tachment 1.

Dr. Alam

Date 30.1.90
7-2.90

ETHICAL REVIEW COMMITTEE, ICDDR,B.

320

Principal Investigator Sufia Islam

Trainee Investigator (if any)

Application No. PCC-001/90

Supporting Agency (if Non-ICDDR,B)

Title of Study Study of absorption promoting oral rehydration solutions animal models.

Project status:
(✓) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population: NA

(a) Ill subjects Yes No
(b) Non-ill subjects Yes No
(c) Minors or persons under guardianship Yes No

Does the study involve: NA

(a) Physical risks to the subjects Yes No
(b) Social Risks Yes No
(c) Psychological risks to subjects Yes No
(d) Discomfort to subjects Yes No
(e) Invasion of privacy Yes No
(f) Disclosure of information damaging to subject or others Yes No

Does the study involve: NA

(a) Use of records, (hospital, medical, death, birth or other) Yes No
(b) Use of fetal tissue or abortus Yes No
(c) Use of organs or body fluids Yes No

Are subjects clearly informed about: NA

(a) Nature and purposes of study Yes No
(b) Procedures to be followed including alternatives used Yes No
(c) Physical risks Yes No
(d) Sensitive questions Yes No
(e) Benefits to be derived Yes No
(f) Right to refuse to participate or to withdraw from study Yes No
(g) Confidential handling of data Yes No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No

5. Will signed consent form be required: NA
   (a) From subjects Yes No
   (b) From parent or guardian (if subjects are minors) Yes No
6. Will precautions be taken to protect anonymity of subjects NA Yes No
7. Check documents being submitted herewith to Committee:

NA Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
NA Protocol (Required).
✓ Abstract Summary (Required)
NA Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
NA Informed consent form for subjects
NA Informed consent form for parent or guardian
NA Procedure for maintaining confidentiality
NA Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:

1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

(PTO)

agree to obtain approval of the Ethical Review Committee for any changes olving the rights and welfare of subjects before making such change.

Sufia Islam
Principal Investigator

Trainee

PCC-001/90
7.2.90

SECTION I : RESEARCH PROTOCOL

Title	: Study of absorption promoting oral rehydration solutions in animal models.
Principal Investigator	: Ms.Sufia Islam
Co-Investigators	: 1. Mr.M.A.Wahed Head,Biochemistry & Nutrition,ICDDR,B 2. Dr.A.S.M.Hamidur Rahman Chief,Research & Treatment Section,Animal Resources Branch,ICDDR,B
Supervisors	: 1. Dr.D.Mahalanabis Associate Director,Clinical Sciences Division,ICDDR,B 2. Professor A.K.Azad Chowdhury Department of Pharmacy University of Dhaka
Starting Date	: April 1, 1990
Completion Date	: March 31, 1991
Total Direct Cost	: US$ 8,428
Scientific Programme Head	: This protocol has been approved by the Clinical Sciences Division

Recommended

---

gnature of Chairman
partment of Pharmacy
versity of Dhaka

e: 29.1.90

Chairman
Department of Pharmacy
University of Dhaka
BANGLADESH

Signature of Associate
Director, CSD
ICDDR,B

Date: 30.1.90

9. Abstract Summary

Glucose-linked enhanced sodium and water absorption from the small intestinal lumen remains largely intact during acute diarrhoea of diverse aetiology and forms the scientific basis of oral rehydration therapy (ORT). Subsequently, ORT has been developed as a powerful intervention for the treatment of dehydration due to acute diarrhoea, an invaluable public health tool, and an essential component of primary health care. The oral rehydration salts solution (ORS) recommended by WHO contains glucose (20 g) and three salts-sodium chloride (3.5 g), trisodium citrate,dihydrate (2.9 g) or sodium hydrogen carbonate (2.5 g), and potassium chloride (1.5 g), to be mixed in one litre of water. This solution has been used in the treatment of acute diarrhoeal illness for over a decade.

Studies have shown that almost all water soluble organic molecules can enhance the absorption of sodium from the small intestine. Examples are D-hexoses, neutral amino acids, dipeptides and tripeptides of neutral amino acids and some water soluble vitamins. It has been hypothesised that optimum exploitation of this phenomenon could lead to the development of an improved ORS formulation that would not only successfully replace the deficit of salts and water in diarrhoea, but also actively induce the reabsorption of endogenous intestinal secretion and thus reduce the volume and duration of diarrhoea. The proposed study intends to examine the net absorption of an ORS containing salts and selected organic solutes in rat and rabbit intestine. The type and combination of organic solutes will be varied to optimise absorption. Candidate organic solutes will include glucose, l-alanine, glutamine, and peptides of alanine and glutamine. Polyethylene glycol (Mol. wt. 4,000) will be used as a non-absorbable marker. The outcome measures will include the magnitude of net absorption of salts and water. Results may be useful in further improvement of the absorption efficiency of ORS formulations.

SECTION II : RESEARCH PLAN

A. INTRODUCTION

1. Objectives and specific aims

Objectives

Absorption promoting oral rehydration solution (ORS) could lead to the development of an improved formulation of ORS. Such an ORS formulation would successfully replace the deficit of salts and water in diarrhoea, induce the reabsorption of endogenous intestinal secretion, and thus reduce the volume and duration of diarrhoea. The reduced severity and duration of diarrhoea will also make it possible to introduce early a liberal and more effective feeding regime. Therefore, the nutritional benefit would be another potential advantage of the intended improved formulation of ORS.

A few organic nutrients likely to be of value in improving absorption efficiency of oral rehydration solution will be evaluated, in an *in vivo* study in animals e.g. rabbits and rats using ligated small intestinal loops. The components will include neutral amino acids like l-alanine, l-glutamine, and their peptides and will be compared with glucose. The results of the study will be used to predict a likely formulation, which will be more absorption efficient than the solution containing glucose. Such formulation can then be evaluated in human subjects with diarrhoea.

Specific aims

Study of the absorption of water and sodium from a solution containing:

1. l-alanine compared to glucose eletrolyte solution;
2. l-glutamine compared to glucose electrolyte solution;
3. combination of alanine and glutamine compared to glucose electrolyte solution;
4. combination of glucose and alanine compared to glucose electrolyte solution; and
5. dipeptides of glucose and alanine compared to glucose electrolyte solution.

Background

Stimulation of sodium absorption across the small intestinal mucosa by glucose was looked at by physiologists (Schultz and Zalusky 1964[1]; Barry et al 1965[2]). In vivo studies in normal human intestine defined the quantitative relationships of glucose linked enhanced sodium and water absorption (Malawer et al 1965[3]; Levinson and Schedl 1966[4]; Fordtran et al 1968[5]).

During the phase of active purging in adult cholera patients, fluid and electrolyte losses were adequately replaced by optimally constituted oral electrolyte solutions containing glucose (Pierce et al 1968 a[6], 1968 b[7], 1969[8]; Hirschhorn et al 1968[9]; Nalin et al 1968[10]). Radioactive tracer studies in cholera patients also showed that glucose (2 g/100 ml) induces net absorption of sodium and water from the small intestine (Taylor et al 1968[11]).

The introduction of oral glucose electrolyte solutions for the treatment of dehydration due to acute diarrhoeas has been a major therapeutic advance of this century. The initial success of oral rehydration therapy (ORT) in adult cholera patients was tested by its vigorous application in treating diarrhoeal disease of diverse aetiology, in various age groups of infants and children. ORT emerged as a powerful therapeutic tool with the ability to correct dehydration due to acute diarrhoea in all but the most severe cases, and in all ages irrespective of aetiological agents (Mahalanabis 1984[12]).

One major area of interest and scientific controversy is the substrate or carrier molecule used in ORS. Sucrose, which in many countries, is a less expensive and more easily obtained sugar than glucose, has proved a possible substitute for glucose[13]. However, a marker perfusion study in rat (Patra et al 1982[13]) showed that an isosmotic sucrose containing electrolyte solution induces a significantly greater Na^+, Cl^- and K^+ absorption compared to glucose electrolyte solution. And the water absorption decreases by 14% due to the osmotic drag of water back into the lumen by the slowly absorbed fructose from the hydrolysis of sucrose.

Use of molasses with common salts simplified ORS with a minimum loss of efficacy. ORS formulations containing 30-50g cooked rice powder (Molla et al 1982[14]; Patra et al 1982[15]) in place of glucose was found to be effective. Cereal-based ORS has been shown to be effective, acceptable, and even superior to standard glucose ORS in reducing the duration and severity of diarrhoea (Patra et al 1982[15]; Molla et al 1989[16]; Molla et al 1985[20]).

In 1984, encouraged by results from several clinical trials, the diarrhoeal disease control programme of the World Health Organisation began supporting research projects on developing improved oral rehydration solution formulations (World Health Organisation. Sixth programme report 1986-1987[17]). Their aim is to compare the standard WHO ORS with other formulations that have the same concentration of salts but may be more effective ([17]). There are two types of alternative formulation. One type contains glucose or a glucose polymer and an aminoacid or peptide, or both, in varying concentrations ([17]): this approach was stimulated by studies in children (Patra et al 1984[18]) and adults (Nalin et al 1970[19]) that used formulations containing glucose and glycine. In the other type of formulation glucose is replaced by a staple food (cereals,legumes,or roots) as a source of starch and protien: this approach was based on studies that showed that a rehydration solution containing 50 or 80g of rice powder per litre in place of glucose can reduce stool volume substantially (Patra et al 1982[15]; Molla et al 1985 [20]).

Oral rehydration solution containing l-alanine was considerably better than standard oral rehydration solution at reducing the severity of symptoms and the need for fluid of male patients with diarrhoea associated with *Vibrio Cholera* and enterotoxigenic *Escherichia Coli* (Patra et al 1989[21]).

Therefore, the proposed study intends to examine the net absorption of an ORS containing salts and organic solutes (glucose, l-alanine, l-glutamine, and peptides of alanine and glutamine) in rat and rabbit intestine.

3. Rationale

The review of theoretical and practical issues surrounding the choice of carrier substances used in ORS suggests that further improvement in the current formulation of ORS for acute diarrhoeal illness is needed. An attempt to increase the efficacy of ORS is therefore necessary. As stated earlier, oral rehydration solution containing l-alanine was considerably better than standard oral rehydration solution (Patra et al 1989[21]). An improved ORS formulation will successfully replace the deficit of salts and water in diarrhoea and will induce the reabsorption of endogenous intestinal secretion, thus reducing the volume and duration of diarrhoea. Such formulation would also offer nutritional benefits and simplicity of management.

B. METHODS

New Zealand white rabbits raised in laboratory environment (av. wt. 1.5 kg) and normal male albino rats (av. wt. 175 g) will be used for the study.

A ligated static loop (10-12 cm long) of small intestine will be constructed after opening the abdomen with a midline incision. The loop which includes jejunum, and ileum will be used to observe net absorption of a solution containing salts and selected organic solutes. Polyethylene glycol (mol. wt. 4,000) will be used as a non-absorbable marker. After injection of the solution the animal will be kept under observation for a specified interval.

After removal of the fluid, the fluid will be analysed for PEG, sodium, potassium, chloride and osmolality and compared with an aliquote of the solution before injection and the net absorption of ORS will be calculated.

The detailed procedures are given below.

1. Construction of the loops

Weight of the rabbit/rat will be taken.In case of rabbit after starvation of 48 hours (with free access to water), anaesthesia will be performed with intravenous pentobarbital sodium (3.25 mg/kg). Midline incision will be given to perform laparotomy; during the experiment body temperature will be maintained by using a lamp. Gently pulled small intestine from the abdominal cavity will be washed with the wash solution (NaCl 120 mmol/l,KCl 4 mmol/l), at a temperature of 37^{o}C. Then the static loops and the interloops will be constructed from the distal to the proximal part (excluding 10-15 cm distal ileum) which include ileum and jejunum.

In case of rat after 24 hours fast (with free access to water), anaesthesia will be performed with intraperitoneal sodium pentobarbital (40 mg/kg), static loops and interloops will be constructed from the proximal to the distal part of the intestine which include jejunum and ileum.

2. Injection of the solution

The test solutions will be injected from the distal to the proximal part of the intestine at a temperature of 37°C. The solution will contain mmol/l Na^+ 120, K^+ 4, Cl^- 124 and PEG (mol. wt. 4,000) 2 g/l. In addition to it the solution will contain 50 mmol/l of selected organic solutes. The intestine will be returned to the peritoneal cavity and abdomen will be closed. The animal will be kept under observation for specified intervals.

3. Removal of the solution

After the observation period the animal will be anaesthetised and the peritoneal cavity will be opened. The solution from each loop will be removed by syringe and the amount of solution will be recorded for each loop. The solutions will be collected in separate containers for estimation of PEG, sodium, potassium, chloride and osmolality. Finally the animal will be sacrificed and the intestinal loops will be removed. After stripping the excess mesentery, length of the loops will be measured (Patra et al 1982[13]; Morin et al 1978[22]).

4. Estimation of polyethylene glycol

Turbidimetric analysis of polyethylene glycol will be done on the development of an oil in-water emulsion when exposed to trichloroacetic acid in the presence of barium ion (Hyden 1955[23]). The optical densities of the standards and the samples will be run against the blank, using a SP8-400 uv/vis spectrophotometer (PYE UNICAM) set at a wavelength of 500nm. A standard curve will be constructed and the unknown samples will be read from the graph.

5. Measurements of electrolytes

Na^+, K^+ and Cl^- will be measured by ISE (Beckman system E4A™) Electrolyte analyser. The system E4A determines sodium, potassium and chloride by measuring electrolyte ions in solution. For sodium, potassium and chloride this is accomplished by using ion-selective electrodes for each of these ions.

6. Measurement of osmolality

Osmolality of the samples will be measured by freezing point depression using an advanced TM osmometer.

7. Calculations

Net transport of water and electrolytes and sugars will be calculated from the changes in the PEG concentration and the solute concentration (Levinson and Schedl 1966[4]).

Calculations will be performed in the following way:

1. PEG ratio (PEG R) = $[PEG_I]/[PEG_F]$
   I and F refers to initial and final concentrations

2. H_2O absorption, % = $100(1-PEG\ R)$

3. H_2O absorption, ml/hr = $\frac{H_2O \text{ absorption},\%}{100}$ X amount of solution injected

4. Na^+(or K^+) absorption, μmoles/hr = $[Na^+_I - (Na^+_F \times PEG\ R)]$ X amount of solution injected

5. Cl^- absorption, μmoles/hr = $[Cl^-_I - (Cl^-_F \times PEG\ R)]$ X amount of solution injected

8. Data analysis

Electrolyte absorption mediated by different organic solutes will be compared
by t test for independent samples
and
by its nonparametric equivalent
(Mannwhitney U test)

Significance of the difference will be assessed ($P<.05$).

C. SIGNIFICANCE OF THE WORK

Reduction of the volume and duration of the diarrhoea by using an improved ORS formulation will greatly simplify the management of dehydration due to acute diarrhoea. This study will examine the net intestinal absorption of different improved ORS formulations and eventually lead to the development of an optimum absorption promoting ORS.

Results of this study will improve our knowledge on the ability of a few potentially important organic nutrients in stimulating absorption of salts and water under *in vivo* conditions in suitable animal models. These information will be of use in designing and formulating absorption efficient oral rehydration solution which can then be tested in human subjects.

D & E. FACILITIES REQUIRED AND COLLABORATIVE ARRANGEMENTS

ICDDR,B shall provide all the laboratory facilities required for the estimation of PEG, measurement of electrolytes and osmolality. The animal resources branch of ICDDR,B will be utilised for the study in animal models.

This is a collaborative study between Department of Pharmacy, University of Dhaka and ICDDR,B. The PI is an M.Phil student of Dhaka University and shall carry out the study at ICDDR,B under the direct supervision of Dr.Dilip Mahalanabis. The study report will eventually be partial fulfilment of the requirement for her M.Phil degree.

SECTION III: BUDGET

1. Personnel services

Name	Time required	Honorarium/Salary P.A US$
Dr.D.Mahalanabis	5%	–
Professor A.K.Azad Chowdhury	5%	–
Mr. M.A. Wahed	5%	–
Dr. A.S.M.Hamidur Rahman	5%	–
Research Fellow (PI)	100%	1600
Animal Technician	5%	–
Animal Attendent	5%	–
	Sub-total:	1600

2. Supplies and materials

a. Disposable syringe, Needle, Glassware etc.			200
b. Chemicals and reagents and laboratory tests			
PEG			1035
Electrolyte and osmolality			3315
c. Animals			
Rabbits	$ 19.80X50	=	990
Rats	$ 1.76X50	=	88
Animal maintenance			100
d. Computer analysis and data entry			500
3. Transport and conveyance			200
4. Overhead, contingency and stationary			400
Grand total			US$ 8,428

BUDGET SUMMARY

	1 year project requirement US$
Salary	1600
Supplies and materials	6228
Other cost	600
Total direct cost	US$ 8,428

REFERENCES

1. Schultz SG, Zalusky R. Ion transport in isolated rabbit ileum.II. The interaction between active sodium and active sugar transport. *J Gen Physiol* 1964; 47:1043 - 59

2. Barry RJC, Smyth DH, Wright EM. Short-circuit current and solute transfer by rat jejunum. *J Physiol* 1965; 181:410 - 431

3. Malawer SJ, Ewton M, Fordtran JS, Ingelfinger FJ. Interrelation between jejunal absorption of sodium, glucose and water in man. *J Clin Invest* 1965; 44: 1072

4. Levinson RA, Schedl HP . Absorption of sodium, chloride, water, and simple sugars in rat small intestine. *Am J Physiol* 1966; 211: 939 - 42

5. Fordtran JS, Rector FC Jr, Carter NW . The mechanisms of sodium absorption in the human small intestine. *J Clin Invest* 1968; 47:884 - 900

6. Pierce NF, Banwell JG, Mitra RC, et al. Oral maintenance of water-electrolyte and acid-base balance in cholera: a preliminary report. *Indian J Med Res* 1968a; 56: 640 - 5

7. Pierce NF, Banwell JG, Rupak DM, et al. Effect of intragastric glucose-electrolyte infusion upon water and electrolyte balance in Asiatic cholera. *Gastroenterology* 1968b; 55: 333 - 43

8. Pierce NF, Sack RB, Mitra RC, et al . Replacement of water and electrolyte losses in cholera by an oral glucose-electrolyte solution. *Ann Intern Med* 1969; 70: 1173-81

9. Hirschhorn N, Kinzie JL, Sechar DB, et al. Decrease in net stool output in cholera during intestinal perfusion with glucose-containing solutions. *N Engl J Med* 1968; 279:176 - 81

10. Nalin DR, Cash RA, Islam R, Molla M, Phillips RA . Oral maintenance therapy for cholera in adults. *Lancet* 1968; 2: 370-3

11. Taylor JO, Kinzie J, Hare R, Hare K . Measurement of sodium flux in human small intestine. *J Clin Invest* 1968; 27:386

12. Mahalanabis D. Oral rehydration therapy. In: Chandra RK,

ed. Critical reviews in tropical medicine. Vol. 2. New York: Plenum, 1984: 77-91

13. Patra FC, Mahalanabis D, Jalan KN. Stimulation of sodium and water absorption by sucrose in the rat small intestine. *Acta Paediatr Scand* 1982; 71: 103 - 7

14. Molla AM, Sarker SA, Hossain M, Moll A, Greenough WB III. Rice-powder electrolyte solution as oral therapy in diarrhoea due to *Vibrio Cholera* and *Escherichia Coli*. *Lancet* 1982; 1: 1317 - 9

15. Patra FC, Mahalanabis D, Jalan KN, Sen A, Banerjee P. Is oral rice electrolyte solution superior to glucose elctrolyte solution in infantile diarrhoea? *Arch Dis Child* 1982; 57: 910 - 2

16. Molla AM, Nath SK, Molla A, Khatun M. Food-based oral rehydration salt solution for acute childhood diarrhoea. *Lancet* 1989; 2: 429 - 31

17. World Health Organisation. Sixth Programme report 1986 - 1987. Programme for control of diarrhoeal disease. Geneva : WHO, 1988: 42 - 6 (WHO/CDD/88.28)

18. Patra FC, Mahalanabis D, Jalan KN, Sen A, Banerjee P. In search of a super solution: Controlled trial of glycine-glucose oral rehydration solution in infantile diarrhoea. *Acta Paediatr Scand* 1984; 73: 18 - 21

19. Nalin DR, Cash RA, Rahman M, Yunus M. Effect of glycine and glucose on sodium and water absorption in patients with cholera *Gut* 1970; 11: 768 - 72

20. Molla AM, Ahmad SM, Greenough WB III. Rice-based oral rehydration solution decreases the stool volume in acute diarrhoea. Bull WHO 1985; 63: 751 - 6

21. Patra FC, Sack DA, Islam A, Alam AN, Majumder RN. Oral rehydration formula containing alanine and glucose for treatment of diarrhoea: a controlled trial. *Br Med J* 1989; 298:1353-6

22. Morin CL, Ling V. Effect of pentagastrin on the rat small intestine after resection. *Gastroenterology* 1978; 75: 224 - 9

23. Hyden SA. Turbidimetric method for the determination of higher polyethylene glycols in biological materials. *Kgl Lantbrunks - Hogsk Ann* 1955; 22: 139 - 45