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The effect of anti-inflammatory drugs on eicosanoid formation in a chronic model of inflammatory bowel disease in the rat

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1 The effects of anti-inflammatory drugs on eicosanoid formation and colonic damage in a chronic model of inflammatory bowel disease (IBD) in the rat were investigated.

2 A single colonic instillation of the hapten, trinitrobenzene sulphonic acid (TNB) resulted in ulceration and inflammation which persisted for 3 weeks.

3 The macroscopic colonic damage, present 3 weeks after TNB, was correlated with an increase in immunoreactive 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and leukotriene B_4 (LTB_4) synthesis by the rat colon.

4 Anti-inflammatory drugs were administered 2 weeks after TNB, when there was substantial colonic damage, and continued for a week. The experimental drug BW755C inhibited the increased formation of 6-keto-$PGF_{1\alpha}$ and LTB_4 by the inflamed colon. Indomethacin and aspirin markedly inhibited prostanoid formation in both inflamed and control colon. Sulphasalazine or prednisolone also inhibited the formation of 6-keto-$PGF_{1\alpha}$ but the effects were less marked.

5 None of the anti-inflammatory drugs significantly reduced the colonic damage induced by TNB.

6 The results suggest that eicosanoids, including LTB_4, have only a minor role in maintaining the chronic macroscopic damage induced in the rat colon by TNB. The role of such eicosanoids in the underlying infiltration and activity of inflammatory cells in this model of IBD, however, is not known.

Introduction

The need for a greater understanding of the aetiology of inflammatory bowel disease (IBD) and the search for more effective and novel therapy for the treatment of the disease, has led to the development of a variety of experimental animal models. A new model of IBD induced in rats by the hapten trinitrobenzene sulphonic acid (TNB) has been described (Morris *et al.*, 1984). In this model, a single instillation of TNB into the rat colon produces a chronic ulceration and inflammation which persists for up to five weeks.

In studies with inflamed colonic tissue from patients with IBD, there is an increase in the formation of both cyclo-oxygenase and lipoxygenase metabolites of arachidonic acid (Harris *et al.*, 1978; Sharon *et al.*, 1978; Boughton-Smith *et al.*, 1983; Sharon & Stenson, 1984). It has been proposed that the drugs used in the treatment of IBD may act by reducing the formation of arachidonic acid metabolites. Particular interest has been focused on the leukotrienes and other lipoxygenase metabolites of arachidonic acid since these products have potent stimulatory activity on inflammatory cells (Samuelsson, 1983; Higgs *et al.*, 1984). At present there is no clinically available drug to test the hypothesis that lipoxygenase metabolites are important in the disease. In the present study, the activity of various inflammatory drugs on the TNB model of IBD in rats has been evaluated. The anti-inflammatory drugs used include the experimental drug, BW755C, which inhibits both cyclo-oxygenase and lipoxygenase enzymes (Higgs *et al.*, 1979) and the therapeutically used drug sulphazalazine, which has been shown *in vitro* to inhibit human colonic

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cyclo-oxygenase (Gould *et al.*, 1981; Ligumsky *et al.*, 1982) and lipoxygenase (Sharon & Stenson, 1984; Hawkey *et al.*, 1985).

The study was designed to evaluate the effects of anti-inflammatory drugs on eicosanoid formation by the chronically inflamed colon induced by TNB in this model of IBD.

A preliminary account of some of these data has been presented to the American Gastroenterological Association (Boughton-Smith *et al.*, 1985).

Methods

Effect of TNB concentration on colonic damage

The concentrations of TNB required to induce a chronic inflammation of the rat colon were evaluated by administering various concentrations of TNB (5, 10, 20 and 30 mg) dissolved in 0.25 ml of 30% ethanol, into the colons of female Wistar rats (225–250 g) as described in detail below. Two weeks later, the rats were killed by CO_2 asphyxiation and the distal colon removed for the assessment of colonic damage. The weight gain of each group of rats was also monitored as a measure of the overall health of the animals.

Damage score

Rats from coded cages were killed in random order by CO_2 asphyxiation 3 to 5 h after the final drug treatment. The distal colon (8 cm) was removed, opened longitudinally and, after cleaning with tap water, pinned out on a wax bed flooded with saline. Inflammation was assessed by use of a stereomicroscope, by two independent observers who were unaware of the animals' treatment. A sketch of each colon was drawn indicating the location of the inflammatory features and the damage scored on a scale ranging from 0 (normal) to 5 (severe) based on the criteria shown in Table 1. The overall score from each colon was taken as the mean of the scores given by the two observers.

Effect of anti-inflammatory drugs on the TNB model

Chronic inflammation of the colon was induced in ether-anaesthetized rats by a single intra-colonic application of TNB (20 mg, dissolved in 0.25 ml of 30% ethanol) using a rubber catheter (8 cm long, external diameter 2 mm). Control rats received intra-colonic application of 0.25 ml of 30% ethanol.

Anti-inflammatory drug treatment to randomly allocated groups of rats was started two weeks after TNB. These drugs were administered orally, twice daily, (09 h 00 min and 17 h 00 min) for 7 consecutive days and on an 8th day at 09 h 00 min only. The anti-inflammatory drugs used were BW755C ($50\,mg\,kg^{-1}$), indomethacin ($0.5\,mg\,kg^{-1}$), aspirin ($100\,mg\,kg^{-1}$) and sulphasalazine ($100\,mg\,kg^{-1}$), and also prednisolone ($0.5\,mg\,kg^{-1}$) which was administered once daily only. These doses were based on previous studies in this laboratory on experimental inflammation and eicosanoid biosynthesis in the rat with these agents (Higgs *et al.*, 1980; Salmon *et al.*, 1983). The drugs were dissolved or suspended in 1% methyl cellulose (celacol) and administered in a volume of $2\,ml\,kg^{-1}$. Control animals (TNB or ethanol treated) received 1% celacol alone.

Eicosanoid formation

Segments (200–300 mg) of colon were taken from involved areas of ulceration and/or inflammation and, because of the discrete nature of the colonic damage, included grossly normal adjacent tissue. The segments were minced finely with scissors (15 s) and after addition of Tris buffer (0.5 ml, 50 mM, pH 7.5 at 0°C), vortexed (10 s) and centrifuged (9000 *g*, 10 s) as described by Whittle (1981). The pellet was resuspended in Tris buffer (1.0 ml) mixed and centri-

Table 1 Criteria for assessment of colonic damage induced by trinitrobenzene sulphonic acid

Score	*Colonic feature*
0	No damage.
1	One region of localized inflammation or thickening. No ulcers.
2	Linear ulceration, but no significant inflammation.
3	Linear ulceration with inflammation at one site.
4	Two or more sites of ulceration and/or inflammation. Ulcers present in at least one site.
5	Two or more sites of ulceration and inflammation or one major site of ulceration and inflammation extending >1 cm along the length of the colon.

fuged as above and the supernatant discarded. These steps were performed to remove adherent mucus and blood from the samples. Finally, the samples were resuspended in Tris buffer, (0.5 ml) vortexed (30 s) and centrifuged (30 s) and the supernatant transferred to microfuge tubes containing indomethacin (final concentration 10 $\mu g\,ml^{-1}$) and frozen (dry ice granules, −20°C) for later specific radioimmunoassay of eicosanoids.

In a further study, the generation of prostacyclin-like activity from minced colonic tissue following 30 s vortex agitation was determined immediately by bioassay utilizing its ability to inhibit ADP-induced human platelet aggregation *in vitro*, as described previously (Whittle, 1981).

Radioimmunoassays

Eicosanoids were measured without prior extraction by specific radioimmunoassays for prostaglandin E_2 (PGE_2), thromboxane B_2 (TXB_2) and 6-keto-$PGF_{1\alpha}$ (diluted 1 : 10 to 1 : 1000 in assay buffer) or leukotriene B_4 (LTB_4) (diluted 1 : 2 to 1 : 5) as previously described (Salmon, 1978; Salmon & Flower, 1979; Salmon *et al.*, 1982) and formation is expressed as $ng\,g^{-1}$ colonic tissue.

Materials

Indomethacin, sulphasalazine, prednisolone 21-sodium succinate, 2,4,6-trinitrobenzene sulphonic acid (picrysulphonic acid) were obtained from Sigma Chemical Co. Ltd. Aspirin, 1-benzyl imidazole hydrogen fumarate and BW755C (3-amino-1-(*m*-(trifluoromethyl)-phenyl)-2-pyrazoline) were from the Wellcome Research Laboratories, Beckenham. Rubber catheters (CH.8 Nelaton 6A) were from Leymed and celacol (methyl cellulose) was from British Celanese Ltd, Derby.

Calculations and statistical analysis

The results were calculated as the mean ± s.e. mean. Differences between the damage scores of the TNB control, ethanol control and drug-treated groups were compared statistically by use of the Mann-Whitney U-test. Correlations between damage scores by different observers and comparison of other parameters were calculated by regression analysis. Differences in eicosanoid generation between control and inflamed tissue and the effects of anti-inflammatory drugs (calculated as % control) were compared by use of Students' *t* test for unpaired data (two tailed). The level of significance for all studies was taken as $P < 0.05$.

Results

Effect of TNB concentration and time on the chronic inflammation and ulceration of the colon

The extent of the colonic damage two weeks after TNB was dependent on the initial concentration instilled into the rat colon (Figure 1a). At TNB concentrations of 5 and 10 mg (in 0.25 ml of 30% ethanol) there was a low level of colonic damage.

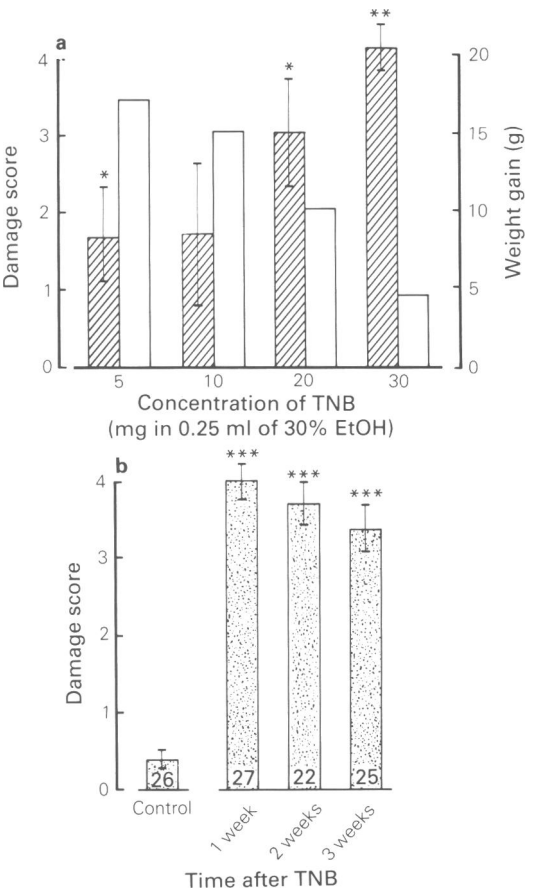

Figure 1 (a) Changes in damage score (hatched columns), and in body weight gain (open columns), 2 weeks after intra-colonic administration of various concentrations of trinitrobenzene sulphonic acid (TNB) to rats. Results are the mean of 5 rats per group. Statistical significance is shown as * $P < 0.05$, ** $P < 0.01$ compared to control. (b) Time-related changes in damage score (stippled columns) after intra-colonic TNB (20 mg in 0.25 ml of 30% ethanol). Results are the mean of *n* (figure in column) rats per group. Statistical significance shown as *** $P < 0.001$ compared to control. In (a) and (b) vertical lines represent s.e. mean.

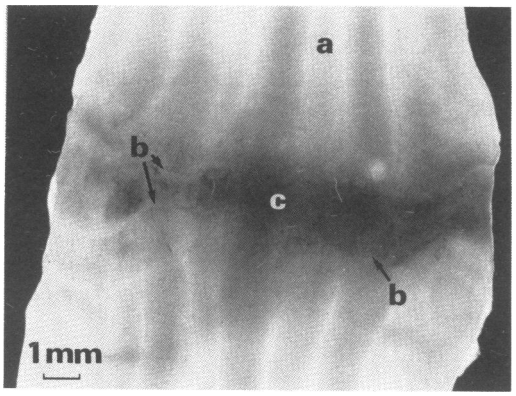

Figure 2 Macroscopic appearance of a transilluminated rat colon showing (a) grossly normal mucosa, (b) linear ulceration, (c) inflammation (thickening) and hypereamia, present 2 weeks after intra-colonic administration of trinitrobenzene sulphonic acid (20 mg in 0.25 ml of 30% EtOH).

The damage was increased with higher TNB concentrations; at 30 mg 0.25 ml^{-1} the colonic damage was significantly greater ($P < 0.005$) than that produced by TNB concentrations at 5 and 10 mg 0.25 ml^{-1}. The increase in damage score produced by TNB was accompanied by a decrease in the normal body weight gain. A TNB concentration of 20 mg 0.25 ml^{-1} was chosen for further experiments because this concentration produced substantial colonic damage without compromising the overall health of the rats, as assessed by body weight gain.

The damage score was maintained for one to three weeks after TNB (20 mg in 30% ethanol), the gradual fall not being statistically significant (Figure 1b).

Characteristics of the inflammation induced by intra-colonic administration of TNB

The major characteristics of the colon 3 weeks after TNB (20 mg in 0.25 ml of 30% ethanol) were open linear ulcers and gross thickening of the colonic wall (Figure 2). The thickening and ulceration was segmented, with bands of grossly normal tissue alternating with areas of damaged tissue. The ulcer sizes varied from small white star-shaped indentations (2–3 mm diameter) in the colonic mucosa, to larger white linear ulcers (2–6 mm length), with very little visually apparent inflammation. In some colons, there were deep fissuring linear ulcers surrounded by thickened, vascular and inflamed tissue. Multiple ulcerations of the colon resulted in a 'cobblestone' appearance of the mucosa. These ulcers often extended around the circumference of the colonic lumen and were associated with strictures resulting in massive dilatation of the proximal colon and narrowing of the distal colon. In cases of severe ulceration, the colon had often adhered to surrounding intestinal tissue or to the uterine horns, and in these areas fat deposits accumulated. Similar patterns of inflammation and damage of the colon were seen at one and two weeks after TNB.

Using the scoring system described in Table 1, there was a highly significant correlation between the damage scores assigned by two independent observers (correlation coefficient $r = 0.98$, $P < 0.001$, from 40 randomly selected scores).

Colonic eicosanoid formation and damage following TNB

The formation of immunoreactive LTB_4 by the control colon (6.1 ± 1.1 ng g^{-1} tissue, $n = 22$), was significantly increased (by $95 \pm 45\%$) in inflamed colon, to 11.9 ± 2.5 ng g^{-1} tissue ($n = 25$, $P < 0.05$), three weeks after TNB. In addition, the formation of immunoreactive 6-keto-$PGF_{1\alpha}$ by control colon (115 ± 19 ng g^{-1} tissue, $n = 22$), was also increased in the inflamed TNB colons, by $56 \pm 14\%$ ($n = 25$, $P < 0.01$) to 168 ± 27 ng g^{-1} tissue (Figure 3).

The overall damage score for the TNB-treated rats after three weeks was 2.9 ± 0.3 ($n = 37$ from 5 experiments) compared to an overall control damage score of 0.5 ± 0.2 ($n = 25$, $P < 0.001$). Regression analysis, using data from TNB and control colons, demonstrated a significant correlation between damage score and the generation of 6-keto-$PGF_{1\alpha}$ ($r = 0.30$, $P < 0.05$, $n = 54$) and LTB_4 ($r = 0.48$, $P < 0.01$, $n = 55$). When damage scores from only TNB colons were analysed, there was a significant correlation with the generation of LTB_4 ($r = 0.41$, $P < 0.05$, $n = 26$) but not with 6-keto-$PGF_{1\alpha}$.

Effect of anti-inflammatory drugs on eicosanoid formation and colonic damage

The anti-inflammatory drugs significantly affected eicosanoid formation by the inflamed colon (Figure 3). The generation of both 6-keto-$PGF_{1\alpha}$ and LTB_4 was reduced to control levels (ethanol control) by BW755C (50 mg kg^{-1}). Sulphasalazine (100 mg kg^{-1}) also reduced the colonic generation of 6-keto-$PGF_{1\alpha}$ to control levels, but had no significant effect on the generation of LTB_4. Both indomethacin (0.5 mg kg^{-1}) and aspirin (100 mg kg^{-1}) substantially inhibited the formation of 6-keto-$PGF_{1\alpha}$, to below control levels, whereas these agents had no effect on LTB_4 formation (Figure 3). In addition, in an experiment in which immunoreactive TXB_2 and PGE_2 were also measured, their formation was similarly inhibited by these doses of indomethacin ($73 \pm 4\%$

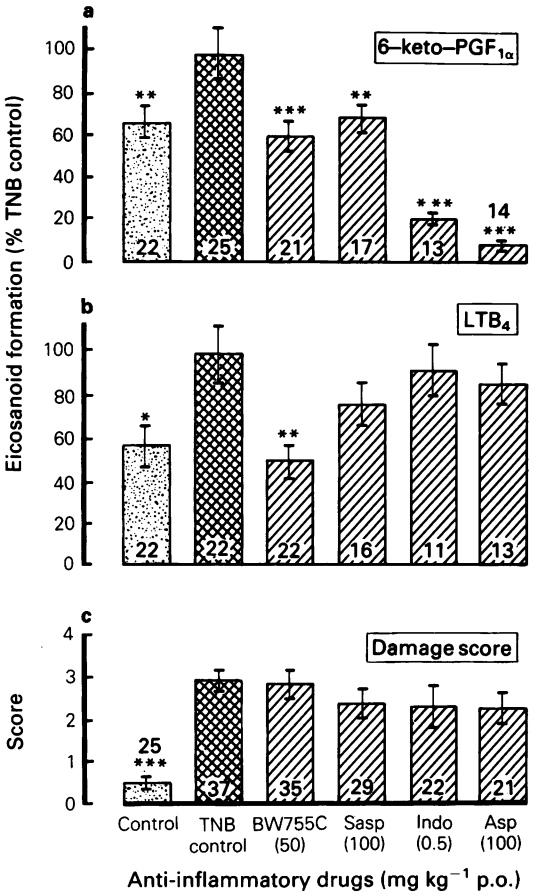

Figure 3 Effect of anti-inflammatory drugs on (a and b) eicosanoid (a, 6-keto-prostaglandin $F_{1\alpha}$; b, leukotriene B_4) formation and (c) on the chronic ulceration and inflammation (damage score assessed macroscopically) induced by trinitrobenzene sulphonic acid (TNB). Drug treatment (orally, twice daily) was started two weeks after intra-colonic TNB (20 mg in 0.25 ml of 30% ethanol) and continued for a week. The formation of eicosanoids (expressed as % of the TNB control group) and the damage score are the mean of n (number in column) rats per group; vertical lines indicate s.e. mean. Statistical significance was calculated by use of Student's t test for eicosanoid formation, or by Mann-Whitney U-test for the damage score, where $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the TNB control group. Sasp = sulphasalazine, Indo = indomethacin and Asp = aspirin.

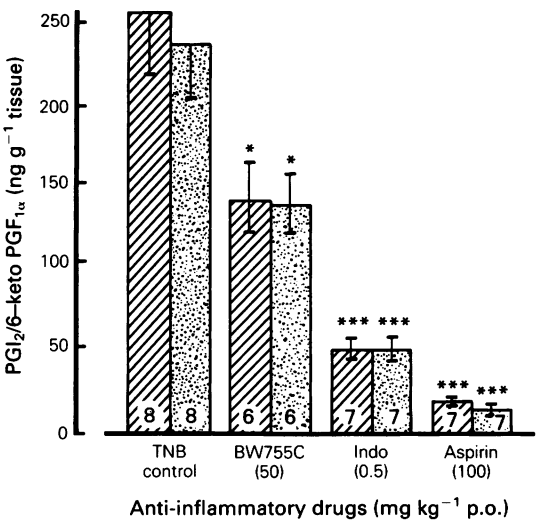

Figure 4 Effect of anti-inflammatory drugs on prostacyclin (PGI_2) formation by rat colon, measured, 3 weeks after trinitrobenzene sulphonic acid (TNB) administration, by bioassay (as inhibition of human platelet aggregation) or by radioimmunoassay as immunoreactive 6-keto-prostaglandin $F_{1\alpha}$. Drug administration (orally twice daily) was started 2 weeks after intra-colonic TNB (20 mg in 0.25 ml of 30% ethanol) and continued for one week. The formation of PGI_2/6-keto-$PGF_{1\alpha}$ expressed as ng g^{-1} tissue, is the mean of n rats per group (figure in column); Vertical lines indicate s.e. mean. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Indo = indomethacin.

and $75 \pm 4\%$ inhibition respectively, $n = 7$, $P < 0.001$) and aspirin ($80 \pm 1\%$ and $95 \pm 1\%$ inhibition, respectively, $n = 7$, $P < 0.001$).

Inhibition of cyclo-oxygenase by the anti-inflammatory drugs was further demonstrated by the determination of prostacyclin formation by a bioassay technique, utilizing its inhibitory actions on ADP-induced human platelet aggregation, as well as by radioimmunoassay (Figure 4). The levels of prostacyclin generated by the inflamed rat colon were comparable whether measured either as immunoreactive 6-keto-$PGF_{1\alpha}$ or as biologically-active prostacyclin, as was the degree of inhibition by the anti-inflammatory drugs.

Prednisolone (0.5 mg kg^{-1}) had no significant effect on the generation of LTB_4 but decreased 6-keto-$PGF_{1\alpha}$ generation ($56 \pm 11\%$ inhibition, $n = 4$, $P < 0.05$).

Although the formation of colonic eicosanoids was thus significantly inhibited, none of the anti-inflammatory drugs had an overall significant effect on the damage score in the five studies (Figure 3).

Discussion

A chronic ulceration and inflammation of the colon was induced in rats by a single intra-colonic administration of TNB. As originally described by Morris *et al.* (1984), the ulceration and inflammation

induced by TNB persisted for up to 3 weeks. The damage was characterized by linear ulceration, gross thickening and hyperaemia and was dependent on the initial concentration of TNB instilled into the colon. There was a highly significant correlation between the damage scores assigned by independent observers, indicating that the scoring system used to assess the macroscopically apparent colonic damage was reproducible.

This experimental model has been used to investigate eicosanoid formation by inflamed colonic tissue and the effects of anti-inflammatory agents. In inflamed colonic mucosa from patients with active IBD there is an increased formation of cyclo-oxygenase and lipoxygenase metabolites of arachidonic acid (Boughton-Smith *et al.*, 1983; Sharon & Stenson, 1984). The prostaglandins may play a role in inflammation by enhancing vasodilatation and oedema formation. Furthermore, the lipoxygenase products, particularly LTB_4, have potent stimulatory actions on leukocytes and therefore may also be important in the colonic inflammation (Higgs *et al.*, 1984). Therefore modulation of arachidonic acid metabolism, particularly lipoxygenase enzymes, could be an important novel therapeutic approach to the treatment of IBD.

In the present study, there was an increase in the generation of immunoreactive 6-keto-$PGF_{1\alpha}$ and LTB_4 by segments of inflamed rat colon taken three weeks after TNB. These increases in eicosanoid formation were correlated with the degree of colonic ulceration and inflammation. Therefore, as in other models of IBD in the guinea-pig (Norris *et al.*, 1982; Boughton-Smith & Whittle, 1985) and the rabbit (Zipser *et al.*, 1987) and in acute colonic damage in the rat (Sharon & Stenson, 1985), increases in the formation of arachidonic acid cyclo-oxygenase and lipoxygenase products accompanied the colonic inflammation.

In the current studies on the actions of anti-inflammatory agents, the increased generation of both LTB_4 and 6-keto-$PGF_{1\alpha}$ by the inflamed colon was reduced to control levels by the dual pathway inhibitor BW755C. Aspirin and indomethacin almost completely inhibited the formation of 6-keto-$PGF_{1\alpha}$, PGE_2 and TXB_2, but did not affect LTB_4, confirming that the immunoreactive prostanoid formation by control and inflamed colon was derived from cyclo-oxygenase enzymes and LTB_4 from the lipoxygenase enzymes. The inhibitory action of these non-steroid anti-inflammatory drugs on immunoreactive 6-keto-$PGF_{1\alpha}$ formation was also demonstrated by the determination of prostacyclin formation using bioassay. Thus, the levels of prostacyclin and the degree of inhibition by anti-inflammatory drugs were very similar to those measured using radioimmunoassay for 6-keto-$PGF_{1\alpha}$.

Overall, these anti-inflammatory drugs had no consistent or significant effect on the chronic ulceration and inflammation induced by TNB as assessed macroscopically by the damage score. Therefore, although the anti-inflammatory drugs were present in the colonic tissue in sufficient concentrations to inhibit substantially the metabolism of arachidonic acid, these tissue levels were not adequate to reduce the colonic damage induced by TNB.

The effects of the anti-inflammatory drugs on colonic eicosanoid formation are similar to those found in an acute model of inflammation in rats, in which a polyester sponge soaked in carrageenin was implanted subcutaneously (Higgs *et al.*, 1980; Salmon *et al.*, 1983). Indomethacin and aspirin, at doses close to those used in the present study, inhibited the formation of prostanoids in the inflammatory exudate, but failed to reduce the synthesis of LTB_4. These drugs had only minimal effects on leukocyte infiltration. In contrast, BW755C, also at a similar dose to that used in the present study, inhibited the formation of both prostanoids and LTB_4 and also considerably reduced the infiltration of leukocytes (Higgs *et al.*, 1980; Salmon *et al.*, 1983). Whether in the present study, the reduction in LTB_4 generation by BW755C was due to an inhibition of lipoxygenase, a reduction in the infiltration of leukocytes, which are probably the primary source of these lipoxygenase products, or to both mechanisms, is not known. However, BW755C did not affect the chronic ulceration and inflammation induced by TNB at a time when eicosanoid formation was significantly inhibited. Therefore, these results suggest that the observed association between inflammation and eicosanoid formation does not reflect a primary role for these eicosanoids in inducing or maintaining gross tissue damage in this model of IBD.

Sulphasalazine and prednisolone both decreased the generation of 6-keto-$PGF_{1\alpha}$ by the rat colon, but these drugs had no effect on LTB_4 generation and did not reduce damage score. As in man, sulphasalazine is cleaved by colonic bacteria to sulphapyridine and 5-amino-salicylic acid (5-ASA) following oral administration to rats (Peppercorn & Goldman, 1972). Hence, the inhibition of 6-keto-$PGF_{1\alpha}$ formation by sulphasalazine could be through the action of the intact molecule or by 5-ASA, both of which have been demonstrated to inhibit prostanoid synthesis (Sharon *et al.*, 1978; Ligumsky *et al.*, 1981).

The lack of effect of sulphasalazine on LTB_4 generation in the TNB model is in contrast to the previously demonstrated inhibition of lipoxygenase in homogenates of both human colonic mucosa (Sharon & Stenson, 1984; Hawkey *et al.*, 1985) and colonic mucosa from a rat model of acute colitis (Sharon & Stenson, 1985). In these studies, however,

exogenous radiolabelled substrate was used and sulphasalazine was incubated with cell-free preparations of colonic tissue. In contrast, in the present study, the drugs were administered *in vivo* and the capacity of the colonic tissue to form eicosanoids from endogenous arachidonic acid measured *ex vivo* in the absence of further added drug. These differences could therefore be explained by drug availability and in this respect, the present *in vivo* study may more accurately reflect the clinical situation.

The anti-inflammatory steroid, prednisolone, which can prevent eicosanoid formation via inhibition of phospholipase A_2 activity following induction of the endogenous peptide, lipocortin, (Flower & Blackwell, 1979; Blackwell *et al.*, 1980), failed to reduce LTB_4 generation by the inflamed rat colon, although 6-keto-$PGF_{1\alpha}$ generation was inhibited. It is feasible that distinct cell-types produce these different eicosanoids, with the cells predominantly forming 6-keto-$PGF_{1\alpha}$ being more susceptible to the actions of corticosteroids than those which produce LTB_4.

In the current experiments, the gross macroscopic features of the colonic damage induced by TNB have been used to assess the inflammation. It is possible that although the anti-inflammatory drugs have no effect on the gross macroscopic damage, they may affect the underlying inflammatory cell infiltration and activity. The anti-inflammatory drugs were administered two weeks after TNB instillation, at a time when there was an existing ulceration and inflammation of the colon. This protocol was used to mimic the clinical situation where initial treatment is started during a period of active inflammation. However, it is possible that the metabolities of arachidonic acid may have a role in the early acute phase of this model of inflammatory bowel disease. Furthermore, any beneficial effects on the underlying inflammatory process may require more than one week to result in any clear improvement in the gross appearance of the colon. This possibility underlines the difficulties in assessing changes in models of chronic inflammation and the need to develop more quantitative markers of inflammatory cell activity.

In the present study using a novel experimental model of IBD in the rat, subsequent administration of anti-inflammatory agents did not reverse the course of the established colonic inflammation and tissue damage, yet substantially altered the profile of eicosanoids generated by the tissue. This could therefore suggest that eicosanoids, particularly LTB_4 have only a minor role in maintaining the chronic macroscopically observed damage induced by the TNB in the rat colon. However, the role of these eicosanoids in modulating the underlying activity of the inflammatory cells is not known. Such a model should therefore be useful to explore further the involvement of both cyclo-oxygenase and lipoxygenase products in the various stages of the colonic inflammatory process and their interactions with other pro-inflammatory mediators including the monokines and lymphokines.

References

BLACKWELL, G.J., CARNUCCIO, R., DI ROSA, M., FLOWER, R.J., PARENTE, L. & PERSICO, P. (1980). Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. *Nature*, **287**, 147–149.

BOUGHTON-SMITH, N.K., HAWKEY, C.J. & WHITTLE, B.J.R. (1983). Biosynthesis of lipoxygenase and cyclo-oxygenase products from [^{14}C]-arachidonic acid by human colonic mucosa. *Gut*, **24**, 1176–1182.

BOUGHTON-SMITH, N.K., WALLACE, J.L., MORRIS, G.P. & WHITTLE, B.J.R. (1985). Evaluation of the role of arachidonic acid metabolites in a rat model of Crohn's Disease. *Gastroenterology*, **88**, A1332.

BOUGHTON-SMITH, N.K. & WHITTLE, B.J.R. (1985). Increased metabolism of arachidonic acid in an immune model of colitis in guinea-pigs. *Br. J. Pharmacol.*, **86**, 439–446.

FLOWER, R.J. & BLACKWELL, G.J. (1979). Anti-inflammatory steroids induce the biosynthesis of a phospholipase inhibitor which prevents prostaglandin generation. *Nature*, **278**, 456–459.

GOULD, S.R., BRASH, A.R., CONNOLLY, M.E. & LENNARD-JONES, J.E. (1981). Studies of prostaglandins and sulphasalazine in ulcerative colitis. *Prostaglandins and Medicine*, **6**, 165–182.

HARRIS, D.W., SMITH, P.R. & SWAN, C.H.J. (1978). Determination of prostaglandin synthetase activity in rectal biopsy material and its significance in colonic disease. *Gut*, **19**, 875–877.

HAWKEY, C.J., BOUGHTON-SMITH, N.K. & WHITTLE, B.J.R. (1985). Modulation of human colonic arachidonic acid metabolism by sulfasalazine. *Dig. Dis. Sci.*, **30**, 1161–1165.

HIGGS, G.A., EAKINS, K.E., MUGRIDGE, K.G., MONCADA, S. & VANE, J.R. (1980). The effects of non-steroid anti-inflammatory drugs on leukocyte migration in carrageenin-induced inflammation. *Eur. J. Pharmacol.*, **66**, 81–86.

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

HIGGS, G.A., MONCADA, S. & VANE, J.R. (1984). Eicosanoids in inflammation. *Ann. Clin. Res.*, **16**, 287–299.

LIGUMSKY, M., KARMELI, F., SHARON, P., ZOR, U., COHEN, F. & RACHMILEWITZ, D. (1981). Enhanced thromboxane A_2 and prostacyclin production by cultured rectal mucosa in ulcerative colitis and its inhibition by steroids and sulfasalazine. *Gastroenterology*, **81**, 444–449.

MORRIS, G.P., REBEIRO, L., HERRIDGE, M.M., SZEWEZUK, M. & DEPEW, W. (1984). An animal model of chronic granulomatous inflammation of the stomach and colon. *Gastroenterology*, **86**, A1188.

NORRIS, A.A., LEWIS, A.J. & ZEITLIN, I.J. (1982). Changes in colonic tissue levels of inflammatory mediators in guinea-pig model of immune colitis. *Agents & Actions*, **12**, 243–246.

PEPPERCORN, M.A. & GOLDMAN, P. (1973). Distribution studies of salicylazosulfapyridine and its metabolites. *Gastroenterology*, **64**, 200–245.

SALMON, J.A. (1978). A radioimmunoassay for 6-keto-prostaglandin $F_{1\alpha}$. *Prostaglandins*, **15**, 383–397.

SALMON, J.A. & FLOWER, R. (1979). Prostaglandins and related compounds. In *Hormones in The Blood*, Vol. 2. ed. Gray, C.H. & James, V.H.T. Chapter IV. Academic Press.

SALMON, J.A., SIMMONS, P.M. & PALMER, P.M.J. (1982). A radioimmunoassay for leukotriene B_4. *Prostaglandins*, **24**, 225–235.

SALMON, J.A., SIMMONS, P.A. & MONCADA, S. (1983). The effects of BW755C and other anti-inflammatory drugs on eicosanoid concentrations and leukocyte accumulation in experimentally-induced acute inflammation. *J. Pharm. Pharmacol.*, **35**, 808–813.

SAMUELSSON, B. (1983). Leukotrienes mediators of immediate hypersensitivity reactions and inflammation. *Science*, **220**, 568–575.

SHARON, P., LIGUMSKY, M., RACHMILEWITZ, D. & ZOR, U. (1978). Role of prostaglandins in ulcerative colitis, enhanced production during active disease and inhibition by sulfasalazine. *Gastroenterology*, **75**, 638–640.

SHARON, P. & STENSON, W.F. (1984). Enhanced synthesis of leukotriene B_4 by colonic mucosa in inflammatory bowel disease. *Gastroenterology*, **86**, 453–460.

SHARON, P. & STENSON, W.F. (1985). Metabolism of arachidonic acid in acetic acid colitis in rats. Similarity to human inflammatory bowel disease. *Gastroenterology*, **88**, 55–63.

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

ZIPSER, R.D., NAST, C.C., LEE, M.H.W. & DUKE, R. (1987). *In vivo* production of Leukotriene B_4 and Leukotriene C_4 in rabbit colitis. Relationship to inflammation. *Gastroenterology*, **92**, 33–39.

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