Library (2)

Attachment 1.

Date _____

ETHICAL REVIEW [illegible] ICDDR,B.

Principal Investigator Thomas Butler

Trainee Investigator (if any) L

Application No. 84-040P

Supporting Agency (if Non-ICDDR,B) _____

Title of Study, Trial of Orally-administered Bovine Colostral Anti-Cholera Toxin in Cholera Patients

Project status:
(X) 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).

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
   (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 NA
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.

Thomas Butler
Principal Investigator

16 SEP 1984

Trainee

84-040P
5/9/84

SECTION I - PILOT RESEARCH PROTOCOL

1.	Title:	Trial of Orally-administered Bovine Colostrum anti-Cholera Toxin in Cholera Patients
2.	Investigators:	T. Butler, R. McClead, G.H. Rabbani, and Paul Ney
3.	Starting Date:	October 1984
4.	Completion Date:	January 1985
5.	Total Incremental:	$4,320
6.	Scientific Program Head:	Ivan Ciznar

This protocol has been approved by the Host Defense Working Group.

Signature of Scientific Program Head: _____

Date: _____

7. Abstract Summary:

Forty-five patients with cholera will be randomly assigned to receive immune bovine colostrum anti-cholera toxin immunoglobulin (IBC) or non-immune bovine colostrum immunoglobulin (NBC) or heat inactivated bovine colostrum immunoglobulin (Control). Patients will be selected for heavy purging and will be hydrated intravenously during the 32-hour study period. Patients will be given 2g of colostrum every 2hr during 16hr or the treatment period. All will receive tetracycline 1g in a single dose. The effects of treatment on purging rates will be assessed by stool volume. The ability of IBC antibodies to survive passage through the human intestine will be tested by measuring anti-cholera toxin antibody in stool.

8. Reviews:

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

1. Objective

This study intends to evaluate a new therapeutic approach to the treatment of cholera by giving orally a bovine colostral antibody preparation with specific activity against cholera toxin. If the bovine antibodies are active in the human gut to neutralize cholera toxin and to reduce the clinical severity of cholera, this approach of oral passive immunization can be further evaluated as both therapeutic and preventive measures for cholera.

2. Background

Cholera patients have characteristically watery diarrhoea which leads to dehydration and metabolic acidosis. If untreated, this fluid loss rapidly leads to death. The disease is caused by an intestinal infection with Vibrio cholerae. These bacteria adhere to and colonize the small intestine and secrete an exotoxin — cholera toxin — that binds to receptors on the mucosal cells and stimulates intestinal adenylate cyclase activity. The resulting increase in cyclic AMP then causes diarrhoea and fluid loss by inhibiting uptake of sodium chloride by the villi as well as by stimulating active chloride secretion by crypt cells.[1]

There is strong evidence that GMI ganglioside is the membrane receptor to which cholera toxin must bind before it can induce intestinal secretion and diarrhea. This suggested the possibility of using isolated GMI ganglioside for the prevention of cholera. In ligated rabbit intestinal loops,

experimentally induced cholera can be completely prevented by the presence of free GM1 ganglioside. However, the interaction between toxin and tissue is essentially irreversible—GM1 ganglioside cannot deactivate toxin already bound to intestinal mucosal. One previous study to bind intralumenal toxin in cholera patients has been carried out at the ICDDR,B. Dr. Stoll and co-workers[2] administered GM1 ganglioside charcoal to patients. These patients showed a reduction in purging rates and showed no toxin remaining in the stools after treatment. However, this therapy had only a transient and partial effect and is not recommended for general use in cholera.

The production of antiboides by the gut in response to Vibrio cholerae cell antigens and cholera toxin occurs after a few days following exposure. It is likely, but not proven, that these antibodies by combining with toxin and other antigens necessary for bacterial attachment and multiplication act to limit the cholera infection and to terminate disease. This principle of gut immunization by natural infection has been applied to the development of new cholera vaccines which can stimulate protective immunity.

Passive immunization by administering preformed antibodies to patients or well persons has not yet been undertaken. There is however, indirect evidence that passive oral immunization has effects through breast feeding when the milk contains anti-cholera antibodies. Glass et al reported that infants being fed breast milk with high titers of antibody against cholera toxin and LPS were protected significantly against cholera diarrhea when compared to a group of infants receiving low titer breast milk.[3]

Colostrum from mothers that is produced by the breast in the few days after birth is a much richer source of immunoglobulin than is the milk which follows. In humans the colostrum contains predominantly IgA, whereas in cows the colostrum contains predominantly IgG. Cows can be immunized during pregnancy in order to obtain colostrum with antibodies of certain specificities. Cows immunized with cholera toxin by Dr. McClead in Columbus, Ohio developed a high titer colostrum and most of the anti-cholera toxin activity was localized in the IgG_1 fraction. Although IgG molecules are more susceptible to proteolysis in the gut than are IgA molecules, the bovine colostral antibodies were demonstrated to be reduced in activity by only 50% after exposure to trypsin and pepsin.[4] When rabbits were fed the bovine colostrum, functionally active anti-cholera toxin antibody was recovered in the caecum. In the infant rabbit prior feeding with the immune bovine colostrum resulted in protection of the ileum against fluid production caused by the injection of cholera toxin.[5]

This demonstrated ability of bovine colostral antibody to protect against cholera disease in an animal model suggests that human application may be fruitful.

B. SPECIFIC AIMS

1. To carry out a clinical trial of immune bovine colostrum immunoglobulin (IBC) with anti-cholera toxin activity for its effect to limit the natural disease process.

2. To evaluate the ability of bovine colostral antibodies to survive passage through the human gut.

C. METHODS OF PROCEDURE:

1. Preparation of immunoglobulin fraction from immune and nonimmune colostrum. In Columbus, Ohio Dr. McClead will salt out the globulin fraction with 35% ammonium sulfate. The precipitated globulins will be dialyzed against distilled water, the immunoglobulin content determined by radioimmunodiffusion, and the specific anti-enterotoxin antibody content determined by quantitative precipitation with purified cholera toxin. The dialyzed globulin preparation will be sterilized by ultrafiltration and this will be lyophilized. Each 200cc dose will contain 2 gram IgG. The control group will receive non-immune immunoglobulin that has been heated to 100°C for 10 min.

2. Patient selection. Adult males over 15 years old who come to the ICDDR,B with watery diarrhea and moderate-severe dehydration will be considered. After the stool has been tested for dark-field examination and found positive for Vibrios, patients will be asked to sign an informed consent form. They will be rehydrated with the intravenous Dhaka solution (Na 133 m mole/L, K 13 m mole/L. Cl 99 m mole/L, and acetate 48 m mole/L). The stool bucket will be placed for measurement of the first 8-hour period. If this 8-hour volume is between 1.6 and 4.0L (200 ml - 500 ml ml per hr), the patient will be selected.

3. Randomization. The study will be double blinded. Dr. McClead will provide vials with serial numbers 1-45 with 3 treatments in a random order.

4. Treatment. Patients in all groups will receive volumes of 200cc oral liquid treatment containing 2g bovine colostrum immunoglobulin starting 8 hr and given 8 times at 2 hr intervals. All patients will receive volumes of intravenous fluid to match the losses in the stool. Patients will not be fed during the 32-hr study period. The antibiotic tetracycline in a 1g single dose will be given to patients after the initial 8-hr observation period.

5. Measurement:

   a. Stool volume. Stool will be collected in buckets during 4 consecutive 8-hr periods and quantitated in a graduated cylinder after each 8-hr period.

   b. Stool cultures will be done for V. cholerae.

   c. Bovine IgG anti-cholera toxin antibody in stool. This antibody will be assayed in stool in the laboratory of Dr. McClead using radioimmunoassay and immunodiffusion.

D. SIGNIFICANCE AND RATIONALE

Cholera is serious health problem in Bangladesh and other developing countries. Our presently available treatments are rehydration and anti-biotics. We do not have optimal treatments directed at neutralizing cholera toxin or antagonizing the biochemical effects of cholera toxin. The use of high-titer immune bovine colostrum to treat cholera is a new promising approach. If successful in binding toxin and limiting the volume of cholera diarrhea, this approach could lead to passive oral immunization to prevent and control cholera in endemic areas of the world.

E. FACILITIES REQUIRED

Existing hospital facilities are adequate for patient care. The laboratories in Microbiology, Immunology, and Biochemistry are sufficient to carry out needed assays.

F. ANALYSIS OF DATA

Data sheets will be kept for every patient. Stool volumes will be recorded and the means and standard deviations compared by Students' T test. The frequencies of finding cholera toxin in stool will be compared among the 3 treatment groups by Chi-square and Fisher Exact Tests. The viable Vibrio counts will indicate whether the anti-cholera toxin also exerted an antibacterial effect.

REFERENCES

1. Holmgren, J. Actions of cholera toxin and the prevention and treatment of cholera. Nature 292:413, 1981

2. Stoll, B.J., Holmgren, J., Bardhan, P.K., Huq, I. Greenough, W.B., Fredman, P., Svennerholm, A.M. Binding of intraluminal toxin in cholera : trial of GM1 ganglioside charcoal. Lancet 2:888, 1980

3. Glass, R.I., Svennerholm, A.M., Stoll, B.J., khan, M.R.. Holmgren, J. Cholera in breast-fed infants: protective effect of milk antibodies. Abstract. International Congress of Infectious and Parasitic Diseases. Stockholm. June 1982

4. McClead, R., Gregory, S., Resistance of bovine anti-cholera toxin IgG, (anti-CT) to invitro and in vivo proteolysis. Abstract. Society for Pediatric Research, May 1982

5. McClead, R., Gregory, S. The effect of orally-fed, specific bovine colostral immunoglobulins (BCI) on the toxicity of cholera enterotoxin (CT) in the infant rabbit. Abstract. Ohio Academy of Science. April 1982

ABSTRACT SUMMARY

(for Ethical Review Committee)

1. This study proposes to test the efficacy of a new therapeutic material in cholera. It is antibody against cholera toxin made by cows' mammary glands. The patients to be studied are adult males with cholera.

2. The risks are minimal. The therapeutic material is bovine colostrum, which is a natural product very similar in composition to cow's milk.

3. Any patient with a history of cow's milk allergy sould be excluded. This will be effective in removing one potential risk.

4. Hospital records are kept anonymous by using a hospital number rather than the name.

5. Written informed consent will be obtained before admission into the study. No information will be withheld.

6. There will be no interview.

7. The potential benefits are those for the society and mankind at large through obtaining useful therapeutic information.

8. This activity requires only the use of stool from patients with cholera.

SECTION III - BUDGET

(A. DETAILED BUDGET)

1. Personnel services:

Name	Position	%Effort	Taka	Dollar
Dr. T. Butler	Prin. Investigator	20%(2mo)	-	3,400
Dr. R. McClead	Co-Investigator	-	-	-
Dr. G.H. Rabbani	Co-Investigator	20%(3mo)	4,000	-
Dr. Paul Ney	Guest Investigator	50%	(no salary/50days Guest House @$30 p/day)	1,500

2. Supplies and materials:

Clinical supplies - needles, gloves, syringes testtubes ..	-	500
Lab tests - cultures @ Tk.80 x 100 =	8,000	-

3. Eqipment - None

4. Hospitalization - 50 x 5d x 150	37,500	-
5. Outpatient care - None		
6. ICDDR,B transport - None		
7. Printing and reproduction -	-	500
8. Construction, renovation - None		
Total	49,500	5,900

Total in US$: 7,880

Incremental Cost : 4,320
(excluding personnel)

Grand Total: 12,200

(Conversion rate US$1 = Taka 25)

B. BUDGET SUMMARY

		US$
1.	Personnel Services	5,060
2.	Supplies & Materials	820
3.	Equipment	-
4.	Hospitaliztion	1,500
5.	Outpatient care	-
6.	ICDDR,B transport	-
7.	Printing & Reporduction	500
8.	Construction/Renovation	-
	Total :	7,880

CONSENT FORM - (Orally-administered Bovine Colostral Anti-Cholera Toxin)

You have been attacked with cholera which may require treatment with intravenous fluid. You are being asked to be admitted into the Research Ward and will stay in hospital till the diarrhea is over. In addition to the routine treatment, you may receive either of two new materials prepared from cow's milk that are being tested for their ability to treat cholera. These drugs will be given in two doses. If you are in the control group, none of these drugs will be given, but rehydration will be continued. 2 cc blood will be taken from an arm vein to determine electrolytes and complete blood counts. Stool, urine and vomitus will be collected, measured and examined through out the course of illness.

After listening all these information, even if you do not want to be admitted into the proposed study, you will be given proper and routine treatment in this hospital. You will always preserve the right of withdrawal from the study at any point in time.

All the treatment records will be kept confidential.

Signature : _____

Date : _____

Relationship to patient : _____

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, ঢাকা

(Trial of Orally-administered Bovine Colostral)

সম্মতি পত্র

(দুগ্ধজাত কলেরা প্রতিষেধক গবেষণা)

আপনি কলেরা রোগে আক্রান্ত হয়েছেন এবং তার জন্য রক্তের শিরায় স্যালাইন প্রয়োগ করে আপনার চিকিৎসা করা হবে। এই গবেষণায় অংশ গ্রহণ করলে আপনাকে ডাইরিয়া ভাল না হওয়া পর্যন্ত হাসপাতালে ভর্তি করা হবে এবং কলেরা রোগের জন্য উপযুক্ত চিকিৎসা আপনাকে দেওয়া হবে। গরুর দুগ্ধ হতে তৈরী দুই ধরণের ঔষধের মধ্যে যে কোন একটি আপনাকে দেওয়া হইবে। এবং তা সাংকেতি চিহ্ন দ্বারা ডাক্তার ও রোগীর কাছ থেকে গোপন রাখা হবে। দুই প্রকার চিকিৎসা দ্বারাই রোগী পূর্ণ সুস্থতা লাভ করে ও কোন প্রকার ঝুঁকির সম্ভাবনা নাই। আট মাত্রায় এই ঔষধগুলী সেবন করতে হবে। যদি আপনি কনটোল দলে নির্বাচিত হন তবে এই ঔষধ ছাড়াই স্বাভাবিক নিয়মে স্যালাইন দ্বারা আপনার চিকিৎসা করা হবে। হাতের শিরা হইতে সামান্য পরিমান রক্ত (২ সি সি) পরীক্ষার জন্য গ্রহণ করা হবে। অসুস্থ থাকাকালীন পায়খানা, পেশাব ও বমি সংগ্রহ করা হবে এবং পরীক্ষা করা হবে।

এই গবেষণায় অংশ না নিলেও আপনাকে এই হাসপাতালের সকল প্রকার চিকিৎসার সুযোগ দেয়া হবে। এবং ইচ্ছা করলে গবেষণা থেকে যে কোন সময় নাম প্রত্যাহার করতে পারবেন।

চিকিৎসা সংক্রান্ত সকল তথ্যাদি গোপন রাখা হবে।

এই সকল জানার পরে যদি গবেষণায় অংশ নিতে সম্মত থাকেন তবে নীচে দস্তখত করুন।

রোগীর নাম/দস্তখত----------

গবেষকের সই---------- হাসপাতালের নম্বর----------

তারিখ----------