Br. J. clin. Pharmac. (1988), **26**, 173–177

Delayed-release mesalazine (5-aminosalicylic acid): coat dissolution and excretion in ileostomy subjects

S. A. RILEY, I. A. TAVARES[1], A. BENNETT[1] & V. MANI[2]
University Department of Medicine, Hope Hospital, Salford, [1]Department of Surgery, King's College School of Medicine and Dentistry, London and [2]Leigh Infirmary, Leigh

1 Delayed-release mesalazine has been formulated to deliver 5-aminosalicylic acid to the colon. We have therefore studied the ileostomy excretion and coat dissolution of this preparation.

2 Following ingestion of a single tablet 88% (range 69–114%) of the 400 mg dose appeared unchanged in the ileosomy effluent over the subsequent 12 h.

3 Ileostomy effluent pH appeared to be a major determinant of 5-aminosalicylic acid release.

4 *In vitro* studies revealed rapid coat dissolution above pH 7.0, slow dissolution between pH 6.0 and 7.0 and non-dissolution at pH 2.0 and 4.0.

Keywords delayed-release 5-aminosalicylic acid Eudragit S pH-dependent release ulcerative colitis ileostomy

Introduction

Sulphasalazine (SSZ) has a well established role in the management of patients with ulcerative colitis (UC). It is of benefit in both active disease (Baron *et al.*, 1962) and in the maintenance of disease remission (Misiewicz *et al.*, 1965).

Unfortunately many patients are unable to take SSZ because of side effects and allergic reactions. Whereas most of these appear to be related to the sulphonamide component of the drug (Das *et al.*, 1973), 5-aminosalicylic acid (5-ASA) seems to be responsible for therapeutic activity (Azad Khan *et al.*, 1977). Clearly it would seem preferable to use only the active ingredient. 5-ASA, however, is unstable in gastric acid and is rapidly absorbed from the jejunum (Nielson & Bondesen, 1983). As it seems likely that the drug has a predominantly topical mode of action (Campieri *et al.*, 1985) a number of new drug formulations have been developed to deliver 5-ASA to the colon.

Delayed-release mesalazine is a pH-dependent 5-ASA delivery system. The amount of 5-ASA delivered to the colon by this method has not previously been determined directly and we have therefore studied the ileostomy excretion and coat dissolution of this formulation.

Methods

Ileostomy study

Eight subjects were studied, four male and four female, whose ages ranged from 21 to 57 years. All had previously undergone colectomy for ulcerative colitis, all were in good health and had normally functioning ileostomies. The study protocol was approved by Leigh Infirmary Ethics Committee and each subject gave written informed consent prior to study.

Correspondence: Dr S. A. Riley, University Department of Medicine, Hope Hospital, Eccles Old Road, Salford M6 8HD

Protocol

After an overnight fast, baseline samples of blood, urine and ileostomy effluent were taken. At 08.00 h a single 400 mg tablet of delayed-release mesalazine (Asacol, Tillotts Laboratories) was taken with 200 ml of water. After 2 h, fluids were allowed freely and subjects ate a normal lunch and dinner at 12.00 and 18.00 h. Ileostomy effluent was collected at 2 hourly intervals for 12 h, examined for evidence of the tablet and the pH was measured using a portable pH meter (Gallenkamp pH stick). Samples were then homogenised (Colworth Stomacher) and an aliquot frozen for subsequent analysis. Urine was collected from 0 to 4, 4 to 8, 8 to 12 and 12 to 24 h following tablet ingestion. Blood samples were drawn at hourly intervals for 9 h, and then at 12 and 24 h.

Measurements of 5-ASA and its acetylated metabolite, *N*-acetyl-5-ASA, were made by high performance liquid chromatography (Waters 6000A pump, Spectra Physics SP 8700 automatic injector) with fluorometric detection (Waters 420AC) using an excitation wavelength of 360 nm and emission wavelength of 425 nm. A reverse phase column (Pye Unicam 5μm ODS) was used with an elution mixture of 0.1M phosphate buffer (pH 7.4), methanol and tetrabutylammonium hydroxide (77.5:22.5:0.1).

All biological samples were mixed with methanol (1:1), centrifuged (1200 *g* for 15 min), mixed with an equal volume of phosphate buffer and injected directly into the system. Peak areas were calculated using a Schimadzu C-R3AA integrator. Calibration curves for 5-ASA and Ac-5-ASA were linear in the range 0 to 50 ng. Detection limits were 5 ng ml^{-1} in plasma and urine, and 0.1 μg ml^{-1} in ileostomy effluent. The interassay coefficient of variation ranged from 4.7 to 2.3 (5 to 50 ng).

Dissolution study

Dissolution of the tablet coat was assessed by adding tablets to isotonic phsophate buffer solutions of varying pH values (pH 2.0 to 8.0) during constant stirring at 37° C. The time to exposure of the pale tablet contents beneath the red acrylic resin was used as an easily recognised estimate of coat dissolution.

Tablet coat microscopy

To examine the nature and thickness of the acrylic resin coat tablets were vacuum-embedded in epoxy resin and unstained 5 μm sections were examined by light microscopy using an eyepiece graticule.

Results

Ileostomy study

All subjects tolerated the study well and no adverse reactions were reported. In three subjects the tablets were passed whole into the ileostomy bag. In each case, however, the Eudragit S coat had split with obvious exposure of the tablet contents. In the other five subjects, whole tablets or discernible tablet fragments were not detected within the ileostomy effluent.

The mean cumulative ileostomy output of 5-ASA is shown in Figure 1. In six subjects 5-ASA first appeared in the effluent between 4 and 6 h and in the remaining two between 6 and 8 h. Within 12 h of taking the tablet 88% (range 69 to 114%) of the 400 mg dose was detected in the ileostomy effluent as unchanged 5-ASA. Ac-5-ASA, however, was detected in the effluent of only two subjects. In one subject Ac-5-ASA

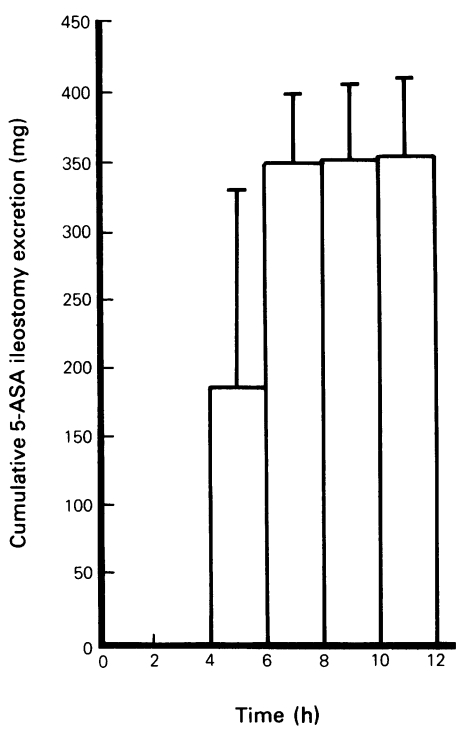

Figure 1 Cumulative 5-aminosalicylic acid ileostomy excretion in the 12 h following ingestion of a 400 mg tablet of delayed-release mesalazine (mean ± s.d., $n = 8$)

accounted for 7% and in the other 31% of the ingested dose of 5-ASA.

Urinary excretion and plasma levels of 5-ASA and Ac-5-ASA also showed marked variability. Mean urinary Ac-5-ASA excretion was 22.8 mg (range 0 to 79 mg) in the 24 h following ingestion. 5-ASA was excreted by only two subjects and in both the amount was less than 3 mg of the ingested 400 mg dose. In two subjects neither Ac-5-ASA nor 5-ASA was detected in the urine. Plasma 5-ASA and Ac-5-ASA were not detected in five subjects. In the remaining three, Ac-5-ASA predominated; levels rose between 3 and 6 h, peaked at 6 to 8 h (0.5 and 2.1 $\mu g\ ml^{-1}$) and had fallen by 8 to 10 h. The mean combined amount of 5-ASA and Ac-5-ASA detected in ileostomy effluent and urine was 387 mg, range 343 to 475 mg (mean 97%, range 86 to 119% of the ingested dose).

The pH of the ileostomy effluent varied considerably, both between subjects and between samples from the same subject during the study. Values ranged from pH 5.9 to pH 8.3 with a mean value of pH 7.1. In the three subjects who had detectable plasma levels of 5-ASA and Ac-5-ASA and in whom 24 h urinary Ac-5-ASA excretion exceeded 20 mg, ileostomy effluent pH never fell below pH 7.0. In a further three subjects pH values fluctuated around pH 7.0 and 24 h Ac-5-ASA excretion ranged from 1.2 to 3.0 mg. In the two remaining subjects values greater than pH 7.0 were infrequent, the tablets were passed whole (although the coatings were split) and Ac-5-ASA was not detected in the urine.

Dissolution study

The results of the *in vitro* dissolution study are shown in Figure 2. At pH values over 7.0, coat dissolution was rapid and exposure of the tablet contents always occurred within 30 min. Indeed, fragments of Eudragit S were seen to drift away from the tablet coat within seconds of immersion in the alkaline buffers leaving the tablets with a moth-eaten appearance (Figure 3a). Between pH 6.0 and 7.0 this phenomenon was not apparent, time to exposure of the contents was considerably delayed (up to 20 h) and invariably occurred by splitting of the tablet coat along one of its edges (Figure 3b). At pH 2.0 and 4.0 the coat remained intact for 3 days at which time the experiments were discontinued.

Tablet coat microscopy

Light microscopy of the tablet coat revealed a mean coat thickness of 69 μm, range 59 to 86 μm.

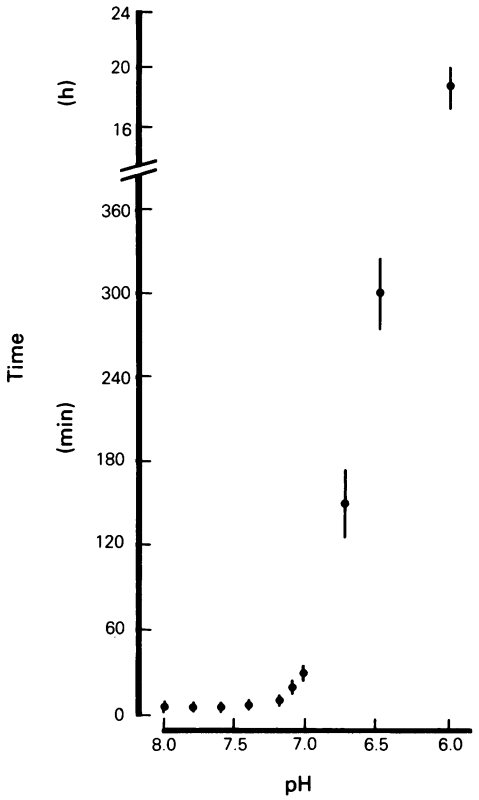

Figure 2 *In vitro* coat dissolution: time to tablet content exposure at varying pH values (mean ± s.d., $n = 6$).

A consistently thinner coat was seen along the edges of the tablet where mean thickness fell to 57 μm, range 52 to 62 μm.

Discussion

Precise and reliable delivery of 5-ASA to the colon is clearly important for patients with ulcerative colitis. 5-ASA released in the proximal small intestine is rapidly absorbed, so increasing the risk of systemic toxicity and decreasing the amount of drug available to the colon. Too distal 5-ASA release, on the other hand, may leave the more proximal colon 'untreated'.

Delayed-release mesalazine comprises 400 mg of 5-ASA within an acrylic resin coat, Eudragit S. Coat dissolution is pH dependent and is reported to occur only above pH 7.0 (Lehmann, 1971). Radiological studies of barium-containing tablets in normal volunteers (120 μm tablet coat) (Dew *et al.*, 1982) and patients with active colitis (80 μm tablet coat) (Dew *et al.*, 1983) suggest

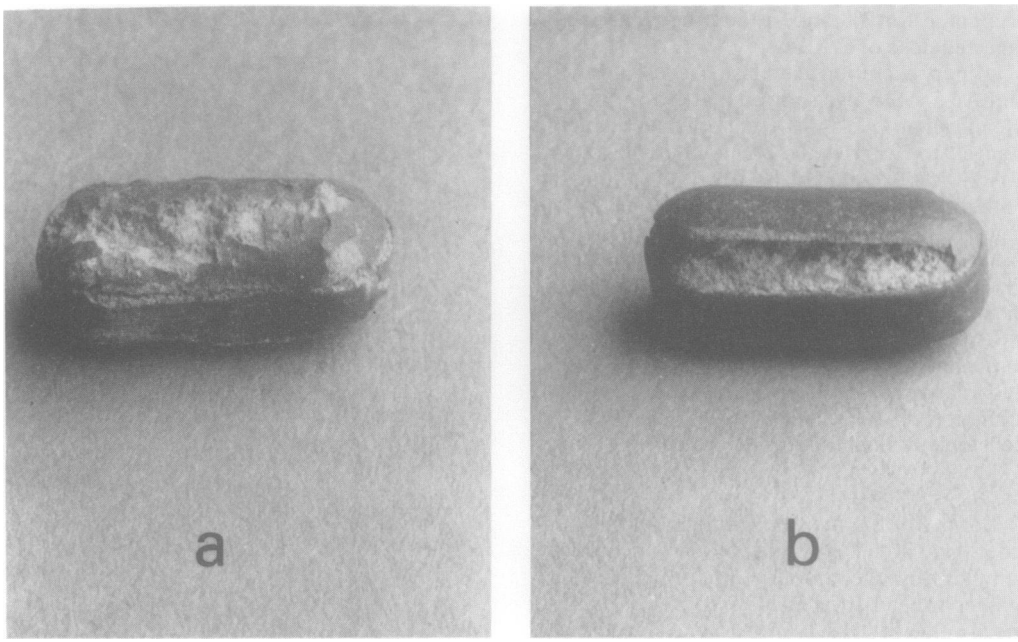

Figure 3 *In vitro* coat dissolution: macroscopic appearances after immersion in (a) buffer pH 7.5, (b) buffer pH 6.5.

that 5-ASA release occurs in the distal ileum or right side of the colon.

The results of the present study show that following the ingestion of a single tablet of delayed-release mesalazine, 88% (range 69 to 114%) of the 400 mg 5-ASA dose appears unchanged in the ileostomy effluent over the subsequent 12 h. Marked variations in plasma levels and urinary excretion of 5-ASA and Ac-5-ASA were also apparent.

In two subjects tablet dissolution seemed premature as approximately 20% of the ingested dose appeared in the urine as Ac-5-ASA. In three other subjects tablets were passed whole into the ileostomy bag. In all three the coat had split and in one Ac-5-ASA was found in the urine confirming *in vivo* dissolution. In the other two cases, however, the possibility of dissolution within the ileostomy bag during the 2 h collection period cannot be excluded.

The present *in vitro* studies confirm rapid dissolution of the tablet coat at or above pH 7.0. Surprisingly, consistent release of the tablet contents was also seen between pH 6.0 and 7.0 although this was always considerably delayed and may be related to splitting of the coat along the tablet edges. In addition, the pH of the ileostomy effluent correlated well with urinary output of Ac-5-ASA and also with the macroscopic appearance of the recovered tablet. It therefore seems likely that luminal pH is a major determinant of variations in colonic delivery. Unfortunately there have been few studies of intestinal pH in man either in health or disease and the influence of factors such as diet and drugs are largely unknown (Fordtran & Locklear, 1966; Bown *et al.*, 1974).

The variations in ileostomy 5-ASA recovery seen in the present study (69 to 114%) are similar to those reported for both SSZ (75 to 90% excreted as SSZ) (Das *et al.*, 1979) and the 5-ASA azo-linked dimer olsalazine (73 to 121% excreted as olsalazine) (Sandberg-Gertzen *et al.*, 1982). However, following colonic delivery these prodrugs must also undergo bacterial azoreduction before releasing 5-ASA. Although this process is usually highly efficient it may be considerably impaired by both rapid transit (van Hees *et al.*, 1979) and antibiotic exposure (Houston *et al.*, 1982), and is therefore a further potential source of reduced bioavailability.

In conclusion, delayed-release mesalazine appears to be an effective method of delivering 5-ASA to the colon. In six of the subjects studied tablet dissolution was probably optimal, but in two premature release occurred. Since luminal pH appears to be a major determinant of 5-ASA release, *in vivo* studies of intestinal pH in patients with ulcerative colitis are indicated. These may be of value in assessing the influence of diet, drugs and changes in bowel function on colonic 5-ASA delivery.

References

Azad Khan, A. K., Piris, J. & Truelove, S. C. (1977). An experiment to determine the active therapeutic moeity of sulphasalazine. *Lancet*, **ii**, 892–895.

Baron, J. H., Connell, A. M., Lennard-Jones, J. E. & Avery-Jones, F. A. (1962). Sulphasalazine and salicylazosulphadimidine in ulcerative colitis. *Lancet*, **i**, 1094–1096.

Bown, R. L., Gibson, J. A., Sladen, G. E., Hicks, B. & Dawson, A. M. (1974). Effect of lactulose and other laxatives on ileal and colonic pH as measured by a radiotelemetry device. *Gut*, **15**, 999–1004.

Campieri, M., Lanfranchi, G. A., Boschi, S., Brignola, C., Bazzocchi, G., Gionchetti, P., Minguzzi, M. R., Belluzzi, A. & Labo, G. (1985). Topical administration of 5-aminosalicylic acid enemas in patients with ulcerative colitis. Studies on rectal absorption and excretion. *Gut*, **26**, 400–405.

Das, K. M., Chowdhury, J. R., Zapp, B. & Fara, J. W. (1979). Small bowel absorption of sulphasalazine and its hepatic metabolism in human beings, cats and rats. *Gastroenterology*, **77**, 280–284.

Das, K. M., Eastwood, M. A., McManus, J. P. A. & Sircus, W. (1973). Adverse reactions during salicylazosulphapyridine therapy and the relation with drug metabolism and acetylator phenotype. *New Engl. J. Med.*, **289**, 491–495.

Dew, M. J., Hughes, P. J., Lee, M. G., Evans, B. K. & Rhodes, J. (1982). An oral preparation to release drugs in the human colon. *Br. J. clin. Pharmac.*, **14**, 405–408.

Dew, M. J., Ryder, R. E. J., Evans, N., Evans, B. K. & Rhodes, J. (1983). Colonic release of 5-aminosalicylic acid from an oral preparation in active ulcerative colitis. *Br. J. clin. Pharmac.*, **15**, 185–187.

Fordtran, J. S. & Locklear, T. W. (1986). Ionic constituents and osmolarity of gastric and small intestinal fluids after eating. *Am. J. Dig. Dis.*, **11**, 503–521.

Houston, J. B., Day, J. & Walker, J. (1982). Azo reduction of sulphasalazine in healthy volunteers. *Br. J. clin. Pharmac.*, **14**, 395–398.

Lehmann, K. (1971). Programmed drug release from oral dosage forms. *Pharmacy International*, **3**, 1–16.

Misiewicz, J. J., Lennard-Jones, J. E., Connell, A. M., Parsons, J. H. & Avery-Jones, F. (1965). Controlled trial of sulphasalazine in maintenance therapy for ulcerative colitis. *Lancet*, **i**, 185–188.

Nielson, O. H. & Bondesen, S. (1983). Kinetics of 5-aminosalicylic acid after jejunal instillation in man. *Br. J. clin. Pharmac.*, **16**, 738–740.

Sandberg-Gertzen, H., Ryde, M. & Jarnerot, G. (1983). Absorption and excretion of a single 1 g dose of Azodisal sodium in subjects with ileostomy. *Scand. J. Gastroenterol.*, **18**, 107–111.

Van Hees, P. A. M., Tuinte, J. H. M., Van Rossum, J. H. & Van Tongeren, J. H. M. (1979). Influence of intestinal transit time on azoreduction of salicylazosulphapyridine (Salazopyrin). *Gut*, **20**, 300–304.

(Received 14 December 1987, accepted 13 April 1988)