The metabolism of salicylazosulphapyridine in ulcerative colitis

I The relationship between metabolites and the response to treatment in inpatients

K. M. DAS¹, M. A. EASTWOOD, J. P. A. McMANUS, AND W. SIRCUS

From the Wolfson Laboratory, Gastrointestinal Unit, Western General Hospital, Edinburgh

SUMMARY The metabolism of salicylazosulphapyridine was studied in 16 patients with ulcerative colitis admitted to hospital. The acetylator phenotype was determined on admission. The mean serum concentration ($\mu\text{g/ml}$) (at steady state eight $\pm$ two days in patients responding to treatment) of SASP, total SP, and 5-ASA were 18.7 ± 12.8 ; 53.7 ± 23.1 ; and 1 ± 0.9 for slow acetylators and 17.6 ± 7.1 ; 31 ± 9.0 and 1 ± 0.9 for fast acetylators respectively. Twenty-four hour urinary excretion of SASP, total SP, and 5-ASA were $4.6\% \pm 3.1$; $52\% \pm 9.6$ and $22.3 \pm 6.7\%$ of the administered dose respectively.

Serum total SP concentration of 20 to $50 \mu\text{g/ml}$ appeared to coincide with clinical improvement in the absence of any side effects related to salicylazosulphapyridine. No such relationship could be shown with serum SASP, individual metabolites, or 5-aminosalicylic acid.

It has been established that salicylazosulphapyridine (sulphasalazine, salazopyrin, SASP) is of therapeutic benefit in the management of ulcerative colitis, both in the active and the quiescent phase (Svartz, 1942, 1948; Baron, Connell, Lennard-Jones, and Jones, 1962; Dick, Grayson, Carpenter, and Petrie, 1964; Misiewicz, Lennard-Jones, Connell, Baron, and Jones, 1965).

The metabolism of SASP is better understood as a result of recent studies in the normal subject (Schröder and Campbell, 1972). Similar information during its clinical use is scant. We have already reported our results in a preliminary communication (Das, Eastwood, McManus, and Sircus, 1972).

It has been suggested that about one third of a given dose of SASP is absorbed from the small intestine, the remainder passes to the colon where SASP is split into its components, presumably by bacteria. Most of the sulphapyridine (SP) thus liberated is absorbed whereas about one third of the 5-aminosalicylic component is also absorbed at this level, the remainder being excreted in the stool. In

the liver, SP undergoes N^4 acetylation and 5'-hydroxylation followed by conjugation with glucuronic acid. The purpose of this paper is to define the metabolism of SASP in patients with ulcerative colitis, during both the active and quiescent phases of the disease and to investigate whether there is any relationship between the clinical state and the serum concentration of the drug and its metabolites.

Patients

Sixteen subjects admitted to the unit with a diagnosis of ulcerative colitis (based on clinical, endoscopic, radiological, and histological criteria) were studied. The clinical assessment was based on the criteria of Jalan, Prescott, Sircus, Card, McManus, Falconer, Small, Smith, and Bruce (1971) and on those of Lennard-Jones (1971).

Thirteen of the patients had received no previous treatment and were studied during the initial introduction of the drug. Three others, previously treated with SASP, were admitted in relapse. Ages ranged from 19 to 79 years, but 10 were within the range 20 to 39 years. The body weight ranged from 47 to 85 kg but 10 weighed between 50 and 70 kg. Table I lists the clinical data of these patients.

¹Presented as part of a PhD thesis, University of Edinburgh
Present address: Nassau County Medical Center, New York, USA.

Received for publication 10 May 1973.

Previously untreated	..	..	..	..	..	..	13
Previously treated	..	..	..	..	..	..	3
Severity of disease							
Mild	..	..	..	..	..	..	6
Moderate	..	..	..	..	..	..	7
Severe	..	..	..	..	..	..	3
Extent of involvement							
Entire colon	..	..	..	..	..	..	3
Distal	..	..	..	..	..	..	13

Table I Clinical data for 16 patients studied

Procedure

During the first 24 hours after admission, the acetylator phenotype was determined in untreated patients by the sulphadimidine method (Evans, 1969). Salicylazosulphapyridine (plain tablet) was given to all patients in a dosage of 3 to 6 g/day. Some patients also received corticosteroids (retention enema 4, oral prednisolone and retention enema 4). Supportive treatment, including iron, water, electrolytes, and blood replacement, was given where indicated. No patient received barbiturates but 10 received nitrazepam (5-10 mg) and four had diazepam (6-10 mg) per day. The initial dose of prednisolone varied from 40 to 60 mg/day but after approximately two weeks the dosage was reduced and subsequently tailed off. Parenteral treatment involved a synthetic corticotrophin (Synacthen Depot) by the intramuscular injection of 0.125 and 0.5 mg at intervals of three or four days.

Blood samples were collected two or three times each day (9 am, midday and early evening) during the acute phase (ie, days 1, 3, 5, 7, and 10, and then at variable intervals until discharge and subsequently at intervals up to one year when the patients attended the review clinic. Twenty-four hour urine collections were obtained from all patients before discharge on the days when blood was obtained and occasionally from six patients at random intervals up to one year.

The response to treatment was assessed on subjective symptoms and sigmoidoscopy.

Clinical Outcome

Of the 13 previously untreated patients, 11 patients responded and behaved in a uniformly consistent fashion. Diarrhoea settled within 10 to 12 days. The mean period of stay in hospital was 13 days. Before discharge sigmoidoscopic examination of all these patients revealed a normal rectum in nine, and marked improvement with only minimal friability in two.

Two patients continued to have active disease despite treatment as outlined before.

Three patients who were admitted with relapse after being established on SASP and with a main-

tenance dose of 2.5 to 3 g/day were treated with 3-4 g of SASP/day and corticosteroids. They went into remission within one week.

Methodology

Each serum and urine specimen was analysed for SASP, free sulphapyridine (SP), N⁴-acetyl sulphapyridine (AcSP), sulphapyridine-O-glucuronide (SP-Gluc), and N⁴-acetyl sulphapyridine-O-glucuronide (AcSP-Gluc).

The methods followed were as described by Sandberg and Hansson (1973) and Hansson and Sandberg (1973). Serum total SP was obtained by adding all the estimated SP metabolites. Urine and representative series of serum from all patients were estimated for 5-aminosalicylic acid (5-ASA) and acetyl-5-aminosalicylic acid (Ac-5-ASA) (Hansson, 1973). The analytical error for each of the methods was found to be not greater than 5%.

Results

Figures 1 and 2 show the serum concentration of SASP and total SP during the study.

Eleven patients who responded to therapy are represented together (mean $\pm$ SEM). The other two who did not respond within 10 days are shown individually.

Serum SASP concentration reached a steady state within three days at a mean level of 10 to 15 μ g/ml (fig 1). No significant difference of serum SASP concentration appears between the patients who responded within 10 days of therapy and those who did not. The serum concentration of SASP decreased after three weeks of treatment, probably because the dosage was reduced (2 to 3 g/day) in most patients during or shortly after discharge from hospital. In the 11 patients who responded to treatment, the steady state in the serum total SP (SP + AcSP + SP-Gluc + AcSP-Gluc) was achieved within five days with a mean level of 43 μ g/ml (fig 2). The two patients who did not improve initially had a significantly lower serum concentration of total SP (< 10 μ g/ml). Following an increase in dosage of SASP in these two patients, coincidental with improvement in the clinical state, the serum concentration of total SP increased to within the range observed in patients who showed an early clinical response.

The SP metabolites appeared in the serum six to eight hours after the first dose though SASP reached its peak concentration within three to five hours. The serum concentration of total SP achieved a peak concentration only after 12 to 24 hours and eventually maintained a steady state from days 3 to 5. The results

Fig 1 Serum SASP concentration related to duration of treatment, $\uparrow$ = change in SASP dosage

Fig 2 Serum total SP concentration related to duration of treatment

for the three patients who were admitted in relapse are shown in table II. The serum total SP concentrations in these patients were less than $20 \mu\text{g/ml}$ and outside 1 standard deviation from the mean for patients who responded to therapy. When clinical improvement was attained then the total SP concentration had increased in all three and was within 1 standard deviation of the mean for patients responding. The serum concentrations of SASP in relapse and remission did not alter. It should be noted, however, that

these patients also received hospital care and were given corticosteroids.

The Influence of Acetylator Phenotype

Among the 16 patients studied, six were fast acetylators and 10 were slow acetylators on the basis of the sulphadimidine test, and the concentration of free and acetylated SP. In slow acetylators most of the total SP was free whereas in the fast acetylators it was AcSP. SP-Gluc and AcSP-Gluc were present only in small concentrations in the serum in both groups (table III). There did not appear to be a diurnal variation in the SP levels in the steady state.

Table III shows the serum concentration of SASP and its metabolites in the slow and fast acetylators, when a steady state had been achieved (8 ± 2 days). There was no significant difference in SASP concentration between the slow and fast acetylators. There was, however, a significant difference in free SP and AcSP and total SP between the two phenotypes.

	Serum Concentrations ($\mu\text{g/ml}$) (Mean $\pm$ SD)	
	On Admission	After Improvement
SASP	11.4 ± 8.3	15.3 ± 8.6
Free SP	9.5 ± 1.5	19.5 ± 12.0
AcSP	6.2 ± 4.7	10.3 ± 6.0
Sp-Gluc	0.4 ± 0.5	3.2 ± 4.3
AcSP-Gluc	2.6 ± 1.9	3.6 ± 1.0
Total SP	18.7 ± 1.9	36.7 ± 13.4

Table II Serum concentrations of SASP and its metabolites in three patients admitted in relapse

SASP and SP Metabolites	Serum Concentration (Mean $\pm$ SD)		t Test
	Slow Acetylators (n = 10)	Fast Acetylators (n = 6)	
Acetylation of SP (%)	25.2 $\pm$ 9.6	62.9 $\pm$ 8.5	P < 0.0005
SASP (μ g/ml)	18.7 $\pm$ 12.8	17.6 $\pm$ 7.1	NS
Free SP (μ g/ml)	42.2 $\pm$ 24.4	8.5 $\pm$ 2.8	P < 0.0005
ACSP (μ g/ml)	7.6 $\pm$ 3.1	15 $\pm$ 5.5	P < 0.005
SP-Gluc (μ g/ml)	2.5 $\pm$ 2.5	2.2 $\pm$ 2.2	NS
ACSP gluc (μ g/ml)	4.4 $\pm$ 3.4	4.4 $\pm$ 3.3	NS
Total SP (μ g/ml)	53.7 $\pm$ 23.1	31.7 $\pm$ 9.0	P < 0.01

Table III Serum concentrations of SASP and its SP metabolites during steady state (eight $\pm$ two days) in patients with ulcerative colitis

Urinary Excretion

The urinary excretion of SASP and SP metabolites at different times are shown in table IV. The daily SASP excretion varied from 1 to 13% (mean 4.6 ± 3.1). Individually there was no significant difference in the excretion of SASP on different days though variations between individuals existed. The total SP metabolites recovered from urine ranged from 9 to 23% of the administered dose on day 1, 31 to 58% on day 3, 38 to 60% on day 5, and 42 to 63% on day 10.

The mean values ($\pm$ 1 SD) are shown in table IV. Individual metabolites of SP did not show much variation once a steady state was achieved.

The proportion of the different metabolites excreted by the slow and fast acetylators is shown in table V. There was no significant difference in the excretion of SASP and total SP metabolites, but

slow acetylators excreted the drug mostly as free SP and SP-Gluc whereas fast acetylators excreted as AcSP and AcSP-Gluc. The differences were statistically significant. The excretion of SP as glucuronides, ie, SP-Gluc + AcSP-Gluc, was more or less the same in the two groups (table V).

Serum Concentration of 5-Aminosalicylic Acid and its Urinary Excretion

The concentration of 5-ASA measured in 48 representative serum samples from 16 patients ranged from 0 to 4 μ g/ml (mean $\pm$ 0.9) and was mainly in the form of free 5-ASA.

The urinary recovery of 5-ASA was mostly as acetylated 5-ASA (> 80%). The urinary excretion ranged from 1 to 10% on day 1, 16 to 39.3% (24.8 ± 9.6) on day 3, 16 to 32% (21.2 ± 7) on day 5, and 5 to 37% (22.3 ± 13.7) on day 10. Of the two

Time of Urine Collection	Percentage of Dose of SASP (m $\pm$ SD) Recovered in 24 Hours	Percentage of Total Sulphapyridine Given (m $\pm$ SD) Recovered in 24 Hours
Day 1 of SASP therapy	3.2 $\pm$ 2.8	16.3 $\pm$ 6.0
Day 3 of SASP therapy	4.5 $\pm$ 4.0	41.7 $\pm$ 9.0
Day 5 of SASP therapy	3.5 $\pm$ 1.8	45.9 $\pm$ 9.4
Day 10 of SASP therapy	4.6 $\pm$ 3.1	52.0 $\pm$ 9.6

Table IV Twenty-four hour excretion of SASP and its SP metabolites in 11 new patients with ulcerative colitis responding to therapy.

	Urinary Excretion (Mean $\pm$ SD)		t Test
	Slow Acetylators (n = 10)	Fast Acetylators (n = 6)	
SASP ¹	4.3 $\pm$ 3.8	4.7 $\pm$ 2.5	NS
Total SP ¹	57.7 $\pm$ 11.3	57.6 $\pm$ 15.9	NS
Free SP ²	36.1 $\pm$ 12.7	13.1 $\pm$ 0.9	P < 0.0005
AcSP ²	23.6 $\pm$ 5.3	39.9 $\pm$ 17.5	P < 0.01
SP-Gluc ²	23.4 $\pm$ 7.7	12.1 $\pm$ 8.2	P < 0.01
AcSP-Gluc ²	16.0 $\pm$ 6.8	35.0 $\pm$ 17.6	P < 0.0005
Proportion of acetylated SP ²	38.5 $\pm$ 10.4	72.8 $\pm$ 8.8	P < 0.0005
Proportion of glucuronidized SP ²	38.4 $\pm$ 7.8	46.2 $\pm$ 14.9	NS

Table V Twenty-four hour urinary excretion of SASP and its metabolites in 16 patients with ulcerative colitis in the steady state (eight $\pm$ two days)

¹Calculated as % of the administered dose

²% of the total recovery of sulphapyridine

NS = not significant

patients who did not improve within the first 10 days of treatment one had very low excretion of total 5-ASA (0.8 to 1%) but the second patient excreted 9 to 19% of the administered dose during the first 10 days.

Follow-up Results of the Patients Studied

Figures 1 and 2 show the mean ($\pm$ SEM) of the serum concentration of SASP and total SP during the follow-up study up to one year. The maintenance dose was found to be adequate both clinically and in terms of serum concentration with 3 g SASP/day in fast acetylators and 2 g/day in slow acetylators. One fast acetylator received 6 g SASP/day for at least one year and yet his serum total SP concentration was less than 50 μ g/ml.

One patient with radiological evidence of total involvement of the colon and a fast acetylator who improved initially with the combined regime did not have a complete remission despite adequate serum concentrations. Mild persistent activity continued over a year, and eventually required a total colectomy. Two other patients stopped treatment themselves (after four months and one-and-a-half years of the introduction of therapy). Both of them relapsed eight and 12 weeks later, respectively.

Side Effects Related to Salicylazosulphapyridine

Side effects, namely, nausea and vomiting (3), rashes (2), haemolysis (1), transient reticulocytosis (2), 'cyanosis' (1), transient leucopenia (1), and in one patient agranulocytosis, were observed and will be reported in detail elsewhere. All patients with side effects (except rashes) had a high serum total SP concentration, > 50 μ g/ml.

Discussion

Our results show that the absorption, metabolism, and excretion of SASP in ulcerative colitis follow the same pattern as in healthy individuals (Schröder and Campbell, 1972). Though the degree of absorption and excretion of SASP as the parent drug was in the same range as in healthy volunteers, the absorption and urinary excretion of the metabolites of SP were lower than found in the volunteers (80% of the dose). However, in a short study in healthy persons (one to five days) in England a comparatively smaller excretion (about 60% of the dose) of SP and its metabolites was noted (Schröder and Evans, 1972a). The SASP serum concentration reaches a peak level within three to five hours after ingestion. Between 1 and 13% only of the drug could be recovered in the urine during its first introduction and also during long-term therapy.

Schröder and Campbell (1972) reported excretion up to 10% as SASP in their experiments in healthy persons (10 days).

Sulphapyridine and its metabolites are only demonstrable in the serum about four to six hours after the ingestion of the first dose of SASP. Since SP itself is more or less completely absorbed from the small intestine (Goodman and Gilman, 1970), this delay may be accounted for by the time taken for bacterial azo-splitting of the drug in the colon, and subsequent absorption of split products. This has recently been shown from the animal experiments (Peppercorn and Goldman, 1972) and the study of SASP metabolism in patients with an ileostomy (Das, 1973).

The Relationship between Clinical States and Serum Drug Concentrations

The patients who improved clinically had a significantly higher concentration of serum total SP > 20 μ g/ml than the two patients who did not improve (< 10 μ g/ml). In these two, coincidental with clinical improvement following an increase in the dose, the serum total SP concentration rose to within the range of patients responding earlier. This was further confirmed by the serum concentration of three patients who were admitted in relapse. Individual SP metabolites did not show any significant correlation with clinical effectiveness whereas the total SP concentration in the serum was found to be highly relevant. No relationship was found between serum SASP concentration and clinical states. Urinary excretion of SASP was low in all patients but in patients with remission the total SP excretion was significantly higher (57.6%) compared with that in the two patients who did not improve (13%).

After absorption SP is distributed throughout the whole body (Frisk, 1941; Hanngren, Hansson, Svartz, and Ullberg, 1963). In the liver it undergoes acetylation which shares the acetylation polymorphism of other compounds, eg, sulphadimidine (Schröder and Evans, 1972b; Das and Eastwood, 1973). In this study 10 patients were slow acetylators and six patients were fast acetylators. Although acetylation polymorphism was determined in 13 patients by the sulphadimidine method before the introduction of SASP, a single estimation of serum and urine for free and acetyl SP in patients taking SASP is sufficient to determine the acetylator phenotype. Acetylation polymorphism did not change in these patients during the various stages of the disease or with prolonged therapy with SASP (Das and Eastwood, 1973).

Hydroxylation of SP is the second path of metab-

olism in the liver. This is followed by conjugation with glucuronic acid. Hydroxylation of SP is variable since the enzyme involved can be induced. There was no significant difference of hydroxylated (glucuronized) amounts of SP excreted in the urine in slow and fast acetylators. Five of the patients with serum total SP above 50 µg/ml were slow acetylators and the hydroxylation capacity in all of them was less than 40%.

Though there was a significant difference in the individual metabolites of SP in the urine among the slow and fast acetylators the total SP excretion did not show significant change during the acute phase. However, the serum concentration of total SP tends slowly to increase in the patient who is a slow acetylator especially if he is also a poor hydroxylator. It was found that the most effective therapeutic concentration of total SP for the achievement of clinical remissions and not associated with side effects, lies within a range of 20 to 50 µg/ml of serum.

The serum concentration of 5-ASA was found to be very low (1 ± 0.9) though urinary excretion was about 22% of the dose. However, 33% excretion was observed in healthy persons (Schröder and Campbell, 1972). In view of such a low serum concentration of 5-ASA both in the active and remission states with wide overlapping, no correlation was found between serum 5-ASA and the clinical state.

It is likely that SASP is serving as a vehicle to carry the two metabolites into the colon, the site of their possible action.

The precise function of the compound drug SASP as such therefore remains uncertain. It also remains to be determined whether the clinical response is in any degree a function of some local action of SASP in the colon, or of the unabsorbed 5-aminosalicylic acid, and whether the therapeutic effect is achieved during the passage of the SP through the mucosa of the colon. What is clear from our studies is that SASP metabolism is similar in normals and in patients with ulcerative colitis and that therapeutic success accompanies a total serum SP concentration of more than 20 µg/ml.

References

- Baron, J. H., Connell, A. M., Lennard-Jones, J. E., and Jones, F. A. (1962). Sulphasalazine and salicylazosulphadimidine in ulcerative colitis. *Lancet*, **1**, 1094-1096.
- Das, K. M. (1973). From PhD Thesis 'Salicylazosulphapyridine metabolism in clinical practice', University of Edinburgh, 1973.
- Das, K. M., Eastwood, M. A. (1973). Acetylation polymorphism of sulphapyridine and its constancy in patients with ulcerative colitis and Crohn's disease. In press.
- Das, K. M., Eastwood, M. A., McManus, J. P. A., and Sircus, W. (1972). Salazopyrin metabolism in ulcerative colitis. *Gut*, **13**, 840.
- Dick, A. P., Grayson, M. J., Carpenter, R. G., and Petrie, A. (1964). Controlled trial of sulphasalazine in the treatment of ulcerative colitis. *Gut*, **5**, 437-442.
- Evans, D. A. P. (1969). An improved and simplified method of detecting the acetylator phenotype. *J. med. Genet.*, **6**, 405-407.
- Frisk, A. R. (1941). Blood concentration, acetylation and urinary excretion of sulphapyridine and sulfathiazole after various sulphapyridine and sulfathiazole derivatives administered by different routes. *Acta med. scand.*, **106**, 369-403.
- Goodman, L. S., and Gilman, A. (1970). *Pharmacological Basis of Therapeutics*, 4th ed., Macmillan, London.
- Hanngren, A., Hansson, E., Svartz, N., and Ullberg, S. (1963). Distribution and metabolism of salicyl-azo-sulphapyridine II. A study with S⁸-salicyl-azo-Sulphapyridine and S⁸-Sulphapyridine. *Acta med. scand.*, **173**, 391-399.
- Hansson, K. A. (1973). Determination of free and acetylated 5-amino salicylic acid in serum and urine after administration of salicylazosulphapyridine. *Acta pharm. suec.*, **10**, 153.
- Hansson, K. A., and Sandberg, M. (1973). Determination of sulphapyridine and its metabolites in biological materials after administration of salicylazosulphapyridine. *Acta pharm. suec.*, **10**, 87-92.
- Jalan, K. N., Prescott, R. J., Sircus, W., Card, W. I., McManus, J. P. A., Falconer, C. W. A., Small, W. P., Smith, A. N., and Bruce, J. (1971). Ulcerative colitis: a clinical study of 399 patients. *J. Roy. Coll. Surg. Edin.*, **16**, 338-355.
- Lennard-Jones, J. E. (1971). Observations on the treatment of colitis. *Schweiz med. Wschr.*, **101**, 701-706.
- Misiewicz, J. J., Lennard-Jones, J. E., Connell, A. M., Baron, J. H., and Jones, F. A. (1965). Controlled trial of sulphasalazine in maintenance therapy for ulcerative colitis. *Lancet*, **1**, 185-188.
- Peppercorn, M. A. and Goldman, P. (1972). The role of intestinal bacteria in the metabolism of salicylazosulphapyridine. *J. Pharmacol. exp. Ther.*, **181**, 555-562.
- Sandberg, M., and Hansson, K. A. (1973). Determination of salicylazosulphapyridine in biological materials. *Acta pharm. suec.*, **10**, 107-110.
- Schröder, H., and Campbell, D. E. S. (1972). Absorption metabolism, and excretion of salicylazosulphapyridine in man. *Clin. Pharmacol. Ther.*, **13**, 539-551.
- Schröder, H., and Evans, D. A. P. (1972a). Acetylator phenotype and adverse effects of sulphasalazine in healthy subjects. *Gut*, **13**, 278-294.
- Schröder, H., and Evans, D. A. P. (1972b). The polymorphic acetylation of sulphapyridine in man. *J. med. Genet.*, **9**, 168-171.
- Svartz, N. (1942). Salazopyrin, a new sulfanilamide preparation: B. Therapeutic results in ulcerative colitis. *Acta med. scand.*, **110**, 580-581.
- Svartz, N. (1948). The treatment of 124 cases of ulcerative colitis with salazopyrin and attempts of desensitization in cases of hypersensitivity to sulfa. *Acta med. scand.*, Suppl., **206**, 465-472.