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5-Aminosalicylic acid in serum and urine after administration by enema to patients with colitis

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5-Amino salicylic acid (5-ASA) is an effective treatment for ulcerative colitis. It is probably the active constituent of sulphasalazine (Azad Kahn et al 1977) and given as a specially coated oral preparation will maintain remission in colitis (Dew et al 1982). Enemas of 5-ASA and steroids are equally as effective in proctitis (Campieri et al 1981) although there is considerable difficulty in producing enema preparations because 5-ASA is unstable in water.

Even when given as sulphasalazine, 5-ASA probably acts topically after its release in the colon; the serum levels of both 5-ASA and its acetylated derivate are low; usually less than 2 μg ml^{-1} (Fischer & Klotz 1979). Between 20 to 30 per cent of the 5-ASA administered as sulphasalazine is excreted in urine within 48 h of administration most being acetylated (>80%) (Das et al 1973). However, a large bolus of 5-ASA in the inflamed rectum and colon could produce high serum and urinary levels of 5-ASA and its metabolites.

This study describes a novel and practical formulation of 5-ASA for enema administration and reports serum and urinary levels of 5-ASA and acetyl 5-ASA after enema administration to seven patients with colitis.

Methods

Patients. Seven patients, mean age 40 years (range 19–68 years), 6 male and 1 female, were studied. Five had total colitis, 1 left sided colitis and 1 proctitis. All had ulcerative colitis except 1 male with total Crohn's colitis. Five were inpatients at the time of study because of active disease and two were outpatients with quiescent colitis. Administration of 5-ASA whether as sulphasalazine or coated 5-ASA was stopped at least 48 h before the enema study. Blood was obtained at time zero, and then at 30 min, 1, 1½, 2, 3 and 4 h. Urine was collected for 8 h after administration of the enema.

Enema preparation. 5-ASA enemas contained 700 mg of 5-ASA (Aldrich Chemicals) mixed with 300 mg of a xanthan hydrophillic gum (Keltrol: ABM Chemicals Limited) which was mixed with warm water at 37 °C before administration and shaken for 3–4 min to ensure uniform mixing and then allowed to stand for a further 20 min. The enemas were well retained despite active disease, mean 3·6 h (range 1–8 h; Table 1).

Analysis. 5-ASA and acetyl 5-ASA were analysed by high pressure liquid chromatography using a modification of the method of Shaw et al (1980). The analysis was performed on a LiChrosorb 10 RP18 bonded silica reversed phase-column (Merck). The mobile phase was acetonitrile—0·05 m potassium dihydrogen phosphate solution (15:85) pH 7·4 containing 0·1 per cent tetrabutylammonium hydroxide. 5-ASA and acetyl 5-ASA were detected using a fluorescent spectrometer (excitation 360 nm, emission 425 nm). Serum and urinary samples were treated with an equal volume of aceto-nitrile to precipitate proteins and after centrifugation the supernatant was injected onto the chromatograph. Levels of 5-ASA and acetyl 5-ASA were read off a calibration curve which was linear over the ranges of 5-ASA and acetyl 5-ASA measured.

Results

The serum levels of 5-ASA after a 700 mg enema were low, 0–0·3 μg m^{-1}, mean 0·1 μg ml^{-1} (Fig. 1). Serum acetyl 5-ASA levels were higher (0–0·8 μg ml^{-1}; mean 0·3 μg ml^{-1}; Fig. 2). Urinary levels of 5-ASA were low 0·7–2·0 mg/8 h; mean 1·0 mg, with most 5-ASA (98%) excreted in the urine as the acetyl form 10·5–77·3 mg/8 h, mean 40·3 mg (Table 1).

Discussion

5-Amino salicylic acid is unstable in water and is difficult to prepare as an enema solution to supply to patients. We have overcome this by supplying the dry powder mixed with a suspension agent (Keltrol) to assist mixing. The patient prepares the enema by adding warm water to the powder immediately before administration.

Serum levels of 5-ASA and acetyl 5-ASA after administration of a 700 mg enema prepared as above were measured over 4 h and found to be low (<1 μg ml^{-1}); acetyl 5-ASA concentrations were

Table 1. Total urinary excretion of 5-ASA and acetyl 5-ASA during 8 h after administration of an enema containing 700 mg of 5-ASA in seven patients with colitis.

Patient	Time enema retained (h)	Urine vol (ml)	Total 5-ASA (mg)	Total AC-5-ASA (mg)
1	4·0	400	2·0	77·3
2	8·0	290	1·1	51·1
3	1·0	125	0·7	36·4
4	3·75	1080	0·9	40·3
5	4·0	950	0·8	50·5
6	3·0	160	1·0	15·9
7	1·5	85	0·6	10·5
Mean	3·6	441	1·0	40·3

* Correspondence.

approximately four times higher than those for 5-ASA. These levels are similar to those obtained from patients taking up to 3 g of sulphasalazine daily (Fischer & Klotz 1979). In the urine, the acetyl form predominated (98%).

These low levels would suggest that the action of 5-ASA may be largely topical rather than systemic and it is probable that 5-ASA rather than the acetyl 5-ASA is the active compound (Binder et al 1981), although both have anti-inflammatory activity.

Renal damage due to 5-ASA has been reported in rats (Calder et al 1972) after intravenous administration of 1·4–5·7 mM kg which might be expected to give higher serum levels than those obtained by our enemas or sulphasalazine administration. As the levels of 5-ASA and acetyl 5-ASA documented after 5-ASA enema administration in our patients (most of whom had active inflammation and might be expected to absorb more of the drug) were low, this should be a safe and non-toxic preparation in man.

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p-Chlorophenylalanine antagonism of the analgesia and increase in brain noradrenaline metabolism produced by morphine

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Although the potential roles of noradrenaline and 5-hydroxytryptamine (5-HT) in opiate analgesia have been extensively studied (Messing & Lytle 1977; Iwamoto & Way 1979), information concerning their interaction in the expression of opiate effects is relatively lacking (Sewell & Spencer 1976). We here report an attempt to establish such a relationship by evaluating the effect of *p*-chlorophenylalanine (PCPA), an inhibitor of 5-HT synthesis (Koe & Weissman 1966), on the analgesia and increase in brain 3-methoxy-4-hydroxyphenylethylene glycol sulphate (MOPEG-SO_4), the major noradrenaline metabolite in rat brain (Schanberg et al 1968), produced by morphine.

Methods

Male Sprague-Dawley rats (Holtzman, Madison, WI) 200–300g were housed in pairs in automatic watering cages with food freely available. *p*-Chlorophenylalanine methyl ester HCl (Sigma, St. Louis, MO) (PCPA), dissolved in distilled water with the pH adjusted to 6·0 with 5 M NaOH, was administered at a dose of 300 mg kg^{-1} base i.p. 72 h before i.p. injection of 10 mg kg^{-1} morphine sulphate (Mallinckrodt, St. Louis, MO) or an equivalent volume of its saline (0·9% NaCl) vehicle (2 ml kg^{-1}). Analgesia was measured by a modification (Bass & Vander Brook 1952) of the tail flick procedure of D'Amour & Smith (1941). Baseline (BL) latency (3–4s) was determined immediately before the injection of morphine or saline and analgesia tested (T) 60 min thereafter. A 12s cut off was employed in the absence of a response and the degree of analgesia (DA) was calculated according to the following formula (Mayer & Hayes 1975): $DA = 100\ (T-BL)/(12-BL)$. The mean and standard errors of these ratios were calculated through the use of an arcsine transformation (Sokal & Rohlf 1969). Animals were decapitated following analgesic testing and whole brain noradrenaline and dopamine were measured in the same samples by the fluorometric procedures of Anton & Sayre (1962) and Carlsson & Waldeck (1958). Separate groups were employed for the fluorometric determination of brain 5-HT (Maickel et al 1968) and MOPEG-SO_4 (Meek & Neff 1972). Statistical comparisons among treatment groups were made with a one way analysis of variance and/or Student's *t*-test ($\alpha = 0{\cdot}05$).

To verify the selectivity of the PCPA treatment and to obtain an indication of the degree of 5-HT depletion, whole brain concentrations of dopamine, noradrenaline, 5-HT and MOPEG-SO_4 were measured in rats given PCPA (300 mg kg^{-1} i.p.) 73 h and saline 1 h before death. PCPA produced a 75% decrease in brain 5-HT ($55 \pm 3{\cdot}9$ vs $215 \pm 12{\cdot}4$ ng g^{-1} in untreated controls; $P < 0{\cdot}01$) but did not significantly alter the concentration of dopamine ($547 \pm 35{\cdot}7$ vs $560 \pm 12{\cdot}3$ ng g^{-1}), noradrenaline ($260 \pm 24{\cdot}4$ vs $265 \pm 7{\cdot}8$ ng g^{-1}) or MOPEG-SO_4 ($423 \pm 18{\cdot}6$ vs $430 \pm 13{\cdot}5$ p mol g^{-1}). This treatment also did not significantly alter baseline tail flick latency in the analgesic test.

The effect of PCPA on the analgesia and increase in

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