

Metabolism and urinary excretion of 5-amino salicylic acid in healthy volunteers when given intravenously or released for absorption at different sites in the gastrointestinal tract

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SUMMARY In six healthy subjects serum concentrations of 5 amino salicylic acid (5ASA) and acetyl 5ASA were measured for up to 24 hours, and urinary excretion over 48 hours. After an intravenous injection of 3.26 mmol 5ASA serum concentrations fell rapidly with a distribution half-life of 17 ± 2 min and an elimination half-life of 42 ± 5 min. After 45 minutes acetyl 5ASA became the dominant compound and after seven hours serum concentrations of both components were almost unrecordable. Orally ingested 5ASA in three preparations to ensure its release in the stomach, small intestine and ileocaecal region respectively gave lower serum concentrations and urinary excretion than those obtained after an intravenous infusion. Bioavailabilities which ranged from 19% for ileocaecal release to 75% for release in the upper gastrointestinal tract, were calculated from areas under the serum concentration curves. Urinary excretion of 5ASA and its acetyl metabolite over 48 hours was 78%, 52%, 55%, and 21% respectively of the dose given intravenously and orally for gastric, small intestinal and ileocaecal release.

Sulphasalazine is an effective treatment for maintenance of clinical remission in ulcerative colitis.¹ In recent years it has been shown that 5-amino salicylic acid (5ASA) is released from this preparation in the human colon and is the active principle responsible for its effect in colitis.² Sulphapyridine which is linked by a diazo bond to 5-amino salicylic acid appears to act only as a carrier molecule, but in many patients is responsible for side effects which could be overcome by delivering 5-amino salicylic acid to the colon in some other way. Several preparations have been investigated recently with claims that the drug is released in the human colon to achieve the desired therapeutic effect.³ During its metabolism 5ASA is rapidly converted to the acetyl form and excreted in the urine.⁴ Studies of preparations which release 5ASA for absorption in the colon are only of limited

value in following the pharmacokinetics of 5ASA; because of this we have carried out the present study. Normal healthy volunteers took part and were given 5ASA in four different formulations – intravenously and orally in three preparations which ensured release in the stomach, the mid small bowel and the colon. Serum concentrations and urinary excretion were followed for 24 and 48 hours respectively.

Methods

SUBJECTS

Six healthy women between 44 and 58 years of age took part in the study. In the four experiments each subject was given the 5ASA preparation and urine was collected for 48 hours during the periods 0–4, 4–12, 12–24 and 24–48 hours.

EXPERIMENT 1: INTRAVENOUS 5ASA

Each subject was given 0.5 g (3.26 mmol) of 5ASA by intravenous infusion over five minutes. The prepara-

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tion, which also contained 400 mg sodium carbonate, had been sterilised by filtration through a micropore filter (0.22 μm) and freeze dried under nitrogen. Blood samples were taken before the infusion and thereafter at 5, 10, 15, 30, 45, and 60 minutes, and at 1½, 2, 3, 4, and 7 hours.

In experiments 2, 3, and 4, 2.4 g of 5ASA (15.7 mmol) was given orally at 900 h. This was encapsulated in six plain no. 1 gelatin capsules each of which contained 400 mg of 5ASA, 300 mg barium sulphate and 100 mg of wheat starch.

EXPERIMENT 2: 5ASA IN UNCOATED CAPSULES

Each subject took six uncoated capsules which were designed to release their contents in the stomach. Blood samples were taken before ingestion of the capsules and thereafter at 2, 2½, 3, 5, and 7 hours.

EXPERIMENT 3: 5ASA IN CAPSULES COATED WITH EUDRAGIT L

Identical capsules of 5ASA were then coated to a thickness of about 80 μ with the acrylic based resin Eudragit L; this was designed to release contents in the midjejunum. Plain abdominal radiographs were taken after two and usually four hours to identify the site at which capsules disintegrated. Blood samples were taken initially and then 2, 3, 4, 6, 9, 12, and 24 hours after the capsules had been taken.

EXPERIMENT 4: 5ASA IN CAPSULES COATED WITH EUDRAGIT S

Identical capsules of 5ASA were then coated with the acrylic based resin Eudragit S, to about 80 μ in thickness; this was designed to delay release of the contents until the terminal ileum or right side of the colon. Plain abdominal radiographs were taken after four and 12 hours to identify where capsules disintegrated. Samples of blood were taken at 3, 6, 9, 12, and 24 hours after the capsules had been taken.

Plasma was separated from the blood samples within an hour and stored at -20°C until measurements were made. Urine volumes were measured and aliquots stored with the plasma samples.

Serum concentrations of 5ASA and acetyl 5ASA were measured by HPLC, but because some concentrations were very low, these samples were concentrated approximately five times by extraction through ether and reconstituted in water. Acetyl 5ASA was extracted directly from serum while 5ASA was acetylated with acetic anhydride; two samples were therefore required, the sum of 5ASA plus acetyl 5ASA being determined from the acetylated sample.

The assay procedure was as follows:— Two 1 ml aliquots were taken from each sample and one acetylated by adding 20 μl acetic anhydride. After standing for five minutes, 20 μl of methanol was

added to both aliquots to quench excess anhydride and then samples again left for five minutes. Forty microlitres of 10 mM 2,4-dihydroxy-benzoic acid was added as an internal standard, followed by 1 ml of 1M HCl. The samples were then extracted with 10 ml diethyl ether while shaking for 10 minutes before separating by centrifugation for two minutes. The organic phase was removed, and then evaporated to dryness. The residue was redissolved in 100 μl phosphate buffer. Fifty microlitres of the buffer solution was used to load a 20 μl sample loop for injection on to the column.

The analysis was undertaken on an Ultrasphere-IP (25 cm \times 4.6 mmID) bonded silica reverse phase column. The mobile phase consisted of 90% 0.05 M phosphate buffer a pH 7.4, 10% acetonitrile and 0.08% tetrabutyl ammonium hydroxide pairing ion. Acetyl-5ASA and the internal standard 2,4-DHBA were detected spectrofluorimetrically (excitation 360 nm, emission 425 nm).

Standard solutions of 5ASA were made up fresh on the day for calibration purposes and samples were acetylated as above. Calibration was linear over the range 0–4 $\mu\text{g/ml}$ acetyl ASA. Using this extraction procedure to concentrate the original plasma 10-fold, a sensitivity of 0.01 $\mu\text{g/ml}$ 5ASA, and acetyl 5ASA was obtained.

Results

After intravenous infusion of 0.5 g of 5ASA high serum concentrations of 5ASA were recorded (mean \pm SD, 330 ± 82 $\mu\text{mol/l}$ at five minutes) but in the first hour levels fell rapidly (Figure), giving a distribution half-life of 17 ± 2 min and an elimination half-life of 42 ± 5 min for the parent compound (Table 1). At 45 minutes the concentration of acetyl 5ASA equalled and thereafter exceeded that of 5ASA (Figure). Seven hours after the injection, serum concentrations of acetyl 5ASA and 5ASA were almost unrecordable. Urinary measurements showed that almost all the 5ASA was excreted within the first four hours, with overall 67% of the total in acetyl form (Table 2). The total amount of 5ASA and acetyl 5ASA recovered in the urine over 48 hours was 2.6 ± 0.8 mmol which was equivalent to $78 \pm 24\%$ of the administered dose.

After ingestion of 5ASA in uncoated gelatin capsules serum concentrations of 234 ± 77 , 172 ± 28 $\mu\text{mol/l}$ for 5ASA, Ac 5ASA respectively were recorded after two hours, and thereafter fell steadily with only 30.5 ± 17.7 $\mu\text{mol/l}$ Ac 5ASA present after seven hours (Figure). Again, almost all of the 5ASA was excreted in the urine within four hours with 59% overall in the acetyl form (Table 2). The total amount of 5ASA plus acetyl 5ASA recovered in the urine

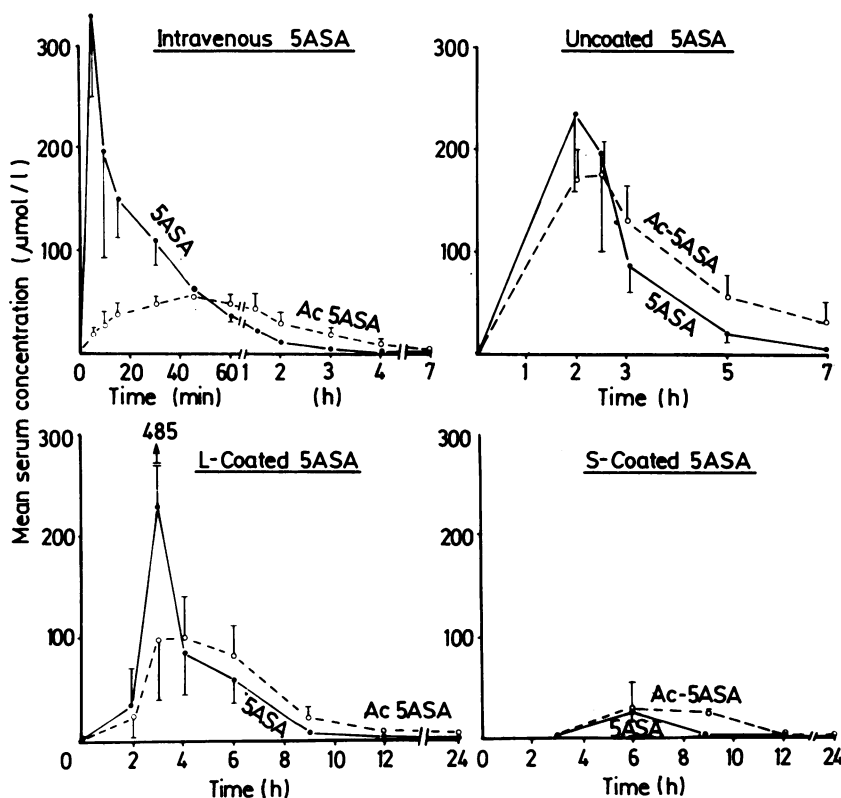


Figure Mean \pm SD serum concentrations ($\mu\text{mol/l}$) of 5-amino salicylic acid (5ASA) and N-acetyl 5ASA in six healthy volunteers after:— (i) a bolus intravenous injection of 0.5 g (3.26 mmol) 5ASA; (ii)–(iv) oral ingestion of 2.4 g (15.7 mmol) 5ASA in hard gelatin capsules designed to release their contents in (ii) the stomach, (iii) the midjejunum, (iv) the ileocaecal region.

within 48 hours was 8.2 ± 2.1 mmol which was equivalent to $52 \pm 13\%$ of the dose given. The bioavailability calculated from areas under the serum concentration curve was 75%.

Most of the capsules of 5ASA coated with Eudragit-L disintegrated in the small intestine (Table 3). Radiographs showed only fragments of disrupted capsules when they were in the terminal ileum; most capsules remained intact in the stomach although three were broken at this site. Serum concentrations of 5ASA and acetyl 5ASA (Figure) were highest at three hours with values of 228 ± 257 and 95 ± 56 $\mu\text{mol/l}$ respectively, falling to low values at 12 hours. Most of the drug was excreted in the first 12 hours, particularly in the period four to 12 hours (Table 2), overall 76% was excreted in the acetyl form. The total urinary excretion over 48 hours was $55 \pm 17\%$ of the oral dose. The bio-availability calculated from serum concentration data was 73%.

Most of the S-coated capsules appeared to disintegrate in the terminal ileum or right colon (Table 3).

No 5ASA or acetyl 5ASA was detected in serum at 3 hours (Figure) and the highest recorded mean value for acetyl 5ASA was at six hours, 29.7 ± 23.6 $\mu\text{mol/l}$ after which it fell steadily in the next 24 hours. None appeared in the urine in the first four hours and the highest urinary excretion was in the period four to 12 hours (Table 2). Total urinary excretion over 48 hours was equivalent to $21 \pm 10\%$ of the oral dose given, 86% as acetyl 5ASA.

Discussion

The intravenous study showed a rapid conversion of 5ASA to the acetyl derivative with an elimination half-life of the parent compound of 42 ± 5 minutes. In the three studies where subjects ingested 15.7 mmol 5ASA to be released in the stomach, midjejunum or ileocaecal region, the pattern and timing of absorption and clearance was related to the site of release. With release in the stomach there was a very high two hour concentration of 5ASA and rapid clearance of

Table 1 Pharmacokinetic parameters calculated from serum concentrations of 5ASA and acetyl 5ASA

	$T_{1/2\beta}$ (min)	AUC/dose	Ratio (%)	Recovery (%)	Cl_p ($l\ h^{-1}$)	V_D (l)
1 After intravenous 5ASA						
5ASA	42±5*	455±98	100	78±24	18±4	18±5
Ac5ASA	78±15	433±110			13±3	23±4
2 After oral 5ASA in uncoated capsules						
5ASA	55±6	339±95	75	52±13	16±4	25±9
Ac5ASA	109±22	446±92	103		7±2	18±3
3 After oral 5ASA in capsules coated with Eudragit-L						
5ASA	58±7	330±156	73	55±17	20±9	27±11
Ac5ASA	100±10	340±83	79		13±4	31±8
4 After oral 5ASA in capsules coated with Eudragit-S						
5ASA	67±15	87±49	19	21±10	32±20	55±39
Ac5ASA	137±45	272±27			7±1	22±8

The pharmacokinetic values are based on serum concentrations of 5ASA and acetyl 5ASA following intravenous (3.26 mmol) and oral administration (15.7 mmol) of 5ASA. $T_{1/2\beta}$ is the elimination half life in minutes. In the intravenous study 5ASA data show two component elimination with $T_{1/2\alpha}$ 17±2 min, and $T_{1/2\beta}$ 42±5 min. AUC/Dose is the area under the curve for serum concentrations against time divided by the dose given (10^{-4} h/l). All values for areas under the curve were extrapolated to infinite time. Ratio % represents the AUC/Dose for each preparation divided by that for the intravenous dose and expressed as a percentage. Recovery % is the percentage of the dose recovered as 5ASA + acetyl 5ASA in urine over 48 hours. Cl_p represents plasma clearance = Dose/AUC. V_D is the volume of distribution.

both 5ASA and the acetyl derivative. A sample was not taken before two hours, however, and an earlier peak may have been missed; furthermore, the bioavailability of the uncoated preparation (75%) may be an underestimate. In contrast, when 5ASA was released in the ileocaecal region, the peak concentration was low and occurred between three and six hours. Release in the midjejunum gave an intermediate pattern. When 5ASA was released in either the stomach or midjejunum the overall recovery in urine over a 48-hour period was about 50% compared with only 21% recovery of the compound after ileocaecal release; the figure of 50% is similar to previous work with jejunal absorption of 5ASA.

We were able to validate the site at which the oral

preparations were released by including barium sulphate in the capsules and taking radiographs at intervals. The serum and urinary measurements of 5ASA and acetyl 5ASA were reproducible against standards using HPLC.

The serum and urinary concentrations obtained after ingestion of capsules coated with Eudragit-S were similar to those obtained on previous occasions⁴ and the overall absorption of the preparation was also similar. 5ASA has not been given intravenously on previous occasions so that the information from this study is new. The release of equivalent doses of 5ASA in the stomach and jejunum showed a marked difference in the concentrations obtained compared with the S-coated preparation which was largely available for absorption in the colon.

Table 2 Urinary excretion of 5-amino salicylic acid (5ASA) and N-acetyl 5ASA (Ac 5ASA)

	Time of collection period in hours				48 hour excretion	Total of 5ASA + Ac 5ASA	Urinary excretion as % of dose
	0-4	4-12	12-24	24-48			
1 After intravenous 5ASA							
5ASA	800±420	40±80	0	0	840±430	2560±770	78±24
Ac5ASA	1350±400	220±270	50±50	100±60	1720±370		
2 After oral 5ASA in uncoated capsules							
5ASA	3250±1240	97±44	2±13	2±1	3350±1240	8150±2080	52±13
Ac5ASA	4290±1020	490±170	17±13	6±5	4800±980		
3 After oral 5ASA in capsules coated with Eudragit-L							
5ASA	740±300	1270±560	18±6	20±15	2050±500	8610±2660	55±17
Ac5ASA	1340±600	4230±1130	380±680	620±1340	6570±2410		
4 After oral 5ASA in capsules coated with Eudragit-S							
5ASA	0	450±550	18±13	0	470±550	3300±1570	21±10
Ac5ASA	0	1740±1250	490±210	610±330	2830±1030		

Urinary excretion of 5-amino salicylic acid (5ASA) and N-acetyl 5ASA (mean±SD μ mol) in 6 healthy volunteers, during 4 collection periods over 48 hours, after an intravenous injection of 0.5 g (3.26 mmol) 5ASA and oral ingestion of 2.4 g (15.7 mmol) 5ASA in hard gelatin capsules, which are either uncoated or coated with Eudragit-L or Eudragit-S.

Table 3 Radiological observations in experiments with delayed release of 5ASA

Experiment 3 Capsules coated with Eudragit-L Time of radiographs and findings			
Subject	2 hours	4 hours	8 hours
BB	Fragments only in distal SI	—	—
MH	Gastric – 2 intact, 1 broken Upper SI – 2 intact, 1 broken	Fragments only in terminal ileum, right colon	—
MI	Mid SI – 4 intact, 2 broken	Non intact, fragments only in terminal ileum, R colon	—
CR	Non intact, fragments in stomach, mid SI, terminal ileum, caecum	—	—
AR	Stomach – 6 intact	All breaking – 4 in stomach 1 in SI, 1 terminal ileum	Fragments only in terminal ileum
DS	Stomach – 4 intact, 1 fragment Jejunum – 1 fragment	Terminal ileum – 1 breaking, remainder fragments only	—
Experiment 4 Capsules coated with Eudragit-S Time of radiographs and findings			
Subject	4 hours	12 hours	
BB	6 intact in terminal ileum	None intact, fragments in terminal ileum and right colon	
MH	6 intact in terminal ileum	None intact, fragments in terminal ileum and right colon	
MI	3 intact and 3 breaking in terminal ileum	None intact, fragments in R colon, L colon and rectum	
CR	1 intact and 5 breaking in terminal ileum	—	
AR	6 intact, 5 terminal ileum and 1 mid SI	3 intact R colon, others fragmented	
DS	6 intact in terminal ileum	None intact, fragments in R colon	

The findings on radiographs taken after ingestion of six capsules coated with Eudragit-L and Eudragit-S in six healthy subjects. The barium outline of capsules was distinct for intact capsules, irregular or angular when breaking or disrupted altogether with fragments of barium only (SI – small intestine).

Because the therapeutic aim in ulcerative colitis is to release 5ASA for a topical effect on inflamed colonic mucosa, it is important that the preparation achieves this. The coating of Eudragit-S ensures release in the ileocaecal region which reduces peak serum concentrations and the overall absorption, but presents a maximum amount of drug for its topical effect on the colonic mucosa. Preparations which are released higher in the gastrointestinal tract are more readily absorbed by the small intestine and will give higher serum concentrations, greater absorption, and a reduction in the 5ASA available for topical action in the colon.

'Slow release' preparations which begin releasing 5ASA in the upper small intestine may not be ideal because they rely on most of the preparation being released in the colon. In patients with a rapid intestinal transit, slow or delayed release preparations may fail to release the drug, although it was found that Eudragit-S worked satisfactorily in a group of patients who had active symptoms and diarrhoea from their colitis.⁷

5-amino salicylic acid appears to be both rapidly absorbed by the small intestine and converted mainly within one hour to the acetyl derivative which is largely excreted within about 12 hours. In contrast, the preparation is only poorly absorbed by the colon and release in the caecal region ensures that serum

concentrations are low and overall absorption and urinary excretion are prolonged over 24 hours.

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