Absorption of a hypotonic oral rehydration solution in a human model of cholera

J B Hunt, A V Thillainayagam, S Carnaby, P D Fairclough, M L Clark, M J G Farthing

Abstract

The development of oral rehydration solutions (ORSs) has been one of the important therapeutic advances of this century. The optimal formulation, however, of ORSs for both cholera and other infective diarrhoeas is still debated. Part of the problem in developing ORSs has been the lack of adequate test systems for the assessment of new formulations before clinical trial. We have developed a jejunal perfusion, cholera toxin induced, secretory model in humans and have compared net water and solute absorption from a hypotonic ORS (HYPO-ORS; sodium 60 mmol/l, glucose 90 mmol/l, osmolality 240 mOsm/kg) and the British Pharmacopoeia recommended ORS (UK-ORS; sodium 35 mmol/l, glucose 200 mmol/l, osmolality 310 mOsm/kg) in six healthy volunteers. A plasma electrolyte solution (PES) was also perfused in all subjects to confirm a secretory state. Only HYPO-ORS reversed sodium secretion to absorption (p<0.01). Both ORSs promoted net water absorption but this was greatest with HYPO-ORS (p<0.01). Glucose and potassium absorption rates were similar for both ORSs whereas chloride absorption mirrored sodium absorption and was greatest from HYPO-ORS (p<0.05). These results, in a biologically relevant model of secretory diarrhoea, suggest it may be possible to achieve improved rates of rehydration by the use of hypotonic ORS with mid range sodium concentrations.

The widespread use of oral rehydration treatment has produced a dramatic decline in the morbidity and mortality of acute infectious diarrhoea throughout the developed and the developing world. While the efficacy of simple glucose electrolyte oral rehydration solutions (ORSs) is well established, these formulations do not reduce stool volume. This desirable effect has been reported with rice based, hypotonic solutions. It is possible that hypotonic monomeric glucose based solutions may also reduce stool volume.

The ORS recommended in the British Pharmacopoeia and until recently the most widely used ORS in the UK (UK-ORS) has a low sodium and high glucose content (sodium 35, glucose 200 mmol/l) and is slightly hypotonic (osmolality 310 mOsm/kg). The composition of UK-ORS and most other ORSs have been developed empirically by extrapolation from perfusion studies in cholera patients. While glucose is central to the proabsorptive action of ORSs high glucose concentrations such as those found in UK-ORS may be deleterious. In acute infective diarrhoea glucose is not always completely absorbed and thus may enter the colon and induce osmotic diarrhoea.

One of the problems in assessing new treatments for acute diarrhoea has been the lack of a biologically relevant human model system. Various groups have assessed ORSs by perfusion studies in normal or secreting animal intestine or normal human small intestine. We have recently used cholera toxin to induce secretion in a short segment of human jejunum and have now assessed water and solute absorption from UK-ORS and an experimental hypotonic ORS (HYPO-ORS) by triple lumen perfusion.

Methods

INDUCTION OF SECRETION

Highly purified cholera toxin was used to induce secretion as described previously. Briefly, six healthy, fasted male volunteers (ages 19–28 years) were intubated with a triple lumen perfusion tube, which incorporated 10 cm mixing and 30 cm test segments. Two inflatable balloons were mounted on the tube, one in the middle of the mixing segment and the second on the tip of the weighted tube. The tube was positioned fluoroscopically, with the infusion port 5 cm distal to the ligament of Trietz. Both balloons were then inflated with air (approximate volume 34 ml) until the subject experienced slight abdominal discomfort. Fifteen µg cholera toxin 10 ml 145 mmol/l sodium chloride was introduced into the test segment for a period of two hours, after which the balloons were deflated. We have shown previously that this dose of cholera toxin induces a stable secretory state for water and sodium after three hours.

Each subject was then perfused double blind with three solutions in a predetermined order. The ORS were always perfused first or third with plasma electrolyte solution (PES) second to ensure adequate washout of the ORS from one study period to the next. Solutions were maintained at 37°C in a water bath for 30 minutes before and during perfusion. Both bicarbonate containing solutions were continuously gassed with 5% CO₂ 51/min 30 minutes before and during perfusion.

Solutions were infused by a Watson Marlow flow indicator at a rate of 15 ml/min. Proximal collection was by hand held syringe aspiration at a rate of 1-5 ml/min and distal collection by passive syphoning. Collection of proximal and distal samples were staggered by 10 minutes. Acceptance criteria of a minimum of 70% recovery of the expected volume for the proximal port and a minimum of 30% of the remaining volume marker from the distal port were set.

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Accepted for publication 7 July 1993
SOLUTION DESIGN

Table I shows the composition of the three solutions studied. UK-ORS had a high glucose (200 mmol/l) and a low sodium concentration (35 mmol/l) and contains bicarbonate (18 mmol/l). We compared UK-ORS with a hypotonic ORS, HYPO-ORS, which we have formulated as a result of previous perfusion studies in secreting animal and normal human intestine. HYPO-ORS contains glucose 90 mmol/l. While this is considerably less than the concentration in UK-ORS it is in the range that has previously been shown to be optimal for promoting water and electrolyte absorption in secretory diarrhoea.19

The sodium concentration in HYPO-ORS was 60 mmol/l. This offers a smaller gradient for passive sodium secretion than UK-ORS. Several clinical trials have suggested that sodium 60 mmol/l is optimal for correction of electrolyte imbalance and minimises the risk of inducing either hyper or hyponatraemia.14,15 While the role of base in ORS is debated, the World Health Organisation has recommended inclusion of citrate,16 which has a proabsorptive effect in normal human jejunum17 and improves both shelf life and palatability.22 Disodium citrate (10 mmol/l) was included in HYPO-ORS. Both ORSs contained potassium 20 mmol/l. These changes in solution composition resulted in a reduction in calculated osmolality to 240 mOsm/kg for HYPO-ORS in comparison with 310 mOsm/kg for UK-ORS.

Plasma electrolyte solution was perfused in all subjects to confirm the presence of a secretory state. All solutions contained polyethylene glycol 4000 (PEG 4000 2·5 g/l and [14C]-PEG 4000 2 μCi) as a non-absorbable marker.23

ANALYSIS

Sodium and potassium were analysed by flame photometry (Instrumentation Laboratory IL943 flame photometer). Bicarbonate content was measured immediately as total CO2 with a Corning 965 CO2 analyser. Glucose concentration was determined on a Beckman glucose analyser 2 and chloride measurement on a Corning 945 chloride meter. Osmolality was established by use of a Wescor 5500 vapour pressure osmometer (Wescor Inc). [14C]-PEG concentration was measured by liquid scintillation spectroscopy using an LKB 1912 liquid scintillation counter, which was calibrated to correct for bile quenching of the signal. Some 0·4 ml of sample was diluted with 4·6 ml of commercial scintillant (Optiphase Safe, Fisons Pharmaceuticals). Each specimen was counted for 10 minutes in triplicate. Results were calculated using standard formulae24 and are expressed as mean (SEM). Statistical analysis was by analysis of variance and t test.

All chemicals were of Analar-R grade, obtained from BDH Chemicals, Poole, Dorset. [14C]-PEG was obtained from Amersham International. Cholera toxin was obtained from the Swiss Serum and Vaccine Research Centre, Basle.

The study was approved by the research ethics committee of the City & Hackney Health District.

Results

All solutions changed in composition during transit along the mixing segment (Table II). The sodium concentration of both ORSs increased. This was greater for UK-ORS than HYPO-ORS. In contrast, the high sodium concentration of PES was unchanged. The potassium concentration of both ORSs decreased but that of PES remained stable. The chloride concentration of all solutions increased, the effect being least noticeable with PES. The glucose concentration decreased in both ORSs, but was greater than the minimum concentration required to maximally stimulate water and sodium absorption in normal jejunum.21 The bicarbonate concentration of both UK-ORS and PES decreased slightly.

The calculated sodium and chloride concentrations in both ORSs increased further with transit down the test segment (Table III). In contrast the potassium concentrations fell slightly and the glucose concentrations fell considerably more. The osmolality of UK-ORS was unchanged while that of HYPO-ORS increased slightly but not sufficiently to render the solution isotonic. PES remained of similar composition in both the mixing and test segment.

### Table I Composition of perfusion solutions

<table>
<thead>
<tr>
<th>Solute (mmol/l)</th>
<th>HYPO-ORS</th>
<th>UK-ORS</th>
<th>PES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>60</td>
<td>35</td>
<td>140</td>
</tr>
<tr>
<td>Potassium</td>
<td>20</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>18</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Citrate</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>60</td>
<td>37</td>
<td>104</td>
</tr>
<tr>
<td>Glucose</td>
<td>90</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Calculated osmolality (mOsm/kg)</td>
<td>240</td>
<td>310</td>
<td>288</td>
</tr>
</tbody>
</table>

All solutions contained polyethylene glycol (PEG) 4000, 2·5 g/l and [14C]-PEG 4000 as a non-absorbable marker. ORS=oral rehydration solution, plasma electrolyte solution.

### Table II Composition of perfusion solutions (mean (SEM)) at entry to test segment

<table>
<thead>
<tr>
<th>Solute (mmol/l)</th>
<th>HYPO-ORS</th>
<th>UK-ORS</th>
<th>PES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>76·6(3·7)</td>
<td>56·9(3·1)</td>
<td>141·9(2·3)</td>
</tr>
<tr>
<td>Potassium</td>
<td>17·9(0·2)</td>
<td>14·4(0·4)</td>
<td>4·4(0·4)</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>ND</td>
<td>15·0(1·3)</td>
<td>28·9(2·3)</td>
</tr>
<tr>
<td>Citrate</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chloride</td>
<td>76·8(3·1)</td>
<td>55·0(1·9)</td>
<td>110·2(0·4)</td>
</tr>
<tr>
<td>Glucose</td>
<td>75(3·7)</td>
<td>154·7(9·9)</td>
<td>208·6(1·6)</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>249(6·7)</td>
<td>292(3·8)</td>
<td>208(6·1)</td>
</tr>
</tbody>
</table>

ND=not determined; other abbreviations as in Table I.

### Table III Calculated composition of perfusion solutions (mean (SEM)) at midpoint of test segment

<table>
<thead>
<tr>
<th>Solute (mmol/l)</th>
<th>HYPO-ORS</th>
<th>UK-ORS</th>
<th>PES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>86·9(4·5)</td>
<td>64·1(3·4)</td>
<td>143·0(2·1)</td>
</tr>
<tr>
<td>Potassium</td>
<td>17·2(0·5)</td>
<td>12·9(0·5)</td>
<td>4·5(0·1)</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>ND</td>
<td>12·7(2·8)</td>
<td>26·7(2·5)</td>
</tr>
<tr>
<td>Citrate</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chloride</td>
<td>83·4(3·1)</td>
<td>60·9(2·0)</td>
<td>112·8(1·0)</td>
</tr>
<tr>
<td>Glucose</td>
<td>61(5·9)</td>
<td>138·6(1·6)</td>
<td>272(6·6)</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>256(7·2)</td>
<td>288(3·9)</td>
<td>272(6·6)</td>
</tr>
</tbody>
</table>

Abbreviations as in Table II.
Absorption of a hypotonic oral rehydration solution in a human model of cholera

Transport Results

Net water secretion occurred with PES, whereas both ORSs promoted absorption (p<0.01), which was greater from HYPO-ORS than UK-ORS (p<0.01) (Fig 1). Net sodium secretion occurred with PES and UK-ORS, but was less noticeable with UK-ORS (p<0.01) (Fig 2). Only HYPO-ORS promoted net sodium absorption, which differed from the secretion seen with both UK-ORS and PES (p<0.01).

Glucose absorption was similar during perfusion of UK-ORS and HYPO-ORS. Net potassium absorption rates were similar with both ORSs but net secretion occurred with PES (p<0.01) (Table IV). Net chloride movement was congruent with sodium movement; secretion occurred with UK-ORS but this was less than with PES (p<0.01). HYPO-ORS promoted net chloride absorption, which was greater than from UK-ORS (p<0.05).

Discussion

Net water absorption was greater from HYPO-ORS than UK-ORS despite similar glucose absorption from the two solutions. Net sodium absorption occurred with HYPO-ORS but net secretion was seen during perfusion of UK-ORS. This shows the importance of sodium concentration in promoting sodium absorption in secreting jejunum, a well recognised feature of normal intestine. The osmolality and sodium concentrations of the two ORSs were different. It is therefore impossible to state at which sodium concentration the combined cholera toxin and concentration gradient induced sodium secretion is overcome by solute stimulated and osmotically induced, solvent drag related, sodium absorption. For a solution with an osmolality of 249 mOsm/kg (osmolality of HYPO-ORS at entry to the test segment) it would seem to be slightly less than 77 mmol/l. The work of other investigators in other secretory systems suggests this value is lower than would be found if an isotonic solution was studied. Sodium secretion was reported by Matuchansky and Bernier during perfusion of a 130 mmol/l sodium chloride and glucose solution in prostaglandin (PG) E1-induced secreting human jejunum.38 Similarly decreased sodium absorption but not secretion has been reported during jejunal perfusion of a 149 mmol/l sodium and 10 mmol/l glucose solution using PG12 as the secretagogue.37 These results suggest a sodium concentration of approximately 140 mmol/l would be necessary to prevent net sodium secretion. It is hazardous to compare these findings, however, with the results of our study as, unlike HYPO-ORS, the solutions were isotonic and the low glucose concentrations (10 mmol/l) may have limited active sodium transport.

The congruence between sodium and chloride transport seen in these experiments is not surprising. Cholera toxin abolishes electroneutral, bicarbonate mediated sodium absorption but does not affect electrogenic, solute stimulated sodium absorption.38 During absorption, chloride movement in the jejunum follows electrogenic sodium transport passively. During secretion, sodium movement is determined by electrogenic chloride movement.31,32 In this model of cholera toxin induced intestinal secretion, the inhibition of electroneutral sodium absorption will have increased the congruence between net sodium and net chloride movement.

The two ORSs contained equal concentrations of potassium both initially and at entry into the test segment. The equivalent net potassium absorption rates for the two solutions, in contrast with the slight net secretion seen with PES, is consistent with potassium absorption being determined by the concentration gradient.38 The equivalent glucose absorption from both ORSs suggest that active glucose absorption was not critical in promoting different net water and sodium absorption rates from the ORSs. If there had been greater active electrogenic glucose/sodium absorption with either of the two ORSs, this would have produced an increased positive charge in the enterocyte. This in turn would have

Table IV: Net solute absorption (μmol/cm²/h)

<table>
<thead>
<tr>
<th>Solute (mmol/l)</th>
<th>HYPO-ORS</th>
<th>UK-ORS</th>
<th>PES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>115-5 (36.8)</td>
<td>111-4 (20.3)</td>
<td>-25-5 (8.7)</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>ND</td>
<td>13-9 (56.1)</td>
<td>ND</td>
</tr>
<tr>
<td>Citrate</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chloride</td>
<td>166-5 (198)</td>
<td>-207 (126)</td>
<td>-679 (177)</td>
</tr>
<tr>
<td>Glucose</td>
<td>782-5 (128)</td>
<td>-1143-3 (178)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Negative values denote secretion into the lumen. Abbreviations as in Table II.
decreased potassium absorption. The presence of equal potassium absorption rates from both ORS suggests, but does not prove, that active glucose/sodium cotransport was similar from both solutions.

In conclusion, we have shown that HYPO-ORS promotes greater water and net sodium absorption than UK-ORS in this human model of secretory diarrhoea. These results suggest that the composition of UK-ORS may not be ideal and that reduction in osmolality of ORSs may result in increased water and solution absorption. This may be of benefit in decreasing morbidity in secretory diarrhoeas.

A degree of caution should, of course, be exercised in extrapolating these findings to the clinical situation. In practice ORSs are taken by mouth and are not infused into the small bowel. The ORS might be significantly diluted by salivary, gastric, and pancreatic secretions (about 4 litres in 24 hours) by the time they reach the jejunum. Once modified in this way the differences in performance we have shown in our model of secretory diarrhoea may not be so striking. Nevertheless, a recent clinical trial has compared a hypertonic ORS (of almost identical composition to HYPO-ORS) with WHO-ORS in the treatment of acute diarrhoea. The hypertonic ORS was associated with lower stool volumes and their findings strongly support the conclusions from our perfusion study.

This study was supported by a grant from Rorer Healthcare Ltd. MJGF gratefully acknowledges financial support from the Wellcome Trust.

31 Dharmsapahophon K, Weynner A, McRoberts JA. Chloride secretion induced by prostaglandin E (PGE2); participation of Na+, K+, Cl- co-transport, Cl channels and K+ channels. Gastroenterology 1983; 88: 1364.
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Gut 1994 35: 211-214
doi: 10.1136/gut.35.2.211

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