Scintigraphic Imaging and Absorption of a 5-Aminosalicylic Acid Enema in Patients with Ileorectal Anastomosis

Dario Lisciandrano, Riccardo Benti, Tullio Ranzi, Anna R. Baldassarri, Andrea Bruno, Paolo A. Bianchi and Paolo Gerundini

Department of Gastroenterology, Istituto di Scienze Mediche, University of Milan, Milan, and Nuclear Medicine Service, Istituto di Ricovero e Cura a Carattere Scientifico Ospedale Maggiore, Milan, Italy

Ileorectal anastomosis (IRA) is a possible surgical treatment for hyperacute and drug-unresponsive forms of ulcerative colitis (UC). UC relapses in the rectal remnant usually are prevented by chronic administration of 5-aminosalicylic acid (5-ASA) in topical formulations. The relationships between intestinal absorption and pattern of luminal spread of 5-ASA enemas are still unknown in patients with IRA. We correlated the absorption of a 5-ASA enema with its spread in the distal bowel of patients with IRA as assessed by ^{99m}Tc radioenema imaging. **Methods:** Eight patients with UC in remission and previous IRA received a therapeutic 50-mL 5-ASA enema labeled with ^{99m}Tc-sulfer colloid. Absorbed 5-ASA and its major metabolite, acetyl 5-ASA, were measured in plasma, and dynamic images of radiolabeled enema were obtained for 6 h. The retrograde ileal spread (RIS) was determined and expressed as percentage of total enema radioactivity. Plasma levels of 5-ASA and acetyl 5-ASA were measured in six healthy volunteers after administration of the same enema volume with no radiolabeling. **Results:** The mean 5-ASA plasma level was 0.70 μg/mL (range 0.37–0.95 μg/mL) in patients and 0.96 μg/mL (range 0.78–1.16 μg/mL) in healthy volunteers (P = not significant), and the mean acetyl 5-ASA plasma levels were 0.89 μg/mL (range 0.44–1.19 μg/mL) and 0.84 μg/mL (range 0.51–1.02 μg/mL), respectively (P = not significant). Radioenema imaging allows RIS assessment of patients with IRA. The mean value was 8.5% (range 2%–19.3%) of administered radioactivity, which correlated significantly with the total absorption of 5-ASA in the IRA group (P = 0.033, linear correlation test). Rectal wall contractions recognized by dynamic radioenema imaging were defined as a common cause of RIS episodes. **Conclusion:** In IRA patients, 5-ASA plasma levels were similar to those in healthy volunteers after administration in enema. Only part of a 50-mL 5-ASA enema reaches the ileum, and radiolabeled imaging shows the degree and number of these RIS episodes. The absorption of 5-ASA can increase in patients compared with healthy volunteers, in the presence of either occasional but significant ileal spread associated with postural factors and abdominal wall contraction or multiple moderate episodes of radioenema backdiffusion related to rectal wall motility.

Key Words: 5-aminosalicylic acid absorption; radioenema imaging; ileorectal anastomosis; ulcerative colitis

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For correspondence or reprints contact: Paolo Gerundini, MD, Servizio di Medicina Nucleare, Ospedale Maggiore, via Francesco Sforza 35, 20122 Milano, Italy.

The most common therapeutic modality for ulcerative colitis (UC) treatment, both in remission and in active phases of the disease, is 5-aminosalicylic acid (5-ASA) (*1–5*). 5-ASA is also administered in topical formulations as suppositories or as enemas in UC patients with colectomy and ileorectal anastomosis (IRA). In unoperated UC patients the reported spread and absorption of topical 5-ASA formulations have varied (*6–14*), and dose-related nephrotoxicity was reported in chronic treatment (*15,16*). Systemic absorption depends on the pH of the enema solution (*9*) and on the inflammatory status of the intestinal mucosa (*6*). The retrograde spread of enemas along the colon is volume dependent (*10*), and a 60-mL radioenema always reaches the splenic flexure in UC patients with unoperated colon (*12*). The anatomic situation after IRA can favor the retrograde spread of enemas toward the distal ileum even when small volumes are administered. At present little is known about the ileal spread of enemas in UC patients with IRA, and it is unclear whether the absorption of topical drugs differs from that in unoperated patients. Experimental data suggest a different absorption rate in small intestine compared with colon (*7,8*). In fact, 5-ASA instillation in the proximal small bowel resulted in higher plasma levels of the drug than that obtained by enema administration. The aim of this study was to assess and correlate the absorption of 5-ASA with its ileal spread after a therapeutic enema by ^{99m}Tc enema imaging in UC patients with IRA.

MATERIALS AND METHODS

Eight patients (six men, two women; mean age 45 y; range 22–76 y) with UC in endoscopic remission and IRA were enrolled in the study after informed consent was obtained by the local ethics committee. Colectomy and IRA had been performed 3–7 y previously in every patient by the same surgical team, and in all patients the length of the rectal remnant, measured by a rigid endoscope, was 13–15 cm. Six patients had two bowel movements a day, and two patients had three to five bowel movements a day. Any treatment with 5-ASA was discontinued at least 48 h before the study. Hemoglobin, leukocyte count, platelet count and erythrocyte sedimentation rate were in the normal range, and the liver and

kidney functions were normal. All subjects were studied in the morning, 60–90 min after a light breakfast. Immediately before administration of the enema, each patient was asked to empty the bowel. The 5-ASA enema used (Pentasa;Yamanouchi Pharma, Milan, Italy) was a buffered solution (pH 4.6–4.7) with moderate-to-prolonged absorption of 5-ASA content (*9*). Radiolabeled colloids were obtained by adding a ^{99m}Tc-pertechnetate solution to a commercial kit containing rhenium sulfide (Sulfotec; Sorin Biomedica, Saluggia, Italy). The suspension was boiled for 10 min and cooled at room temperature according to the manufacturer's instructions. The final pH of the labeled product ranged from 3.5 to 6.0. A small volume (0.5–0.7 mL) of radiolabeled colloids (30–40 MBq) was diluted in 50 mL Pentasa 4% enema, heated to 35°C and administered to subjects in the left lateral decubitus position. The patients lay supine for 60 min during early imaging and were then free to walk or sit. After 4 h each had a light meal to standardize the effect of gastrointestinal reflex during the study. At 6 h the patients were asked to defecate.

Six healthy volunteers (two men, four women; mean age 28.5 y; range 22–32 y) served as the control group for only the pharmacokinetic part of the study. After giving informed consent, each subject was given the same enema formulation without the addition of radiolabeled colloids. Blood samples were obtained as in the patient group.

Analysis of Samples

Heparinized blood samples (7 mL) were taken immediately before enema administration, at 30 and 60 min and then hourly for the following 5 h. Blood samples were centrifuged immediately for 10 min at 3000 rpm, and the plasma obtained was stored at −80°C to prevent 5-ASA degradation (*14*). 5-ASA and its major metabolite, acetyl 5-ASA (ac 5-ASA), were measured in plasma by high-performance liquid chromatography (HPLC). For all measurements of both compounds the sensitivity limit was 0.1 μg/mL. Fresh standard solutions of 5-ASA (Giuliani SpA, Milan, Italy) and ac 5-ASA were prepared on the day of the HPLC calibration procedure. The latter compound was produced by adding acetic anhydride to a 5-ASA solution and checking the solution with spectrometry. The two compounds were separated on a Waters Nova-Pak C_{18} (3.9 × 150 mm, 4 μm) column with a Waters Guard-Pak module with μBond-Pak as the guard column (Waters Corp., Milford, MA). The pump was a Waters 510 model and the detector was a Waters spectrofluorometer (emission wavelength 515 nm, excitation wavelength 305 nm). The detector signals were recorded and analyzed by Maxima 820 software (Waters Corp.). The mobile phase was acetonitrile and water (10:90) containing 0.005 mol hexan-sulfonic acid and adjusted to pH 2.5 by orthophosphoric acid; this was pumped through the column at 1 mL/min. All assays were performed at room temperature.

Plasma samples (1 mL), after the addition of 100 μL concentrated perchloric acid to precipitate the serum proteins, were vortexed and centrifuged for 5 min at 3000 rpm; the supernatant was then filtered through a 0.45-μm filter and injected into the HPLC device. 5-ASA absorption in patients and healthy volunteers was expressed as area under the curve (AUC), which represents the integral of the plasma concentration curve of each subject in the period studied.

Scintigraphic Imaging and Analysis of Enema Spread

^{99m}Tc-sulfer colloid in enema is not absorbed or metabolized by the colonic mucosa (*13*) and was therefore selected for assessment of the volume distribution of enemas along the colonic lumen (*10,11*). Because of the lack of visualization of other abdominal organs, the spread of the enema can be measured in segments of the intestine proximal and distal to the IRA in each image of the study. Scintigraphic images (128 × 128 matrix) of the abdomen were acquired by a large-field-of-view gamma camera (General Electric 400 AC; General Electric Medical Systems, Milwaukee, WI) equipped with a low-energy, high-resolution collimator and processed using a dedicated computer (General Electric 3000). Images of the abdomen were acquired by positioning the detector over the supine patient immediately after enema administration. The supine position is the standard posture that allows the overnight action of 5-ASA administered in UC patients with IRA or unoperated colon before sleep. A dynamic sequence (two images per minute centered over the rectal and ileal regions) was obtained during the first hour (in anterior projection) to study this condition. Static images of the abdomen were taken in the same position every 60 min for the next 5 h. A final static image of the abdomen was obtained after rectal emptying. In all acquired frames, radioactivity counts were determined in rectal and ileal regions of interest (ROIs) delimited manually by two observers. The mean counts in ROIs (two measurements per observer) were averaged and used to estimate the retrograde reflux of radiolabeled enema from the rectal to the ileal compartment. The averaged counts in the ileal ROI were divided by the sum of ileal and rectal counts in ROIs to obtain the percentage of the ileal spread. The percentage of radioenema spread in the ileal region (%IS) was calculated in the first hour from the early dynamic images; the mean %IS values of the second, third, fourth, fifth and sixth hour were obtained from late static images (Fig. 1). Rectal wall movements and related retrograde ileal spread (RIS) episodes were also assessed by screen analysis of the first-hour sequence displayed in slow cine loop. The number of these events was recorded in all patients in the first hour. The IRA was localized properly in all patients at this time. The efficacy of final rectal evacuation was expressed as the percentage of radioactivity removed from the rectal lumen; residual ileal radioactivity, if detectable, was also measured in postevacuation images.

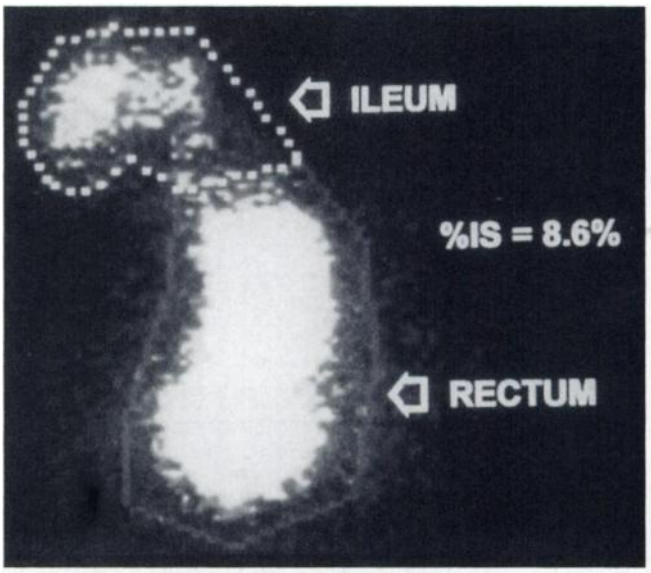

FIGURE 1. ROIs and percentage of repartition of enema in ileal (%IS) and rectal compartments in patient 2.

Statistical Methods

The plasma concentrations of 5-ASA and ac 5-ASA in patient and control groups were expressed as mean ± SE. Ileal spread episodes per hour and rectal wall movement per hour were expressed in patients as mean ± SD. Correlation coefficients between variables were determined by linear regression analysis. Differences were considered significant at the $P \geq 0.05$ level.

RESULTS

All patients and healthy volunteers retained the enema for the 6 h of the study without discomfort. As shown in Figure 2, the plasma levels of ac 5-ASA were higher than those of 5-ASA from 1 to 6 h. Peak concentrations of both compounds were observed at 3 h, and the subsequent decline in the values was similar for both compounds. The mean ac 5-ASA plasma level during the study was 0.89 μg/mL (range 0.44–1.19 μg/mL) in patients and 0.84 μg/mL (range 0.51–1.02 μg/mL) in healthy volunteers. As expected, the 5-ASA absorption varied moderately in healthy volunteers (AUC range 3.78–8.62 μg/mL/h) and widely in patients (AUC range 0.74–13.3 μg/mL/h) (Fig. 3). However, the mean absorption values of the two groups were not significantly different, although these values were slightly lower in the patient group.

The mean %IS was 8.5% (SE 1.9; range 2%–19.3%) in the 6-h period (Fig. 4). An early peak at 1 h was followed by a second peak at 2 h; from the third hour onward the value remained stable. In some patients and at the single time points, the %IS varied more markedly than did the AUC (range of mean %IS: first h, 2%–32.6%; third h, 2.3%–15.8%) (Fig. 5). The %IS varied widely in only two patients (Fig. 5). One of them, patient 6, suddenly sat up on the gamma camera couch immediately after enema administration. This movement determined the reflux of about 70% of enema in the ileal lumen, as shown on scintigraphic images (Fig. 6). Later, at 3 h, peristalsis took most radioactivity to the rectum with a minimal ileal residue. By excluding this patient from the calculation of the mean %IS, the early peak at 1 h was abolished, whereas the peak at 2 h remained. The data are consistent with a mild-to-moderate degree of RIS in most patients; five of the eight patients had no %IS values >10% and reached the %IS peak 2 h after enema administration. A significant correlation was observed between the mean %IS and 5-ASA AUC ($P = 0.033$) during the study (Fig. 7).

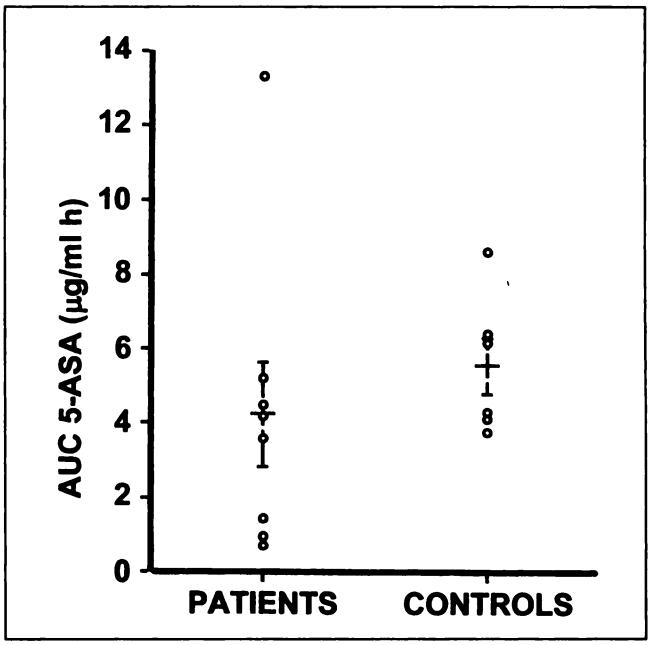

FIGURE 3. 5-Aminosalicylic acid (5-ASA) plasma levels expressed as area under plasma concentration curve (AUC) in patients and healthy volunteers (mean ± SE).

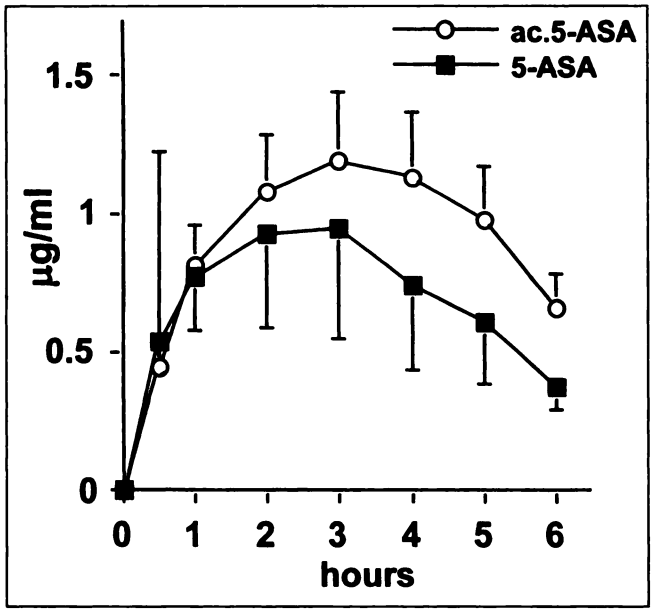

FIGURE 2. Mean plasma levels ± SE of 5-aminosalicylic acid (5-ASA) and acetyl-5-aminosalicylic acid (ac 5-ASA) in IRA patients.

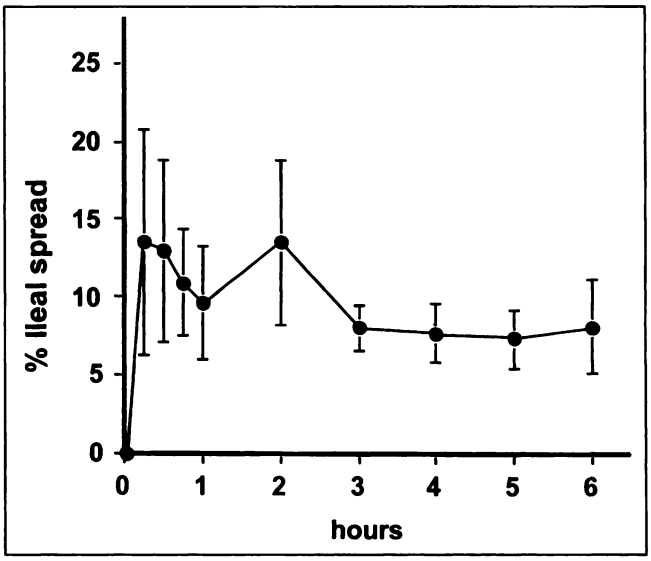

FIGURE 4. %IS of enema (mean ± SE) in patients.

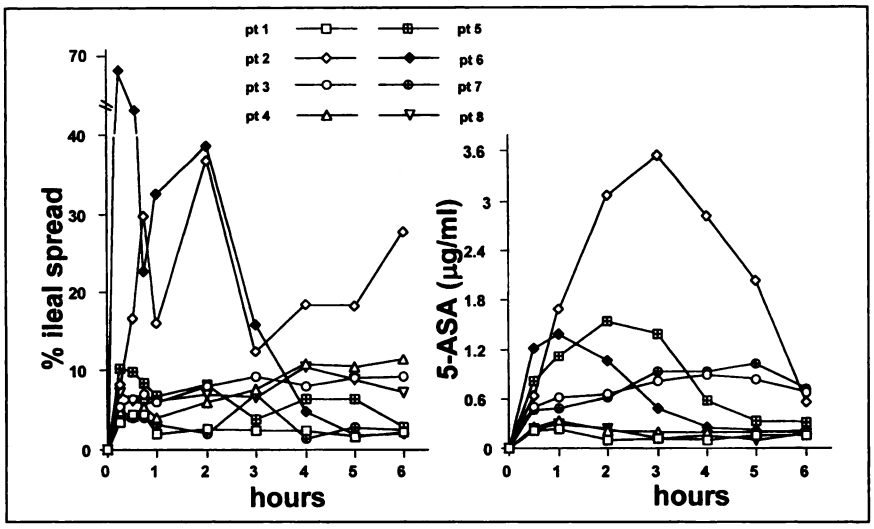

FIGURE 5. %IS and 5-aminosalicylic acid (5-ASA) plasma levels (AUC) for each patient.

Rectal wall movement is defined scintigraphically as the change of shape and diameter in a segment of the roughly cylindric volume of the radioenema distributed between the IRA and anus. The pattern is frequently cyclic: a first concentric contraction is followed within 1–2 min by recovery of the original appearance of the rectal segment, possibly related to muscular wall relaxation. The functional efficacy of these cyclic contractions to produce an ileal spread episode was graded by the amount of the radioenema observed above the IRA, thus defining rectal wall movements with associated ileal spread episodes (RWM+) and without ileal spread (RWM−). The number of rectal contractions without ileal spread during the first hour varied moderately in supine patients (mean 12.1, range 9–21) and was higher than the number of RWM+ (mean 3.8, range 2–11); the latter represented only 29% (range 22%–43%) of the total rectal movements shown by scintigraphic imaging.

The small patient number precludes further correlation between RWM+ episodes and AUC values, even though the highest AUC value was observed in the patient with the most RWM+ episodes (Fig. 8).

The mean rectal evacuation efficacy at 6 h was 86.7% ± 8.8% (range 25%–100%). Rectal evacuation values were >88% in seven of the eight patients. Residual ileal radioactivity (1.7% and 7.9%) was observed in two patients after rectal evacuation.

DISCUSSION

IRA is a therapeutic option used in the surgical treatment of UC in some patients, mainly in those patients in whom an

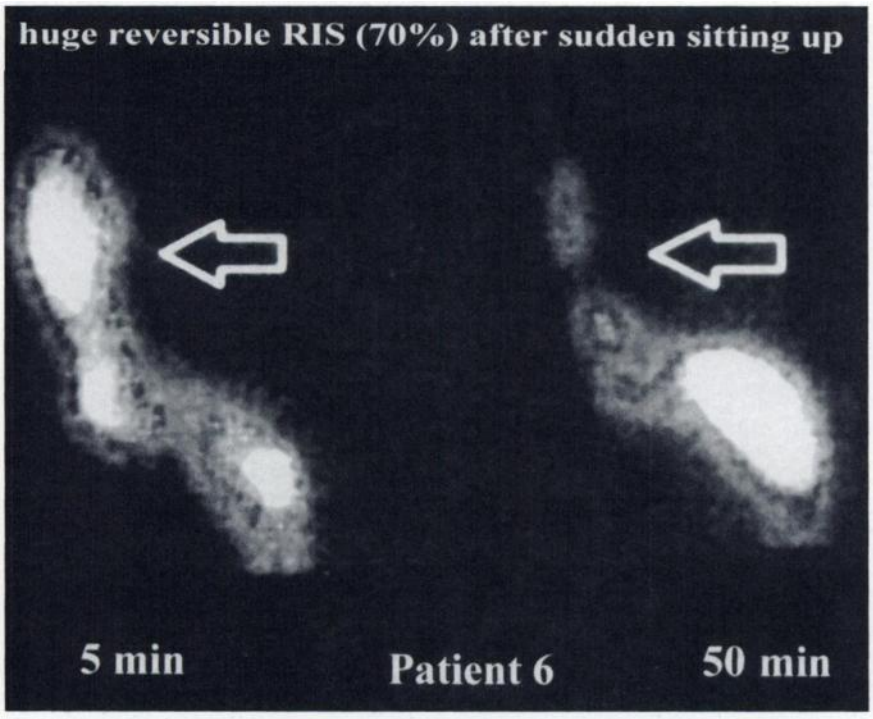

FIGURE 6. Scintigraphic images of patient 6 obtained in first hour. Large but transient retrograde ileal spread (RIS) in first minutes after radioenema administration was caused by sudden sitting up on camera couch.

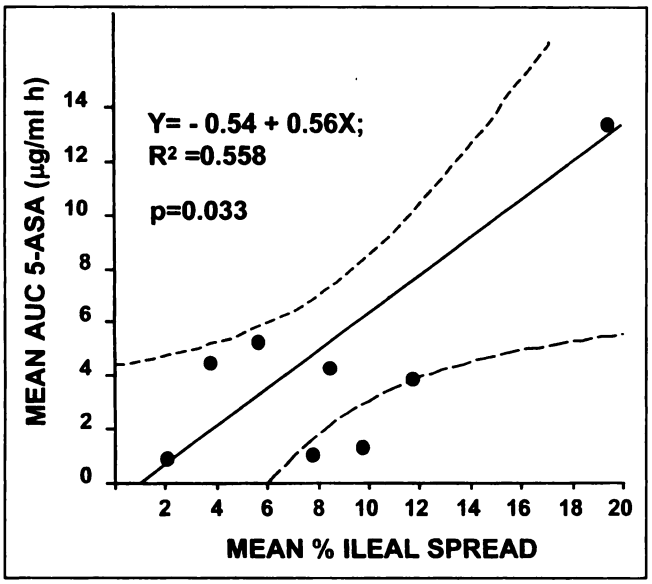

FIGURE 7. Correlation between absorption of 5-aminosalicylic acid (5-ASA), expressed as AUC (area under plasma concentration curve), and RIS, expressed as mean %IS of total counts in distal bowel.

ileoanal anastomosis with ileal pouch is not indicated because of clinical or individual reasons. Almost all patients with IRA are still treated with 5-ASA in enema or suppository formulations to prevent UC relapse in the rectal remnant. To our knowledge, the absorption and spread of 5-ASA enemas have not been studied in these patients.

The efficiency of 5-ASA transport is greater in the ileal epithelium than in the colonic epithelium, which suggests that abundant ileal spread after IRA could cause a considerable increase in 5-ASA absorption from the ileum and possible long-term side effects (*15,16*). Some variability of the 5-ASA absorption rate was observed in our healthy volunteers, possibly related to different degrees of retrograde enema distribution along the colon at different times than those reported in UC patients (*11,12*). The variability in 5-ASA levels in plasma seems higher in this study with IRA and is possibly related to anatomic factors, such as the reduced absorption surface of the rectal remnant or the reduced molding activity in its lumen (or both). These anatomic factors could explain a normal or reduced absorption rate of 5-ASA enema but not the transient or stable increase, reaching levels twice as high as the mean normal value, observed in some patients with IRA (e.g., patient 2, Fig. 5). 5-ASA plasma levels and ileorectal repartition of the radioenema volume, derived from scintigraphic images, correlated in patients up to 6 h after administration, i.e., lower AUC values were seen in patients with less numerous and less extensive RIS episodes.

Apparently, IRA reduced or did not influence the mean 5-ASA intestinal absorption in the patient group compared with that of the control group with unoperated colon. However, the occurrence of increased absorption in a minority of patients after 5-ASA enema and the significant correlation between %IS and 5-ASA AUC (Fig. 7) led us to hypothesize that ileal spread of enema increases the absorption surface for the drug and also causes a net increase in the absorption rate of 5-ASA from the ileal lumen. This hypothesis agrees with experimental data that attribute a nearly sevenfold higher efficiency for 5-ASA transport to ileal mucosa than to colonic mucosa (*17*). Because the enema diffusion in the rectal remnant and along the unoperated colon differs as well, radiocolloids were not administered to the healthy volunteers. Moreover, the volumes of therapeutic enemas (200–250 mL) given to UC patients with unoperated colon differ from those administered after IRA (30–100 mL), and radioenema imaging never showed ileal spread in UC patients with unoperated colon (*11,12*). For

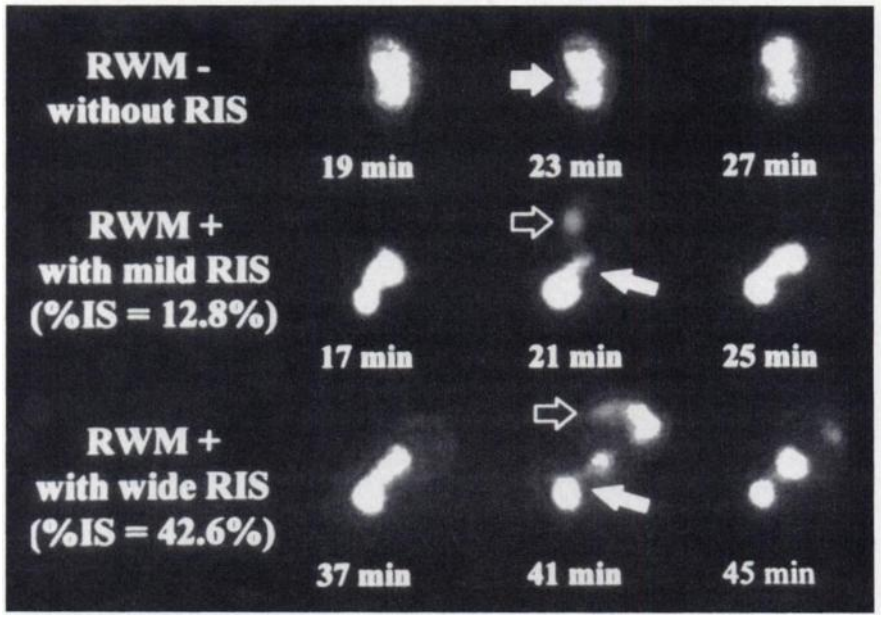

FIGURE 8. Scintigraphic images of patient 2 obtained during first hour show rectal wall movement with retrograde ileal spread (RIS) (RWM+) or without RIS (RWM−). Rectal wall movement is indicated by solid arrows; possible ileal spread is indicated by open arrows.

these reasons the scintigraphic patterns reported for UC patients with unoperated colon and for patients with IRA in this study cannot be compared. The scintigraphic pattern of ileal reflux in these patients was easily defined in static and dynamic images obtained with a gamma camera. The spread of enema aliquots in the ileal lumen is properly distinguishable from the rectal content on scintigraphic images because of the different caliber and shape of the ileum versus the rectum. Diffuse radiation from the rectal content to the ileal ROI was estimated 2 min after enema administration in six of seven patients, excluding patient 6 because of early ileal visualization. Scattered radiation contribution to final decay-corrected counts in the ileal ROI ranged from 0.1% to 1.2% (mean 0.5%) up to 6 h. The minimal %IS value observed in this study was 2%. The interobserver variability of counts in ileal ROIs was acceptable along the study time (mean 0.8%, range 0.2%–3.6% of total counts in the abdomen). Therefore, photon scattering and the ROI drawing variability between observers seem to be minor factors affecting the correct calculation of the degree of enema ileal spread from scintigraphic images in this series.

The individual variability in the number and degree of RIS episodes observed appears to be caused by multiple factors related to the intrinsic contractility of the rectum, postural changes and voluntary contraction of the abdominal muscles (i.e., sudden sitting up, as in patient 6, Fig. 6). The final effect of these factors on retrograde radiolabeled enema spread is well defined by %IS, which correlates positively with 5-ASA absorption. This correlation between intestinal absorption and retrograde spread in these patients is in agreement with the increased 5-ASA absorption observed in the ileal mucosa versus colonic mucosa reported in healthy volunteers in whom 5-ASA was absorbed better orally than in enema (*8*). In this study the %IS peak occurred at 2 h, whereas the peak of 5-ASA plasma levels occurred slightly later, at 3 h. Thus, a significant ileal spread is possibly followed by a proportional increase in 5-ASA absorption, with increasing plasma levels, in the following hour. Scintigraphic data also show the intrinsic motility of the rectal wall as an important cause of ileal spread in supine IRA patients. However, only a minority of scintigraphically assessed rectal wall contractions led to an RIS episode (mean 29%) in this series; when occurring, these episodes were generally moderate and transient (i.e., %IS $< 10\%$). The observed high number of RWM− is probably related to the physiologic molding movement of the rectal content, and this scintigraphic pattern varies less than does RWM+ in IRA. On the contrary, the patients with more (>8 RWM+/h) or more extensive enema ileal spread (%IS $> 15\%$) had the highest 5-ASA plasma levels. This finding can be attributed either to more intense involuntary contractions of the rectal wall or to voluntary contraction of abdominal muscles. Further statistical evaluation of rectal wall contraction and AUC values was precluded by the small number of patients studied. Postural changes determining the degree of RIS were not studied specifically in this protocol, but the incidental occurrence of a major episode soon after sitting up underlines their importance and merits further attention, even if small-volume enemas are administered. In this case, an increased value of plasma 5-ASA was observed only in the following 2 h (patient 6, Fig. 5); subsequently, the 5-ASA level as well as the %IS values decreased.

The rectal evacuation efficiency at the end of the study was assessed by pre- and postevacuation images. The radioactive enema was completely eliminated from the rectum (>97%) in six of the eight IRA patients. This result confirms the cessation of the topical effect of 5-ASA enema after rectal emptying in most subjects. By contrast, UC patients with unoperated colon, treated with large-volume enemas, had increased colonic retention after emptying, thus protracting the topical effect of 5-ASA for hours (*11–13*). The best topical effect of 5-ASA on the rectal remnant of IRA patients could be achieved by giving 5-ASA in enema or suppository early after each bowel movement.

CONCLUSION

Ileal absorption of 5-ASA after administration by enema seems to be a relevant feature in UC patients with IRA. The intestinal spread and volume distribution of enemas in subjects with IRA can be effectively assessed by nuclear imaging after labeling the enema with ^{99m}Tc-sulfer colloid. A therapeutic buffered 50-mL 5-ASA formulation showed a variable but significant RIS in the patients studied. Major episodes of retrograde spread were shown and graded by scintigraphic imaging in some patients, in whom 5-ASA plasma levels were higher than those of healthy volunteers during the early and late phases of the study. Ileal spread values derived from scintigraphic images correlated positively with 5-ASA plasma levels in the patient group, supporting the hypothesis that the 5-ASA absorption increases when part of the enema is moved proximally to the IRA by rectal contractions. The mean 5-ASA plasma level during enema retention was slightly reduced in the patients compared with the control group, although occasionally a twofold increase with respect to the mean normal values was observed in the presence of extensive or multiple episodes (or both) of ileal reflux. The intrinsic motility of the rectal wall remnant and some postural changes can be major causes of ileal spread, recognized by radiolabeled enema imaging in UC patients in remission with IRA.

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REFERENCES

1. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. *N Engl J Med.* 1987;317:1625–1629.
2. Riley SA, Mani V, Goodman MJ, et al. Comparison of delayed release 5-aminosalicylic acid (mesalazine) and sulphasalazine as maintenance treatment for patients with ulcerative colitis. *Gastroenterology.* 1988;94:1383–1389.

3. Campieri M, Lanfranchi GA, Bazzocchi G, et al. Treatment of ulcerative colitis with high-dose 5-aminosalicylic acid enemas. *Lancet.* 1981;2:270–271.
4. Biddle WS, Greenberger NJ, Swan JT, McPhee MS, Miner PB. 5-amino-salicylic acid enemas: effective agent in maintaining remission in left-sided ulcerative colitis. *Gastroenterology.* 1988;94:195–199.
5. Campieri M, De Franchis R, Bianchi Porro G, Ranzi T, Brunetti G, Barbara L. Mesalazine (5-aminosalicylic acid) suppositories in the treatment of ulcerative proctitis or distal proctosigmoiditis: a randomized controlled trial. *Scand J Gastroenterol.* 1990;25:663–668.
6. Campieri M, Lanfranchi GA, Boschi S, et al. Topical administration of 5-amino-salicylic acid enemas in patients with ulcerative colitis: studies on rectal absorption and excretion. *Gut.* 1985;26:400–405.
7. Bondesen S, Brønnumschou J, Pedersen V, et al. Absorption of 5-amino-salicylic acid from colon and rectum. *Br J Clin Pharmacol.* 1988;25:269–272.
8. Nielsen OH, Bondensen S. Kinetics of 5-aminosalicylic acid after jejunal instillation in man. *Br J Clin Pharmacol.* 1983;16:738–740.
9. Bondesen S, Haagen Nielsen O, Jacobsen O, et al. 5-aminosalicylic acid enemas in patients with ulcerative colitis: influence of acidity on the kinetic pattern. *Scand J Gastroenterol.* 1984;19:677–682.
10. Campieri M, Lanfranchi GA, Brignola C, et al. Retrograde spread of 5-aminosalicylic acid enemas in patients with active ulcerative colitis. *Dis Colon Rectum.* 1986;29:108–110.
11. Vitti RA, Meyers F, Knight LC, Siegel JA, Malmud LS, Fisher RS. Quantitative distribution of radiolabeled 5-aminosalicylic acid enemas in patients with left-sided ulcerative colitis. *Dig Dis Sci.* 1989;34:1792–1797.
12. Chapman NJ, Brown ML, Phillips SF, et al. Distribution of mesalazine enemas in patients with active distal ulcerative colitis. *Mayo Clin Proc.* 1992;67:245–248.
13. Tiel van Buul M, Mulder C, van Royen E, Wiltink E, Tytgat G. Retrograde spread of mesalazine (5-aminosalicylic acid) containing enema in patients with ulcerative colitis. *Clin Pharmacokinet.* 1991;20:247–251.
14. Brendel E, Meineke I, Stüwe, Osterwald H. Stability of 5-aminosalicylic acid and 5-acetylaminosalicylic acid in plasma. *J Chromatogr.* 1988;432:358–362.
15. Metha RP. Acute interstitial nephritis due to 5-aminosalicylic acid. *Can Med Assoc J.* 1990;143:1031–1032.
16. Masson EA, Rhodes JM. Mesalazine-associated nephrogenic diabetes insipidus presenting as weight loss. *Gut.* 1992;33:563–564.
17. Grisham MB, Granger ND. 5-aminosalicylic acid concentration in mucosal interstitium of cat small and large intestine. *Dig Dis Sci.* 1989;34:573–578.