

Concentrations of 5-ASA and Ac-5-ASA in human ileocolonic biopsy homogenates after oral 5-ASA preparations

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Abstract

Intramucosal 5-aminosalicylic acid (5-ASA) and acetylated 5-ASA (Ac-5-ASA) concentrations were determined in ileocolonic biopsy specimens from 61 patients with irritable bowel syndrome treated for one week with near equimolar doses of different slow release preparations of 5-ASA (Claversal, Asacol, or Pentasa) or azo-bound drugs (Salazopyrin, Dipentum). The transit time in these patients was accelerated by a laxative, metoclopramide, and colonic lavage. The presence of 5-ASA in the mucosa was confirmed by autofluorescence. The highest concentrations of 5-ASA were obtained after Asacol (mean (SEM), 298.5 (37.3) ng/mg wet wt), followed by Claversal 500 mg (108.8 (11.7) ng/mg wet wt) and Pentasa (25.7 (2.2) ng/mg wet wt). Very low concentrations only were observed after Claversal 250 mg (0.3 (0.03) ng/mg wet wt), Salazopyrine (1.2 (0.1) ng/mg wet wt), and Dipentum (11.0 (3.2) ng/mg wet wt). The results for Ac-5-ASA were similar but the concentrations were generally lower. Serum concentration-time curves over eight hours were obtained from 34 healthy volunteers after a single oral dose of 400 to 500 mg of the different drugs. For the slow release forms, an apparently inverse relationship was found between the area under the curve of the serum concentrations and the intramucosal concentrations, supporting the importance of the local availability of the drug. This inverse relationship was absent for the azo-bound drugs. Colonic washout induced mechanical removal of intraluminal 5-ASA with a secondary disturbance in absorption resulting in a rapid decline in the serum concentrations. However, only for Dipentum did this result in significantly lower 5-ASA mucosal concentrations. This is the first reported attempt to evaluate the mucosal availability of 5-ASA after different oral preparations. It shows that where transit time is accelerated higher mucosal concentrations occur after slow release preparations (except for Claversal 250 mg) than after azo-bound drugs. Additional studies are necessary to correlate these concentrations with clinical effects.

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5-Aminosalicylic acid (5-ASA) is the active moiety of sulphasalazine in inflammatory bowel disease.¹⁻³ Because orally ingested, uncoated 5-ASA is almost completely absorbed in the stomach and the proximal small intestine,⁴ several methods have been used to delay and

prevent this proximal absorption. In delayed release preparations (Asacol, Claversal), 5-ASA is coated with an acrylic based resin that dissolves at a pH greater than 6. In a slow release preparation (Pentasa), 5-ASA is encapsulated in microspheres of ethyl-cellulose that gradually dissolve in the small intestine. Finally, in azo-bound drugs, bacterial azoreductases liberate two 5-ASA molecules (Dipentum) or split off 5-ASA from sulphapyridine (Salazopyrin).

The presumptive topical mode of action of 5-ASA^{5,6} suggests that it would be best to obtain a maximal concentration at the site of disease (the terminal ileum and colorectum). The urinary output of 5-ASA reflects the fraction that is absorbed in the small intestine, and that is not therefore available for the topical effect; the faecal concentrations represent the residual 5-ASA present in the bowel lumen. An 'ideal' 5-ASA containing preparation would give a low plasma concentration and urinary recovery but high faecal concentration. Faecal concentrations, however, provide only an indirect estimate of the drug available in the tissues. We have therefore developed a high performance liquid chromatography (HPLC) system to determine 5-ASA and its metabolite acetylated 5-ASA (Ac-5-ASA) in biopsy specimen homogenates. We confirmed the presence of 5-ASA in the mucosa by autofluorescence and correlated these concentrations with serum concentration-time curves in healthy volunteers. At the same time we determined Ac-5-ASA concentrations to evaluate the mucosal acetylation.

Finally, we analysed the influence of oral colonic washout by comparing serum concentration-time curves in the steady state condition with those after the start of the lavage. We excluded this effect as sole explanation for the very low mucosal concentrations after Claversal 250 mg tablets or azo-bound drugs by comparing rectosigmoidal concentrations in patients with and without lavage.

Methods

SUBJECTS AND STUDY DESIGN

Patients

Sixty one patients (27 men and 34 women) (mean age: 41.8 years; range: 17-74 years) with irritable bowel syndrome were treated for one week with one of the following drugs:

Salazopyrin (Pharmacia AB, Uppsala, Sweden), 500 mg × 6 per day (n=22);

Pentasa (Gist Brocades, Delft, Netherlands) 250 mg × 6 per day (n=7);

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Dipentum (Pharmacia AB, Uppsala, Sweden) 250 mg × 6 per day (n=6);

Claversal (Smith Kline Dauelsberg, Göttingen, Germany) 500 mg × 3 per day (n=9), 250 mg × 6 per day (n=8);

Asacol (Byk Cedona, Haarlem, Netherlands) 400 mg × 3 per day (n=9).

These regimens provide near equal daily doses of 5-ASA. On days five to seven, 7.5 mg sodium-picosulfate (Laxoberon – Boehringer Ingelheim) were given to facilitate colonic washout. The last dose of 5-ASA was given at 6 am on day eight, after overnight fasting. No food or liquid was allowed until 9 am, when oral colonic lavage was started with an Endopeg solution (Vanden Bogaerde, Brussels, Belgium). Before each litre, 5 mg metoclopramide were given orally. Most patients needed 3 l (range 2–5 l) to obtain complete evacuation of the gut. Ileocolonoscopy was performed between 2 and 3 pm with an Olympus CF 10 endoscope under sedation with 5 mg midazolam and 50 mg pethidine. Two adjacent biopsy specimens were taken from the terminal ileum, the ileocaecal valve, and from eight different sites in the colon. The specimens were weighed immediately, frozen in liquid nitrogen, and stored at –80°C. The interval between taking and freezing the biopsy specimens was less than 30 minutes. In eight patients taking slow release preparations, the influence of colonic washout on the serum concentration-time curve was analysed by comparing the curves on days seven and eight. The influence of lavage was evaluated by comparing the corresponding concentrations on both days.

In 13 patients, the influence of the washout on the mucosal Ac-5-ASA and 5-ASA concentrations was analysed by determining the concen-

trations in rectal and sigmoid biopsy specimens from patients who had not undergone lavage. A similar time interval was respected between the intake of the last dose of drug and the endoscopy. Its influence was analysed for the preparations with very low mucosal concentrations (Dipentum (n=6), Salazopyrin (n=4) Claversal 250 mg tablets (n=3)). Six biopsies specimens were taken from every patient.

Finally, in a few patients treated with Asacol (n=2), Claversal 500 mg tablets (n=2), Salazopyrin (n=2), and Claversal 250 mg tablets (n=1) additional biopsy specimens were taken from the ileum, sigmoid, and rectum. The specimens were frozen immediately in liquid nitrogen and subjected to autofluorescence microscopy.

Volunteers

Serum concentration-time curves over eight hours were obtained from 34 healthy volunteers after a single oral intake of 500 mg Pentasa (n=5), two Claversal 250 mg tablets (n=5), one Claversal 500 mg tablet (n=8), 1000 mg Salazopyrin (n=5), 500 mg Dipentum (n=5), or 400 mg Asacol (n=6).

ASSAY METHODS

HPLC

HPLC was used to determine 5-ASA and Ac-5-ASA in serum and in biopsy specimen homogenates as previously described.⁷ The detection limit for both 5-ASA and Ac-5-ASA in biopsy homogenates was approximately 1 ng; the results were expressed as ng/mg wet wt. The detection limit for serum was 2 ng/ml.

TABLE I Steady state concentrations of 5-aminosalicylic acid (5-ASA) in biopsy specimen homogenates (ng/mg wet wt). (Values are mean (SEM))

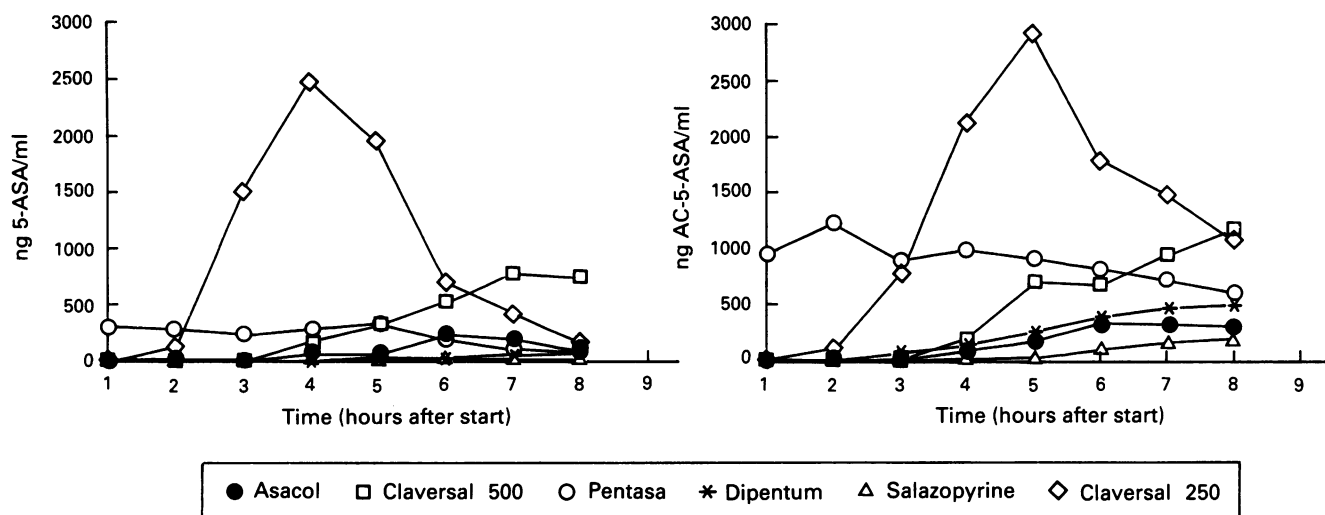
Product	ILE	ICV	CAE	ASC	HEP	TRA	SPL	DES	SIG	REC	Total
Salazopyrin (n=22)	1.8 (0.5)	1.0 (0.3)	1.2 (0.6)	1.5 (0.6)	0.8 (0.2)	1.2 (0.4)	1.2 (0.3)	1.1 (0.3)	0.9 (0.2)	1.4 (0.4)	1.2 (0.1)
Pentasa (n=7)	33.6 (7.8)	26.7 (4.0)	25.7 (5.1)	29.4 (6.7)	19.5 (4.6)	33.2 (12.5)	27.5 (8.0)	17.8 (3.5)	29.8 (7.8)	14.2 (2.3)	25.7 (2.2)
Dipentum (n=6)	9.1 (5.7)	6.6 (4.2)	4.4 (2.9)	27.2 (19.0)	17.6 (16.7)	10.2 (9.7)	13.9 (9.2)	8.7 (5.7)	7.2 (5.0)	4.0 (2.5)	11.0 (3.2)
Claversal 500 (n=9)	173.5 (58.1)	110.1 (30.0)	96.3 (35.3)	117.1 (37.8)	82.1 (23.6)	125.8 (41.4)	111.7 (33.4)	105.0 (39.3)	81.5 (28.4)	91.5 (31.1)	108.8 (11.7)
Claversal 250 (n=8)	0.5 (0.1)	0.4 (0.2)	0.2 (0.06)	0.2 (0.09)	0.2 (0.06)	0.2 (0.06)	0.2 (0.09)	0.2 (0.06)	0.2 (0.1)	0.2 (0.08)	0.3 (0.03)
Asacol (n=9)	477.3 (188.6)	576.6 (166.8)	525.9 (162.5)	370.0 (125.6)	237.7 (56.1)	320.1 (130.0)	300.0 (125.0)	123.2 (31.4)	118.0 (34.0)	108.5 (37.2)	298.5 (37.3)

ILE=terminal ileum; ICV=ileocaecal valve; CAE=caecum; ASC=ascending colon; HEP=hepatic flexure; TRA=transverse colon; SPL=splenic flexure; DES=descending colon; SIG=sigmoid colon; R=rectum.

TABLE II Steady state concentrations of Ac-5-ASA in biopsy specimen homogenates (ng/mg wet wt). (Values are mean (SEM))

Product	ILE	ICV	CAE	ASC	HEP	TRA	SPL	DES	SIG	REC	Total
Salazopyrin (n=22)	3.0 (0.6)	1.4 (0.3)	2.0 (0.8)	2.1 (0.5)	1.7 (0.4)	2.4 (0.4)	2.6 (0.5)	2.7 (0.5)	2.4 (0.5)	2.9 (0.7)	2.3 (0.2)
Pentasa (n=7)	12.3 (0.8)	12.0 (1.3)	19.1 (2.3)	24.9 (5.3)	24.2 (5.2)	26.2 (4.5)	20.4 (2.8)	23.9 (3.2)	25.5 (4.0)	17.4 (2.1)	20.6 (1.2)
Dipentum (n=6)	1.5 (0.7)	1.5 (0.7)	1.6 (0.7)	5.2 (3.0)	4.7 (4.0)	5.4 (4.4)	7.2 (4.3)	6.9 (3.8)	5.4 (2.9)	3.6 (1.7)	4.4 (1.0)
Claversal 500 (n=9)	25.3 (5.3)	24.1 (5.7)	29.4 (4.0)	36.6 (5.4)	38.6 (4.9)	42.8 (6.0)	38.9 (4.8)	34.0 (4.6)	27.4 (3.5)	27.9 (4.7)	32.4 (1.6)
Claversal 250 (n=8)	0.8 (0.3)	0.6 (0.2)	0.7 (0.2)	0.7 (0.3)	0.6 (0.3)	0.5 (0.2)	0.4 (0.1)	0.5 (0.2)	0.7 (0.3)	0.5 (0.2)	0.6 (0.07)
Asacol (n=9)	19.3 (3.8)	26.7 (6.1)	40.8 (6.8)	37.1 (4.8)	36.0 (4.3)	30.1 (3.8)	32.7 (4.1)	37.6 (6.3)	35.5 (6.4)	28.2 (5.5)	32.8 (1.7)

See Table I for abbreviations.



Mean plasma concentrations of Ac-5-ASA and 5-ASA in healthy subjects after a single dose of different aminosalicylates (400–500 mg 5-ASA).

Autofluorescence microscopy

All samples were snap frozen in liquid nitrogen. Cryostat sections (6 μ) were cut within one hour (Frigocut model 2700 Reichert-Jung). The slides were air dried for 10 minutes at room temperature and then embedded in Entellan.

The slides were examined immediately with an Orthomat microscope equipped with a Leitz Wetzlar fluorescence light source for epifluorescence. Any delay was avoided since the lack of fixation and instability of 5-ASA at room temperature and daylight exposure promotes the fading of the autofluorescence. Since 5-ASA fluoresces when excited with a light source at 300–400 nm, a heat filter (4 mm BG38 suppression filter), an excitation light filter (broad band incident light 2 mm UG1) and an emission light filter (suppression filter K 430) were applied.

Results

The mean (SEM) concentrations of 5-ASA and Ac-5-ASA in biopsy homogenates from different sites of the ileum and colorectum after drug intake are given in Tables I and II. The concentrations after the intake of Asacol, Claversal 500 mg tablets, and Pentasa are significantly ($p < 0.05$; Mann-Whitney U test) higher than after Claversal 250 mg tablets, Dipentum, and Salazopyrin. The serum concentration-time curves of 5-ASA and Ac-5-ASA after a single dose of the different preparations in healthy volunteers are given in the Figure. T_{max} , C_{max} , and AUC are listed in Table III. For the slow release preparations an apparent inverse

relationship is found between the AUC (Table III) and the mucosal concentrations (Table I). This inverse relationship is absent for the azo-bound drugs. Colonic washout reduces rapidly and significantly the serum concentrations of 5-ASA and Ac-5-ASA as seen in Table IV. The effect of the colonic washout on the mucosal 5-ASA concentrations after the intake of Salazopyrin and Claversal 250 mg tablets was only moderate. In contrast, a much higher effect was found for Dipentum (Table V).

Autofluorescence

Biopsy specimen homogenates from patients taking Claversal 250 mg tablets and Salazopyrin show only a faint fluorescence. In contrast, patients on Claversal 500 mg tablets and Asacol show considerable staining of the cytoplasm of the covering enterocytes and the presence of fluorescence in single cells in the lamina propria with irregular cytoplasmic extensions. These cells are probably macrophages.

Discussion

In steady state conditions, 5-ASA and Ac-5-ASA concentrations in biopsy homogenates depend on the galenic formulation and the chemical structure of the oral preparations tested. Significantly higher concentrations are found after most slow release preparations (Asacol, Pentasa, and Claversal 500 mg tablets) than after azo-bound preparations (Dipentum and Salazopyrin). A large intra- and interindividual variation is found, as reported in all previous pharmacokinetic studies of the different 5-ASA formulations.

The apparent inverse relationship between the AUC and the intramucosal concentrations of the slow release drugs supports the hypothesis that the local availability of the drug is the major determinant of the mucosal concentrations. After the intake of azo-bound drugs, the mucosal concentrations are very low despite a low systemic absorption. This suggests impaired delivery of 5-ASA, also found in the study of Rijk *et al*⁸ in which the influence of the acceleration of

TABLE III Area under the curve (AUC), time of maximal serum concentrations (T_{max}), and maximal concentrations (C_{max}) of 5-ASA and Ac-5-ASA after oral intake of single dose (400–500 mg) in healthy volunteers. (Values are mean (SEM))

Product	AUC (ng \times h/ml)		T_{max} (h)		C_{max} (ng/ml)	
	5-ASA	Ac-5-ASA	5-ASA	Ac-5-ASA	5-ASA	Ac-5-ASA
Salazopyrin (n=5)	63 (31)	382 (166)	7	8	25 (14)	190 (54)
Pentasa (n=5)	1842 (488)	6805 (1144)	5	4	304 (123)	987 (177)
Dipentum (n=5)	203 (67)	1550 (572)	8	8	76 (23)	504 (107)
Claversal 500 (n=8)	2133 (786)	2989 (900)	7	8	790 (590)	1160 (454)
Claversal 250 (n=5)	7270 (2850)	9699 (2248)	4	5	2484 (1271)	2910 (754)
Asacol (n=6)	496 (265)	1074 (483)	6	6	208 (145)	338 (171)

TABLE IV Influence of colonic washout on steady state serum concentrations (ng/ml) of Ac-5-ASA and 5-ASA four to six hours after the intake of the last dose (corresponding with one to three hours after start of washout) (n=8 patients on release modified preparations)

Interval (h)	Serum concentrations (mean (SEM))			
	5-ASA		Ac-5-ASA	
	Day 7	Day 8	Day 7	Day 8
4 (1)	1219 (664)	154 (79)	1704 (548)	799 (183)
5 (2)	678 (241)	68 (27)*	1521 (390)	491 (91)*
6 (3)	916 (368)	55 (34)**	1646 (318)	348 (109)**

Wilcoxon rank test: *p<0.05; **p<0.01.

Day 7 represents steady state serum concentrations without washout; day 8 represents serum concentrations on the day of the washout.

the intestinal transit on urinary and faecal excretion of several 5-ASA preparations was analysed.

The diarrhoea induced by the colonic washout in our study provokes mechanical removal of the intraluminal 5-ASA. This results, even in steady state, in a rapid decline in serum concentrations. This reduction excludes early dissolution of the coat by Endopeg (pH 7.4). Although this washout interferes with the pharmacokinetic profiles of the preparations, it is essential for the endoscopy and prevents the contamination of the biopsy specimens with the (Ac-)5-ASA from the stools. Furthermore, we can assume that this mechanical effect is similar for all preparations tested and does not greatly influence the conclusions about the results of the mucosal concentrations.

More difficult to study and quantify is the effect of the washout on the cleavage of the azo-bond. The acceleration of transit shortens the time available for the bacteria to split the azo-bond. The sterilisation of the gut by the washout can even prevent the cleavage. This effect seems more important for Dipentum than for Salazopyrin resulting in significantly higher mucosal concentrations in the rectum and sigmoid in patients who had not had previous washout. In contrast, mucosal concentrations after Salazopyrin remain low in both situations.

To minimise the effects of the washout we observed a delay of three hours between the intake of the last dose and the start of the washout to permit drug transit to the ileum, particularly because transit had already been accelerated in the previous three days by laxatives. This delay was based on our serum curves after single doses and on the studies of scintigraphic images of tablet disintegration showing a correlation with serum blood levels of 5-ASA.⁹ Our serum curves show the start of the release after one to three hours. Apparently no correlation is seen between the time of maximal release (T_{max}) and the mucosal concentrations suggest-

ing that although the release pattern of the different drugs has certainly influenced our results, this influence should not be overestimated.

A concentration gradient through the colon is not observed except for a slight predominance in the ileum and right hemicolon after Asacol and Claversal 500 mg corresponding to a large release of the drug.

To our knowledge, no previous studies of mucosal concentrations are available. Faecal concentrations are used as indirect estimates of the local concentrations. Comparative studies of faecal concentrations after different amino-salicylates are few and provide contradictory results. Staerk Laursen *et al*¹⁰ estimated colonic concentrations by means of in vivo dialysis of faeces and found that in patients in steady state with inactive ulcerative colitis concentrations after olsalazine (Dipentum) and Asacol were almost double those seen with equimolar doses of Pentasa and salofalk (Claversal). In contrast, Rijk *et al*⁸ evaluated the faecal excretions of healthy volunteers with accelerated transit time after a single dose. The trend in their results was similar to our findings: faecal excretion of 5-ASA was highest after Asacol, followed by Pentasa and Claversal, and lowest after Dipentum and Salazopyrin.

In our study, autofluorescence microscopy confirmed that 5-ASA penetrates the mucosa and does not only cover its surface. Moreover, this covering is reduced to a minimum by the colonic washout. The important differences in intramucosal concentrations after Claversal 250 and 550 mg tablets were intriguing. Both drugs differ not only in terms of the dosage but also in the composition of the coating, giving rise to a different bioavailability.

Finally, we found significant Ac-5-ASA concentrations in the biopsy homogenates. In contrast with serum, the ratios of 5-ASA to Ac-5-ASA are higher than 1, reflecting the abundant supply of 5-ASA. This acetylation is an irreversible¹¹ and partially local¹² process. However, the role of this probably therapeutically inactive metabolite¹³ remains unclear.

In conclusion, it can be said that during accelerated intestinal transit, slow release preparations, except for Claversal 250 mg tablets, provide higher steady state mucosal concentrations than azo-bound drugs. These concentrations are inversely related to the AUC in serum supporting the role of the local availability of the drugs. Because of the proved clinical efficacy of Salazopyrin, despite very low mucosal concentrations, studies to correlate these concentrations with clinical benefit are necessary.

TABLE V Comparison of steady state Ac-5-ASA and 5-ASA concentrations (ng/ml) in rectosigmoidal biopsy homogenates with and without colonic washout

Product	Without washout			With washout		
	No	5-ASA	Ac-5-ASA	No	5-ASA	Ac-5-ASA
Dipentum	4	19.7 (4.3)	40.2 (6.4)	6	5.5 (2.7)***	4.5 (1.7)***
Salazo	5	1.9 (0.5)	6.6 (1.1)	22	1.2 (0.1)*	2.3 (0.2)***
Claversal 250	3	2.5 (1.1)	8.4 (3.2)	8	0.2 (0.06)*	0.6 (0.2)**

Mann-Whitney U test: *NS; **p<0.05; ***p<0.01.

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