

Inhibition of prostaglandin synthetase in human rectal mucosa

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SUMMARY Miniaturised methods have been used to construct dose-reponse curves for the effects of inhibitory drugs on prostaglandin synthesis using individual rectal biopsies obtained from patients with ulcerative colitis. The potency of different drugs has been compared. Sulphasalazine, 5 amino salicylic acid (5-ASA) and N-acetyl 5-ASA inhibited prostaglandin synthesis at high concentration, but sulphapyridine and prednisolone did not. Indomethacin and flurbiprofen were considerably more potent inhibitors. These data imply that sulphasalazine does not act by simple inhibition of prostaglandin synthesis but leave open the possibility that sulphasalazine or 5-ASA may be inhibitors of the synthesis of related lipoxygenase products.

Although sulphasalazine is widely used in the management of ulcerative colitis its mode of action remains unclear. Increased prostaglandin synthesis occurs during active ulcerative colitis,¹⁻⁵ and it has been suggested that sulphasalazine or one of its cleavage products – 5 amino salicylic acid (5-ASA) or sulphapyridine – may exert anti-inflammatory activity by inhibiting prostaglandin synthesis.²

In organ culture, 5-ASA inhibited synthesis of PGE₂ to a greater extent than sulphasalazine and sulphapyridine,² but this result could have arisen through a non-specific effect on cell viability. In another report, a specific ability to inhibit the prostaglandin synthetase (cyclooxygenase) of human rectal mucosa was shown for sulphasalazine but its cleavage products were not investigated.³

In the present study we compared the potency of sulphasalazine and its cleavage products with indomethacin and flurbiprofen as inhibitors of prostaglandin synthetase using individual rectal biopsies obtained from untreated patients with quiescent ulcerative colitis.

Methods

PREPARATION OF INHIBITORY DRUGS

Prednisolone phosphate (Organon Laboratories), sulphapyridine (Sigma Chemical Co), and N-acetyl

5-ASA (Pharmacia) were dissolved directly in Tris HCl. Dilutions of indomethacin (Merck, Sharp and Dohme) were made from a 2 mg/ml stock solution prepared in sodium carbonate. Flurbiprofen (Boots Chemical Co) and sulphasalazine (Pharmacia) were dissolved in Tris HCl at pH 8.5 to make stock solutions.

For 5-ASA a modified procedure was used. Solutions of 5-ASA (5.95×10^{-2} M (1 g/100 ml)) prepared under argon for enema administration (Ferring Pharmaceuticals) were used as the source of active drug. Placebo enemas with identical additives (sodium chloride 3.45×10^{-2} M (200 mg/100 ml) disodium orthophosphate 3.09×10^{-2} M (500 mg/100 ml) potassium metabisulphite 4.50×10^{-3} M (100 mg/100 ml) sodium EDTA 5×10^{-3} M (20 mg/100 ml)) were used for homogenising tissue and making drug dilutions. Before use, the pH of each solution was manipulated using Tris and HCl solutions to achieve a final Tris HCl concentration of 0.15 M and a pH of 7.4.

INCUBATION CONDITIONS

Single rectal biopsies (weighing 17–45 mg) were excised from patients with inactive ulcerative colitis who were receiving no treatment. They were frozen in liquid nitrogen and stored at -70°C until used. The frozen biopsy was then added to 1.0 ml of ice cold Tris HCl, pH 7.4, 0.15 M with sodium EDTA 5×10^{-3} M in a Dual ground glass homogeniser and homogenised using a standardised action.

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Microscopy showed the suspension to consist largely of broken cells, with fibrous tissue from the submucosa unhomogenised.

The volume of the homogenate was adjusted to contain 5–20 mg (wet weight) of tissue per ml. 200 μ l aliquots were mixed with serial dilutions of inhibitory drugs in Tris HCl and preincubated on ice for 20 minutes. PGE₂ synthesis during homogenisation was measured in aliquots extracted immediately after homogenisation and without incubation.

The reaction was started by the addition of 50 μ l arachidonic acid (final concentration 6.1×10^{-5} M (20 μ g/ml)) in Tris HCl containing cofactors (adrenaline 3.0×10^{-3} M (1 mg/ml) and reduced glutathione 1.3×10^{-3} M (400 μ g/ml) final concentrations). The reaction tubes were incubated at 37°C for 30 minutes in a rocking water bath. The reaction was stopped by the addition of 1 volume of ice cold ethanol containing indomethacin 10^{-3} M (0.35 μ g/ml, final concentration) and (H₃)PGE₂ (c. 500 cpm, 0.23 mCi/tube, specific activity 160 Ci/mmol, Radiochemical Centre, Amersham) for estimation of recovery. The concentration of the drug under investigation was adjusted in each tube to the maximum concentration used during incubation. Each reaction mixture was then extracted into chloroform and resuspended for assay in phosphate buffered saline. PGE₂ was measured by radioimmunoassay as described elsewhere.⁶

CONSTRUCTION OF DOSE RESPONSE CURVES

Three of four individual dose response curves using rectal mucosa from different patients and single incubation per drug concentration were constructed for indomethacin, flurbiprofen, N-acetyl 5-ASA, prednisolone, and sulphapyridine. For sulphasalazine, 5-ASA, and sulphapyridine, individual dose response curves were obtained using mucosa from two to four different patients with incubations in duplicate or triplicate. With each drug the results from individual patients were used to construct overall mean values; here each point therefore represents the mean of up to nine observations.

Results

CHARACTERISTICS OF THE REACTION

The coefficient of variation for duplicate observations was 16.9% (n=48). There was a modest effect on pH with maximum synthesis around physiological pH. PGE₂ synthesis by boiled biopsies was usually less than 10% of that by unboiled biopsies. Both adrenaline and reduced glutathione stimulated PGE₂ synthesis; with both

cofactors there was an additive effect with stimulation to $243 \pm 48\%$ of that seen without cofactors (n=4).

EFFECT OF INHIBITORS

The Table shows individual data for all drugs studied. No inhibition could be seen for prednisolone ($101.2 \pm 9.8\%$ control values at 10^{-2} M (3.6 mg/ml), n=3) or sulphapyridine (mean $115.0 \pm 15.2\%$ of control values at 10^{-2} M (2.5 mg/ml), n=3, tested at highest concentration only). High concentrations of 5-ASA inhibited synthesis of PGE₂ (mean IC₅₀ 2.29×10^{-2} M (3.9 mg/ml)). As shown in Fig. 1 its potency was similar in all four individuals investigated. The potency of N-acetyl 5-ASA and sulphasalazine was similar to that of 5-ASA but indomethacin and flurbiprofen were about five orders of magnitude more potent. The overall mean values for all the subjects have been used to compare these drugs graphically in Fig. 2.

Discussion

These data show that sulphasalazine and 5-ASA but not sulphapyridine can inhibit the prostaglandin synthetase activity of human rectal mucosa. The

Table PGE₂ synthesis by human rectal mucosa: effect of drugs in individual patients

	IC 50 (molar)	Maximum inhibition	
		% Inhibition*	Concentration
Indomethacin	7.0×10^{-8}	78.0	10^{-5} M
	8.9×10^{-8}	95.6	
	2.0×10^{-7}	96.4	
Flurbiprofen	8.2×10^{-7}	94.6	10^{-4} M
	2.9×10^{-7}	86.3	
	3.0×10^{-7}	96.2	
	6.1×10^{-7}	86.5	
Sulphasalazine	6.5×10^{-7}	79.9	10^{-3} M
	$c 3.0 \times 10^{-2}$	48.4	
	$>3.0 \times 10^{-2}$	40.0	
5-ASA	1.7×10^{-2}	81.1	3×10^{-2} M
	2.4×10^{-2}	63.0	
	2.6×10^{-2}	55.7	
	2.6×10^{-2}	55.7	
N-acetyl 5-ASA	1.4×10^{-3}	67.2	3×10^{-3} M
	3.0×10^{-3}	58.0	
	$>3.0 \times 10^{-3}$	31.1	
Sulphapyridine	—	8.4	3×10^{-2} M
	—	3.4	
	—	-21.8	
Prednisolone	—	15.0	10^{-2} M
	—	0.3	
	—	-19.0	

Individual IC 50 values are shown in the first column. The maximum inhibition achieved and inhibitor concentration used are shown in the last two columns.

* Negative values indicate enhanced synthesis.

Fig. 1 Dose response curves for the effect of 5-ASA on the rectal mucosa of four different individuals. In each case the mean values of two or three duplicate observations (with SEM) are shown.

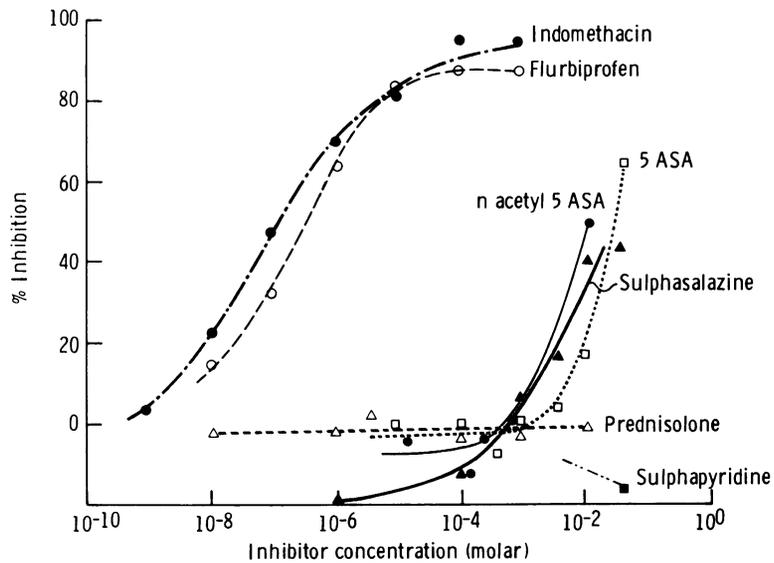
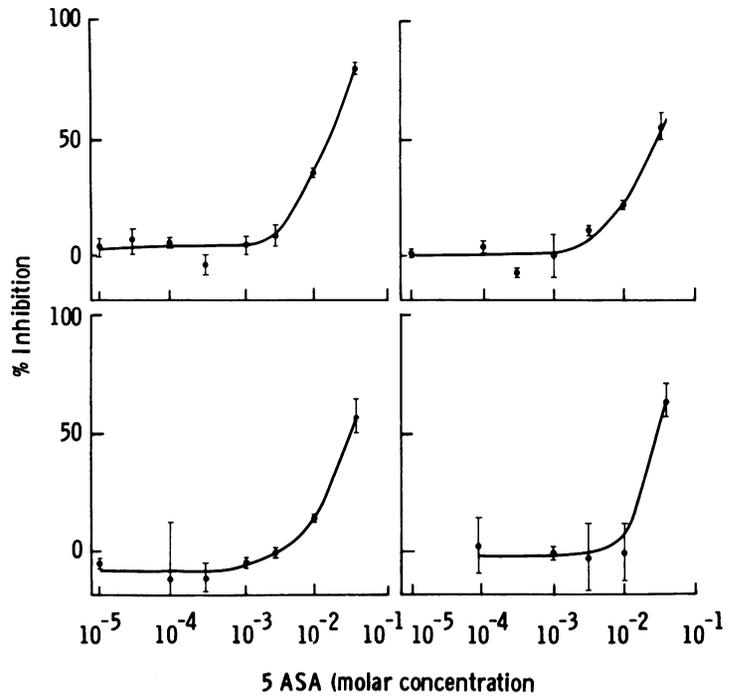


Fig. 2 Overall mean dose response curves for the drugs studied. In each case results from two, three or four individuals (shown in Table) have been averaged for each point shown.

results parallel the clinical observation that sulphasalazine and 5 amino salicylic acid when administered by enema to patients with active disease are therapeutically potent, while sulphapyridine is not.⁷ Prednisolone, which has anti-inflammatory properties in ulcerative colitis, exerts no pharmacological inhibition of prostaglandin synthetase but has previously been shown to reduce basal prostaglandin synthesis in intact human rectal mucosa maintained in organ culture.⁵ This may be attributed to its ability in living cells to induce synthesis of a polypeptide, macrocortin, which inhibits the release of free arachidonic acid.⁶ In addition, one of us has proposed that it may also induce synthesis of a local cyclooxygenase inhibitor.⁶ Neither of these properties would, however, apply with homogenates *in vitro*.

The concentrations of 5-ASA and sulphasalazine required to inhibit prostaglandin synthesis are high and the data are open to the possible criticism that this may not reflect true competitive inhibition. The concentrations of 5-ASA required for inhibition, however, are similar to those measured in the faeces of patients taking sulphasalazine.⁸ N-acetyl 5-ASA is a derivative of 5-ASA which is therapeutically active when administered topically;⁹ its potency as an inhibitor of prostaglandin synthesis is similar to that of 5-ASA.

There are at present two main views as to the role of prostaglandins in ulcerative colitis. The present data support the interpretation that prostaglandin synthesis contributes to an excessive inflammatory reaction and that inhibition of this synthesis is therefore desirable. The data also show, however, that indomethacin and flurbiprofen are more potent than 5-ASA as inhibitors of prostaglandin synthesis by human rectal mucosa but they do not appear to be effective agents in the treatment of ulcerative colitis.^{10 11} A detailed analysis of treatment of active ulcerative colitis by flurbiprofen showed that this drug had a number of deleterious effects.¹²

Such results have led to the alternative view that prostaglandins may be 'cyto-protective' in ulcerative colitis. In support of this proposal, it has been shown that sulphasalazine (but not 5-ASA) can inhibit prostaglandin metabolism in rabbit colon,¹² and that 5-ASA can lead to enhanced prostacyclin (PGI₂) synthesis by human rectal mucosa.¹³ These data show that, in some circumstances, sulphasalazine and 5-ASA might lead to enhanced rather than reduced levels of mucosal prostaglandins. There is at present, however, very little direct evidence that this would lead to a 'cyto-protective' effect in the colon such as occurs with prostaglandins in the stomach. Furthermore, where 5-ASA was shown to

enhance prostacyclin synthesis, inhibition was observed at the higher concentrations which were similar to those found in the faeces, and to those used in the experiments reported here.¹³

A third theory can be proposed as to the mode of action of sulphasalazine in ulcerative colitis. Weak inhibitors of prostaglandin synthetase have been shown to inhibit lipoxygenase pathways which may lead to the production of non-prostaglandin hydroxy acid products of arachidonic acid metabolism;¹⁴ this is associated with an ability to reduce the accumulation of white cells at sites of inflammation.¹⁵ By contrast, potent prostaglandin synthetase inhibitors have little effect on white cell accumulation and can divert arachidonic acid metabolism along lipoxygenase pathways¹⁶ which have been shown to exist in human colonic mucosa.¹⁷ The possibility that sulphasalazine or one of its cleavage products may inhibit lipoxygenase activity is therefore currently under investigation.

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