62 Pilot Study

tachment 1. Date 29/7/82

ETHICAL REVIEW COMMITTEE, ICDDR,B.

rincipal Investigator R. McGlaughlin Trainee Investigator (if any) _____

pplication No. 82-033 (P) Supporting Agency (if Non-ICDDR,B) _____

itle of Study Evaluation of Antisecre- Project status:
tory Effects of Verapamil
(X) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

ircle 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)

. Does the study involve:
(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:
(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)

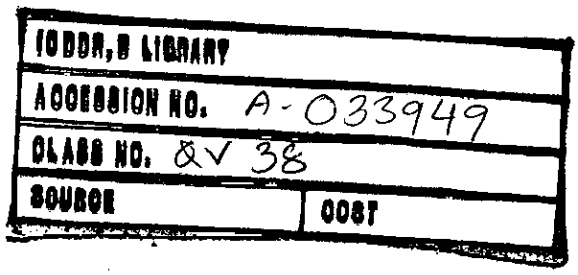
5. Will signed consent form be required:
(a) From subjects Yes (No)
(b) From parent or guardian (if subjects are minors) Yes (No)
6. Will precautions be taken to protect anonymity of subjects Yes (No)
7. Check documents being submitted herewith to Committee:
___ Umbrella proposal - Initially submit an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

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

R. McGlaughlin
Principal Investigator

Trainee



82.033(P)

Rec. 3.8.82

SECTION I - PILOT STUDY PROTOCOL

1. Title: Evaluation of Antisecretroy Effects of Verapamil

2. Principal Investigator: R. McGlaughlin

   Co-Investigator: S. Sanyal, T. Butler

3. Starting Date: August 1982

4. Completion Date: December 1982

5. Total Direct Cost: $940

6. Scientific Program Head: T. Butler

This protocol has been approved by the Pathogenesis-Therapy Working Group.

Signature of the Scientific Program Head: T Butler

Date: 29/7/82

7. Abstract Summary:

Twenty rabbits will undergo ileal loop injections with cholera toxin (CT) in varying doses. Five will serve as controls; three groups of 5 rabbits each will receive intravenous verapamil before, during, or after CT administration, and every six hours for the next twenty-four hours. The rabbits will then be sacrificed and the anti-secretory effects of verapamil determined.

8. Reviewe:

a. Research Involving Human Subjects: _____

b. Research Review Committee: _____

c. Director: _____

d. B M R C: _____

e. Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

The aim of this study is to determine whether verapamil has an antisecretory effect.

At the ICDDR,B a large number of cholera patients are seen. Most can be treated with ORS + AB. However, a minority have such high purging rates that ORS and antibiotics alone are not adequate treatment, and intravenous hydration is required. This therapy is more expensive than ORS, and, there is limited access to it. Thus development of an effective antisecretory agent has therapeutic implications.[1]

A short discussion of cholera toxin's effects and the regulators of secretion is necessary background. The massive watery diarrhea of cholera is due to the elaboration of cholera toxin. The B subunit of this molecule binds enterocyte GM1 ganglioside and the A subunit enters the cell. There it activates adenylate cyclase and causes a rise in intracellular CAMP. This activates specific protein kinases which phosphorylate membrane proteins. These membrane changes result in active anion secretion, inhibition of coupled Cl and Na^+ absorption.[2] Secretion may also be due to an increase in cytosal free calcium arising from cyclic nucleotide dependent mobilization within the cell, or from increased gating of calcium across the basolateral membrane.[3] Thus calcium flux plays a role in the absorption and secretion of electrolytes, and increased intracellular free calcium has a secretory effect.[4]

Intracellular calcium is regulated via two main mechanisms; calmodulin and slow channel influx Calmodulin is a membrane-bound tetramer found in all eukaryotes so far tested; it is capable of binding four calcium ions at once. Rising intracellular calcium levels cause saturation of calmodulin and an alteration in its configuration leads to activation of phospholipase A. This enzyme cleaves membrane triglycerides to form arachidonic acid. This fatty acid is the substrate for prostaglandin synthesis. Elevated PG levels are thought to stimulate nucleotide cyclases, with the ensuing chain of events leading to secretion.[6]

This sequence depends on calcium flux into the cell through the slow channel. It follows that inhibitors of this flux might reduce the secretory effect of cholera toxin. The calcium channel blockers Nifedipine and diltiazem have been demonstrated to diminish secretory responses to E.coli heat stable enterotoxin.[7]

Verapamil, a calcium channel antagonist, has been used in Europe for about 8 yrs, and has recently been released in the U.S. It is a safe, nontoxic drug used mainly to control supraventricular tachycardia, but also for hypertension. It is thought to work by binding cellular membranes near the calcium channels, altering the channel configuration and blocking uptake of calcium.[8] No reports of its effect on secretion are available, but it is logical to assume it may have an antisecretory effect.

B. SPECIFIC AIMS

To inject rabbit ileal loops with CT and assess the effect of IV verapamil on the resultant secretion.

C. METHODS

Cholera toxin will be prepared as previously described.[9] Briefly, a subculture of Vibrio cholerae 569B will be grown on trypticase soy agar. Five or six colonies will then be inoculated into brain heart infusion broth in a 50 ml conical flask containing 10 ml of medium. This flask will be placed in a shaker bath at 37°C for 16 hours. The solution will be centrifuged at 22,000 g at 4°C for 30 min. The supernatant will then be decanted and filtered through a 0.45 u millipore filter. A portion of the filtrate will be used as a positive control. The remainder will be saturated with ammonium sulfate (crystalized) up to 95% at 4°C with cons constant stirring. This solution will be dialyzed against distilled water at 4°C with repeated changes of the dialysis fluid until the ammonium sulfate disappears.

The protein content of the material within the dialysis bag will be estimated. The minimal reacting dose (1 ml/cm of rabbit ileal loop) per gram of protein will be determined. Serial dilutions of this fluid will be made, with resultant protein concentrations of 1,2,4,8,16,32, and 64.

Four groups of five rabbits each will be studied. The first group will serve as a control.

The second group will receive a large dose of verapamil (0.3 mg/kg) intravenously at the beginning of the study and every 6 hours until the conclusion 24 hours later.

Should the high dose of verapamil be effective, lower doses will be given until the standard dose used in humans (0.3 mg/kg) is reached. The rabbits will be anesthetized with phenobarbital by ear vein. A midline incision will be made and the ileum isolated. Endarterial regions of the ileum will be dissected out and ligated. Sixteen sections will be ligated. Alternate sections of bowel will be injected with cholera toxin of sequential dilution. The ileum will be replaced and the abdomen sewn up. Verapamil will be given by ear vein then and every 6 hours for a total of 4 doses. The rabbits will be sacrificed after 24 hr by an overdose of phenobarbital and their abdomens re-opened. The ileum will be removed. The net secretion in ml/cm of ileum will be measured for each section.

The each group, means, standard deviations and statistical significance will be calculated in the usual manner.

D. SIGNIFICANCE AND RATIONALE

A hypothesized mechanism of cellular secretory regulation will be tested, with possible application to cholera patients who are heavy purgers.

REFERENCES

1. Greenough WB III (1978) Principles and Prospects in the treatment of holera and dehydrating diarrheas. In Cholera and Related Diarrheas 43rd Nobel Symposium. Ed Ouchterlong O and Holmgren J, and S Karger. Basel. 1980 P211

2. Field, M: "Intracellular mediation of secretion in the small Intestine". In Mechanisms of Intestinal Secretion. Binder, HJ, ed. AR Liss,Inc. New York, 1979

3. Field, M., Fordtran JS and Schultz (eds)- SECRETORY DIARRHEA American Physiological Society, Bethesda, MD Ch.15

4. Thomas DD, Knoop F. "The Effect of Calcium and Prostaglandin Inhibitors on the Intestinal Fluid Response to Head Stable Enterotoxin of E.coli." Journal of Infectious Diseases, Vol145, No.2, Feb, 1982

5. Cheung WY. "Calmodulin plays a pivotal role in cellular Regulation" SCIENCE 207: 19-27, 1980

6. Brasitus TA, Fired M,, Kimberg DV. Intestinal mucosal cyclic GMP: regulation and relation to ion transport Am.J. Physiol: 231:275-282, 1974

7. Livesly B et al. Mode of action of verapamil in Man. British Journal of Med 2:50, 1 April 1972

8. Leitch GJ, Twert ME, and Burrows W. Experimental Cholerain rabbit ligated ileal loops: Toxin-produced water and ion movement. J. of Infectious Disease. 116: 303-312, June 1966

Summary for Ethical Review Committee

This is a pilot animal investigation to study the effects of a new drug to prevent fluid production in the intestine following exposure to cholera toxin. Rabbits will be anesthetized before surgery and kept as pain-free as possible for a period of up to 24 hr before sacrifice.

Information from this study may lead to more effective therapies for human disease.

This project involves no interviews, physical, psychological, social, legal, or any other risks. Personnel of the ICDDR,B will not be exposed to infectious hazards or other risks.

SECTION III - BUDGET SUMMARY

1. Personnels:

Investigators	%Effort	Taka	Dollars
Dr. R. McGlaughlin	40%	-	-
Dr. S. Sanyal		-	-
Dr. T. Butler		-	-
Technician 2	25%	12,500	-
Animals : Rabbits 100 x 120		1,200	-
Reagents : Syrings, needles		2,000	
Culture broth		300	
Anesthetic		2,200	
Verapamil		600	
	Total	18,800	

US Dollar 940 (Conversion $1=Tk.20)

7. Furazolidone : /__/ 55

8. Metronidazole : /__/ 56

9. Nystatin (Mycostatin) : /__/ 57

10. Vitamin A : /__/ 58

11. Other (specify) : _____ _____ /__/ 59

Duration of stay : _____ Days _____ Hours /__/__/__/__/ 60 61 62 63

Outcome :

1=Cured/recovered
2=Illness continued/discharge on risk bond
3=Referred
4=Expired

/__/ 64