

SHORT COMMUNICATION

GUM ARABIC ENHANCES PARACELLULAR TRANSPORT OF WATER IN AMPHIBIAN EVERTED SMALL INTESTINAL SEGMENTS

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Abstract : Gum Arabic (GA) is known for its proabsorbent activity in normal intestine as well as in animal models of diarrhea. The aim of the study was to find the effect of GA on intestinal transport of water and possible route of absorption in frog everted gut sacs. D-Mannitol was used as a marker of paracellular transport to find the route of absorption. Everted gut sacs (n=4,5) were placed in Ringer containing GA (2.5 g/L) with or without D-Mannitol (0.5 g/L), incubated for 1 hour and analysed for change in weights of the sacs and D-Mannitol uptake. There was significant increase in uptake of water and D-Mannitol in the presence of GA compared to controls (P<0.05). Gum Arabic improves water uptake by the intestinal mucosa, possibly by opening the paracellular pathways.

Key words : gum arabic
everted gut sacs

D-Mannitol
paracellular transport

INTRODUCTION

Gum Arabic (GA) has been investigated for its proabsorbent activity in animal models of normal and diarrheic intestine. The results obtained were equivocal. In normal rats, Wapnir et al demonstrated that in the presence of GA, even though sodium

absorption was increased, net water absorption remained unaffected (1). When rat intestine was perfused with oral rehydration solution containing GA, a net decrease in water absorption was noticed (2). A small but insignificant improvement in sodium and glucose absorption occurred. However, when tried on animal models of

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diarrhea, GA promoted water and sodium uptake by jejunum, when incorporated in oral rehydration solutions (ORS) (3). GA helped in optimal recovery from diarrhea compared to plain ORS(4). When tight junction modifiers like Chenodeoxy cholic acid were present in the perfusate, a decline was noted in water and electrolyte absorption and the addition of GA restored the absorption to normal (5).

No human studies have been reported on effect of GA on the intestinal absorption. It has recently been reported that isolated frog intestinal sacs can be used for predicting peroral absorption in humans for passively absorbed compounds and drugs (6, 7). Therefore we employed this amphibian preparation to study the effect of GA on intestinal transport of water. We also used D-Mannitol, the marker of paracellular transport, to identify the route of transportation. D-Mannitol, a commonly used intestinal probe was chosen due to its negligible intracellular uptake and possible paracellular transit (8).

METHODS

Frogs (*Rana Tigrina*) weighing about 25 g obtained from the animal house of Kasturba Medical College, Manipal were used for this study, with the prior approval of Institutional Animal Ethics Committee. Proximal five centimeter segments of the gut were dissected out of pithed frogs and were everted to prepare the sacs. The sacs were filled with 1 ml of Ringer which contained (117 mM NaCl, 3 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 0.8 mM Na₂HPO₄ and 0.2 mM NaH₂PO₄). They were then placed in flasks containing 5 ml of the Ringer along with GA (2.5 g/L).

The flasks were aerated for 2 min, then stoppered and incubated in a shaker water bath (frequency 100 oscillations/min) for 1 hour at 30 degree C. Control samples were placed in Ringer without GA. The sacs were weighed before (initial weight) and after (final weight) incubation. The total gain in the weight of the sac was determined by the difference between the initial and final weights. This was taken as the uptake of water by the sac.

In order to find the route of absorption, D-Mannitol was used. The everted sac was weighed before and after filling with 1ml of Ringer. The everted sacs were placed in Ringer with GA (2.5 g/L) and D-Mannitol (0.5 g/L) and incubated in shaker as described above. Control samples were placed in Ringer with D-Mannitol (0.5 g/L) only. The uptake of water was measured as described above. After draining their contents, the empty sacs were weighed again. The fluid samples (both inside and outside the sacs) were analysed for the concentration of D-Mannitol using Mannitol estimation kits (Megazyme International, Ireland). The amount of D-Mannitol taken up by the tissue was calculated and expressed as micro grams per sac per hour of incubation. Statistical analysis were done using student's t test. The P value of < 0.05 was considered significant.

RESULTS

There was significant increase in uptake of water in the presence of GA as shown in Table I. When D- Mannitol was added without GA, there was significant reduction of water uptake. In presence of GA, addition of D-Mannitol significantly increased the uptake

TABLE I: Effect of Gum Arabic on the uptake of water by the everted sacs of frog intestine.

	<i>Additives</i>	<i>Without GA</i>	<i>With GA</i>	<i>P value</i>
Water uptake (g/sac/h)	None	222.5±86.17	305.0±123.96*	0.032
Water uptake (g/sac/h)	D-Mannitol	110.0±57.73**	282.5±75.0*	0.012*0.029**

All the values are expressed as mean±SD of 4 to 5 observations. The values marked * are significantly higher than the corresponding values noted in the absence of GA ($P<0.05$). Value marked ** is significantly less than the value above it ($P<0.05$).

of water by the sacs. As shown in Table II, there was significant increase in D-Mannitol uptake in the presence of GA. The weight of the sac was also significantly elevated in the group incubated with GA in presence of D-Mannitol.

TABLE II: Effect of Gum Arabic on D-Mannitol uptake and weight of the sac after incubation.

	<i>Ringer with D Mannitol only</i>	<i>Ringer with GA and D-Mannitol</i>	<i>P value</i>
D-Mannitol uptake (ug/sac/h)	412.5±12.0	875.0±59.7	0.001
Weight of the empty sac (mg/sac/h)	1027.5±135.9	1515.0±332.1	0.048

The values are expressed as mean±SD of 4 observations. The value obtained in the presence of GA is significantly higher than the corresponding value noted in the absence of GA ($P<0.05$).

DISCUSSION

Our study clearly showed an increase in water uptake in the presence of Gum Arabic. This is contrary to the findings by Wapnir et al and Rehman et al where either net water transport was either unaffected or decreased in normal rats (3, 4). Addition of D-Mannitol to the mucosal compartment has reduced the water uptake significantly in the

absence of GA. This is due to the osmotic effect exerted by D-Mannitol. Similar results were obtained by Naftalin & Tripathi (9) who added various osmotically active substances to the fluid bathing the mucosal compartment, in their studies on paracellular transport of water. Gum Arabic significantly elevated the water taken up by the sacs, in the presence of D-Mannitol. The uptake of this paracellular marker by the everted sacs, was also significantly elevated by GA. This indicates the possible opening of paracellular pathways in presence of the gum. Water in these newly opened paths has led to a significant elevation of the weights of empty sacs after incubation due to trapping. Teichberg et al had earlier indicated fluid retention by GA in rats induced with chronic osmotic diarrhea by exhibiting the expansion of basolateral intercellular spaces between the villus absorptive epithelial cells and lamina propria (4).

Mannitol is an established probe of paracellular transport, transported from the mucosal to the serosal side of the sac tissue showing linear transport over a wide range of concentrations (0.025– (10 mM) (8). Krugliak et al have demonstrated that addition of tight junction modifier like CDC to the rat intestinal perfusate resulted in a significant decrease in absorption of water and a corresponding decrease in mannitol

permeability substantiating its role as a paracellular marker (10).

These results support the claim that GA improves water uptake by the intestinal mucosa, possibly by opening the paracellular pathways. In view of the similarity of the frog intestine to human gut, our study suggests that human trials of oral rehydration solution fortified with innocuous Gum Arabica should be planned.

Passage of water across the epithelia –

transcellular or paracellular - continues to be a much debated subject (11). Our results indicate that across the gut epithelium of frog, paracellular route remains an important conduit of water.

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