Attachment 1.

Date 25 Jan 1980

REVIEW BOARD ON THE USE OF HUMAN SUBJECTS, ICDDR,B.

LJ

Principal Investigator Dr Yunus

Trainee Investigator (if any) _____

Application No. 80-008

Supporting Agency (if Non-ICDDR,B) _____

Title of Study Evaluation of Tetracycline therapy in patients with cholera due to tetracycline resistant cholera.

Project status:
( ) 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) None
   (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 None
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 Board:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   X Protocol (Required)
   X 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)
   X Informed consent form for subjects
   X Informed consent form for parent or guardian
   X 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 Board for review.

We agree to obtain approval of the Review Board on the Use of Human Subjects for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator

80-008
Rec'd 28/1

ABSTRACT SUMMARY

1. The subjects will be patients coming either to the Dacca or Matlab treatment center for treatment of cholera. We will include patients age 3-60 years. The children must be included since they are the main age group at risk.

2. The risks from this study are minimal. The treatment for cholera will be given (e.g. i.v. hydration) though patients who have sensitive V. cholerae and who are randomized to the placebo group will continue to purge for a longer time than if they were given tetracycline.

3. There are no special risks. All patients will be treated with I.V. hydration to avoid dehydration.

4. Patients will be identified by number on the data sheets.

5. Signed informed consent will be obtained.

6. N.A.

7. The subject will receive benefit in that he will be treated for his cholera. Society will benefit in that the knowledge of the treatment of antibiotic resistant enteric infections will be increased and this could have much broader implications that only the treatment of resistant V. cholerae infections. Since the risk consists only of increased duration of purging, it would seem the benefits out weigh the risk.

8. The study will utilize data collected during the study and recorded on the chart. Stool and rectal swab specimens will be collected but no other specimens will be collected for the study.

SECTION A - RESEARCH PROTOCOL

Title: Evaluation of Tetracycline therapy in patients with cholera due to tetracycline resistant cholera.

Principal Investigator: Dr. Yunus

Co-Investigators: Dr. Roy, Dr. Bardhan, Dr. Glass, Dr. Baqui,

Starting Date: Feb 1980

Completion Date: Dec 1980

Total Direct Cost:

Abstract Summary:

V. cholerae resistant to multiple antibiotics (MARV) are now causing about 20% of cases of cholera in Matlab. From a retrospective analysis, these patients with MARV's do not have as good a clinical response to tetracycline as patients infected with sensitive V. cholerae. We would like to document the clinical response of the disease caused by MARV in a controlled tetracycline-placebo study to 1) compare the clinical disease patterns in a placebo group 2) determine if there is any clinical effect of tetracycline in the MARV group and 3) compare the effectiveness of tetracycline in the resistant and sensitive cases. This study should be important in planning therapy for the present outbreak of resistant cholera but will also be important in testing the hypothesis that tetracycline may have some beneficial effect clinically though at sub-M.I.C. levels in the intestine.

Reviews:

(a) Research Involving Human Subjects: _____

(b) Research Committee: _____

(c) Director: _____

(d) BMRC: _____

(e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

INTRODUCTION

1. Objective: The objective of this protocol is to determine the effectiveness of tetracycline treatment on the clinical and bacteriological course of patients infected with multiple antibiotic resistant V. cholerae (MARV).

2. Background: V. cholerae, the bacteria which causes cholera, has until 1978 remained sensitive to most antibiotics including tetracycline the drug most commonly used for treatment and prophylaxis of the disease. Only a few reports of antibiotic resistant V. cholerae appeared in the literature, but as these cases did not appear frequently, or in clusters, it seemed the antibiotic resistant were not of any public health importance. Also in the laboratory it seemed that V. cholerae was not able to retain plasmids well;e.g. R factors passed into V. cholerae were lost quickly. The absence of a clinical problem with antibiotic resistance for more than 20 years and the reassurance from genetic bacteriologists led physicians to conclude that antibiotic resistant V. cholerae would not become a problem.

However in 1978, in Tanzania during a cholera epidemic, an El Tor Ogawa V. cholerae resistant to many antibiotics including tetracycline emerged and by March 1978, these MARV's represented

76% of the V. cholerae clinical isolates. The Tanzanian MARV isolates were resistant to Ampicillin, chloramphenicol, neomycin, sulfa, and tetracycline but were sensitive to trimethoprim and furoxone. This resistance was conferred by a stable R factor.

There were no further reports of MARV's until 1979 when, in Matlab, some patients were noted to be responding poorly to the usual dose of tetracycline. (250mg Q6H x 4 days). These patients continued to purge longer, and remained in the hospital longer than the usual cholera patients. Sensitivity tests done on the V. cholerae isolated from these patients showed multiple antibiotic resistance including resistance to tetracycline and trimethoprim - sulfamethoxazole. A preliminary case-control study confirmed the initial clinical impression of a poor clinical response. Bacteriologic sensitivity testing of a sample of V. cholerae isolates from Matlab showed that the first MARV appeared in August 1979, that they increased during the fall cholera season and that they represent about 20% of cases in Matlab. In Dacca, scattered cases of cholera due to MARV are now occurring though in both Matlab and Dacca, few cases of cholera are being seen during this cold season.

It would seem that since many V. cholerae are now resistant to tetracycline that an alternate drug should be selected. Furoxone (furazolodone) would be the alternate first choice drug

and further studies are being done to determine if the MARV is sensitive to foroxone. Chloramphenicol is another antibiotic which has known efficacy in sensitive cholera and since the Bangladesi MARV (unlike the Tanzanian MARV) is chloramphenicol sensitive this would be an alternative drug. There is some evidence however that even tetracycline might be clinically effective in bacterial intestinal diseases resistant to tetracycline. The mechanism of this effectiveness might be through inhibition of motility or adhesiveness even though the concentration of tetracycline is not sufficient to kill the bacteria. For instance if the MIC of the V. cholerae for tetracycline is 30 µg/ml and intestinal levels reach 20 ng/ml, the bacteria might be inhibited in one of its functions and thereby the patient will have a clinical response. If the spread between MIC and actual intestinal levels is too great (> 4 fold), then no effect would be expected.

Tetracycline is actually being recommended by some to treat tetracycline resistant shigellosis because of an "adequate clinical response" with the drug. In cholera, we have a disease with much more objective ways of determining clinical response, hence, it would seem important to determine if an antibiotic could be useful even though not present in concentrations greater than the MIC

3. Rationale. The clinical efficacy of tetracycline in the treatment of patients with cholera due to MARV will be determined. This is important to document the possible effect of an antibiotic, in sub MIC levels, on the clinical and bacteriologic course of the disease.

SPECIFIC AIMS

1. Compare the natural course of cholera in patients taking placebo infected with tetracycline resistant and sensitive Vibrio cholerae

2. Determine the clinical and bacteriologic effect of tetracycline therapy (500mg QID x 3 days) on cholera due to MARV

METHODS

1. Subjects: Approximately 150 patients from Matlab and Dacca will be admitted into the study who meet the following criteria.

   a. age between 3 and 50 years.
   b. clinical history and physical exam suggestive of cholera, with $\geq$ 7.5% dehydration.
   c. dark field positive examination of stool
   d. either sex
   e. purging rate > 1.25 ml/kg/hr during a 4 to 8 hour observation period.
   f. No antibiotics received during previous 1 week.
   g. No other medical complication other than dehydration due to diarrhea.

2. Clinical procedures

Patients who are possible candidates for this study will be rehydrated with standard cholera solution and rehydration will be maintained intravenously. They will be observed for purging during a 4 to 8 hour observation period and if they meet criteria for admission, will be placed in the study ward where special care will be made to maintain accurate intake and output measurements. In addition to the I.V. fluids, the patients will receive either tetracycline or a multivitamin every 6 hours for 3 days. The dose of tetracycline will be 500mg per dose (10 mg/kg) for children. Randomization to the treatment group will be done by drawing from an envelope.

A stool specimen will be collected from patients upon entrance into the study for quantitative culture, and this will be repeated every 12 hours for 48 hours and daily thereafter until negative twice during hospitalization. Antibiotic sensitivity testing will be done on Vibrios isolated on admission, at 48 hours and on the last Vibrio positive culture. One stool specimen will be collected the morning after admission for measurement of tetracycline concentration. Patients will be discharged when they have had no diarrhea for 24 hours or have passed a formed stool.

Followup visits will be made to patients who have a positive stool culture at discharge to collect rectal swab specimens daily until they have two Vibrio negative cultures.

These visits will continue daily until the rectal swab culture is negative twice. (These follow up visits are also part of the epidemiologic studies of MARV).

Patients will be allowed a normal diet and water but no oral therapy solutuon will be used. No blood samples will be collected unless indicated by the specific clinical conditions.

5. Analysis of data:

a. Clinical evaluation. The four groups of patients (placebo-sensitive (PS), pacebo-resistant (PR), tetracycline sensitive (TS), tetracycline-resistant (TR)) will be compared in the following way. The mean duration of diarrhea and mean duration of hospitalization mean i.v. fluids used, mean purging volume, mean purging rate for each of the first 3 days will be determined and the significance of difference will be tested with the T test. The numbers of patients purging greater than 0.25 ml/kg/hr at 48 hours, and the numbers of patients purging watery stool (i.e. no change in consistency) at 48 hours will be determined and differences will be tested using chi Square Analysis.

b. Bacteriologic evaluation:

The four groups will be evaluated for the rate of disappearance of V. cholerae. The mean number of days until the stool is ng for V. cholerae will be determined and significance tested with T test. The number of patients who have a positive stool specimen at 48 hours will be tested (Chi Square) and the different rate of stool clearance will be tested using the Mann Whitney test.

In addition we will search for evidence of reversion of resistant to sensitive or sensitive to resistant Vibrio's in an individual patient.

. SIGNIFICANCE

Now that antibiotic resistant vibrio are causing disease in Bangladesh, we should know if the present antibiotic treatment has any clinical effect. Furthermore, this protocol should give an answer to the more general question of the effectiveness of antibiotics in sub-MIC concentrations in enteric diseases which might apply to E. coli diarrhea and shigellosis as well.

FACILITIES REQUIRED

1. Office space - already provided
2. Lab space - already provided
3. Hospital resources - 150 patients x 6 days = 900 days
4. Animal resources - none
5. Logistic support - speed boat service for followup of cases in the VTS area.
6. Equipment - none
7. Other requirements - Bacteriology branch will be required to do multiple quantitative stool cultures for V. cholerae (estimate 6/patient or 900 cultures)

COLLABORATIVE ARRANGEMENT - None

REFERENCES

O'Grady, F., Lewis, M.S., Pearson, N.I. Global Surveillance of antibiotic sensitivity of Vibrio cholerae. Bull WHO 54:181-185, 1976.

Yokota, T, et al. Genetic behavior of R factors in Vibrio cholerae. J. Bacterial 109:440-442, 1972.

Davey, R.B., Pittard. J. Potential for in vivo acquisition of R plasmids by one strain of Vibrio cholerae Biotype El Tor. Antimicorbial Agents Chemotherapy. 8:111-116, 1975.

Prescott, L.M. et al. R factors in Calcutta strains of Vibrio cholerae and members of the Enterobacteriaceae Bull WHO 39:971-973, 1968.

Hedges, R.W., Jacob, A.E., A 98 megadalton R factor of compatability Group C in a Vibrio cholerae El Tor isolate from Southern USSR. J. Gen Microbiol 89:383-386, 1975.

Mhalu, F.S., Mmari, P.W., Ijumba, J. Rapid Emergency of El Tor vibrio cholerae resistant to antimicrobial agents during first six months of fourth cholera epidemic in Tanzania. Lancet i:345-347, 1979.

Pickering, L.K., Dupont, H.L., Otorte, J. Single dose tetracycline therapy for shigellosis in adults. JAMA 239: 853-854, 1978.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% of effort or no. days	Annual Salary	Project Requirement TAKA	Project Requirement DOLLARS
Dr. Yunus	Investigator	25%	5409	1352	
Dr. Roy	Investigator	40%	3119	1247	
Dr. Baqui	Co-Investigator	40%	2851	1140	
Dr. Bardhan	Co-Investigator	25%	2985	746	
Dr. Glass	Co-Investigator	10%	35,000		3500
Mr. Alim	Sr.Research Tech	25%	3387	846	
Bacteriology	Tech	25%	1536	384	
Field Assistants(2)	equivalent to	50%	1693	846	
				6561	3500

2. SUPPLIES AND MATERIALS

900 cultures, 450 sensitivity tests 45000

3. Equipment - None

4. Patient Hospitalization 900 patient days @150/day 135,000

5. Outpatient - None

6. CRL Transport

Speed boat - 200 hours @ Tk100/hr 20,000

Dacca-Matlab trips (12 trips) 3,600

7. Travel and Transportation of Persons

Presentation to International Meeting 2,500

8. Transport of things 500

9. Rent, Communication, Utilities - None

		TAKA	DOLLARS
10.	Printing		
	Mimeo & Xerox	2000	
	Publication		300
11.	Other - None		
12.	Construction - None		

BUDGET SUMMARY

CATEGORY		TAKA	DOLLARS
1.	Personnel	6561	3500
2.	Supplies	45000	
3.	Equipment	-	-
4.	Hospitalization	135,000	-
5.	Outpatients	-	-
6.	CRL Transport	23,600	-
7.	Travel Persons	-	2500
8.	Transport Things	-	500
9.	Rent/Communication	-	-
10.	Printing/Reproduction	2000	300
11.	Contractual Service	-	-
12.	Construction	-	-
		212,161	6800

(US$ 14,144)

TOTAL: $20,944

CONSENT FORM - TETRACYCLINE TREATMENT OF CHOLERA

The International Center for Diarrhoeal Disease Research is carrying out a study to determine the best treatment for cholera. Since some of the cholera germs which have recently been found seem to be resistant of the usual antibiotics. If you agree to participate in this study you can expect the following:

1. We will treat your cholera with appropriate intravenous fluids.

2. In addition to the fluids which are the main treatment for cholera we will give you either tetracycline antibiotic or vitamin medicine.

3. We will test your stool daily while you are in the hospital and will come to your house 1-3 times after you have recovered to get other stool specimens to test.

4. There is no risk to your health from joining the study. No special tests will be done. No blood will be drawn from the body.

5. Your medical records will be kept confidential.

6. You do not have to participate in the study. If you do enter the study, you are free to leave the study at any time. If you do not join the study, or you leave it later, you may still be treted for your diarrhea at the cholera Hospital. Your diesion regarding the study will not jeopardize your medical care.

7. We will answer any questions you have.

If you agree to participate, please sign your name here

_____

_____ date

_____ Child's name

For parents of children _____ Parent's Signatur

"টেট্রাসাইক্লিন" দ্বারা কলেরা চিকিৎসা করার সম্মতি পত্র

কলেরা রোগের উৎকৃষ্ট ঔষধ নিরূপন করার জন্য ICDDR,B একটি পরীক্ষা চালাইতেছে যেহেতু সাধারন 'এনটিবাইওটিক' কোন কোন ধরনের কলেরা জীবানুতে কার্যকরী নয়। যদি আপনি এই পরীক্ষায় অংশ গ্রহন করিতে চান তবে আপনাকে নিম্ন বর্নিত কাজ করিতে হইবে:-

১। আমরা আপনার কলেরা যথাযথ ভাবে অন্তঃশিরা স্যালাইন দিয়া চিকিৎসা করিব।

২। অন্তঃশিরা স্যালাইন (যাহা কলেরার প্রধান চিকিৎসা) ছাড়াও আমরা আপনাকে "টেট্রাসাইক্লিন" এনটিবাইওটিক অথবা এরিথ্রোমাইসিন ঔষধ দিব।

৩। হাসপাতালে থাকা কালীন, প্রত্যহ আমরা আপনার পায়খানা পরীক্ষা করিব এবং সুস্থ হইয়া বাড়ী যাওয়ার পর, আমরা এক থেকে তিন বার আপনার বাড়ী গিয়া পরীক্ষার জন্য আপনার পায়খানা আনিব।

৪। এই পরীক্ষায় অংশ গ্রহনে আপনার কোন বিপদের ঝুকি নেই। বিশেষ কোন পরীক্ষা করা হইবে না। আপনার শরীর থেকে কোন রক্তও নেওয়া হইবে না।

৫। আপনার চিকিৎসা তথ্যের গোপনীয়তা রক্ষা করা হবে।

৬। এই পরীক্ষায় অংশ গ্রহন করাতে আপনার কোন বাধ্যবাধকতা নেই। যদি আপনি অংশ গ্রহন করেনও, তবু যে কোন সময় আপনি ইচ্ছা হইতে বিরত থাকিতে পারিবেন। এই পরীক্ষায় অংশ গ্রহন করেন বা না করেন

২য় পৃষ্ঠায় দ্রষ্টব্য

না করিয়াথাকি পরবর্তী সময়ে ছাড়িয়া দেন তবু আপনাকে
আমাদের হাসপাতালে ডাই বিয়ার চিকিৎসা করা হবে।
আপনার সিদ্ধান্ত যেন তাবে আপনার চিকিৎসার
কোন রকম ব্যত্যয় হবে না।

৭। আমরা আপনার যে কোন প্রশ্নের উত্তর
দিব।

যদি আপনি উহা পরীক্ষায় অংশ গ্রহন করিতে চান
তবে আপনি নিম্নে সই করুন।

_____

_____ তারিখ

_____ রোগীর নাম

রোগীর পিতা/মাতার জন্য _____ পিতা/
মাতা স্বাক্ষর