Effect of bicarbonate on efficacy of oral rehydration therapy: studies in an experimental model of secretory diarrhoea

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SUMMARY *In situ* perfusion of rat intestine was used to evaluate the effect of bicarbonate on the efficacy of a low sodium (35 mmol/l) glucose-electrolyte oral rehydration solution in normal and cholera toxin-treated rat small intestine. In normal intestine, absorption of water was greater (108 (8.1) $\mu\text{l}/\text{min}/\text{g}$; $p < 0.01$) and sodium secretion less (-4.3 (0.3) $\mu\text{mol}/\text{min}/\text{g}$; $p < 0.01$) from the oral rehydration solution containing bicarbonate than from the solution in which bicarbonate was replaced by chloride ions (59.5 (7.2) $\mu\text{l}/\text{min}/\text{g}$ and -7.8 (0.8) $\mu\text{mol}/\text{min}/\text{g}$, respectively). Glucose absorption in normal intestine was similar with both solutions. In the secreting intestine, both oral rehydration solutions reversed net water secretion to absorption, but inclusion of bicarbonate resulted in significantly less net absorption of both water (2.18 (6.9) $\mu\text{l}/\text{min}/\text{g}$; $p < 0.05$) and glucose (18.7 (2.1) $\mu\text{mol}/\text{min}/\text{g}$; $p < 0.001$) compared with bicarbonate free oral rehydration solution (19.4 (3.9) $\mu\text{l}/\text{min}/\text{g}$ and 35.8 (3.7) $\mu\text{mol}/\text{min}/\text{g}$, respectively). Net sodium secretion occurred in normal and secreting intestine but was significantly less with the bicarbonate containing oral rehydration solution. These findings suggest that the demonstrable advantage of bicarbonate in promoting water absorption from this oral rehydration solution in normal rat intestine does not apply to cholera toxin treated secreting intestine.

Glucose-electrolyte oral rehydration solutions (ORSs) are effective in the treatment of dehydration caused by acute diarrhoeal illnesses including cholera,¹ and their widespread use has contributed significantly to the decrease in morbidity and mortality associated with these infections.² Controversy still exists regarding the value of an individual base or base precursor in ORSs. Historically, bicarbonate or a base precursor (lactate, acetate, citrate) has been included in ORSs on the assumption that it is required for correction of the metabolic acidosis associated with acute gastroenteritis.³ Their inclusion has been further justified by the observation that bicarbonate, acetate and citrate promote water and sodium absorption in the normal human⁴⁻⁷ and rat⁸ small intestine. There is no evidence, however, that

bicarbonate or the base precursors have similar effects on water and sodium absorption in the secreting intestine.

Recent clinical studies suggest that bicarbonate free ORSs are effective in correcting the dehydration and acidosis associated with acute gastroenteritis.⁹⁻¹¹ Rehydration alone allows the kidney to correct acidosis in all but the most severe cases, when exogenous base may be required.¹² In addition, there are practical difficulties associated with the use of bicarbonate. It is often unobtainable in developing countries, adds bulk and expense to prepackaged oral rehydration powders and may discolour solutions containing glucose by the formation of furfural compounds.¹³ Thus there would be economic and production advantages if bicarbonate could be omitted. For these reasons the World Health Organisation (WHO) has recently substituted citrate for bicarbonate in their ORS¹⁴ although its advantage over a similar solution without citrate has not been

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substantiated. Despite this, ORSs containing bicarbonate are still widely used in the United Kingdom and elsewhere.

The purpose of this study was to evaluate the effect of bicarbonate on the ability of the most commonly used ORS in the United Kingdom to promote water and solute absorption. To investigate this we have undertaken *in situ* perfusion of the entire rat small intestine excluding the duodenum using methods described previously.¹⁵ Studies were done in normal intestine and in intestine after induction of a secretory state with cholera toxin.

Methods

IN SITU STEADY-STATE PERFUSION OF RAT SMALL INTESTINE

Male Wistar rats of 180–240 g in weight were fasted for 18 hours with free access to water, then anaesthetised with intraperitoneal sodium pentobarbitone (60 mg/kg) and maintained with 30 mg/kg *im* as required. At laparotomy cannulae were inserted into the proximal jejunum and terminal ileum. The proximal cannula was used to deliver the test solution and the distal cannula to collect the effluent. Body temperature was maintained at $37 \pm 0.5^\circ\text{C}$ by a heating pad and overhead lamp. Before perfusion residual small intestinal contents were removed by gentle lavage with isotonic saline at 37°C .

In all experiments the entire small intestine apart from the duodenum, to exclude pancreatic secretions, was perfused using a Braun ED2 pump at a rate of 0.5 ml/min.¹⁵ After an equilibration period of 60 minutes with the test solution, three successive 10 minute collections were made by siphonage from each rat *via* the distal cannula. The variation in polyethylene glycol (PEG) concentration was <10% about the mean in the three successive collections, showing that a steady state had been achieved. At the end of the experiment rats were killed and the perfused segment of small intestine removed, dried for 12 hours in a hot air oven at 100°C and weighed.

A secretory state was induced in the intestine of half the rats before perfusion by instillation of 75 μg pure cholera toxin (Sigma Chemical Co, Poole, Dorset, product no. C-9025, lot 14F-4054) in 5 ml 150 mmol/l sodium chloride into the intestine *via* the distal cannula, which was then clamped.¹⁵ Cholera toxin saline was distributed throughout the small intestine and the abdomen closed for two hours. The clamp was then removed and the intestine drained. Induction of a secretory state was reliably and reproducibly achieved and confirmed in this model by determination of water and sodium secretion during perfusion with isotonic saline before and after exposure to cholera toxin. Comparisons between the

different perfusion solutions were always made during the same time period after exposure of the intestine to cholera toxin.¹⁵

Seventy two rats were studied. Three groups of 12 rats received either 150 mmol/l sodium chloride, or the ORS with bicarbonate, or the ORS without bicarbonate after two hour exposure to cholera toxin saline. Another three groups of 12 rats were not exposed to cholera toxin, but were perfused with the same three solutions. Only one solution was studied in each animal to ensure the small intestine remained functional throughout the perfusion, which had a total duration of 1.5 h. Our previous study using this method confirmed satisfactory absorptive function after three hour perfusion.¹⁵

TEST SOLUTIONS

The ORS containing bicarbonate (HCO_3^- 18, Na 35, Cl 37, K 20, glucose 202 mmol/l; 312 mOsm/kg; pH 8.15) was compared with an ORS in which bicarbonate was replaced by chloride ions (Na 35, Cl 55, K 20, glucose 202 mmol/l; 312 mOsm/kg; pH 5.89). Polyethylene glycol (PEG; MW 4000) was added as a non-absorbable marker (5 $\mu\text{Ci/l}$ [^{14}C]-PEG with 2.5 g/l unlabelled PEG).¹⁶ The pH of the ORSs was not adjusted to the physiological range for the small intestine.

This particular ORS was chosen as it has been recommended in the British National Formulary since the mid-1970s,¹⁷ is commercially available, and is currently the ORS most widely used in the United Kingdom. It has a lower osmolality, contains more glucose and less sodium, chloride and bicarbonate than the solution recommended by the WHO.¹³ The 150 mmol/l sodium chloride solution was rendered isotonic to rat serum osmolality (308 mOsm/kg) by addition of 25 mmol/l mannitol. All chemicals used were AnalaR grade from British Drug House Chemicals Ltd, Poole, England.

ANALYTICAL METHODS AND CALCULATIONS

Bicarbonate concentration of the effluents was measured immediately as total CO_2 using a Corning 965 carbon dioxide analyser. Glucose content was estimated by the glucose oxidase method using a Technicon Autoanalyzer. Sodium and potassium were determined by flame emission spectroscopy (Instrumentation Laboratory 943), osmolality by freezing point depression and chloride by coulombometric titration (CCMI chloride meter). [^{14}C]-PEG was measured by liquid scintillation spectroscopy using an LKB 1210 Ultrabeta counter. pH was measured immediately using a PTI-6 Universal digital pH meter.

Three consecutive 10 minute collections were made from each rat and were analysed. The mean of

these results was used to calculate net movement of solute. Water movement was determined from the ratio of PEG concentrations in the perfusate and effluents.¹⁸ Net absorption (+) indicates movement of water or solute out of the lumen; net secretion (−) indicates movement into the lumen. Polyethylene glycol recovery was 100.3 (3) % (mean (SE); n=100). Differences in water and solute fluxes were examined statistically using the non-parametric Mann-Whitney U test for unpaired data.

Results

WATER AND SOLUTE MOVEMENT DURING

PERFUSION WITH ISOTONIC SODIUM CHLORIDE
Minimal net water absorption (+1.38 (5.0) $\mu\text{l}/\text{min}/\text{g}$ dry wt) and net sodium absorption (+5.24 (0.8) $\mu\text{mol}/\text{min}/\text{g}$) occurred during perfusion of normal small intestine (n=12) with isotonic 150 mmol/l sodium chloride (Fig. 1). Perfusion with the same solution after exposure to cholera toxin (n=12) resulted in net secretion of water (−47.8 (11.8) $\mu\text{l}/\text{min}/\text{g}$; $p<0.001$) and sodium (−3.7 (2.1) $\mu\text{mol}/\text{min}/\text{g}$; $p<0.001$) and decreased absorption of chloride (+6.6 (1.1) v +1.4 (1.7) $\mu\text{mol}/\text{min}/\text{g}$; $p<0.01$) compared with normal intestine (Fig. 1), confirming induction of a secretory state.

EFFECT OF BICARBONATE ON WATER MOVEMENT

In the normal intestine, perfusion with bicarbonate

Fig. 1 Effect of bicarbonate on water movement in normal and secreting rat small intestine.

Table 1 Effect of bicarbonate in ORS on solute movement in normal and secreting rat small intestine

Net small intestinal transport	Normal small intestine		Secreting small intestine	
	ORS without bicarbonate	ORS with bicarbonate	ORS without bicarbonate	ORS with bicarbonate
Sodium	−7.8 (0.8)	−4.3 (0.9)*	−15.8 (1.2)	−9.7 (1.4)†
Chloride	+0.3 (1)	−4.6 (1)†	−6.8 (0.9)	−6.0 (1.3)‡
Potassium	+4.9 (0.3)	+7.0 (0.3)*	+3.9 (0.3)	+4.2 (0.6)‡
Bicarbonate	−2.0 (0.1)	+5.8 (0.4)*	−3.8 (0.8)	+0.5 (0.5)*

Net movement of water is expressed as $\mu\text{l}/\text{min}/\text{g}$ dry weight of small intestine and of solutes as $\mu\text{mol}/\text{min}/\text{g}$. + denotes absorption, − denotes secretion. Results are the mean (SE) (n=12). The level of significance of the differences between movement during perfusion with ORS with and without bicarbonate are shown by: * $p<0.01$; † $p<0.001$; ‡NS.

free glucose saline ORS resulted in net water absorption (Fig. 1). This almost doubled with the addition of 18 mmol/l of bicarbonate to the solution ($p<0.01$). In the secreting intestine, net water secretion was reversed to net absorption by bicarbonate free glucose saline ORS (Fig. 1), but net absorption was less than with the same solution in the normal intestine ($p<0.001$). In contrast with the normal intestine, the addition of bicarbonate to the glucose saline ORS did not enhance water absorption, but resulted in a 10-fold reduction in water absorption ($p<0.05$, Fig. 1).

EFFECT OF BICARBONATE ON SOLUTE MOVEMENT

Sodium movement

Perfusion with both test ORSs resulted in net sodium secretion in normal and secreting intestine despite net water absorption. The addition of bicarbonate to the ORS significantly reduced the degree of sodium secretion in the normal intestine ($p<0.01$) and secreting intestine ($p<0.001$), although net sodium absorption never occurred (Table).

Chloride movement

In the normal intestine net chloride absorption occurred from the bicarbonate free ORS and secretion from the ORS containing bicarbonate ($p<0.001$). Secretion of chloride by the secreting intestine was similar with the bicarbonate free and bicarbonate containing solutions (Table).

Potassium movement

Although net potassium absorption was less from the bicarbonate free than from the bicarbonate containing ORS in the normal intestine ($p<0.01$), absorption was similar from both solutions in the secreting intestine (Table).

Fig. 2 Effect of bicarbonate on glucose absorption in normal and secreting rat small intestine.

Bicarbonate movement

In the normal and secreting intestines, net bicarbonate secretion occurred during perfusion of the bicarbonate free ORS, and absorption of bicarbonate occurred from the ORS containing bicarbonate. Bicarbonate absorption from ORS containing bicarbonate was severely impaired in the secreting intestine, being 10-fold less than in normal intestine ($p<0.001$).

EFFECT OF BICARBONATE ON GLUCOSE ABSORPTION

In normal intestine there was no significant difference between glucose absorption from the bicarbonate free or bicarbonate containing solution (Fig. 2). In the secreting intestine, however, the addition of bicarbonate to the ORS almost halved glucose absorption ($p<0.001$).

pH and osmolality

The mean pH of the ORS with bicarbonate was 8.15 (0.02) and of the ORS without bicarbonate was 5.89 (0.21) and these differed significantly ($p<0.01$). The mean intraluminal pH, however, differed less, suggesting that pH equilibrates rapidly in the small intestine. In the normal intestine the intraluminal pH was similar in those who received the ORS with and without bicarbonate respectively (7.08 (0.03) v 7.01 (0.03); $p>0.05$). In the secreting intestine, the effluent pH was higher (7.13 (0.04)) in those perfused with ORS with bicarbonate than in those perfused with ORS without bicarbonate (6.84 (0.05); $p<0.05$).

The mean ORS osmolality was 316 (0.7) mOsm/kg. The mean intraluminal osmolality was similar in all groups. In normal intestine, osmolality was 297.3

(2) and 290.5 (2) mOsm/kg in those perfused with ORS with and without bicarbonate, respectively. In secreting intestine, values were 292 (2.7) and 305.8 (2.4) mOsm/kg in those perfused with ORS with and without bicarbonate, respectively.

Discussion

Perfusion of normal rat intestine has been used previously to evaluate the potential efficacy of established and experimental ORS.^{8,19,20} We have developed this rat model of intestinal secretion as we considered it might reflect more closely the pathophysiology of acute diarrhoeal disease, particularly that due to enterotoxin.¹⁵ The validity of using an animal model should always be questioned but our findings on the pattern of water absorption in normal intestine in our model confirm previous observations in man that: (i) little or no net jejunal sodium or water absorption occurs from an isotonic sodium chloride solution,¹⁸ (ii) the addition of glucose to an isotonic saline solution enhances water absorption,¹⁸ and (iii) the addition of bicarbonate to a saline solution enhances small intestinal water and sodium absorption.^{4,6} We have also recently shown that there is close parallelism between the way in which normal rat and human small intestine handle a variety of ORSs (unpublished observations), although comparisons in the secreting intestine have as yet not been made.

In our experiments in the normal rat intestine we confirmed that bicarbonate enhances water and sodium absorption. Net absorption of sodium, however, was not achieved in any of the experiments, and probably relates to the low sodium concentration (35 mmol/l) of this ORS. It has been shown in the human jejunum that sodium movement is critically dependent on the luminal sodium concentration and that net absorption does not occur until a luminal concentration of 90 mmol/l is reached.²¹ Our own work in the rat whole small intestine confirms this although a luminal concentration of >120 mmol/l was necessary for net sodium absorption in this secreting model.^{15,22} Net water absorption occurred despite net sodium secretion emphasising the importance of osmotic forces in determining water absorption. In this situation water absorption probably occurs as a result of the osmotic drive generated by the hyperosmolar region at the tip of the villus.²³

In the secreting intestine, however, bicarbonate failed to enhance water absorption. Indeed water absorption was actually reduced. Bicarbonate did, however, reduce net sodium secretion (Table, Fig. 1). The reason for this disjunction of sodium and water absorption is not clear and requires further investigation. It is of interest though that bicarbonate

absorption was markedly decreased in the secreting intestine compared with the normal intestine. A further unexpected observation was that glucose absorption was decreased in the secreting intestine in the presence of bicarbonate whereas in the normal intestine bicarbonate as expected had no effect on glucose absorption (Fig. 2). Thus in our model of the secreting intestine bicarbonate inhibits water and glucose absorption.

The pH of the ORSs perfused was not adjusted to the physiological range for the small intestine as no such adjustment is made when ORSs are given therapeutically. We do not consider that this difference in initial pH can explain our findings because the stimulatory effect of bicarbonate on sodium and water absorption is independent of luminal pH.⁴ Measurement of luminal pH in our study suggests that ORS pH equilibrates rapidly in the small intestine.

Luminal osmolality did not differ substantially from the original ORS osmolality, suggesting that the observed water absorption is closely related to overall absorption of osmotically active particles. Thus, in the secreting intestine, the fall in water absorption is caused primarily by the fall in glucose absorption and this might explain the disjunction of sodium and water absorption.

The adverse effect of bicarbonate seen in the secreting intestine may relate to an adverse effect on enterocyte function by an alkaline solution. This seems unlikely, however, as a similar effect was not observed in the normal intestine. Further, the jejunum is constantly exposed to large quantities of bicarbonate rich endogenous secretions from the liver and pancreas.

These data show that in this model bicarbonate does not enhance the action of this ORS and indeed appears to have a detrimental effect with respect to water movement. Our findings also emphasise the fact that the normal and secreting intestines behave differently and that results obtained in the normal gut do not necessarily apply to disease states. One must be cautious when extrapolating these findings to man. When taken by mouth the solute concentration and osmolality of an ORS is almost certainly altered before it enters the jejunum and so these studies may not be an accurate reflection of the clinical situation. There is, however, support for our observations from clinical trials. ORSs without bicarbonate have been shown to be effective for correction of both dehydration and acidosis.⁹⁻¹¹ Furthermore, in some studies comparing bicarbonate and citrate, stool volume was higher when bicarbonate was included in ORS.^{14,24}

Thus there seems little experimental or clinical evidence to justify the addition of bicarbonate to ORS³ particularly as its addition increases the cost,

and decreases the shelf life of ORSs. Bicarbonate is probably required only in the treatment of acute gastroenteritis when acidosis is severe, although it seems likely that on these occasions parenteral fluid replacement would also be necessary.

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