The effect of L-glutamine on salt and water absorption: a jejunal perfusion study in cholera in humans

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Objectives: To assess the efficacy of an L-glutamine solution on jejunal salt and water absorption in cholera patients.

Design: A randomized double-blind jejunal perfusion study.

Setting: International Centre for Diarrhoeal Disease Research, Bangladesh.

Patients: Nineteen adults with acute cholera.

Interventions: Perfusion of balanced salt solutions alternated with defined glucose salt solution and glutamine glucose salt or alanine glucose salt solutions.

Main outcome measures: Net jejunal water and sodium secretion.

Results: Perfusion of glutamine in the presence of glucose significantly reduced net water secretion ($J_{net}^{H_2O} = -2.6 \pm 1.3$ ml/h/cm) and also reduced net sodium secretion ($J_{net}^{Na} = -213 \pm 153$ µmol/h/cm). Similar results were observed during the perfusion of solutions that contained alanine in addition to glucose ($J_{net}^{H_2O} = -4.2 \pm 1.1$ ml/h/cm and $J_{net}^{Na} = -444$U ± 142 µmol/h/cm, respectively) or glucose alone ($J_{net}^{H_2O} = -4.3 \pm 1.7$ ml/h/cm and $J_{net}^{Na} = -452 \pm 212$ µmol/h/cm, respectively). In addition, a higher basal secretion was associated with a greater stimulation of water absorption ($F = 17$, $P < 0.001$).

Conclusion: Glutamine in the presence of glucose significantly reduces net water secretion and also reduces sodium secretion; higher basal secretion is associated with greater water absorption. As glutamine is able to stimulate water absorption to the same degree as glucose and alanine, and because it has the theoretical advantage of providing fuel for the mucosa, the inclusion of glutamine as the sole substrate in oral rehydration solution warrants further study.

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Introduction

An oral rehydration solution containing glucose (glucose-ORS) is the reference solution for rehydrating patients with cholera. During the past 25 years, researchers have tried to improve the efficacy of glucose-ORS by adding substrates or substituting glucose for substrates. The substrates were chosen for their ability to stimulate glucose absorption, essentially through amino acid or glucose–sodium cotransport [1]. Most of these studies did not bring substantial clinical improvement [2–7]. Glutamine is worth considering for three main reasons: it stimulates sodium absorption [8–11]; it is safe [12]; and it is an important source of energy for the intestinal epithelial cells [13–20]. Therefore, if it is as effective as glucose on rehydration, it could be used in oral rehydration solutions as a source of energy. This may be relevant for cholera patients, many of whom are malnourished. However, even if glutamine is not as effective as glucose in promoting sodium absorption, it is probably worth looking at the metabolic consequences of adding glutamine to ORS. The aim of the study was to assess the

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effect of a glutamine solution on jejunal salt and water absorption in patients with cholera. Glucose and alanine were also studied for comparison [1,8,21–24]. The results indicate that solutions containing glutamine plus glucose, alanine plus glucose, or glucose alone, stimulate the absorption of water and salt equally.

Patients and methods

On admission to the Clinical Research Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh, the following patients were eligible for the study: males more than 16 years of age who had had watery diarrhoea for less than 24 h, in whom cholera had been confirmed by darkfield microscopy and culture, and who had a purging rate of more than 5 ml/kg/h during 4 h of observation or who were severely dehydrated (defined as having a weak radial pulse or no radial pulse and at least one additional sign of dehydration), who had not taken antibiotics, and who had given their written informed consent.

Initial severe dehydration was corrected by intravenous administration of the Dacca solution (containing Na 133 mmol/l, K 13 mmol/l, Cl 98 mmol/l, acetate 48 mmol/l) at a rate of 100 ml/kg in 4 h. Over this period stool losses were measured hourly in graduated cylinders, and were replaced by equal amounts of intravenous solution.

After the patients were completely rehydrated, intestinal perfusion was started. During perfusion the intravenous infusion already described was maintained, and serum specific gravity was measured by bedside refractometry. Hourly losses of stool minus the 600 ml volume of perfused solution were replaced intravenously during the following hour.

Tetracycline (500 mg every 6 h) was given after the perfusion was over [25].

Steady-state segmental intestinal perfusion

After complete rehydration the subjects swallowed a triple-lumen radio-opaque polyvinyl tube with a mercury bulb at the tip. Under fluoroscopy the tube's final position (its infusion port located at the ligament of Treitz) was checked. The proximal and distal collection sites 15 cm and 45 cm farther down allowed for a 15-cm mixing segment and a 30-cm study segment [26–28]. The perfusion was carried out at a rate of 10 ml/min with a peristaltic pump (Criticon Inc., St Louis, Missouri, USA), a rate sufficient to minimize any confounding effects of intestinal motility changes. Manual sampling at the collection ports secured uninterrupted flow. A 30-min period at a perfusion rate of 10 ml/min had proved sufficient to achieve steady state in a pilot study of six patients who had acute cholera. Steady state was defined by: (a) the absence of a significant variation in absorption/secretion rates with time; and (b) a coefficient of variation (CV = SD/mean) of less than 10% for the considered parameter over the last 30 min of each infusion period. Polyethylene glycol (PEG-5000) was added to each solution (5 g/l) and acted as the non-absorbable marker.

Each patient was perfused with four solutions in sequence for 1 h each (Table 1). To validate the perfusion method and to control for sequential changes, we conducted perfusions of balanced salt solution during the first hour and the last hour, thus sandwiching the study and the control solutions into the second and third hours. The solutions were transparent, clear and identical in appearance, and were delivered according to a master chart containing the random sequence of perfusion for control and study solutions [29]. The sequence was as follows: glucose salt solution was the second solution, and then either of the amino acid solutions was the third solution; or either of the amino acid solutions was the second solution and then glucose salt solution was the third solution. The purpose of randomizing the sequence of study amino acid solutions and control (glucose salt) solutions was that variations in jejunal fluid composition could then be ascribed only to the effect of the perfusion solution under study, not to the natural course of the disease. The codes were not disclosed until all studies were completed, thus safeguarding the double-blinded nature of the study.

Preparation of the salt mixtures

The balanced salt solution, the glucose salt solution (control) and the two study solutions were prepared at the ICDDR Biochemistry Laboratory just before the perfusions began; osmolality was kept at 300 mmol/l (isotonic): Na^+ 100 mmol/l, K^+ 5 mmol/l, Cl^- 75 mmol/l, bicarbonate 30 mmol/l, and either alanine and glucose, or glutamine and glucose, 90 mmol/l, i.e. 45 mmol/l of either component (Table 1). To reduce the difference in concentration of salts between perfusion solution and plasma, we increased the sodium concentration to 100 mmol/l and decreased the potassium concentration to 5 mmol/l. For the same reason we decreased the substrate concentration to 90 mmol/l.

Biochemical assays

Electrolytes were measured by ion-selective electrodes. Serum osmolality was determined by the freezing point depression method. Specific gravity was measured by refractometry, PEG by the Hyden turbidometric method.

Analysis of samples, calculations and statistics

After measuring the PEG and salt concentration of each sample we calculated net water and salt absorption, and

Table 1. Composition of perfusion solutions (mmol/l).

	Na	K	Cl	HCO_3	Glucose	Alanine	Glutamine
Balanced salt solution	145	5	135	15	–	–	–
Glucose salt solution	100	5	75	30	90	–	–
Alanine glucose salt solution	100	5	75	30	45	45	–
Glutamine glucose salt solution	100	5	75	30	45	–	45

Table 2. Clinical characteristics of the study patients by amino acid solution perfused.

	Glutamine glucose salt solution ($n=8$)	Alanine glucose salt solution ($n=11$)
Age (years)*	28.6±7.1	29.2±9.5
Weight (kg)*	43.0±4.7	41.1±4.9
Dehydration		
Severe (*n*)	5	6
Moderate (*n*)	3	5
Stool volume (ml)		
4 h before perfusion*	2004±899	1652±1014
Stool volume (ml)		
4 h after perfusion*	1439±773	1209±1056
Pathogens isolated		
El Tor Inaba	3	6
El Tor Ogawa	5	3
Classical Ogawa	0	2

* Mean ± 2 SD.

we calculated flow rates at the proximal and distal collecting sites by using non-absorbable marker equations [30–32].

Data were analysed by using SAS software (SAS Institute Inc., Cary, North Carolina, USA). The effect of adding the amino acids to glucose was assessed by non-parametric and parametric tests.

Results

A total of 19 valid perfusions were conducted. The tandem combinations (a) control glucose salt solution followed by glutamine glucose salt solution, and (b) glutamine glucose salt solution followed by control glucose solution, were each perfused in four patients; the control glucose salt solution followed by alanine glucose salt solution was perfused in five patients, and the combination alanine glucose salt solution followed by control glucose salt solution was perfused in six patients. Steady state for each solution was checked by variance analysis of three successive 10-min samples collected at each site.

Glucose salt solution and glutamine glucose salt solution were randomly perfused in eight patients of the glutamine group, and glucose salt solution and alanine glucose salt solution were perfused in 11 patients of the alanine group.

Table 2 shows the clinical characteristics of the patients, all of whom had a stool output of more than 5 ml/kg/h or 20 ml/kg/4 h on admission and were severely or moderately dehydrated. *Vibrio cholerae* 01 El Tor Inaba grew in the stool culture of nine patients, El Tor Ogawa in eight, and Classical Ogawa in two.

During initial perfusion of balanced salt solution a state of net secretion existed for sodium, potassium, chlorine, bicarbonate and water (Table 3). When balanced salt solution was perfused following the amino acid and glucose solutions, the rate of secretion remained essentially the same except for a reversal of bicarbonate flux toward absorption. Thus, during the four periods of perfusion it can be assumed that the basal secretory rate induced by cholera remained unaltered.

The solutions that contained substrates reduced water secretion (positive values) or stimulated water absorption (negative values). Non-parametric analysis, using the Mann–Whitney U – Wilcoxon rank sum W test, indicated that the movements of water were not significantly different for the different solutions ($P=0.076$). None of the other variables showed statistically significant differences between groups.

Because the magnitude of the basal secretion may modify the effect of solutions that contain substrates, the change in water transport and electrolyte fluxes was analysed for each substrate perfused.

The effects of substrates on water and electrolyte transport were calculated using Student's *t*-test, and are presented in Table 4 as the difference between solutions that contained substrates, and balanced salt solution. In general, solutions that contained substrates stimulated water and electrolyte absorption (Table 4). There was, however, a great variability in individual response, reflecting the changes in water (δJ^{H_2O}) and sodium (δJ^{Na}) transport rates induced by the substrates (Fig. 1). Higher basal secretion rate was associated with greater stimulation of water absorption by substrates ($\delta J^{H_2O} = 0.43\ (\pm 1.5) - 0.81\ (\pm 0.20)\ J^{H_2O}$ ml/h/cm; $R^2 = 0.50$; $P < 0.001$) (Fig. 2), but again no significant difference emerged between the solutions that contained substrates. In fact, in nine perfusions glucose ($n=5$), glutamine ($n=3$) or alanine

Table 3. Jejunal water and salt transport rates* by perfusion solution in study patients ($n = 19$)

	H_2O (ml/h/cm)	Na (mmol/h/cm)	K (mmol/h/cm)	Cl (mmol/h/cm)	HCO_3 (mmol/h/cm)
BSSi ($n=19$)	5.0 (±4.3)	768 (±636)	27.8 (±35.6)	750 (±614)	59.7 (±71.5)
BSSf ($n=19$)	5.7 (±5.2)	876 (±745)	31.9 (±31.5)	843 (±704)	48.2 (±164.0)
GSS ($n=19$)	1.4 (±5.7)	470 (±772)	21.4 (±66.0)	448 (± 878)	6.7 (±73.3)
AGSS ($n=11$)	2.4 (±4.2)	558 (±566)	32.7 (±47.3)	576 (±549)	40.0 (±81.2)
GGSS ($n=8$)	0.2 (±4.4)	233 (±551)	18.3 (±26.0)	274 (±460)	35.2 (±86.6)

Note: Positive values denote net secretion into jejunal lumen, negative values denote net absorption from lumen.
BSSi, balanced salt solution, initial perfusion; BSSf, balanced salt solution, final perfusion; GSS, glucose salt solution; AGSS, alanine glucose salt solution; GGSS, glutamine glucose salt solution.
*Mean (±SD).

Table 4. Changes in the water and salt transport rates (means±SD) induced by the various substrates, expressed as the difference with balanced salt infusion.

Variables	Groups			
	Alanine ($n=11$)		Glutamine ($n=8$)	
	Glucose salt solution	Alanine glucose salt solution	Glucose salt solution	Glutamine glucose salt solution
δH_2O.¶	−4.3 (±1.7)*	−4.2 (±1.1)*	−2.4 (±1.7)	−2.6 (±1.3)*
δNa^+.§	−452 (±212)*	−444 (±142)*	−127 (±243)	−213 (±153)
δK^+.§	0.4 (±12)	−4.6 (±8.5)	1.4 (±8.3)	3.4 (±8.8)
δCl^-.§	−359 (±214)	−396 (±134)*	−64 (±241)	170 (±139)
δHCO_3^-.§	−61 (±22)*	−41 (±19)*	41 (±27)	5.5 (±28)

¶. J^{H_2O} ml/h/cm.
§δJ^{H_2O} mmol/h/cm.
*Significantly different from zero ($P<0.05$).

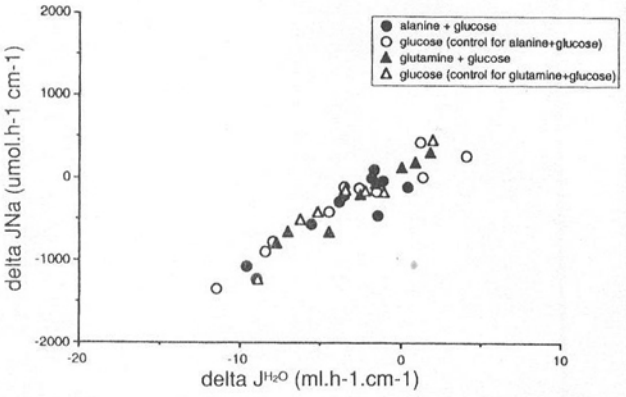

Fig 1. Relationship between changes in water (δJ^{H_2O}) and sodium (δJ^{Na}) transport induced by substrate-containing solutions.

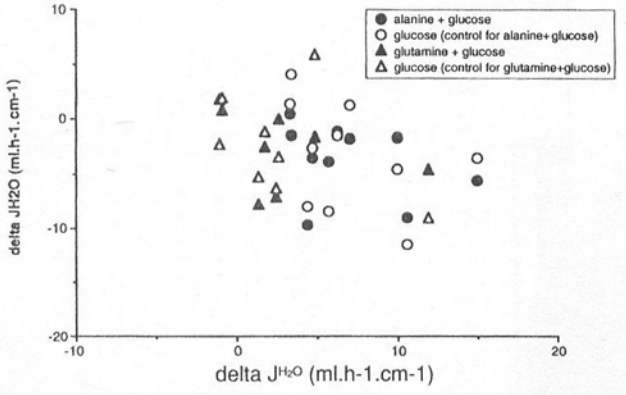

Fig 2. Relationship between changes in water transport (δJ^{H_2O}) and basal water transport (J^{H_2O} basal).

($n=1$) stimulated water secretion. Solutions that contained glutamine or alanine produced no better effect than solutions that contained glucose only. Similarly, the effects of glutamine versus alanine on water secretion did nor differ statistically (Table 4).

In addition, whichever substrate was studied, the basic and highly significant linear relationship between the movements of water and sodium was conserved, as indicated by the linear regression in which all the fluxes are included. The slope of the line representing the sodium concentration in the solution absorbed or secreted across the intestinal wall did not differ significantly among the three solutions that contained substrates ($\delta J^{Na} = 115(\pm 34) + 126(\pm 6)\ \delta J^{H_2O}$ µmol/h/cm); ($R^2 = 0.92$; $P<0.001$) (Fig. 1).

Finally, comparison of 4-h stool outputs before and after the perfusions and between the two study populations did not reveal statistically significant differences (not shown).

Discussion

The significant differences in transport rates for salt and water between glucose salt solution and balanced salt solution (Table 4) agree with those in earlier studies [32,33] and confirm the validity of the perfusion method used. The jejunal transport rates for water and salts during perfusion of balanced salt solution are higher than the rates in earlier studies in acute cholera [31–35].

The lower absorption rate of alanine glucose salt solution compared to the rate of glucose salt solution is, however, statistically not significant, and is at variance with a clinical trial of 98 patients who had watery diarrhoea and in whom an oral glucose salt solution that contained hypertonic alanine reduced stool output significantly more than did oral rehydration solution that contained glucose and salt only [3]. In contrast to that trial, our study population was small, homogeneous and confined to cholera patients; moreover, all our perfusion solutions were isotonic (300 mOsmol/l).

The substrate-induced changes in net water movement were linearly related to changes in sodium movement. This is in agreement with an earlier in-vivo jejunal perfusion study in healthy volunteers using an isotonic solution of the elemental diet Vivonex,, or solutions containing amino acid and carbohydrate components of Vivonex, which demonstrated a linear relationship between net Na^+ absorption and initial Na^+ concentration for all the amino acid and carbohydrate solutions studied [36]. Because in the intestine most water movement is passive and coupled to the movement of electrolytes only, this finding further attests to the accuracy of the perfusion procedures used.

One of main observations was that the basal intestinal secretory rate of cholera patients influenced the absorption of water and electrolytes; higher initial secretion was associated with higher stimulation of water absorption by substrates. Although unexpected, this observation is supported by evidence from studies in animals and in human intestinal cell line cultures. Cholera toxin has been shown to stimulate sodium-coupled glucose absorption across the rat ileum [37]. Similarly, in a chloride secretory intestinal carcinoma cell line HRT-18, which lacks absorptive properties under basal conditions, sodium-coupled glucose absorption became apparent after stimulation of chloride secretion by cholera toxin and dibutyryl adenosine cyclic monophosphate (DbcAMP) [38,39]. The activation of secretion does, in fact, induce absorption in these cells. Taken together, the results of in-vitro studies and our in-vivo perfusion study suggest that stimulated secretory processes may be associated with enhanced intestinal absorption, a mechanism in which increased cellular cAMP, metabolic acidosis or volume regulation will be involved [37–41].

The present study could not demonstrate a net advantage of using glutamine over alanine or glucose in oral rehydration solutions in terms of intestinal absorption of electrolytes and water. Nevertheless, the natural L-glutamine remains the preferred energy substrate for intestinal epithelial cells, notably when the structural and functional integrity of the mucosa is compromised [13–20]. Therefore, glutamine as a component of ORS would be able to provide the additional energy required for mucosal repair and growth, whereas the incorporation of other organic substrates into ORS would fail to do so. Additional studies in different types of diarrhoeal disease and utilizing a different study design, including perfusion of a solution containing glutamine only, are necessary to conclusively demonstrate the potential benefit of utilizing glutamine in ORS either as the principal organic substrate or as an adjuvant to other substrates currently in use. Inclusion of glutamine in the form of a dipeptide such as alanylglutamine seems less advantageous than as a monopeptide, as glutamine is unique among amino acids in its capacity to serve as a fuel for mucosal metabolism [19].

So far, we have lacked accurate quantitative data on jejunal salt and water transport rates during the natural course of acute cholera. In our study we did not observe a significant difference when we compared the transport rates for salt and water during the first hour of perfusion of balanced salt solution with the transport rates during the fourth hour. This implies that during the 4-h perfusion no changes in salt and water transport could be ascribed to the natural evolution of the disease.

That we could not establish a significantly reduced stool output between the glutamine perfusion and the alanine perfusion in the 4 h after the study period may be attributable to the adjacent perfusion of glucose ORS in all patients.

Although the purging rate during the initial 4 h of observation differed in the glutamine and the alanine groups, perfusion with balanced salt solution at the beginning and at the end of the study did not show significant differences in water and electrolyte absorption rates between the two groups.

In conclusion, our results confirm that salt solutions containing glucose stimulate the absorption of electrolytes in cholera patients. In addition, they indicate that equimolar salt solutions that contain glucose (90 mmol/l), glucose (45 mmol/l) plus alanine (45 mmol/l) or glucose (45 mmol/l) plus glutamine (45 mmol/l) perfused in the jejunum of these patients equally stimulate intestinal water (and sodium chloride) absorption, despite individual variations due to the initial secretory state of the intestine.

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