Dose loading with delayed-release mesalazine: a study of tissue drug concentrations and standard pharmacokinetic parameters

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Aims Tissue concentrations of 5-aminosalicylic acid (5ASA) and its metabolites may influence the clinical course of inflammatory bowel disease. Since the factors that determine tissue drug concentrations are unknown we have studied the relationships between the oral dose of delayed-release mesalazine, rectal tissue drug concentrations and standard pharmacokinetic parameters.

Methods Twelve healthy volunteers were studied following 7 days treatment with 1.2, 2.4 and 4.8 g of delayed-release mesalazine daily. 5-aminosalicylic acid and *N*-acetyl 5-aminosalicylic acid concentrations were measured in serum, urine, stool and rectal tissue biopsies.

Results Serum concentrations and 24 h urinary excretion of 5ASA and *N*-acetyl 5ASA increased as the oral dose of mesalazine was increased from 1.2 g through 2.4 g to 4.8 g daily (serum area under curve (AUC): 5ASA = 3.9, 15.4 and 46.8 $\mu\text{g ml}^{-1}$ h, $P < 0.0001$; *N*-acetyl 5ASA = 17.2, 30.9 and 57.8 $\mu\text{g ml}^{-1}$ h, $P < 0.0001$; urinary excretion: 5ASA = 1.8, 85.5 and 445 mg, $P < 0.0001$; *N*-acetyl 5ASA = 250, 524 and 1468 mg, $P < 0.0001$, respectively). Faecal 5ASA excretion increased as the oral dose increased from 1.2 g to 2.4 g but did not increase further with 4.8 g daily dosing whereas faecal *N*-acetyl 5ASA excretion was similar at all three doses. Rectal tissue concentrations of 5ASA increased markedly, and *N*-acetyl 5ASA increased modestly, as the dose of oral mesalazine increased from 1.2 g to 2.4 g daily but neither increased further with 4.8 g daily dosing.

Conclusions The relationship between the ingested dose of delayed-release mesalazine and rectal tissue drug concentrations is complex. Factors other than dose are likely to be important determinants of rectal tissue drug concentrations.

Keywords: 5-ASA, dose-loading, mesalazine, pharmacokinetics, tissue concentration

Introduction

In patients with ulcerative colitis, it is well established that mesalazine (5ASA) is the active ingredient of sulphasalazine whereas sulphapyridine appears therapeutically inert [1]. Since most of the adverse effects of sulphasalazine are thought to be due to sulphapyridine, a range of new formulations has been developed to deliver 5ASA to the colon without the toxic sulphapyridine carrier. These

agents appear as effective as sulphasalazine and cause fewer side-effects [2–4]. This reduced toxicity permits the use of higher doses, but the results of high-dose treatment have been somewhat disappointing, particularly in the maintenance of ulcerative colitis remission [5–11].

The precise mode of action of 5ASA in ulcerative colitis remains unknown. It seems to act locally within the colonic mucosa, since systemic concentrations are low following both oral and rectal dosing [12, 13]. *In vitro* studies show that many of the actions of 5ASA are dose-related [14–17] suggesting that tissue drug concentrations may be a determinant of therapeutic efficacy. Since factors that determine tissue drug concentrations are unknown we have studied the relationships between oral mesalazine dose, rectal tissue drug concentrations and standard pharmacokinetic parameters in healthy volunteers.

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Methods

Clinical protocol

Twelve healthy volunteers were recruited to the study. Five were female and their ages ranged from 18 to 30 years. All gave written consent prior to study and the protocol was approved by the Northern General Hospital Ethics Committee.

Each volunteer was studied on three separate occasions, following daily dosing with 1.2, 2.4 and 4.8 g delayed-release mesalazine (Asacol, Smith Kline Beecham) for 7 days. The drug was taken in divided doses; with breakfast (08.00 h), lunch (13.00 h) and the evening meal (18.00 h). A minimum drug-free interval of 7 days separated each study period.

Subjects were admitted for sampling on the seventh day of each study period. Blood samples were taken at 90 min intervals over 24 h and the serum was separated and stored at -30°C . Urine and faeces were also collected over 24 h. Faeces were passed directly into 500 ml methanol to prevent acetylation of 5ASA by faecal flora [18]. Faecal collections were weighed and homogenized using a Colworth Stomacher (Seward Medical, London, UK.).

Rectal tissue biopsies were taken from the anterior wall of the rectum at 09.00, 12.00, 15.00, 18.00 and 21.00 h. In order to minimize the effect of faecal contamination, the mucosa to be biopsied was washed with 20 ml 0.9% saline immediately prior to biopsy. We have previously shown that washing will remove more than 97% (5ASA 99.4–100%, *N*-acetyl 5ASA 97.7–100%) of the surface contamination [19]. Biopsies were immediately frozen in liquid nitrogen and stored at -80°C .

Analytical methods

The analytical method has been described in detail elsewhere [19]. A Waters (Watford, UK) 510 pump, 717 plus autosampler and 470 fluorescence detector (excitation 315 nm, emission 430 nm) were employed and the data was analysed using a 746 data module. A Supelcosil ABZ column (150 $\times$ 4.6 mm i.d., 5 μm silica particles) purchased from Sigma (Poole, UK) was protected by a Supelco (Sigma) guard column (20 $\times$ 4.6 mm i.d., 5 μm silica particles). The mobile phase consisted of 0.1 M acetic acid and acetonitrile and triethylamine (1600: 114: 6) at pH 4.3. The flow-rate was 1.5 ml min $^{-1}$, with a resulting pressure of 10.35 Mpa, and the analysis was performed at ambient temperature. Samples were derivitized using propionic anhydride to enhance the fluorescence characteristics of 5-aminosalicylic acid. Triethylamine was used as an ion-pairing agent to improve peak symmetry.

Stock solutions of 5-aminosalicylic acid and *N*-acetyl-5-aminosalicylic acid, were prepared by dissolving 20 mg of each compound in 200 ml of water (heated to 70°C). Standard solutions of different concentrations were obtained by serial dilution of the stock solution and each was stored at -80°C . A 4-aminosalicylic acid solution (60 $\mu\text{g ml}^{-1}$) was prepared for use as the internal standard using a similar protocol. Calibration samples were prepared by adding standard solutions to blank serum, biopsy homogenate, urine, faeces and 0.05 M phosphate buffer (pH 7.4). Calibration curves were constructed, using unweighted linear regression, for each analyte from the peak area ratios of the respective analyte to the internal standard *vs* the amount of analyte. Recovery is expressed as the percentage response of a processed spiked matrix standard compared with pure standard.

Rectal biopsy samples were weighed and then crushed. A 100 μl volume of internal standard solution (15 $\mu\text{g ml}^{-1}$ 4-ASA), 400 μl of 0.05 M phosphate buffer (pH 7.4), 500 μl of methanol and 20 μl of propionic anhydride were added. Tissue cells were disrupted ultrasonically using a micro-probe (Jencons Scientific, Leighton Buzzard, UK) inserted into the suspension for 60 s at 40 W. After Vortex mixing, samples stood for 30 min at room temperature, to permit protein precipitation, and were then centrifuged at 5000 *g* for 15 min. The supernatant was filtered using 0.5 μm Millex LCR filters (Millipore, Watford, UK) and 20 μl samples were injected directly into the h.p.l.c. system.

Urine was diluted 10 fold in 0.05 M phosphate buffer (pH 7.4). To a 0.1-ml aliquot, 0.3 ml of 0.05 M phosphate buffer, 0.1 ml of internal standard (60 $\mu\text{g ml}^{-1}$ 4-ASA), 20 μl of propionic anhydride and 0.5 ml methanol was added. Following Vortex mixing, samples stood for 30 min and were then centrifuged at 5000 *g* for 10 min. The supernatant was filtered and 20 μl injected directly into the h.p.l.c. system.

Faecal samples were prepared using the protocol employed for urinary drug analysis.

Samples of serum (0.5 ml) were added to 0.5 ml of 0.05 M phosphate buffer, 50 μl of internal standard (60 $\mu\text{g ml}^{-1}$ 4-aminosalicylic acid), 20 μl of propionic anhydride and 1.5 ml methanol. After standing for 30 min at room temperature, the mixture was centrifuged at 1500 *g* for 15 min. The supernatant was filtered and 20 μl samples were injected directly into the h.p.l.c. system.

All calibration curves were linear and median correlation coefficients exceeded 0.99. The lower limits of detection, for both 5ASA and *N*-acetyl 5ASA, were less than 0.1 $\mu\text{g ml}^{-1}$ for urine and faeces, less than 0.005 $\mu\text{g ml}^{-1}$ for serum and less than 0.2 ng mg $^{-1}$ for rectal biopsy tissue. Intra- and interassay coefficients of variation when 5ASA and *N*-acetyl 5ASA were added to rectal tissue, serum, urine, and faeces did not exceed 10%. Recoveries ranged from 95.4% to 108.3%.

Statistical analysis

All results are expressed as median (range) unless otherwise stated. Paired data from the same matrix were analysed using Wilcoxon signed rank test whereas the Friedman two-way analysis of variance test was used to analyse trends across the three oral doses. Correlations between different matrices (e.g. serum and tissue concentrations) were sought using Spearman's rank correlation coefficient.

Power calculations, based on intrasubject variability noted in our previous studies (intrasubject CV 40%), suggested that sample sizes of 6 and 12 would detect changes in rectal tissue *N*-acetyl 5ASA of 55% and 33%, respectively, at 80% power ($P < 0.05$, one tailed *t*-test).

Figure 1 (a) Serum 5ASA time concentration profiles (median and interquartile range) and (b) serum *N*-Acetyl 5ASA time concentration profiles (median and interquartile range). ○ mesalazine 400 mg three times daily; ■ mesalazine 800 mg three times daily; △ mesalazine 1600 mg three times daily.

Results

The serum concentration time profiles of 5ASA and *N*-acetyl 5ASA following oral dosing with delayed-release mesalazine 1.2, 2.4 and 4.8 g daily are shown in Figure 1. Peak concentrations of 5ASA and *N*-acetyl 5ASA were found in the morning irrespective of the ingested dose [5ASA t_{max} : 1.2 g daily equals; 09.00 h (07:30–15:00), 2.4 g daily equals; 07.30 h (06.00–22.30 h) and 4.8 g daily = 09.00 h (04.30–18.00 h), *N*-acetyl 5ASA t_{max} : 1.2 g daily = 06.00 h (06.00–15.00 h), 2.4 g daily = 09.00 h (07.30–21.00 h) and 4.8 g daily = 09.00 h (04.30–21.00 h)]. With increasing oral dose peak concentrations (C_{max}), trough concentrations (C_{min}) and the area under the serum concentration time curve (AUC) increased significantly [Table 1]. A disproportionate (greater than four fold) increase in both 5ASA serum AUC and C_{min} was seen with successive doubling of the oral dose. *N*-acetyl 5ASA was found to predominate in the serum at all doses but as the oral dose of mesalazine increased the percentage of drug present in the serum as *N*-acetyl 5ASA decreased [1.2 g daily = 79.7 (59.5–92.0)%, 2.4 g daily = 64.3 (55.8–75.7)% and 4.8 g daily = 53.0 (45.0–73.4)%; $P < 0.0001$].

The 24 h urinary and faecal excretion of 5ASA and *N*-acetyl 5ASA are shown in Figure 2. The urinary excretion of both 5ASA and *N*-acetyl 5ASA increased significantly with increasing oral dose. Again, a disproportionate (greater than four fold) increase in urinary 5ASA was seen with successive doubling of the oral dose whereas *N*-acetyl 5ASA increased in proportion to increases in the oral dose. Urinary *N*-acetyl 5ASA was found to predominate at all doses but with increasing oral dose the percentage of drug present in the urine as *N*-acetyl 5ASA decreased [1.2 g daily = 98.7 (76.9–100)%, 2.4 g daily = 88.7 (53.8–95.7)% and 4.8 g daily = 75.8 (61.6–81.0)%; $P < 0.0001$].

The 24 h faecal excretion of 5ASA increased substantially as the oral dose of mesalazine increased from 1.2 g to 2.4 g daily. However, the rise in faecal 5ASA excretion when the oral dose increased further to 4.8 g daily was modest and failed to reach statistical significance though this may have been partly due to variability in the data and the small sample size. In contrast, faecal *N*-acetyl 5ASA excretion was similar at all three ingested doses.

With increasing oral mesalazine dose the total drug recovery (5ASA plus *N*-acetyl 5ASA) from the urine increased [1.2 g daily = 21.2 (4.7–103)%, 2.4 g daily = 29.1 (15.7–90.5)% and 4.8 g daily = 40.3 (18.4–104)%, $P < 0.05$] with a corresponding fall in recovery from the stool [40.8 (14.1–66.2)%, 28.5 (1.9–82.6)% and 20.3 (1.6–149)%]. Total recovery of the ingested dose was similar at all doses [1.2 g daily = 69.3 (37.4–154)%, 2.4 g daily = 56.1 (32.7–173)% and 4.8 g daily = 66.5 (36.3–234)%].

Figure 2 (a) 24 h urinary excretion of 5ASA (□) and N-acetyl 5ASA (▨) (box and whisker plots) * $P < 0.05$ and (b) 24 h faecal excretion of 5ASA and N-acetyl 5ASA (box and whisker plots) * $P < 0.05$.

Figure 3 shows the rectal tissue concentration time profiles for 5ASA and N-acetyl 5ASA.

Rectal tissue concentrations were similar at all time points studied. On increasing the oral mesalazine dose from 1.2 g to 2.4 g daily both 5ASA and N-acetyl 5ASA tissue concentration increased. The rise in tissue 5ASA concentrations was substantial (four fold or more) at all time points and achieved statistical significance at 12.00 h (1.1(0–5.1) ng mg⁻¹ vs 4.3(0.8–9.6) ng mg⁻¹), 18.00 h (0.4(0–3.4) ng mg⁻¹ vs 9.3(0.3–36.7) ng mg⁻¹) and 21.00 h (0.7(0–12.8) ng mg⁻¹ vs 8.5 (1.3–29.3) ng mg⁻¹). An increase in tissue N-acetyl 5ASA was also seen on increasing the oral dose of mesalazine from 1.2 g to 2.4 g daily (1.5 fold or more) but these differences did not achieve statistical significance. On increasing the dose of mesalazine to 4.8 g daily neither rectal tissue 5ASA nor N-

acetyl 5ASA increased further. Tissue N-acetyl 5ASA was found to predominate at all doses but as the oral dose increased the percentage of tissue N-acetyl 5ASA fell [1.2 g dai1y = 77.5(70.6–86.2)%, 2.4 g dai1y = 65.3(40.3–69.1)% and 4.8 g dai1y = 57.7(33.0–69.6)%, $P < 0.01$].

Highly significant correlations were apparent between serum AUC and the corresponding 24 h urinary drug excretion (5-ASA $r = 0.84$, $P < 0.0001$ and N-acetyl 5-ASA $r = 0.74$, $P < 0.0001$; Figure 4) but no correlations were found between serum and tissue concentration of either 5-ASA or N-acetyl-5ASA.

Discussion

Despite its widespread clinical use in patients with ulcerative colitis, the mode of action of 5ASA remains unknown. The drug probably acts locally since systemic concentrations of 5ASA are low following both oral and rectal dosing [12, 13]. Its fluorescent properties enable it to be localized microscopically, and following both oral and rectal dosing, fluorescence is seen within colonic epithelial cells and throughout the lamina propria. Cells with the morphological characteristics of macrophages seem to take up the drug avidly [20].

Getting oral 5ASA to its site of action, however, necessitates some form of colonic delivery system since the drug is unstable in gastric acid and is rapidly absorbed from the small intestine [21]. Slow-release and delayed-release coatings and azo-bonding to an inert carrier are the usual methods and the relative merits of the two systems are still a subject of interest [22].

A considerable body of pharmacokinetic literature demonstrates marked variability in 5ASA metabolism and distribution following oral dosing. Depending on the dose and type of formulation ingested 15–67% of the drug is absorbed and excreted in the urine, mostly in the acetylated form, while 24–67% is passed in the stool, approximately half in acetylated form and up to 45% of the ingested dose cannot be accounted for [23–26]. Comparative studies suggest the systemic absorption of 5ASA is lower from azo-bonded preparations than from coated formulations [12, 27–29].

5ASA is extensively metabolized *in vivo* but the principal site of acetylation and its relevance to the drug's mode of action remain unclear. Colonic epithelial cells absorb and acetylate 5ASA rapidly [30] but the amount of drug excreted in the stool in acetylated form correlates with transit time suggesting a role for luminal metabolism [31]. N-acetyl 5ASA, however, is poorly absorbed by epithelial cells [30] and this may explain its apparent lack of therapeutic efficacy when administered as topical treatment [32, 33].

Since many of the *in vitro* actions of 5ASA appear dose-related [14–17], tissue drug concentrations may be an

Table 1 Serum pharmacokinetic variables following oral mesalazine dosing.

Oral dose		1.2 g day ⁻¹	2.4 g day ⁻¹	4.8 g day ⁻¹
$C_{\max}$ ($\mu\text{g ml}^{-1}$)	5ASA	1.1(0.1–8.6)	*3.3(0.4–9.2)	*6.0(1.4–4.2)
	NA5ASA	2.2(1.4–8.7)	4.0(0.8–8.5)	*5.9(1.8–10.6)
$C_{\min}$ ($\mu\text{g ml}^{-1}$)	5ASA	0.03(0–0.2)	*0.2(0.03–1.1)	*1.3(0.4–2.8)
	NA5ASA	0.4(0.1–1.0)	*0.8(0.3–2.2)	*2.2(1–3.9)
AUC ($\mu\text{g ml}^{-1} \text{ h}$)	5ASA	3.9(0.6–4.3)	*15.4(2.5–66)	*47(8.5–139)
	NA5ASA	17.2(7–64)	*31(6–84)	*58(17–117)

All values expressed as median (range).

*denotes significant ($P < 0.05$) between dose differences.

important determinant of therapeutic response. The factors that govern these concentrations, however, are unknown. Although serum 5ASA concentrations increase with increasing oral 5ASA dose [23, 27], the relationship between oral dose and tissue concentrations has not been studied. Furthermore, clinical trials utilising high-dose oral 5ASA in the maintenance of ulcerative colitis remission have yielded somewhat disappointing results [5–11].

In the present study, we have shown that increasing the oral dose of delayed-release mesalazine from 1.2 g to 2.4 g daily is associated with a substantial rise in rectal tissue concentrations of 5ASA (four fold or more) whilst increasing the oral dose further to 4.8 g daily had no significant additional effect. An increase in rectal tissue concentrations of *N*-acetyl 5ASA was also apparent on increasing the oral dose from 1.2 g to 2.4 g daily but this failed to reach statistical significance. No significant correlations between tissue and serum concentrations were apparent but it should be noted that intersubject variability was high.

The total recovery of 5ASA and *N*-acetyl 5ASA from urine and stool was similar at all three oral doses ($\approx 60\%$) and comparable with other studies [26, 28]. With increasing dose, both the absolute amount and the proportion of drug appearing in the urine increased whereas the proportion appearing in the stool decreased. In addition, the proportion of drug present as *N*-acetyl 5ASA in both urine and stool decreased with increasing dose suggesting saturation of the acetylation processes. Disproportionate increases in serum concentrations and urinary 5ASA with higher oral doses of delayed-release mesalazine have been noted by others [23, 27]. At all three doses, peak serum concentrations were found during the morning and it has been suggested that this may relate to the night-time clearance of tablets from the stomach with subsequent release in the distal small intestine and colon [34].

In contrast to the wealth of traditional pharmacokinetic data, there is little information relating to tissue concentrations of 5ASA and *N*-acetyl 5ASA. De Vos *et al.* measured 5ASA and *N*-acetyl 5ASA tissue drug concentration in biopsies taken during colonoscopy from patients

with irritable bowel syndrome [20]. The concentrations reported were somewhat higher than those found in the present study but this may relate to differences in bowel preparation since we have found that washing the mucosal

Figure 3 (a) Rectal tissue time-concentration profiles of 5ASA (median and interquartile range) and (b) rectal tissue time-concentration profiles of *N*-acetyl 5ASA (median and interquartile range). ○ mesalazine 400 mg three times daily; ■ mesalazine 800 mg three times daily; △ mesalazine 1600 mg three times daily.

Figure 4 (a) Correlations between 5ASA serum AUC and 24 h urinary excretion (5ASA, $r=0.84$, $P<0.0001$) and (b) correlations between *N*-acetyl 5ASA serum AUC and 24 h urinary excretion (*N*-acetyl 5ASA, $r=0.74$, $P<0.0001$).

surface prior to biopsy brings about a significant fall in apparent tissue drug concentrations by removing surface faecal contamination [19].

Since increasing the oral dose of mesalazine does not result in a *pro rata* increase in tissue concentrations other determinants of tissue absorption, metabolism and excretion merit study. Pharmaceutical variables, such as formulation type and route of administration, may be important. Green *et al.* [22] for example, have recently reported that the azo-bonded formulation, balsalazide, is more effective than delayed-release mesalazine in active colitis and this may reflect differences in drug delivery and hence tissue concentrations. Furthermore, patients with active distal colitis who fail oral treatment frequently respond to mesalazine enemas and rectal formulations are

increasingly used as maintenance therapies in patients who suffer frequent relapse [36, 37] suggesting that route of administration may also be important. The influence of physiological factors such as intestinal transit, luminal pH and epithelial permeability has yet to be studied but such factors are likely to be of considerable importance as they determine absorption of 5ASA from the colonic lumen where drug concentrations are 100 fold greater than in the tissues.

Not surprisingly, we have demonstrated that higher doses of oral mesalazine are associated with higher serum concentrations and urinary excretion and this clearly increases the potential for systemic toxicity. Although, on present evidence, much of the toxicity of mesalazine appears independent of dose [38, 39], a recent study suggests an

increased prevalence of tubular proteinuria during high-dose maintenance therapy [40] and it is clearly appropriate to minimize systemic absorption wherever possible.

We recognize that the present study has limitations. Firstly, we have studied healthy volunteers rather than patients with ulcerative colitis. Since active colitis is associated with changes in transit [41], luminal pH [42] and epithelial permeability [43] our results probably have more relevance to patients with quiescent colitis. It is worth noting that for a given oral dose of delayed-release mesalazine healthy volunteers and patients with inactive colitis have similar tissue concentrations of 5ASA and *N*-acetyl 5ASA [19]. Secondly, tissue drug concentrations were measured only in the rectum. This study design was prompted by ease of access and a concern that bowel preparation prior to colonoscopy may invalidate interpretation of tissue drug concentrations [35]. Finally, we have measured drug concentrations in whole tissue biopsies. The relevance of drug concentrations in cellular, subcellular and noncellular tissue compartments remain to be determined.

In conclusion, we have shown that the relationship between oral mesalazine dose and rectal tissue concentrations is complex. Tissue concentrations of 5ASA increased markedly when the dose of delayed-release mesalazine was increased from 1.2 to 2.4 g daily but no further increase was seen with 4.8 g daily dosing despite a marked increase in systemic concentrations. Our results may explain why trials of high-dose oral 5ASA in the maintenance of ulcerative colitis remission have yielded disappointing results.

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