



Mesalazine vanishing time from rectal mucosa following its topical administration

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Abstract

To investigate how long and how much Mesalazine (M) is available inside the rectal mucosa following its topical instillation, in patients (pts) with Ulcerative Colitis (UC). Two rectal biopsies for M concentration were obtained from 45 UC pts in clinical remission and on oral M treatment (OT), before a 4 g enema randomly given to consentient pts every day (Group A, 15 pts), every 2 days (Group B, 15 pts) and every 3 days (Group C, 15 pts). Two additional biopsies were taken 1, 2 and 3 days after the last enema in group A, B and C respectively, at least 10 days later. All biopsies were immediately frozen at -80°C for later assay by means of high-performance light chromatography (HPLC). Data were analyzed using Student's *t*-test. Mean values \pm standard deviation of M mucosal concentration (ng/mg of tissue) were 1.32 ± 1.41 , 56.1 ± 39.2 , 9.65 ± 6.60 , and 6.39 ± 5.03 in pts receiving OT alone, groups A, B and C, respectively. Values in Group A were statistically higher ($p < 0.001$) than those in Groups B and C while no differences were found between Groups B and C. Values of OT were lower than groups A, B and C. M mucosal concentration rapidly decreases 2 days after a 4 g enema, but after three days is still higher than OT alone. These results may provide data which would be useful to plan topical therapy and improve adherence to treatment.

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1. Introduction

Salicylates have played a central role in Inflammatory Bowel Disease since 1942, when Nanni Svartz described the efficacy

of Salazopyrine (SASP) in improving diarrhoea and haematochezia in patients with UC.¹ Later, the discovery of 5-amino-salicylate acid as the active moiety of SASP, allowed the introduction of new oral drugs with fewer side-effects and the possibility of the topical treatment.² More recently, it has been demonstrated that the anti-inflammatory activity of the drug is related to its mucosal concentration and that topical treatment greatly increases M availability in the left colon

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mucosa with respect to oral treatment alone.^{3–5} These findings explained the good results obtained in clinical trials exploring the efficacy of rectal M administration in treating and maintaining not only mild to moderate forms of UC, but even the most severe, frequent relapsing diseases.^{6–12}

However, the need of topical treatment does not match patients' compliance, especially with a daily therapeutic scheme. Therefore, different, discontinuous treatment schedules have been tested in clinical trials, such as the bi- or tri-weekly, or one week monthly administration.^{13–16}

All these clinical trials have been empirically designed, then the "fate" of M into the intestinal mucosa still remains unknown, as well as no data are yet available concerning the tissutal pharmacokinetics of M into the colonic mucosa. In other words, no studies have, so far, investigated how long and how much the drug remains into the intestinal mucosa following rectal instillation. These data could be of some interest when defining the administration time table of the drug.

The aim of the present study was to measure the residual concentration of M 1, 2 and 3 days after the administration of 4 gM enemas in the rectal mucosa of 3 groups of pts assuming M enemas: *i*) once a day; *ii*) every 2, or *iii*) every 3 days.

2. Materials and methods

All consecutive UC pts, seen for regular endoscopic follow-up and in clinical remission for at least one month (Truelove and Witts's criteria), were asked to take part in the study.¹⁷ All pts were on oral M at a dose of 2.4–3.2 g/day, none had been on steroids, immunosuppressive drugs or topical M for at least 3 months. None of these pts had immunological, renal or other hepatic disorders. All pts assumed 4 l of PEG solution for intestinal cleansing during the previous afternoon and took their oral tablets regularly. Informed consent was obtained from all but 2 pts.

If, at endoscopy, no signs of active mucosal inflammation were found, (score: 0, according to Baron's criteria),¹⁸ the patient was included in the study and then, in addition to routine biopsies for histological examination, another two biopsies were taken in the rectum in order to measure M concentration. These specimens were immediately frozen at -80°C for later assay. A complete blood assessment was also carried out in order to evaluate the inflammatory parameters and the hepatic/renal functions. A total of 45 patients were enrolled in the study (16 pan-colitis, 29 distal colitis) in about eight months.

Upon enrolment pts received a 100 ml enema containing 4 g of M (Asacol, Giuliani-Bracco), randomly assigned to be assumed every day (Group A, 15 pts), every 2 days (Group B, 15 pts) or every 3 days (Group C, 15 pts), at bedtime, for at least 10 days

as shown in Table 1. All patients filled a diary sheet reporting the time of enema administration, the time of the following evacuation, stool consistency and eventual abdominal symptoms. OT was not modified. The diary was completed by patients every day and was checked at the end of the treatment period during the control visit. Patients who did not retain the enema for at least 6 h were excluded from the study. All patients were seen by the same staff of physicians.

Following the treatment period, the patients were again submitted to rectoscopy, 2 h after a tap-water cleansing enema 1, 2, or 3 days after the last enema for Groups A, B and C, respectively (Table 1). Two biopsies were taken in the rectum for M measurement and immediately frozen at -80°C . This endoscopy was performed in the late afternoon, at a time as close as possible to that of the next scheduled enema. In this way we would measure the residual concentration of M in the rectal mucosa just before its further topical instillation.

2.1. HPLC mesalazine measurement

After thawing, biopsies specimens were placed in tubes containing 2 mL of methanol with internal standard. After sonication, the supernatants were collected and evaporated to dryness. The samples were then reconstituted with 100 mL of mobile phase and aliquots of each sample (5 mL) were chromatographed on an analytical column. The mobile phase was a mixture of 0.01 M Na_2HPO_4 (pH=3.0) and methanol (85:15, v-v) and delivered at a flow rate of 1 mL/min. The standard curve was linear in the selected range with an inter-assay coefficient of variation less than 4.6%. Quality control samples were also run on each day of the sample analysis. The limit of detection for M was 1 ng/mg at a signal to noise ratio of 5.¹⁹ The physician performing the M measurement was unaware of the treatment schedule of the patients.

A two-tailed Student *t*-test was used for the statistical analyses.

3. Results

The clinical and demographic characteristics of the 3 groups of patients were similar and shown in Table 2. Of the 45 patients, 4 (1 pts in Group A, 2 in Group B, 1 in Group C) were excluded from the study: 1 patient had retained the enemas for less than 3 h for 2 nights, 2 patients had not observed the treatment schedule, 1 was lost to follow-up.

Table 2 Characteristics of patients and disease: no differences were obtained among the three groups.

	Patients			Disease	
	Age, X (SD)	M/F	Excluded	Oral dosage X (SD)	Pan/ distal yrs (SD)
Group A	37 (11)	5/9	1	2.83 (0.41)	3/11 4.6 (2.6)
Group B	32 (10)	6/7	2	2.88 (0.41)	5/8 5.7 (3.2)
Group C	38 (9)	3 2/3	1	2.67 (0.39)	5/9 6.3 (2.7)

Table 1 Times of enema administration (+) and measurement of Mesalazine mucosal concentration (=).

Day	1	2	3	4	5	6	7	8	9	10	11	12	13
Group A			+	+	+	+	+	+	+	+	+	+	=
Group B			+		+		+		+		+		=
Group C	+			+			+			+			=

The diary, checked during the control visit, showed that no patients had experienced abdominal pain, diarrhoea, urgency or any other abdominal symptoms during the treatment period. The retention time of the M enemas ranged from 5 to 9 h. No statistical difference was found between the 3 groups as far as concerns mean retention time of enemas. The endoscopic score of the rectal mucosa, following treatment was similar to that observed at enrolment for all patients.

The mean values \pm SD of M concentration (ng/mg of tissue) in the rectal mucosa before treatment were 1.32 ± 1.41 for the whole group of patients and 1.34 ± 1.38 , 1.30 ± 1.48 , 1.30 ± 1.42 for Group A, B and C respectively. No statistical differences were found between the three groups. Following treatment, the mean values \pm SD of M concentration (ng/mg of tissue) in the rectal mucosa were: 56.10 ± 39.2 , 9.65 ± 6.6 , 6.39 ± 5.03 for Group A, B and C respectively. Statistical differences ($p < 0.001$) were observed between mean values of M concentration obtained in Group A compared to Group B and C. Group B did not differentiate from Group C. The three groups studied were significantly different $p < 0.001$ from OT (Fig. 1).

The mean values \pm Standard Deviation of rectal concentration of Acetyl-5 ASA concentration (ng/mg of tissue), available for only 10 pts for each group, before treatment were 1.34 ± 1.38 , 1.30 ± 1.42 , 1.29 ± 1.42 , for groups A, B and C respectively. No significant differences were found between the three groups.

After the treatment, rectal concentration (ng/mg of tissue) of Acetyl-5-ASA was 256.39 ± 151.88 , 46.94 ± 27.80 , 29.53 ± 17.53 for groups A, B and C respectively. A significant difference ($p < 0.001$) was found between group A vs B and A vs C. No difference was found between group B vs C. All data obtained after topical treatment were significantly higher than OT ($p < 0.0001$, 0.0002 and 0.001 for group A, B and C respectively).

4. Discussion

The rectum is the earliest intestinal segment affected by inflammatory lesions in UC and is invariably involved throughout the entire clinical history of the disease.²⁰ Moreover,

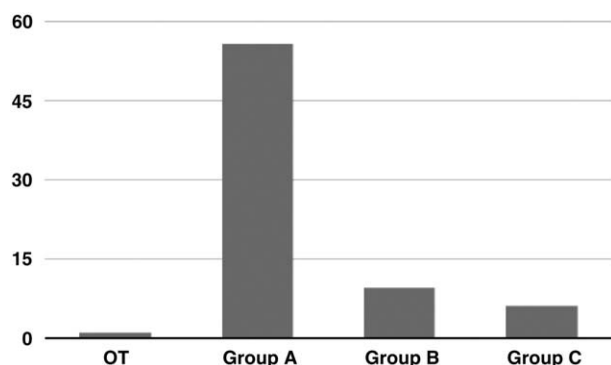


Figure 1 Comparison of mean values of mesalazine concentration (ng/mg of tissue) obtained during oral treatment (OT) and 1, 2, and 3 days after a 4 g drug enema administration (group A, B and C). Statistical significance: A vs B, C and OT; B and C vs OT. No difference between group B and C.

rectal inflammation alone may often be the only responsible for the patient's disabling symptoms. Thus, the rectum should be a constant target of anti-inflammatory treatment even in extensive UC.

The best way to guarantee optimal treatment of the rectum and distal colon with M is the trans-anal administration of the drug directly in the rectum since it offers a mucosal concentration significantly higher than that reached by the oral administration alone.^{5,11} Enemas, foams, gel, suppositories, at variable dosages, are now commercially available, all of which have demonstrated their efficacy in the distal treatment of UC. However, how long and how much M remains in the mucosa following rectal instillation is not yet known. So far, the data available regarding pharmacokinetics only refers to vanishing time of plasma M, following intravenous, oral or intra-rectal administration. These studies have provided important information on M metabolism, but are of limited utility in clinical practice since M has an exclusive topical action.^{21–23}

The present study, therefore, focused on the vanishing time of M from the rectal mucosa. Pharmacokinetic studies usually require a regular pick-up of information, from the same sample, at different times. Since it was ethically impossible to take two biopsies every day, for three days, from the same patient, we adopted the method of the group pharmacokinetic study in which a homogeneous cohort of patients is split into three different groups, assigned to different schedules of treatment. The mucosal concentration was measured when the patient would take the next enema, 1, 2, or 3 days after rectal instillation. This indirect method offers data concerning the residual mucosal concentration before the new medication ensues.

Results of the present study have shown that M concentration, in the rectal mucosa, decreases 5-fold after 2 days, with respect to residual values measured one day after drug administration. These data reflect results of plasma pharmacokinetics in which, 2 days after M enema, no trace of the drug is found in the blood.^{23–25} It is well known that M is rapidly metabolised to acetyl-5-ASA, already in the intestinal mucosa, and subsequently in the liver.^{22,26,27} Therefore, the presence of residual M in the rectal mucosa, 2 days after the enema is, somewhat, surprising.

However, the really unexpected result was that, three days after its administration, M is still present in the rectal mucosa. Moreover, its concentration is significantly higher than that reached by oral treatment alone, even if this result was obtained after a different intestinal cleansing. Whether this finding has some clinical relevance is not known, but it is possible that this residual availability of M, can guarantee a higher protection of the rectal mucosa than that assured by oral treatment alone. Good results, in clinical studies that tested the effects of intermittent topical treatment in the maintenance of remission, may be due merely to the prolonged presence of mucosal M up to 3 days after its rectal instillation.

Since topical treatment is one of the conditions responsible for poor compliance to treatment, these data could appear to justify a less frequent administration that could, at the same time, overcome this problem assuring some clinical benefit.²⁸

In conclusion, results of the present study have shown that rectal mucosal M concentration drops 5-fold, 2 days after rectal instillation but that, on the third day, it is still higher

than mucosal concentrations obtained with oral treatment alone. These data could be useful not only to improve patients' compliance but also in designing different therapeutic programmes for different severity of the disease.

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