Agenda 1.

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Date 24/5/86

ETHICAL REVIEW COMMITTEE, ICDDR,B. 26/

Principal Investigator Dr. G.H. Rabbani
Dr. F.P.L. Van Loon

Trainee Investigator (if any)

Application No. 86-017

Supporting Agency (if Non-ICDDR,B)

Title of Study "The antisecretory role of 5-Ht antagonist (Ketanserin) in patients with diarrhoea due to Vibrio cholerae"

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.

(PTO)

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

MAY 27 1986

Principal Investigator

Trainee

86-017
2615

SECTION I - RESEARCH PROTOCOL

1.	Title:	THE ANTISECRETORY ROLE OF 5-HT ANTAGONIST(KETANSERIN) IN PATIENTS WITH DIARRHOEA DUE TO VIBRIO CHOLERAE
2.	PRINCIPAL INVESTIGATORS	DR. G.H. RABBANI, DR. F.P.L. VAN LOON
	Co-investigator	Dr. Mamun Shahrier
	COLLABORATING INVESTIGATORS	DR.J.RASK-MADSEN DR.K.BUKHAVE (UNIVERSITY OF COPENHAGEN)
3.	STARTING DATE	AUGUST 1986
4.	COMPLETING DATE	MARCH 1987
5.	TOTAL DIRECT COST	US $ 26900
6.	SCIENTIFIC PROGRAMME	This protocol has been approved by the Pathogenesis and Therapy Working Group

Signature of Scientific Programme Head

Date 14/1/86

7. ABSTRACT SUMMARY ...

A number of intracellular chemical mediators have recently been identified that appear to be involved in the production of diarrhoea due to enterotoxins of V.Cholerae and E.Coli. 5-Hydroxytryptamine(5-HT), a neurotransmitter, has been reported to be associated with diarrhoea due to carcinoid syndrome; 5-HT induces intestinal secretion in experimental animals and in man. Ketanserin is a potent antagonist of 5-HT and acts by inhibiting 5-HT2 receptor sites on the cell membrane; in animal studies ketanserin has appeared to be effective in inhibiting cholera induced fluid secretion. Ketanserin is clinically safe and useful in the treatment of hypertension and peripheral vascular diseases.
It is proposed to study 16 cholera patients by means of small intestinal perfusion technique, in the context of the development of antisecretory drugs currently conducted at the ICDDRB.

8. REVIEWS

(i) Ethical Review Committee.....
(ii) Research Review Committee.....
(iii) Director.....

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives

To examine the therapeutic antisecrtory role of Ketanserin a 5- hydroxytryptamine (5-HT)-antagonist in Cholerapatients

2. Background

The development of pharmacological approaches to inhibit intestinal secretion due to toxin producing enteropathogen is an interesting concept recently developed from increasing understanding of the mechanisms of intestinal ion-transport. A number of intracellular chemical messengers have been identified in animal tissues which mediate the response of secretory stimuli due to enterotoxins of Vibrio Cholerae and Escherichia Coli(1). Thes are cyclic AMP, cyclic GMP, adenylate cyclose, calcium ions, and neurotransmitters including 5-HT(2). Normally, large amounts of 5-HT are present in myenteric plexus of the gut (3) and increased amounts are found in the blood of patients with diarrhoea associated with carcinoid syndrome (4). The role of 5-HT as mediator of toxin-induced intestinal secretion has increasingly been recognised. It has been suggested that 5-HT causes diarrhoea of carcinoid syndrome by its modulatory effect on intestinal motility (5,6). 5-HT also affects intestinal water and electrolyte transport and this can contribute to the diarrhoea of carcinoid syndrome (7,8,9). This experimental evidence suggests that 5-HT may play a regulatory role on motility and water and electrolyte transport. It has been shown that 5-HT acts as an intestinal secretagogue in the jejunum and ileum of rabbits given by intravenous infusion (10) and in man by triple lumen perfusion studies (5). It has been postulated that prostaglandins(PG) are involved in cholera toxin induced diarrhoea and PG formation occurs in response to secretagogues like 5-HT (11,12). In patients with carcinoid syndrome PGE2 levels in the jejunal fluid were markedly raised, and both indomethacin and ketanserin reduced the diarrhoeal volume and the local intestinal PGE2 concentrations. Furthermore treatment with methysergide a 5-HT inhibitor significantly decreased the diarrhoea and a repeat triple lumen perfusion study indicated that the intestinal water and electrolyte transport had returned to normal.
The mechanism by which 5-HT stimulates intestinal secretion is less clear. However, experimental evidence suggests that 5-HT affects Na and Cl transport, specifically decreasing neutral NaCl absorption (13). In addition 5-HT does not appear to affect (a)adenylate cyclase cAMP system and Na-K-ATPase activity in certain animal tissues (14,15) but not in rabbit intestine (6).

We have reported that in patients with severe cholera, the jejunal PG levels are significantly increased which returns to normal levels during convalescence(11). This finding indicates that PG's may be involved in the mechanism of choleric diarrhoea. However, a possible role of 5-HT can not be ruled out.
In this study we propose to evaluate the probable role of 5-HT in the inducement of V.Cholerae diarrhoea, specifically examining the therapeutic effect of 5HT antagonists like such as ketanserin. A recent observation indicates that ketanserin is ten times more effective than indomethacin as an inhibitor of intestinal secretion induced by prostaglandin (Rask-Madsen, 1986, personal communication).
Ketanserin (+)-3-[2-(4-(4-fluorobenzoyl)-1-piperidinyl)]ethyl]-2, (1H,3H)-quinazolinedione, a new selective 5-HT2 receptor antagonist is currently being used in the clinical management of hypertension and peripheral vascular disease (16,17,18). After oral ingestion of 40 mg ketanserin tablet, the active compound is rapidly liberated, reaching a peak of 103.8 ng/ml after 0.9 hours. An average terminal eliminate (19) halflife of 15.4+/- 4.2 was found after administration of 40 mg ketanserin solution.
In extensive clinical pharmacological and therapeutic studies ketanserin was found not to be associated with orthostatic hypotension but supine heart rate was slightly decreased. Some patients complained of adverse reactions, mostly vertigo and unsteadiness . Clinical safety data indicate that there is no haematological or biochemicalg abnormalities other than transient and slight elevation of triglycerides and serum glucose (20).

METHODS

Patient selection

Adult patients, male and female, presenting to the ICDDR,B treatment centre with a history of acute watery diarrhoea (duration less than 24 hours) are eligible for the study. Patients should at least be moderately dehydrated. Only those patients with initially a purging rate of 200 ml/hour or more will be eligible for the study. No prior medication is allowed.
Fresh faecal specimen will be examined by darkfield microscopy for the presence of V.Cholerae and a specimen will be sent for culture. The study will be explained to the patient by a local Bangladeshi doctor before the patient will be invited to participate.
As soon as informed written consent has been obtained the patient will be transferred to the study ward. A complete physical examination will be done and rehydration will be performed with intravenous fluid. No oral rehydration solution will be used during the study period.

Perfusion studies

Patients will undergo jejunal intubation by an oral or nasogastric triple lumen tube. The intubation will preferably carried out in morning, the patient being in a fasting state. The position of the tube(distal aspiration port 20 cm distal to the ligament of Treitz) will be checked under fluoroscopy. Ten ml of jejunal fluid will be aspirated for determination of PG's and 5-HT. Hereafter a "slow marker"(21) or a "steady state" perfusion (22) of the jejunum segment will be performed, using BSP as a non-absorbable marker.
In the "slow marker" perfusion procedure the test segment will be perfused with a rate of 1 ml/min for approximately 60 minutes for equilibration. Then 10 ml of jejunal fluid will be sampled for determination of fasting intestinal flow rates, and 5-HT. Herafter the response to a bolus injection of ketanserin 40 mg will be studied collecting samples at 30, 60 and 90 minutes.
The equilibration period in the "steady state" perfusion technique will also be 60 minutes with an infusion rate of 10 ml/min. After the equilibration period sequential 15 min. collections of 10 ml will be used for determination of transport rates of fluid, Na, Cl, K, ketanserin, and 5-HT. Hereafter the response to a bolus injection of ketanserin (40 mg) will be studied collecting samples at 30, 60, and 90 minutes.
In summary, 16 patients will be investigated during acute cholera and convalescence. In 8 patients the effect of ketanserin will be studied during "slow marker" perfusion; 8 other patients will be studied during "steady state" perfusion. All patients studied will be requested to return to the hospital one week after discharge for jejunal intubation, sampling of jejunal fluid, and control perfusion studies without administration of drugs.

Laboratory analyses

5-HT measurements

Radioimmunological measurements (RIA) will be performed for determination of 5-HT in jejunal fluids aspirating during "slow marker" perfusion and in fluids collected during "steady state" perfusion of the small intestine. Determination of 5-HT which include purification by extraction and column chromatography before the quantification are performed by RIA (23), are currently carried out in the Danish laboratory. The addition of the relevant internal standards to the biological samples will be performed at the biochemical laboratories of ICDDRB prior to the preliminary extraction in order to correct for losses of unstable 5-HT during storage and transport of samples. Determination of 5-HT will be performed by HPLC according to Sperk (24). The samples will be mixed immediately with cold perchloric acid (final concentration 0.2 M) and ascorbic acid (final concentration 0.01 mM).

Degradation during storage and transport from Dhaka may provide some problems. Any storage problem, however, can be solved by adding radiolabelled 5-HT to the samples immediately following their collection in ICDDRB --thus providing an internal standard for correction of decay.

Data analysis

The data will be analysed using relevant parametrical statistical methods, such as the Student's t-test for paired and impaired variates and the analysis of variance or non-parametrical statistical analyses preferentially Wilcoxon's test for paired variates and Mann Withney's U-test. Subjects will serve as their own controls whenever possible.

SIGNIFICANCE

Definition of the role of 5-HT in intestinal secretion in patients with diarrhoea may provide a rationale for the clinical use of potential anti-diarrhoeal drugs that inhibit 5-HT metabolism or interfere with the action of secretagogues.

FACILITIES REQUIRED

Existing ICDDRB facilities will be utilized

COLLABORATIVE ARRANGEMENTS

This proposal is a part of a collaborative research project between ICDDRB and Dr.J.Rask-Madsen, University of Copenhagen, Denmark.

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ABSTRACT SUMMARY for RESEARCH and ETHICAL REVIEW COMMITTEES

The antisecretory role of a 5-Hydroxy-tryptamine antagonist(Ketanserin) in patients with diarrhoea due to Vibrio Cholerae

1. This study will be conducted in 16 adult male cholera patients. We will test the 5-Hydroxy-tryptamine(=serotonine) antagonist ketanserin since it has been shown to be an effective anti-secretory agent in V.Cholerae toxin induced fluid secretion in a number of animal experiments and human trials. Widely being marketed in Europe as a safe treatment for hypertension and vessel diseases, it will here be given to only the acute patients in a single dose (40 mg iv.).

2. Out of the 16 patients eight will undergo slow marker perfusion and eight steady state perfusion in both the acute stage of the illness and during convalescence (a week after discharge). The Centre has been acquainted with these procedures since the early sixties (Greenough, Gilman, Speelman, Van Loon) that have appeared to be harmless in this hospital setting. The patients will be rehydrated by intravenous route initially and then with oral rehydration solution combined with antibiotics. Hourly stool measurements will be performed during the eight hours before and those after drug administration. It is not expected that serious adverse effects of the treatment will be found.

3. Not applicable

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

5. Informed consent will be obtained from each patient('s guardian)

6. Medical history will lege artis be taken.

7. Benefits to the patient participating in the study will be the cost-free treatment of diarrhoeal illness. General benefits to society include the possible identification of a valuable antisecretory drug for cholera.

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

SECTION III - BUDGET

1. Personnel service

Name	Position	% time	Project Requirement USD
Dr GH Rabbani	Princ. Invest.	50	8000
Dr FPL VanLoon	Princ. Invest.	70	8800
Dr M Shahrier	Co-Investigator	10	800
Nursing staff(3)		20	800
Clinical clerk		10	100
Biochemist technician		20	800

2. Supplies and materials		
BSP standard and ketanserin		1500
Clinical supplies and materials		1000
Glassware		300
Minor equipment		1100
Equipment maintenance		300
Data analysis		1000
3. Patient hospitalization		3500
4. Transport local		100
5. Outpatient care		100
6. Shipment of samples		1000
7. Printing, Publication		300
8. X-Ray/IV Fluid/ Media/ Pathology		500
	total (USD)	26700

The prostaglandin and serotonin analysis is furnished from official and private funds through Drs Rask-Madsen/Bukhave

Funding of the above proposal by WHO is pending.

Consent Form

You have been attacked with cholera which provokes a very important loss of water from your body and requires replacement of lost water by intravenous fluids. We want to study your intestinal fluid. Therefore we want to introduce a small tube through your mouth or nose to the gut to collect this fluid. This procedure will take about half a day. Hereafter the tube will be removed. This procedure is completely safe may cause some discomfort in nose or throat.
During this procedure a single dosage of ketanserin will be administered -- a drug whose efficacy and safety has been established in vessel diseases and hypertension but that is strongly suggested to be effective in controlling cholera induced diarrhoea as well.
We will request you to come back to the hospital two weeks after discharge. When you come back we will reimburse your travel expenses and a daily income.
If you don't want to be included in this study, you will not be penalized in any way but you you will receive the same proper treatment. You may also decide to withdraw from the study at any time.
If you accept to join the study, please sign the consent form here below.

Signature of Investigator

Signature/Thumb impression of Patient..Date ..-..-..

সম্মতি পত্র

আপনি কলেরা রোগ দ্বারা আক্রান্ত হয়েছেন। এ রোগের মাধ্যমে আপনার শরীর হতে প্রচুর পানি চলে যায়, যেটাকে ইনজেকশান স্যালাইনের মাধ্যমে পূরন করতে হয়। আমরা আপনার অন্ত্রের মধ্যে যে পানি রয়েছে তা পরীক্ষা করতে চাই। খুতক্ষ আমরা এ পানি সংগ্রহ করবার উদ্দেশ্যে আপনার মুখ অথবা নাক দিয়ে একটি ছোট নল ঢুকাতে চাই। এ পদ্ধতি প্রায় আর্ধেক দিন লাগতে। তারপর নলটি বের করে নেয়া হবে। এ প্রক্রিয়া সম্পূর্ন ভাবে নিরাপদ কিন্তু আপনার নাক অথবা গলায় কিছু অসুবিধা হতে পারে।

আমরা আপনাকে এ হাসপাতাল হতে ছুটি দেয়ার দু সপ্তাহ পর আবার আসতে অনুরোধ করবো। আবার আসলে আপনার দৈনিক মজুরি বাবদ এবং যাতায়ত বাবদ অর্থ প্রদান করবো।

যদি এ গবেষনায় অংশগ্রহনে আপনার সম্মতি না থাকে, তাহলে আপনাকে হাসপাতালে যথা চিকিৎসাই করা হবে। যে কোন সময় আপনি এ গবেষনা হতে আপনার নাম প্রত্যাহার করতে পারেন।

যদি এ গবেষনায় অংশগ্রহনে আপনার সম্মতি থাকে, তাহলে নিম্নে আপনার সম্মতি সূচক স্বাক্ষর অথবা টিপসহি প্রদান করুন।

গবেষকের স্বাক্ষর

রোগীর স্বাক্ষর অথবা বৃদ্ধাঙ্গুলীর ছাপ

তারিখ— _____