Br. J. Pharmac. (1982), **76**, 157–161

ULCERATIVE COLITIS: EFFECT OF SULPHASALAZINE, ITS METABOLITES AND INDOMETHACIN ON THE ABILITY OF HUMAN COLONIC MUCOSA TO METABOLIZE PROSTAGLANDINS *in vitro*

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1 Homogenates of mucosa from human colon metabolize [^{3}H]-prostaglandin E_1 in the presence of nicotinamide adenine dinucleotide to 15-oxo prostaglandin E_1 or 15-oxo, 13,14 dihydro prostaglandin E_1.

2 Metabolic capacity of tissue from patients with active ulcerative colitis under treatment with sulphasalazine (0.021 ± 0.004 nmol/mg protein $\pm$ s.e.mean) did not differ from mucosa of normal patients (0.02 ± 0.004 nmol/mg protein) during 1 h incubation.

3 Sulphasalazine inhibits prostaglandin E_1 metabolism by mucosal homogenates in a dose-dependent manner with an ID_{50} of 125 μM. Its therapeutically active metabolite, 5-aminosalicylic acid (2.6 mM) was without significant inhibitory activity.

4 Indomethacin inhibits prostaglandin E_1 metabolism by colonic mucosa with an ID_{50} of 388 μM.

5 At present we cannot clearly relate the therapeutic benefit of sulphasalazine and its therapeutically active metabolite, 5-aminosalicylic acid, in ulcerative colitis to their effects on prostaglandin E synthesis or metabolism *in vitro*.

Introduction

Ulcerative colitis is characterized by acute attacks of diarrhoea, pain and rectal bleeding, interspersed with periods of remission. Sulphasalazine is of therapeutic benefit in treating the disease, particularly in reducing the relapse rate (Truelove, Watkinson & Draper, 1962; Misiewicz, Lennard-Jones, Connell, Baron & Avery-Jones, 1965).

The use of sulphasalazine is empirical, which is not surprising considering that the aetiology of the disease is largely unknown. However, it has recently been observed that colonic mucosa from colitic patients can synthesize larger amounts of prostaglandins *in vitro* than mucosa from normal patients. Thus, elevated prostaglandins have been implicated in the symptoms and perhaps the aetiology of the disease. Moreover, it has been suggested that sulphasalazine, probably acting via its metabolite, 5-aminosalicylic acid (5-ASA), is therapeutically effective by reducing the pathologically enhanced colitic mucosal prostaglandin synthetic capacity (Sharon, Ligumsky, Rachmilewitz & Zor, 1978; Gould, Brash, Conolly & Lennard-Jones, 1981).

Some of the known properties of the prostaglandins support this concept. High doses can cause accumulation of fluid in the bowel with resulting effects on diarrhoea (Robert, 1976); in some circumstances prostaglandins can exhibit proinflammatory actions (Weissmann, 1980) and sulphasalazine and 5-ASA, which benefit patients with ulcerative colitis, clearly, but relatively weakly, inhibit prostaglandin synthetase activity (Collier, Francis, McDonald-Gibson & Saeed, 1976; Sharon *et al.*, 1978). If prostaglandins are mediators of the inflammatory processes of ulcerative colitis, more powerful inhibitors of their synthesis would be of benefit. However, the converse has been demonstrated; flurbiprofen exacerbated the disease (Rampton & Sladen, 1980) and indomethacin did not produce clinical improvement (Gould *et al.*, 1981).

Prostaglandins and sulphasalazine possess additional properties which may be important. Prostaglandins have a cytoprotective effect and are able to prevent intestinal damage by a variety of noxious stimuli (Miller & Jacobson, 1979; Robert, 1980); moreover, prostaglandins can inhibit some aspects of the inflammatory process (Bonta & Parnham, 1978). Furthermore, Hoult & Moore (1980) have shown that sulphasalazine can inhibit prostaglandin metabolism in a variety of laboratory animal tissues, suggesting the alternative hypothesis that enhanced mucosal prostaglandins may not adversely affect the disease state, but may be beneficial.

This paper describes the results of a study on the prostaglandin metabolic capacity of normal and ulcerative colonic mucosa and the effect of sulphasalazine, 5-ASA and indomethacin on prostag-

0007-1188/82/050157–05 $01.00

landin metabolism by the human colonic mucosa.

A preliminary account of this work was presented to the British Pharmacological Society (Hillier, Mason & Smith, 1981).

Methods

To study the metabolism of prostaglandins by colonic mucosa, rectal mucosal biopsies were taken from 20 patients, 6 with ulcerative colitis. The patients without colitis were either suffering from irritable bowel disease or were normal and were receiving a variety of drugs, mainly antacids and chlordiazepoxide which are not known to affect prostaglandin synthesis or metabolism. The 6 colitic patients were in the active phase of the disease as indicated sigmoidoscopically and histologically (with inflammatory infiltrate, crypt abscesses and mucus depletion). Five were being treated with sulphasalazine and/or prednisolone; one was not receiving drugs.

In assessing the effect of sulphasalazine, 5-ASA and indomethacin on the metabolism of prostaglandins by the colonic mucosa, biopsy tissue, or samples from hemicolectomies for rectal carcinoma, were used. Five normal tissues and 7 with ulcerative colitis were studied. The biopsies from all patients were examined histologically. Tissue was stored at −70°C for less than one month before use. Biopsy specimens were homogenized at 4°C in 0.1 M phosphate buffer containing 20 mM disodium edetate (EDTA) and 20% glycerol; aliquots containing 0.3–0.8 mg protein (estimated by the method of Lowry, Rosebrough, Farr & Randall, 1951) were incubated with 1 mM nicotinamide adenine dinucleotide (NAD) and 0.0217 nmol (1 μCi) tritiated prostaglandin E_1 for 1 h at 37°C. The presence of NAD was mandatory; in 3 experiments without NAD, metabolism was less than 5% of that seen in the presence of NAD. Following acidification to pH 4 with 1 M citric acid and extracting twice with 3 times volume of ether, the evaporated organic phase was streaked on to silica gel thin layer chromatography (t.l.c.) plates in 100 μl of 20% methanol in chloroform and developed in the organic phase of the solvent system ethyl acetate, *iso*-octane, acetic acid, water (11:5:2:10) in equilibrated tanks; the plates were scanned radiochromatographically. The radioactivity in zones corresponding to authentic standards which were run in parallel were scraped and counted using standard scintillation counting techniques, and converted by appropriate quenching corrections to degradations per minute (d/min). Standard PGE_2; 15-oxo PGE_2; 15-oxo 13,14 dihydro PGE_2 were used and visualized by spraying with phosphomolybdic acid.

Different concentrations of drug or control vehicle (0.05 N HCl or 0.02 N NaOH) were added to homogenized aliquots of tissue which were then incubated for 1 h and treated as already described. The size of samples was variable and with some, relatively few points were obtained. Because the amount of tissue used differed between experiments the total [^{3}H]-PGE_1 metabolized varied in control tubes. However, the percentage metabolism by consecutively sampled amounts within an experiment was very reproducible. A continuous check was made on this by including (if tissue size permitted) control tubes at the beginning, middle and end of each experimental run; however, at least 2 controls were always run. In 6 experiments where 3 controls were used per experiment the mean intra-assay coefficient of variation of the amount metabolized in control tubes was 3.4%. In another experiment where 6 identical aliquots were incubated, the intra-assay coefficient of variation was 5.7%.

Materials

[5,6(*n*)-^{3}H]-prostaglandin E_1 (specific activity 50 Ci/mmol) was obtained from the Radiochemical Centre, Amersham and silica gel coated (0.2 mm) plastic t.l.c. plates (Schleicher & Schull) from Anderman Ltd, London.

Sulphasalazine was prepared as a 0.1–0.5% solution in 0.02 N NaOH kept at 4°C and renewed weekly. 5-Aminosalicylic acid (5-ASA) was stored as a dry powder at −20°C in the dark. A 2.5 mg/ml solution in 0.05 N HCl was prepared fresh before each experiment. Indomethacin (Sigma Ltd) was prepared as a 10 mg/ml solution in ethanol, stored at 4°C and renewed fortnightly; ethanol was evaporated before adding reagents and incubating.

Sulphasalazine and 5-ASA were gifts from Pharmacia Ltd and the authentic prostaglandin standards from Upjohn Ltd, U.S.A. All other chemicals were of Analar grade from Sigma Chemicals, London or BDH Ltd, Dorset.

Results

Incubation of radioactive prostaglandin E_1 with biopsy specimens resulted in a major metabolite with an R_F value of 0.4. This corresponded to authentic standards of 15-oxo-PGE_1 and 15-oxo-13,14-dihydro PGE_1 which were not separable in this system. Other minor unidentified peaks with R_F values of 0.08 and 0.055 were present in some experiments when 5-ASA was included. The amount of prostaglandin metabolized in mucosa from ulcerative colitic patients was 0.021 ± 0.004 nmol/mg protein $\pm$ s.e.mean ($n = 6$) and in mucosa from normal patients was 0.02 ± 0.004 nmol/mg protein $\pm$ s.e.mean ($n = 20$).

Sulphasalazine inhibited metabolism in a dose-dependent way in concentrations up to 250 μM (Figure 1). The percentage metabolism in control tubes was 60.7% ± 2.7 ($n = 45$). Higher concentrations (500 μM) did not further decrease the amount of prostaglandin metabolized (not shown). Fifty per cent inhibition of metabolism was observed with about 125 μM sulphasalazine. The percentage inhibition of PGE_1 metabolism by sulphasalazine in colonic mucosa from colitics compared with normal patients was assessed at different dose levels. At 50 μM the inhibition as a percentage of control was 56.7% ± 1.26 in normal patients and was significantly greater than in colitics, 66.1% ± 3.44 ($P < 0.05$). No significant difference was apparent at other dosage levels.

In concentrations up to 2.6 mM, 5-ASA had no consistent effect on the metabolism of added [^{3}H]-PGE_1. Control metabolism was 65.4% ± 4.9 ($n = 26$); at 350–400 μM metabolism (as a percentage of control) was 109 ± 4.1 ($n = 10$), at 650–700 μM it was 96.4 ± 3.3 ($n = 5$); at 1000–1500 μM it was 95.6 ± 5.3 ($n = 10$) and at 1650–2600 μM it was 98.5 ± 3.9 ($n = 16$).

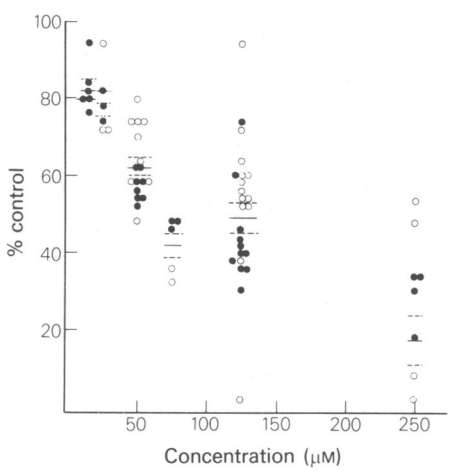

Figure 1 Effect of sulphasalazine on metabolism of [^{3}H]-prostaglandin E_1 ([^{3}H]-PGE_1) by homogenates of colonic mucosa from normal patients (●) and patients with ulcerative colitis (○). The radioactivity (in d/min) in the zone on the t.l.c. plate corresponding to the metabolite was divided by the total radioactivity in zones corresponding to the metabolite and parent (PGE_1) compound. The value at different concentrations of drug was expressed as a percentage of the metabolism in control tubes without drug addition. Data from 5 normal patients and 7 with ulcerative colitis are included in this study. Where duplicate determinations were carried out at one dose level these are shown separately. All tissues were assessed at 125 μM and 50 or 62.5 μM concentration. Means are shown by solid bars and dotted bars indicate the s.e.mean. The linear regression coefficient (r) is −0.69 ($P < 0.001$).

Indomethacin inhibited metabolism dose-dependently (Figure 2); control metabolism with added PGE_1 was 51% ± 6.3 ($n = 11$). Fifty per cent inhibition of metabolism was noted with approx. 388 μM.

In two experiments, sulphapyridine in doses of 0.625 and 1.25 mM did not affect the amount of prostaglandin metabolized.

Discussion

We have demonstrated *in vitro* that human rectal mucosa metabolizes prostaglandins, the main metabolite being 15-oxo-PGE_1 or 15-oxo-13,14-dihydro PGE_1 (which were not chromatographically separated in our analytical system). The former metabolite results from the action of 15-hydroxy prostaglandin dehydrogenase on the parent PGE and the latter by further reduction of 15-oxo-PGE_1 by PG Δ13-reductase. NAD is a cofactor for 15-hydroxy-prostaglandin dehydrogenase but not PG Δ13-reductase. It is likely, therefore, that the identified metabolite is 15-oxo-PGE_1. While this work was in progress, Smith, Dawson & Swan (1980) published a preliminary report in 4 patients showing that the supernatant from homogenates of colonic mucosa centrifuged at 100,000 *g* metabolized prostaglandins.

We found no difference in metabolic capacity be-

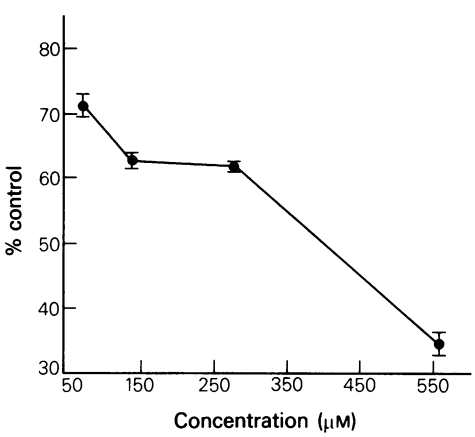

Figure 2 Effect of indomethacin on [^{3}H]-prostaglandin E_1 metabolism by homogenates of human colonic mucosa. Handling of data is as described in Figure 1. Values shown are mean; vertical lines indicate s.e.mean. Data are derived from 4 different tissues; at 70 μM $n = 6$ and at other concentrations $n = 5$. The linear regression coefficient (r) is −0.82 ($P < 0.001$). The ID_{50} derived from the linear regression curve is 388 μM.

tween biopsies from control patients and samples from patients with active disease. However, apart from one patient, the latter group was receiving sulphasalazine treatment which we have shown inhibits prostaglandin metabolism *in vitro*; as yet, we have insufficient data from untreated patients with active ulcerative colitis to make a comparison. In contrast, the mucosa from patients with ulcerative colitis synthesizes greater amounts of prostaglandins *in vitro* and *in vivo* than mucosa from normal patients (Gould, Brash & Connolly, 1977, Harris, Smith & Swan, 1978; Sharon *et al.*, 1978; Smith, Dawson & Swan, 1979), and clinical remission in patients treated with sulphasalazine, codeine and prednisolone was associated with a return to normal of the prostaglandin synthetic capacity of the mucosa (Smith *et al.*, 1979). Using rectal dialysis bags, Rampton, Sladen & Youlten (1980) similarly showed enhanced prostaglandin synthesis in patients with active disease.

The above studies show that prostaglandin synthetase activity is elevated in active ulcerative colitis but do not confirm the increase as a cause or result of the inflammatory process. Notwithstanding this dilemma, it is important to try to clarify whether prostaglandins exacerbate the disease state or are increasingly produced as a protective measure. If high concentrations of prostaglandins are detrimental, then inhibitors of their synthesis would be beneficial; yet the prostaglandin synthesis inhibitors, sodium salicylate (delivered as an enema) and flurbiprofen, were not useful; indeed, the latter resulted in a worsening of the disease (Campieri, Lanfranchi, Bazzocchi, Brignola, Corazza, Cortini, Michelini & Labo, 1978; Rampton & Sladen, 1980). It appears that inhibition of prostaglandin E_2 and $F_{2\alpha}$ synthesis is not an important property of drugs useful in the treatment of ulcerative colitis, particularly as sulphasalazine and 5-ASA only weakly inhibit the synthetase enzymes (Collier *et al.*, 1976; Sharon *et al.*, 1978).

Is the inhibition of prostaglandin metabolism in colonic mucosa important in ulcerative colitis? We were unable to show a difference between patients with active disease and controls in ability of mucosal tissue to metabolize prostaglandins; however, our group of patients with ulcerative colitis were in the main being treated with sulphasalazine. Sulphasalazine (125 μM) inhibited prostaglandin E metabolism in colonic mucosa *in vitro* by 50% whereas 250 μM was required to inhibit synthesis by 65% (Ligumsky, Karmeli, Sharon, Zor, Cohen & Rachmilewitz, 1981). If an increase in net prostaglandin concentrations occurs as a result of sulphasalazine treatment this may have a beneficial cytoprotective action (Robert, 1976; 1980) possibly by ameliorating the disturbed electrolyte transport noted in ulcerative colitis (Hawker, McKay & Turnberg, 1980; Schiessel, Mathews, Barzilai, Merhav & Silen, 1980).

However, 5-ASA which has also been shown to be effective in treating ulcerative colitis (Azad Kahn, Piris & Truelove, 1977) did not affect [^{3}H]-PGE_1 metabolism *in vitro* in doses up to 2.6 mM. Recently, Hoult & Page (1981) showed that 5-ASA enhanced 6-keto $PGF_{1\alpha}$ synthesis in human colonic mucosal biopsies when a dose of 500 μM was used but synthesis was inhibited by 5000 μM. This contrasts with the results of Ligumsky *et al.* (1981) and Sharon *et al.* (1978) who showed that 5-ASA inhibited prostaglandin synthesis including 6-keto $PGF_{1\alpha}$ by human biopsy specimens with doses of 326–1307 μM. The lowest dose inhibited 6-keto $PGF_{1\alpha}$ and PGE_2 synthesis by approximately 50%.

Schlenker & Peskar (1981) have suggested that the effect that sulphasalazine has on human colonic prostaglandin synthesis *in vitro* depends upon the substrate arachidonic acid concentration present. At low substrate concentrations, PGE_2 synthesis was inhibited whereas at high substrate concentrations, it was enhanced.

In the colonic mucosa there appears to be no clear correlation between the ability of drugs to inhibit tissue prostaglandin synthesis and metabolism and their therapeutic usefulness.

We thank Pharmacia (UK) Ltd for financial support and Upjohn Co. Ltd for generous gifts of prostaglandins. S.P. is supported by the British Council.

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(Received October 20, 1981.
Revised January 12, 1982.)