ıment 1. 6

Date 22/6/85

ETHICAL REVIEW COMMITTEE, ICDDR,B.

cipal Investigator Dr. F.C. Fatoa Trainee Investigator (if any) x

ıication No. 85-022P Supporting Agency (if Non-ICDDR,B) x

e of Study Studies on the effect of pate on the absorption of sodium water in the rat small intestine in vivo means of luminal perfusion technique

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

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

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

Does the study involve:
(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

es the study involve:
) 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:
(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:
(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 Yes No

7. Check documents being submitted herewith to Committee:
___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
✓ Protocol (Required)
✓ Abstract Summary (Required)
___ 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)
___ Informed con ent form for subjects
___ Informed consent form for parent or guardian
___ Procedure for maintaining confidentiality
___ 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)

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

Principal Investigator

Trainee

85-022P
24.7.85.

SECTION 1 - RESEARCH PROTOCOL

(Pilot protocol)

1. TITLE: STUDIES ON THE EFFECTS OF CITRATE ON THE ABSORPTION OF SODIUM AND WATER IN THE RAT SMALL INTESTINE BY AN IN-VIVO MARKER PERFUSION TECHNIQUE.

2. PRINCIPAL INVESTIGATOR: Dr. F.C. Patra

CO-INVESTIGATORS: Dr. K.A. Al-Mahmud
Dr. A.S.M. Hamidur Rahman
Mr. M.A. Wahed

3. STARTING DATE: As soon as possible

4. COMPLETION DATE: 6 months after approval

5. TOTAL INCREMENTAL COST: US $ 2,600.00

6. SCIENTIFIC PROGRAMME: This protocol has been approved by the Pathogenesis and Therapy Working Group.

Signature of Acting Associate Director in-charge, PTWG: _____

Date: _____

7. ABSTRACT SUMMARY:

One important disadvantage of oral rehydration salts containing bicarbonate is that when the constituents are mixed together in a packet in humid atmosphere bicarbonate reacts with glucose to form brownish furfural compounds. To overcome this problem sodium citrate is being now increasingly used in place of sodium bicarbonate as a constituent of oral rehydration salts. Citrate has been claimed to promote absorption of sodium and chloride in the rabbit ileum. The proposed research plan intends to further study the effect of citrate on the absorption of sodium, water, potassium and chloride from the rat jejunum and ileum by an in vivo perfusion technique.

8. REVIEWS:

(a) Research involving human subjects: ----------

(b) Research Review Committee: ----------

(c) Director: ----------

SECTION II - RESEARCH PLAN

A. INTRODUCTION:

1. Objective

To study the effect of citrate on the absorption of sodium and water in the rat small intestine by an in vivo perfusion technique.

2. Background

Oral rehydration therapy for acute diarrhoeal dehydration is a major therapeutic advance. This is based on the finding that glucose mediated sodium absorption remains largely intact during diarrhoea due to various aetiological agents. Presently used WHO recommended oral rehydration solution contains three salts i.e. sodium chloride 3.5 gram , sodium bicarbonate 2.5 gram , potassium chloride 1.5 gram and glucose 20 grams per litre of water. Sodium bicarbonate is used mainly for the correction of metabolic acidosis. Apart from correction of metabolic acidosis it also helps in the absorption of sodium and water independent of glucose (1,2,3,4). But one important disadvantage of oral rehydration salts containing sodium bicarbonate is that when the constituents are mixed together in a packet in humid atmosphere bicarbonate reacts with glucose to form brownish furfural compounds (5). This is a serious constraint for the implementation of oral rehydration therapy in developing countries. So research is being carried out to

find an appropriate alternative to sodium bicarbonate. Sodium citrate being a stable salt (5) has been choosen to this effect and many clinical trials have been conducted using sodium citrate in place of sodium bicarbonate in the oral rehydration solution (5,6). The results of these studies indicate that sodium citrate is as effective as sodium bicarbonate for the correction of metabolic acidosis due to acute diarrhoea (5,6). Apart from correction of metabolic acidosis it has been observed that in some of the studies the stool output was less in the citrate-ORS treated group compared to the bicarbonate-ORS treated group and in one study the difference was statistically significant (6). This phenomenon of reduced stool output could be attributed to an effect of citrate in increasing the intestinal absorption of sodium. Sodium citrate has been shown in vitro to stimulate sodium and chloride absorption by rabbit ileal mucosa both under basal conditions as well as during a secretory state induced by heat stable enterotoxin of Escherichia coli, this effect on ion absorption was dose dependent and the absorption of citrate was shown to be an active process (7).

The proposed research plan intends to study the effects of citrate on the sodium and water absorption from the rat jejunum and ileum by an in vivo perfusion technique.

3. Rationale.

The proposed study will provide us with further information regarding the effect of citrate on the absorption of sodium and water from the rat small intestine.

B. SPECIFIC PLAN:

- to use the existing in vivo perfusion model using the entire rat jejunum and ileum.

- to obtain baseline net flux (lumen⇒serosa) values of sodium, water, chloride and potassium from an glucose electrolyte solution similar to the one used for oral rehydration therapy.

- to study the effect of citrate on the absorption of sodium and water i.e. whether citrate enhances the absorption of sodium and water from an glucose electrolyte solution, the composition of which is similar to the presently used glucose electrolyte solution for the treatment of acute diarrhoeal dehydration.

C. MATERIAL AND METHODS:

Analytical grade sodium chloride, potassium chloride, glucose, tribasic sodium citrate dihydrate and polyethylene glycol (PEG) mol wt 4000 (Sigma) will be used for the study. These will be available from the biochemistry laboratory.

Composition of the perfusion solution.

The control solution will contain sodium 90, potassium 15, chloride 105 and glucose 90, all in mmoles per litre. The test solution will contain sodium 90, potassium 15, chloride 75, citrate 30 and glucose 90, all in mmoles per litre.

Polythylene glycol (PEG) mol wt. 4000 (Sigma) 2 gram per litre will be added to both the control and study solution as a non-absorbable marker.

Perfusion technique.

General.

Male adult rats will be used as subjects. Rats will be kept in specially designed cages during fasting to prevent coprophagia. After fasting (water day-1 and 5% glucose day-2 allowed ad lib) for a period of 48 hours the rat will be anaesthetised by intraperitoneal injection of Nembutal, 40 mg /kg. On laporatomy a canula (proximal canula) will be introduced into the jejunum about 2 to 4 cm distal to the duodeno-jejunal flexure and tied securely. Similarly the distal canula will be introduced about 2 to 4 cm proximal to the ileo-caecal junction. The canulated segment will be washed gently using the perfusion solution. Perfusion experiment will be started by infusing perfusion solution through the proximal canula at the rate of 0.4 ml/min at a temp. of about 37°C. The rate of infusion will be maintained by using an peristaltic pump. After allowing 45 minutes to achieve a steady state the perfusate will be collected from the distal canula over ice. Details of the perfusion technique have been described elsewhere (8).

Study sequence.

(a) For standardisation and to obtain baseline values

Perfusion technique will be similar as mentioned above (general). Here the control solution will be used. After 45 minutes of equillibration perfusate will be collected for another 45 minutes.

(b) for testing the effects of citrate

Perfusion technique will be similar as mentioned above (general). Here the test solution containing the citrate will be used. After 45 minutes of equillibration perfusate will be collected for another 45 minutes.

Calculation of sample size.

for 1 test group and 1 control group

$$n = \frac{(Z\alpha/2\sigma)^2}{d}$$

Where d is the difference between the test mean and the control mean which will be detected as significant with probability $1-\alpha$. σ is the standard deviation of the test sample. From previous experience (4) σ could be approximately equal to 144. We assumed d could be as large as 75.

Taking α = 0.05

$Z\alpha/2$ is the standard normal deviate for two tailed test = 1.96

$$\therefore \quad n = \frac{(1.96 \times 144)^2}{75} = 15$$

So 15 successful experiments in each group will be conducted.

Calculations

Net transport of water and electrolytes can be calculated from the change in PEG concentration and the electrolyte concentration by the following formula (9).

The subscript I and F refer to the initial and final concentration. IR refer to the infusion rate (in ml/min). In these studies absorption is the measured disappearance of a substance from the intestinal lumen during the time interval of perfusion. Entry is the measured appearance (secretion) of a substance during a time interval in luminal fluid. Calculations are performed as follows:

1. PEG ratio (PEGR) = (PEG I) / (PEG F)
2. H_2O absorption % = 100 (1 - PEGR)
3. H_2O absorption ml/45 min = $\frac{H2O\%\ (IR)\ (45)}{100}$
4. Na^+, K^+ or Cl^- absorption μmol/45 min

$$\left[Na^+_I - (Na^+_F \times PEGR)\right] (45) \times IR$$

Statistical calculations.

If the samples reasonably behave like a normal distribution then the statistical significance will be measured by the Student's t test. Otherwise Wilcoxon's rank sum test will be applied. The only assumption needed for this test is that the two samples have come from a common population which we would like to reject.

Analysis:

Both the perfusion solution and the perfusate will be analysed. PEG will be measured by turbidimetric method. Na and K will be measured by using flame photometer and chloride by a chloridometer. Osmolality will be measured by freezing point depression using an Osmette automatic osmometer (Precision System,Inc) and glucose will be measured by glucose oxidase method.

Facilities required.

The existing facilities at the animal lab and the biochemistry lab will be utilised.

REFERENCES:

1. Fordtran JS, Rector FC, Carter NW. The mechanism of sodium absorption in the human small intestine. J Clin Invest., 1968, 47:884-900.

2. Turnberg LA, Fordtran JS, Carter NW and Tector FC Jr. Mechanism of bicarbonate absorption and its relationship to sodium transport in human jejunum. J Clin Invest., 1970; 49 : 548-56.

3. Turnberg LA, Bieberdart FA, Morawski SG and Fordtran JS. Interrelationship of chloride bicarbonate, sodium and hydrogen transport in the human ileum. J Clin Invest., 1970; 49:557-67.

4. Patra FC, Mahabanabis D and Jalan KN. Bicarbonate enhances sodium absorption from oral electrolyte solution in the presence of glucose or glycine: an in vivo perfusion marker study over the whole length of rat small intestine (unpublished).

5. Islam MR, Samadi AR, Ahmed SM, Bardhan PK and Ali A. Oral rehydration therapy: efficacy of sodium citrate equals to sodium bicarbonate for correction of acidosis in diarrhoea. Gut, 1984; 25: 900-904.

6. Mahalanabis D. Personal communication.

7. Newsome PM, Borgess MN and Homan GD. Stimulation of ileal absorption by sodium citrate. Scand J Gastroenterol., 1983; 18 (suppl. 87), 119-121.

8. Patra FC, Mahalanabis D and Jalan KN. Stimulation of sodium and water absorption by sucrose in the rat small intestine. Acta Paediatr Scand., 1982; 71 : 103-107.

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

SECTION III - BUDGET

Detailed Budget

1. Personnel service

Name	Position	% effort	Annual salary	Project requirement Taka	Dollar
Dr. F.C. Patra	Principal Investigator	40%	-	-	-
Dr. K.A. Al-Mahmud	Co-Investigator	10%	-	-	-
Dr. A.S.M. Hamidur Rahman	-do-	20%	-	-	-
Mr. M.A. Wahed	-do-	20%	-	-	-

2. Supplies and Materials:

Name	Unit cost	No. required	Project requirement Taka	Dollar
Rat adult	Tk. 20	100	2000.00	-
Laboratory tests				1500.00
Anoesthetic agents and Chemicals			2000.00	
			4000.00	1500.00

3. Equipment

Surgical instruments	1 set	1000.00	-
Glass ware		5000.00	-
Syringe, Needle etc.		-	100.00
		6000.00	100.00

4. Patient hospitalization	-	None
5. Outpatient care	-	None
6. Transport ICDDR,B	-	None
7. Travel and Transport of persons	-	None
8. Transport of materials	-	Nil
9. Communications	-	Nil
10. Printing and Reproduction	-	Tk. 5000.00
11. Miscellaneous	-	Tk.10000.00