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AZO REDUCTION OF SULPHASALAZINE IN HEALTHY VOLUNTEERS

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1 A comparison of total sulphapyridine saliva concentrations resulting from administration of sulphapyridine and sulphasalazine at equimolar doses has been carried out. It is concluded that the extent of azo reduction of sulphasalazine to release sulphapyridine is complete in healthy volunteers.

2 An ampicillin course of treatment alters the azo reduction of sulphasalazine. The extent of cleavage is reduced by one third on average. There is no change in the rate of absorption of the released sulphapyridine.

3 An ampicillin course of treatment does not alter the disposition of sulphapyridine. The changes noted in acetyl conjugate plasma concentrations are entirely consistent with reduced availability of sulphapyridine.

Introduction

Sulphasalazine (Salazopyrin) has proved a useful drug in the long term management of ulcerative colitis (Dissanyake & Truelove, 1976; Azad Khan *et al.*, 1980) and Crohn's disease (Summers *et al.*, 1979). Only a small fraction of orally administered sulphasalazine is absorbed (Schroder & Campbell, 1972). The majority of the ingested dose travels down the small intestine to the colon where it undergoes bacterial azo reduction to liberate sulphapyridine and 5-aminosalicyclic acid (Schroder & Campbell, 1972; Peppercorn & Goldman, 1972). The majority of sulphapyridine is absorbed from the large intestine and it is subject to extensive metabolism prior to urinary excretion; *N*-acetylsulphapyridine being the other major circulating metabolite derived from sulphasalazine (Das & Dubin, 1976). In contrast only a small percentage of aminosalicyclic acid is absorbed, the faecal recovery of this sulphasalazine metabolite is extensive (Das & Dubin, 1976).

Current evidence suggests that 5-aminosalicylic acid is the active moiety of sulphasalazine (Azad Kahn *et al*, 1977; Van Hees *et al.*, 1980; Kotz *et al.*, 1980). Sulphasalazine may only be a vehicle to achieve high concentrations of aminosalicylic acid in the large bowel where it can act locally on the inflamed mucosa. It is therefore paradoxical that sulphapyridine plasma concentrations have proved useful in dosage adjustment for sulphasalazine in patients with inflammatory bowel disease (Das & Dubin, 1976). It would appear that sulphonamide plasma monitoring gives an indirect measure of the extent of azo reduction and therefore the availability of aminosalicylic acid from sulphasalazine.

The purpose of the present investigation was first to obtain a precise estimate of the extent of azo reduction of sulphasalazine by comparing the pharmacokinetics of sulphapyridine after ingestion of both sulphasalazine and sulphapyridine *per se*. Secondly, the influence of a commonly prescribed antibiotic, ampicillin, on the extent of azo reduction of sulphasalazine to sulphapyridine was determined.

Methods

Healthy male volunteers participated in the investigations. No history of sulphonamide sensitivity or gastrointestinal disease was apparent and each subject gave his informed consent. No other drugs were ingested within a 7 day period prior to the investigations. On the days of drug administration, only a liquid breakfast was allowed and solid food taken 4 h after drug ingestion.

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To determine extent of azo reduction

Five subjects provided mixed saliva samples at regular intervals following ingestion of either 2 g sulphasalazine (length of study 72 h) or 1.25 g sulphapyridine (length of study 48 h).

Samples were subjected to the assay procedure of Hansson & Sandberg (1973). This procedure gave total salivary sulphonamide concentrations, that is the sum of sulphapyridine and its acetyl conjugate.

To determine the influence of ampicillin on azo reduction

Five subjects provided blood samples over a 72 h time period following sulphasalazine (2 g) ingestion on two occasions. Six days after completion of the control study each subject started an ampicillin course (250 mg Penbritin four times daily) which lasted for 5 days prior to the second sulphasalazine ingestion and continued through the 3 days of the study.

Plasma samples were assayed for both sulphapyridine and acetyl-sulphapyridine by the Hansson & Sandberg (1973) methods.

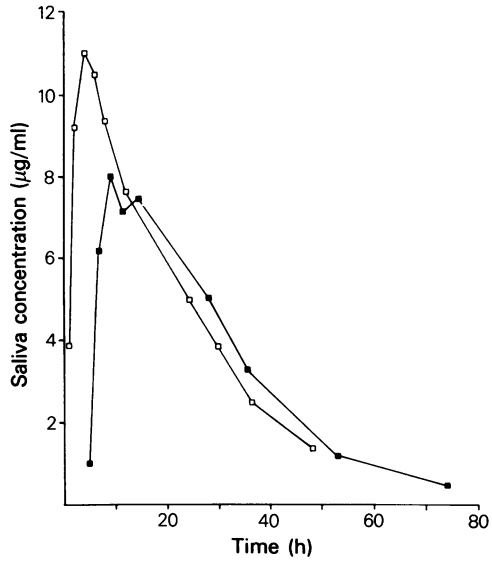

Figure 1 Saliva concentration-time profiles for total sulphapyridine following administration of equimolar doses of either sulphapyridine (□) or sulphasalazine (■) in a typical subject.

Results

In order to determine the extent of azo reduction of sulphasalazine, five subjects were administered sulphapyridine and on a separate occasion its precursor sulphasalazine. Saliva samples were analysed for total sulphapyridine (sulphapyridine plus acetyl-sulphapyridine) following ingestion of the above equimolar doses and a saliva concentration-time profile in a typical subject is shown in Figure 1. Administration of sulphasalazine rather than sulphapyridine *per se* results in a significant reduction in the maximum saliva concentration and a significant increase in the time taken to attain this concentration (Table 1). In contrast, neither the area under saliva concentration-time curve between onset of absorption and time infinity nor the terminal half-life are altered.

Table 2 summarises the effect of ampicillin on the absorption and disposition of sulphapyridine and its acetyl conjugate following ingestion of sulphasalazine. The changes observed are consistent with a decrease in the extent of absorption following antibiotic treatment. No effect is observed in the rate of absorption or in the disposition of sulphapyridine.

As illustrated in Figure 2, ampicillin treatment reduced the AUC for sulphapyridine. The times for the onset of absorption and the attainment of maximum plasma concentration, and the absorption

Table 1 Disposition of sulphapyridine based on saliva concentration-time profiles obtained following oral administration of equimolar doses of sulphapyridine and sulphasalazine.

Parameter	*After sulphapyridine*†	*After sulphasalazine*†
Area under curve ($\mu g\ ml^{-1}\ h$)	258 ± 108	244 ± 105
Maximum saliva concentration ($\mu g\ ml^{-1}$)	12.1 ± 1.1	6.9 ± 0.9*
Time of maximum concentration (h)	4 ± 1	12 ± 4*
Terminal half-life (h)	14.0 ± 4.8	15.5 ± 5.6

† Mean of 5 subjects ± s.d.

* Significantly different by paired t-test, $P < 0.02$.

Table 2 Absorption and disposition of sulphapyridine based on plasma sulphapyridine and acetylsulphapyridine concentration-time profils obtained following sulphasalazine administration under control conditions and after pretreatment with ampicillin.

Parameter	*Control conditions*†	*After ampicillin*†
Area under curve (μg ml^{-1} h)	370 ± 181	239 ± 136*
Onset of absorption (h)	4 ± 2	4 ± 1
Time of maximum concentration (h)	12 ± 4	13 ± 2
Absorption half-life (h)	2.3 ± 0.9	2.5 ± 0.9
Terminal half-life (h)	14.3 ± 6.0	14.7 ± 6.9
Conjugate area under curve (μg ml^{-1} h)	212 ± 130	127 ± 94*
Time of maximum conjugate concentration (h)	19 ± 8	17 ± 5

† Mean of 5 subjects ± s.d.
* Significantly different by paired t-test, $P < 0.05$

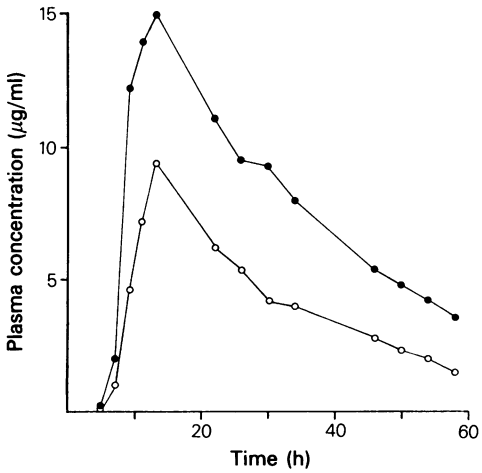

Figure 2 Plasma concentration-time profiles for sulphapyridine following administration of sulphasalazine under control conditions (●) and after pretreatment with ampicillin (○) in a typical subject.

half-lives are not significantly altered. The conjugate AUC is decreased after antibiotic treatment (see Table 2). There is a strong ($r = 0.9642$) and statistically significant ($P < 0.01$) positive correlation between the decrease in sulphapyridine AUC and decrease in acetyl conjugate AUC for the five subjects studied.

Other parameters which described the disposition of sulphapyridine are unaltered; that is the terminal half-life, the time of maximum acetyl conjugate concentrations and the AUC ratio. In each study the comparison of plasma half-lives of sulphapyridine and its acetyl conjugate for each subject showed no significant difference and therefore only the former is reported. This behaviour indicates that the disposition of the acetyl conjugate is formation rate limited.

The ratio of conjugate AUC to sulphapyridine AUC is a measure of acetylator status (Day & Houston, 1980). In four subjects this ratio is less than 0.6 which is consistent with slow acetylator status. The remaining subject has an AUC ratio of approximately one and is classed as a fast acetylator.

Discussion

The saliva:plasma concentration ratio for sulphapyridine and its acetyl conjugate has been shown to be constant (Bates *et al.*, 1978; Day & Houston, 1980). Therefore the saliva data presented may be regarded as representative of plasma concentration-time relationships. Total sulphapyridine concentrations in saliva was selected to compare sulphonamide availability from its precursor, sulphasalazine, with that from administration of sulphapyridine *per se*. *N*-acetylation of certain drugs is readily saturable and this conjugation may exhibit absorption rate-dependency (Drucker *et al.*, 1964). The determination of total sulphonamide minimises any complications

which might arise in the assessment of azo reduction if sulphapyridine acetylation is also readily saturable.

There is a delay in the appearance of total sulphapyridine in the body when this compound is administered as sulphasalazine. The decrease in the maximum saliva concentration and the increase in the time taken to achieve this maximum, reported here, confirms earlier observations by Schroder & Campbell (1972). Unlike the previous investigators we have shown that the extent of absorption of the sulphonamide from both forms is the same since plasma samples were taken over a sufficiently protracted period to allow accurate assessment of the total area under the curve. Hence azo reduction would appear to be complete in the healthy subject.

Administration of an ampicillin course of treatment, as might be prescribed for a respiratory or urinary tract infection, decreases the area under the concentration-time curves for sulphapyridine and acetylsulphapyridine. The following equations show the determinants of both areas.

$$AUC = \frac{F.\,D}{CL} \qquad (1)$$

$$AUC\,(m) = \frac{fm.\,F.\,D}{CL(m)} \qquad (2)$$

where AUC and AUC(m) are the areas under the plasma concentration-time curve between zero and infinity for sulphapyridine and its acetyl metabolite, respectively, F and fm are the fractions of the sulphasalazine dose administered (D) undergoing azo reduction (hence absorption) and subsequently acetylation, respectively, CL and CL(m) are the plasma clearance of sulphapyridine and its acetyl conjugate, respectively. Equations 1 and 2 may be combined to give the area ratio —

$$\frac{AUC\,(m)}{AUC} = \frac{fm.\,CL}{CL(m)} \qquad (3)$$

Ampicillin does not alter the area ratio suggesting that the clearance of neither sulphapyridine nor the acetyl conjugate changes. Substantiation of this claim lies in the observation that the terminal half-lives of these lowly cleared compounds (Bates *et al.*, 1978) is not altered by the antibiotic.

Furthermore no parameter associated with the rate of absorption of sulphapyridine changes after ampicillin treatment. Hence the effect of this antibiotic would appear to be solely to decrease the extent of absorption via a decrease in the extent of azo reduction by the bacterial microflora.

The results of this study confirm the importance of the intestinal microflora in the azo reduction of sulphasalazine in man. Any change in microflora, as exemplified by ampicillin treatment, may alter sulphasalazine disposition and by inference the amount of aminosalicylic acid released in the lower bowel. Thus the variability in response to sulphasalazine in patients with inflammatory bowel disease may in part be a function of individual intestinal microflora status.

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