Attachment 1. (10) d

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Date November 7, 1982

82

ETHICAL REVIEW COMMITTEE, ICDDR,B: [illegible]

Principal Investigator Rose Glass, M.H. Hossain

Trainee Investigator (if any) M M Hossain

Application No. 82-055

Supporting Agency (if Non-ICDDR,B) SAREC

Title of Study GM1 Ganglioside Binding of cholera toxin

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

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

1. Source of Population:
   (a) Ill subjects (Yes) No
   (b) Non-ill subjects (Yes) No
   (c) Minors or persons under guardianship (Yes) No
2. 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)
3. 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
4. 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 NA
   (d) Sensitive questions Yes No NA
   (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.

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

Principal Investigator

Trainee

82-055
24/11/82

SECTION I - RESEARCH PROTOCOL

1. **Title** : Cellulose GM1 binding of cholera toxin in family contacts of cholera patients

2. **Principal Investigators** : M.M. Hossain, Roger Glass

   Co-investigators : J. Holmgren
   W.B. Greenough
   B.J. Stoll
   M.U. Khan
   M.I. Huq

3. **Starting Date** : December 1, 1982

4. **Completion Date** : December 31, 1983

5. **Total Direct Cost** :

6. **Scientific Program Head** :

This protocol has been approved by the Disease Transmission Working Group

Signature of Scientific Program Head : [signature]

Date : 22/11/82

7. **Abstract Summary :**

GM1 ganglioside when given to patients with severe cholera has been shown to specifically bind free cholera toxin in the intestinal lumen and reduce diarrhoea fluid loss. Since family contacts of cholera patients have a 20% chance of infection with V. cholerae 0:1 in the 6 days after a family member is hospitalized, we propose to examine the toxin-binding effect of GM1 ganglioside among this high risk population. Two hundred cholera patients from the Matlab VTS area will be entered into the study. Their families will be visited in 24 hours of making a culture confirmed diagnosis, enrolled in the study, and if they consent, be visited daily for 6 days. On the first day, each member will be randomized to a treatment

or placebo group to receive 3 tablets per day for 4 days. On day 1 and 21, a fingerstick blood will be drawn for antitoxin assay. On day 1 through 6, contacts will be cultured for V. cholerae 0:1 and queried about symptoms of diarrhoea. Selected stool specimens from contacts having cholera will be assayed for stool toxin. Analysis will involve comparing contacts receiving GM1 ganglioside or placebo for their rates of diarrhoea once infected and levels of stool toxin in those contacts infected.

This study will improve our understanding of the role of toxin-binding agents in the prophylaxis of toxin mediated diarrhoeas and of the role and kenetics of toxin in the pathogenesis of disease. As antibiotic related problems of drug resistance and alteration of gut flora become better appreciated, more specific non-toxic treatments for toxin-mediated diarrhoeas would be desirable.

8. Reviews :

a. Ethical Review Committee : _____

b. Research Review Committee : _____

c. Director : _____

d. BMRC : _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. **Objective :** To determine if orally administered cellulose - GM1 ganglioside can prevent cholera or decrease its severity in household contacts of index cases.

2. **Background :**

Previous studies at ICDDR,B have shown that GM1 ganglioside attached to medical charcoal, when given to patients with severe cholera specifically bound all the free cholera toxin in the intestinal lumen, significantly reduced the diarrhoeal fluid loss in these patients during the early stage of the disease and caused no adverse side effects. (Stoll, 1980). This is probably the first instance in which a specific receptor has been used to interfere with an infectious disease. However, it should be emphasized that the observed effect of the GM1-charcoal was too transient to be practically useful in the treatment of cholera. As GM1 can not deactivate toxin that has already bound to the intestine, it would be expected to have a preventive rather than a curative effect. The results with charcoal-GM1 suggest that a suitable preparation of GM1 ganglioside given orally might be useful for prophylaxis in high risk groups such as family contacts of cholera patients.

In the previously used GM1-charcoal preparation, the ganglioside was non-covalently adsorbed to the charcoal through the strong hydrophobic interaction of the ceramide (lipid) moiety of GM1 with the matrix. While this preparation was highly useful to prove that matrix-bound

receptor ganglioside could efficiently bind cholera toxin in the human intestinal lumen under "natural conditions" it had certain practical limitations, too :

a. much of the GM1 ganglioside had attached to pockets in the charcoal that were inaccessible for cholera toxin resulting in less than optimal binding capacity;

b. charcoal is known to unspecifically bind various substances including tetracycline which could interfere with the efficiency of tetracycline treatment of cholera;

c. the GM1-charcoal preparation was not in a tablet form and hence caused some momentary discoloration of the teeth.

A new preparation of matrix-coupled GM1 ganglioside has therefore been prepared, which has none of these limitations. The GM1 ganglioside has been covalently epoxy-coupled to very fine cellulose particles, 1 micromol GM1/g of cellulose, and after extensive washing of the coupled preparation tablets 0.75 g and containing 50% cellulose-GM1, and 50% of sucrose, vanilla, starch and magnesium stearate have been prepared. These tablets have been shown to rapidly dissolve at neutral or alkaline pH, to rapidly dissolve in human small intestinal fluid, to be entirely stable in human intestinal fluid for incubation periods of at least 24 h. While underivitized cellulose does not bind any detectable amounts of cholera toxin, the cellulose-GM1 preparation binds about 10 mg of cholera toxin per gram when tested either in intestinal fluid - mimicking artificial solutions (containing lecithine and thaurocolate) or in samples of aspirated small intestinal fluid of Swedish volunteers. This binding capacity for cholera

toxin is only slightly less than that of purified, free GM1 ganglioside, and per quantity of attached GM1 ganglioside, the cellulose-GM1 preparation is about five times more potent. At the same time it is also a more specific cholera toxin binder than the previously used charcoal-GM1 preparation. In vivo studies in rabbit intestinal loops have shown that the biological cholera toxin neutralizing activity of cellulose-GM1 equals its cholera toxin binding capacity as calculated from the in vitro binding tests. Furthermore, the studies have shown that cellulose-GM1 efficiently protects animals against challenge with live Vibrio cholerae as tested both with classical and El tor strains. Finally, it can be mentioned that in similar experiments cellulose-GM1 has been shown to efficiently neutralize heat-labile enterotoxin from Escherichia coli as well as prevent fluid loss from challenge with LT-producing E. coli strains. The GM1-cellulose tablets have been prepared by the Institute Merieux, Lyon, France and the various stability, binding and protection tests performed in Goteborg, Sweden. Swedish volunteers have also taken the recommended amounts of GM1-cellulose tablets without any side-effects at all.

It is now proposed to study the possible cholera prophylactic effect of cellulose-GM1 tablets in family contacts of cholera index cases in Matlab, Bangladesh. In a double-blind, randomized way family contacts would receive 1 tablet three times per day for a 4 day period and cholera infection and symptoms be monitored daily during this period in the same way as has been done for evaluating possible blocking of receptors by means of oral B subunit.

3. Rationale : The recent occurrence of multiply drug resistant V. cholerae 0:1 in Bangladesh and general problems inherent in antibiotic therapy such as changes caused in the normal gut flora suggest that in future prophylactic therapy for some infections might be more effectively directed at the mechanisms related to pathogenicity. GM1 is one of the first specific toxin-binders identified which has already been shown in humans to be effective in absorbing toxin and decreasing the severity of disease. This study will look specifically at toxin-binding and prevention of cholera in the 20% of family contacts who become infected with V. cholerae (half of whom go on to develop diarrhoea) in the 6 day period after an index case is identified. This study will be important both to clarify the mechanisms of toxin-binding and to investigate the feasibility of toxin-binding therapy for prevention of disease.

B. SPECIFIC AIMS

To determine whether Cellulose-GM1 ganglioside administered to family contacts of cholera patients can block cholera 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 diarrhoea 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. Family members of index cases (with 4 or more family members at home) will be randomized to an intervention or a control group and visited within 24 hours of admission. Children under 1 year and pregnant women will be excluded from the study.

After explaining the study and receiving their informed consent each family member will be cultured for cholera immediately and daily for six days. Members will be queried daily about symptoms of diarrhoeal illness and anyone who is ill will be offered treatment either at home or in the hospital depending upon the severity. They will also be queried about their compliance for taking the 3 tablets in 24 hours, un used tablets will be collected and any possible adverse side effects will be reported. At the first visit and again 21 days later, a finger stick blood (100 lambda) will be taken to (1) identify family contacts who show arise in antitoxin titre but whose cultures have remained negative and (2) to determine whether by binding toxin in the gut, an individuals' antitoxin response is altered. Stool specimens from selected patients who develop diarrhoea will be collected if cholera present and the amount of unbound toxin will be measured in selected cases.

2. Treatment Procedure

Adult family members will receive 1 tablet of Cellulose-GM1 ganglioside or placebo three times per day on the first four days of the study. Children 1-4 will receive ½ tablets crushed for easy administration. Any side effects of the Cellulose-GM1 ganglioside will be monitored by field workers using a separate questionnaire. Patients having any severe side effects will be referred to physicians at Matlab Hospital or the study physicians.

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 diarrhoea will have a stool specimen collected for assay of cholera toxin and culture of other pathogens if V. cholerae is not present. All will receive appropriate treatment. Toxin will be assayed quantitatively for the presence of toxin by Dr.Holmgren's laboratory.

Serology :

Fingerstick bloods (100 lambda) will be diluted in 9 parts normal saline and frozen. They will be assayed later for a rise in antitoxin antibody using the standard Elisa technique.

4. Data Analysis

Four endpoints will be observed among family contacts- (1) frequency of V. cholerae isolation, (2) the duration and severity of symptoms, (3) toxin levels in stools of patients with cholera vs non-cholera diarrhoea by intervention group(GM1 vs placebo). (4)antitoxin responses in patients with severe vs mild disease. Data analysis will proceed with the same computer programs used in the B subunit study. In this study, severity of diarrhoea is assessed in the field using both the response of the contact (or their parent) to the question "how severe is your diarrhoea", quantitation of stool frequency(number of motions/24 hours) stool consistency(formed, loose, liquid-watery, bloody), and duration of episode. These are checked for internal consistency and a weighted severity code is formed by the combination of these observations. This method has been tested and used in the B subunit studies.

5. Sample Size

In the first 4 days of followup, we expect 1 new infection in every 2 families. Half of these patients would be expected to have symptoms. If GM1 ganglioside reduces cholera symptoms by 50%, a sample size of 200 families should be adequate to prove efficacy at the $P < .01$ level.

	GM1	Placebo	
Families	100	100	10% infected between day 2-5
Families contacts	560	560	
Infected individuals Day 2-5	56	56	
Expected no. with diarrhoea	14	28	50% of those infected develop diarrhoea GM_1 decreases diarrhoea by 50%
Expected no. without diarrhoea	41	28	
95% Confidence interval	(14-42)	(20-36)	$X^2 = 7.11$, $P < .01$
99% Confidence interval	(11-45)	(10-30)	

D. SIGNIFICANCE

This study would improve our understanding of the role of toxin-binding agents in the treatment of toxin-mediated diarrhoeas and of the role and kinetics of toxin in the pathogenesis of disease. As antibiotic-related problems of drug resistance and alteration of gut flora became better appreciated, a new, different more specific non-toxic treatment for toxin-mediated diarrhoeas would be desirable.

E. FACILITIES REQUIRED

No new facilities will be required.

COLLABORATIVE ARRANGEMENTS

Full collaboration with Dr. Jon Holmgren, Inst. of Medical Microbiology, Dept of Bacteriology, University of Goteborg, Goteborg Sweden has been agreed upon.

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. Cellulose-GM1 has been tested volunteers and no adverse effects have been noted to the orally administered preparation. Charcoal-GM1 has been used effectively for treating cholera patients in Dacca.

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. Cellulose-GM1 offers possibilities for treatment as a binding agent. Should cellulose GM1 be an effective binding agent, the direction towards non-antibiotic modes of therapy for diarrhoeal diseases caused by toxins would be more clear.

8. This study requires collection of fingertip blood.

SECTION III

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% or # of days	Project Requirements TAKA	DOLLARS
Dr. Hossain	Asstt.Scientist	75%	50,000	-
Dr. Glass	Scientist	10%		5,000
Dr. Greenough	Scientist }			
Dr. Holmgren	Scientist } Consultants			
Dr. B. Stoll	Scientist }			
Dr. M.I. Huq	Scientist	5%		2,500
Dr. M.U. Khan	Scientist	5%		2,500
Dr. M.R. Khan	Supervisor (Matlab)	(75%x58,000x9m)	43,000	
	2 Sr.H.Asstt.	(100%x49,000x9m)	98,000	
	3 Health Asstt.	(3x100%x25,000) 9 months	75,000	
Mr. Belayet	Res.Technician	(30%x25,800)	7,740	
	Lab.Technician	(30%x20,000)	6,000	
	Key Punch Operator	10%	1,600	
	Operation Researcher/ Programmer/Research Associate		60,000	
	Sub total :		Tk. 331,340	$ 10,000

2. SUPPLIES AND MATERIALS

Item			
Rectal swabs for vibrio - 3000	11tk	33,000	
Cholera antitoxin tests - 500	5tk	2,500	
Stationary, office supplies, forms computer paper, xerox		10,000	
Sub total :		Tk. 45,500	-

3. EQUIPMENT

None

	TAKA	DOLLAR
4. PATIENT HOSPITALIZATION		
No additional costs		
5. OUTPATIENT CARE		
ORS packets - 400 - 11th/packet	400	
6. ICDDR,B TRANSPORT		
Dacca-Matlab - 20 round trips 400T/trip	8,000	
Speed boats - 3 boats - 3 run hours/ day/boat at 100T/hr x 240 days	210,000	
7. TRAVEL AND TRANSPORTATION OF PERSONS		
International travel (2 follow-up visits and per diem)		5,000
8. TRANSPORTATION OF THINGS		
Supply shipment		200
9. RENT, COMMUNICATION & UTILITIES		
None		
10. PRINTING AND REPRODUCTION		200
11. OTHER CONTRACTUAL SERVICES		
Daily wagers - 3 female helpers and 3 porters - as required (22-25th/day) x 180 days	23,760	
12. CONSTRUCTION, RENOVATION, ALTERATIONS		
None		

B. BUDGET SUMMARY

	Category	TAKA	DOLLAR
1.	Personnel	331,340	10,000
2.	Supplies	45,500	-
3.	Equipment	-	-
4.	Hospitalization	-	-
5.	Outpatients	400	-
6.	ICDDR,B Transport		
7.	Travel persons	-	5,000
8.	Transportation of things		200
9.	Rent/Communication	-	-
10.	Printing/Reproduction	-	-
11.	Contractual Services	23,760	-
12.	Construction	-	-
	Total Tk.	625,000	$ 15,200
	= $	28,000	
	Total $	43,200	

REFERENCES

Holmgren J. Actions of cholera toxin and the prevention and treatment of cholera. Nature 1981; 292:413-417.

Stoll BJ, Holmgren J, Bordhan PK, Huq I, Greenough WB, Fredman P, Svennerholm L. Binding of intraluminal toxin in cholera : Trial of GM1 Ganglioside charcoal. Lancet 1980; 2:888-891.

Holmgren J, Lönnroth I, Mansson J-E, Svennerholm L. Interaction of cholera toxin and membrane GM1 Ganglioside of small intestine. Proc Nat Acad Sci USA 1975; 72:2520-24.

Holmgren J, Lannroth J. Structive and function of enterotoxins and their receptors. in Cholera and Related Diarrhoeas. 43rd Nobel Symp. Stockholm 1978, pp 88-103(Karger, Basel 1980).

CONSENT FORM

Yesterday, a member of your family became ill with cholera and was hospitalized at the cholera hospital in Matlab. In the next 6 days, others of you may become ill with cholera as well. Doctors of the Cholera Hospital are studying a new substance called GM1 Ganglioside which they feel may prevent or decrease the severity of disease caused by cholera. The material has previously been effective in reducing the severity of diarrhoea in patients with severe cholera but its role for prevention has not been tested. We would like you to participate in this study. If you decide to do so, we will give you and members of your family 3 tablets daily for 4 days of either GM1 Ganglioside or a placebo with no active drug. Regardless of which you receive, we will visit your family every day for 6 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

যেহেতু আপনার পরিবারের একজন সদস্য কলেরা রোগে আক্রান্ত হয়ে মতলব কলেরা হাসপাতালে ভর্তি হয়েছেন, আগামী ১০ দিনের মধ্যে আপনারা সকলেই ঐ রোগে আক্রান্ত হতে পারেন। কলেরা হাসপাতালের ডাক্তারগন GM1 GANGLIOSIDE নামে একটি ঔষধ দিয়ে গবেষনা করছেন যা কলেরা রোগ প্রতিরোধ বা উহার তীব্রতা কমাতে সাহায্য করতে পারে। এই ঔষধটি পূর্বেও ডায়রিয়া বিশেষত: আশঙ্কাজনক কলেরা রোগের তীব্রতা কমানোর জন্য ব্যবহার করা হয়েছে। কিন্তু উহা কলেরা রোগ দমন করার কাজে ব্যবহার করা হয় নি। আমরা আপনাকে এই গবেষনায় অংশ গ্রহন করতে অনুরোধ করছি। যদি আপনি সম্মত হন তাহলে আমরা আপনাকে এবং আপনার পরিবারের সকলকে GM1 GANGLIOSIDE অথবা PLACEBO (যার মধ্যে কোন কার্যকরী ঔষধ নেই) দেয়া হবে এবং আপনারা প্রতিদিন ৩টা করে ট্যাবলেট ৪দিন পর্যন্ত খাবেন। যে কোন ঔষধই দেয়া হোক না কেন আমরা আপনার বাড়ীতে পরপর ৬দিন আসব, আপনাদের অসুখ হলে খোঁজ করব এবং অন্য কারও কলেরা রোগ হয়েছে কি না দেখার জন্য কাঠি দিয়ে মলদ্বার হতে পায়খানা নেব। এই ঔষধের সঠিক কার্যকারিতা দেখার জন্য আমরা প্রথম ও শেষ দিন আঙ্গুল থেকে সামান্য পরিমান রক্ত নেব।

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

যদি আপনি গবেষনায় সহযোগীতা করতে চান তবে নিম্নে স্বাক্ষর করুন।

স্বাক্ষর:

SCHEDULE FOR GM1 FAMILY STUDY

ACTIVITY OF FIELD STAFF	DAY 1	2	3	4	5	6	7	8	9	10	21
Ask informed consent											
Administer Drug	+	+	+	+							
Interview*	+	+	+	+	+	+					
Rectal swab	+	+	+	+	+	+					
Finger stick blood	+										+
Collect stool specimen	(Stool toxin assayed for selected cholera & non-cholera cases)										

*Interview includes questionnaire for symptoms, monitor compliance taking drug, questionnaire for adverse side effects, and decision regarding need for treatments with ORS in village or hospital