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Sulphasalazine and PhCL28A inhibit the formation of ethanol- and phenylbutazone-induced rat gastric ulcers: lack of involvement of endogenous prostaglandins?

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1 The effects of sulphasalazine (SZP) and PhCL28A on macroscopic lesion formation and *ex vivo* prostaglandin inactivation were studied in the ethanol (ETOH) and phenylbutazone (PBT) models of gastric ulcers in the rat. Prostaglandin 'synthesis' during homogenisation of the stomachs was also studied in the latter model.

2 Both PhCL28A and SZP when injected i.p. prevented the formation of ETOH- and PBT-induced gastric ulcers with ED_{50} values of 13 and 41 mg kg^{-1} (vs ETOH) and 3 and 32 mg kg^{-1} (vs PBT) for PhCL28A and SZP respectively. However, neither compound was active orally in the dose ranges used (up to 30 mg kg^{-1} for PhCL28A and 100 mg kg^{-1} for SZP).

3 Irrespective of the route of administration, SZP (100 mg kg^{-1}) and PhCL28A (30 mg kg^{-1}) produced slight but statistically significant decreases in *ex vivo* prostaglandin inactivation by 100,000 *g* cytosolic supernatants prepared from stomachs not receiving ulcerogen. When tested *in vitro*, PhCL28A (IC_{50} = 230 nM) was approximatively 480 times more potent than SZP (IC_{50} = 110 μM) against rat stomach cytosolic prostaglandin inactivation.

4 Both ETOH (50%, 5 ml kg^{-1}, orally) and PBT (200 mg kg^{-1}, orally) significantly decreased *ex vivo* gastric cytosolic prostaglandin inactivation. PhCL28A (30 mg kg^{-1}, orally or i.p.) decreased prostaglandin inactivation still further after ulcerogen treatment except when given i.p. before ETOH treatment. SZP (100 mg kg^{-1}) had a similar effect when given orally before PBT treatment.

5 When the prostaglandin content of the stomach homogenates was used as a measure of *ex vivo* prostaglandin synthesis in the PBT experiments, PhCL28A 30 mg kg^{-1} orally (but not i.p.) produced an 88% increase in prostaglandin E_2 (PGE_2) levels, but had no effect on 6-keto-$PGF_{1\alpha}$ or thromboxane B_2 formation during homogenization. SZP (100 mg kg^{-1} i.p. or orally) was without effect.

6 We conclude from these results that the anti gastric ulcer activity of SZP and PhCL28A is independent of prostaglandin inactivation and endogenous prostaglandin formation is probably not involved.

Introduction

Sulphasalazine (SZP) is used extensively for the maintenance of the remission of ulcerative colitis (Miscewicz *et al.*, 1965; Goldman & Peppercorn, 1975). One of several mechanisms postulated for this activity is the inhibition of the enzyme 15-hydroxy-prostaglandin dehydrogenase (PGDH; Hoult & Moore, 1978; 1980). Such an action would tend to increase the levels of tissue prostaglandins, leading to cytoprotection of the mucosa (as reviewed extensively by Miller, 1983). Moreover in the rat, SZP is active orally against indomethacin-induced intestinal ulcers in rats (Del Soldato *et al.*, 1985), and prevents stress-induced gastric ulceration after sub-cutaneous injection (Ogle & Cho, 1985).

In the light of these findings, we investigated the activity of SZP against two other models of gastric ulcers, namely ethanol (ETOH)- and phenylbutazone (PBT)-induced lesions. We also examined the antiulcer activity of an azobenzone analogue of SZP, PhCL28A (2-hydroxy-5-(3,5-dimethoxycarbonyl-benzoyl) benzene acetic acid, which has been shown to be a highly potent inhibitor of PGDH *in vitro* (K_i = 19 nM) and in the perfused rat lung (Berry *et al.*, 1985).

In addition, we explored the role of PGDH inhibition in the antiulcer activity of these compounds by

measuring cytosolic prostaglandin degradation in stomachs taken from rats pretreated with SZP or PhCL28A both in the absence or after subsequent treatment with the aforementioned ulcerogens. We also studied the prostaglandin 'synthesis' during homogenization of stomachs in the PBT gastric ulcer model. Furthermore, given that SZP is susceptible to azo-splitting by the colonic flora (Goldman & Peppercorn, 1975; Eastwood & Das, 1975) and given that PhCL28A which also possesses an azo linkage (Figure 1) is likely to suffer the same fate, we studied the effects of both oral and parenteral administration of these drugs on ulcer formation.

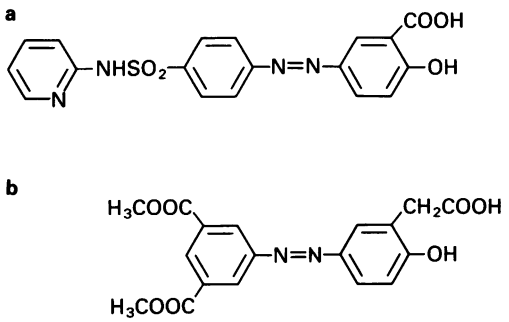

Figure 1 Structures of (a) sulphasalazine (SZP) and (b) PhCL28A.

A preliminary account of this work has been presented to the British Pharmacological Society (Berry & Lloyd, 1987).

Methods

Treatment protocol and assessment of ulcers

Female Wistar rats (180–250 g; 6 per group fasted for 22 h) were treated with either SZP, PhCL28A or the vehicle (1% Tween 80) in which the drugs were suspended, 30 min before oral injection of the ulcerogen; the ulcerogen being either 50% ethanol (1 ml per rat) or phenylbutazone (200 mg kg^{-1} dissolved in equimolar NaOH).

Two hours after PBT or 1 h after ETOH, the animals were killed by chloroform and the stomachs were removed and scored for the presence of lesions by two independent 'blind' observers. The lesions were scored qualitatively as follows: (1) in the case of ethanol ulcers a scale of 0–5 was used whereby a score of 5 represented multiple ulcers following almost the entire length of the gastric folds, 4 = lesions which followed approximately 80% of the folds, 3 = ulcers 1–4 mm in length on 80% of the folds, 2 = at least 2 ulcers of approximately 2 mm in length, 1 = the presence of 1 ulcer and generalised erythema and 0 = no visible damage; (2) in the case of PBT ulcers a scale of 0–3 was used where 3 represented multiple ulcerations of between 1–4 mm in diameter, 2 = at least 2 ulcers of 2 mm in diameter (the ulcers being round in appearance), 1 = 2 to 3 haemorrhagic petechiae or 1 or 2 small ulcers of approximately 1 mm in diameter and 0 = no visible signs of damage. In each case, where the appearance of the stomach was difficult to assign a unitary value, a score of 0.5 was added to the lower score (for example 1.5 would represent 3–4 ulcers of 1 mm in diameter in the PBT model). From these scores the ulcer index was obtained, which is the product of the mean lesion score and the percentage of animals presenting lesions.

After the ulcer score was noted, the stomachs were moistened with 0.9% saline and stored at −80°C for prostaglandin synthesis and degradation studies.

Preparation of rat stomach cytosolic 100,000 g supernatants

The stomachs were thawed, weighed and then homogenized (Ultra-Turrax 18/2N homogenizer) for 15 s in 5 ml of 50 mM phosphate buffer, pH 7.5 (containing 1 mM EDTA and cysteine). The 100,000 *g* supernatants were obtained as described by Hoult & Moore (1977). In the PBT ulcer experiments 0.7 ml of the homogenate was added to the same volume of ETOH (to stop further endogenous prostaglandin synthesis) and the samples were centrifuged for 2 min at 10,000 *g* in an Eppendorf bench micro centrifuge. The prostaglandins were extracted and assayed by radioimmunoassay as described below.

Prostaglandin breakdown in 100,000 g rat stomach supernatants

This was assessed by use of a radiochemical assay fully described by Hoult & Moore (1977, 1978). Briefly, 0.19 ml (or 0.18 ml plus 0.01 ml drug or vehicle in the *in vitro* experiments) rat stomach supernatant was incubated (37°C, 60 min) with 0.01 ml NAD^+ (final concentration 5 mM) and 2 μg (5.7 nmol) prostaglandin $F_{2\alpha}$ containing 0.1 μCi radiolabel (= 2.4 ng). After ethyl acetate extraction and solvent evaporation, the metabolites and remaining substrate were separated by thin layer chromatography (solvent: upper phase of water, ethyl acetate, 2,2,4 trimethyl pentane and glacial acetic acid; 100: 110: 50: 20). After localisation by co-chromatographed authentic standards the extent of prostaglandin breakdown was calculated by comparing the proportions of counts in the relative zones of the chromatogram after correcting for any radioactive substrate impurities found in the metabolite zone (usually 4–9%, determined by the use

of time zero preparations, where the reactions were stopped by adding the enzyme preparation to tubes containing 0.2 ml ethanol plus the substrate and other reagents; these tubes were then incubated for the same time as the others).

Prostaglandin 'synthesis' during homogenization

The prostaglandin content of the homogenates in PBT experiments, which reflects mostly *de-novo* prostaglandin synthesis during homogenization (Bennett *et al.*, 1977) was measured in dried ethyl acetate extracts of the crude homogenates. The extracts were resuspended in 1 ml distilled water and aliquots of 10–20 µl from 20 fold dilutions were radioimmunoassayed for PGE_2, 6-keto-$PGF_{1\alpha}$ and thromboxane (TX) B_2 by a double antibody method (Dighe *et al.*, 1975) using commercially available antibodies. The sensitivity of these assays was 2–5 pg, and the coefficients of variation between and within assays were as follows (percentages): PGE_2 7.6, 15.2; 6-keto-$PGF_{1\alpha}$ 15.3, 13.8 and TXB_2 6.4, 13.9. Percentage cross reactivites against PGE_2, 6-keto $PGF_{1\alpha}$ and TXB_2 for anti PGE_2 were 100, 2.0 and <1.0 respectively; for anti 6-keto-$PGF_{1\alpha}$ were 4.0, 100 and 0.5 respectively and for anti TXB_2 were <0.1, <0.1 and 100 respectively. Extraction efficiencies obtained with radiolabelled prostanoid tracers (6 determinations) were: PGE_2 94.3 ± 0.9%, TXB_2 88.2 ± 1.0% and 6-keto-$PGF_{1\alpha}$ 85.6 ± 3.4% and accordingly the measured values in the reconstituted extracts have not been corrected.

Statistics

Results are expressed as means ± calculated s.e.means. In the ulcer studies, tests for significance between treatments were performed by use of the non parametric Kruskall-Wallis test. The other significance tests used were Student's unpaired *t* tests. Differences with a probability value of 5% or less were regarded as being significant.

Materials

PhCL28A was synthesized by Dr T. Purcell, Chemistry Dept., LERS. Sulphasalazine, NAD^+, PBT, arachidonic acid and all prostaglandin standards were obtained from Sigma chemicals. The multitritiated ^{3}Hn prostanoids used for radioimmunoassays had specific activities of 140–180 Ci mmol^{-1} and were from Amersham International, as was [9β-^{3}H]-prostaglandin $F_{2\alpha}$ (specific activity 14–17 Ci mmol^{-1}). Antibodies against PGE_2 and TXB_2 were purchased from Institut Pasteur Productions and the anti 6-keto $PGF_{1\alpha}$ antibody was obtained from Byosis, Compiegne.

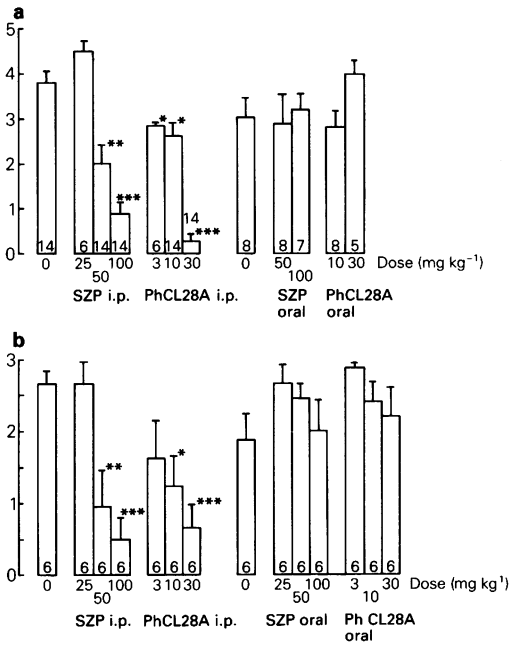

Figure 2 Effect of oral and i.p. treatment with sulphasalazine (SZP) and PhCL28A on gastric ulcers induced by (a) ethanol and (b) phenylbutazone (as described in Methods). The results are expressed as mean ulcer score (s.e.mean shown by vertical lines) with the number of animals indicated in the columns. The asterisks denote the level of statistical significance, where $*P<0.05$; $**P<0.01$ and $***P<0.001$.

Results

Effects of sulphasalazine and PhCL28A on rat gastric ulcer formation

In naïve animals both ETOH and PBT produced marked gastric lesions, with ulcer indices ranging from 322 to 380 for ETOH and 156–267 for PBT. Oral pretreatment with either SZP (up to 100 mg kg^{-1}) or PhCL28A (up to 30 mg kg^{-1}) did not protect the stomach from the ulcerogenic effects of either ETOH or PBT (Figure 2). However, when administered by the i.p. route, both compounds showed a marked dose-dependent inhibition of macroscopic lesion formation (Figure 2). ED_{50} values obtained from the ulcer indices (Table 1) show that PhCL28A is approximately 3 times more potent than SZP against ETOH ulcers, estimated ED_{50} values being 13 and 41 mg kg^{-1} respectively. A similar profile of activity was observed against PBT ulcers (Figure 2b) namely that SZP had an approximate ED_{50} of 32 mg kg^{-1} with PhCL28A

Table 1 Antiulcer actions of sulphasalazine (SZP) and PhCL28A given i.p. 30 min before treatment with either ethanol (ETOH) or phenylbutazone (PBT) (for details see methods)

Treatment	*Ulcerogen*	*Ulcer score* (Mean ± s.e.mean)	*Ulcer index*	ED_{50} (mg kg^{-1})
Vehicle	ETOH	3.8 ± 0.3 (14)	380	
SZP 25 mg kg^{-1}		4.5 ± 0.4 (6)	450	
50 mg kg^{-1}		2.0 ± 0.4 (14)**	171	41
100 mg kg^{-1}		0.9 ± 0.3 (14)***	58	
PhCL28A 3 mg kg^{-1}		2.8 ± 0.1 (6)	283	
10 mg kg^{-1}		2.5 ± 0.3 (14)*	252	13
30 mg kg^{-1}		0.3 ± 0.2 (14)***	6	
Vehicle	PBT	2.7 ± 0.3 (6)	270	
SZP 25 mg kg^{-1}		2.7 ± 0.3 (6)	270	
50 mg kg^{-1}		1.0 ± 0.3 (6)**	48	32
100 mg kg^{-1}		0.5 ± 0.3 (6)***	17	
PhCL28A 3 mg kg^{-1}		1.6 ± 0.5 (6)*	135	
10 mg kg^{-1}		1.3 ± 0.4 (6)*	83	3
30 mg kg^{-1}		0.7 ± 0.3 (6)**	44	

The results show the mean ulcer scores, ulcer indices and ED_{50} values estimated from these indices. The asterisks denote the values of statistical significance by the Krunskall-Wallis test: *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$, the number of animals in each group are given in parentheses.

being in this case 10 times more potent (estimated $ED_{50} = 3$ mg kg^{-1}).

Inhibition in vitro *of prostaglandin breakdown by sulphasalazine and PhCL28A*

As is shown in Figure 3, both SZP and PhCL28A elicited dose-dependent inhibition of [9β-^{3}H]-$PGF_{2\alpha}$ degradation when incubated with control rat stomach cytosolic supernatants. PhCL28A ($IC_{50} = 230$ nM) was 478 times more potent than SZP ($IC_{50} = 110$ μM).

Ex vivo *prostaglandin degradation studies*

When compared with controls, gastric cytosolic [9β-^{3}H]-$PGF_{2\alpha}$ degradation was significantly ($P < 0.001$ in each case) reduced in both the PBT and ETOH treatment groups (Figure 4). The decrease in PGDH activity was similar after either ulcerogenic stimuli, being 35% after ETOH and 31% after PBT.

Irrespective of the route of administration both SZP (100 mg kg^{-1}) and PhCL28A (30 mg kg^{-1}) (doses which when given by the i.p. route produced more than 80% inhibition of macroscopic lesion formation), inhibited *ex vivo* [9β-^{3}H]-$PGF_{2\alpha}$ breakdown to slight but statistically significant extents. Thus, when the compounds were given by the oral route, control prostaglandin degradation was 1153 ± 39 ng h^{-1} g^{-1} tissue (mean ± s.e.mean, $n = 6$ animals) which was reduced to 937 ± 53 and 938 ± 47 ng h^{-1} g^{-1} tissue for PhCL28A and SZP respectively ($n = 6$ animals per group, $P < 0.01$ in each case). Likewise, in the i.p. experiments a control [9β-^{3}H]-$PGF_{2\alpha}$ degradation was reduced from 977 ± 62 ng h^{-1} g^{-1} tissue to 798 ± 57 ($P < 0.01$) and 833 ± 51 ng h^{-1} g^{-1} tissue after PhCL28A and SZP respectively ($n = 6$ animals per

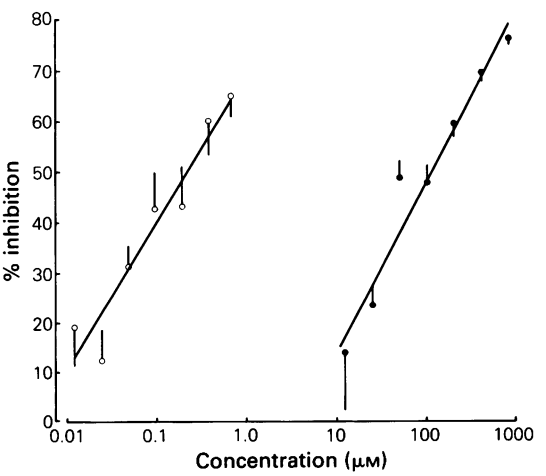

Figure 3 Effects of sulphasalazine (●) and PhCL28A (○) on [9β-^{3}H]-prostaglandin $F_{2\alpha}$ catabolism in rat stomach 100,000 *g* cytosolic supernatants. The points show mean ($n = 4$) percentage inhibition of control catabolism measured by radio t.l.c., where the ordinate scale = percentage inhibition and abscissa scale = concentration (μM); vertical lines show s.e.mean.

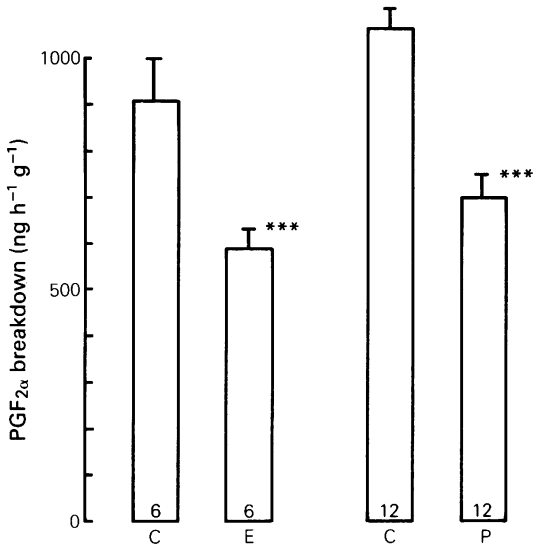

Figure 4 Effect of gastric ulceration on stomach cytosolic prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) breakdown. The columns represent mean (vertical lines show s.e.mean) [9β-^{3}H]-$PGF_{2\alpha}$ breakdown (expressed as ng broken down $h^{-1}g^{-1}$ tissue) in 100,000 *g* supernatants prepared from control stomachs (C) or stomachs presenting macroscopic lesions induced by ethanol (E) or phenylbutazone (P). The asterisks denote the levels of statistical significance compared with the respective controls where $***P < 0.001$ with the number of animals shown in the columns.

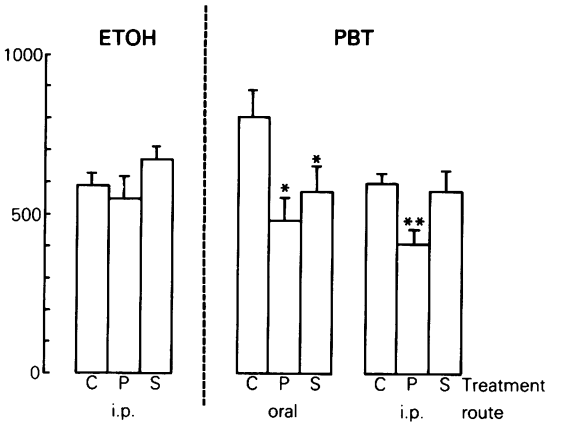

Figure 5 Effects of PhCL28A (P) and sulphasalazine (S) pretreatment on *ex vivo* cytosolic prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) breakdown in animals receiving either ethanol (ETOH) or phenylbutazone (PBT). The columns represent prostaglandin degradation (mean with s.e.mean shown by vertical lines, $n = 6$ animals per treatment group) expressed as ng $PGF_{2\alpha}$ catabolised per hour per g wet weight of tissue. The columns marked C represent animals receiving ulcerogen without pretreatment with the test compound. The asterisks denote the levels of statistical significance as compared to C, where $*P < 0.02$ and $**P < 0.001$.

treatment group). In some cases, the reduction in *ex vivo* PGDH activity caused by ulcerogen treatment was reduced still further after treatment with PhCL28A and SZP. In the case of PBT ulcers, the anti-PGDH activity of PhCL28A was observed irrespective of the route of administration and thus irrespective of whether or not the compound protected the animal from macroscopic gastric lesion formation (Figure 5).

Ex vivo *prostaglandin synthesis studies*

In the PBT-induced ulcer studies, a portion of each homogenate (700 μl) was used for the determination of 6-keto-$PGF_{1\alpha}$, PGE_2 and TXB_2 after ethyl acetate extraction by radioimmunoassay. The results are shown in Table 2 and four main points emerge. Firstly, in both the i.p and oral studies, PBT reduced 6-keto-$PGF_{1\alpha}$ and PGE_2 synthesis although this was only statistically significant in the i.p. study. Moreover, it has been previously shown that PBT is a relatively weak inhibitor of cyclo-oxygenase when given *in vivo* (Strub & Muller, 1979). Secondly irrespective of the route of administration, neither SZP (100 mg kg^{-1}) nor PhCL28A (30 mg kg^{-1}) pretreatment had any effect on the synthesis of 6-keto-$PGF_{1\alpha}$, whether or not the animal subsequently received PBT. Thirdly, when given orally PhCL28A caused nearly a twofold increase in gastric PGE_2 synthesis. This effect was not observed when the compound was given i.p., nor was it seen after oral treatment in stomachs which subsequently received PBT. Finally, TXB_2 formation was unaltered by any of the treatments studied.

Discussion

This study demonstrates that both SZP and the potent PGDH inhibitor PhCL28A prevent gastric ulceration induced by either PBT or ETOH, and that this effect is dependent upon the route of administration. The fact that the compounds had to be administered systemically (i.e. i.p.) to produce their antiulcer effects in the dose ranges used suggests very strongly that it is the parent compound which is active and not products of splitting the diazo linkages by intestinal flora. Moreover, in the only other published study (to our knowledge) of the effects of SZP on gastric ulceration (stress-induced ulcers, Ogle & Cho, 1985), the compound was administered sub-cutaneously. In addition,

Table 2 Effects of PhCL28A and sulphasalazine (SZP) pretreatment on prostaglandin synthesis during homogenization in stomachs taken from either control animals or animals which subsequently received phenylbutazone (PBT) by oral administration.

Treatment	*Route*	n	*Prostaglandin* ($ng\,g^{-1}$) *6-keto-PGF$_{1\alpha}$*	*PGE$_2$*	*TXB$_2$*
Control	Oral	5	1320 ± 240	180 ± 24.5	39.3 ± 6.1
+ PhCL28A 30 mg kg^{-1}	Oral	5	1360 ± 150	340 ± 26.4**	35.6 ± 2.9
+ SZP 100 mg kg^{-1}	Oral	5	1010 ± 140	187 ± 33.6	30.1 ± 2.7
PBT	Oral	5	1070 ± 200	117 ± 33	35.4 ± 2.8
PBT + PhCL28A 30 mg kg^{-1}	Oral	5	810 ± 150	157 ± 23.6	30.3 ± 2.1
PBT + SZP 100 mg kg^{-1}	Oral	5	1730 ± 460	123 ± 13.4	47.2 ± 7.3
Control	i.p.	6	640 ± 60	117 ± 13.3	23.9 ± 4.5
+ PhCL28A 30 mg kg^{-1}	i.p.	6	610 ± 70	110 ± 16.0	21.9 ± 4.0
+ SZP 100 mg kg^{-1}	i.p.	6	680 ± 110	99 ± 7.0	23.6 ± 4.8
PBT	i.p.	6	350 ± 100*	61.5 ± 5.1*	20.6 ± 3.4
PBT + PhCL28A 30 mg kg^{-1}	i.p.	6	280 ± 80*	74.0 ± 14.0*	20.5 ± 3.9
PBT + SZP 100 mg kg^{-1}	i.p.	6	330 ± 90*	72.2 ± 5.3*	24.1 ± 3.4

The results show mean ± s.e.mean prostaglandin synthesis per g wet weight of tissue, where *n* represents the number of animals in each treatment group and statistical significance compared to control values was determined by Student's *t* test: $*P<0.05$; $**P<0.01$.

the oral doses of SZP necessary to inhibit indomethacin-induced intestinal ulcers in the rat were at least 4 times greater than the active i.p. doses used in this study (Del Soldato *et al.*, 1985). Furthermore, given that SZP is systemically active in a similar dose-range in both ulcer models described here and in the stress ulcer model cited above, it seems likely that its effect is independent of gastric acid secretion, since cimetidine, which is a potent inhibitor of gastric acid secretion (Carmichael *et al.*, 1978) is inactive against ETOH-induced ulcers (Matsuo *et al.*, 1986). These observations suggest that the antiulcer actions of SZP are independent of the ulcerogenic stimulus.

These investigations also confirm the previously reported findings that SZP and PhCL28A inhibit PGDH *in vitro* (Hoult & Moore, 1978; Berry *et al.*, 1985) and extend these observations to rat stomach PGDH (in the case of PhCL28A). In this preparation, PhCL28A was less potent than it has been found to be in other sources of PGDH, with an IC_{50} of 230 nM as opposed to between 20 and 70 nM (Berry *et al.*, 1985). However, it is unlikely that this particular action *in vitro* can be proposed as a mechanism for the antiulcer effects of the two compounds presented above for the following reasons: firstly, PhCL28A is nearly 500 times more potent as a PGDH inhibitor than SZP *in vitro*, whereas there is only a 3 fold difference in potency between the two compounds *in vivo* against ulcer formation; secondly, *in vivo* both compounds reduce cytosolic prostaglandin breakdown independently of the route of the administration whereas the antiulcer effect was only seen after i.p. treatment; and finally in both ulcer models there was a decrease in PGDH activity in the animals which had received the ulcerogens when compared to control animals. It could be argued, however, that this latter effect may be part of a homeostatic response to increase the levels of gastric prostanoids in the presence of ulcerogen, especially since it has been found that colonic PGDH activity is reduced in a guinea-pig model of ulcerative colitis (Hoult *et al.*, 1979) a disease in which a role for endogenous 'cytoprotective' prostaglandins has been implicated (reviewed in Hawkey & Rampton, 1985).

Since exogenously administered prostaglandins of the E and I series protect the gastric mucosa against various ulcerogens (Whittle *et al.*, 1978; Robert, 1979; Miller, 1983 *inter alia*), prevention of the breakdown of endogenous prostaglandins seems an attractive therapeutic proposition. Furthermore, it has recently been claimed by Konturek *et al.* (1986) that the flavonoid solon owes its anti-ulcer action to PGDH inhibition. However, in the latter study the proposition was based on *in vitro* rather than *ex vivo* data. Such an extrapolation must be made with caution as we have shown with SZP and PhCL28A where the *in vivo* effect is dependent on the route of administration.

It has previously been shown that both SZP and PhCL28A are inactive at 1 mM on sheep seminal vesicle cyclo-oxygenase *in vitro* (Berry *et al.*, 1985). Our experiments show that i.p. administration of either SZP or PhCL28A had no effect on cyclo-oxygenase activity during subsequent homogenization of stomachs taken from naïve or ulcerogen (PBT)-treated animals. This observation, however, does not eliminate a possible *ex vivo* effect on cyclo-oxygenase (which, to lead to ulcer prevention would be of a

stimulatory nature, as cyclo-oxygenase inhibitors actually provoke gastric lesions), since we did not perform the experiments on the mucosal layer itself, the latter being generally believed to be the principle site of gastroprotective prostaglandin synthesis. Nevertheless, we believe that, taken together, both the lack of effects *in vitro* and our results would make it difficult to implicate a modification of cyclo-oxygenase activity as a mechanism for the anti-ulcer actions of either SZP or PhCL28A.

It has been suggested that inhibition of thromboxane synthesis may be a possible mechanism for the antiulcer actions of BW755C (Wallace & Whittle, 1985). Moreover, SZP at 50 and 100 μM inhibits human colonic TXB_2 formation *in vitro* (Boughton-Smith *et al.*, 1983). However, we found that at doses which almost completely inhibited ulcer formation neither PhCL28A nor SZP had any effect on gastric TXB_2 formation during homogenization, irrespective of the route of administration. Thus it seems that despite the great importance of prostaglandins in maintenance of gastric mucosal integrity (recently emphasised by the occurrence of gastroduodenal ulcers in rabbits used to raise antibodies to 6-keto-$PGF_{1\alpha}$ and PGE_2, Olsen *et al.*, 1985), neither SZP nor PhCL28A appear to owe their anti ulcer effects to changes in endogenous prostaglandin formation or degradation as could be measured in the present studies.

In addition to the actions discussed above, SZP also inhibits mast cell degranulation (Ogle & Cho, 1985), the binding of f-MetLeuPhe to its receptor on neutrophils (Stenson *et al.*, 1984) and various lipoxygenases including soybean lipoxygenase (Sircar *et al.*, 1983) and neutrophil 5 and 12 lipoxygenases (Stenson & Lobos, 1982). All of these properties, if applicable *in vivo*, have potential beneficial effects in the prevention of gastric ulceration. Of particular interest is the effect of SZP on lipoxygenase, despite the high concentrations (1–2 mM) required to inhibit 5-lipoxygenase *in vitro*. The importance of lipoxygenase products, especially the peptido-leukotrienes, in the pathogeneseis of gastric ulceration is becoming increasingly apparent. Leukotriene C_4 has recently been shown to cause vasoconstriction and stasis of the gastric submucosal microcirculation (Whittle *et al.*, 1985). Instillation of ETOH enhances rat gastric mucosal leukotriene C_4 formation, an enhancement which correlates with ulcer formation (Dreyling *et al.*, 1986). In addition in a preliminary study, we have found that peptido-leukotriene formation, measured as immunoreactive leukotriene C_4 with commercially available radioimmunoassay kits, was increased from $3.8 \pm 0.82\,ng\,g^{-1}$ (mean ± s.e.mean, $n = 5$ animals) to $8.7 \pm 1.09\,ng\,g^{-1}$ (mean ± s.e.mean, $n = 5$ animals, $P < 0.01$) during homogenizing of stomachs taken from control rats and rats with PBT-induced ulcers (C.N. Berry, unpublished experiment). Furthermore, Ogle & Cho (1986) have recently reported that the leukotriene antagonist, FPL 55712 protects rats from stress-induced gastric ulcers. Thus, there is an obvious need to examine the effects of SZP and PhCL28A treatment on subsequent *ex vivo* lipoxygenase activity; this is currently being investigated.

In conclusion, we have shown that SZP and PhCL28A have antiulcer properties when given systemically. This effect appears to be independent of a prostaglandin-mediated mechanism and the mechanism of action of these two compounds remains unclear.

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