achment 1.

Date 16. 9. 82

ETHICAL REVIEW COMMITTEE, ICDDR,B.

99

incipal Investigator P. SPEELMAN

Trainee Investigator (if any) _____

plication No. 82-048(P)

Supporting Agency (if Non-ICDDR,B) _____

tle of Study

PGE_2 -levels in cholera-patients.

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

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

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

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)

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

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

N.A

NA

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.

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

P. Speelman
Principal Investigator

Trainee

82-048(P)
22/9/82

PILOT

SECTION I - RESEARCH PROTOCOL

1. Title: PGE_2 - levels in cholera-patients

2. Principal Investigators: P. Speelman and J. Rask-Madsen

3. Co-Investigators: G. H. Rabbani, F.T. Black and K. Bukhave

4. Starting Date: mid November, 1982

5. Completion Date: mid January, 1983

   Duration: 2 months

6. Total Direct Cost: US $ 3,410

7. Scientific Program Head:

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

   Signature of Scientific Program Head: Thomas C. Butler

   Date: 20 Sept 1982

8. Abstract Summary:

Prostaglandins (PG's) may have an important effect on intestinal ion transport. Measurements of PG's - concentrations in peripheral plasma have been made to clarify their possible significance. However, the results are misleading due to PG's - release by aggregating thrombocytes Moreover, measurement of stable plasma-metabolites reflects the total body production. Determination of PG levels in the luminal fluids might prove to be the most accurate index of intrinsic intestinal PG formation. In this study we will sample 10 cc of jejunal fluid through intubation, from 20 adult cholera patients.

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objectives

The main objective of this study is to find out if the concentration of PGE_2 in the intestinal fluids of cholera patients is raised.

2. Background

It has been evident over the last two decades that abnormalities in the active transport of water and electrolytes in the intestines play a fundamental role in diarrhoeal diseases(1). This is particularly true for secretory diarrhoeas although important interactions between intestinal motility, mucosal blood flow, and epithelial transport play a significant role, too.

Active ion transport is regulated by numerous substances, which include "classical" hormones, paracrine mediators, neuro-transmitters, and luminal secretagogues. Although the role of the arachadonic acid (AA)-prostaglandin (PG) system in the regulation of intestinal transport has not yet been established (and many experiments have been contradictory and have led to much confusion) there is now ample evidence to suggest that this system makes up an additional mode of regulatory control (2,3). Hormones, paracrine mediators, andneurotransmitters interact with a cell surface receptor which in turn initiates the enzymatic release of AA from the phospholipid pool. The released AA can then be oxygenized by the enzyme

cyclo-oxygenase to unstable cyclic endoperoxides which are further enzymatically converted to the "classical" PGs, PGE_2 and PGF_2a, prostacyclin (PGI_2), and thromboxanes (TX).

In addition to specific hormonal mediators and neurotransmitters, non-specific stimuli, for example mechanical damage, ischaemia, or irradiation can led to de novo synthesis and release of PGs through mechanisms which do not involve a cell surface receptor.

Pharmacological doses of both E- and F-compounds may evoke copious watery diarrhoea in humans following oral,(4), intrajejunal (5) or parenteral administration (6). In the small intestine exogenous PGs of the E type stimulate secretion by inhibiting active Na-absorption and eliciting active Cl-secretion. Conversely PGI_2 appears to promote absorption of fluid and electrolytes (3). Possible effects on the colonic epithelium have not yet been defined. Exogenous PGs of the F-type primarily affect motility throughout the gastro-intestinal tract, without decreasing the transit time of the luminal contents significantly (7). In addition, sub-threshold doses of all PGs tested have the ability to protect the gastrointestinal epithelium against noxious agents which would otherwise produce cellular damage and necrosis (3). This action, which has been named cytoprotection, seems independent of the effect on secretion of some PGs (8).

PGs of the E-and F-type, PGI_2, and TXs are synthetized throughout the gastro-intestinal tracts where different regions are characterized by differing profiles of AA-metabolizing enzymes (9). Although the named actions of exogenous PGE and PGF upon epithelial transport and intestinal motility may be related to the diarrhoeagenic properties of these PGs,

previous studies in vitro (10) have been handicapped by the inability of isolated intestinal mucosa to respond to PG-concentrations which may be considered physiological.

Therefore, the effects of low (physiological) doses (10^{-11} to 10^{-7}M) of PGE_2 were assessed in vitro in the Ussing chamber preparation of human jejunal mucosa during blockade of endogenous PG-production by indomethacin (2.9×10^{-5}M). It was shown that serosal application of PGE_2 causes a prompt rise in the short-circuit current (I_{sc}), decrease net Na-flux, and elicits Cl-secretion resulting in a slightly depressed conductance.

Rask-Madsen and K. Bukhave have shown that the inability of untreated tissues to respond to so-called "physiological" concentrations of exogenous PGE_2 may be explained by the presence of preformed PGE_2. They have reported that pretreatment with indomethacin practically abolished PGE_2 formation in vitro and at the same time increased the sensitivity of the tissue to exogenous PGE_2.

Prior studies in vitro and in vivo (1) have demonstrated that both indomethacin and salicylates, besides enhancing "basel" intestinal ion and fluid absorption (11 & 12), decrease the amount of fluid that accumulates in response to cholera toxin (11, 12, 13 & 14) and E. coli enterotoxin (15). Further, in one study in vivo indomethacin enhanced "basel" absorption and, although net secretion was observed in the presence of cholera toxin, it did not significantly reduce the change in fluid transport caused by cholera toxin (11). Therefore, it is not clear whether anti-inflammatory agents simply offset net secretion by

blocking endogenous PG-synthesis or interfere with the secretory process per se. Thus, indomethacin may act directly on a mechanism responsible for anionic transport.

The extremely low dose-response to exogenous PGE_2 during suppression of PG-biosynthesis by indomethacin suggests a "true" physiological role of PGs in the modulation of ion and fluid transport by the human small intestine. The molecular basis for the actions of PGs is only poorly understood.

Since PGs may have pathophysiological, in addition to pharmacological and physiological effects on epithelial transport much effort has been spent in assaying concentrations of PGs in peripheral plasma to clarify their possible darrhoeagenic properties in humans with diarrhea. However, the correlation between diarrhoea and plasma PG levels is poor in most cases reported, and the absolute value of PG-concentrations determined are misleading due to the fact that PGs are released by aggregating thrombocytes during the sampling procedure (15). In addition, determination of plasma levels (i.e., even of stable metabolites) would at best reflect the total body production (16). Since PGs are also released on the mucosal side of jejunal mucosa, (17) determination of PG-levels in the intestinal fluids might prove the most accurate index of intrinsic intestinal PG-formation.

To validate this assumption the relationship between endogenous PGE_2 levels in the intestinal lumen and the clinical symptoms has been studied in patients with diarrhoea before and, if relevant, during systemic treatment with a PG synthetase inhibitor.

Recently Bukhave and Rask-Madsen (17) have reported a study of PGE_2 - levels in purified samples of jejunal fluids, aspirated at the ligament of Treitz, in healthy volunteers, alcohol addicts with diarrhoea or steatorrhoea, patients with chronic diarrhoea and patients with irritable bowel syndrome. In healthy volunteers (n=22) the PGE_2 concentration range was 5-205 pg/ml. The alcohol addicts had PGE_2 levels within the normal range. Ten out of 17 patients with chronic diarrhoea and 2 out of 15 patients with irritable bowel syndrome had raised PGE_2 - levels (205 - 340 pg/ml). In 6 patients with high PGE_2 - concentrations indomethacin treatment (25 mg x 4 daily) halved the associated diarrhoea and reduced PGE_2 concentrations to normal levels.

In another recent publication (10) these authors have presented clinical and biochemical evidence that PGE_2 may be the mediator of fluid and electrolyte secretion by villous adenomas of the rectum.

Indirect evidence suggests that prostaglandins may be involved in the pathogenesis of fluid transport induced by cholera toxin in vivo, and recent observations suggest that also nerves play a role in cholera-induced secretion since cholera toxin in experimental animals - besides activating the adenylcyclase activity - triggers the release of 5-hydroxy-tryptamine from the enterochromaffine cells which in turn via intestinal reflexes probably stimulates prostaglandin formation. Thus not only cyclo-oxygenase inhibitors but also the new selective $5\text{-}HT_2$ receptor antagonists may have therapeutic implications in the treatment of cholera patients (19, 20).

The above mentioned knowledge should encourage the study of the relationship between endogenous PGE_2 - levels in the intestinal lumen of patients with cholera and other acute severe diarrhoeal syndromes.

Rationale:

It has been shown that prostaglandins may play an important role in the mechanism of secretory diarrhoea (S. typhimurium). The role of PGE_2, measured in intestinal fluid, in acute cholera-patients has not adequately been investigated. The results of such PGE_2- measurements may have clinical applications.

B. Specific aims:

To measure PGE_2 concentrations in the jejunal fluid of adult cholera patients, in acute and convalescent stage.

C. Methods of procedure:

Patient selection: adult patients, male and female, presenting to ICDDR,B treatment centre with a history of acute watery diarrhoea (duration less than 24 hours) are eligible for the study. Patients should be, at least moderately, dehydrated. No prior medication is allowed. Darkfield examination will be performed and, if positive, the patient will be transferred to the study ward, where informed written consent will be obtained. Hereafter a complete physical examination will be done. Rehydration with I.V. fluids will be given. If the clinical condition of the patient requires immediate antibiotic treatment, the patient will be transferred to the general ward. If not, the patient will undergo duodenal jejunal intubation by an oral - or nasogastric tube, after rehydration has been completed.

The intubation will be carried out in the morning, the patient being in a fasting state. The position of the distal part of the tube will be checked under fluoroscopy. Ten ml. of jejunal fluid will be collected hereafter antibiotic treatment will be started. The patient will be discharged as soon as his clinical condition allows this.

The patient will be asked to return for the same investigation after 10 - 14 days. Travel expenses and a daily income will be reimbursed.

The 10 ml of jejunal fluid, obtained in a sterile container, will be deepfrozen immediately and will be transported to Denmark for PGE_2 - concentration measurements.

The radio-immunoassay for determination of PGE_2 will be performed conform the extensive description in the European Journal of Clinical Investigation (1981) 11, 191 - 197.

D. SIGNIFICANCE

This study will provide valuable information about the possible role of PGE_2 in patients with cholera. This knowledge may be useful in the development and selection of antisecretory drugs for the treatment of high purging cholera patients.

E. FACILITIES REQUIRED

1. Office space is already provided.
2. Laboratory space is already provided.
3. Hospital resources: about 20 patients will be hospitalized during a period of 2-7 days.
4. No animal resources are required.
5. Logistic support: a nurse has to assist during the intubations.
6. No major items of equipment are required.

F. COLLABORATIVE ARRANGEMENT

This study will be a collaborative study between

- ICDDR,B (P. Speelman) and
- University of Odense

  Department of Medical Gastroenterology

  DK 5000 Odense C

  Denmark (J. Rask-Madsen M.D.)

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SECTION III - BUDGET

1. Personnel Services: - (2 months)

Name	%Effort	Dollar	Total
Dr. P. Speelman	10	600	
Dr. Rask-Madsen	-	-	
Dr. to be named	10	50	
Study Nurse	10	50	700

2. Supplies and Materials:

Clinical supplies		
(I.V. fluids, tubes, needles, etc.)	100	
Laboratory tests	50	
X-ray facilities	50	200

3. Patient hospitalization:

20 patients x 4 days x US$8	640
4. Transportation of samples:	200
5. Printing/Xerox:	200
Total :	$ 1,790
6. Travel expenses (J. Rask-Madsen)	$ 1,620
Grand Total :	$ 3,410

ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

1. The study will be done in 20 adult patients with proven infection with V. cholerae. No special groups will be used; no pregnant women will be enrolled in the study.

2. The only procedure in this protocol is duodenal-jejunal intubation by an oral or nasogastric tube. This intubation is a common procedure without any substantial risk. I have never heard about complications due to this procedure. However, intubation produces some discomfort to the patient. The position of the tube will be checked under fluroscopy. The time of radiological exposure will be kept as short as is possible.

3. Not applicable.

4. Confidentiality will be maintained by locking the files in the cabinet until completion of the study. Data will be published without reference to the subjects name and identity.

5. Signed consent will be obtained.

6. Medical histories will be obtained.

7. There will be no benefit for the individual subject. The society in general may benefit in the near future from new antisecretory drugs for high purging cholera patients. Knowledge about prostaglandins might be extremely helpfull for future development of antisecretory drugs.

8. The protocol requires sampling of 10 c.c. of jejunal fluid.

FYNS AMTS SYGEHUSVÆSEN
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Dept. of Med. Gastroenterology

Telf. (09) 11 33 33 lokal 2750

Dato 13th Aug., 1982

Ra/em

ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMITTEE

The present study on prostaglandin E_2 levels in small intestinal fluids of adult patients with toxigenic diarrhoea is considered important - not only from a pathophysiological point of view - but also due to the potential therapeutic benefits which may emerge as a consequence of having developed a safe rationale for the selection of new antisecretory drugs in the treatment of patients with high purging cholera.

Recent observations in experimental animals suggest that prostaglandins may play an important role in choleratoxin induced diarrhoea. Determination of prostaglandins released into the gut lumen appears presently to provide the most reliable index of the balance between their local production and degradation. Using this atraumatic approach it will be possible to define whether prostaglandins are important in patients with cholera, and if so, to monitor the effect of drugs inhibiting prostaglandin formation by blocking enzymes or specific receptors in the gut wall.

It is impossible to set up the complex and resource demanding methology of radioimmunoassay for prostaglandin measurements for a small pilot study - and even for measurements in large scale it would not be advisable since a highly specialized biochemist and "check-up" by gas chromatography-mass spectrometry besides routine purification procedures including chromatography are necessary for valid results. Furthermore, the use of commercial antibodies cannot yet be recommended due to lack of specificity.

Sincerely Yours,

Jørgen Rask-Madsen, M.D.
Department of Medical Gastroenterology
Odense University Hospital
DK-5000 Odense C, Denmark

CONSENT FORM

Title: PGE_2- levels in cholera patients

Statement to be read to the patient or guardian before consent is obtained

I understand that my diarrhoea is caused by cholera-bacilli. To restore my body fluids I have got an intravenous drip. I understand that the doctor wants to collect a small amount of fluid from my intestines for investigation. This may be helpfull for the development of drugs needed to treat patients with serious diarrhea in the future.

I have given permission to the doctor to pass a small tube through my nose or mouth to my intestines to collect some fluid. I am informed that this procedure is completely safe but may cause some discomfort in my nose or throat. The investigation will not take more than one hour, usually not more 15 minutes. Two weeks after discharge from the hospital I will probably return to the hospital for the same investigation. Travel expenses and one daily income will be reimbursed. I know very well that I have the right of withdrawal from this study at any point. Refusal of further cooperation with the study will in no way influence my treatment.

_____ Signature of the Investigator

_____ Signature or L.T.I. of patient or guardian.

Date: _____

Case No.: _____

সম্মতি পত্র

পি জি ২-২ স্টাডি

আমি বুঝিতে পারিলাম যে আমার কলেরা জীবাণুজনিত ডায়রিয়া হয়েছে। শরীরের পানি পূরণ করার জন্য আমি শিরার মাধ্যমে স্যালাইন পেয়েছি। আমি বুঝিতে পারিলাম যে রোগ নির্ণয়ের জন্য ডাক্তার সাহেবের - আমার অন্ত্র নিঃসৃত কিছু তরল পদার্থের প্রয়োজন। এই পরীক্ষার ভবিষ্যতে মানুষের কলেরা ডায়রিয়া রোগের নতুন চিকিৎসা উদ্ভাবনে সহায়তা করিবে।

এই জন্য আমি আমার নাক বা মুখের মাধ্যমে অন্ত্রে একটি সরু টিউব প্রবেশ করানোর অনুমতি ডাক্তার সাহেবকে দিলাম। আমাকে জানানো হয়েছে যে এই টিউব প্রবেশ করার সময় আমার নাক বা ~~মুখে~~ গলায় কিছু অস্বস্তি সৃষ্টি হইতে পারে। ইহাও জানানো হয়েছে যে এই টিউব প্রবেশ করাইতে ১ ঘন্টার বেশী লাগিবে না। সাধারণতঃ ১৫ মিনিটে ইহা সম্পন্ন হয়। দুই সপ্তাহ পর আমি সম্ভবতঃ একটি পরীক্ষার জন্য পুনরায় হাসপাতালে আসিব। যাতায়াত খরচ ও একদিনের আয়ের সমপরিমাণ অর্থ আমাকে প্রদান করা হইবে। আমি বেশ ভালভাবে নিশ্চিত যে, যে কোন মুহূর্তে আমি এই গবেষণা হইতে নাম প্রত্যাহার করিয়া নিতে পারি। এবং ইহাতে আমার চিকিৎসার কোনরূপ ব্যাঘাত ঘটিবে না।

গবেষকের স্বাক্ষর - ________ রোগী/অভিভাবকের স্বাক্ষর / টিপসই ________

তারিখ ________