Disposition of 5-aminosalicylic acid by olsalazine and three mesalazine preparations in patients with ulcerative colitis: comparison of intraluminal colonic concentrations, serum values, and urinary excretion

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Abstract

To compare the disposition of 5-aminosalicylic acid (5-ASA) and its acetylated metabolite during treatment with olsalazine and mesalazine, 14 patients with inactive ulcerative colitis were randomly assigned to olsalazine (1 g twice daily) and the mesalazines, Asacol (800+400+800 mg daily), Pentasa (750+500+750 mg daily), and Salofalk (750+500+750 mg daily) in a crossover design trial so that all received each drug for seven days. Intraluminal colonic concentrations of 5-ASA were estimated after five days by the method of equilibrium in vivo dialysis of faeces. A predose serum sample and a 24 hour urine collection were obtained on day seven. The 5-ASA and acetyl-5-aminosalicylic acid (Ac-5-ASA) values were determined by high performance liquid chromatography. Olsalazine almost doubled the colonic concentrations (mean 23.7 (SEM) (1.9) mmol/l) of its therapeutically active ingredient (5-ASA) compared with equimolar doses of Pentasa (12.6 (2.2) mmol/l; $p<0.0003$) and Salofalk (15.0 (2.0) mmol/l; $p<0.003$). At the same time, olsalazine treatment was associated with lower serum concentrations and urinary excretions ($p<0.05$) of 5-ASA and Ac-5-ASA compared with the mesalazine preparations. The low systemic load of 5-ASA provided by olsalazine reduces the potential risk of nephrotoxicity during long term treatment.

foundation of rational pharmacotherapy with this drug, whether it depends on the activity of bacterial azoreductases or the physicochemical properties of the drug. Thus, knowledge of the disposition is mandatory for exploring dose response effects, in addition to being of potential toxicological interest. The purpose of the present study was, therefore, to compare the efficiency of delivery of the active principle 5-ASA to the colon and the systemic load as a basis for potential long term toxicity during treatment with olsalazine and three controlled release mesalazine preparations (Asacol, Pentasa, and Salofalk) in patients with inactive ulcerative colitis.

Methods

PATIENTS

Fourteen patients with ulcerative colitis in remission took part in the study (Table I). Apart from the study drugs, and in some cases oral contraceptives, no medication was given during the study. The diagnosis of ulcerative colitis had previously been established on the basis of symptoms, sigmoidoscopic appearance, histology of the rectal mucosa, and radiologic appearance.¹⁸ Patients were considered in remission when they were symptom free – that is, stool frequency less than two a day, no discharge of blood, pus, or mucus from the rectum, normal sigmoidoscopic appearance, and no appreciable inflammation seen on rectal biopsy specimen. Each participant gave informed consent. The study was done in accordance with the Helsinki Declaration II and was approved by the regional ethics committee.

Before entry and on each study day laboratory screening was done. This included blood haemoglobin concentration, reticulocyte count, erythrocyte sedimentation rate, leucocyte count, leucocyte differential count, platelet count, serum concentrations of Na, K, Ca, albumin, orosomucoid, creatinine, urea, alanine amino-

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Since it became clear that 5-aminosalicylic acid (5-ASA) is the therapeutically active moiety of sulphasalazine in treating ulcerative colitis and that sulphapyridine simply acts as a carrier to ensure that the active part is released within the bowel,^{1,2} a 'second generation of sulphasalazine' has emerged.³ Orally ingested plain 5-ASA is rapidly and completely absorbed from the upper gastrointestinal tract.⁴⁻⁶ By contrast, it is poorly absorbed in the colon, where it probably acts from the luminal side.^{4,7} Independent of acetylator phenotype, 5-ASA is subsequently acetylated, mainly in the gut wall,⁸⁻¹⁰ to N-acetyl-5-aminosalicylic acid (Ac-5-ASA). This metabolite is considered to be therapeutically inert.¹¹ A number of 5-ASA delivery systems has, therefore, been developed. These are either sulphapyridine-free azobond analogues of sulphasalazine, such as olsalazine,^{12,13} or enteric coated (delayed release) or slow release 5-ASA (mesalazine) preparations, such as Asacol,^{14,15} Pentasa,¹⁶ and Salofalk.¹⁷

Control of the disposition of 5-ASA is the

TABLE I Patients' details

Sex, male/female	7/7
Age (yrs), mean (range)	44 (25-69)
Height (cm), mean (range)	169 (152-191)
Weight (kg), mean (range)	74 (53-97)
Duration of disease (yrs), mean (range)	9 (2-17)
Duration of remission (mths), mean (range)	16 (1-50)
Location of disease:	
Proctosigmoiditis	7
Left sided colitis	4
Pancolitis	3

transferase, lactate dehydrogenase, alkaline phosphatase, bilirubin, and plasma prothrombin, in addition to urine analyses for protein and glucose and microscopy for erythrocytes, leucocytes, and casts.

FORMULATION OF OLSALAZINE AND MESALAZINE

Olsalazine (disodium azodisalicylate; Dipentum; Pharmacia AB, Uppsala, Sweden), which is one molecule of 5-ASA diazotised to another, was provided either in enteric coated tablets containing 500 mg of the drug or in gelatin capsules, each of which contained 250 mg of the drug.¹² Olsalazine has been shown to be poorly absorbed from the small intestine and reaches the colon intact,¹⁹ where it is activated into two molecules of 5-ASA.¹²

Asacol (Tillotts Laboratories, Henlow, UK) was provided as tablets containing 400 mg of 5-ASA coated by a 120 µm thick acrylic based resin (Eudragit S). Asacol has been designed to disintegrate at a pH above 7, and most has been shown to break up in the terminal ileum or the ascending colon.¹⁵

Pentasa (Ferring A/S, Copenhagen, Denmark) was provided as tablets containing 250 mg of 5-ASA in microgranules coated with a semipermeable membrane of ethylcellulose.¹⁶ Because of the amphoteric properties of ethylcellulose, 5-ASA is slowly released from the granules into the intestine continuously over time, but enhanced with pH above 6.¹⁶

Salofalk (Dr Falk GmbH & Co, Freiburg, FRG; in some countries marketed as Claversal or Mesasal) was provided as tablets containing 250 mg of 5-ASA in a buffer system of sodium-carbonate and glycine protected by ethylcellulose and an outer coating of the resin Eudragit-L.¹⁷ Salofalk has been shown to break up at pH above 5.5, delivering its contents mainly to the distal part of the ileum and the colon.

EXPERIMENTAL DESIGN

Olsalazine versus mesalazine. In the first part of the study olsalazine was compared with each of the three mesalazine preparations – Asacol, Pentasa, and Salofalk. A crossover design was applied. According to a computer generated randomisation list, all patients received each of the following regimens in random order for seven days:

Olsalazine, 500 mg enteric coated tablets, two tablets in the morning and two in the evening;

Asacol, 400 mg tablets, two tablets in the morning, one at noon, and two in the evening;

Pentasa, 250 mg tablets, three tablets in the morning, two at noon, and three in the evening; and

Salofalk, 250 mg tablets, three tablets in the morning, two at noon, and three in the evening.

These regimens (2 g daily) provided near equimolar doses of 5-ASA – that is, 11.6 mmol of 5-ASA by olsalazine (disodium azodisalicylate) and 13.1 mmol of 5-ASA by each of the mesalazine preparations.

Intraluminal colonic concentrations of 5-ASA and Ac-5-ASA were determined in free faecal

water during each treatment period after five days using the method of equilibrium in vivo dialysis of faeces (see below). Faeces were collected until the dialysis bags had been recovered and the stool output was quantified. The 24 hour faecal excretion of 5-ASA and Ac-5-ASA was estimated as the 24 hour stool output × concentration in free faecal water × 0.8 (this fraction²⁰ being used to approximate free faecal water). On day seven, a morning predose serum sample and a 24 hour urine collection were obtained for determination of 5-ASA and Ac-5-ASA and for calculation of their 24 hour urinary excretions.

Patient compliance was checked after each treatment period by counting the amount of unused study drug. A diary had been provided for recording the number of bowel movements, the presence or absence of blood, and unexpected events, if any.

Olsalazine enteric coated tablets versus olsalazine capsules. After the above four treatment periods had been completed the patients were assigned in random order to each of two olsalazine formulations for 21 days: 500 mg enteric coated tablets, one tablet in the morning and one in the evening and 250 mg gelatin capsules, two capsules in the morning and two in the evening. At the end of each study period in vivo dialysis of faeces, the quantity of stool output, a morning predose serum sample, and a 24 hour urine collection were obtained for analyses and calculations as described above. Furthermore, the serum samples were analysed for olsalazine and olsalazine sulphate.

EQUILIBRIUM IN VIVO DIALYSIS OF FAECES

In vivo dialysis of faeces was done as previously described in detail¹³ by pooling the contents of five swallowed dialysis bags after their intestinal transit (faecal dialysates). The bags had been made by tying off 4 cm segments of Visking seamless cellulose tubing 8/32 filled with Rheomacrodex (Pharmacia) containing 10% dextran (mean mol wt 40 000) in saline. The faecal dialysates were stored immediately at –20°C. The samples were analysed for concentrations of 5-ASA, Ac-5-ASA, and olsalazine (the latter only performed on samples obtained during the two three week regimens on olsalazine). The validations to ensure that these compounds had reached an equilibrium have previously been described.¹³ Finally, the dialysates were analysed for concentrations of prostaglandin E₂ and leucotriene B₄ as estimates of disease activity.^{21,22}

ANALYTICAL PROCEDURES

Determination of olsalazine, 5-ASA, and Ac-5-ASA in serum, urine, and faecal dialysates were done by high performance liquid chromatography as previously described.^{12,13} The lower detection limits in serum, urine, and faecal dialysates were 0.3 µmol/l, 6 µmol/l, and 6 µmol/l respectively. All analyses were done on coded samples to retain blindness until the data collection had been completed.

STATISTICAL ANALYSIS

All values were expressed as mean (SEM). The data comparing olsalazine and the mesalazine preparations were analysed by the SAS computer programme²³ using Friedman's one way analysis of variance and multiple range test (general linear models procedure). To ensure overall protection level, preplanned comparisons were defined as comparisons between olsalazine and the mesalazine preparations, and only these probabilities are reported.²³ Data comparing the two olsalazine formulations were also analysed by a general linear models procedure reporting the effect of period, treatment, and sequence.²³ Values of $p < 0.05$ were considered significant.

Results

DRUG TOLERANCE AND COMPLIANCE

All drug formulations were well tolerated and all patients completed the study according to the protocol. Based on tablet counts, all patients had complied with the drug regimen. Based on the diaries, all remained in remission throughout the study and no adverse events were reported. The laboratory screening was uneventful and the concentrations of prostaglandin E₂ and leucotriene B₄ concentrations in the faecal dialysates remained within the ranges observed in normal subjects (< 1.0 ng/ml).

CONCENTRATIONS IN FREE FAECAL WATER AND FAECAL RECOVERY OF 5-ASA AND Ac-5-ASA

Figure 1 shows the concentrations of 5-ASA and Ac-5-ASA in faecal dialysates. Administration of olsalazine almost doubled the colonic concentrations of 5-ASA (23.7 (1.9) mmol/l; $p < 0.0003$) or Salofalk (15.0 (2.0) mmol/l; $p < 0.003$), but not when compared with that of Asacol (23.3 (3.1) mmol/l; $p > 0.05$). Nonetheless, a greater interindividual variation in faecal concentrations of 5-ASA was noted during Asacol treatment as indicated in Figure 1 (SEM). In contrast to olsalazine and Asacol, the major proportion of faecal 5-ASA provided by Pentasa or Salofalk was recovered as the acetyl-inactivated form (Fig 1). The 24 hour faecal

Figure 1: Concentrations in faecal dialysates of 5-aminosalicylic acid (□) and acetyl-5-aminosalicylic acid (▨) after ingestion (2 g/day) of olsalazine (Dipentum) or the mesalazines, Asacol, Pentasa, and Salofalk, for 7 days. Values are mean (SEM).

excretions and the percentage recovery of 5-ASA (of daily ingested dose) provided by the various formulations are listed in Table II and show a similar pattern. The faecal weight was unaltered by treatment or period.

SERUM CONCENTRATIONS OF 5-ASA AND Ac-5-ASA

Table II shows the morning predose concentrations of 5-ASA and Ac-5-ASA in serum. The concentrations of both 5-ASA and the acetylated metabolite were significantly lower ($p < 0.05$) during olsalazine treatment than during treatment with Asacol or Salofalk, but the higher concentrations obtained during Pentasa treatment did not reach statistical significance compared with olsalazine.

TABLE II Mean (SEM) serum concentrations and 24 hour faecal and urinary excretions of 5 aminosalicylic acid (5-ASA) and acetyl-5-aminosalicylic acid (Ac-5-ASA) and recovery of daily ingested dose during administration of olsalazine and the mesalazines, Asacol, Pentasa, and Salofalk for 7 days

Daily ingested dose (equivalent to 2 g/day)	Drug			
	Olsalazine (11.6 mmol)	Asacol (13.1 mmol)	Pentasa (13.1 mmol)	Salofalk (13.1 mmol)
Serum concentration				
5-ASA (mmol/l)	0.0024 (0.0006)	0.0064 (0.0015)*	0.0026 (0.0008)	0.0137 (0.0065)*
Ac-5-ASA (mmol/l)	0.0054 (0.0010)	0.0115 (0.0022)*	0.0081 (0.0016)	0.0183 (0.0047)*
24 hour faecal excretion				
Faecal weight (g/day)	235 (36)	206 (26)	188 (28)	241 (39)
5-ASA (mmol)	4.2 (0.6)	3.7 (0.5)	1.7 (0.3)*	2.8 (0.6)*
Ac-5-ASA (mmol)	1.9 (0.3)	2.0 (0.3)	3.3 (0.4)*	2.9 (0.4)
24 hour urinary excretion				
Urine volume (l/day)	1.525 (0.129)	1.413 (0.107)	1.353 (0.144)	1.439 (0.105)
5-ASA (mmol)	0.3 (0.1)	0.8 (0.2)*	0.8 (0.3)*	1.7 (0.5)*
Ac-5-ASA (mmol)	2.2 (0.4)	3.2 (0.7)*	4.0 (1.0)*	5.4 (1.2)*
Recovery (5-ASA + Ac-5-ASA)				
Urine (%)	22 (3)	31 (7)*	36 (10)*	54 (12)*
Faeces (%)	53 (7)	44 (5)	38 (5)	44 (6)
Total (%)	75 (8)	74 (10)	74 (12)	98 (12)

* $p < 0.05$ compared with olsalazine.

Figure 2: 24 Hour urinary excretion (% of intake) of 5-aminosalicylic acid (5-ASA) and its acetylated metabolite (Ac-5-ASA) after ingestion (2 g/day) of olsalazine (Dipentum) or the mesalazines, Asacol, Pentasa, and Salofalk for 7 days. Values are mean (SEM).

URINARY EXCRETIONS AND TOTAL RECOVERY OF 5-ASA AND Ac-5-ASA

Figure 2 shows that the urinary excretion of free and acetylated 5-ASA was noticeably lower during olsalazine treatment than during administration of any of the mesalazine preparations. The 24 hour urinary excretions, in addition to the total percentage recovery (of daily ingested dose), are listed in Table II.

OLSALAZINE FORMULATIONS

Table III shows the serum concentrations of 5-ASA, Ac-5-ASA, olsalazine, and its sulphated metabolite, in addition to the concentrations of 5-ASA, Ac-5-ASA, and unsplit olsalazine in faecal dialysates and the 24 hour faecal and

TABLE III Mean (SEM) concentrations of 5-aminosalicylic acid (5-ASA), acetyl-5-aminosalicylic acid (Ac-5-ASA), olsalazine, and olsalazine sulphate in serum and concentrations in faecal dialysates and 24 hour faecal and urinary excretions of 5-ASA, Ac-5-ASA, and olsalazine during administration of two different formulations of olsalazine for 21 days

	Olsalazine (0.5 g twice daily)	
	Enteric coated tablets	Capsules
Serum concentration		
5-ASA (mmol/l)	0.0011 (0.0002)	0.0008 (0.0002)
Ac-5-ASA (mmol/l)	0.0035 (0.0007)	0.0035 (0.0004)
Olsalazine (mmol/l)	0.0006 (0.0001)	0.0008 (0.0001)
Olsalazine-sulphate (mmol/l)	0.0032 (0.0005)	0.0099 (0.0009)*
Concentrations in faecal dialysates		
5-ASA (mmol/l)	11.4 (2.2)	10.0 (1.3)
Ac-5-ASA (mmol/l)	9.7 (1.5)	10.1 (1.5)
Olsalazine (mmol/l)	0.133 (0.069)	0.031 (0.013)
24 hour faecal excretion		
Faecal weight (g/day)	183 (23)	186 (20)
5-ASA (mmol)	1.5 (0.3)	1.5 (0.3)
Ac-5-ASA (mmol)	1.2 (0.2)	1.4 (0.2)
Olsalazine (mmol)	0.020 (0.010)	0.005 (0.002)
24 hour urinary excretion		
Urine volume (l/day)	1.318 (0.117)	1.409 (0.100)
5-ASA (mmol)	0.03 (0.01)	0.03 (0.01)
Ac-5-ASA (mmol)	0.96 (0.19)	0.93 (0.13)
Olsalazine + olsalazine-sulphate (mmol)	0.0035 (0.0003)	0.0094 (0.0013)*

*p<0.05.

urinary excretions during administration of the two different formulations of olsalazine. The statistical analyses did not show any effects of period or drug sequence. The serum concentrations of olsalazine sulphate and the urinary excretions of olsalazine (free+sulphated) were higher during treatment with olsalazine formulated in capsules than when enteric coated tablets were given (p<0.001). Otherwise, no effects of the formulation on the disposition of olsalazine, 5-ASA, and Ac-5-ASA were shown. In particular, the low concentrations of olsalazine in the faecal dialysates indicated almost complete azoreduction of this compound regardless of the formulation.

Discussion

The results of the present study show that treatment with olsalazine almost doubles the colonic concentrations as estimated by the method of in vivo dialysis of faeces when compared with equimolar doses of the slow release mesalazine preparation Pentasa and the delayed release preparation Salofalk. By contrast, the colonic concentrations provided by the delayed release preparation Asacol were similar to those provided by olsalazine. The interindividual variation was larger, however, indicating either a peak concentration when the tablets disintegrate or inconsistency in the site of disintegration.²⁴⁻²⁶ The present findings also show a significantly lower systemic absorption of 5-ASA and its acetylated metabolite during the administration of olsalazine in comparison with any of the mesalazine preparations. These results agree with previous non-comparative data on olsalazine,^{12 13 22} Asacol,¹⁵ Pentasa,^{16 27} and Salofalk,^{17 28 29} and with results from studies in healthy volunteers and comparisons of the disposition of 5-ASA provided by single⁶ or repeated²⁶ doses of various mesalazine formulations^{6 26} or single oral doses of mesalazine and olsalazine.³⁰

The Pentasa preparation provided the highest colonic concentrations of Ac-5-ASA in the present study. The 5-ASA released during intestinal transit of the preparation is continuously inactivated by acetylation.¹⁰ Only 22-45% of the ingested dose remains in the lumen after the intestinal contents have reached the colon and half is retained in the microgranules,^{16 26} which may further account for a lower local concentration of 5-ASA. Treatment with Salofalk was associated with the highest serum concentrations and urinary excretions of 5-ASA and Ac-5-ASA. These data suggest an early liberation of 5-ASA in the small intestine, but the great variation indicate inconsistency in the site of disintegration.

The potential toxicity of 5-ASA should be considered, in particular during long term treatment with 5-ASA delivering compounds in doses above 1 g/day. Most interest has been focused on the potential renal abnormalities due to 5-ASA. The drug has structural similarities to phenacetin,³¹ and has caused papillary necrosis when given intravenously in high doses to rats.³² A putative nephrotic syndrome and an interstitial nephritis after treatment with Asacol and

Salofalk, respectively, have been reported.³³⁻³⁴ Finally, a two year follow up of patients on Asacol has shown an incidence of pyuria of more than 50%.³⁵ Hence, the lowest possible systemic load of 5-ASA should be sought in the choice of a system for effective delivery of 5-ASA to the inflamed colon.

Previous studies on sulphasalazine maintenance treatment (2 g/day) showed colonic concentrations of 5-ASA between 3.9 mmol/l and 12 mmol/l.¹² In the present series with the use of 2 g/day, which is comparable with 4.6 g of sulphasalazine, the mean concentration of 5-ASA in faecal dialysis fluid ranged from 12.6 mmol/l (Pentasa) to 23.7 mmol/l (olsalazine). Based on these findings, a clinical efficacy in patients with ulcerative colitis comparable with that of sulphasalazine would be expected for all four preparations. Indeed, evidence is accumulating that all four delivery systems are effective in mildly and moderately active disease³⁶⁻⁴⁰ and about as effective as sulphasalazine in relapse prevention.⁴¹⁻⁴⁶ By contrast, the detection of a clinically relevant (but small) difference between these treatments with an adequate statistical power in controlled clinical trials would require a large number of patients in each treatment group. As yet no studies comparing clinical efficacy of these drugs have been published. In particular, there is no evidence that any of the new compounds is superior to sulphasalazine. Although some studies suggest a dose response effect,³⁷⁻³⁹ the initial hope, also based on experience with rectal applications,⁴⁷ has not yet been fulfilled, and certainly not as regards maintenance treatment.⁴⁸

The high intraluminal colonic concentrations and the low systemic load of 5-ASA provided by olsalazine make this compound the one best suited for long term treatment and further exploration of the dose response in patients with ulcerative colitis. Prolonged administration of doses up to 4 g/day seems well tolerated in active disease too,²² although loose stools occur transiently in some patients with extensive disease. Regardless of the formulation of olsalazine (enteric coated tablet *versus* capsule), the present data show a nearly complete splitting of the azobond. In theory, the enteric coated formulation seems the more attractive because of the reduced systemic load of the unsplit compound and its sulphated metabolite.

In conclusion, olsalazine almost doubles colonic concentrations of its therapeutically active ingredient 5-ASA compared with equimolar doses of the mesalazine preparations, Pentasa and Salofalk. At the same time the low systemic load of 5-ASA provided by olsalazine reduces the potential risk of nephrotoxicity. Hence, olsalazine is particularly well suited for maintaining remission and exploring a dose response efficacy of 5-ASA through controlled trials in patients with ulcerative colitis.

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