...ent 1.

Date July 19, 1988

ETHICAL REVIEW COMMITTEE, ICDDR,B.

...r Investigator Roger Glass

Trainee Investigator (if any) _____

...ation No. 80-028

Supporting Agency (if Non-ICDDR,B) _____

...f Study B Subunit Blocking of Gut Toxin in Family contact children pairs

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

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

...ct of Population:
- Ill subjects (Yes) No
- Non-ill subjects (Yes) No
- Minors or persons under guardianship (Yes) No

...es the study involve:
- Physical risks to the subjects Yes (No)
- Social Risks Yes (No)
- Psychological risks to subjects Yes (No)
- Discomfort to subjects (Yes) No
- Invasion of privacy (Yes) No
- Disclosure of information damaging to subject or others Yes (No)

...the study involve:
- Use of records, (hospital, medical, death, birth or other) Yes (No)
- Use of fetal tissue or abortus Yes (No)
- Use of organs or body fluids (Yes) No

...subjects clearly informed about:
- Nature and purposes of study (Yes) No
- Procedures to be followed including alternatives used (Yes) No
- Physical risks Yes No NA
- Sensitive questions Yes No NA
- Benefits to be derived (Yes) No
- Right to refuse to participate or to withdraw from study (Yes) No
- Confidential handling of data (Yes) No
- 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.

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

...rincipal Investigator

Trainee

Received
21.7.80.

SECTION I - RESEARCH PROTOCOL

1) Title: B Subunit blocking of cholera Toxin in family contacts of cholera patients.

2) Principal Investigators: Roger Glass, Jan Holmgren and W.B. Greenough

3) Starting Date: Sept, 1, 1980

4) Completing Date: Sept 1, 1981

5) Total Direct Cost:

6) Scientific Program Head:

This protocol has been approved by the Disease Transmission Working Group

Signature of Scientific Program Head: WBG 15/7/80

7) Abstract Summary: In animals, B subunit, the binding portion of cholera toxin, has been shown to give good protection from a subsequent challenge dose of active cholera toxin and thereby prevent disease. Furthermore, human studies with orally administered B subunit have shown no toxicity. Since family members of cholera patients have a 24% chance of contracting cholera vibrios in the 10 days after the first member of a family is hospitalized, this study proposes to examine the blocking effect of B subunit among this high risk population.

300 cholera patients from the Matlab UTS area will be entered into the study and randomized into a treatment or placebo group. The families will be visited within 24 hours, enrolled in the study if they consent, and visited daily for 10 days. On day 1 & 2, they will receive treatment with either B subunit or a placebo. Each day, symptoms of diarrhea will be recorded and a rectal swab will be taken and cultured for V. cholera. Fingerstick bloods drawn on day 1 & 2 will help identify culture negative cases and to assess response to B subunit. Analysis will involve comparing incidence of V. cholera isolation, presence or absence and duration of symptoms between the treated and placebo groups.

8) Review:

(a) Ethical Review Committee: _____

(b) Research Review Committee: _____

(c) Director: _____

(d) BMRC: _____

(e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: To determine if orally administered B subunit reduces the incidence and duration of excretion of cholera vibrios and the severity and duration of illness in household contacts of index cases in Matlab.

2. Background: Cholera disease is dependent on the action of the enterotoxin of V. cholerae on the intestinal epithelium. The initial step in intoxication of the epithelium is the binding of the toxin to GM1 ganglioside receptors on the luminal surface of the epithelial cells.[1] Studies in animals have shown that it is possible to block these receptors by occupying them with the nontoxic binding part of the toxin, the B subunit protomer[2,3]. In the rabbit pretreatment of the gut with 0.5 µg B subunit per cm completely protects the animal from experimental cholera after challenge with high doses of active cholera toxin[2,3]. To obtain this blocking it is necessary to give the B subunit prior to or simultaneously with the toxin since the toxin (as well as the B subunit) binds irreversibly to the membrane[1].

   It is attractive to think that in humans as well, orally administered B subunit might effectively block the intestinal receptors for the toxin and thus be used as a prophylactic agent in high-risk groups. Chemical determination of the GM1 ganglioside content in human small

intestinal mucosa has shown considerably lower values than in other species, 0.1 nmole/g wet tissue[4]. This value, supported by determination of the number of toxin receptors on human intestinal cells[4] ($\sim 10^4$ toxin molecules can bind per cell), indicates that even if all of the GM1 was exposed luminally as little as about 100 µg of B subunit could block all available receptors.

With this in mind, and because B subunit is also a promising immunogen against cholera, a method has been devised by which large quantities of pure B subunit, totally devoid of toxicity, can be prepared. The method is based on isolation of cholera toxin and partial elimination of "toxic" A subunit by affinity chromatography on a GM1 ganglioside affinity column[5] followed by complete purification of the B subunit by gel filtration in acid buffer[6]. After oral administration or systemic injection the purified B subunit is completely nontoxic in animals.

Furthermore, studies in human volunteers in Sweden and Bangladesh have shown that single or repeated oral administration of B subunit does not give rise to any detectable side-effects, and i.m. injection only to mild local reactions at the injection sites which are less than those noted after vaccination with whole-cell cholera vaccine[7]. Therefore, B subunit appears entirely

safe if take twice by mouth in a dose of 500 µg. In addition to blocking the available GM1 receptors in the gut, B subunit would later boost a local IgA antitoxin antibody response[8] and thus could have a dual beneficial effect on resistance against cholera.

The present protocol proposes to study the possible receptor-blocking protective effect of B subunit in family contacts of cholera index cases. These persons have a 24% risk of developing cholera within a few days, as compared to about 0.3% in the average population[9] Roughly one half of these patients have symptoms and 10% require hospitalization. Apart from being a group of persons in which the new scientific approach can be evaluated in a limited number of individuals, this high-risk population could benefit much if a safe, specific, non-antibiotic agent were available for prevention during the short period of highly increased risk. Antibiotic prophylaxis is not adviseable especially since tetracycline-resistant V. cholerae strains have appeared in the Matlab area. B subunit is both safe and specific, and might prevent disease while still allowing the infection to boost an antibacterial immune response and by itself boosing local antitoxin immunity.

3. B subunit - Reactogenecity:

The B subunit to be used has been studied previously in human volunteers in Sweden and Bangladesh. It has been extensively purified from contaminating A subunit as well as from other bacterial components, and the methods of preparation and testing have been described in ICDDR,B protocol 79-009 .

The reactogenecity of B subunit in this protocol was studied by Drs Sack & Svennerholm. Despite intense daily surveillance for side effects, none were observed in the 19 women who received a total of 26 oral doses. Although some mild local reaction were observed in women receiving injections, no toxicity was observed in Swedish volunteers receiving the same preparation. Orally administered B subunit is an immunogen inducing a significant rise in anti B subunit antibody.

4. Rationale: There is a great need for effective treatment, prophylaxis and immunization against cholera. This study will provide improved understanding of the role of cholera toxin in the pathogenesis of the disease and in the development of immune response. A blocking agent recently tested at ICDDRB , the GM1 gangliosi le, was shown to have some effect in reducing the volume of purging in patients who already had

cholera. This study will allow us to further our understanding of the possible role of a blocking agent - B subunit - in preventing disease and/or diminishing the severity of disease in a high risk group of patients. While antibiotics have been an important part of therapy before, problems with antibiotic resistance and changes in the normal gut flora caused by these drugs would make a more specific treatment desireable.

B. SPECIFIC AIMS

To determine whether B subunit administered orally to family contacts of cholera patients can block infection with V. cholerae or decrease the severity of illness.

C. METHODS OF PROCEDURE

1. Subjects.

The stools of all VTS patients presenting to the Matlab Treatment Center with watery diarrhea will be screened for V. cholerae using dark field microscopy with type-specific sera. The same specimens will be cultured for V. cholerae using standard procedures of the field laboratory. Patients found to be positive on Dark-Field

examination will be identified as index cases. Index cases with 4 or more family members at home will be randomized to an intervention or a control group and their families will be visited within 24 hours of admission.

After explaining the study and receiving their informed consent each family member will be cultured for cholera immediately and daily for ten days or until all are culture negative for 3 days. Members will be queried about symptoms of diarrheal illness and anyone who is ill will be offered treatment either at home or in the hospital depending upon the severity. At the first visit and again 12 days later, a finger stick blood (100 lambda) will be taken to 1) identify family contacts who seroconvert but whose cultures have remained negative and 2) to determine the immunogenecity of the blocking agent. Children under 1 year and pregnant women will be excluded from the study.

2. Immunization Procedure

Family members will receive B subunit or placebo on the first and second days of the study. On each occasion a measured volume of $NaHCO_3$ (7.5g/l) will be given

first followed by five minutes later by an equal volume of $NaHCO_3$ with B subunit or without (placebo). The dosage schedule will be:

DOSAGE SCHEDULE

AGE GROUP	PRIMER[1]	INTERVENTIONS		
	$NaHCO_3$	B subunit[2]		Placebo[3]
	Vol(cc)	Vol(cc)	ug	Vol(cc)
Adults 10 years	150	150	500	150
Children 5-10 years	100	100	330	100
Children 1-4 "	75	75	250	75

Excluded - children 0-1 and pregnant women

1. Primer given 5 minutes before interventions
2. B subunit 3.3 ug/subunit/cc mixed in 7.5mg/l $NaHCO_3$
3. Placebo solution same as primer -

Any side effects of the B subunit of placebo will be noted on the same form used in the volunteer study.

3. Laboratory Support

Bacteriology:

Rectal swabs (R/S) will be placed in Carey Blair media for transport and plated in the laboratory on TCBS agar. Patients complaining of diarrhea will have a stool specimen collected for microscopic examination and culture of all pathogens and will receive appropriate treatment.

Serology:

Fingerstick bloods (100 lambda) will be diluted in 19 parts normal saline and frozen. They will be assayed later for a rise in vibriocidal antibody and a selection will be tested by elisa for anti-B subunit titer.

4. Data Analysis:

Four endpoints will be observed among the family contacts - 1) frequency of V. cholerae isolation, 2) duration of vibrio excretion, 3) presence or absence of symptoms among contacts who are culture positive or who show seroconverson of vibricidal antibody and 4) duration of symptoms.
Since blocking should occur within hours of administering

B subunit, its effect will be most evident on days 2 through 8. On day 1, blocking could only be expected to decrease symptoms in contacts infected at the same time as the index case.

Estimates of sample size are based on the family study of Spira. Each family had an average of 4.2 people excluding the index case, 24% of family contacts were positive in the 10 days after an index case was found and 45% of these had some symptoms of diarrhea. 39% of cases which occurred on day 1 and 4% which occurred after day 8 would be excluded from analysis. This study design would provide adequate statistical power to assess a 30-50% improvement in symptoms by the blocking agent and a 20% reduction in incidence of V. cholerae at the p=.05 level.

D. SIGNIFICANCE

This study would improve our understanding of the role of blocking agents in the treatment of toxin mediated diarrheas and of the role and kinetics of toxin in the pathogenesis of the disease. As antibiotic-related problems

of drug resistance and alteration of gut flora become better appreciated, a new, different, more specific, non-toxic treatment for toxin mediated diarrheas would be desirable.

E. FACILITIES REQUIRED:

1. No new office space is required

2. Personnel:

   4 Field Assistants - full time

   1 Field Supervisor - 30%

   1 Sr. Research Assistant - 10%

   1 Research Technician - 20%

   1 Keypunch Operator - 10%

   1 Programmer - 10%

   1 Statistical Assistant - 10%

3. No new lab space is required

4. Hospital Support:

   It is estimated that 200 patient-days of hospitalization will be required.

5. Logistical Support:

   Transport, Dacca - Matlab - Dacca, as outlined in budget will be needed.

6. Major items of equipment - No new item is required

7. Other - None

F. COLLABORATIVE ARRANGEMENTS:

Full collaboration with Dr. Jan Holmgren, Institute of Medical Microbiology, Department of Bacteriology, University of Goteborg, Goteborg, Sweden, has been agreed upon.

REFERENCES

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2. Holmgren, J.: Comparison of the tissue receptors for Vibrio cholerae and Escherichia coli enterotoxins by means of gangliosides and natural cholera toxoid. Infect. Immun. 8:851-859 (1973).

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5. Tayot, J.-L., Holmgren, J., Svennerholm, L., Tardy, M. and Lindblad, M.: Receptor-specific large scale purification of cholera toxin on silica beads derivatized with lyso-GM1 ganglioside. Eur. J. Biochem., in press (1980).

6. Holmgren, J., Svennerholm, A.-M., Lonnroth, I., Fall-Persson, M., Markman, B., and Lundback, H.: Development of improved cholera vaccine based on subunit toxoid. Nature, Lond. 269: 602-604 (1977).

7. a) Holmgren, J. et al.: Unpublished information described protocol 79-009.

   b) Sack, D.: Reactogenicity of cholera B subunit antigen. ICDDR,B memorandum, 21 November 1979.

8. Svennerholm, A.-M., Sack, D., Bardhan, P.K. and Holmgren, J. to be published.

9. Spira, W.M., Saeed Y.A., Khan, M.U.,Sattar, M.A. Microbiological Surveillance of intra-neighborhood El Tor cholera transmission in Rural Bangladesh. (in press, 1980).

ABSTRACT SUMMARY

1. The population to be studied includes family members of cholera patients from the VTS area. Children under 1 year of age and pregnant women would be excluded. These family contacts have a hundred-fold greater risk of getting cholera than the normal population.

2. The risks related to this study are minimal. B subunit has been tested in animals & volunteers and no adverse effects have been noted to the orally administered preparation.

3. Daily surveillance of these families will allow any patients developing cholera to be identified early and be treated in the field or in the hospital.

4. Index cases and family contacts will be identified only by a study number so confidentiality can be maintained. Data forms with the names attached will be locked in a file in the investigators office and names will be deleted at the end of the study.

5. Verbal informed consent will be obtained from the head of the household, and all adult members. A statement explaining the study (attached) will be read to each member and every effort will be made to insure that each understands the facts of informed consent.

6. A daily interview covering daily signs and symptoms of illness will take place.

7. Cholera is endemic in Matlab and in other areas of Bangladesh and new approaches to therapy and prevention are needed particularly if antibiotic resistance persists. B subunit offers possibilities for treatment both as a blocking agent as well as a possible vaccine candidates. Should B subunit be an effective blocking agent, the direction forwards non-antibiotic toxin specific modes of therapy for diarrheal diseases caused by toxins would be more clear.

8. This study requires collection of fingertip blood.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% or # of Days	Project Requirements Taka	Dollar
Dr. Glass	Scientist	20%	-	7,000
Dr. Holmgren	Scientist	-	-	-
Dr. Yunus	Physician-in-Charge Matlab	10%	7,575	-
Mr. I. Huq	Microbiologist	10%	9,500	-
Mr. Khan	Supervisor	30%	15,350	-
(To be named)	Field Asst.(4)	100%	94,808	-
Mr. Alim	Sr. Research Asst.	10%	4,930	-
Mr. Belayet	Research Technician	20%	4,300	-
(To be named)	Keypunch Operator	10%	1,600	-
"	Programer	10%	3,990	-
"	Statistical Asst.	10%	3,600	-
	Sub-Total		145,653	

2. SUPPLIES AND MATERIALS

Item	Unit Cost	
Rectal swabs for vibrio - 10,000	Tk. 11.00	110,000
Cholera antitoxin tests - 1,000	5.00	5,000
Vibricidal assays - 1,000	5.00	5,000
Stationery, office supplies, Computer paper and cards		2,500
	Sub-Total	122,500

3. EQUIPMENT

None

4. PATIENT HOSPITALIZATION

Number of patient days (50 patients x 4 days)	200 @ 130T/d	10,000
	Sub-Total	10,000

	Unit Cost	Project Requirements Taka	Dollar
5. OUTPATIENT CARE			
ORS packets - 150 patients x 2 packets each @ 1Tk/packet		300	
6. ICDDR,B TRANSPORT			
Dacca-Matlab - 20 round trips	400T/trip	8,000	
Hours water transport - 4 run hrs/day z 180 days	100T/hour	72,000	
Sub-Total		80,000	
7. TRAVEL AND TRANSPORTATION OF PERSONS			
International travel		-	3,500
Sub-Total			3,500
8. TRANSPORTATION OF THINGS			
Importation of supplies		-	200
Sub-Total			200
9. RENT, COMMUNICATIONS & UTILITIES			200
Sub-Total			200

	Project Requirements	
	Taka	Dollar
10. PRINTING AND REPRODUCTION		200
Sub-Total		200

11. OTHER CONTRACTUAL SERVICES

None

12. CONSTRUCTION, RENOVATION, ALTERATIONS

None

B . BUDGET SUMMARY

	Category	Taka	Dollars
1.	Personnel	145,653	7,000
2.	Supplies	122,500	-
3.	Equipment	-	-
4.	Hospitalization	10,000	-
5.	Outpatients	300	-
6.	ICDDR,B Transport	80,000	-
7.	Travel Persons	-	3500
8.	Transportation Things	-	200
9.	Rent/Communication	-	200
10.	Printing/Reproduction	-	200
11.	Contractual Services	-	-
12.	Construction	-	-
		358,153	11,100

Total (US$23,107)

Grand Total : US$34,207

CONSENT FORM

Yesterday, a member of your family became ill with cholera and was hospitalized at the cholera hospital in Matlab. In the next 10 days, others of you may become ill with cholera as well. Doctors of the Cholera Hospital are studying a new substance called B subunit which they feel may prevent or decrease the severity of disease caused by cholera. The material is a natural substance which has previously been tested in man and has caused no adverse reactions. We would like you to participate in this study. If you decide to do so, we will give you and members of your family either 2 doses of B subunit or 2 doses of a bicarbonate solution. Regardless of which you receive, we will visit your family every day for 10 days, inquire about any illness, and take a rectal swab which will help us determine if others have cholera. On the first and the last day of the study, we will collect a small sample of blood from your finger which will help us determine if you might have had cholera but not have suffered any symptoms and if you responded appropriately to the new material.

If you or anyone should become ill during this period, we will either provide treatment for you at home or bring you to the hospital.

During this study, and afterwards, your names and any information on illness that you provide will be held confidential. You will not be specifically named or identified in connection with this study. If you chose not to participate, you may still receive treatment at the cholera hospital should you become ill. If you agree to participate, you may withdraw from the study at anytime.

সম্মতি পত্র

গতকাল আপনার পরিবারের একজন সদস্য কলেরা রোগে আক্রান্ত হয়ে আমাদের কলেরা হাসপাতালে ভর্তি হয়েছেন। আগামী ১০ দিনের মধ্যে আপনারা সকলেই এই রোগে আক্রান্ত হতে পারেন। কলেরা হাসপাতালের ডাক্তারগন B-subunit নামে একটি ঔষধ দিয়ে গবেষনা করছেন যেটা কলেরা রোগ প্রতিরোধ বা উহার তীব্রতা কমাতে সাহায্য করতে পারে। এই ঔষধটি পূর্বে মানুষের উপর ব্যবহার করা হয়েছে এবং উহার দ্বারা কোন বিরূপ প্রতিক্রিয়া দেখা যায় নাই। আমরা আপনাকে এই গবেষনায় অংশ গ্রহন করতে অনুরোধ করছি। যদি আপনি সম্মত হন তাহা হইলে আমরা আপনাকে এবং আপনার পরিবারের সকলকে এক গ্লাস B-subunit বা bicarbonate মিকশ্চার দেওয়া হবে। সে কোন ঔষধই দেওয়া হউক না কেন আমরা আপনার বাড়ীতে পরপর ১০ দিন আসব, আপনাদের কোন অসুবিধা হলে খোঁজ নিব এবং অন্য কারো কলেরা রোগ হয়েছে কি-না দেখার জন্য রাত্রি দিয়ে অলন্দার হতে পায়খানা নেব। এই ঔষধের সঠিক কার্যকারিতা দেখার জন্য আমরা প্রথম ৩ দিন আপনার থেকে পায়খানা পরিমানে রক্ত নেব।

এই সময়ের মধ্যে যদি আপনি বা পরিবারের কেহ রোগে আক্রান্ত হন, তবে আমরা আপনার বাড়ীতে বা হাসপাতালে চিকিৎসার ব্যবস্থা করবো। গবেষনা চলাকালীন বা পরবর্তী সময়ে আপনার নাম এবং রোগের যাবতীয় তথ্য গোপন রাখা হবে। গবেষনার রিপোর্টে আপনার নাম প্রকাশ করা হবেনা। আপনি যে কোন সময় গবেষনা থেকে আপনার নাম প্রত্যাহার করতে পারবেন। ইহাতে হাসপাতালে চিকিৎসার আপনার কোন ব্যাঘাত হবেনা।

যদি আপনি গবেষনায় সহযোগীতা করতে চান তবে নিম্নে স্বাক্ষর করুন।

স্বাক্ষর