Br. J. Pharmacol. (1988), **93**, 937–943

Effects of non-steroidal anti-inflammatory drugs on rat gastric mucosal leukotriene C_4 and prostanoid release: relation to ethanol-induced injury

* B.M. Peskar, [1] U. Hoppe, [2] K. Lange & B.A. Peskar

* Department of Experimental Clinical Medicine, Ruhr-University of Bochum, and Department of Pharmacology and Toxicology, Ruhr-University of Bochum, Im Lottental, D-4630 Bochum 1, F.R.G.

1 The effects of oral and subcutaneous administration of the non-steroidal anti-inflammatory drugs sodium salicylate, aspirin and indomethacin on *ex vivo* gastric mucosal release of leukotriene C_4 (LTC_4) prostaglandin E_2 (PGE_2), 6-oxo-$PGF_{1\alpha}$ and thromboxane B_2 (TXB_2) were investigated in rats under basal conditions as well as after challenge with ethanol.

2 Basal release of PGE_2, 6-oxo-$PGF_{1\alpha}$ and TXB_2 was inhibited by oral administration of aspirin (0.6–400 mg kg^{-1}) and indomethacin (4 or 20 mg kg^{-1}), but not by sodium salicylate (up to 400 mg kg^{-1}), in a dose-dependent manner. Oral administration of aspirin in the dose range 3.2–400 mg kg^{-1} and of indomethacin (20 mg kg^{-1}) additionally inhibited release of LTC_4, while sodium salicylate (up to 400 mg kg^{-1}) had no effect. Indomethacin (20 mg kg^{-1}) and aspirin (400 mg kg^{-1}) administered subcutaneously inhibited generation of cyclo-oxygenase products of arachidonate metabolism, but did not significantly affect LTC_4 synthesis.

3 Oral instillation of ethanol caused gastric mucosal damage and simultaneously induced a selective increase in the *ex vivo* release of LTC_4 from rat gastric mucosa, while release of cyclo-oxygenase products of arachidonate metabolism was not significantly affected. Oral pretreatment of rats with sodium salicylate protected the gastric mucosa and simultaneously inhibited the ethanol-stimulated gastric mucosal LTC_4 release in a dose-dependent manner. Sodium salicylate had no effects on the release of PGE_2 and TXB_2, while that of 6-oxo-$PGF_{1\alpha}$ was slightly increased.

4 Pretreatment with indomethacin (4 or 20 mg kg^{-1} p.o.) or aspirin in doses up to 25 mg kg^{-1} p.o. prior to oral instillation of ethanol did not inhibit gastric mucosal damage and had no effect on the stimulatory action of ethanol on LTC_4 release. Higher doses of aspirin (100 mg kg^{-1} or 400 mg kg^{-1} p.o.) reduced the mucosal damaging effect of ethanol and simultaneously inhibited LTC_4 release.

5 The results suggest that aspirin and indomethacin in concentrations higher than those necessary to inhibit the cyclo-oxygenase pathway of arachidonate metabolism additionally inhibit gastric mucosal LTC_4 synthesis under basal conditions, while sodium salicylate has no such effect. On the other hand, sodium salicylate, but not indomethacin or low doses of aspirin (up to 25 mg kg^{-1}), by an unknown mechanism inhibits stimulation of LTC_4 biosynthesis by ethanol and simultaneously protects the gastric mucosa against ethanol-induced damage. Similar effects of high oral doses (>100 mg kg^{-1}) of aspirin might be due to significant formation of salicylate. These results suggest that there is a causal relationship between enhanced LTC_4 biosynthesis and the development of ethanol-induced gastric injury.

Introduction

In contrast to aspirin, sodium salicylate is not ulcerogenic in rats (Glenn *et al.*, 1979; Whittle *et al.*, 1980). Moreover, sodium salicylate was found to be gastroprotective against various noxious agents including ethanol (Robert, 1981). The mechanism of this protection remained unknown, but did not appear to be due to increased formation of prostaglandins by the rat gastric mucosa. The protective

[1] Present address: Nattermann & Cie. GmbH., D-5000 Cologne 30, F.R.G.
[2] Present address: BAYER AG., D-5600 Wuppertal, F.R.G.

effect of sodium salicylate was not blocked by indomethacin and thus differed from the protection afforded by mild irritants such as 20% ethanol (Robert, 1981).

A mediator role for lipoxygenase products of arachidonate metabolism and/or oxygen radicals in ethanol-induced rat gastric damage has been suggested by Wallace & Whittle (1985). We have recently demonstrated that, indeed, oral instillation of ethanol stimulates rat gastric mucosal leukotriene C_4 (LTC_4) formation parallel to the production of haemorrhagic mucosal lesions (Peskar *et al.*, 1986). Furthermore, we have shown that inhibition of gastric LTC_4 formation by a number of drugs such as nordihydroguaiaretic acid (NDGA), cysteamine and diethyl maleate is accompanied by protection against ethanol-induced gastric mucosal injury (Peskar *et al.*, 1986; Lange *et al.*, 1987). From these results we have concluded that the mechanism of action of such gastroprotective drugs is inhibition of cysteinyl-leukotriene biosynthesis, in contrast to the gastroprotection afforded by other drugs, such as exogenous prostaglandin E_2 (PGE_2), that do not affect gastric LTC_4 biosynthesis (Lange *et al.*, 1987).

We have now investigated whether the mode of action underlying the protective effect of sodium salicylate against ethanol involves effects on gastric mucosal LTC_4 formation. In these experiments we have compared the effects of sodium salicylate with those of two other non-steroidal anti-inflammatory drugs (NSAIDs), aspirin and indomethacin, on *ex vivo* mucosal eicosanoid release under basal conditions and after oral instillation of ethanol, as well as on ethanol-induced gastric damage. Some of the results have been presented at the American Gastroeneterological Association Meeting (Peskar *et al.*, 1987).

Methods

Male Wistar rats (180–200 g) were deprived of food for 24 h with free access to water. Groups of 6–14 rats were treated orally with sodium salicylate (6.25–400 mg kg^{-1}), aspirin (0.4–400 mg kg^{-1}) or indomethacin (4 or 20 mg kg^{-1}). All drugs were suspended in 0.25% w/v carboxymethylcellulose. Control rats received the vehicle (2.0 ml kg^{-1}). After 30 min, ethanol (1.5 ml) was instilled into the stomach and the rats were killed 5 min later. The stomachs were removed and opened along the greater curvature and the severity of gastric damage was evaluated by calculation of a lesion index (Peskar *et al.*, 1986). Briefly, the number of necrotic bands of more than 4 mm in length was multiplied by the factor 3, lesions of 2–4 mm were multiplied by the factor 2 and lesions of less than 2 mm by the factor 1. The lesion index was calculated as the total number of lesions multiplied by the respective severity factors.

In further experiments groups of 6–10 rats were treated orally with sodium salicylate (25–400 mg kg^{-1}), aspirin (0.6–400 mg kg^{-1}), indomethacin (4 or 20 mg kg^{-1}) or the vehicle 0.25% w/v carboxymethylcellulose and were killed 30 min later without prior ethanol instillation. In another series of experiments the highest dose of each drug used (400 mg kg^{-1} sodium salicylate, 400 mg kg^{-1} aspirin, 20 mg kg^{-1} indomethacin) was administered to groups of 6 rats by subcutaneous injection (2.5 ml kg^{-1}). All drug solutions were freshly prepared. Indomethacin was dissolved in 1% w/v sodium bicarbonate and sodium salicylate was dissolved in water. Aspirin was dissolved in 0.1 N NaOH and the solution was immediately adjusted to pH 5.0. Control rats received one of the three solvents, which did not differ in their effects on gastric mucosal eicosanoid release. In these experiments the rats were killed 1 h after drug administration. No evidence of macroscopically visible mucosal damage was observed after oral or subcutaneous administration of non-steroidal anti-inflammatory drugs.

Fragments of gastric corpus mucosa were excised, blotted, weighed and aliquots of 40 mg wet weight were incubated in 0.5 ml oxygenated Tyrode solution at 37°C for 10 min. LTC_4, prostaglandin E_2 (PGE_2), 6-oxo-$PGF_{1\alpha}$ and thromboxane B_2 (TXB_2) in the incubation media were determined radioimmunologically as described previously (Peskar *et al.*, 1986). For the LTC_4 determination aliquots of the media were boiled immediately after the incubations in order to eliminate material that interferes non-specifically in the radioimmunoassay (Peskar *et al.*, 1986). Validation of the radioimmunological data on gastric mucosal LTC_4 release by reversed phase high performance liquid chromatography (h.p.l.c.) and bioassay has been described previously (Peskar *et al.*, 1986). By use of h.p.l.c. it was demonstrated that the LTC_4-like immunoreactivity released from the gastric mucosa *ex vivo* under basal conditions, as well as after stimulation with ethanol, comigrated almost exclusively with standard LTC_4, while only minor amounts of LTD_4 and LTE_4 were found.

Results were calculated as means ± s.e. mean. Statistical analysis of eicosanoid release was performed by use of Student's *t* test. Ethanol-induced gastric mucosal damage was evaluated by the Wilcoxon rank test.

Materials

Sodium salicylate, aspirin and indomethacin were from Sigma, St. Louis, Mo., U.S.A.

Results

Basal and ethanol-induced gastric mucosal eicosanoid release

Fragments of gastric corpus mucosa from rats that had not received ethanol released about equal amounts of PGE_2 and 6-oxo-$PGF_{1\alpha}$ and smaller amounts of TXB_2 (Table 1). The amounts of LTC_4 detected in the incubation media were lower than those of the cyclo-oxygenase-derived products of arachidonate metabolism (Table 1). Instillation of 1.5 ml ethanol 5 min before removal of the stomach caused extensive haemorrhagic mucosal necrosis resulting in a lesion index of 48 ± 2 ($n = 48$). Subsequent *ex vivo* incubation of the gastric mucosa of these rats demonstrated a significant ($P < 0.001$) increase in the release of LTC_4 as compared to that in control rats (Table 1). On the other hand, the *ex vivo* release of the cyclo-oxygenase products of arachidonate metabolism, PGE_2, 6-oxo-$PGF_{1\alpha}$ and TXB_2, was not significantly altered by ethanol treatment, although release of PGE_2 tended to be slightly increased (Table 1).

Table 1 Effect of ethanol instillation on gastric mucosal eicosanoid release *ex vivo*

	Controls	*Ethanol-treated rats*
LTC_4	82 ± 11	429 ± 34*
PGE_2	890 ± 123	1016 ± 55
6-oxo-$PGF_{1\alpha}$	805 ± 87	760 ± 51
TXB_2	327 ± 36	286 ± 15

Results are given in $ng\,g^{-1}$ wet weight $10\,min^{-1}$ and represent means ± s.e. mean of $n = 25$ for the controls and $n = 48$ for the ethanol-treated group. * $P < 0.001$ as compared to controls. Abbreviations used in this and subsequent tables: LTC_4, leukotriene C_4; PGE_2, prostaglandin E_2; TXB_2, thromboxane B_2.

Effects of sodium salicylate

Sodium salicylate administered orally in doses up to $400\,mg\,kg^{-1}$ did not significantly affect release of LTC_4 and the cyclo-oxygenase-derived products of arachidonate metabolism, PGE_2, 6-oxo-$PGF_{1\alpha}$ and TXB_2, from gastric mucosal fragments of rats that had not additionally received ethanol (Table 2). Similarly, subcutaneous administration of $400\,mg\,kg^{-1}$ sodium salicylate had no significant effect on *ex vivo* gastric mucosal eicosanoid release (Table 3).

On the other hand, oral pretreatment with sodium salicylate inhibited in a dose-dependent manner the stimulatory action of ethanol on gastric mucosal LTC_4 release (Figure 1). This effect paralleled the protection afforded by the drug against ethanol-induced injury (Figure 1). Similar to basal conditions release of the cyclo-oxygenase products of arachidonate metabolism PGE_2 and TXB_2 was not significantly altered by sodium salicylate in ethanol-treated rats. Release of 6-oxo-$PGF_{1\alpha}$ tended to be increased,

Table 2 Effect of oral treatment with sodium salicylate on *ex vivo* eicosanoid release from gastric mucosa of rats not additionally treated with ethanol

	Controls	*Sodium salicylate*		
		$25\,mg\,kg^{-1}$	$100\,mg\,kg^{-1}$	$400\,mg\,kg^{-1}$
LTC_4	67 ± 19	57 ± 18	48 ± 20	51 ± 18
PGE_2	859 ± 127	697 ± 140	1082 ± 154	956 ± 75
6-oxo-$PGF_{1\alpha}$	676 ± 43	856 ± 143	849 ± 85	808 ± 115
TXB_2	245 ± 16	251 ± 25	230 ± 17	288 ± 30

Results are given in $ng\,g^{-1}$ wet weight $10\,min^{-1}$ and represent means ± s.e. mean of $n = 6$ for each group.

Table 3 Effect of subcutaneous administration of sodium salicylate ($400\,mg\,kg^{-1}$), aspirin ($400\,mg\,kg^{-1}$) and indomethacin ($20\,mg\,kg^{-1}$) on *ex vivo* eicosanoid release from gastric mucosa of rats as determined 1 h later

	Controls	*Sodium salicylate*	*Aspirin*	*Indomethacin*
LTC_4	49 ± 14	65 ± 16	30 ± 9	33 ± 6
PGE_2	786 ± 132	773 ± 148	75 ± 3*	105 ± 12*
6-oxo-$PGF_{1\alpha}$	725 ± 116	542 ± 62	63 ± 1*	90 ± 12*
TXB_2	216 ± 28	244 ± 28	26 ± 2*	26 ± 2*

Results are given in $ng\,g^{-1}$ wet weight $10\,min^{-1}$ and represent means ± s.e. mean of $n = 6$ for each group. * $P < 0.001$ as compared to controls.

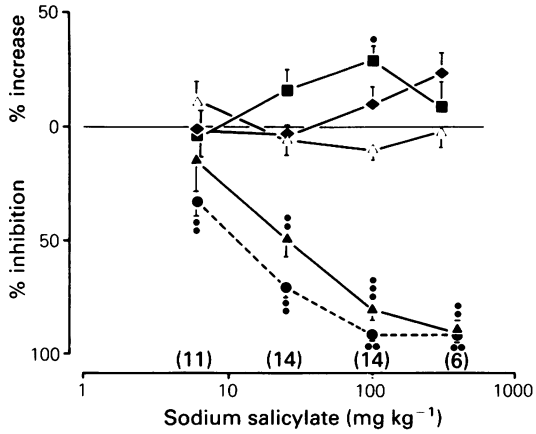

Figure 1 Effects of pretreatment with sodium salicylate on ethanol-induced gastric mucosal damage, determined as lesion index (●), and release of leukotriene C_4 (LTC_4, ▲), prostaglandin E_2 (PGE_2, ◆), 6-oxo-$PGF_{1\alpha}$ (■) and thromboxane B_2 (TXB_2, △) from gastric mucosal fragments incubated *ex vivo*. Each point represents the mean of (n) rats; vertical lines indicate s.e. mean. Lesion index in rats pretreated with vehicle before ethanol was 51 ± 2 and was taken as 100%. Release of eicosanoids from gastric mucosa of vehicle-pretreated rats was (in $ng\,g^{-1}$ wet weight $10\,min^{-1}$, $n = 13$): LTC_4 347 ± 50, PGE_2 722 ± 69, 6-oxo-$PGF_{1\alpha}$ 693 ± 49, TXB_2 253 ± 15. These values were taken as 100%. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$

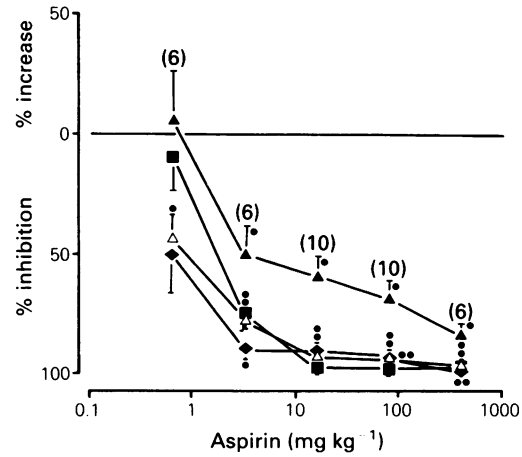

Figure 2 Effect of treatment with aspirin on the release of leukotriene C_4 (LTC_4, ▲), prostaglandin E_2 (PGE_2, ◆), 6-oxo-$PGF_{1\alpha}$ (■) and thromboxane B_2 (TXB_2, △) from rat gastric mucosal fragments incubated *ex vivo*. Each point represents the mean of (n) rats; vertical lines indicate s.e. mean. Release of eicosanoids from gastric mucosa of control rats was (in $ng\,g^{-1}$ wet weight $10\,min^{-1}$, $n = 13$): LTC_4 83 ± 18, PGE_2 671 ± 114, 6-oxo-$PGF_{1\alpha}$ 921 ± 86 and TXB_2 435 ± 53. These values were taken as 100%. $^{**}P < 0.01$; $^{*}P < 0.05$.

and the effect was significant ($P < 0.05$) for the dose of $100\,mg\,kg^{-1}$ (Figure 1).

Effects of aspirin

Contrary to sodium salicylate, oral administration of aspirin to rats not additionally treated with ethanol resulted in a significant and dose-dependent reduction of the release of the cyclo-oxygenase products of arachidonate metabolism, PGE_2, 6-oxo-$PGF_{1\alpha}$ and TXB_2, from gastric mucosal fragments incubated *ex vivo* (Figure 2). Interestingly, oral aspirin treatment also reduced the release of LTC_4, the effect being significant ($P < 0.05$) with a dose as low as $3.2\,mg\,kg^{-1}$ (Figure 2). With none of the doses of aspirin studied was an increased release of LTC_4 observed. Subcutaneous administration of aspirin ($400\,mg\,kg^{-1}$) resulted in a highly significant reduction of the *ex vivo* gastric mucosal release of the cyclo-oxygenase products of arachidonate metabolism. Release of LTC_4 tended also to be decreased. However, the effect was not significant ($P > 0.05$) (Table 3).

When rats were treated orally with various doses of aspirin 30 min before intragastric instillation of ethanol, inhibition of the cyclo-oxygenase pathway of arachidonate metabolism was observed (Figure 3); this was quantitatively similar to the experiments without ethanol treatment (Figure 2). Thus, formation of PGE_2, 6-oxo-$PGF_{1\alpha}$ and TXB_2 was significantly reduced by oral pretreatment with an aspirin dose as low as $1.6\,mg\,kg^{-1}$. The *ex vivo* release of LTC_4 as well as the ethanol-induced gastric mucosal damage, however, were not significantly affected by oral pretreatment with aspirin in doses between $0.4\,mg\,kg^{-1}$ and $25\,mg\,kg^{-1}$. On the other hand, the highest doses of aspirin studied ($100\,mg\,kg^{-1}$ and $400\,mg\,kg^{-1}$) induced a dose-dependent and significant reduction of LTC_4 release and simultaneously protected against ethanol-induced gastric damage (Figure 3).

Effects of indomethacin

Oral administration of indomethacin ($4\,mg\,kg^{-1}$ or $20\,mg\,kg^{-1}$) 30 min before *ex vivo* incubation of gastric mucosa resulted in a dose-dependent decrease in basal LTC_4 release (Table 4). The inhibitory effect was significant ($P < 0.025$) for the higher dose of indomethacin only. Simultaneously, indomethacin inhibited the release of PGE_2, 6-oxo-$PGF_{1\alpha}$ and

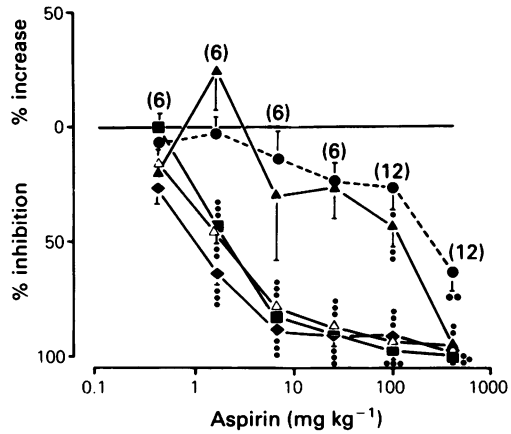

Figure 3 Effects of pretreatment with aspirin on ethanol-induced gastric mucosal damage, determined as lesion index (●), and release of leukotriene C_4 (LTC_4, ▲), prostaglandin E_2 (PGE_2, ◆), 6-oxo-$PGF_{1\alpha}$ (■) and thromboxane B_2 (TXB_2, △) from gastric mucosal fragments incubated *ex vivo*. Each point represents the mean of (n) rats; vertical lines indicate s.e. mean. Lesion index in rats pretreated with vehicle before ethanol was 55 ± 4 and was taken as 100%. Release of eicosanoids from gastric mucosa of the vehicle-pretreated rats was (in $ng\,g^{-1}$ wet weight $10\,min^{-1}$, $n = 14$): LTC_4 330 ± 119, PGE_2 794 ± 86, 6-oxo-$PGF_{1\alpha}$ 855 ± 87, TXB_2 282 ± 32. These values were taken as 100%. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

TXB_2 in a dose-dependent manner (Table 4). Subcutaneous administration of $20\,mg\,kg^{-1}$ indomethacin resulted in a highly significant reduction of the gastric mucosal release of the cyclo-oxygenase products of arachidonate metabolism, while synthesis of LTC_4 was slightly, but not significantly, inhibited (Table 3).

Table 4 Effect of oral treatment with indomethacin on *ex vivo* eicosanoid release from gastric mucosa of rats not additionally treated with ethanol

	Controls	*Indomethacin*	
		$4\,mg\,kg^{-1}$	$20\,mg\,kg^{-1}$
LTC_4	39 ± 9	28 ± 7	13 ± 3*
PGE_2	765 ± 269	329 ± 141	53 ± 19*
6-oxo-$PGF_{1\alpha}$	875 ± 118	348 ± 132*	141 ± 47***
TXB_2	208 ± 36	77 ± 16**	17 ± 6***

Results are given in $ng\,g^{-1}$ wet weight $10\,min^{-1}$ and represent means ± s.e. mean of $n = 6$ for each group.
*** $P < 0.001$, ** $P < 0.01$, * $P < 0.025$ as compared to controls.

Table 5 Effect of indomethacin on lesion production and *ex vivo* eicosanoid release from gastric mucosa of rats challenged with ethanol

	Controls	*Indomethacin*	
		$4\,mg\,kg^{-1}$	$20\,mg\,kg^{-1}$
Lesion index	48 ± 4	46 ± 3	45 ± 4
LTC_4	354 ± 36	420 ± 89	309 ± 83
PGE_2	889 ± 71	172 ± 32**	90 ± 10**
6-oxo-$PGF_{1\alpha}$	983 ± 186	209 ± 39*	101 ± 35*
TXB_2	326 ± 27	121 ± 18**	53 ± 17**

Results are given in $ng\,g^{-1}$ wet weight $10\,min^{-1}$ and represent means ± s.e. mean of $n = 8$ for the controls and $n = 6$ for each indomethacin-treated group.
** $P < 0.001$, * $P < 0.005$ as compared to controls.

Oral pretreatment with indomethacin ($4\,mg\,kg^{-1}$ or $20\,mg\,kg^{-1}$) did not affect gastric damage caused by oral instillation of ethanol (Table 5). Furthermore, while such pretreatment significantly reduced the *ex vivo* formation of the cyclo-oxygenase products of arachidonate metabolism, PGE_2, 6-oxo-$PGF_{1\alpha}$ and TXB_2, neither dose of indomethacin affected the ethanol-stimulated release of LTC_4 (Table 5).

Discussion

The present results confirm the observation by Robert (1981) that sodium salicylate protects rat gastric mucosa against ethanol-induced damage. Our data further support the view (Robert, 1981) that this effect is not mediated by increased formation of prostaglandins in the mucosa. Although sodium salicylate in a dose of $100\,mg\,kg^{-1}$ increased the *ex vivo* formation of 6-oxo-$PGF_{1\alpha}$, significant protection was observed with lower doses of the drug, which did not stimulate prostaglandin synthesis. Furthermore, high doses of aspirin ($100\,mg\,kg^{-1}$ and $400\,mg\,kg^{-1}$) were found almost to abolish gastric mucosal prostaglandin and TXB_2 synthesis, but were still protective. Thus, an active cyclo-oxygenase is not a prerequisite for protection against ethanol-induced injury. We had reached this conclusion previously from data obtained with cysteamine (Lange *et al.*, 1987). This drug was shown to be protective against ethanol, although it inhibited formation of cyclo-oxygenase products of arachidonate metabolism (Lange *et al.*, 1987). However, cysteamine inhibited in addition mucosal LTC_4 formation in a dose-dependent manner and parallel to its protective action (Lange *et al.*, 1987). We had suggested previously from the protection observed with NDGA, carbenoxolone and PGE_2 (Peskar *et al.*,

1986; Dreyling *et al.*, 1986) that inhibition of synthesis or functional antagonism of effects of cysteinyl-leukotrienes might be a mechanism of action of a number of gastroprotective drugs. The present results suggest that sodium salicylate confers protection against ethanol-induced gastric damage by an inhibitory action on the activation by ethanol of the gastric mucosal cysteinyl-leukotriene system. The exact mechanism of this effect of sodium salicylate remains unknown, but from the lack of effect on basal LTC_4 release does not seem to involve direct enzyme inhibition. Sodium salicylate could rather interfere with an early step involved in the ethanol-induced activation of LTC_4-synthesizing enzymes and/or with a stimulus-dependent increase in substrate availability. The lack of effect on basal enzyme activity correlates with the lack of effect of sodium salicylate on gastric mucosal cyclo-oxygenase (Whittle *et al.*, 1980).

In contrast to sodium salicylate, oral administration of aspirin or indomethacin reduced release of both cyclo-oxygenase products of arachidonate metabolism and LTC_4 from gastric mucosa obtained from rats not treated with ethanol. The inhibitory action of aspirin and indomethacin on LTC_4 formation was, however, less pronounced than on cyclo-oxygenase activity. The mechanism of inhibition of gastric LTC_4 formation by aspirin and indomethacin under basal conditions remains to be elucidated. This inhibition could be due to direct effects on the enzymes phospholipase A_2 and/or 5-lipoxygenase. Such enzyme inhibition has, in fact, been observed for high concentrations of indomethacin in rat peritoneal leukocytes (Ahnfelt-Ronne & Arrigoni-Martelli, 1982). In this context it is of interest that due to the pharmacokinetics of acidic NSAIDs, particularly high concentrations of some of these compounds can be reached in the gastric mucosa within short periods of time after oral administration (Brune & Lanz, 1985). The view that inhibition of LTC_4 formation may be related to particularly high local concentrations of aspirin and indomethacin is further supported by the fact that parenteral administration of these NSAIDs resulted only in a tendency for reduced LTC_4 release, as compared to the significant effects of identical doses administered orally. Similarly, it has been demonstrated by Robert (1981) that the dose-response curve for the inhibitory action of subcutaneous sodium salicylate on ethanol-induced gastric damage is far to the right of that depicting the effects of oral drug administration.

Interestingly and contrary to basal conditions, no inhibition of LTC_4 synthesis by oral doses of aspirin lower than $100\,mg\,kg^{-1}$ and by indomethacin was observed after administration of ethanol. This potent stimulus of gastric mucosal LTC_4 synthesis might overcome the inhibitory effect of the two NSAIDs on LTC_4 synthesis. Alternatively, LTC_4 released under basal or stimulated conditions, respectively, could originate from different mucosal cell types of which the 5-lipoxygenase pathway of arachidonate metabolism could be differentially affected by NSAIDs such as indomethacin, aspirin and sodium salicylate.

Work by Robert (1981) has shown that aspirin administered 20 min before ethanol does not prevent development of gastric damage. Nevertheless, a slight (about 25%) decrease in the number of lesions per stomach seemed to occur in these experiments with the highest dose of aspirin used ($100\,mg\,kg^{-1}$). Under our experimental conditions with time intervals of 30 min between drug administration and instillation of ethanol, doses of $100\,mg\,kg^{-1}$ and $400\,mg\,kg^{-1}$ aspirin caused significant protection. After oral administration aspirin is rapidly hydrolysed in the gastrointestinal tract, liver and plasma (Flower *et al.*, 1985). It seems, therefore, possible that the protective effect of high doses of aspirin observed in our experiments is mediated by the formation of significant amounts of the metabolite salicylic acid. Thus, aspirin seems to exert its effects via two different mechanisms. Basal LTC_4 biosynthesis is inhibited by the drug itself, but not by the metabolite salicylic acid. Conversely, ethanol-stimulated biosynthesis is inhibited – most probably indirectly (see above) – by salicylic acid.

While indomethacin and non-protective doses of aspirin did not prevent the ethanol effect on mucosal LTC_4 synthesis, protective doses of aspirin, like sodium salicylate, inhibited the mucosal generation of LTC_4. These results further support the view that there is a causal relationship between enhanced LTC_4 biosynthesis and the development of ethanol-induced gastric injury. It cannot be completely excluded that reduced formation of LTC_4 after treatment with various drugs is, at least in part, a consequence of, rather than a cause for gastroprotection. However, several drugs such as exogenous PGE_2 (Dreyling *et al.*, 1986; Lange *et al.*, 1987) and a low dose ($7\,mg\,kg^{-1}$, unpublished observation) of the cysteinyl-leukotriene receptor antagonist L-649,923 (sodium (βS*, γR*)-4-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)-propylthio)-γ-hydroxy-β-methyl-benzene-butanoate) (Jones *et al.*, 1986) have been shown to afford protection against ethanol without significantly affecting LTC_4 synthesis. These results support the concept that LTC_4 formed in the gastric mucosa is, indeed, an important mediator of ethanol-induced gastric damage and that drugs such as sodium salicylate exert a protective effect by inhibition of LTC_4 formation.

We thank Miss K. Braun and Mr K.-H. Ehrlich for excellent technical assistance.

References

AHNFELT-RONNE, I. & ARRIGONI-MARTELLI, E. (1982). Multiple effects of a new anti-inflammatory agent, timegadine, on arachidonic acid release and metabolism in neutrophils and platelets. *Biochem. Pharmacol.*, **31**, 2619–2624.

BRUNE, K. & LANZ, R. (1985). Pharmacokinetics of non-steroidal anti-inflammatory drugs. In *Handbook of Inflammation*, Vol. 5: *The Pharmacology of Inflammation*. ed. Bonta, I.L., Bray, M.A. & Parnham, M.J., pp. 413–449. Amsterdam: Elsevier Science Publishers.

DREYLING, K.W., LANGE, K., PESKAR, B.A. & PESKAR, B.M. (1986). Release of leukotrienes by rat and human gastric mucosa and its pharmacological modification. *Br. J. Pharmacol.*, **88**, 236P.

FLOWER, R.J., MONCADA, S. & VANE, J.R. (1985). Analgesic-antipyretics and anti-inflammatory agents: drugs employed in the treatment of gout. In *The Pharmacological Basis of Therapeutics*, ed. Goodman Gilman, A., Goodman, L.S., Rall, T.W. & Murad, F., pp. 674–715. New York: Macmillan Publishing Co.

GLENN, E.M., BOWMAN, B.J. & ROHLOFF, N.A. (1979). Anomalous biological effects of salicylates and prostaglandins. *Agents & Actions*, **9**, 257–264.

JONES, T.R., YOUNG, R., CHAMPION, E., CHARETTE, L., DENIS, D., FORD-HUTCHINSON, A.W., FRENETTE, R., GAUTHIER, J.Y., GUINDON, Y., KAKUSHIMA, M., MASSON, P., MACFARLANE, C., PIECHUTA, H., ROKACH, J. & ZAMBONI, R. (1986). L-649,923, sodium (βS*, γR*)-4-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)-propylthio)-γ-hydroxy-β-methyl-benzene-butanoate, a selective orally active leukotriene receptor antagonist. *Can. J. Physiol. Pharmacol.*, **64**, 1068–1075.

LANGE, K., PESKAR, B.A. & PESKAR, B.M. (1987). Stimulation of rat gastric mucosal leukotriene C_4 formation by ethanol and effect of gastric protective drugs. In *Advances in Prostaglandin, Thromboxane and Leukotriene Research*, Vol. 17, ed. Samuelsson, B., Paoletti, R. & Ramwell, P., pp. 299–302. New York: Raven Press.

PESKAR, B.M., LANGE, K., HOPPE, U. & PESKAR, B.A. (1986). Ethanol stimulates formation of leukotriene C_4 in rat gastric mucosa. *Prostaglandins*, **31**, 283–293.

PESKAR, B.M., HOPPE, U., LANGE, K. & PESKAR, B.A. (1987). Effect of non-steroidal anti-inflammatory drugs (NSAID) on rat gastric leukotriene formation. *Gastroenterology*, **92**, 1573.

ROBERT, A. (1981). Gastric cytoprotection by sodium salicylate. *Prostaglandins*, **21**, (Suppl.), 139–146.

WALLACE, J.L. & WHITTLE, B.J.R. (1985). Role of prostanoids in the protective actions of BW755C on the gastric mucosa. *Eur. J. Pharmacol.*, **115**, 45–52.

WHITTLE, B.J.R., HIGGS, G.A., EAKINS, K.E., MONCADA, S. & VANE, J.R. (1980). Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. *Nature*, **284**, 271–273.

(Received June 19, 1987
Revised October 7, 1987
Accepted November 3, 1987)