Evaluation of the efficacy of oral rehydration solutions using human whole gut perfusion

D D K Rolston, S N Zinzuvadia, V I Mathan

Abstract
Whole gut perfusion in humans was used to compare the effect on intestinal water and electrolyte transport of the World Health Organisation oral rehydration solution (solution II, composition in mmol/l: glucose 111, sodium 90, bicarbonate 30, potassium 20; 308 mOsm/kg); a hypertonic commercial oral rehydration solution (solution III, glucose 188, sodium 50, bicarbonate 20, potassium 20 mmol/l; 335 mOsm/kg); and three experimental bicarbonate free, hypotonic oral rehydration solutions: solution IV (glucose 111, sodium 60, potassium 20 mmol/l; 260 mOsm/kg), solution V (glucose 80, sodium 60, potassium 20 mmol/l; 219 mOsm/kg), and solution VI (glucose 80, sodium 30, potassium 20 mmol/l; 177 mOsm/kg). Perfusion of the intestine with a standard cleansing solution (solution I, sodium 125, potassium 10, bicarbonate 20, sulphate 40, mannitol 80 mmol/l; 275 mOsm/kg) confirmed published data on minimal water and sodium absorption. Experimental solution VI produced maximum water absorption (mean (SE) 16600·0 (29·8) ml/h) significantly greater than solution II (1195·3 (79·5) ml/h), III (534·7 (140·3) ml/h), IV (1498·0 (42·7) ml/h), and V (1327·7 (24·4) ml/h; p < 0·05). Sodium absorption was significantly greater with solution IV (97·4 (7·9) mmol/h) compared to VI (43·3 (7·8) mmol/h; p < 0·01) but not compared to IV (67·2 (13·0) mmol/h). A hypotonic oral rehydration solution such as solution VI may provide optimal replacement treatment for patients with acute diarrhoea.

The effectiveness of oral rehydration solutions in increasing intestinal water and electrolyte absorption has been studied in clinical trials and in vivo segmental intestinal perfusion studies. Clinical trials are tedious to conduct, require large numbers of patients if meaningful data are to be obtained, and do not permit systematic optimisation of the constituents of oral rehydration solutions. In contrast, evaluation of the relative effects of these constituents is possible in animal models. Applying data derived from animal studies to humans must be done with caution because of the known differences between human and animal intestinal transport of substances. For instance, D-xylene absorption in the human jejunum occurs predominantly by passive diffusion while in the hamster small intestine absorption is by active transport. Human segmental perfusion studies suffer from the disadvantage that changes observed in one intestinal segment cannot be extrapolated to the entire intestine because functional differences exist between different parts of the small and large intestines.

These problems with methods currently used for evaluating the efficacy of oral rehydration solutions led us to use human whole gut perfusion in vivo in healthy subjects to compare the effect of the World Health Organisation (WHO) bicarbonate oral rehydration solution, a commercial solution (Pedital, Searle (India) Ltd) and three experimental glucose-electrolyte solutions on water and electrolyte transport. The composition of the experimental solutions is based on the following observations: (i) segmental perfusion studies in healthy Indian subjects have shown that 80 mmol/l glucose stimulates jejunal water absorption maximally; (ii) WHO recommends as optimal 111 mmol/l glucose in oral rehydration solutions; (iii) bicarbonate in oral rehydration solutions may not be necessary to correct acidosis; and (iv) hypotonic glucose-electrolyte solutions result in greater water absorption than isotonic glucose-electrolyte solutions.

During whole gut perfusion these solutions were instilled directly into the third part of the duodenum. In practice, however, oral rehydration solutions are taken by mouth. It is possible that when taken orally the glucose concentration in these solutions greatly decrease by the time the solution reaches the distal duodenum because of dilution with gastric, pancreatic, biliary, and intestinal secretions and glucose absorption by the duodenum. To ascertain whether this occurred, the glucose concentration profile of the small intestine was determined after the subject had drunk solutions containing different glucose concentrations.

Methods
WHOLE GUT PERFUSION
Thirty healthy subjects (17 men, 13 women; median age 27 years) were studied using a modification of the whole gut perfusion technique of Davis et al. After a six hour fast the distal end (infusion port) of a mercury weighted polyvinyl tube was positioned fluoroscopically in the third part of the duodenum. The collection port was a 24 F Foley’s catheter introduced 10 cm from the anal verge.

Five minutes after the start of perfusion, at a constant flow rate of 30 ml/min, a bolus of sulphobromophthalein (50 mg in 10 ml of the perfusate) was rapidly injected into the duodenum through the polyvinyl tube and the perfusion continued. Steady-state was achieved as evidenced by constant polyethylene glycol concentrations in the four 30 minute collections (polyethylene glycol range, expressed as a per-
TABLE I Composition of solutions perfused* (mmol/l)

<table>
<thead>
<tr>
<th>Solution</th>
<th>Glucose</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>HCO₃</th>
<th>Cl</th>
<th>Osmolality (mOsm/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-</td>
<td>125</td>
<td>10</td>
<td>20</td>
<td>35</td>
<td>275</td>
</tr>
<tr>
<td>II</td>
<td>111</td>
<td>90</td>
<td>20</td>
<td>30</td>
<td>80</td>
<td>308</td>
</tr>
<tr>
<td>(WHO-oral rehydration solution)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>188</td>
<td>50</td>
<td>20</td>
<td>20</td>
<td>50</td>
<td>335</td>
</tr>
<tr>
<td>IV</td>
<td>111</td>
<td>60</td>
<td>20</td>
<td>-</td>
<td>80</td>
<td>260</td>
</tr>
<tr>
<td>V</td>
<td>80</td>
<td>60</td>
<td>20</td>
<td>-</td>
<td>80</td>
<td>219</td>
</tr>
<tr>
<td>VI</td>
<td>80</td>
<td>30</td>
<td>20</td>
<td>-</td>
<td>50</td>
<td>177</td>
</tr>
</tbody>
</table>

*All solutions contained polyethylene glycol 4000 (2.5 g/l) as a non-absorbable volume marker.
†Also contains SO₄ 40 mmol/l and mannitol 80 mmol/l.
‡Searle (India) Ltd.

centage of the mean, was <±8%). This occurred half an hour after no sulphobromophthalein was detected in the rectal effluent, as judged by the absence of a colour change on alkalising the effluent with saturated sodium hydroxide.

The subject was perfused with solution I (Table I) in the evening to clean the intestinal lumen of food debris and faecal matter. This solution has been shown to result in minimal water and electrolyte fluxes during whole gut perfusion, and is an effective means of cleansing the intestine of particulate matter. The polyvinyl tube was left in place overnight and the subject was permitted only water. The next morning the subject was perfused with one of the glucose-electrolyte solutions (Table I).

GLUCOSE CONCENTRATION PROFILE OF THE SMALL INTESTINE
Six healthy volunteers were studied to determine the glucose concentration profile of the small intestine after the subject had ingested solutions containing glucose. Each subject swallowed four polyvinyl tubes glued together and weighted with a mercury bag. These tubes were positioned fluoroscopically such that the proximal tube opening was in the jejunum immediately distal to the ligament of Treitz and the openings of the other three tubes were at 45, 90, and 135 cm, respectively, from the ligament of Treitz. The subject then drank 600 ml of a glucose solution in water over a 10 minute period. Solutions containing glucose in concentrations of 100, 140, 250, and 350 mmol/l were studied. Some 1-2 ml of the ingested solution was aspirated from each tube and stored at -20°C until analysed for glucose. Three hours later the subject ingested another glucose solution and intestinal fluid was aspirated and stored as described above. All reagents used were of analytical grade.

This study was approved by the Research and Ethics Committee of the Christian Medical College Hospital, Vellore.

TABLE II Glucose, potassium, chloride, and bicarbonate transport (mmol/l) during whole gut perfusions (mean (SE))

<table>
<thead>
<tr>
<th>Solution</th>
<th>Glucose</th>
<th>K⁺</th>
<th>Cl</th>
<th>HCO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>-</td>
<td>+4.7(0.6)</td>
<td>-2.5(1.8)</td>
<td>+11.9(1.6)</td>
</tr>
<tr>
<td>II</td>
<td>+182.7(0.7)</td>
<td>+25.6(1.0)</td>
<td>+89.1(8.8)</td>
<td>+31.4(2.8)</td>
</tr>
<tr>
<td>III</td>
<td>+217.1(4.2)</td>
<td>+17.1(2.4)*</td>
<td>+32.7(5.3)*</td>
<td>+9.2(2.3)*</td>
</tr>
<tr>
<td>IV</td>
<td>+183.9(1.2)</td>
<td>+26.2(2.7)</td>
<td>+99.0(16.5)</td>
<td>-10.4(2.0)*</td>
</tr>
<tr>
<td>V</td>
<td>+127.9(0.2)</td>
<td>+26.3(1.2)</td>
<td>+101.0(6.1)</td>
<td>-10.4(1.6)*</td>
</tr>
<tr>
<td>VI</td>
<td>+129.5(0.1)</td>
<td>+30.3(0.4)*</td>
<td>+78.8(1.8)</td>
<td>-9.1(3.4)*</td>
</tr>
</tbody>
</table>

* = net absorption; † = net secretion.
**p<0.01 compared to solution II.

ANALYSIS OF SAMPLES AND CALCULATIONS
Aliquots from each collection period during the whole gut perfusions were stored at -20°C until analysed for glucose by the glucose oxidase method, polyethylene glycol by the turbidimetric method of Hyden, sodium and potassium by flame photometry, chloride by an EEL chloridometer, and total CO₂ in an automated Corning 965 CO₂ analyser (Corning Ltd, Halstead, Essex, UK). Osmolality was measured in a Wescor vapour pressure osmometer (Wescor Inc, St Logan, USA). Glucose, water, and electrolyte fluxes were calculated using standard formulas. Net absorption (+) denotes net transfer from the intestinal lumen; net secretion (-) denotes transfer into the lumen.

STATISTICAL METHODS
Data are presented as the mean (SE). Differences were considered significant at p<0.05 by the Mann-Whitney U test.

Results
WHOLE GUT PERFUSION
Water and electrolyte fluxes
All solutions containing glucose (II–VI) stimulated water absorption (Fig 1). Hypotonic experimental solutions IV and VI enhanced water absorption to a significantly greater extent than the bicarbonate WHO solution (solution II; p<0.05) and solution VI to a greater extent than solution IV (p<0.05). The commercial hypertonic solution (Pedital; solution III) produced significantly less water absorption (p<0.01) compared to all other solutions containing glucose.

Sodium absorption occurred with all solutions (Fig 2). Maximal sodium absorption occurred with solution II (WHO), which contained the

Figure 1: Net water transport during whole gut perfusion with a lavage solution (I) and glucose-electrolyte solutions (II–IV).
*p<0.05, and †p<0.01 compared to solution II; ‡p<0.05, and §p<0.01 compared to solution VI.
highest initial sodium concentration (90 mmol/l). Solution I resulted in minimal sodium absorption.

Potassium and chloride absorption also occurred with all solutions containing glucose. Bicarbonate absorption occurred with solutions containing bicarbonate (II and III), whereas bicarbonate secretion occurred when bicarbonate free solutions (IV–VI) were perfused (Table II). At the end of the study, however, none of the subjects was clinically acidotic.

Other results
The time required for the rectal effluent to become free of sulphobromophthalein after injection into the infusion port (equilibration period) ranged from 1 hour 5 minutes to 4 hours 30 minutes (Table III).

Rectal flow rate was significantly greater with solution I compared to II, IV, V, and VI (p<0.001, Table III). It was least when solution VI was perfused which produced the greatest water absorption.

SMALL INTESTINAL GLUCOSE CONCENTRATION PROFILE
After the subject had drunk a solution containing glucose the glucose concentration in the proximal jejunum was not statistically different from the glucose concentration in the ingested solution (Fig 3). Glucose was almost completely absorbed in the first 135 cm of the small intestine distal to the ligament of Treitz.

Discussion
The glucose concentration profiles in the small intestine obtained after solutions with different concentrations of glucose were drunk show that the glucose concentration does not change appreciably until the proximal jejunum. This observation justifies the use of the whole gut perfusion technique (in which solutions were perfused into the distal duodenum) to compare the effects of different glucose-electrolyte solutions, which in practice are taken orally, on water and electrolyte transport.

In attempting to design an oral rehydration solution which could produce maximum water absorption, we took advantage of data from human and animal studies which showed that the glucose concentration, osmolality, and the glucose:sodium ratio are important determinants of intestinal water absorption from a glucose-electrolyte solution. The present study supports jejunal segmental perfusion data in that solutions with glucose concentrations of ~200 mmol/l resulted in diminished water absorption compared to lower glucose concentrations. It is not surprising that the effect of glucose on water absorption derived from human and animal jejunal segmental and human whole gut perfusions are similar, because glucose exerts its maximum effect on water transport in the jejunum and is not a potent stimulant of water absorption in the ileum and colon. The glucose concentration profile in the small intestine, after solutions containing glucose were drunk, confirms that nearly all the ingested glucose is absorbed in the jejunum irrespective of the initial concentration.

In contrast to glucose, there is no parallel in sodium transport between human and animal segmental intestinal perfusions and human...
whole gut perfusions. Small intestinal perfusions in both rats and humans have shown that sodium absorption usually occurs at concentrations >90 mmol/l. It is possible that this is a peculiarity of the segmental perfusion system as here the role of the colon in sodium homeostasis is completely ignored. Indeed, the human colon has an equilibrium concentration for sodium absorption as low as 30 mmol/l and it is probable that the relatively inefficient sodium absorption by the jejunal is offset by efficient sodium absorption by the colon. Furthermore, the higher flow rate in the whole gut perfusion study could result in a better mixed, but thinner unstirred water layer than at the lower flow rates used in the segmental perfusions and allow for more efficient sodium absorption.

The glucose:sodium ratio is believed to influence water absorption from a glucose solution containing sodium. It has been suggested that glucose:sodium ratios of 2:1 to 2:8:1 produce maximum water absorption. In the present study, solution VI, which has a glucose:sodium ratio of 2:7, did stimulate water absorption at a significantly greater rate than the other solutions (II to V) which have glucose:sodium ratios of either <2:1 or >2:8. These solutions, however, have markedly different osmolalities which could have contributed to the results obtained. It is impossible to determine from the present study the relative contributions of osmolality and glucose:sodium ratio on net water transport.

One of the reasons for including base precursors – for example, bicarbonate, acetate, and citrate – in oral rehydration solutions is that they stimulate water and sodium absorption. The present study suggests that a glucose solution containing bicarbonate (solution II) does not provide an advantage in water absorption compared to a bicarbonate free solution with a comparable glucose concentration (solution IV), confirming the results of our rat intestinal perfusion studies. Bicarbonate loss did occur during whole gut perfusion with bicarbonate free solutions, but did not result in clinical acidosis. This is probably because the kidneys are capable of correcting (within limits) bicarbonate loss without base precursor supplementation so long as normal renal perfusion is maintained. Indeed, clinical trials which compared bicarbonate free oral rehydration solutions with solutions containing bicarbonate showed that there was no difference in the morbidity, mortality, and purging rates in these two groups studied. Excluding bicarbonate from oral rehydration salt packets would decrease cost and simplify packaging, which are important considerations in developing countries.

The effect that flow rate has on water and solute transport should be considered when interpreting the results of perfusion studies. This is particularly important in the present study because although the initial flow rates were identical (30 ml/min), the flow rates of the solutions as they moved down the intestine were different as evidenced by the different rectal flow rates (Table III). Flow rate directly determines the solute load (initial solute concentration × flow rate). This in turn has a pronounced effect on glucose absorption and a less striking effect on water absorption during segmental perfusions. As water is absorbed the flow rate further down the intestinal segment decreases and luminal glucose concentrations tend to remain high because of the relative concentrating effect. The luminal glucose concentration, in turn, has a profound effect on water absorption. It is therefore important to bear in mind that the results of the present study, where a high flow rate was used to obtain reasonable rectal effluent flow rates, might have been different if a different flow rate had been used.

Our data provide evidence that solution VI, which is a hypotonic bicarbonate free solution containing 80 mmol/l glucose, could be the oral rehydration solution of choice in combating dehydration due to acute diarrhoea in the tropics. The results of this study, which are derived from intestinal perfusions performed on healthy subjects, should, however, be extrapolated with caution to patients with acute diarrhoea because during acute diarrhoea oral rehydration solutions are ingested as boluses and the intestine is in a net secretory state.

The technical assistance of S Jayakumar and the financial support of Searle (India) and Searle (UK) Ltd in carrying out this study is gratefully acknowledged. The Welcome Research Unit is supported by the Wellcome Trust, London, UK, in association with the Christian Medical College, Vellore, India.


Evaluation of the efficacy of oral rehydration solutions using human whole gut perfusion

18 Davis RG, Santa Ana CA, Morawski SG, Fordtran JS. Development of a lavage solution associated with minimal water and electrolyte absorption or secretion. Gastroenterology 1980; 78: 991-5.


