Effect of azodisal sodium and sulphasalazine on ileostomy output of fluid and PGE₂ and PGF_{2α} in subjects with a permanent ileostomy

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SUMMARY Azodisal sodium is a highly effective means of oral delivery of 5-amino-salicylic acid to the colonic mucosa. Administration of this drug to patients intolerant of sulphasalazine, however, occasionally results in liquid stools. In preliminary experiments, which comprised 10 healthy volunteers treated with colectomy for ulcerative colitis, ileostomy fluid output increased ($p < 0.001$) during oral intake of azodisal sodium (1 g/day). In a double blind, placebo controlled crossover study, comprising eight similar volunteers, ileostomy fluid output increased ($p < 0.05$) in a dose related manner during intake of azodisal sodium (1 g/day vs 2 g/day) compared with placebo or sulphasalazine (2 g/day). Concentrations of prostaglandin (PG)F_{2α} in free ileal water determined by equilibrium *in vivo* dialysis of ileostomy contents decreased ($p < 0.05$) during intake of azodisal sodium (2 g/day), whereas concentrations of PGE₂ and the output of PGE₂, PGF_{2α}, and 'PGE₂+PGF_{2α}' remained unchanged. Thus increased formation of PGs is apparently not the cause of increased ileostomy fluid output associated with azodisalicylate intake.

Sulphasalazine has proved useful in mild or moderately active attacks of ulcerative colitis¹⁻³ and is the mainstay of maintenance therapy for the prevention of relapse.⁴⁻⁵ This compound is split in the colon by bacterial azoreductases into sulphapyridine and 5-aminosalicylic acid (5-ASA),⁶⁻⁷ the latter being considered the therapeutic moiety of sulphasalazine.⁸⁻¹⁰ In contrast, the major side effects of sulphasalazine are considered to be caused by sulphapyridine,¹¹ which is readily absorbed, even in the colon.

Azodisal sodium (ADS, disodium azodisalicylate, see Fig. 1) provides an attractive means of reducing serious adverse reactions, because it is sulpha-free and delivers twice the amount of 5-ASA on a molar basis.¹²⁻¹³ This compound has recently been given the generic name of olsalazine sodium. We have earlier demonstrated that azodisal sodium is a highly

effective means of oral delivery of 5-ASA to the colon,¹⁴⁻¹⁵ because small intestinal absorption and metabolism are minimal and a single oral dose is completely recovered from ileostomy fluid.¹⁴ In patients with inactive ulcerative colitis, as well as in healthy volunteers, complete azoreduction of azodisal sodium occurs and concentrations of 5-ASA in faecal dialysates double when sulphasalazine is replaced by the same dosage of azodisal sodium.¹⁵

Exacerbation of diarrhoea and inflammation has occasionally been reported after the administration of sulphasalazine or 5-ASA to patients with ulcerative colitis.¹⁶⁻²⁰ In a tolerance study of azodisal

Fig. 1 Structural formula of azodisal sodium (ADS).

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sodium in 160 patients, intolerant of or allergic to sulphasalazine, the most commonly reported complaints were loose or watery stools.²¹ This study comprised a highly selected group of patients. Diarrhoea was the cause of sulphasalazine intolerance only in seven (4.4%) of the patients, and six of them experienced the same reaction on azodisal sodium. Of the remaining 153 patients 9.8% had diarrhoea during azodisal sodium intake. Because the diarrhoea associated with azodisal sodium intake was watery rather than bloody, and most often occurred in patients with extensive colitis we considered the possibility that azodisal sodium might affect small intestinal transport of fluid and electrolytes. The present study was designed, therefore, to test whether administration of azodisal sodium to patients having had colectomy for ulcerative colitis was associated with increased ileostomy output and, if so, to study in a double blind, placebo controlled crossover design the effect of sulphasalazine and azodisal sodium on the release of PGE₂ and PGF_{2α} into the small intestinal lumen by the method of equilibrium *in vivo* dialysis^{15 22} of ileostomy contents.

Methods

PATIENTS

The subjects volunteering in the study had previously had a colectomy for ulcerative colitis. All had a well functioning, conventional ileostomy and were in good health. Apart from one subject treated with a beta-adrenergic blocker no other drug was taken, except for the study medicine, during the investigations. The pilot study comprised five men and five women with a median age of 39 years (range 31–73), three of whom had previously proven to be intolerant of or allergic to sulphasalazine. The controlled study comprised five men and three women with a median age of 41 years (range 24–62 yr). One woman took part in both studies.

The investigation was carried out in accordance with the Helsinki Declaration II and approved by the Regional Ethical Committee in Örebro. Before the studies all volunteers were informed verbally and in writing and gave their written consent to participate.

EXPERIMENTAL DESIGN

Ileostomy output was collected in containers allowing volume measurement. Volume and consistency of ileostomy fluid, as well as time for meals, intake of study medicine, and emptying of bags were registered in a diary by the volunteers, who were also asked to note factors which they considered might influence the fluid output.

In the pilot study pretreatment measurements of the daily ileostomy output were carried out for one week and continued during one week's intake of azodisal sodium (Dipentum, Pharmacia AB, Uppsala, Sweden) 0.5 g bid.

In the controlled study the volunteers were randomised in blocks of four. During four different five day periods one of the following was administered in gelatin capsules of identical appearance on blister cards to secure adequate control: azodisal sodium 2 g/day, azodisal sodium 1 g/day, sulphasalazine (Salazopyrin, Pharmacia) 2 g/day, or placebo. During each five day period ileostomy output was measured and diary notes recorded as described above. Between each medication there was a nine day washout period.

The compliance was tested by analysis of ileostomy fluid for the presence of azodisal sodium or sulphasalazine. In the pilot study compliance was tested twice during the seven days of azodisal sodium intake. In the controlled study compliance was tested once during each medication period.

EQUILIBRIUM IN VIVO DIALYSIS OF ILEOSTOMY CONTENTS

The rationale for using the equilibrium *in vivo* dialysis of intestinal contents, originally described by Wrong *et al*,²³ was to measure concentrations of prostaglandins in free intestinal water. The validation of the method has been described elsewhere.^{22 23} Dialysis bags (volume 0.5 ml) were made by tying off 2 cm segments of Visking seamless cellulose tubing 8/32 filled with Rheomacrodex (Pharmacia) containing 10% dextran (mean mol wt 40 000) in saline. On the third day in each medication period of the controlled study the participants swallowed 15 dialysis bags. As soon as the dialysis bags had appeared in the ileostomy sack they were removed and cleaned with dry Kleenex tissue (Kimberly-Clark Corporation, Neenah, Wis.). The dialysis bags appeared in the ileostomy sacks either all at the same time or in intervals. The contents of the dialysis bags removed at the same occasion were pooled in one test tube and immediately stored at –20°C. Thus the number of vessels for prostaglandin analysis could vary between one to four in different subjects. Analyses for PGE₂ and PGF_{2α} were carried out within two weeks. Each pool was analysed separately and the daily PG concentration for each participant was the result of the mean values of these measurements. Samples for prostaglandin determination from one patient on sulphasalazine medication and one patient on azodisal sodium (2 g/day) medication were lost during transport.

ANALYTICAL PROCEDURES

PGE₂ and PGF_{2α} were measured as previously described in detail by a radioimmunoassay method²⁴ validated by gas chromatography-mass spectrometry.²⁵ The methods included purification by extraction with ethylacetate/cyclohexane=1:1 and chromatography on microcolumns of Sephadex LH-20 (Pharmacia) before doing the RIA on the relevant eluate fractions. In samples containing azodisal sodium this drug was extracted with prostaglandins, but did not separate with these compounds nor did it cross-react with the antibodies. Concentrations of prostaglandins were expressed as pg/ml and output of 'PGE₂+PGF_{2α}' as ng/day, calculated from the sum of the mean concentrations times the volume of ileostomy output within the same day.

STATISTICAL ANALYSES

The pilot study was planned as a matched pair design and was analysed by Wilcoxon's signed rank method.

In the controlled study the results of the fluid volumes were expressed as mean ($\pm$ SEM), whereas the prostaglandin concentrations were given as median (ranges) of mean values observed in individual patients within the same day. The study was designed as a latin square in which rows represent patients and columns represent the order of administration of treatments. Each subject received the various treatments in random order. The comparison between treatments was performed using analysis of variance and Duncan's multiple range test. Before analysis the data were normalised using the logarithmic transformation. In both

studies *p* values less than 0.05 were considered significant.

Results

PILOT STUDY

Azodisal sodium was present in all samples studied, thus confirming good compliance. The ileostomy output increased significantly during the period of medication (*p*=0.001) in all volunteers whether they were tolerant, intolerant, or allergic to sulphasalazine (Fig. 2). The net increase was approximately 250 ml/day in all but one subject (KE), who possibly had had a gastroenteritis during the control period.

CONTROLLED STUDY

The analyses of ileostomy samples for study medication disclosed no cases of non-compliance. The study drugs were well tolerated by all participants, but more liquid ileostomy contents were noted during periods of azodisal sodium intake. The ileostomy output increased significantly (*p*<0.05) during azodisal sodium (1 g/day) administration compared with placebo (Table 1), whereas sulphasalazine (2 g/day) caused no change (*p*>0.05). A dose related increase in ileostomy output was observed during azodisal sodium medication (*p*<0.05).

The transit time of the dialysis bags given by mouth varied considerably, but exceeded in all cases the period of four hours necessary for reaching an equilibrium of diffusion.²² No relationship was found between the PG-concentrations and the transit time of the dialysis bags. Table 2 gives the median values of the average PGE₂ and PGF_{2α}

Fig. 2 Daily ileostomy output in the pilot study during the control and the medication periods in each subject. Numbers above the bars represent days during which output measurements were undertaken. Values are expressed as ml/24 h (mean $\pm$ SEM). The difference between the two periods was statistically significant (*p*<0.001); Wilcoxon's matched pairs signed rank test.

Table 1 Ileostomy output during the four five day periods of medication

Patient	Placebo	Medication		
		SASP (2 g/day)	ADS (1 g/day)	ADS (2 g/day)
1	854±80	748±98*	1000±85	1124±105
2	824±82	746±102	1046±70	930±75
3	292±23	252±15	530±32	796±115
4	282±19	360±26	466±24	658±27
5	488±58	462±33	1184±90	1524±137
6	468±36	508±40	636±50	670±35
7	380±13	324±9	732±76	730±31
8	450±6*	545±38*	605±41*	1010±44*
Mean	505±78	493±65	775±94	930±103

Values represent mean±SEM in ml/day. SASP=sulphasalazine; ADS=azodisal sodium. Differences between ADS and placebo or SASP and between the two doses of ADS were statistically significant ($p<0.05$, Duncan's multiple range test).

*These calculations are based on four days measurements.

Table 2 Concentrations of PGE_2 and $PGF_{2\alpha}$ in free ileostomy water and the output of ' $PGE_2+PGF_{2\alpha}$ ' during the four five day periods of medication

		Placebo (n=8)	Medication		
			SASP (2 g/day) (n=7)	ADS (1 g/day) (n=8)	ADS (2 g/day) (n=7)
PGE_2	pg/ml	140 (30–1030)	55 (40–565)	85 (40–215)	85 (30–290)
$PGF_{2\alpha}$	pg/ml	225 (130–600)	170 (85–700)	145 (95–540)	100 (65–660)*
' $PGE_2+PGF_{2\alpha}$ '	ng/day	215 (105–1340)	210 (40–1080)	255 (105–770)	145 (70–710)

Values represent median (range. SASP=sulphasalazine; ADS=azodisal sodium.

* $p<0.05$ (Duncan's multiple range test).

concentrations as well as those of ' $PGE_2+PGF_{2\alpha}$ ' output observed in each individual during intake of placebo, sulphasalazine (2 g/day), and azodisal sodium (1 or 2 g/day), respectively. PGE_2 and $PGF_{2\alpha}$ levels varied considerably, even within the same individual during the same day. During medication with ADS (2 g/day), however, there was a significant ($p<0.05$) reduction in $PGF_{2\alpha}$ concentrations compared with the placebo periods. Significant changes in the outputs of PGE_2 , $PGF_{2\alpha}$, and ' $PGE_2+PGF_{2\alpha}$ ' were not observed.

Discussion

Our results suggest that the parent azodisal sodium molecule was responsible for the increase in ileostomy output in patients having had a colectomy for ulcerative colitis. The sulphasalazine and azodisal sodium doses, however, were not matched exactly for salicylate content (sulphasalazine 2 g contains 5-ASA 750 mg). By extrapolation back to a deduced effect of ADS 750 mg per day, six out of eight patients would have had a greater ileostomy output

than whilst on placebo or sulphasalazine. The increased ileostomy output appears, therefore, to be specifically associated with azodisal sodium, which is not metabolised in the small intestine and completely recovered from ileostomy fluid after a single oral dose.¹⁴ Also recent experiments in the rat have shown that azodisal sodium, but not sulphasalazine or the common therapeutically active split product, 5-ASA, causes impaired net absorption and at high concentrations net secretion of fluid, chloride, and sodium in the ileum and the colon.²⁶

In the present study high dose azodisal sodium caused a significant decrease in $PGF_{2\alpha}$ concentrations in free ileal water, but PGE_2 concentrations were not affected. Also outputs, ' $PGE_2+PGF_{2\alpha}$ ' were unchanged. Thus, the increased ileostomy output during intake of azodisal sodium is apparently not mediated by any of these two prostaglandins.

The clinical significance of our observations is at present purely speculative. The effect of ileostomy – that is, adaptive changes, on the handling of fluid and electrolytes by the small intestine might under-

estimate the azodisal sodium induced secretion. In health the human colon is capable of compensating for even a large overload of fluid from the small intestine,²⁷ which may result in diarrhoea if the colon is inflamed and its absorptive capacity decreased.²⁸ Bacterial azoreduction of azodisal sodium is almost complete in healthy volunteers as well as in patients with inactive ulcerative colitis,¹⁵ but the efficacy of bacterial azoreduction of azodisal sodium in patients with active ulcerative colitis and/or accelerated intestinal transit is totally unknown, although indirect evidence points to a reduced azoreduction of sulphasalazine.²⁹ The choice of an alternative dispensing formulation to the disodium salt in gelatin capsules used in the present study, which avoids the exposure of the small intestinal epithelium to azodisal sodium, might prove clinically relevant. Clearly, more work is required to determine if drug induced intestinal secretion involves significant tolerance problems in a general population of patients with ulcerative colitis, thus limiting the use of azodisal sodium. It has also to be worked out whether the secretory effect observed in the present study is confined to azodisal sodium or if it is a property shared by other new azo-bound pro-drugs of 5-ASA, currently undergoing clinical investigations.

In conclusion, the results of the present study clearly show that azodisal sodium, unlike sulphasalazine, causes increased ileostomy output, which is not due to increased formation of prostaglandin. This may implicate tolerance problems in patients with extensively located ulcerative colitis, but further work is required to clarify the clinical relevance of the observation.

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