp. 357—360,
20. Konturek S, Bilski J, Dembinski A, Warzecha Z, Beck G, Jendralla H. Effects of leukotrienes on gastric acid and alkaline secretions. Gastroenterology 1987; 92: 1209—1214.
21. Schepp W, Kath D, Tatge C et al. Leukotrienes C and D potentiate acid production by isolated rat parietal cells. Gastroenterology 1989; 97: 1420—1429.
22. Pihan G, Rogers C, Szabo S. Vascular injury in acute gastric mucosal damage. Mediatory role of leukotrienes. Dig Dis Sci 1988; 33: 625—632.
23. Konturek S, Brzozowski T, Drozdowicz D, Beck G. Role of leukotrienes in acute gastric lesions induced by ethanol, taurocholate, aspirin, platelet activating factor and stress in rats. Dig Dis Sci 1988; 33: 806—813.
24. Oates P, Hakkinen UP. Studies on the mechanism of ethanol-induced gastric damage in rats. Gastroenterology 1988; 94: 10—21.
25. Peskar AM, Lange K, Hope V, Peskar BA. Ethanol stimulates formation of leukotrienes C_4 in rat gastric mucosa. Prostaglandins 1986; 31: 283—292.
26. Wallace J, Beck PL, Morris GP. Is there a role for leukotrienes as mediators of ethanol-induced gastric mucosal damage? Am J Physiol 1988; 254: G117—123.
27. Boughton-Smith NK, Whittle BJR. Failure of the inhibition of rat gastric mucosal 5-lipoxygenase by novel acetohydroxamic acids to prevent ethanol-induced damage. Eur J Pharmacol 1988; 95: 155—162.
28. Wallace J, Whittle BJR. Role of prostanoids in the protective actions of BW755c on the gastric mucosa. Eur J Pharmacol 1985; 115: 45—52.
29. Konturek S, Brzozowski T, Drozdowicz, Garlicki J, Beck G. Role of leukotrienes and platelet activating factor in acute gastric mucosal lesions in rats. Eur J Pharmacol 1989; 164: 285—292.
30. Peskar BM. Leukotrienes in mucosal damage and protection. J Physiol Pharmacol 1991; 42: 135—145
31. Peskar BM, Hoppe V, Lange K, Peskar BA. Effects of non-steroidal antiinflammatory drugs on rat gastric mucosal leukotriene C_4 and prostaglandin release relation to ethanol — induced injury. Br J Pharmacol 1988; 93: 937—943.
32. Peskar BM. Lipooxygnase products in gastric damage and protection. Adv Prostaglandin Thromboxane Leukotriene Res 1990; 21: 753—760.
33. Holzer P, Pabst MA, Lippe IT, et al. Afferent nerve-mediated protection against deep mucosal damage in the rat stomach. Gastroenterology 1990; 98: 838—848.
34. Osada T, Goto H, Tsukamoto Y, et al. Role of leukotrienes in hydrochloric acid--induced gastric lesions in rats. Dig Dis Sci 1990; 35: 186—192.
35. Wallace JL, McKnight GW, Keenan CM, et al. Effects of leukotrienes on susceptibility of the rat stomach to damage and investigation of the mechanism of action. Gastroenterology 1990; 98: 1178—1186.
36. Wallace JL. Lipid mediators of inflammation in gastric ulcer. Am J Physiol 1990; 258: G1-G11.

37. Wallace JL, Whittle BJR. Acceleration of recovery of gastric epithelial integrity by 16,16 dimethyl prostaglandin E_2. Br J Pharmacol 1985; 86: 837—842.
38. Schmidt KL, Bellard RL, Smith GS, Hanagan JM, Miller TA. Influence of prostaglandin on repair of rat stomach damaged by absolute ethanol. J Surg Res 1986; 41: 367—377.
39. Kuroiwa M, Sugiyama S, Goto H, et al. The role of mucosal prostaglandin levels in healing of water immersion-induced gastric ulcers in rats. Scand J Gastroenterol 1990; 25: 59—65.
40. Brzozowski T, Drozdowicz D, Konturek SJ et al. Leukotrienes in healing of acute gastric lesions induced by water immersion and restraint stress. Digestion (in publication)
41. Benveniste J. PAF-a new mediator of anaphylaxis and immune complex deposition from rabbit and human basophlis. Nature 1974; 249: 581—582.
42. Chignard M, LeCouedic IP, Tence M, Vargaftig BB, Benveniste J. The role of PAF in platelet aggregation. Nature 1979; 279: 799.
43. Lynch IM, Lotner G, Betz S, Henson PM. The release of a platelet activating factor by stimulated rabbit neutrophiles. J Immun 1979; 123: 1219.
44. Chilton FH, O'Flattery IT, Wash CE, Thomas MJ, Wykle RL, Dechatelet IR, Waite BM. Platelet activating factor. Stimulation of the lipoxygenase pathway in polymorphonuclear leukocytes by 1-0-ally-2-0-acetic-ns-glycerin-3-phosphocholine. J Biol Chem 1982; 257: 5402—5407.
45. Blackwell G, Carnuccio R, Dirks M, Flower R, Parent L, Perish P. Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocortycoids. Nature 1980; 287: 147—149.
46. Wallace IL. Glucocortycoids-induced gastric mucosal damage: Inhibition of leukotriene, but not prostaglandin biosynthesis. Prostaglandins 1987; 34: 311—323.
47. Heymans F, Michel E, Borrel W et al. New total synthesis and high resolution HNMR spectrum of platelet activating factor; its enantiomer and racemic mixture. Biochim Biophys Acta 1981; 666: 230—240.
48. Flower RJ, Blackwell GJ Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation. Nature 1979; 278: 456—459.
49. Whittle BJR, Morisiten T, Ohya Y, Lung F, Guth PH. Microvascular actions of platelet activating factor on rat gastric mucosa and submucosa. Am J Physiol 1986; 251: 6722—6778.
50. Terashita Z, Imura Y, Nishikawa K, Sumida S. Is platelet activating factor (PAF) a mediator of endotoxin shock? Eur J Pharmacol 1985; 109: 257—262.
51. Wallace JL, Steel G, Whittle BJR, Lagemente V, Vargaftic B. Evidence for platelet activating factor as a mediator of endotoxin-induced gastrointestinal damage in the rat: effects of three platelet-activating factor antagonists. Gastroenterology 1987; 93: 765—773.
52. Doebber TW, Wu MS, Robbins IC, Choy BM, Chang MN, Shen TY. Platelet activating factor (PAF) involvement in endotoxin-induced hypotension in rats. Studies with PAF-receptor antagonist kadsurenone. Biochem Biophys Res Comm 1985; 127: 799—802.
53. Wallace JL, Whittle BJR. Prevention of endotoxin-induced gastrointestinal damage by TV-3988 an antagonist of platelet activating factor. Eur J Pharmacol 1986; 124: 209—210.
54. Wallace JL, Whittle BJR. Gastrointestinal damage induced by platelet activating factor. Inhibition by the corticoid, dexamethasone. Dig Dis Sci 1988; 33: 225—232.

55. Braquet P, Paubert-Braquet M, Bessin P, Vargaftig B. Platelet activating factor a potential mediator of shock. In; Samuelsson B (ed) Advances in Prostaglandin, Thromboxane and Leukotrienes Research. New York, Raven 1986; 17 pp. 823—829.
56. Braquet P, Paubert-Braquet M, Koltai M, Bourgain R, Bussolino F, Hosford D. Is there a case for PAF antagonists in the treatment of ischemic states? TiPS 1989; 10: 23—30.
57. Rosam AC, Wallace JL, Whittle BJR. Potent ulcerogenic actions of platelet activating factor on the stomach. Nature 1986; 319: 54—56.
58. Etienne A, Hecquet F, Soulard C, Spinnewyn B, Closutre F, Braquet P. In vivo inhibition of plasma protein leakage and salmonella antagonist: BN 52021. Agents and Actions 1985; 17: 368—370.
59. Wallace JL, Whittle BJR. Effects of inhibitors of arachidonic acid metabolism on PAF-induced gastric mucosal necrosis and haemoconcentration. Br J Pharmacol 1986; 89: 415—422.
60. Wallace JL, Whittle BJR. Picomole doses of platelet activating factor predispose the gastric mucosa to damage by topical irritants. Prostaglandins 1986; 31: 1989—1998.
61. Braquet P, Etienne A, Mencia-Huerta JM, Clostre F.. Effects of the specific platelet activating factor antagonists, BN 52021 and BN 52063 on various experimental gastrointestinal ulcerations. Eur J Pharmacol 1988; 150: 269—276.
62. Dembinska-Kiec A, Peskar BA, Muller OK, Peskar AM. The effects of platelet activating factor on flow rate and eicosanoid release in the isolated perfused rat gastric vascular bed. Prostaglandins 1989; 37: 69—91.
63. Hsueh W, Gonzales-Crussi F, Arroyave J. Platelet activating factor induced ischemic bowel necrosis. An investigation of secondary mediators in its pathogenesis. Am J Path 1986; 122: 231—239.
64. Hsueh W, Gonzales-Crussi F, Arroyave J. Release of leukotriene C_4 by isolated prefused rat small intestine in response to platelet activating factor. J Clin Invest 1986; 78: 108—114.
65. Cucala M, Wallace J, Salas A, Guarner F, Rodriguez R, Malagelada J-R. Central regulation of gastric acid secretion by platelet activating factor in anesthesized rats. Prostaglandins 1989; 37: 275—285.

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