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Inhibition of folate-dependent enzymes by non-steroidal anti-inflammatory drugs

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Many non-steroidal anti-inflammatory drugs (NSAIDs) (including sulphasalazine, sulindac, indomethacin, naproxen, salicylic acid, ibuprofen, piroxicam and mefenamic acid) were found to be competitive inhibitors (with respect to folate) of avian liver phosphoribosylaminoimidazolecarboxamide formyltransferase (AICAR transformylase, EC 2.1.2.3) and bovine liver dihydrofolate reductase (EC 1.5.1.3). In contrast, aspirin and the antipyretic–analgesic drugs acetaminophen and antipyrine were weak inhibitors of these enzymes. Structure–activity correlation suggests that an aromatic ring with a side chain containing a carboxylic acid is a requirement for competitive inhibition of the transformylase. The above-listed NSAIDs also inhibited the folate-coenzyme-mediated biosynthesis of serine from glycine and formate (i.e., the C_1 index) by human blood mononuclear cells (BMCs) in experiments where the drug was added to a culture of BMCs. Acetaminophen had a weak inhibitory effect on the C_1 index. Consistent with the results obtained *in vitro* is the observation that the C_1 index of BMCs from rheumatoid-arthritis patients treated with drugs which possess little antifolate activity (e.g. acetaminophen) is higher than the C_1 index of BMCs from rheumatoid-arthritis patients treated with NSAIDs possessing more potent antifolate activity (e.g. sulindac, sulphasalazine, naproxen and ibuprofen). The mean activity of the transformylase in BMCs taken from healthy humans was 1.98 nmol of product/h per 10^6 cells and the activity was positively correlated with BMC folate levels. These results are consistent with the hypothesis that (1) the antifolate activity of NSAIDs, and hence cytostatic consequences, are important factors in producing anti-inflammatory activity and (2) aspirin exerts its anti-inflammatory effects after its conversion into salicylic acid, which possesses greater antifolate activity than its parent compound.

INTRODUCTION

It is generally accepted that non-steroidal anti-inflammatory drugs (NSAIDs) are inhibitors of prostaglandin biosynthesis and that this property alone is sufficient to explain many of their clinical effects. Yet, discrepancies exist between the extent of inhibition of prostaglandin biosynthesis and the anti-inflammatory activity of certain NSAIDs and NSAID-like drugs. For example, relatively small concentrations of aspirin are required to inhibit prostaglandin biosynthesis *in vitro* and, consequently, small doses produce antipyretic and analgesic effects. In contrast, only large doses of aspirin have anti-inflammatory activity (Smith, 1975; Abramson *et al.*, 1985; Abramson & Weissmann, 1989). Aspirin is irreversibly and rapidly converted into salicylic acid (Rainsford, 1985) which, although not an inhibitor of prostaglandin biosynthesis *in vitro*, is clinically equivalent to aspirin as an anti-inflammatory agent (Rainsford, 1985; Paulus, 1989). On the other hand, antipyrine, a good inhibitor of prostaglandin biosynthesis *in vitro*, has little anti-inflammatory activity (Brune *et al.*, 1976). Finally, some agents useful in the treatment of inflammatory disorders, including sulphasalazine and azathioprine, are not known to be direct inhibitors of prostaglandin biosynthesis (Flower *et al.*, 1972; Rainsford, 1985). It seemed evident that inhibition of prostaglandin biosynthesis *per se* does not satisfactorily explain the activity of NSAIDs. Furthermore, it seemed unlikely that all NSAIDs would have this as their main single property (Flower, 1974; Vane, 1978).

During a study of the folate-requiring enzyme phosphoribosylaminoimidazolecarboxamide formyltransferase (AICAR transformylase; EC 2.1.2.3), naproxen and ibuprofen were found to be inhibitors (Ha *et al.*, 1990). This finding suggested that anti-inflammatory and antifolate activities might be related. Therefore, the ability of other NSAIDs and NSAID-like drugs to inhibit AICAR transformylase, dihydrofolate reductase (5,6,7,8-tetrahydrofolate:$NADP^+$ oxidoreductase, EC 1.5.1.3) and the folate-coenzyme-mediated biosynthesis of serine by human blood mononuclear cells (BMCs) was investigated.

MATERIALS AND METHODS

All NSAIDs and other drugs, NADPH and AICAR, were purchased from Sigma Chemical Co. and were used without further purification. AICAR transformylase (from chicken liver) and 10-formyltetrahydrofolate (10-CHO-H_4folate) were prepared, quantified and assayed as described previously (Baggott *et al.*, 1986). The AICAR concentration was 1 mM and the 10-CHO-H_4folate concentration was varied from 12 to 200 μM in the assays. AICAR transformylase was assayed in human blood mononuclear cells (BMCs) with 100 μM concentrations of AICAR and 10-CHO-H_4folate as described previously (Ha *et al.*, 1990). The activity of the transformylase in BMCs is expressed as the amount of the folate-degradation product, *p*-aminobenzoylglutamate (pABG) produced/h per 10^6 cells.

Dihydrofolate (H_2folate) was prepared by the method of Futterman (1963). Dihydrofolate reductase from bovine liver was purchased from Sigma Chemical Co. and was used without further purification. Dihydrofolate reductase was assayed with H_2folate as the substrate by the method of Scheufler (1981), with the following modifications: the pH of the assay was 7.4; [NADPH] was 100 μM; H_2folate concentration was varied from 1.3 to 40 μM; the reaction was monitored continuously.

Folic acid was purchased from Sigma Chemical Co., purified by butanol extraction (three times) of an aqueous solution (pH 6.5). Dihydrofolate reductase was assayed with folic acid as

Abbreviations used: NSAID, non-steroidal anti-inflammatory drug; AICAR transformylase, phosphoribosylaminoimidazolecarboxamide formyltransferase (EC 2.1.2.3); BMCs, blood mononuclear cells; 10-CHO-H_4folate, 10-formyltetrahydrofolate; *p*ABG, *p*-aminobenzoylglutamate; H_2folate, dihydrofolate; RBC, red blood cell; RA, rheumatoid arthritis.

Table 1. Competitive inhibition of folate-dependent enzymes by drugs used in diseases of inflammation, daily drug doses and peak plasma and tissue concentrations

Drug	AICAR transformylase* (avian liver)		Dihydrofolate reductase (bovine liver)				Daily dose to treat inflammation (g)††	Concentration (mM)	
			Folic acid substrate†		H_2folate substrate‡				
	[I]/[S]§	K_i (mean ± S.E.M.) (mM)	[I]/[S]	K_i (mean ± S.E.M.) (mM)	[I]/[S]	K_i (mean ± S.E.M.) (mM)		Peak plasma††	Tissue
Sulphasalazine	3	0.022 ± 0.002		(0.23)‖			3–4	0.05	
Methotrexate	15	0.11 ± 0.01		S.I.¶		S.I.	≈ 0.002	0.0006‡‡	0.0002‡‡
Sulindac	15	0.13 ± 0.03	240	0.47 ± 0.12			0.3–0.4	0.02	0.06–0.28§§
Indomethacin	21	0.35 ± 0.08	180	0.22 ± 0.05			0.15–0.2	0.02	0.06‖‖
Naproxen	210	0.99 ± 0.13	540	3.1 ± 0.7	1100	2.6 ± 0.8	0.5–1	0.4	
Salicylic acid	110	1.1 ± 0.3	2200	35 ± 10	4600	9.3 ± 2.6	5–8	2.0	4–67¶¶
Ibuprofen	140	1.5 ± 0.1	600	2.5 ± 0.4	2200	1.4 ± 0.3	1–3	0.4	0.9***
Mefenamic acid	180	3.8 ± 1.2	90	1.1 ± 0.4	100	0.072 ± 0.010	1–2	0.08	1.3†††
Aspirin	700	11 ± 1	1000	Inactive	2500	Inactive	5–8	2.0	12‡‡‡
Piroxicam	60	Inactive**	60	0.45 ± 0.14			0.02	0.02	
Acetaminophen	430	Inactive	1200	8.2 ± 2.0	6900	4.9 ± 0.60	1–4	0.5	
Sulphapyridine	80	Inactive							
Phenylbutazone	60	Inactive	180	Inactive	220	Inactive	0.6	0.5	
Antipyrine	140	Inactive			520	Inactive			
Penicillamine	760	Inactive			3100	Inactive	1.5	0.2	
Chloroquine	12	Inactive							
Hydroxychloroquine					460	Inactive	0.4	0.001	

* K_m of 10-CHO-H_4folate was 118 μM.
† K_m of folic acid was 4.6 μM.
‡ K_m of H_2folate was 0.53 μM.
§ [I]/[S] = the maximum drug-to-substrate (i.e. 10-CHO-H_4folate, folic acid or H_2folate) concentration ratio tested.
‖ From Selhub *et al.* (1978).
¶ S.I., stoichiometric inhibition.
** 'Inactive' means that less than 10% inhibition was found at highest [I]/[S] concentration ratios.
†† From Physicians Desk Reference (1990) and Flower *et al.* (1985).
‡‡ In serum and RBCs of RA patients treated with low dose (Kremer *et al.*, 1986); polyglutamates of methotrexate are better inhibitors of the transformylase when compared with methotrexate itself (Baggott *et al.*, 1986).
§§ Estimated from the results obtained by Dugan (1981), using reported tissue/plasma concentration ratios of 3–10.
‖‖ Estimated from the results obtained by Weissenborn *et al.* (1985), using a reported tissue/plasma concentration ratio of 3.
¶¶ Palmoski & Branot (1985); Raghoebar *et al.* (1988).
*** Simchowitz *et al.* (1979).
††† Estimated from the results obtained by Kemmelmeir & Bracht (1989), using a reported tissue/plasma concentration ratio of 16.
‡‡‡ Raghoebar *et al.* (1988).

the substrate by using the following method. Each assay tube contained 0.01 unit of enzyme and (final concentrations) 0.05 M-potassium phosphate, pH 7.4, 200 μM-NADPH and 13–130 μM-folic acid in a total volume of 0.5 ml. The reaction was initiated by the addition of the enzyme and allowed to proceed for 4 h at 37 °C. The reaction was stopped by the addition of 0.6 ml of 5 M-H_2SO_4, followed by 1.1 ml of 0.3 M-NaCl with mixing. Under these conditions, any H_4folate formed is quantitatively degraded with the formation of pABG, which was measured by using a modified Bratton–Marshall reaction. The tube was placed in a water/ice bath, and 0.2 ml of 0.1% $NaNO_2$ was added with mixing. After 5 min, 0.2 ml of 0.5% ammonium sulphamate was added with mixing, followed by 0.2 ml of 0.1% *N'*-napthylethylene diamine. The tubes were removed from the water/ice bath and the colour was allowed to develop overnight. Absorbance at 560 nm was read against a blank containing all components except NADPH.

NSAIDs and other drugs were dissolved in 0.005 M-potassium phosphate buffer and the pH was adjusted to 7.4. Except for sulphasalazine, none of the drugs developed colour with the modified Bratton–Marshall assay method and none altered the colour yield of an assay tube containing a 400 μM concentration of pABG.

Initial velocities and corresponding substrate concentrations were estimated by the method of Waley (1981) in the non-continuous spectrophotometric assay. All kinetic parameters were estimated by the method of Cleland (1979), and at least two drug concentrations were used to estimate K_i values.

The assay that measures the BMC incorporation of [^{14}C]-formate into serine in the presence of excess glycine (i.e. the C_1 index) was performed as described previously (Morgan *et al.*, 1987). Whole blood was obtained from rheumatoid-arthritis (RA) patients and healthy volunteers. The RA patients have been described previously (Morgan *et al.*, 1990). In experiments using BMCs from healthy volunteers, NSAIDs were added (without diluting or changing the pH of the medium) to the culture medium at the beginning of the assay. Red-blood-cell (RBC) and BMC folate levels were measured as described previously (Morgan *et al.*, 1987).

RESULTS AND DISCUSSION

Inhibition of AICAR transformylase and dihydrofolate reductase by NSAIDs is shown in Table 1. High drug-to-substrate concentration ratios (i.e. [I]/[S] ratios) were attempted because the daily dose of many of these agents is large (i.e. gram

quantities) compared with the usual daily folate intake (i.e. microgram quantities). The [I]/[S] ratios were limited by drug solubility or its high absorbance at 340 nm or 312 nm.

Inhibition of AICAR transformylase and dihydrofolate reductase

Most NSAIDs tested were inhibitors of AICAR transformylase. The following observations suggest that this property may be related to anti-inflammatory activity.

(1) Sulphasalazine, a compound which possesses substantial anti-inflammatory activity, was found to be a potent inhibitor of AICAR transformylase. On the other hand, its sulpha-drug metabolite, sulphapyridine, was found to be inactive. Thus the inhibition of the transformylase by sulphasalazine is not simply a general property shared by sulpha drugs, but is found in this particular compound which possesses anti-inflammatory properties. Selhub *et al.* (1978) have also found that sulphasalazine, but not sulphapyridine, is an inhibitor of other folate-requiring enzymes.

(2) Salicylic acid is a better inhibitor of the transformylase than is aspirin. This may account for the fact that equal doses of salicylic acid and aspirin have similar anti-inflammatory effects, since, *in vivo*, aspirin is irreversibly hydrolysed to salicylic acid.

(3) Penicillamine and chloroquine, which are not primarily anti-inflammatory agents, were inactive as inhibitors of the transformylase. It must be noted, however, that chloroquine could only be tested at relatively low [I]/[S] ratios and that weak inhibition could have been missed.

(4) Both antipyrine and acetaminophen have analgesic and antipyretic activities, but little anti-inflammatory activity. Both of these drugs were inactive as inhibitors of the transformylase.

(5) Inhibition was always competitive with respect to 10-CHO-H_4folate, suggesting that it was not due to non-specific binding or denaturation.

Dihydrofolate reductase was assayed with both H_2folate and folic acid as substrates (Table 1). Most of the NSAIDs tested were inhibitors of the reductase. This property may be related to the anti-inflammatory activity as suggested by the following observations:

(*a*) Penicillamine and hydroxychloroquine were also inactive as dihydrofolate reductase inhibitors, even at very high [I]/[S] ratios. Antipyrine was inactive, whereas acetaminophen proved to be a relatively weak inhibitor. This again suggests that drugs with weak or no antifolate activity have little anti-inflammatory effect, and that the antipyretic and analgesic effects are not related to antifolate activity.

(*b*) Aspirin was inactive as an inhibitor of the reductase, in contrast with salicylic acid. This suggests, again, that aspirin, when considered as an anti-inflammatory agent, is a pro-drug and that salicylic acid is the active compound.

(*c*) The inhibition was always competitive (with respect to H_2folate or folic acid), suggesting that this was not a non-specific effect.

Comparisons of K_i values with peak plasma concentrations

Table 1 shows that the K_i values of sulphasalazine and salicylic acid for AICAR transformylase are lower than the peak plasma concentrations achieved with the usual daily dosages of these drugs. With the exception of piroxicam, other drugs investigated had at least one K_i value within an order of magnitude of its peak plasma concentration or its intracellular concentration (Table 1). Therefore inhibition of one or both of these enzymes could occur *in vivo* under routine levels of drug administration.

Effect of NSAIDs *in vitro* on the C_1 index

The incorporation of [^{14}C]formate into serine in the presence of excess glycine requires catalytic amounts of folate coenzymes; all three enzyme activities on the trifunctional folate-metabolizing protein [i.e. 10-CHO-H_4folate synthetase (EC 6.3.4.3), methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9) and methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5)] and the enzyme serine hydroxymethyltransferase (EC 2.1.2.1). The amount of ^{14}C incorporated/1×10^6 peripheral BMCs is reported as the C_1 index and is shown in Table 2. The effect on the C_1 index of NSAIDs was not due to a general cytotoxic activity, since cell viability at the end of the 3 h assay was 70–100 % and comparable with that of the controls. Both acetaminophen and phenylbutazone, which were weak inhibitors of the transformylase and reductase, were also weak inhibitors of the C_1 index. Aspirin was a relatively strong inhibitor of the C_1 index, which is somewhat inconsistent with its weak inhibition of the transformylase and dihydrofolate (Table 1). Intracellular conversion of aspirin into salicylic acid or acylation of the enzymes could account for these results. Also, the C_1 index requires only catalytic amounts of folate coenzymes to be present, in contrast with stoichiometric amounts used in the other enzyme assays, thus [I]/[S] ratios may be very high.

Rowe *et al.* (1985) have demonstrated that the β-carbon of

Table 2. Inhibition of the C_1 index by NSAIDs

The C_1 index measures the incorporation of [^{14}C]formate into serine by cultured human BMCs. Assay conditions are described in the Materials and methods section.

Drug	Concn. tested (mM)	C_1 index (% of control value)
Salicylic acid	2.0	7
Mefenamic acid	0.1	11
Sulindac	0.1	11
Ibuprofen	1.0	12
Aspirin	2.0	15
Indomethacin	0.1	22
Piroxicam	0.1	25
Sulphasalazine	0.5	46
Acetaminophen	2.0	72
Phenylbutazone	0.5	100

Table 3. C_1 indices of RA patients receiving NSAIDs and of healthy controls

The C_1 index measures the incorporation of [^{14}C]formate into serine by cultured human BMCs. Assay conditions are described in the Materials and methods section.

Current NSAID use	No. of patients	C_1 index (average) (d.p.m./10^6 cells)
Acetaminophen	2	2984
Piroxicam	2	1769
Aspirin and ibuprofen	1	1283
Aspirin	8	1111
Aspirin and piroxicam	3	874
Naproxen	5*	687
Ibuprofen	2	580
Aspirin and naproxen	4*	498
Aspirin and sulindac	2*	457
Aspirin and sulphasalazine	1	116
Healthy controls not using prescription drugs	21	905

* One patient in the group had RBC folate below 150 ng/ml.

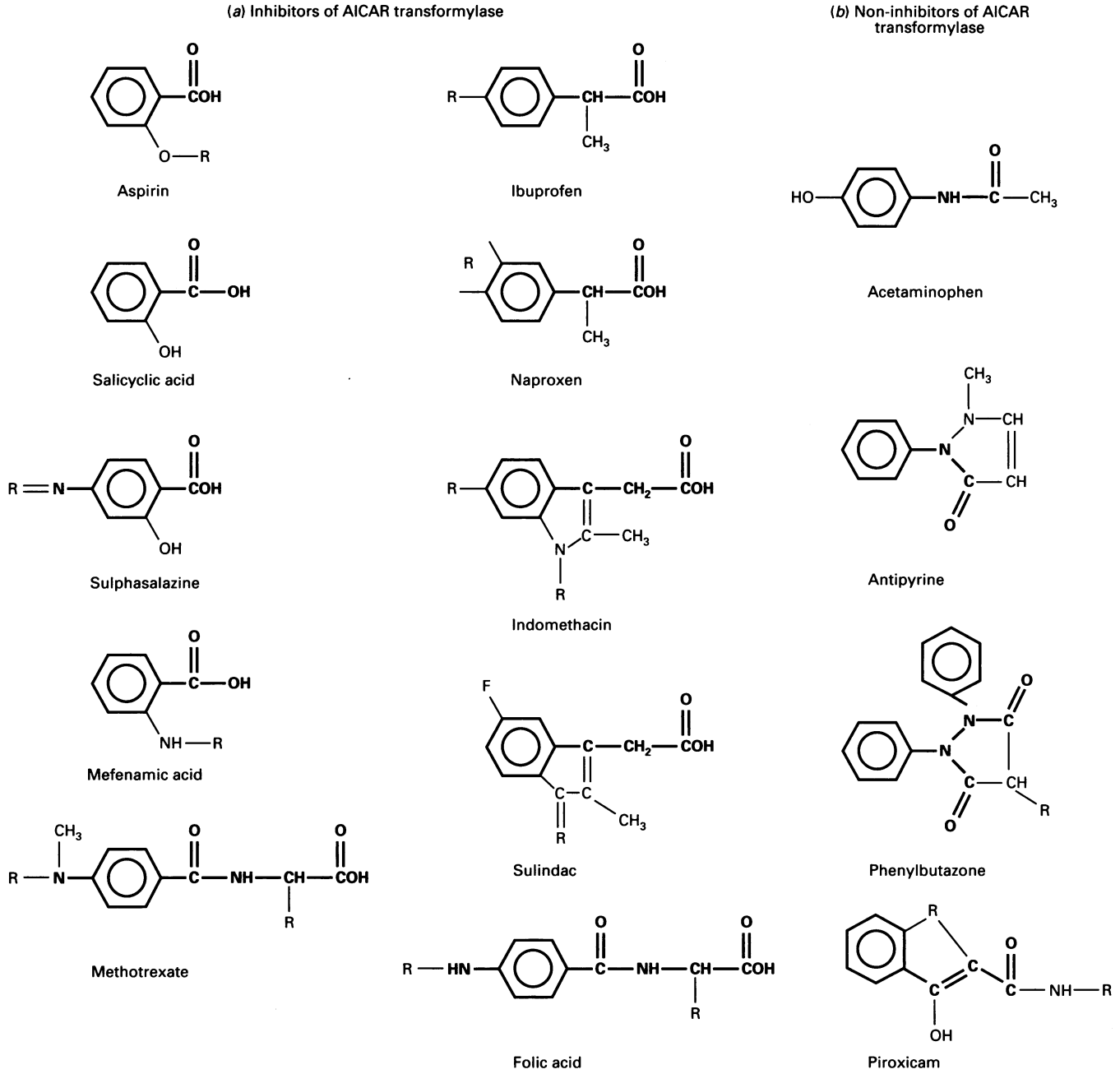

Fig. 1. Chemical structure of some drugs used in diseases of inflammation: aromatic and carbonyl moieties of some agents which are (*a*) and are not (*b*) inhibitors of AICAR transformylase

The aromatic group and the side chain containing the carboxylic acid or other carbonyl moiety are drawn with heavy lines.

serine is the major source of one-carbon fragments required for the biosynthesis of purine nucleotides and thymidylate by mitogen-activated lymphocytes. This leaves the folate-mediated reactions of the C_1-index pathway as the main, and perhaps the only, metabolic route to the synthesis *de novo* of one-carbon fragments. Inhibition of these enzymes may, therefore, blunt lymphocyte mitogen activation. Panush (1976) has, in fact, demonstrated that a number of NSAIDs, including aspirin, indomethacin and naproxen, are inhibitors of mitogen and antigen-mediated lymphocyte activation *in vitro*, whereas acetaminophen and penicillamine were found to be comparatively weaker inhibitors. These results could be explained by the relatively potent antifolate effect of naproxen and indomethacin, in contrast with the weak effect of acetaminophen and penicillamine.

NSAIDs were tested at, or above, peak plasma concentration in the '*in vitro*' C_1 index assay. Under these conditions, mechanisms other than direct inhibition of the C_1-index enzymes may have influenced the results. For example, NSAIDs may have interfered with the cellular transport of glycine or [^{14}C]formate. Thus the data is only suggestive of a direct effect of NSAIDs on the C_1 index and it is necessary to test their effect *in vivo*.

Effect of NSAIDs *in vivo* on the C_1 index

The C_1 index was measured in patients with RA receiving a variety of NSAIDs, as shown in Table 3. There is a consistent

trend to lower C_1 indices in patients using the more potent anti-inflammatory drugs (e.g. sulphasalazine, sulindac and naproxen) versus those with weak anti-inflammatory activity (.e.g acetaminophen), with aspirin use (as a single therapy) falling somewhere in between. Folate-replete healthy controls (not using prescription drugs) also had intermediate C_1 indices. The possible confounding effects of drug transport and metabolism are eliminated, since all patients had been receiving the drug(s) for extended periods before the assay. The relatively low C_1 indices in patients taking the NSAIDs with more potent anti-inflammatory activity was not simply due to a systemic depletion of folate (i.e. low RBC folate). The rank order in Table 3 is unchanged if patients with RBC folates less than 150 ng/ml are removed from the data analysis. The finding of low RBC folate in 10% (i.e. 3 of 30) of the RA patients is not, however, unexpected, since Alter *et al.* (1971), Gough *et al.* (1964) and Omer & Mowat (1968) have shown low folate levels among RA patients treated with NSAIDs.

The inhibition of the C_1 index by NSAID therapy is consistent with the finding of Hine *et al.* (1990) that BMCs from both methotrexate-treated RA patients and NSAID-treated RA patients have a blunted proliferative response to phytohaemagglutinin when compared with a population not using prescription drugs. The blunted response was more pronounced when the test was conducted in a medium containing physiological levels of folic acid as opposed to a medium with very high folate levels. This finding is mechanistically consistent with high intracellular folate coenzyme concentrations relieving, in part, the competitive inhibition produced by the NSAIDs.

AICAR transformylase activity in BMCs

The activity of the transformylase was assessed in isolated human BMCs from healthy volunteers. The mean activity was found to be 1.98 ± 0.68 μM nmol of pABG/h per 10^6 cells (range 1.23–4.14). This cellular activity is greater than the rate of biosynthesis of purine nucleotides in resting BMCs (0.03 nmol/h per 10^6 cells) or in mitogen-stimulated BMCs (0.59 nmol/h per 10^6 cells), suggesting that the transformylase is not rate-limiting in the biosynthetic pathway (McCairns *et al.*, 1983). However, the assay contains high concentrations of both 10-CHO-H_4folate and AICAR, a situation unlikely to occur *in vivo*. For example, the K_m for AICAR is approx. 15 μM for both the chicken liver and mouse BMC transformylase (Ha *et al.*, 1990), which is below the concentration in the assay (i.e. 100 μM). McCairns *et al.* (1983) failed to detect any intermediate of the pathway in human BMCs actively synthesizing purine nucleotides, and Bokkerink *et al.* (1986) detected only 1–5 μM intracellular levels of AICAR in cultures of acute-lymphoblastic-leukaemia cells. In addition, human BMC folate-coenzyme concentrations may be as low as 25 pg/10^6 cells or approx. 0.05 μM (Morgan *et al.*, 1987), which is well below the concentration used in the assay (i.e. 100 μM). Thus relatively low concentrations *in vivo* of both AICAR and the folate coenzyme may make the transformylase rate-limiting in purine-nucleotide biosynthesis, especially in activated BMCs.

AICAR transformylase in human BMC activity was positively correlated [Spearman's ρ (rank correlation coefficient) = 0.66; $n = 20$; $P = 0.002$] with BMC folate levels (range 92–3500 pg/10^6 cells). There was essentially no correlation of the BMC transformylase activity with the C_1 index ($\rho = 0.22$; $P = 0.3$) or with RBC folate ($\rho = 0.18$; $P = 0.4$), which argues that its correlation with BMC folate levels does not merely reflect a general-activity folate-dependent enzyme in these cells or the general level of folate nutriture in the body. The transformylase from BMCs may be stabilized, or protected from catabolism, by relatively high intracellular folate levels.

Structure–activity correlation

There is only a weak correlation between the inhibitory potency against AICAR transformylase when compared with dihydrofolate reductase. For example, piroxicam is inactive as an inhibitor of the transformylase, but it is a fairly potent inhibitor of the reductase.

All inhibitors of AICAR transformylase listed in Table 1 contain a carboxylic acid group and an aromatic ring. The carboxylic acid group may be proximal (e.g. salicylic acid) or distal (e.g. sulindac) from the aromatic ring (Fig. 1). The data suggest that both a carboxylic acid moiety and an aromatic ring are necessary for competitive inhibition of AICAR transformylase, since penicillamine has a carboxylic acid moiety but not aromatic ring; piroxicam, acetaminophen, phenylbutazone, antipyrene and chloroquine all have aromatic rings, but no carboxylic acid moiety, and none of the above agents is an effective inhibitor. A nitrogen in the *para* position to the carboxylic acid group (i.e., sulphasalazine and methotrexate) produced relatively tight binding, possibly better mimicking the structure of the *p*-aminobenzoylglutamate moiety of folate coenzymes (Fig. 1).

In contrast with those of AICAR transformylase, inhibitors of dihydrofolate reductase may have no carboxylic acid group (e.g. piroxicam). This finding is not unexpected. For example, both pyrimethamine and trimethoprim are good inhibitors of the reductase and neither have carboxylic acid moieties.

Conclusion

The results presented are consistent with the hypothesis that the anti-inflammatory properties of NSAIDs results in part from interference with folate-coenzyme metabolism. Interference of folate metabolism will have a cytotoxic or cytostatic effect and may explain why general cytotoxic agents (e.g. cytoxan) are useful in the treatment of diseases of inflammation. This finding may also explain why a variety of non-prostaglandin-mediated cytostatic and anti-proliferative effects are observed when cells are exposed to NSAIDs (Panush, 1976; Hine *et al.*, 1990; Weissman, 1991). Antifolate properties are not observed in drugs which have only antipyretic or analgesic activity. NSAID antifolate activity, anti-prostaglandin activity and other properties (Weissman, 1991) may work together to produce the entire complement of anti-inflammatory effects.

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