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SELECTIVE ACTIONS OF ASPIRIN- AND SULPHASALAZINE-LIKE DRUGS AGAINST PROSTAGLANDIN SYNTHESIS AND BREAKDOWN

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Abstract—Groups of ten aspirin- and five sulphasalazine-like drugs were tested as inhibitors of prostaglandin synthesis (rabbit renal medulla 14,000 *g* supernatants) and prostaglandin breakdown (rabbit colon 100,000 *g* supernatants). The aspirin-like drugs exhibited selectivity against synthesis but all also inhibited breakdown at higher doses. The sulphasalazine-like drugs (including diphloretin phosphate and carbenoxolone) exhibited selectivity against breakdown and some, but not all, inhibited synthesis at higher doses. It is proposed that there are accordingly two pharmacologically distinct groups of drugs, and that they can be characterised by the ratios of ID_{50}s against breakdown and synthesis (M/S ratio).

Much evidence shows that non-steroidal anti-inflammatory drugs of the aspirin type (NSAIDs) directly inhibit the activity of membrane bound cyclooxygenase, thus reducing the formation of prostaglandins (PGs) and related eicosanoids such as prostacyclin and thromboxane A_2 [1–7]. There is a good correlation between the clinical effectiveness of different NSAIDs as anti-inflammatory agents and their relative potencies as cyclooxygenase inhibitors [2, 5, 8].

It has also been occasionally noted [5, 9–11] that at higher doses certain NSAIDs inhibit the enzymatic breakdown of prostaglandins, presumably by an action on 15-hydroxyprostaglandin dehydrogenase (PGDH), as this is the first enzyme in the catabolic pathway. However, there are no detailed comparisons of the activities of a series of NSAIDs on both PG synthesis and breakdown.

We have shown recently that sulphasalazine and its methylene homologue homosulphasalazine are potent inhibitors of PG degradation both in cytosolic supernatants and in intact cell systems, but are much weaker inhibitors of PG synthesis [12, 13]. It was proposed on the basis of this and other evidence that this action against breakdown may explain the prophylactic benefit of sulphasalazine for preventing relapse of ulcerative colitis [14, 15]. Carbenoxolone — a drug useful for the treatment of gastric and duodenal ulcers — also appears to be a potent inhibitor of PGDH action, at least in human gastric mucosa preparations [16]. Taken together, these facts suggest that drugs which are selective inhibitors of PG breakdown rather than of synthesis form a separate pharmacological group with correspondingly different therapeutic properties.

This paper describes a comparison of the inhibitory potencies *in vitro* of several different aspirin and sulphasalazine-like drugs against PG synthesis and breakdown. It is suggested that the two 'families' of drugs represent distinct pharmacological types.

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MATERIALS AND METHODS

Drugs. Indomethacin, aspirin (acetylsalicylic acid), arachidonic acid, NAD^+ and reduced glutathione were purchased from Sigma (London) Ltd, Poole, U.K. The following drugs were kind gifts from the listed pharmaceutical companies: flurbiprofen and ibuprofen (Boots Company Ltd, Nottingham, U.K.), benorylate (Winthrop Laboratories, Surbiton, U.K.), mefanamic acid and flufenamic acid (Parke, Davis and Company, Pontypool, U.K.), naproxen (Syntex Laboratories Inc., Palo Alto, CA), ketoprofen and carbenoxolone disodium (M & B 36153A) (May & Baker Ltd., Dagenham, U.K.), phenylbutazone (Geigy Pharmaceuticals, Macclesfield, U.K.), diphloretin phosphate (Leo AB, Helsingborg, Sweden), sulphasalazine, homosulphasalazine and dihomosulphasalazine (Pharmacia AB, Uppsala, Sweden).

Inhibition of prostaglandin synthesis by rabbit kidney medulla supernatants. Kidney medulla from male New Zealand White rabbits (2–4 kg) was separated from cortex, homogenised in 4 vol. 50 mM phosphate buffer pH 7.5 (containing 1 mM EDTA and cysteine) and centrifuged at 14,000 *g* for 1 min. The resulting supernatants contained large amounts of prostaglandin-like activity (2.55 ± 0.22 μg $PGF_{2\alpha}$ equivalents g wet wt tissue, $n = 22$), but were also capable of efficiently converting added arachidonic acid to prostaglandins.

Samples containing 180 μl homogenate, 2 μl arachidonic acid (final concentration 10 μg/ml), reduced glutathione (final concentration 3 mM, to favour conversion of arachidonate to classical PGs) and 20 μl drug solution (or equivalent volume of the vehicle) were incubated for 60 min at 37° and extracted twice with 0.8 ml ethyl acetate after adding 0.2 ml absolute alcohol and 0.2 ml 1 M formic acid.

After removing solvent and resuspending the dried extracts in 0.2 ml Krebs solution, the resulting prostaglandins were assayed in terms of $PGF_{2\alpha}$ on the isolated rat fundus strip preparation [18].

The effects of added drugs were expressed as percentage inhibition of PG formation after correction for intrinsic PG content. Each drug was tested 4 times at four doses (after estimating its likely potency in preliminary tests) and the dose required for 50% inhibition of synthesis (ID_{50}) estimated from plots of $\log_{10}$ dose versus per cent inhibition.

Inhibition of prostaglandin breakdown in rabbit colon supernatants. Rabbit colon contains large amounts of PGDH [17]. 100,000 *g* supernatants were prepared in the same phosphate pH 7.5 buffer and the breakdown of 10 μg/ml labelled $PGF_{2\alpha}$ (containing 0.05–0.1 μCi [^{3}H-9β]$PGF_{2\alpha}$, Radiochemical Centre, Amersham, specific activity 19.4 Ci/mmole) was determined after extraction by radiochromatography [18]. The tubes also contained 5 mM NAD^+ and varying concentrations of inhibitory drug or appropriate volumes of vehicle and were incubated for 60 min at 37°. Inactivation of $PGF_{2\alpha}$ in various colon preparations was 78.2 ± 4.6% (n = 12) under these conditions. The experimental design and calculation of results were as described above for synthesis inhibition.

RESULTS

The molar ID_{50} values for the 15 drugs tested are given in Table 1.

All the aspirin-like drugs inhibited PG synthesis by renal medulla homogenates, although as expected the potencies varied widely, with nearly five orders of magnitude separating the most potent (flurbiprofen) from the weakest inhibitor (aspirin).

Diphloretin phosphate and sulphasalazine were very weak inhibitors of microsomal PG synthesis but homosulphasalazine, dihomosulphasalazine and carbenoxolone did not inhibit synthesis in the range 50–2000 μM. Diphloretin phosphate and carbenoxolone are not structurally related to sulphasalazine, but like sulphasalazine they effectively inhibit PG inactivation by PGDH.

The aspirin-like drugs all inhibited PG breakdown in the rabbit colon supernatant, presumably by a direct inhibitory action on PGDH. These effects occur at higher doses than those needed to inhibit synthesis. Moreover, there was not such a large spread of potencies (rather more than two orders of magnitude separating most from least potent), although the ID_{50} values of several very weakly active compounds could not be measured accurately.

The sulphasalazine-like drugs were better inhibitors of PG breakdown than the aspirin-like drugs and these effects occurred at much lower doses than needed for synthesis inhibition by the same drugs.

We propose using the M/S ratio to distinguish the selectivity of these two types of drugs on a quantitative basis. It is obtained simply by dividing the molar ID_{50} against breakdown (*Metabolism*) by the ID_{50} against *Synthesis*. Values greater than unity denote preferential action against synthesis; values less than unity show selectivity against breakdown.

DISCUSSION

This paper gives comparative data for fifteen compounds which are selective inhibitors either of microsomal PG synthesis (aspirin-like drugs) or cytosolic PG breakdown (sulphasalazine-like drugs). The M/S ratio shows the degree of selectivity.

It was interesting that all the aspirin-like drugs inhibited PG breakdown (although phenylbutazone, naproxen and aspirin were very weak), and that the range of potencies was much narrower than against synthesis. These findings confirm earlier scattered reports (e.g. Refs 5, 9–11) of this action of certain drugs. However, it is unlikely to underly any of the therapeutic effects of NSAIDs because PG depletion

Table 1. Selectivity of inhibitors of prostaglandin synthesis and breakdown

Drug	ID_{50} (molar) Synthesis	ID_{50} (molar) Breakdown	M/S ratio*	Relative inhibitory potency Synthesis†	Relative inhibitory potency Breakdown‡
Aspirin-like					
Flurbiprofen	7.5×10^{-9}	8.9×10^{-5}	11867	34.7	0.56
Ibuprofen	1.1×10^{-8}	2.6×10^{-4}	23636	23.6	0.19
Flufenamic acid	2.9×10^{-8}	1.1×10^{-4}	3793	8.9	0.45
Mefanamic acid	4.6×10^{-8}	1.5×10^{-4}	3261	5.6	0.33
Ketoprofen	5.2×10^{-8}	8.4×10^{-5}	1615	5.0	0.59
Indomethacin	2.6×10^{-7}	2.4×10^{-4}	923	1.0	0.21
Naproxen	2.7×10^{-7}	8.2×10^{-4}	3037	0.96	0.06
Phenylbutazone	9.5×10^{-6}	3.9×10^{-4}	41	0.027	0.13
Benorylate	2.1×10^{-5}	2.5×10^{-4}	12	0.012	0.20
Aspirin	3.8×10^{-4}	1.4×10^{-2}	37	0.0007	0.004
Sulphasalazine-like					
Sulphasalazine	6.0×10^{-4}	5.0×10^{-5}	0.083	0.0004	1.0
Homosulphasalazine§	$\geqslant 2 \times 10^{-3}$	6.7×10^{-6}	$\leqslant$0.0034		7.5
Dihomosulphasalazine§	$\geqslant 2 \times 10^{-3}$	4.0×10^{-5}	$\leqslant$0.02		1.3
Carbenoxolone	$\geqslant 2 \times 10^{-3}$	2.0×10^{-4}	$\leqslant$0.1		0.25
Diphloretin phosphate	7.0×10^{-4}	2.0×10^{-5}	0.029	0.0004	2.5

* Calculated as ID_{50} breakdown divided by ID_{50} synthesis; values > 1 show selective anti-synthesis action.

† Indomethacin = 1.

‡ Sulphasalazine = 1.

§ Homo- and dihomosulphasalazine contain the substituents -CH_2COOH or -CH_2CH_2COOH respectively at the 1-COOH substituent in the aminosalicylic acid moiety (see Ref. 13).

would presumably occur before concentrations sufficient to affect breakdown could be obtained.

We attribute the inhibitory effects seen in our *in vitro* tests to inhibition of the cyclooxygenase component of prostaglandin synthetase (anti-synthesis effect) or 15-hydroxyprostaglandin dehydrogenase (PGDH, anti-breakdown effect). That all these drugs should affect both enzymes is perhaps not surprising as one would expect some features of the active sites or substrate binding regions of both enzymes to be similar. However, studies of the structure–activity relationships of the aspirin-like drugs have not so far offered much insight into the stereochemical features or structure of cyclooxygenase. In any case, such studies would be best carried out using purified enzyme preparations rather than the crude systems used here and by many other authors; nevertheless, the effects observed in the crude systems may well be closer to those in the whole animal.

It was recently confirmed* that the prevention of PG breakdown observed in this study is due to a direct inhibitory effect of the sulphasalazine-like drugs on PGDH. Thus experiments using semi-purified bovine lung or human placental PGDH (both type I, NAD^+-dependent) showed evidence for powerful inhibition by sulphasalazine and its homo- and dihomo-analogues, with inhibitory potency greatest with the homo-compound as observed here (Table 1).

The identification of a group of drugs which are selective against prostaglandin breakdown is of pharmacological interest, especially as those members of the group which are established therapeutic agents have very different uses to the aspirin-like drugs. Thus carbenoxolone and sulphasalazine do not have well-defined anti-inflammatory actions but both find places in prophylactic therapy against ulcerative diseases of the gastrointestinal tract [19, 20]. By contrast, a common problem with chronic (or even acute) therapy with aspirin-like drugs is their tendency to cause ulceration and damage to the gastrointestinal mucosa [21–23]. It has been suggested [14, 15] (but remains to be proved) that the gastric protective effects of sulphasalazine and carbenoxolone might be due to preservation or potentiation of the cytoprotective effects of prostaglandins [24, 25] consequent to reduced degradation.

We therefore suggest that these drugs which interact with enzymes of the prostaglandin system are members of two distinct pharmacological 'families' and propose that the term 'sulphasalazine-like' be used to designate those which selectively inhibit PGDH and the breakdown of prostaglandins.

It should be noted that several other familiar drugs with established potent actions on various other enzyme or transport systems (such as certain xanthines, thyroid hormones and the diuretics furosemide, mersalyl acid and ethacrynic acid) also have been shown to inhibit PGDH in cytosolic supernatants or semi-purified enzyme preparations (reviewed in Refs 9, 26, 27). Since these effects on PGDH generally occur at high doses they probably have little to do with the diverse pharmacological effects of these substances *in vivo*. Moreover, these drugs do not have established anti-ulcer effects. Accordingly, they can be excluded from the category 'sulphasalazine-like'.

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