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Prevention of post-operative recurrence of Crohn's disease requires adequate mucosal concentration of mesalazine

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SUMMARY

Background: Surgical resection of Crohn's disease is followed by early recurrence in a high percentage of patients. Mesalazine has been shown to be effective in the prevention of post-operative recurrence, but some 50% of patients under treatment recur at 3 years of follow-up.
Aim: To establish whether the mucosal concentration of mesalazine might affect the development of post-operative recurrence.
Methods: Colon-ileoscopy was performed in 25 consecutive patients resected for Crohn's disease. The mean time from surgery was 14 months. After the operation, all patients were taking oral mesalazine (Asacol, 2.4 g/day). Ten patients showed signs of endoscopic recurrence (apthae, ulcers, narrowing of the lumen) in the neoterminal ileum, five of whom also showed juxta-anastomotic colonic involvement. Fifteen patients were free of recurrence. At endoscopy, four biopsies were taken from the perianastomotic area (two specimens at the ileal site and two specimens at the colonic site of the anastomosis). The specimens were weighed and immediately frozen at −80 °C. Mesalazine concentration (ng/mg) was measured in tissue homogenates by high-performance liquid chromatography with electrochemical detection. Fisher's exact test was used for the statistical analysis.
Results: The mean value of mucosal mesalazine concentration, expressed as ng/mg of tissue, was significantly lower in patients with recurrence than in those without recurrence both in the ileum (mean ± s.d.: 21.6 ± 28.3 vs. 70.9 ± 47.4; $P = 0.007$) and in the colon (25.8 ± 26.4 vs. 60.3 ± 32.5; $P = 0.010$).
Conclusions: The mucosal conentration of mesalazine in the juxta-anastomatic area is significantly lower in patients with recurrence than in those free of recurrence. These data could suggest an association between mucosal mesalazine concentrations and the clinical effectiveness of the drug.

INTRODUCTION

In the natural history of Crohn's disease, intestinal resection is a necessary step because some 80% of patients require surgery during their lifetime.[1] Whilst surgical treatment is effective in removing the lesions, it has the disadvantage of early and frequent reappearance of perianastomotic recurrence. Therefore, because at least 80% of patients with Crohn's disease undergo

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surgery, prevention of post-operative recurrence is, nowadays, one of the most demanding challenges in the management of this condition.[2, 3]

Several randomized controlled trials have demonstrated that mesalazine is effective in reducing the frequency of post-operative recurrence and in decreasing the severity of the lesions.[4–6] Results of a recent meta-analysis including 401 patients resected for Crohn's disease showed that the pooled risk difference significantly favoured mesalazine treatment in the post-surgical setting ($P = 0.0026$; $Z = -3.01$).[7] However, about half of the patients under prophylactic treatment with mesalazine show recurrence at the 3-year follow-up.

Considering the topical mode of action of mesalazine, the aim of this study was to evaluate the relationship between mucosal concentration of the drug and post-operative recurrence.

MATERIALS AND METHODS

From 1 October 1995 to 30 January 1996, 25 consecutive patients operated for Crohn's disease were enrolled in an Italian multicentre cross-sectional study. Patients underwent surgery for disease limited to the terminal ileum with or without involvement of the caecum-ascending colon. The intestinal resection consisted of complete removal of the macroscopically involved intestinal segment. Patients with one or more previous resections were not excluded from the study. The mean time since surgery was 14 months. Thirteen patients had an end-to-end anastomotic configuration, 12 patients had another type of anastomosis (seven side-to-end; five side-to-side). The characteristics of the patients are shown in Table 1. All patients had been taking prophylactic oral mesalazine (Asacol, Procter & Gamble Pharmaceutical; 2.4 g/day) since 2 weeks after the operation and were on regular endoscopic follow-up. Only patients with no signs of endoscopic recurrence in the previous examination were included in the study. Patients under treatment with steriods, immunosuppressive agents, antibiotics, H_2-receptor antagonists or proton pump inhibitors were excluded.

Methods

All patients were prepared for colonoscopy by intestinal cleansing using 3 L of oral PEG solution. The last tablet of mesalazine was taken by the patient 3 h before starting intestinal preparation and colonoscopy was preformed 5–6 h later. During endoscopy, multiple biopsies were taken from the perianastomotic area: two specimens 3 cm proximal to the anastomosis (ileal site) and two 3 cm distal to the anastomosis (colonic site). The specimens were weighed and immediately frozen at −80 °C. Recurrence was defined as the presence of typical Crohn's disease inflammatory mucosal lesions at the perianastomotic area, according to the criteria proposed by Rutgeerts *et al.*[8] Hyperaemia and oedema alone were not considered to be signs of recurrence. All endoscopists taking part in the trial were given preliminary training on the definition criteria of recurrence.

Analytical methods

Mucosal concentrations of mesalazine and acetyl-5-ASA were measured using the high-performance liquid chromatographic method previously described.[9] Analyses were performed on a chromatographic apparatus (Waters, Milford, USA) consisting of a Model 510 solvent delivery system, a Model U6K injector valve, and an electrochemical detector coulochem (ESA,

Table 1. Patient characteristics

Characteristics	Groups		
	No recurrence (15 patients)	Recurrence (10 patients)	*P*
Age (mean ± s.d)	33.0 ± 10.6	33.5 ± 6.9	N.S.
Gender (M/F)	11/4	7/3	N.S.
Smokers	3	2	N.S.
Ex smokers	5	4	N.S.
Anastomosis			
end-to-end	8	5	N.S.
other types	7	5	N.S.
Previous resections	5	4	N.S.
Distance from surgery months (mean ± s.d.)	12.9 ± 9.9	15.9 ± 11.1	N.S.

Bedford, USA) Model 5100A equipped with a conditioning cell (Model 5021) and analytical cell (Model 5011) connected to a Model 746 integrator. Briefly, after thawing, the biopsy specimen was 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 μL of mobile phase and aliquots of each sample (5 μL) were chromatographed on an analytical column Erbasil S C18 (250 × 4.6 mm, i.d.; particle size 10 μm) (Farmitalia, Carlo Erba, Italy). The mobile phase was a mixture of 0.01 M Na_2 HPO_4 (pH = 3.0) (containing 0.1 mM EDTA, 0.1 M citric acid and 0.1 mM heptanesulphonic acid) and methanol (85:15 by volume) and delivered at a flow rate of 1 mL/min. The standard curve was linear in the selected range with an inter-assay coefficient variation of < 4.6%. Quality control samples were also run on each day of sample analysis. The limit of detection for mesalazine and acetyl-5-ASA was 1 ng/mg at a signal-to-noise ratio of 5.

Statistical analysis

The general, therapeutic and biochemical characteristics in recurring and unrecurring patients were compared by means of Fisher's exact test and Student's *t*-test where appropriate. An ANOVA model was used to identify the sources of variability of tessutal concentrations of mesalazine and acetyl-5-ASA. The model included demographic characteristics, duration of the disease, type of anastomosis, time elapsing after surgery and presence of recurrence (presence vs. absence) as dependent variables (sources of variability).

RESULTS

Patients with or without recurrence of Crohn's disease showed no significant differences with respect to prestudy characteristics (Table 1).

The mean value of mucosal mesalazine concentrations (expressed as ng/mg of tissue) was significantly lower in the specimens taken from patients with recurrence than in those from patients free of recurrence for both ileal (mean ± s.d: 21.6 ± 28.3 vs. 70.9 ± 47.4, respectively; $P = 0.007$) and colonic sites (25.8 ± 26.4 vs. 60.3 ± 32.5, respectively; $P = 0.010$) (Figure 1).

Mucosal concentration of acetyl-5-ASA (ng/mg of tissue) was also significantly lower in the specimens taken from the ileum of patients with recurrence than in

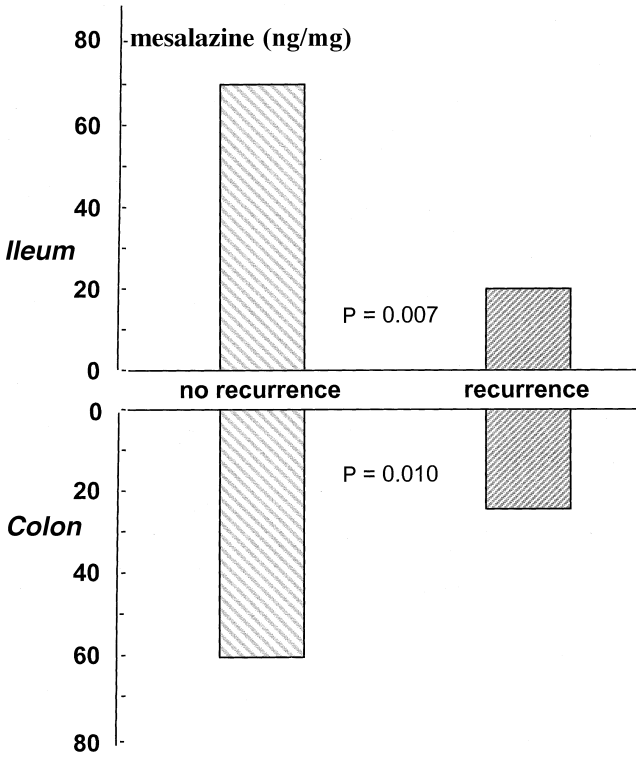

Figure 1. Mean values of mucosal mesalazine concentration in patients with and without recurrence.

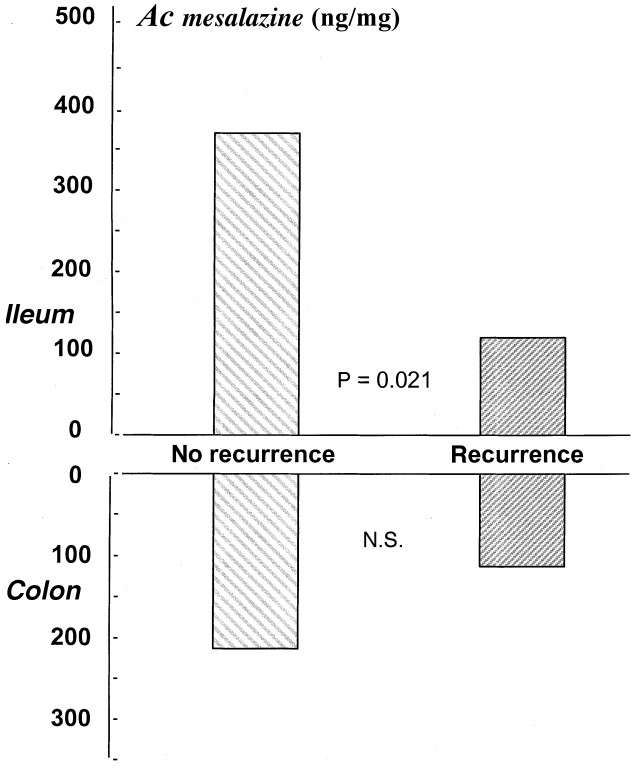

Figure 2. Mean values of mucosal acetyl 5-ASA concentration in patients with and without recurrence.

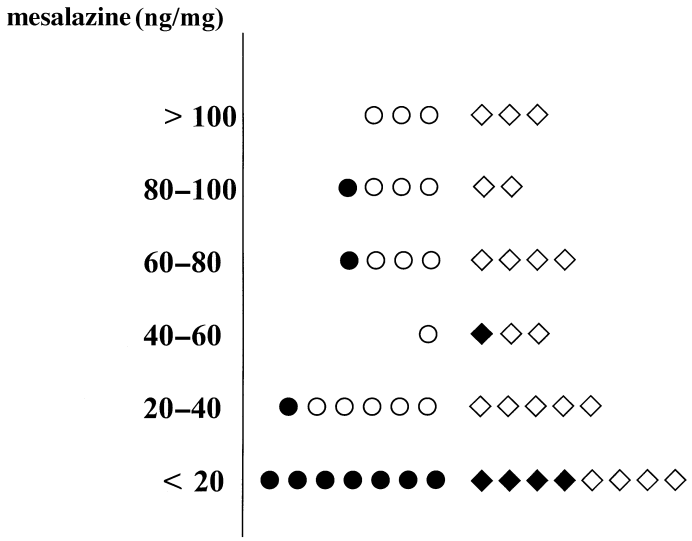

Figure 3. Distribution of mucosal mesalazine concentration in the ileal (○) and colonic (◇) perianastomotic sites of patients with (full symbols) and without (empty symbols) recurrence.

those free of recurrence (123.0 ± 124.1 vs. 370.7 ± 351.5, respectively; $P = 0.021$). With regard to colonic concentrations, no significant difference was observed between the two groups (Figure 2).

The distribution of mesalazine concentration in the ileum and colon, according to the absence or presence of recurrence, is shown in Figure 3. Seven out of 10 patients (70%) with ileal recurrence and four out of five patients (80%) with colonic recurrence showed a mucosal mesalazine concentration lower than 20 ng/mg. When the concentration was between 29 and 100 ng/mg, the recurrence rate was 17% for the ileum and 6% for the colon. No recurrence was observed at a concentration of > 100 ng/mg.

DISCUSSION

Data from this study show that in patients resected for Crohn's disease under treatment with oral mesalazine, recurrence is associated with a low mucosal concentration of the drug at the peri-anastomotic site. The data also provide evidence of a relationship between mucosal concentration and clinical effectiveness of mesalazine in the prevention of post-operative recurrence.

Morphological, biochemical and immunological findings have demonstrated that, despite radical resection, Crohn's disease is an ongoing process in which intestinal inflammation is premanently present. Modifications in the mucosal architecture, such as epithelial bridge formation and goblet cell hypertrophy or hyperplasia, were found by means of electron microscopy scan in histopathologically unaffected specimens of ileum and colon resected for Crohn's disease.[10] An increased phospholipase A2 activity in morphologically normal ileal mucosa after ileocolonic resection has also been reported.[11] A marked expression of adhesion molecules (ICAM 1, LFA 1, LFA 3) by endothelial cells, lymphocytes and monocytes has been observed in histologically unaffected areas of patients operated for Crohn's disease.[12] Finally, Reimund *et al.* have shown an increased release of TNF-alpha, IL-1 and IL-6 in organ culture of intestinal biopsy specimens taken from unaffected areas in patients operated for Crohn's disease.[13] Taken together, these date indicate that a sustained immune stimulation precedes edoscopically detectable inflammation and suggest the need for adequate prophylactic treatment of these early changes to prevent development of the lesions.

Studies performed in cellular and molecular models have demonstrated that mesalazine affects many inflammatory pathways and mediators involved in the pathogenesis of inflammatory bowel diseases. The drug is able to scavenge toxic reactive oxygen metabolites[14–19] and inhibit the production of prostaglandins and leukotrienes from the intestinal mucosa[20–25] and prevent leucocyte recruitment into the bowel wall by interfering with adhesion molecule expression. Moreover, mesalazine inhibits the synthesis of many cytokines such as IL-1, TNF-alpha IFN-gamma, which are primarily involved in the pathogenesis of Crohn's disease.[26–28] All these anti-inflammatory effects require an *in vitro* threshold concentration of the drug and are dose-dependent.[29–32] *In vivo* data are lacking.

In the present study, all the patients with a mucosal mesalazine concentration lower than 20 ng/mg showed recurrence at the neo-ileal site, while no patients showed recurrence when the concentration of the drug was >100 ng/mg of tissue. These observations suggest that the effectiveness of the drug is related to its mucosal concentration and that patients with mesalazine concentration lower than 20 ng/mg are at higher risk of post-operative recurrence.

The reasons for such inter-individual variability are not known. Data obtained in blood, urine and faecal samples have shown analogous inter-individual variability,[33, 34] probably due either to pharmacodynamic (dosage and delivery system of the drug) or to

pharmacokinetic factors (gastric emptying, intestinal transit time and intraluminal pH).[35–38] In the present investigation drug dosage and delivery system were constant and thus the inter-individual variability in mucosal concentration may simply be due to the complex mesalazine pharmacokinetics.

The low mucosal concentration of mesalazine observed in patients with recurrence could also represent the consequence rather than the cause of tissue inflammatory lesions. However, the widely recognized effectiveness of mesalazine in treating intestinal inflammatory lesions means that the drug is absorbed and concentrated in intestinal mucosa, site of inflammatory changes, enough to exert its therapeutic effect. Moreover, pharmacokinetic studies have shown indirect evidence that the presence of an active disease does not significantly influence the mucosal absorption of the drug.[39, 40] Therefore, the presence of inflammation may only play a secondary role in mucosal mesalazine concentration, if any.

Also, acetyl-5-ASA has been found in low concentrations in patients with recurrence even if, at the colonic site, the difference was not statistically significant. The role of this metabolite in the anti-inflammatory action of mesalazine has not yet been fully elucidated and therefore its role in preventing post-operative recurrence is not clear.

In summary, the mucosal concentration of mesalazine may be important in preventing post-operative recurrence. It is possible that an increase in mucosal mesalazine concentration may lead to a further reduction in the post-operative recurrence rate. Prospective studies are needed to establish the modalities required to reach an optimal tissue concentration of the drug.

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