Br. J. Pharmac. (1983), **80**, 545–551

The potentiation of taurocholate-induced rat gastric erosions following parenteral administration of cyclo-oxygenase inhibitors

B.J.R. Whittle

Department of Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS

1 Subcutaneous administration of anti-inflammatory doses of aspirin, indomethacin, naproxen and flurbiprofen inhibited prostacyclin formation *ex vivo* in the luminally-perfused gastric mucosa of anaesthetized rats.

2 These doses of anti-inflammatory compounds potentiated the formation of gastric mucosal erosions following 3 h luminal perfusion of the topical irritant, acidified sodium taurocholate (2 mM in 100 mM HCl).

3 The increase in luminal acid-loss during gastric perfusion of acidified taurocholate was not significantly enhanced by these anti-inflammatory agents.

4 A correlation was found between the increase in gastric erosion formation and the inhibition of mucosal prostacyclin formation *ex vivo* by intravenous injection of aspirin or ketoprofen during acid-taurocholate perfusion.

5 BW755C, which failed to inhibit mucosal prostacyclin formation *ex vivo*, did not significantly augment acid-taurocholate induced gastric damage.

6 The present findings support the potentiating interactions between topical irritation and inhibition of gastric cyclo-oxygenase in the genesis of the gastric lesions.

Introduction

The presence of bile acids or their salts in the gastric lumen enhances the formation of gastric mucosal erosions following intragastric administration of aspirin (Semple & Russell, 1975) or parenteral administration of indomethacin (Abtahi & Djahanguiri, 1975; Whittle, 1976). In a study in rats on the underlying mechanisms, it was proposed that a decrease in mucosal blood flow by indomethacin resulting from prostaglandin synthesis inhibition (Vane, 1971) and the increase in acid back diffusion induced by the topical irritant sodium taurocholate interact to produce this extensive gastric damage (Whittle, 1977). In the present study, the potentiation of bile salt-induced damage by aspirin and other anti-inflammatory agents has been correlated with the ability of these drugs to inhibit the production of gastric mucosal prostacyclin *ex vivo*. Since prostacyclin is a major cyclo-oxygenase product in the rat gastric mucosa (Whittle, 1981; Whittle & Salmon, 1983), its generation was taken as an index of cyclo-oxygenase activity.

A preliminary account of this work has been presented to the British Pharmacological Society (Lidbury, Steel & Whittle, 1982.

Methods

Gastric perfusion and gastric acid-loss

Male Wistar rats (180–220 g body weight), deprived of food for 18 h but allowed water, were anaesthetized with urethane (1.6 g kg^{-1} s.c.). The gastric lumen was perfused (0.2 ml min^{-1}) for 3 h with acidified-saline (100 mM HCl and 50 mM sodium chloride solution) or acidified-saline containing sodium taurocholate (2 mM), using techniques previously described in detail (Whittle, 1976). Drugs were administered by subcutaneous injection, by intravenous injection through a tail vein, or infused via a cannula inserted into a femoral vein. Acid-loss from the gastric lumen during perfusion was determined by titration to pH 7

© The Macmillan Press Ltd 1983

of aliquots of the perfusing fluid and the gastric perfusate collected over 30 min periods using an autoburette (Radiometer, Copenhagen). The decrease in the titratable acid concentration following gastric perfusion was expressed as the acid loss (μmol) over this 3 h period.

Assay of prostacyclin production ex vivo

Prostacyclin is the most potent endogenous inhibitor of platelet aggregation known (Moncada, Gryglewski, Bunting & Vane, 1976) and this property has been utilized in its bioassay. Strips of gastric mucosa (0.3 g), freed from underlying muscle were removed, washed in Tris buffer (50 mM; pH 8.4 at 4°C) and weighed. The tissues were chopped in 1 ml buffer, centrifuged in a fixed-speed Eppendorf bench centrifuge (10 s at 9000 *g*), rewashed in 1 ml buffer, re-centrifuged, and finally incubated in 0.5 ml of buffer by vortex mixing for 1 min at room temperature. After a 15 s centrifugation (9000 *g*), aliquots (5–50 μl) of the supernatant were immediately tested for their ability to inhibit adenosine-diphosphate (3–9 μM)-induced aggregation of human platelets (0.5 ml of citrated platelet-rich plasma) in a Payton dual-channel aggregometer and assayed against authentic prostacyclin. Prostacyclin as the sodium salt was dissolved in 1 M Tris buffer (pH 9.5, 4°C) and freshly diluted (50 mM Tris buffer, pH 8.4 at 4°C) when required. The anti-aggregating activity was characterized as prostacyclin by its pH stability profile and inactivation by a prostacyclin-directed antiserum as described before (Whittle, 1981). Dose-response curves to prostacyclin were determined at regular intervals throughout the assay period and the concentration of prostacyclin in each sample was determined from a 2–3 point assay. The calculated limits of error ($P = 0.05$) for prostacyclin generation in the control tissue samples were ± 7%.

Experiments were carried out using 3–8 animals in each group, while the prostacyclin formation was assessed in animals taken in a randomized manner. For the gastric perfusion experiments, the daily protocol always included a control group (acid-taurocholate perfusion alone), while prostacyclin formation in the other drug-treated groups was expressed as % of the mean control for that day.

Assessment of gastric mucosal lesions

The perfused stomachs were removed at the end of the 3 h perfusion, opened along the lesser curvature and coded to prevent observer bias. Gross erosions, which formed only the glandular mucosa, were measured and each one given a severity rating (0.2 mm length = 1; 2–4 mm = 2; 4–6 mm = 3). The total score was the erosion index for that stomach.

Drugs and materials

The non-steroid anti-inflammatory drugs indomethacin (Sigma Chemical Co.), aspirin (Wellcome Research Laboratories), naproxen (Syntex), flurbiprofen (Boots Ltd), ketoprofen (May & Baker Ltd) were dissolved (5–40 mg ml^{-1}) in sodium bicarbonate solution (5% w/v) following warming and vortex mixing, diluted to the appropriate concentration if necessary, and injected immediately. For intravenous administration, the stock was diluted 1:4 in distilled water (since sodium bicarbonate solution, 1.25% w/v, is isotonic). These compounds were then administered as slow intravenous infusion at a rate of 0.2 ml min^{-1} over a 5 min period.

BW755C(3-amino-1[*m*-(trifluoromethyl)-phenyl] -2-pyrazoline) as the hydrochloride (synthesized by Dr F.C. Copp of the Chemical Research Laboratories, Beckenham) was dissolved in saline when required (0.9% w/v sodium chloride solution). Sodium taurocholate (BDH, England) was dissolved freshly when required in an acid-saline solution (100 mM HCl in 50 mM sodium chloride).

Statistical analysis

Results are expressed as the mean ± s.e.mean, where *n* is the number of values in the group. The statistical significance of the data was evaluated using Student's *t* test for non-paired data and the significance of the erosion data was confirmed using the non-parametric Mann-Whitney U-test. $P < 0.05$ was taken as significant. Linear regression and the correlation coefficients were calculated using a Wang desk-top computer (Model PSCII).

Results

Inhibition of gastric prostacyclin formation

From dose-response studies on the actions of aspirin (50–200 mg kg^{-1}), indomethacin (1.25–10 mg kg^{-1}), naproxen (2.5–10 mg kg^{-1}) and flurbiprofen (0.1–1 mg kg^{-1}) following subcutaneous administration in unanaesthetized rats, doses of these compounds which caused 75–90% inhibition of gastric mucosal prostacyclin generation *ex vivo* were chosen for further study (Table 1). Subcutaneous administration of aspirin (50 mg kg^{-1}), indomethacin (5 mg kg^{-1}), naproxen (5 mg kg^{-1}) and flurbiprofen (0.5 mg kg^{-1}) likewise significantly ($P < 0.01$) inhibition prostacyclin generation *ex vivo* from the gastric mucosa of anaesthetized rats which had been luminally-perfused with acidified-taurocholate. The degree of cyclo-oxygenase inhibition by these anti-inflammatory doses of the compounds in both series of experiments was comparable (Table 1).

Table 1 Inhibition of *ex vivo* gastric mucosal prostacyclin formation 3 h following s.c. administration of anti-inflammatory agents in anaesthetized rats with gastric luminal perfusion (2 mM taurocholate plus 100 mM HCl) and in unanaesthetized rats (non-perfused)

		% Inhibition of prostacyclin formation	
	(mg kg^{-1})	*Non-perfused*	*Perfused*
Indomethacin	5	90± 4(5)	94± 6(5)
Flurbiprofen	0.5	83± 2(3)	89± 3(5)
Aspirin	50	73± 8(5)	79± 8(5)
Naproxen	5	87± 4(3)	74± 9(5)
BW755C	100	5±25(5)	−38±25(5)

Results are expressed as % inhibition of control prostacyclin formation and shown as mean±s.e.mean of *n* experiments (number in parentheses). In all cases, except for BW755C, there was a significant inhibition ($P<0.01$) compared to control prostacyclin formation.

These doses are within the anti-inflammatory dose-range for each compound, as determined by the reduction in oedema following subplantar injection of carrageenin in the hind paw of rats (see Whittle, Higgs, Eakins, Moncada & Vane, 1980). In contrast, BW755C (100 mg kg^{-1} s.c.) in a dose ten-fold the anti-inflammatory dose (Higgs, Flower & Vane, 1979), failed to inhibit significantly prostacyclin formation *ex vivo* in either series of experiments (Table 1).

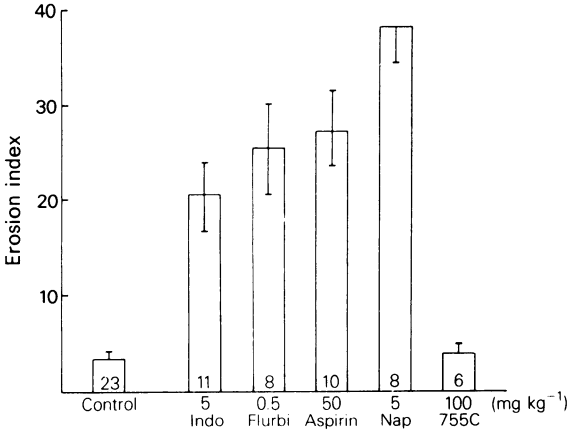

Figure 1 Effect of subcutaneous administration of the anti-inflammatory drugs, indomethacin, aspirin, naproxen, flurbiprofen and BW755C on the degree of gastric lesions 3 h following luminal perfusion of acidified-taurocholate. Results are expressed as erosion index and show a significant increase ($P<0.01$) in gastric damage, compared with acidified-taurocholate alone, with all compounds except BW755C. The number of experiments are shown in the columns, where the bars represent s.e.mean.

Induction of gastric erosions

During luminal perfusion of acid-saline (100 mM HCl) in the anaesthetized rat, subcutaneous injection of the anti-inflammatory agents had little action in increasing the low control erosion index (1.0±1.0, $n=5$); the erosion index with aspirin (50 mg kg^{-1}) was 1±0.5 ($n=3$); with indomethacin (5 mg kg^{-1}), 1.3±0.8 ($n=4$); with naproxen (5 mg kg^{-1}), 0.7±0.7 ($n=3$); with flurbiprofen (0.5 mg kg^{-1}), 3±1 ($n=3$); and with BW755C (100 mg kg^{-1}), 0.5±0.5 ($n=3$).

Perfusion of the gastric lumen for 3 h with acidified bile salts (2 mM taurocholate, 100 mM HCl) likewise induced only a low incidence of gastric erosions (Figure 1). However, subcutaneous injection of these doses of aspirin, indomethacin, naproxen and flurbiprofen, at the start of the acid-taurocholate perfusion led to a substantial ($P<0.01$ for each) potentiation of the erosion index when assessed after 3 h (Figure 1).

Correlation between prostacyclin inhibition and erosion induction

To study more closely the relationship between the potentiation of acid-taurocholate induced gastric erosions and the inhibition of mucosal prostacyclin formation, the dose-response relationship following intravenous injection of ketoprofen (0.125–10 mg kg^{-1}) was assessed. As shown in Figure 2a, significant inhibition (62±3%; $P<0.05$) of prostacyclin formation with ketoprofen (0.5 mg kg^{-1}) was accompanied by a significant elevation of the erosion index. Over the dose-range of ketoprofen studied, there was a significant correlation between these two parameters with a correlation coefficient of 0.64 ($P<0.05$). Likewise, intravenous injection of aspirin (7.5 mg kg^{-1}), in a dose inhibiting mucosal prostacyclin production (by 66±10%; $P<0.05$), significantly increased the erosion index during acid-taurocholate perfusion (Figure 2b). Over the dose-range of aspirin (7.5–60 mg kg^{-1}), there was a correlation between both parameters with a correlation coefficient of 0.88 ($P<0.001$).

When the data for mucosal prostacyclin inhibition and erosion index, taken from these series of experiments with both aspirin and ketoprofen following intravenous injection were pooled, the overall correlation coefficient for these parameters was 0.75 ($P<0.001$), as shown in Figure 3. In these studies on gastric prostacyclin production *ex vivo*, the calculated ID_{50} (dose causing 50%) inhibition) was 0.4 mg kg^{-1} for i.v. ketoprofen and 5.2 mg kg^{-1} for i.v. aspirin.

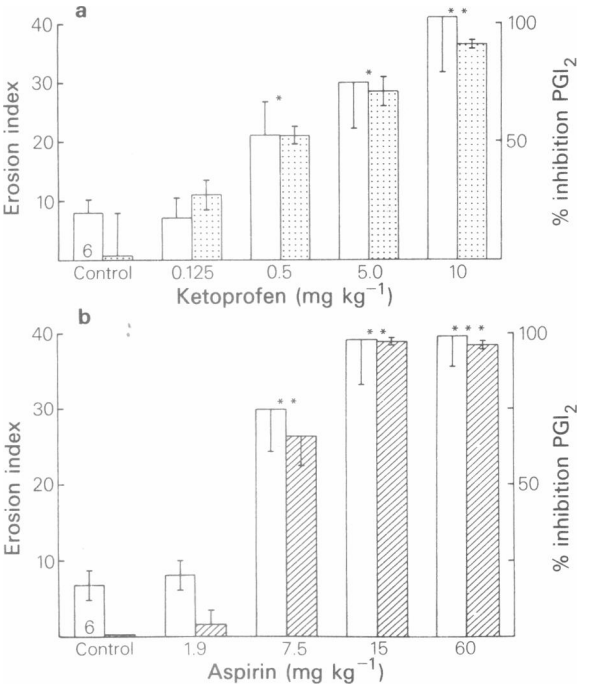

Figure 2 The gastric erosion index (open columns) and inhibition of mucosal prostacyclin (PGI_2) generation *ex vivo* (filled columns) 3 h following intravenous injection of (a) ketoprofen and (b) aspirin during luminal perfusion of acidified-taurocholate. Results are mean ± s.e.mean of 3–6 experiments for each value, where level of significance is shown as $*P<0.05$, $**P<0.01$, $***P<0.001$.

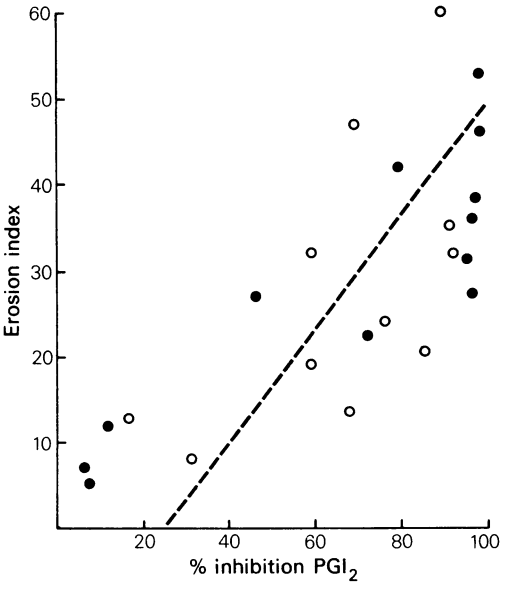

Figure 3 Correlation between the gastric erosion index and inhibition of mucosal prostacyclin (PGI_2) generation 3 h following intravenous administration of ketoprofen 0.125–10 mg kg^{-1} (○) and aspirin 1.9–60 mg kg^{-1} (●), during luminal perfusion of acidified-taurocholate in 24 experiments. The correlation coefficient was 0.75 ($P<0.001$).

Gastric acid loss

There was a significantly greater titrable acid loss during gastric perfusion of acid-taurocholate (2 mM in 100 mM HCl) than during acid-saline alone (576 ± 135 μmol ($n=8$) and 237 ± 109 μmol ($n=5$) over 3 h respectively, $P<0.05$) as found previously in this model (Whittle, 1977). However, administration of the anti-inflammatory agents in the doses used to investigate erosion formation and prostacyclin inhibition, failed to augment this acid loss significantly during the 3 h perfusion of acid-taurocholate (Table 2).

Actions of noradrenaline

The action of the vasoconstrictor, noradrenaline, was also investigated in this gastric perfusion model. Intravenous infusion of noradrenaline (5 and 20 μg kg^{-1} min^{-1}) in doses which elevated systemic arterial blood pressure (by 10 ± 3 mm Hg ($n=3$) and 25 ± 5 mm Hg ($n=3$) respectively) caused a significant increase in the erosion index when infused throughout the 3 h perfusion period of acid-taurocholate (Figure 4). Noradrenaline (20 μg kg^{-1} min^{-1} i.v.) failed to damage the mucosa significantly ($P>0.05$) during perfusion of acid-saline alone (Figure 4).

Table 2 Loss in tritratable acid from the rat gastric lumen during 3 h perfusion with acidified-taurocholate (2 mM in 100 mM HCl)

		Acid loss	
	(mg kg^{-1})	(μmol $^{-1}$ $3h^{-1}$)	n
Control	—	576 ± 135	8
Indomethacin	5	587 ± 136	8
Flurbiprofen	0.5	527 ± 161	5
Aspirin	50	556 ± 63	7
Naproxen	5	520 ± 41	5
BW755C	100	480 ± 36	3

Results expressed as acid-loss, μmol $^{-1}$ $3h^{-1}$, are mean ± s.e.mean of n experiments. There was no significant difference between the control acid-taurocholate perfusion group and those additionally receiving subcutaneous administration of the anti-inflammatory agents.

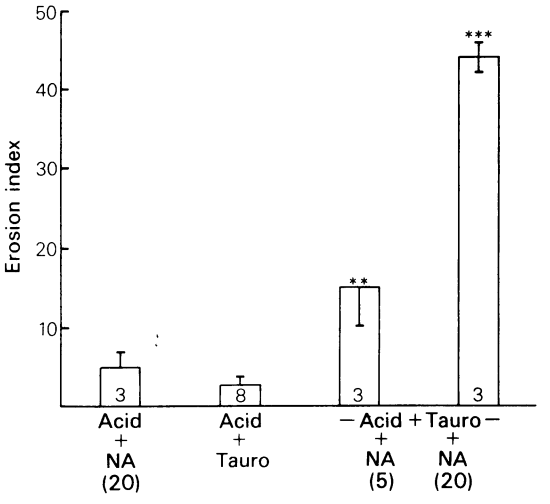

Figure 4 Effect of intravenous infusion of noradrenaline ($5-20\,\mu g\,kg^{-1}\,min^{-1}$) on gastric mucosal erosion formation following 3 h luminal perfusion of acidified-taurocholate. Results, expressed as an erosion index, show significant potentiation of taurocholate-induced gastric damage by noradrenaline infusion. The figures in the columns represent number of experiments, the bars represent s.e.mean, while level of significance shown as $^{**}P<0.01$, $^{***}P<0.001$.

Discussion

The present findings confirm the observations that indomethacin, administered parenterally, can significantly augment the degree of gastric erosion formation induced by acidified bile salts (Abtahi & Djahanguiri, 1975; Whittle, 1976). They also indicate that aspirin, naproxen, flurbiprofen and ketoprofen can similarly augment taurocholate-induced damage, in anti-inflammatory doses which inhibit gastric mucosal cyclo-oxygenase as assessed by an *ex vivo* technique which generates prostacyclin. In previous studies, indomethacin has been shown to inhibit the generation, by the rat gastric mucosa, of prostacyclin determined by bioassay and of 6-oxo-prostaglandin $F_{1\alpha}$ and prostaglandin E_2 determined by radioimmunoassay to a comparable degree (Whittle & Salmon, 1983).

The experimental anti-inflammatory agent BW755C failed to inhibit prostacyclin formation *ex vivo* in gastric mucosal tissue in doses shown previously to reduce significantly prostaglandin levels in inflammatory exudates, and confirms previous studies on gastric fundic mucosal tissue from the rat following its oral administration (Whittle *et al.*, 1980; Peskar, Weiler & Peskar, 1982). BW755C also failed to augment the level of gastric erosions induced by acidified-taurocholate, again supporting the concept that the other non-steroid anti-inflammatory agents enhance such damage by inhibition of gastric cyclo-oxygenase. Furthermore, in the study with intravenous injection of incremental doses of two such agents, aspirin and ketoprofen during perfusion of acidified-taurocholate, there was a significant correlation between the extent of gastric erosions and the degree of prostacyclin inhibition. Cyclo-oxygenase inhibition of 60% or greater following either subcutaneous or intravenous administration of these anti-inflammatory compounds is thus associated with a significant enhancement of gastric damage.

In a study on the mechanisms underlying gastric damage in the rat, the increase in acid-back diffusion in the presence of acidified-taurocholate, failed to induce extensive damage, the accompanying rise in mucosal blood flow being thought to prevent acid accumulation within the mucosal tissue (Whittle, 1977). This protective hyperaemic response may be mediated in part by local prostaglandin production since it could be attenuated by concurrent administration of indomethacin (Whittle, 1977), an inhibitor of prostaglandin biosynthesis (Vane, 1971). Such a reduction in the prostaglandin-mediated component of hyperaemia would be expected to reduce the ability of the mucosa to dispose of the acid diffusing back into the tissue, leading eventually to acid accumulation, a fall in tissue pH and subsequent necrosis. It is proposed that the other anti-inflammatory drugs studied, aspirin, naproxen, ketoprofen and flurbiprofen which inhibit gastric mucosal cyclo-oxygenase, act on the microcirculation in a comparable manner. Studies in the dog have shown that similar potentiation of bile-salt induced gastric necrosis can be achieved with intravenous administration of indomethacin (Lewi & Carter, 1980; Whittle & Moncada, 1983). This again could reflect a local vascular involvement since indomethacin can decrease gastric blood flow (Gerkins, Shand, Flexner, Nies, Oates & Data, 1977; Kauffman, Aures & Grossman, 1980) and increase gastric vascular resistance in the dog (Whittle & Moncada, 1983).

Potentiation of taurocholate-induced gastric damage has also been demonstrated by direct vasoconstriction with vasopressin in the dog (Ritchie, 1975). In other studies, the actions of thromboxane A_2, generated from arachidonic acid in blood flowing through an incubation coil (Whittle, Kauffman & Moncada, 1981) or a stable epoxy-methano endoperoxide analogue were also investigated (Whittle & Moncada, 1983). Both these prostanoids were potent vasoconstrictors in the canine gastric circulation, and induced acute gastric erosion formation in the presence of luminally-applied acidified taurocholate. Furthermore, in the current study in the rat, intravenous infusion of the vasopressor agent noradrenaline likewise augmented taurocholate-induced

gastric erosions. Thus both inhibition of mucosal prostaglandin synthesis and direct gastric vasoconstriction can exacerbate gastric damage by this topical irritant in the dog and rat.

Inhibition of endogenous prostaglandin formation may also remove or alter other endogenous factors involved in the protection of the gastric mucosa. The actions on mucus-bicarbonate secretion which may be modulated by endogenous cyclo-oxygenase products (Allen & Garner, 1980) have not yet been evaluated in this model. The contribution of any changes in gastric alkaline secretion in the assessment of titrable acid-loss in the current preparation is also not yet known. However, preliminary studies in the lumen-perfused anaesthetized rat suggest that even at the maximally-achieved rates of stimulation, gastric alkaline secretion could only account for 5% of the acid-loss detected in the present study (Whittle & Kauffman, 1981).

No further increase in luminal acid-loss with these parenterally-injected anti-inflammatory compounds could be detected during acid-taurocholate perfusion, confirming previous studies with indomethacin (Whittle, 1977). Although localized increases in acid back-diffusion cannot be entirely excluded it thus seems unlikely that an elevation in acid back-diffusion by these parenterally-administered anti-inflammatory agents is an underlying mechanism of the enhanced gastric damage. In other studies, intravenous administration of aspirin or indomethacin in doses sufficient to inhibit gastric prostaglandin formation also failed to alter hydrogen-ion flux across the gastric mucosa of cat and dog (Bugat, Thompson, Aures & Grossman, 1976; Kauffman *et al.*, 1980) although in another study in the Heidenhain-pouch dog, intravenously-injected indomethacin did augment taurocholate (5 mM)-induced ion flux across the mucosa, and led to bleeding (Lewis & Carter, 1980). The bulk of these findings suggest that inhibition of gastric cyclo-oxygenase does not itself cause substantial changes in hydrogen-ion back-diffusion, supporting the proposal that endogenous prostanoids do not directly regulate mucosal 'barrier' function (Whittle, 1977; Kauffman *et al.*, 1980).

In this anaesthetized rat model, parenterally-injected indomethacin, as well as naproxen, flurbiprofen, ketoprofen and aspirin did not induce marked gastric damage when the gastric lumen was perfused with acidified-saline alone. This contrasts with the ability of such agents as indomethacin to induce gastric erosions when administered parenterally to non-operated, un-anaesthetized rats (Whittle, 1981) and emphasises that inhibition of gastric cyclo-oxygenase alone in this model is insufficient to induce extensive erosion formation (Whittle, 1977). Intravenous administration of aspirin can, however, induce gastric damage during the gastric perfusion of acid-saline in unanaesthetized rats (Kauffman & Grossman, 1978) and this gastric damage is accompanied by inhibition of mucosal prostaglandin formation (Konturek, Piastuki, Brzozowski, Radecki, Dembinska-Kiec, Zmuda & Gryglewski, 1981). It is not known whether these differences between anaesthetized and unanaesthetized rats reflect differences in either basal acid and bicarbonate secretion, the degree of acid back-diffusion or the presence of endogenous bile or other topical irritants. Such differences could also be linked to a greater ability of the gastric mucosa in anaesthetized rats to withstand acid back-diffusion or topical irritation, which could in turn be related to relative rates of gastric mucosal blood flow. These studies thus suggest that the genesis of gastric lesions following mucosal cyclo-oxygenase inhibition is greatly exacerbated by the concurrent action of other aggravating factors, and that the presence of such factors may be required for the acute development of gastric erosions by anti-inflammatory drugs.

Agents such as salicylate, taurocholate and ethanol which disrupt mucosal integrity and allow acid back-diffusion can induce gastric ulceration and bleeding (Davenport, 1964). However, it is likely that the gastric damage following the application of topical irritants is not directly related to the degree of 'barrier disruption' and the concentration of hydrogen ions back-diffusion itself, but depends more on the ability of the mucosa to withstand or clear this acid which diffuses into the tissue. The present findings confirm that cyclo-oxygenase inhibition can reduce the ability of the gastric mucosa to withstand such challenge. Furthermore, they support the concept of a potentiating interaction between topical irritation, as seen with acidified-taurocholate, and the inhibition of gastric mucosal prostaglandin formation.

I am grateful for the excellent technical assistance provided by Mr Paul Lidbury and Mr Graham Steel.

References

ABTAHI, F.S. & DJAHANGUIRI, B. (1975). Decreased incidence of indomethacin-induced gastric ulceration in rats by bile duct diversion. *Br. J. Surg.*, **62**, 113–114.

ALLEN, A. & GARNER, A. (1980). Mucous and bicarbonate secretion in the stomach and their possible role in mucosal protection. *Gut*, **21**, 249–262.

BUGAT, R., THOMPSON, M.R., AURES, D. & GROSSMAN, M.I. (1976). Gastric mucosal lesions produced by in-

travenous infusion of aspirin in cats. *Gastroenterology*, **71**, 754–759.

DAVENPORT, H.W. (1964). Gastric mucosal injury by fatty and acetylsalicylic acids. *Gastroenterology*, **46**, 245–253.

HIGGS, G.A., FLOWER, R.J. & VANE, J.R. (1979). A new approach to anti-inflammatory drugs. *Biochem. Pharmac.*, **28**, 1959–1961.

KAUFFMAN, G.L. & GROSSMAN, M.I. (1978). Prostaglandin and cimetidine inhibit the formation of ulcers produced by parenteral salicylate. *Gastroenterology*, **75**, 1099–1102.

KAUFFMAN, G.L., AURES, D. & GROSSMAN, M.I. (1980). Intravenous indomethacin and aspirin reduce basal gastric mucosal blood flow in dogs. *Am. J. Physiol.*, **238**, G131–134.

GERKINS, J.F., SHAND, D.G., FLEXNER, C., NIES, A.S., OATES, J. & DATA, J.L. (1977). Effect of indomethacin and aspirin on gastric blood flow and acid secretion. *J. Pharmac. exp. Ther.*, **203**, 646–652.

KONTUREK, S.J., PIASTUKI, I., BRZOZOWSKI, T., RADECKI, T., DEMBINSKA-KIEC, A., ZMUDA, A. & GRYGLEWSKI, R. (1981). Role of prostaglandins in the formation of aspirin-induced gastric lesions. *Gastroenterology*, **80**, 4–9.

LEWIS, H.J. & CARTER, D.C. (1980). Intravenous prostaglandin synthetase inhibitors potentiate the effect of topical taurocholate on transmucosal ion flux. In *Gastrointestinal mucosal blood flow*, ed. Fielding, L.P. pp. 192–201. London: Churchill-Livingstone.

LIDBURY, P., STEEL, G. & WHITTLE, B.J.R. (1982). Potentiation of taurocholate-induced gastric damage by parenteral administration of cyclo-oxygenase inhibitors. *Br. J. Pharmac.*, **75**, 10P.

MONCADA, S., GRYGLEWSKI, R.J., BUNTING, S. & VANE, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature*, **263**, 663–665.

PESKAR, B.M., WEILER, H. & PESKAR, B.A. (1982). Effect of BW755C on prostaglandin synthesis in the rat stomach. *Biochem. Pharmac.*, **31**, 1652–1653.

RITCHIE, W.P. (1975). Acute gastric mucosal damage induced by bile salts, acid and ischemia. *Gastroenterology*, **68**, 699–707.

SEMPLE, P.F. & RUSSELL, R.I. (1975). Role of bile acids in the pathogenesis of aspirin-induced gastric mucosal haemorrhage in rats. *Gastroenterology*, **68**, 67–70.

VANE, J.R. (1971). Inhibition of prostaglandin biosynthesis as a mechanism of action of aspirin-like drugs. *Nature (New Biol.)*, **231**, 232–235.

WHITTLE, B.J.R. (1976). Relationship between the prevention of rat gastric erosions and the inhibition of acid secretion by prostaglandins. *Eur. J. Pharmac.*, **40**, 233–239.

WHITTLE, B.J.R. (1977). Mechanisms underlying gastric mucosal damage induced by indomethacin and bile salts, and the actions of prostaglandins. *Br. J. Pharmac.*, **60**, 455–460.

WHITTLE, B.J.R. (1981). Temporal relationship between cyclo-oxygenase inhibition, as measured by prostacyclin biosynthesis, and the gastrointestinal damage induced by indomethacin in the rat. *Gastroenterology*, **80**, 94–98.

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.

WHITTLE, B.J.R. & KAUFFMAN, G.L. (1981). Prostacyclin analogues stimulate gastric alkaline secretion in rat and dog. *Gastroenterology*, **80**, 1315A.

WHITTLE, B.J.R., KAUFFMAN, G.L. & MONCADA, S. (1981). Vasoconstriction with thromboxane A_2 induces ulceration of the gastric mucosa. *Nature*, **292**, 472–474.

WHITTLE, B.J.R. & MONCADA, S. (1983). Ulceration induced by an endoperoxide analogue and by indomethacin in the canine stomach. In *Advances in Prostaglandin, Thromboxane and Leukotriene Research*, Vol. 12, ed. Samuelsson, S., Paoletti, R. & Ramwell, P. pp. 373–378. New York: Raven Press.

WHITTLE, B.J.R. & SALMON, J.A. (1983). Biosynthesis of prostacyclin and prostaglandin E_2 in gastro-intestinal tissue. In *Proc. of III B.S.G./SK&F International Workshop 'Intestinal Secretion'*, ed. Turnberg, L.A. pp. 69–73. Welwyn Garden City: Smith Kline and French Publications.

(Received May 25, 1983. Revised July 15, 1983.)