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Original Article

Absorption of Water and Electrolytes from a Liposome Based Oral Rehydration Solution: An *in vivo* Perfusion Study on Mucosal Injured Small Intestine Rat Model

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ABSTRACT: In the present study, rats were used to identify the efficacy of three types of Oral Rehydration Solutions (ORSs); standard ORS (S-ORS), hydrolyzed starch ORS (HS-ORS) and Liposomal ORS (Lipo-ORS) using an *in vivo* perfusion technique on a 5-Fluorouracil mediated mucosal injured rat small intestine model. This study comparess the absorption of electrolytes (Na^+, K^+, Cl^-) and water from these three types of ORSs and compared among these substances from each other. It was found that liposome based ORS is associated with significantly higher Na^+, K^+, Cl^- and water absorption compared to hydrolyzed starch ORS or standard ORS. It can be concluded that liposomes enhance more electrolyte absorption from a carbohydrate electrolytes solution in mucosal injured small intestine of rat than normal small intestine of rats. These findings underscore the potential clinical importance of liposome based electrolyte solution for rehydration.

KEYWORDS: Rat, Liposome based ORS, perfusion Study, mucosal injured intestine of rat, 5-Fluorouracil.

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INTRODUCTION

Diarrhoeal disorders constitute a leading cause of morbidity and mortality globally and continue to be a major concern, particularly for developing countries[1]. Based upon the pathogenic mechanisms, the diarrhoeal organisms may be broadly divided into two groups, secretory and invasive. *Vibrio cholera* is the prototype pathogen causing secretory diarrhea and on the other hand *Shigella* the prototype invasive pathogen causing mucosal gut injury. Similar to this, different research groups orally administrated 5-fluorouracil to induce small intestinal injury, which resembles bacterial injury.

5-Fluorouracil (5-FU) is an antimetabolite that acts as a pyrimidine antagonist[6-7]. Major side effects of 5-FU include leukopenia, thrombocytopenia and diarrhea[6-7]. However, we know that chemotherapy alters the state of the intestinal microbiome[3-4]. 5-FU and irinotecan cause severe mucositis manifesting as diarrhea, and changes to the microflora are observed following administration of these drugs[8-9].

The development of ORS for the treatment of dehydration is based upon the observations that even in a secreting small intestine it is possible to achieve a positive gut balance of fluid and

electrolytes by adding glucose to the salt solutions. It is estimated that ORS alone can successfully rehydrate 90% of patients with dehydration from acute diarrhoea who previously would have required intra-venous (i.v.) therapy[13]. However, Oral Rehydration Therapy (ORT) with the present ORS formulation has certain limitations-ORT does not reduce the volume, frequency or the duration of diarrhoea[14]. These limitations prompted the concept of developing an improved ORS (initially named 'super ORS')[15].

Conceptually, an improved ORS should (a) reduce stool volume, (b) shorten duration of diarrhoea and (c) reduce failure rate of ORT particularly in patients with high purging rate. Recently recommended reduced osmolarity ORS is similar to the original ORS but has a lower concentration of sodium (75 mmol rather than 90) and glucose (75 mmol rather than 111 mmoles/liter) yielding a solution with a toal osmolality of 245 rather than 311. Though the new solution reduces the frquency of vomiting, however, the duration of diarrhoea is not shortened, and there are still ORT failures in which patients initially rehydrated and place on ORT, become dehydrated again and require additional IV infusions. Thus it is very necessary to improve the formulation and administration of the current ORT.

In this study, we use liposomes to improve the delivary of ORS. When phospholipids are mixed with water, they spontaneously rearrange into concentric bilayer structures, termed liposomes separated by aqueous compartments[16]. Incorporating ORS components into liposomes, as opposed to simply having salts and substrate in solution, has several potential advantages: (a) it may add an additional mechanism of absorption of solutes to that already present with glucose mediated transport, (b) it may become especially important in patients who have severe purging or who have damaged intestinal epithelium (severe malnutrition, persistent diarrhoea), and (c) the solutions will taste less salty and will have lower osmolarity. This liposome based ORS solution also have the potential advantage compared to all cereal based ORSs, i.e. slow release of substrate avoiding osmotic drag or load.

The aim of the present study is to determine the absorption of water and electrolytes from liposome based glucose–containing solution over the whole length of mucosal injured small intestine of rat under *in vivo* conditions. The whole length of rat small bowel was chosen to obtain results close to those in an intact animal and therefore relevant to the design of an improved ORS formulation[17].

The present study has been reviewed and approved by the institutional Research Review Committee (RRC), Ethical Review Committee (ERC) and Animal Experimentation Ethics Committee (AEEC) of International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

MATERIALS AND METHODS

Adult male Long Evans rats were selected for the present experiment. In this study ORS was used to perfuse isolated rat intestine. The electrolyte concentrations are shown in table 1. Polyethylene glycol (PEG) was used as unabsorbable control molecule. Three types of ORS were used as perfusion solution. These were Standard ORS (S-ORS), Hydrolyzed starch ORS (HS-ORS) and Hydrolyzed starch with Liposome based ORS (Lipo-ORS). In the liposome based ORS, approximately half of the total amount of the electrolytes were estimated to be microencapsulated within the liposomes.

Experimental Procedure

Sixty adult male rats, with body weight of 250-300g, were selected and studied for the experiment. They were fasted for 24 hours with free access to water.. The rats were anaesthetized with intra-peritoneal sodium pentobarbital (40 mg/kg) injection. Rats were grouped into two experimental groups: (i) control group and (ii) 5-Fluorouracil treated experimental group (5FU-treated). Both group of rats were further treated with one of three types of oral rehydration solution (Table 2).

Healthy rat was anesthesized and abdomen was opened after a midline-incision (3-4 cm in length). The intestine along with stomach and caecum was taken outside and placed on the lap-sheet. Thereafter, two incisions were performed into the intestine. The first was on the stomach 3-4cm proximal to the duodeno-jejunal flexure and the second was into the ileum 3-4 cm before the ileocaecal junction for cannulation. The small

intestine was then cannulated with two polyvinyl tubes (2mm in diameter). A proximal cannula was introduced into the distal stomach through the incision and gently guided into the duodenum through the pylorus. The tip of the cannula was placed 2-3 cm distal to the pylorus, and the pylorus was tied externally to prevent backflow of the perfusate into the stomach. The distal cannula was inserted through the other incision and the ileum was tied just before the ileocaecal junction. The isolated and cannulated small intestine was gently rinsed to clear residual contents with the perfusion solution by gravity drainage. Prior to the final wash, the intestine was returned to the abdominal cavity and abdominal cavity was closed by suturing the incision, keeping the cannulas out of the abdomen.

In control experiment, only the comparison of absorption rates of water and electrolytes were observed among three types of ORS (S-ORS, HS-ORS and Lipo-ORS) in normal small intestine. On the other hand in 5-Fluorouracil-treated experimental study, firstly a mucosal injured intestine was established and thus absorption rates of water and electrolytes from S-ORS, HS-ORS and Lipo-ORS in mucosal injured small intestine were observed.

Table 1. Composition of solution used to perfuse the rat intestines.

Electrolytes	S-ORS (g)	HS-ORS (g)	Lipo-ORS (g)
Sodium (mmol)	75	75	75
Potassium (mmol)	20	20	20
Chloride (mmol)	65	65	65
Citrate (mmol)	10	10	10
Carbohydrate (gram per liter)	Glucose (13.6)	Hydrolyzed tapioca starch (25)	Hydrolyzed tapioca starch (25)
Osmolality	245	210 (approx)	125 (approx)

Types of experiment	Types of group according to used ORS	Type of ORS	No of rats
1) Control experiment	1. Control S-ORS group	S-ORS	10
	2. Control HS-ORS group	HS-ORS	10
	3. Control Lipo-ORS group	Lipo-ORS	10
5-FU-treated experiment	1. 5-FU -treated S-ORS group	S-ORS	10
	2. 5-FU -treated HS-ORS group	HS-ORS	10
	3. 5-FU -treated Lipo-ORS group	Lipo-ORS	10

Oral administration of 5-Fluorouracil (5-FU)

To produce mucosal injured intestine of rats using 5-fluorouracil by oraladministration. Administration of 5-FU produces mucosal injury as well as markedly reduces absorptive and enzymatic activities of the rat small intestine[19-21]. After 24

Figure 1. Oral administration of 5-FU with orogastric tube.

hours of fasting (water was allowed ad lib), rats were administration of 5-FU (300mg/kg body wt) intra-gastrically (Fig 1). Food (rat chow) was allowed and the animals were monitored for 2 days, then again after 1 day of fasting (water was allowed) the rats were anaesthetized to construct the small intestinal perfusion segment and the perfusion studies was conducted.

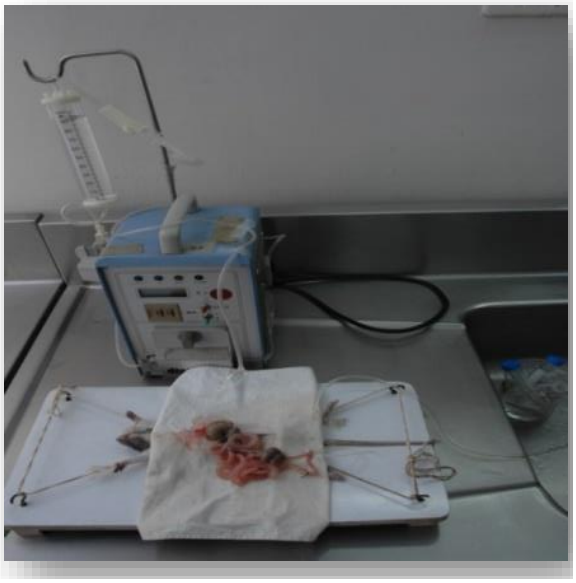

Figure 2. Rat after dissection to perform perfusion with ORS

Then intestinal perfusion were started at a rate of 0.5 ml/min with Oral Rehydration Solutions (ORS).

a **b**

Figure 3. a. Normal intestinal segment
b. Mucosal injured intestine treated with 5-FU

Each rat was perfused with only one of the three solutionsas shown in Table 2. The perfusion was performed by attaching the proximal cannula to a constant infusion pump using a measuring burette as a reservoir. Each solution was infused at a constant rate of 0.5 ml/min. The distal cannula was extended to aid drainage of effluent by gravity. After 30 minutes of equilibration to achieve a steady state, the effluent was collected for 3 consecutive 15 minute-collections (total 45 minutes) in Falcon tubes kept on ice. During the experiments the body temperature of the rats was maintained by controlling the ambient temperature using a spot lamp with thermostatic control and monitored by rectal thermometers.

After completing perfusion, aliquots of infusion and perfusion solutions were transferred from Falcon tubes to Eppendorf tubes by micro-pippette. The samples were stored at -50ºC for up to 48 hours before analysis of net water and electrolyte movement. At the end of each experiment, the rats were sacrificed with overdose of pentobarbital. After sacrificing the perfused small intestinal segment was removed and stripped of excess mesentery. The segment wet weight was taken by digital measuring scale (Metler Toledo, College). The dry weight of the

perfused segment was obtained after desiccation in an oven at 100ºC for 18 hours.

Analytical methods

Sodium (Na^+), potassium (K^+), chloride (Cl^-) were measured by flame emission spectroscopy, and PEG was measured by spectro-photometry.

Calculations[22]

Net transport of water and electrolytes were calculated from the changes in the PEG concentration and the solute concentration[23,24]. The calculation for the net transport of water and ions was done as follows:

Net transport=$\{F\times([S_1]-[S_2])([PEG_1]/[PEG_2])\}/W$

Where:

F is the flow rate

S_1 is the solute concentration in the perfusate

S_2 is the solute concentration in the effluent

PEG_1 is the PEG concentration in the perfusion fluid

PEG_2 is the PEG concentration in the effluent and

W is the dry weight of the small intestinal segment used for perfusion.

Statistical Analysis

Results were presented as mean±SD used in tables and median used in graphs and values were expressed as $\mu l.gm^{-1}.min^{-1}$ for water and $\mu mol.gm^{-1}.min^{-1}$ for electrolytes while calculation performed on the dry weight of perfused segment. Positive results indicate net absorption and negative results indicate the net secretion into the lumen. Analysis of variance (Anova) was performed to test the statistical significance of the differences between the groups.

RESULT

Net transport of water and electrolytes were calculated from the changes in the PEG and solute concentration[23-24]. Net sodium, potassium, chloride and water absorption were significantly higher from the Lipo-ORS compared to the other two solutions, S-ORS and HS-ORS.

In case of water absorption, for both in the control and the experimental studies absorption rates from Lipo-ORS were the highest 0.33±0.10 $\mu l.gm^{-1}.min^{-1}$ and 0.48±0.05 $\mu l.gm^{-1}.min^{-1}$ **(Table 3)** and the differences among three ORSs were statistically significant not only in control group but also in the 5-FU treated group (Table 3). In another way of analysis, the difference between two ORS, S-ORS and Lipo-ORS were significant (P=0.01* and P=0.01*), in both groups (Table 4). Also the difference between HS-ORS and Lipo-ORS were significant (P=0.04 and P=0.01*), in both groups (Table 5). Water absorption rate were highest from Lipo-ORS than other two types of ORSs in both groups (Fig 4.Graph-1).

On the other hand, secretion of sodium ion was observed in all experiments. In the control group net secretion of sodium was lowest from HS-ORS in control group and from Lipo-ORS in 5-FU treated group. The differences among three ORSs were not statistically significant in both groups (Table 3). Sodium ion secretion were lower from Lipo-ORS than S-ORS in both groups **(Fig 4.Graph-2)**.

Moreover, in case of potassium ion for both in control and experimental group absorption occurred from three ORS. In control group absorption of K^+ from Lipo-ORS was highest 2.08±0.92 $\mu mol.cm^{-1}.min^{-1}$ **(Table 3)** and the difference was not statistically significant. In 5-FU treated group absorption rate from Lipo-ORS (1.49±1.23 $\mu mol.gm^{-1}.min^{-1}$) was highest and the difference among three ORS was not statistically significant **(Table 3)**. Potassium ion absorption rate was highest from Lipo-ORS than other two types of ORS in control group and similar to HS-ORS in 5-FU treated group **(Fig 4.Graph-3).**

Table-3: Comparative analysis through analysis of variance (F- Test) absorption rates of water and electrolytes from three types of ORSs in perfusion study on whole small intestine of rat using dry weight of perfused segment.

Name of the group		Control group				5-FU treated group			
No of rats		10	10	10	P (F test)	10	10	10	P (F test)
Dry Weight of segment (gm)									
Types of ORS / Sample Concentration[1]		S-ORS	HS-ORS	Lipo-ORS		S-ORS	HS-ORS	Lipo-ORS	
H_2O	Mean±SD	0.23±0.05	0.25±0.08	0.33±0.10	**0.02***	0.34±0.06	0.36±0.04	0.48±0.05	**0.01***
Na^+	Mean±SD	-3.39±3.11	-1.11±2.14	-1.79±3.07	0.20	-6.68±2.24	-5.56±3.07	-4.14±6.63	0.44
K^+	Mean±SD	1.67±0.66	2.13±0.85	2.08±0.92	0.39	1.14±0.78	1.08±1.44	1.49±1.23	0.71
Cl^-	Mean±SD	-1.07±2.04	1.93±1.10	2.96±1.99	**0.00***	-3.49±1.92	-3.70±2.66	-1.94±5.74	0.54

[1]Sample concentration: $\mu l.gm^{-1}.min^{-1}$ for water absorption & $\mu mol.gm^{-1}.min^{-1}$ for electrolytes net absorption
* the mean difference is significant at the 0.05 level.
Legend: S-ORS= Standard Oral Rehydration Solution
HS-ORS= Hydrolyzed Starch Oral Rehydration Solution
Lipo-ORS= Liposomal Oral Rehydration Solution

In control group absorption rate of Cl- was highest from Lipo-ORS and the difference between three ORS was statistically significant (P=0.00*). Whereas in the 5-FU treated group, Cl- secretion were higher from S-ORS and Lipo-ORS and the difference between three ORS was not statistically significant. By another way of analysis Post Hoc test, in control group, net absorption of Cl- from Lipo-ORS was higher than S-ORS and the difference between S-ORS and Lipo-ORS was statistically significant P=0.00* **(Table 4**). Net absorption of Cl- from HS-ORS and Lipo-ORS groups were not statistically significant in both control group and 5-FU treated group (Table 5). Highest absorption occurred from Lipo-ORS in control group and highest secretion occurred from Lipo-ORS in 5-FU treated group (**Fig 4.Graph-4**).

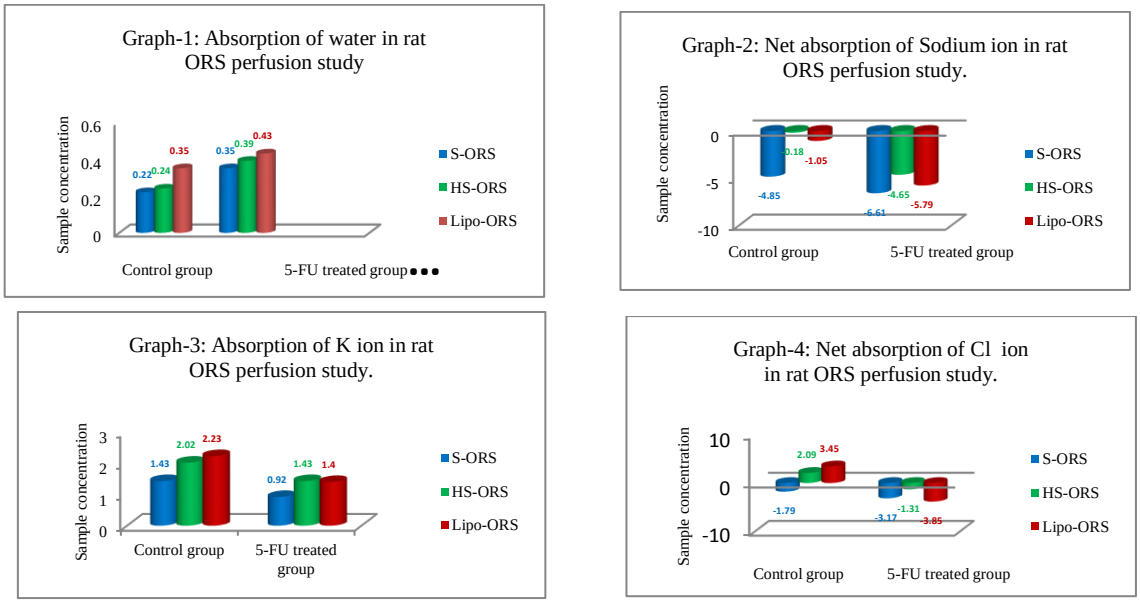

Figure 4. Graph-1, 2, 3 and 4 represents the absorption and secretion rates of water and electrolytes in rats treated with the three types of ORS.

Legend of figures:
S-ORS= Standard Oral Rehydration Solution
HS-ORS= Hydrolyzed Starch Oral Rehydration Solution
Lipo-ORS= Liposomal Oral Rehydration Solution

Table: 4. Comparative analysis by Post Hoc Test, between the absorption rates of water and electrolytes from Lipo-ORS and S-ORS in perfusion study on whole small intestine of rat.

Name of the group		Control group			5-FU treated group		
No of rats		10	10		10	10	
Dry Weight of segment (gm)		1.34 gm	1.27 gm		1.68 gm	1.63 gm	
Types of ORS / Sample Concentration[1]		Lipo-ORS	S-ORS	P (Post Hoc test)	Lipo-ORS	S-ORS	P (Post Hoc test)
H_2O	Mean±SD	0.33±0.10	0.23±0.05	**0.01***	0.48±0.05	0.34±0.06	**0.01***
Na^+	Mean±SD	-1.79±3.07	-3.39±3.11	0.22	-4.14±6.63	-6.68±2.24	0.21
K^+	Mean±SD	2.08±0.92	1.67±0.66	0.26	1.49±1.23	1.14±0.78	0.51
Cl^-	Mean±SD	2.96±1.99	-1.07±2.04	**0.00***	-1.94±5.74	-3.49±1.92	0.37

[1]Sample concentration: $\mu l.gm^{-1}.min^{-1}$ for water absorption & $\mu mol.gm^{-1}.min^{-1}$ for electrolytes net absorption
* the mean difference is significant at the 0.05 level.
Legend: S-ORS= Standard Oral Rehydration Solution
HS-ORS= Hydrolyzed Starch Oral Rehydration Solution
Lipo-ORS= Liposomal Oral Rehydration Solution

Table 5. Comparative analysis by Post Hoc Test, between the absorption rates of water and electrolytes from Lipo-ORS and HS-ORS in perfusion study on whole small intestine of rat.

Name of the group		Control group			5-FU treated group		
No of rats		10	10		10	10	
Dry Weight of segment (gm)		1.44 gm	1.27 gm		1.63 gm	1.63 gm	
Types of ORS / Sample Concentration[1]		Lipo-ORS	HS-ORS	P (Post Hoc test)	Lipo-ORS	HS-ORS	P (Post Hoc test)
H_2O	Mean±SD	0.33±0.10	0.25±0.08	**0.04***	0.48±0.05	0.36±0.04	**0.01***
Na^+	Mean±SD	-1.79±3.07	-1.11±2.14	0.59	-4.14±6.63	-5.56±3.07	0.48
K^+	Mean±SD	2.08±0.92	2.13±0.85	0.91	1.49±1.23	1.08±1.44	0.45
Cl^-	Mean±SD	2.96±1.99	1.93±1.10	0.20	-1.94±5.74	-3.70±2.66	0.31

[1]Sample concentration: $\mu l.gm^{-1}.min^{-1}$ for water absorption & $\mu mol.gm^{-1}.min^{-1}$ for electrolytes net absorption
* the mean difference is significant at the 0.05 level.
Legend: S-ORS= Standard Oral Rehydration Solution
HS-ORS= Hydrolyzed Starch Oral Rehydration Solution
Lipo-ORS= Liposomal Oral Rehydration Solution

DISCUSSION

The implementation of World Health Organization ORS (WHO-ORS) has resulted in decreased mortality associated with acute diarrhoeal illnesses in children, although in general stool volume and diarrhoea durations were not reduced. Some limitations in Oral rehydration therapy (ORT) prompted the development of the concept of improved ORS (initially named 'super-ORS')[16]. Several strategies were used to develop and test improved ORS. Though the new hypo-osmolar ORS has advantages over the previously used standard ORS, the duration of diarrhoea was still not shortened, and there were failures with ORT[25].

Various modifications to the standard ORS have been derived. These modification have included hypo-osmolar or hyperosmolar solutions, use of rice-based ORS, and the use of amino acids, including glycine, alanine, and glutamine[26]. Some of these variations have been successful, some have not, and others are still under investigation.

ORS has also been used to decrease intravenous (IV) fluid infusion to patients with short bowel syndrome (SBS) who require parenteral nutrition[27].

The present study demonstrated that a liposome-based ORS induced a significantly greater electrolytes (Na^+, K^+ and Cl^-) and water absorption compared to standard-ORS and hydrolyzed starch-ORS solution. As expected rat intestines treated with 5-Fluorouracil (5-FU) by oral administration was used to creat mucosal injured intestine. The three types of ORSs were then evaluated to determine their absorptive characteristics. In both the control rats as well as the 5-FU treated rats, the liposome based ORS solution induced a significantly greater water and electrolyte absorption compared to other two ORSs.

In the control (no cholera toxin) rats, the highest absorption of water, K^+ and Cl^- as well as lowest secretion of Na^+ were observed in the rats perfused with Lipo-ORS. In the CT treated rats highest absorption of water and K^+ as well as lowest secretion of Na^+ were observed. One of the interesting findings of this study was that the median values of net Na^+ transport were negative in all groups, suggesting net intestinal secretion; however the Na^+ secretion was observed lowest among the Lipo-ORS group. In contrast, the median values of net K^+ transport were positive in all groups, suggestive net intestinal absorption; again, the highest K^+ absorption was noted in the Lipo-ORS group. In case of chloride ion absorption the difference among three ORSs was significant.

In 1990, Patra *et al* , studied that the effect of citrate on sodium, potassium, chloride and water absorption in the presence of glucose from the whole rat small intestine by an *in vivo* marker perfusion technique. The perfusion solutions contained glucose and were similar in their electrolyte composition to the currently recommended oral rehydration solution for the treatment and prevention of diarrhoeal dehydration. Significantly more sodium and water absorption occurred from the citrate-containing solution than from the one without citrate[17].

Clinical studies showed improved absorption with alternative types of ORS. In a non-randomized open trial, children receiving an oral rehydration salt solution with a lower concentration of glucose and was hyptonic had reduced frequency of diarrhoeal stools and could be discharged sooner than other children who received the standard ORS which was isotonic[28]. Faruque *et al* (1996) compared a hypo-osmolar ORS with sucrose replacing glucose (Na^+ 60, K^+ 15, Cl^- 60, citrate 5, sucrose 58 mmoll-1, calculated osmolality 198 mOsm kg-1) with mildly hyperosmolar glucose ORS (WHO) in 46 children aged 6-30 months with acute diarrhoea and dehydration. In the hypo-osmolar sucrose ORS group (n=18) faecal output was 30% less during the initial 24 and 48 h compared with controls.

In a randomized controlled clinical trial, Dutta *et al* found that children, aged 2-10 years with severe cholera who were treated with a rice-based hypo-osmolar ORS had reduced ($p<0.05$) stool output, ORS consumption and diarrhea duration than patients who received either WHO-ORS or glucose-based hypo-osmolar ORS. In another randomized controlled trial, Ramakrishna tested a hypotonic ORS in which the carbohydrate was an amylase resistant starch in adults with acute dehydrating diarrhoea. Compared to hypo-osmolar (HO-ORS) ORS, amylase resistant starch -ORS reduced diarrhoea duration by 55% and significantly reduced fecal weight after the first 12 hours of ORS therapy in adults with cholera like diarrhoea.

Various liposome-based medications are already used in diverse clinical situations. Various pharmacological agents of varying solubility and size (anti-tumour and antimicrobial agents, enzymes, peptides, hormones, vaccines and genetic materials) have already been encapsulated in either the aqueous or the lipid phase of the liposomes[16]. Proteins and other non-lipid molecules can be incorporated into the lipid membranes. Drug ligands (e.g. antibodies) can also be linked with the outer bilayer. In fact, liposomes can be designed to satisfy particular needs in a variety of applications ranging from biochemical and immunological assay kits and diagnostic reagents to therapeutic preparations for enteral and parenteral uses as well as vaccines[29-34].

CONCLUSION

In the present investigation liposomes incorporated into ORS for intestinal perfusion to stimulate absorption of water an

significantly enhanced absorption of water, K^+, Cl^- and reduced secretion of Na^+ when compared with other ORSs. The improved lipsome based ORS (Lipo-ORS) may have potentials in reducing stool

volume, duration of diarrhoea and failure rate of ORT in patients with high purging rate. Future RCTS are therefore warranted to evaluate its clinical efficacy in infectious diarrhea.

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been reviewed and approved by the institutional Research Review Committee (RRC), Ethical Review Committee (ERC) and Animal Experimentation Ethics Committee (AEEC) of International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

REFERENCES

1.Bern, C., Martines, J., Zoysa, D. I. and Glass, R.I. 1992. The magnitude of the global problem of diarrhoeal disease: a ten-year update. *Bulletin of the World Health Organization.* **70:** 705-14.

2.Gibson, R.J. and Keefe, D.M.K. 2006. Cancer Chemotherapy-induced diarrhea and constipation: mechanisms of damage and prevention strategies, *Support CareCan*.**14:** 890-900.

3.Stringer, A.M., Gibson, R.J., Logan, R.M., Bowen, J.M., Yeoh, A.S.J. and Burns, J. 2007a.Chemotherapy-induced diarrhea is associated with changes in the luminal environment in the DA Rat. *ExpBiol Med.* **232:** 96-107.

4.Stringer, A.M., Gibson, R.J., Logan, R.M., Bowen, J.M., Yeoh, A.S.J. and Keefe, D.M. 2007b. Chemotherapy-induced mucositis: the role of gastrointestinal microflora and mucins in the luminal environment. *J Support Oncol.* **5:** 259-67.

5.Haenal, H. 1970. Human normal and abnormal gastrointestinal flora. *Am J ClinNutr*. **23:** 1457.

6.Gutheil, J.C., Kearns, C.M. and Antimetabolites in Perry, M.C. 1997. *The Chemotherapy Source Book, Williams and Wilkins, Baltimore*. 317-44.

7.MacDonald, D. R. and Neurotoxicity of chemotherapeutic agents in Perry, M.C. 1997. *The Chemotherapy Source Book, Williams and Wilkins, Baltimore.*745-66.

8.Stringer, A. M., Gibson, R. J., Logan, R. M., Bowen, J. M., Yeoh, A. S. J. and Keefe, D. M. K. 2009a. Gastrointestinal microflora and mucins play a role in the development of 5-Fluorouracil-induced gastrointestinal mucositis in rats. *ExpBiol Med.* **234:** 430-441

9.Stringer, A. M., Gibson, R. J., Logan, R. M., Bowen, J. M., Laurence, J. and Keefe, D. M. K. 2009b. Irinotecan-induced mucositis is associated with changes in intestinal mucins. *Cancer Chemo Pharmacol.* **64(1):**123-32.

10.Hirschhorn, N., Kinzie, J.L., Sachar, D.B., Northrup, R.S., Taylor, J.O. and Ahmad, S.Z. 1968. Decrease in net stool output in cholera during intestinal perfusion with glucose-containing solutions. *The New England journal of medicine.***279(4):**176-81.

11.Pierce, N.F., Banwell, J.G. and Rupak, D.M. 1968. Effect of intragastric glucose-electrolyte infusion upon water and electrolyte balance in Asiatic cholera. *Gastroenterology*. **55:** 333-343.

12.Fordtran, J.S. 1975. Stimulation of active and passive sodium absorption by sugars in the human jejunum. *J Clin Invest*. **55:**728-37.

13.Hirschhorn, N. and Greenough, W.B. 111. 1991. Progress in oral rehydration therapy. *Scient Amer*.**264:**50-56.

14.Mahalanabis, D. 1996. Current status of oral rehydration as a strategy for the control of diarrhoeal diseases. *The Indian journal of medical research.* **104:**115-24.

15.Mahalanabis, D. and Merson, M.H. 1986. Development of an improved formulation of oral Rehydration solution (ORS) with antidiarrhoeal and nutritional properties: a 'Super ORS'. In Holmgren J, Lindberg A and Molby R, eds.

16.Development of vaccines and drugs against diarrhoea; 11th Nobel Conference, Stockholm, 1985. *Lund Student Literature*. 240-256.

17.Gregoriadis, G. 1991. Overview of liposomes. *J Antimicrob Chemother*. **28 (Suppl.B):** 39-48

18.Patra, F.C., Rahman, A.S.M.H., Wahed, M.A. and Al-Mahmud, K.A. 1990. Enhanced sodium absorption by by citrate: an *in vivo* perfution study of rat small intestine. *J Ped Gastroenterol Nutr*.**11:** 385-388.

19.Rolston, D.D.K., Borodo, M.M., Kelly, M.J., Dawson, A.M. and Farthing, M.J.G. 1987. Efficacy of oral rehydration solutions in a rat model of secretory diarrhea. *Journal of pediatric Gastroenterology and Nutrition*. **6:** 624-630.

20.Bounos, G., Hugon, J. and Gentile, J.M. 1971. Elemental diet in the management of the intestinal lesion produced by 5-Fluorouracil in the rat. *Can J Surg*. **14:** 298-311.

21.Levin, R. J.1968. Anatomical and functional changes of the small intestine induced by 5-Fluorouracil. *J Physiol (Lond).* **197:**73P-74P.

22.Tanaka, H., Miyamoto, K. I. and Morla, K. 1998. Regulation of PepTI peptide transporter in the rat small intestine in response to 5-Fluorouracil-induced injury. *Gastroenterology*. **114:** 714-23.

23.Sladen, G.E. and Dawson, A.M. 1968.An evaluation of perfusion techniques in the study of water and electrolyte absorption in man: the problem of endogenous secretions. *Gut* **9:** 530-535.

24.Gray, G.M. and Ingelfinger, F.J. 1966. Intestinal absorption of sucrose in man: Interrelation of hydrolysis and monosaccharide product absorption. *J Clin Invest*. **45:**388-398.

25.Levinson, R.A. and Schedl, H.P. 1966. Absorption of sodium, Chloride, water and simple sugars in rat small intestine. *Am J Physiol*.**211:**939-942

26.Alam, N.H., Mazumder, R.N. and Fuchs, G.J. 1999. Efficacy and safety of oral rehydration solution with reduced osmolarity in adults with cholera: a randomized double-blind clinical trial. *Lancet*; **354 (9175)**: 296-299.

27.Rhoads, J. M., Keku, E. O., Bennett, L. E., Quinn, J. and Lecce, J. G. 1990. Development of L-glutamine-stimulated

electroneutral sodium absorption in piglet jejunum. *Am J Physiol*. **259:** G99-G107.
28.Atia, A. N. and Buchman, A. L. 2009. Oral rehydration solutions in non-cholera diarrhea: a review. *Am J gastroenterol*. **104 (10):** 2596-604.
29.Rautanen, T., El-Radhi, S. and Vesikari, T. 1993. Clinical experience with a hypotonic oral rehydration solution in acute diarrhoea. *Acta Paediatr*. **82:** 52-54.
30.Gregoriadis, G. 1988. Liposomes as drug carriers: recent trends and progress. Wiley, Chichester.
31.Lopez-Berestein, G. and Fidler, I. J.1989. Liposomes in the therapy of infectious diseases and cancer. Alan Liss, New York.
32.Baemner, A.J., Schlesinger, N.A., Slutzki, N.S., Romano, J., Lee, E.M. and Montagna, R.A. 2002. Biosensor for dengue virus detection: sensitive, rapid and serotype specific. *Anal chem.***74(6):**1442-8.
33.Wu, J., Nantz, M.H. and Zern, M.A. 2002. Targeting hepatocytes for drug and gene delivery: emerging novel approaches and applications. *Front Biosci*.**7:** 717-25.
34.Dima, V. F., Lonescu, M. D., Palade, R., Balotescu, C., Becheanu, G. and Dima, S. V. 2001. Stimulation of mucosal immune response following oral administration of enterotoxigenic *Escherichia coli* fimbriae (CFA/1) entrapped in liposomes in conjunction with inactivated whole-cell *Vibrio cholerae* vaccine. *Roum Arch Microbiol Immunol*. **60 (1):** 27-54.
35.Brouwers, A. H., De Jong, D. J., Dams, E. T., Oyen, W.J., Boerman, O.C., Laverman, P., Naber, T.H., Storm, G. and Corstens, F.H. 2000. Tc-99m-PEG-Liposomes for the evaluation of colitis in Crohn's disease. *J Drug Target.***8**: 225-33.