Library (2)

Attachment I.

ICDDR,B LIBRARY

Date 13/09/87

ETHICAL REVIEW COMMITTEE, ICDDR,B - DHAKA - 12

26

Principal Investigator DR P.K. BARDHAN Trainee Investigator (if any)

Application No. 87-020 Supporting Agency (if Non-ICDDR,B) M/s Sandoz, Switzerland

Title of Study SMS 201-995 and ICS 205-930 as antisecretory agent in secretory diarrhoea: trials in animal models.

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

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

Source of Population: NA
(a) Ill subjects Yes No
(b) Non-ill subjects Yes No
(c) Minors or persons under guardianship Yes No

Does the study involve: NA
(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

Does the study involve: NA
(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

Are subjects clearly informed about: NA
(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: NA
(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 NA

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)
NA 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)
NA Informed consent form for subjects
NA 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)

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

Pradip Bardhan
Principal Investigator

Trainee

OCT 28 1987

87-020
13.09.87

SECTION I: RESEARCH PROTOCOL

1.	Title	:	SMS 201-995 and ICS 205-930 as antisecretory agent in secretory diarrhoea: trials in animal models.
2.	Principal investigator	:	Dr P K Bardhan
	Co-investigators	:	Dr F C Patra Dr K A Al-Mahmud Dr A S M Hamidur Rahman Mr M A Wahed
3.	Starting date	:	October 1987
4.	Completion date	:	March 1988
5.	Total direct cost	:	US $9,953.00
6.	Source of fund	:	M/s. Sandoz, Switzerland
7.	Scientific programme	:	This protocol has been approved by Clinical Sciences Division

Signature of the Acting Associate Director, Clinical Sciences Division

Date: 10.9.1987

8. Abstract Summary

The use of an antisecretory agent to reduce stool output in secretory diarrhoea is an attractive idea. Because of the lack of a suitable and useful drug, the search is continuing. Somatostatin (SST) has been shown to inhibit intestinal secretion stimulated by various agents, though found ineffective in cholera in humans. Different possibilities include inadequate dose, inability of the drug, or the inherent difficulties of SST, such as very short half-life. SMS 201-995 is a synthetic analogue of SST, which is more potent, has longer half-life, and was found useful in managing secretory diarrhoea in human. Again, serotonin (5-HT) receptors have been found to be involved in cholera. ICS 205-930 is a potent and selective 5-HT receptor blocker which was found to be effective in cholera toxin (CT) induced diarrhoea in mice. The proposed study intends to examine the effects of these two drugs on intestinal secretion induced in animal (rabbits and rats) by CT and Deoxycholic acid (DCA). Jejunal perfusion technique will be used in rats, and ileal and colonic loops will be used in rabbits. The duration of the study will be six months.

9. Reviews:

(i) Ethical Review Committee:_____

(ii) Research Review Committee: _____

(iii)Director's signature & remark, if any:_____

SECTION II: RESEARCH PLAN

INTRODUCTION

1. Objective: To examine the antisecretory effects of two synthetic drugs -- ICS 205-930 and SMS 201-995 on intestinal secretion, experimentally induced in animals.

2. Background: Among the many drugs tested as antisecretory agents in cholera or other enterotoxigenic diarrhoea only a few (e.g. chlorpromazine, nicotinic acid) showed same effects. However, they have not proved to be useful in a clinical setting, and thus the search for a suitable agent is ongoing.

SMS 201-995

Somatostatin (SST) a tetradecapeptide, has been shown to inhibit intestinal secretion stimulated by prostaglandin (PG_1) and theophylline[1], glucagon[2], serotonin[3], and vasoactive intestinal polypeptide (VIP[4]) in different species including man, without altering basal jejunal water and electrolyte fluxes. Thus both cyclic AMP (cAMP) and non-cAMP mediated intestinal hypersecretion can be inhibited by SST. However, it was found ineffective in reducing stool output in human cholera[5]. Diarrhoea in cholera occurs due to cholera toxin (CT) induced cAMP mediated intestinal hypersecretion. One possible explanation suggested was the inability of the SST to influence the intestinal hypersecretion once the CT is irreversibly bound to the mucosal GM_1 ganglioside receptors. Nevertheless, data in favour of such a hypothesis are not available for CT induced intestinal secretion. Another distinct possibility is that the dose of SST might not have been enough.

The very short half-life of SST and the observation of rebound phenomenon at the end of i.v. application makes SST unsuitable for long-term clinical use. SMS 201-995 is a synthetic analogue of SST[6]. It is a cyclic octapeptide, has longer half-life, and can be given intramuscular, sub-cutaneous or even orally[6]. It also seems to be more potent than SST in animal models[7]. SMS has been found useful in the management of secretory diarrhoea in human patients[8].

ICS 205-930

5-Hydroxytryptamine (5-HT) receptors have been shown to be involved in the pathophysiology of cholera[9]. 5-HT itself is a potent stimulater of intestinal secretion[10]. It was suggested that about 60% of the effect of cholera toxin (CT) on intestinal fluid transport could be ascribed to 5-HT mediated nervous mechanisms[11].

Among the two subclasses, 5-HT-D receptors seem to be of minor importance in the gut[12]. Recently ICS 205-930 [(1H)-indol-3-carbonic-acid-tropine ester hydrochloride], a highly potent and selective 5-HT-M receptor blocker has been developed[13]. Effects of this agent on the GI tract have been investigated in different animals[14]. It successfully inhibited 5-HT induced changes in gut motility and enhanced gastric emptying in guinea pigs, without influencing the normal GI motility, as seen in mice. It was also found to be effective in 5-HTP and CT induced diarrhoea in mice[14]. Acute and chronic toxicity studies in mice, rats, and dogs did not reveal any specific drug-induced abnormalities. It was also found safe given in single doses in healthy human volunteers. A recent report has shown its effectiveness in reducing diarrhoea in human carcinoid syndrome[15]. If found effective, this drug may prove to be a clinically useful

antisecretory agent for treatment of secretory diarrhoeas where 5-HT mechanisms may be involved.

The proposed research plan intends to study the effect of ICS 205-930 given in 4 different single doses (0.1, 0.2, 0.4, and 0.8 mg/kg) i.p., and SMS 201-995 given i.m. and intraluminally in the dose of 10 µg/kg and 50 µg/kg respectively on intestinal secretion induced in rats and rabbits by CT and Deoxycholic acid (DCA), both of which induce intestinal secretion mediated by cAMP, using jejunal perfusion technique in rats and jejunal and colonic loops in rabbits.

3. Rationale:

Development of an effective antisecretory agent will greatly simplify management of acute dehydrating secretory diarrhoeas such as cholera. This trial is designed to test two such candidates in experimental animal models.

SPECIFIC AIMS

1. To compare the effect of SMS 201-995 with that of controls on net water and electrolyte transport in CT induced diarrhoea in rats in vivo.

2. To compare the effect of ICS 205-930 with that of controls on fluid accumulation in experimentally induced diarrhoea in rabbits in vivo.

3. To examine the effects of these drugs on intestinal mucosal cAMP levels in the same animals.

METHODS AND PROCEDURE

PHASE I

Sample size:

With 95% confidence limit (alpha=.05) and 80% power (beta=0.2) the sample size in each group was calculated according to the following formula:

$$\frac{2 \times SD^2}{(\text{Difference of means})^2} \quad X \ f\ (\text{alpha,beta}) \quad [f(.05,\ .2)=7.9]$$

Rats:

Expecting 50% reduction in net water secretion induced by cholera toxin (14.28 ± 4.02 μl/20 min/cm)[16] sample size becomes

$$\frac{2 \times 4.02^2}{7.14^2} \quad X\ 7.9 = 5$$

Rabbits:

Similary, with expectation of 50% reduction in luminal fluid accumulation stimulated by CT (0.9 ± 0.3 ml/cm)[18], sample size in each group is:

$$\frac{2 \times 0.3^2}{0.45^2} \quad X\ 7.9 = 7$$

Anticipating about 20% experimental failures, the number of animals will be 6 rats and 8 rabbits in each group.

Rat perfusion model:

Normal male albino rats weighing 250-350 grams, after overnight fast (with free access to water) will be anaesthetised with pentobabital (6.5 mg/100 g) and two 15 cm ileal loops, 5 cm apart beginning 40 cm proximal to ileocaecal valve will be constructed after opening the abdomen with a midline incision. Each loop will be ligated both proximally and distally

and will be inoculated either by 1 ml of 0.9% of NaCl or 75 ug CT. This will be left in situ in the peritoneal cavity for 2 hours after which the perfusion will be set up[16]. After washing with 50 ml of warm saline, both the ileal loops will be cannulated and perfused at constant temperature ($37^{o}C$) and rate (0.5 ml/min) with a peristaltic pump. Body temperature will be maintained with the help of a heating lamp. The perfusion solution will be a balanced electrolyte solution consisting of Nacl 115, $NaHCO_3$ 25, K_2PO_4 2.4, KH_2PO_4 0.4, $CaCl_2$ 1.2, $MgCl_2$ 1.2, mannitol to bring final osmolality to 300 mosmoles/l, PEG 5 g/l as unabsorbable marker.

After 30 minutes to reach steady state, 3 collections each of 20 mins will be obtained. After the perfusion studies, the length of both the loops will be measured. Concentrations of Na^+, K^+, Cl^-, and PEG and osmolality of the perfusates will be determined. Net water and electrolyte transport will be calculated using standard formulas[17], and expressed as µl, uosmol, or ueq/20 min per cm length of the loop as follows:

Net water movement: $PR\ [1-(PEG)_{In}/(PEG)_{Eff}]$

Net ion movement: $PR\ [I_{In} - I_{Eff}(PEG)_{In}/(PEG)_{Eff}]$

PR = Perfusion rate in ml/min

$(PEG)_{In}$ = PEG concentration in infusion solution

$(PEG)_{Eff}$ = " " " effluent "

I_{In} = Ion " " infusion "

I_{Eff} = Ion " " effluent "

Net absorption from the lumen will be expressed as positive value, not secretion into the lumen as negative value. These rats will be receiving the drug or placebo (0.9% NaCl) as a single subcutaneous (SC) injection or intraluminal dose 1 hour before being challenged with CT.

Six rats will be included in each group. The total number of groups are 11.

SMS - S.C. route (placebo, two doses) = 3

Intraluminal " = 3

ICS - Placebo, 4 doses = 5

Rabbit jejunal loops:

New Zealand white rabbits raised in laboratory environment and weighing 2-2.5 kg will be fasted for 48 hours before operation. After anaesthesia (pentobarbital), the peritoneal space will be entered through a mid-abdominal incision. 10 ml of 0.9% NaCl will be injected into the jejunum and colon distal to two ligatures to cleanse those segments of intestine of their contents. Four mid-jejunal loops and four loops in the ascending colon (approx 6-7 cm each) will be constructed in each of the animals[18]. One ml of CT (10 ug/ml) or 0.9% NaCl pH 7.5 as basal controls will be injected in the mid-jejunal loops, whereas 1 ml of 6 mM DCA (Deoxycholic acid) or 0.9% NaCl will be injected into the colonic loops in random order. The animals will be sacrificed after 5 hours. The dose of CT will be doubled if the stated dose fails to induce intestinal hypersecretion.

The volume of fluid will be measured after making a small incision in each loop and collecting the fluid in a container, separately for each loop. The results will be expressed as mls of fluid per cm of the length

of each loop.

One hour before operation, the rabbits will receive the drug or the placebo in a single injection, SC or intraluminal dose. Eight rabbits will be included in each of the 11 groups.

SCHEDULE I

[Hours]

	-1	0	1	2	3	4
Challenge (CT or DCA)		x				
Loop preparation in rats and rabbits:						
- (Subcutaneous injection experiments)		x				
- (Intraluminal instillation experiments)	x					
Drugs (ICS or SMS)	x					
Fluid collection (rabbits only)						
Perfusion (rats only)				Steady state	Study	

cAMP assay:

cAMP will be determined by a competitive protein-binding assay[19], commercially supplied as a kit from Amersham/Searle Corporation, Arlington Heights, Illinois. After gentle separation from muscularis, fresh intestinal mucosa will be immediately homogenized in an ice-cold 0.05 M Tris buffer, pH 7.5 using a glass tissue homogenizer. The cold homogenate will be deproteinized with 0.1 M $ZnSO_4$ and 0.1 M $Ba(OH)_2$ and centrifuged at 10,000 rpm for 15 min. The supernatant will be lyophilized and the residue will be redissolved in 0.05 M Tris buffer for the cAMP determination.

PHASE II

If the drugs are found to be effective in inhibiting intestinal secretion, their capability of reversal of CT-induced intestinal secretion will be examined. The experimental design will be similar to the previous experiments. However, instead of giving the drugs 1 h before challenge, they will be given 2 h after challenge.

SCHEDULE II

	[Hours]					
	0	1	2	3	4	5
Loop preparation	x					
Challenge	x					
Drug			x			
Fluid accumulation (rabbits)						x
Perfusion (rats)				Steady state	Study	

SIGNIFICANCE

Reducing the magnitude of fluid and electrolyte loss in cholera will simplify management. Identification of effective and useful antisecretory drugs is expected to be of immense help in optimal management. This study will examine the antisecretory effects of two such promising candidates -- SMS 201-995 and FCS 206-930 in experimental cholera in animal models. Moreover, by determining mucosal cAMP levels, insights in their mode of actions may be gained.

DATA ANALYSIS

Statistical analysis will be performed by Student's 't' test for paired and unpaired data and will be two-tailed. Significance will be accepted at 0.05 level. All results will be expressed as mean $\pm$ S.E.M.

FACILITIES REQUIRED

Help of animal branch of ICDDR,B will be required.

Existing ICDDR,B laboratory facilities will be utilized.

REFERENCES

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SECTION III: BUDGET

SMS 201-995 AND ICS 205-930 AS ANTISECRETOLRY AGENT IN SECRETORY DIARRHOEA: TRIALS IN ANIMAL MODELS

LOCAL SALARIES

Name	Position	%	Rate (m/m)	Total Fund
Dr P K Bardhan	Principal Inv.	20	830 US$	996
Dr K A Al-Mahmud	Co-Investigator	10	1262	757
Dr A S M H Rahman	"	20	667	800
Mr M A Wahed	"	10	782	470
Dr F C Patra	"	5%	-	-
	Sub-total:			3023

SUPPLIES AND MATERIALS

Rat adults	@ $1.60 X 100 =	160
Rabbit adults	@ $18.00 X 90 =	1620
Equipments		150
Chemicals [including chemical agents, DCA & cholera toxin]		250
cAMP kits		400
Stationery		50
Non-stock supplies		50
Sub-total:		2680

OTHER COSTS

Rent, communication and utilities	50
Printing and reproduction	200
Sub-total:	250

INTERDEPARTMENTAL SERVICES

Laboratory tests [PEG, Electrolytes, Osmolality, cAMP assays]	4000
Sub-total:	4000

BUDGET SUMMARY

	6 months project requirement
Local salaries	US $ 3,023
Supplies and materials	2,680
Other costs	250
Interdepartmental services	4,000
Total direct cost:	$ 9,953