Studies on the mechanism of action of dioctyl sodium sulphosuccinate in the human jejunum

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SUMMARY An intestinal perfusion technique has been used to investigate the mechanism of action of the laxative, dioctyl sodium sulphosuccinate, in the human jejunum. Dioctyl sodium sulphosuccinate stimulated net secretion of water, sodium, chloride and potassium and inhibited net absorption of glucose and bicarbonate. These changes in water and solute transport were partially reversed by administration of indomethacin (4 mg/kg/day orally for three days), which suggests that they are mediated in part by endogenous prostaglandins.

Dioctyl sodium sulphosuccinate (DSS) is a laxative which has been shown to stimulate water and electrolyte secretion in mammalian intestine. Possible mechanisms contributing to this secretagogue action include active electrolyte secretion, mediated by increased intracellular concentrations of cyclic AMP, impaired solute absorption due to mucosal cell damage, altered mucosal permeability and deranged intestinal motility. These effects of DSS may be produced by a direct action of the laxative on the intestinal mucosa, or they may be mediated by endogenous prostaglandins, synthesised and released locally from the intestine after contact with DSS. The purpose of the present studies was to investigate the mechanism of action of DSS in the human jejunum, and in particular to assess the role of endogenous prostaglandins in mediating changes in water and solute transport.

Methods

Subjects
Ten healthy subjects gave written informed consent for the study which was approved by the Ethical Committee of St Bartholomew's Hospital, London.

Intestinal perfusion
After an eight hour fast, each subject swallowed a double lumen intestinal perfusion tube, incorporating a proximal occluding balloon, a 30 cm test segment and a mercury bag. The tube was positioned under fluoroscopic control such that the balloon was situated at the ligament of Treitz with the infusion orifice located in the first 5 cm of jejunum. Using a peristaltic pump, the test solutions at 37°C were perfused through the infusion orifice at a rate of 15 ml/min. The control perfusate (control) contained (mmol/l): Na, 149; Cl, 124; HCO₃, 25; glucose, 10; polyethylene glycol (PEG), 2.5 g/l and 1 μCi/l [¹⁴C]PEG as a non-absorbable marker. The composition of the DSS-containing perfusate (DSS) was identical, except that it also contained 0.5 mmol/l DSS. The osmolality of both perfusates was 290 mosmol/kg. The solutions were continuously gassed throughout each experiment with 95% O₂-5% CO₂. After a 30 minute equilibration period, during which the aspirates were discarded, three successive 10 minute aspirates were collected by siphonage. Aliquots were taken for immediate bicarbonate estimation and samples for the determination of other solute concentrations were stored at -20°C before analysis. The net transport of water and solutes for each perfusate were calculated as the mean of the values measured in each of the three aspirates.

Experiment 1
In all 10 subjects, the control and DSS solutions were perfused in random order to determine their effects on water and solute transport.

In order to assess whether the action of DSS was reversible, the order of perfusion in three of these subjects was control, DSS, control for a final 80 minute period, during which sequential 10 minute
aspirates were collected.

In six subjects, the mean transit time for the transport of luminal fluid through the test segment for the control and DSS perfusates was calculated, under steady state conditions, by injecting a one millilitre (50 g/l) bolus of bromsulphalein through the infusion orifice and measuring the appearance and subsequent disappearance of the dye from the aspirates.10

EXPERIMENT 2

In six of the 10 subjects studied in experiment 1, repeat perfusion with both the control and DSS perfusates was carried out after the administration of the cyclooxygenase inhibitor, indomethacin (4 mg/kg body wt/day orally for three days). This was taken in four divided doses, the last dose being ingested one hour before the repeat perfusion was started.

CHEMICALS

The following chemicals were used: Dioctyl sodium sulphosuccinate (Sigma Chemical Company, St Louis, Missouri, USA); Indomethacin (Merck, Sharp & Dohme Ltd, Hoddesdon, Herts).

ANALYSIS OF SAMPLES AND CALCULATIONS

The concentrations of [14C]PEG, glucose, sodium, potassium, chloride, bicarbonate, urea, and the alkaline phosphatase activities in the aspirates were measured. [14C]PEG was measured in an LKB 1210 Ultrobeta liquid scintillation counter.11 Glucose was determined using a modified glucose oxidase method.12 Sodium and potassium concentrations were measured using an EEL 227 flame photometer (Evans Electroelenium Ltd, Halstead, Essex) and chloride by an EEL chloridometer. Bicarbonate concentrations were measured as total CO2 using a Corning 965 CO2 analyser (Corning Ltd, Halstead, Essex), whilst urea concentrations were measured on a Technicon II system. Alkaline phosphatase activity was measured spectrophotometrically. This enzyme liberates p-nitrophenol (PNP) from the substrate p-nitrophenylphosphate (PNPP), in the presence of the co-factors, magnesium, zinc and cobalt.13

Absorption rates of water and solutes from the test segment were calculated from their measured concentrations in the perfusate and aspirates.14 Net absorption (+) indicates a net transfer of water or solute from the lumen; net secretion (−) indicates net transfer of water or solute into the lumen.

STATISTICAL METHODS

Statistical comparisons were done using the Student’s t test and the Mann-Whitney U test.15

Results

EFFECT OF DIOCTYL SODIUM SULPHOSUCCINATE ON NET WATER AND SOLUTE TRANSPORT

(Table 1)

Dioctyl sodium sulphosuccinate stimulated net secretion of water (p<0.001), sodium (p<0.001), chloride (p<0.001) and potassium (p<0.001), inhibited net absorption of bicarbonate (p<0.01) and glucose (p<0.01), but had no effect on net transport of urea.

EFFECT OF INDOMETHACIN ON WATER AND SOLUTE TRANSPORT FROM THE CONTROL AND DSS PERFUSATES (Table 2)

Indomethacin pretreatment had no significant effect on net water and solute transport from the control solution, but it partially reversed the effect of DSS on jejunal water and solute transport, enhancing net absorption of water (p<0.01), sodium (p<0.01), chloride (p<0.01), bicarbonate (p<0.01) and glucose (p<0.02), and reducing net potassium secretion (p<0.05). The effects of DSS were, however, not completely inhibited by indomethacin pretreatment, in that a comparison between DSS post-IND and control pre-IND reveals that the net absorption of water (p<0.01), sodium (p<0.01), chloride (p<0.01), bicarbonate (p<0.05) and glucose (p<0.02) were significantly inhibited and net potassium secretion (p<0.05) was significantly greater from DSS post-IND.

REVERSIBILITY OF THE EFFECTS OF DIOCTYL SODIUM SULPHOSUCCINATE

In the three subjects in whom the control solution was perfused both before and after the DSS perfusate, net absorption of water during the second control perfusion returned to values which did not differ significantly from those observed during the

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DSS</th>
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</thead>
<tbody>
<tr>
<td>Water</td>
<td>+174±9.0</td>
<td>-120±19.4*</td>
</tr>
<tr>
<td>Sodium</td>
<td>+27.3±1.6</td>
<td>-16.3±2.9*</td>
</tr>
<tr>
<td>Chloride</td>
<td>+18.2±1.6</td>
<td>-19.2±2.7*</td>
</tr>
<tr>
<td>Potassium</td>
<td>-1.62±0.06</td>
<td>-2.53±0.08*</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>+11.6±0.7</td>
<td>+2.4±1.1+</td>
</tr>
<tr>
<td>Glucose</td>
<td>+8.44±0.1</td>
<td>+6.73±0.36+</td>
</tr>
<tr>
<td>Urea</td>
<td>-1.46±0.17</td>
<td>-1.50±0.20</td>
</tr>
</tbody>
</table>

Net transport of water is expressed in ml/30 cm/h and of solutes in mmol/30 cm/h. + = absorption, − = secretion. Results are the mean±SEM of observations in 10 subjects. * p<0.001, + p<0.01. p values refer to the level of significance of the difference between the control and DSS perfusion solutions.
Table 2  Effect of indomethacin (IND) on net water and solute transport

<table>
<thead>
<tr>
<th></th>
<th>Control pre-IND</th>
<th>Control post-IND</th>
<th>DSS pre-IND</th>
<th>DSS post-IND</th>
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</thead>
<tbody>
<tr>
<td>Water</td>
<td>+175±13.6⁺</td>
<td>+222±28.4</td>
<td>-132±25</td>
<td>+65±18*</td>
</tr>
<tr>
<td>Sodium</td>
<td>+27.9±2.5⁺</td>
<td>+33.6±5.4</td>
<td>-18±1.3</td>
<td>+10.8±1.6*</td>
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<tr>
<td>Chloride</td>
<td>+19.8±2.5⁺</td>
<td>+22±4.1</td>
<td>-18±3.8</td>
<td>+3.7±2.5⁺</td>
</tr>
<tr>
<td>Potassium</td>
<td>-1.67±0.08⁺</td>
<td>-1.63±0.08</td>
<td>-2.51±0.1</td>
<td>-2.14±0.11</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>+11.7±1.1⁺</td>
<td>+13.7±1.0</td>
<td>+0.7±1.3</td>
<td>+8.7±0.6⁺</td>
</tr>
<tr>
<td>Glucose</td>
<td>+8.51±0.13⁺</td>
<td>+8.64±0.11</td>
<td>+6.6±0.5</td>
<td>+8.0±0.2⁺</td>
</tr>
<tr>
<td>Urease</td>
<td>-1.54±0.20</td>
<td>-1.86±0.19</td>
<td>-1.52±0.12</td>
<td>-1.80±0.12</td>
</tr>
</tbody>
</table>

Net transport of water is expressed in ml/30 cm/h and of solutes in mmol/30 cm/h. + = absorption. − = secretion. Results are the mean±SEM of observations in six subjects. * p<0.01. † p<0.02. ‡ p<0.05, where the p values refer to the level of significance of the differences between DSS pre-IND and DSS post-IND. § p<0.01. ¶ p<0.02. ‰ p<0.05, where the p values refer to the level of significance of the differences between control pre-IND and DSS post-IND.

first control period (Figure). This occurred within 30–40 minutes of discontinuation of the DSS perfusion and was paralleled by the net movement of glucose.

EFFECT OF DSS ON ALKALINE PHOSPHATASE ACTIVITY

The effect of DSS on the luminal release of the brush border enzyme, alkaline phosphatase, as determined by the liberation of p-nitrophenol (PNP) from the substrate p-nitrophenylphosphate (PNPP), is shown in Table 3. The release of alkaline phosphatase into the luminal fluid was significantly greater from the DSS perfusate than from the control solution, both before (p=0.004) and after (p=0.047) indomethacin pretreatment. Indomethacin had no significant effect on alkaline phosphatase activity in either the control or DSS perfusates.

EFFECT OF DSS ON JEJUNAL MEAN TRANSIT TIME

There was no significant difference between the mean transit time of luminal fluid through the test segment during the control (5.6±1.0 min) and DSS (4.7±0.8 min) perfusions.

Discussion

Diocetyl sodium sulphosuccinate was perfused at a concentration of 0·5 mmol/l because it has been calculated that concentrations of this order are achieved in the jejunum if the recommended therapeutic dose is ingested.1 At this concentration, DSS stimulated net secretion of water, sodium, chloride, and potassium and inhibited net absorption of glucose and bicarbonate in the human jejunum in vivo. In vitro, DSS produced an increase in the short-circuit current and PD and decreased net sodium absorption across the intestinal mucosa.2 Mucosal concentrations of cyclic AMP were increased after exposure to the secretagogue, and it is now known that, in vitro, cyclic AMP inhibits

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DSS</th>
<th>p</th>
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<tbody>
<tr>
<td>pre-IND</td>
<td>40.0±6.7</td>
<td>86.1±11.8</td>
<td>0.004</td>
</tr>
<tr>
<td>post-IND</td>
<td>47.4±6.5</td>
<td>95.5±20.7</td>
<td>0.047</td>
</tr>
</tbody>
</table>

Alkaline phosphatase activity is measured in mmol/l p-nitrophenol liberated by the aspirates/30 cm/min. Results are the mean±SEM of six observations. p values refer to the level of significance of the differences between the control and DSS perfusates.
coupled sodium chloride entry\textsuperscript{16} and stimulates active chloride secretion,\textsuperscript{17} while in vivo, cyclic AMP-mediated processes inhibit bicarbonate absorption in the human jejunum.\textsuperscript{18} The changes induced by DSS in the present study in sodium, chloride and bicarbonate movement, which provide the driving force for the passive secretion of water and potassium, suggest that they may be mediated by increased intracellular cyclic AMP.

The mechanism by which DSS increases cyclic AMP concentrations in the intestinal mucosa is controversial, because of species variation in enzyme activities. In the rat, DSS activated adenylate cyclase in the colon but not in the jejunum, whilst the activity of cyclic AMP-dependent phosphodiesterase in both the small and large intestine was unchanged.\textsuperscript{19} Diocetyl sodium sulphonate, however, had no effect on adenylate cyclase activity in either human small or large intestinal mucosa, although it competitively inhibited cyclic AMP-dependent phosphodiesterase activity in colonic mucosa.\textsuperscript{20} These effects on mucosal cyclic AMP may be due to a direct action of DSS or could be mediated by prostaglandins of the E series released locally from the intestine following contact with DSS.\textsuperscript{3,19}

The effects of DSS on water and solute transport were partially reversed when perfusion was repeated after indomethacin pretreatment. The mechanism of this antisecretory action of indomethacin is, however, uncertain. In the present study, indomethacin had no significant effect on water or solute transport from the control solution, indicating that its inhibition of the secretory effects of DSS was not caused by the stimulation of an independent absorptive process.

Indomethacin inhibits fluid secretion mediated by cyclic AMP,\textsuperscript{21-24} and also inhibits cyclic AMP-dependent protein kinase,\textsuperscript{25} which suggests that its site of action is distal to cyclic AMP production. Intestinal secretion mediated by both cyclic GMP\textsuperscript{26} and calcium\textsuperscript{27} is also inhibited by indomethacin, indicating that the drug might inhibit stimulus-secretion coupling generally.

Because it has been proposed that arachidonic acid metabolites, including prostaglandins, are involved in the regulation of all forms of stimulus-secretion coupling,\textsuperscript{27-30} then blockade of arachidonic acid metabolism, particularly via the cyclooxygenase pathway, may be the mechanism for the antisecretory effect of indomethacin.\textsuperscript{30} Indomethacin has been shown to inhibit the biosynthesis of prostaglandins in the intestine.\textsuperscript{31,32} and it is possible that this inhibition, particularly of PGE\textsubscript{2}, may be the mechanism by which indomethacin inhibits fluid secretion.\textsuperscript{33-35}

Doses of indomethacin of the order used in the present study have been shown to inhibit endogenous prostaglandin synthesis by about 90\%, as evidenced by the reduced urinary excretion of the major metabolite of PGE\textsubscript{1} and PGE\textsubscript{2}, 7α-hydroxy-5,11-di-keto tetranorprostane-1,16-dioic acid.\textsuperscript{36} The finding that indomethacin pretreatment did not completely reverse the effects of DSS on water and solute transport suggests that mechanisms other than prostaglandin release may be involved in mediating its action.

Glucose absorption may be inhibited by histological damage to the jejunal mucosa. In the rat, concentrations of DSS of up to 2 mmol/l in the jejunum\textsuperscript{1} and up to 6 mmol/l in the large intestine\textsuperscript{2} did not produce histological damage, whilst in the human jejunum, 2 mmol/l DSS caused increased loss of DNA into the aspirates, although it was not clear whether this finding represented increased cell loss because of mucosal damage or desquamation secondary to fluid secretion.\textsuperscript{4} In the present study, DSS increased the output into the lumen of the brush border enzyme, alkaline phosphatase, and this increase was unaffected by indomethacin treatment. This suggests that DSS increases the rate of desquamation of epithelial cells, particularly villi, into the lumen, which might contribute to the reduced glucose absorption. Mucosal damage may also lead to the release of arachidonic acid with the formation of prostaglandins.\textsuperscript{37} The rapid recovery of both water and glucose absorption after discontinuation of DSS, however, argues against a major contribution of mucosal damage to the effects of DSS on glucose and water transport.

Changes in mucosal permeability have been implicated in the pathophysiology of bile acid- and laxative-induced intestinal secretion.\textsuperscript{38} Sodium ricinoleate, a long chain fatty acid similar to DSS, increases mucosal permeability in the rabbit intestine, as evidenced by increased secretion of urea into the lumen.\textsuperscript{39} In the present study, luminal urea secretion was similar for the control and DSS perfusates, both before and after indomethacin. The absence of a change in urea permeability, however, does not exclude changes in mucosal permeability to other macromolecules induced by DSS.

Likewise, the effects of DSS on water and solute transport were not contributed to by a decrease in the mean transit time of luminal fluid along the perfusion segment. Results obtained in the jejunal perfusion system, however, cannot be extrapolated to whole gut transit time, and DSS has been shown to slow the transit of both a meal and dye through the intestine.\textsuperscript{7,8}

In summary, DSS is a potent secretagogue in the human jejunum. Its mechanism of action is multifac-
torial, including a stimulation of electrolyte secretion, decreased absorption of glucose and bicarbonate and a possible minor contribution from mucosal damage. These effects are likely to be caused by a combination of a direct effect of DSS on the jejunal mucosa and an indirect effect of DSS through the release of endogenous prostaglandins.

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