Attachment 1. 66

Date 6/4/83

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Drs. Knight Trainee Investigator (if any) _____

Application No. 83-015(P) Rabbani Supporting Agency (if Non-ICDDR,B) _____

Title of Study Trial of Berberine as An anti secretory drug in human 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
   (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.

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

Joseph Knight
Principal Investigator

Trainee

83-015(P)
25/4/83

SECTION I - RESEARCH PROTOCOL

(Limited Study)

1. Title: Trial of Berberine As An Anti-Secretory Drug in Human Cholera

2. Principal Investigators: Dr. J. Knight and Dr. G.H. Rabbani

3. Consultant: Dr. T. Butler

4. Starting Date: 1st May, 1983

5. Completion Date: 31 July, 1983

6. Total Direct Cost: $ 2815

7. Scientific Program Head: Dr. T. Butler

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

Signature of the Scientific Program Head: Thomas Butler, M.D.

Date: 25-4-83

8. Abstract Summary:

We will attempt to study the effect of Berberine in reducing secretory diarrhea in patients with severe cholera. The study and control groups will each consist of 10 adult patients with darkfield positive stools for V. cholera. After a period of 8 hours of observation to ascertain the basal purging rate, patients in the study group will be given a dose of Berberine Sulfate equal to 10 mg/kg. Purging rate will then be quantified over the next 24 hours and rates in the treatment and control groups compared. Side effects of treatment if any will be noted.

9. Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) B M R C: _____

3) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives:

To investigate the clinical efficacy of Berberine as an antisecretory agent in cholera.[1]

To determine what if any clinically important side effects of berberine exist.[2]

2. Background

Berberine is an alkaloid (berberine sulfate -mol.wt.384.4) derived from the plants Berberis aristata, the Indian Barberry or Tree-turmeric. It has been known to indigenous Indian and Chinese medicine for several thousand years. The yellow alkaloid berberine is a bitter extract of the root, bark, and wood. It has been traditionally used as a tonic, astringent, diaphoretic, antipyretic, and purgative, in cases of splenomegaly and jaundice, in remittent and intermittent fevers, in neuralgia, in bilious complaints, and in diarrhea. Mixed with honey it is applied externally to aphthous sores, abrasions, and ulcerations of the skin.[2]

The pioneer study of berberine in experimental cholera was Dutta and Panse's 1962 work.[3] They used infant rabbits and found that berberine alone among several indigenous plants tested prevented diarrhea and death when given both before and 8 hours after infection. The authors did not, however, find berberine to be vibriocidal.

Subbaiah and Amin in 1967 found berberine sulphate useful in the prevention of experimental Entamoeba histolytica infection in 3-4

week-old golden hamsters, and tolerated by the animals up to a dose of 100 mg/kg.[4] Amin, Subbaiah, and Abbasi assessed the antimicrobial activity of berberine sulfate in 1969, and found that it was more potent than chloramphenicol and tetracycline against V.cholerae and was also bacteriocidal against this organism. Datta, however, found that berberine was less active than tetracycline and chloramphenicol,[6] and Nair, Modak, and Venkatraman found it to be vibriostatic rather than vibriocidal.[7]

In clinical trials in 1967 berberine was shown to be more effective than chloramphenicol in the treatment of cholera and nonspecific diarrhea.[8] Further clinical studies in India in the late 1960's and early 1970's confirmed the drug's clinical efficacy in diarrhoeal disorders - these include studies by Kamath (1967),[9] Deshpande (1969)[10] Sharda (1970),[11] Sharma et al (1970)[12] and Desai et al (1971)[13]. In 1975 Gupte showed that berberine was effective and well tolerated at a dose of 10 mg/kg/day in the treatment of giardiasis, with efficacy somewhat less than that of standard therapies.[14]

Raswat, a traditional crude dried preparation of Berberis aristata, was also effective against choleratoxin-induced diarrhea at a dose of 1-2 g/kg.[18]

Earlier Dutta and co-workers had shown that oral administration of berberine to infant rabbits 18-24 hours before the intra-intestinal administration of cholera toxin prevented the development of diarrhea or significantly prolonged survival time, whereas berberine given

later was ineffective.[6] They related this result to the finding by Bhide et al that the concentration of berberine in the blood of infant rabbits reaches a maximum 8 hours after G.I. tract administration with some drug still detectible after 72 hours.[19] Mekawi showed that 0.3 mg of berberine injected I.M. protected mice from death from cholera infection, and also showed that the drug protected them against cholera toxin.[20]

Further studies by Akhter and coworkers showed that berberine given orally significantly prolonged the latent period and reduced the frequency and severity of diarrhea in dogs provoked by Ipomoea turpethum root, a potent traditional purgative preparation. Berberine did not prevent diarrhea caused by magnesium sulfate or castor oil. At a dose of10 mg/kg it reduced intestinal motility in mice, and was more effective when given intra-peritoneally than when given orally.[21]

Recent experimental work has continued to confirm berberine's efficacy as an anti-secretory agent. Swabb and co-workers tested the effects of luminal berberine in the cannulated, perfused rat ileum using (^{14}C) polyethylene glycol as a nonabsorbable marker. They found that berberine reduced cholera toxin-induced secretion of water, Na, Cl, and HCO_3 in a concentration - dependent manner, but did not alter normal ileal water and electrolyte transport. The effect of berberine on toxin-induced secretion became evident 60-80 minutes after exposure and was reversed 60-80 minutes after removal of berberine from the perfusate. Berberine also prevented the development of cholera toxin - induced villous tip edema.[22] Sack and Froehlich found that berberine sulfate inhibited

by approximately 20% secretory responses to the heat-labile enterotoxins of V. cholerae and E. coli in rabbit ligated intestinal loop. The drug was effective when given both before and four hours after toxin administration, and by both intraluminal and parenteral administration. It did not inhibit the stimulation of adenylate cyclase by cholera enterotoxin and caused no histological damage to intestinal mucosa. Berberine also inhibited secretion due to the E. coli heat-stable enterotoxin in the infant mouse.[23, 24]

The study of berberine pharmokinetics in humans is now possible thanks to the development of sensitive and specific assay methods for detecting the drug in fluids (e.g., urine).[25, 26]

In summary, berberine has been shown to be an effective anti-secretory agent in cholera and other diarrhoeal syndromes by a great deal of experimental work in various animal models. Some evaluation in humans was also carried out with encouraging results, but there has been no published study of a human trial in more than ten years. Berberine is marketed in India and Japan, and presumably widely used in those countries as an anti-diarrheal medication as well as in other disease states. The published reports suggest that it is safe in humans but no definitive knowledge of possible side-effects exists. These may include some or all of the effects observed in animal studies, including hypotension[27] and sedation.[28] It would seem reasonable to study the efficacy and spectrum of clinical effects of berberine in a controlled trial for if it is really a potent, safe anti-secretagogue it may make a significant contribution to our management of cholera and other severe secretory diarrheal diseases.

2. Rationale:

Cholera causes a severe secretory diarrhea in human beings. Berberine has been shown to be an effective antisecretory agent and to inhibit the actions of cholera toxin in a number of experimental animal models and in some human trials. A controlled randomized study of berberine's efficacy in cholera - induced diarrhea may substantiate this work and provide sound justification for using this drug in the treatment of cholera.

B. SPECIFIC AIMS: See objectives

C. METHODS AND PROCEDURES:

The study will be carried out in 20 adult cholera patients with severe purging. Patients will be selected from the Treatment Centre and will satisfy the following admission criteria:

1. Onset of watery diarrhea within previous 24 hours
2. Darkfield positive stools
3. No history of current use of Tetracycline
4. Voluntary agreement to participate in the study
5. Baseline purging rate of at least 200 ml per hour

as observed in the Treatment Centre over a period of 4-8 hours.

Randomization - After admission to the study ward patients will be randomly assigned to either - a) treatment group or b) control group.

Drug administration - After purging has been observed for 8 hours, patients assigned to group(a) will receive berberine sulfate at a dose of 10 mg/kg.

Monitoring and Treatment - All patients will receive IV hydration to match their stool losses. The latter will be quantitated by 8 hours periods for as long as each patient has significant diarrhea. Stool cultures will be done. Oral feedings will be minimal (e.g., bread, milk) for the first 24 hours, after which regular meals will be provided. Any parasites found on stool culture will be treated appropriately. At discharge patients will receive a course of tetracycline to eliminate

any residual intestinal vibrios. Each patient's B.P. and level of consciousness will be recorded for every 8 hour period, and any significant changes in physical condition will be noted. Vibrio stool counts will be done on admission and after 24 hours.

D. SIGNIFICANCE:

The aim of the study will be to test the efficacy of berberine as an antisecretory agent in cholera in a controlled, randomized manner, as well as to identify any negative side effects of the drug.

Data analysis - Patients in the treatment and control groups will be compared with regard to baseline purging rate, purging rate after treatment, and duration of diarrhea. Each patient's baseline purging rate will be compared with his rate after treatment. Appropriate tests of statistical significance will be employed, e.g., students' T test for comparison of means and S.D.'s of stool volumes.

E. FACILITIES REQUIRED:

1. Office space: present office space will used
2. Laboratory space: the laboratory will be utilized only for carrying out stool cultures to isolate V. cholerae
3. Hospital resources: 20 adult patients will be studied
4. Animal resources: None
5. Logistic support: None
6. Major equipment: None
7. Others: None
8. Transport: None

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ABSTRACT SUMMARY

Controlled trial of Berberine as an antisecretory agent in cholera

1. This study will be conducted with 20 adult patients with cholera and severe purging. It will test Berberine Sulfate, which has been shown to be an effective anti-secretory agent in cholera and other diarrhoeas in a number of animal experiments and some human trials. Berberine is synthesized and marketed in India and Japan and is believed to be safe for human use; it has been found to be essentially free of side effects at a dose of of 10 mg/kg/day.

2. The patients will be randomly assigned to treatment and control groups. They will be observed for 8 hours so that their baseline purging rate may be ascertained. Those assigned to the treatment group will then be given a dose of Berberine equal to 10 mg/kg body weight. Stool volumes will thereafter be measured during 8 hours periods until the cessation of heavy purging. Patients will be rehydrated by the IV route. The study should not subject either treatment or control group patients to any significant risks. Any intestinal parasites found on stool exam will be treated, and at discharge all patients will receive a course of tetracycline. It is not expected that serious side effects of berberine administration will be found.

3. Not applicable

4. Patient confidentiality will be maintained. All data will be abbreviated and will be published without reference to the subjects' name and identity.

5. Informed consent will be obtained from each patient enrolled in the study.

6. No personal interview is required

7. Benefits to the patients involved in the study will be the cost-free treatment of any parasitic diseases found. General benefits to society include the possible identification of a valuable anti-secretory drug in cholera.

8. No retrospective hospital records will be used. No biological specimens except stool will be taken from the subjects.

SECTION III - BUDGET
(LIMITED BUDGET)

1. Personnel:

Investigators	Position	% Time used	Annual Salary Taka	Annual Salary Dollor	Remarks
Dr. Joseph Knight	Investigator	50%	-	-	
Dr. G.H. Rabbani	Investigator	20%	48,000	-	
Dr. T. Butler	Consultant	-	-	-	

2. Patients hospitalization:

20 patients x Tk.150 x 3 days	=	9,000	360
Laboratory tests (R*S for C/S)	=	-	30
Drugs	=	-	50
Grand Total:		Tk.57,000	$440

Total US$-2815

(Converted rate of $1 = Taka:24)

Trial of
Berberine

The International Centre for Diarrheal Disease Research, Bangladesh

(CONSENT FORM)

I understand that I have diarrhea from cholera and that I may need to be treated with intravenous fluids. I also understand that I am to be admitted to the hospital research ward where I will remain until the diarrhea is over. I will be treated either with Berberine, which is a drug, which in India is used to treat diarrhea, or with intravenous fluids alone. The purpose of treating me with Berberine is to find out if it really is effective in helping to stop dairrhea caused by cholera. My stool will be examined in the laboratory and if any worm or other parasite is found I will be treated for it.

I understand that I do not have to participate in this study and if I do not want to, I will not be refused proper treatment in this hospital. I also may withdraw from this study at any time without any penalty.

The records of my treatment will be kept confidential.

_____
Signature of the patient

_____
Date

_____
Signature of the Investigator/
Co-Investigator

_____
Date

বন্ধ্যাত্বের চিকিৎসা গবেষণা

(সম্মতি পত্র)

আমি অবগতে আছি যে আমার কন্যার হইয়াছে আমাকে বিনামূল্যে প্রয়োজনীয় ঔষধ আমাকে হাসপাতালে ভর্তি করা হইবে এবং ডায়ুবিটিস না থাকা হওয়া আবশ্যক নয়। আমাকে বন্ধ্যাত্বের ঔষধ খাওয়া অথবা সুই প্রয়োজন করা চিকিৎসা করা হইবে। এই ঔষধ খাইবার জন্য প্রয়োজন করা হয়। আমার বন্ধ্যাত্বের চিকিৎসা দেওয়ার উদ্দেশ্য হইল হইবে কিনা তাহা কার্যকারিতা পরীক্ষা করিয়া দেখা। আমার পরীক্ষার করা হইবে এবং কোন প্রকার ত্রুটি ধরা পড়িলে তার চিকিৎসা দেওয়া হইবে।

আমি আরও অবগতে আছি যে আমি এই গবেষণার নাও অংশ নিতে পারি, ইহাতে আমার স্বাভাবিক চিকিৎসার কোন প্রভাব পড়বে না। আমার ইচ্ছামত আমি এই গবেষণা হইতে নাম প্রত্যাহার করিয়া লইতে পারিব। আমার গুরুত্বপূর্ণ পরীক্ষার সকল কাগজ পত্র গোপন রাখা হইবে।

গবেষকের স্বাক্ষর
তারিখ:

রোগীর স্বাক্ষর
পরিবর্তে
নং