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ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator DR. IVAN CIENAR

Application No. 85-017

Title of Study IMMUNOGENICITY OF ORAL B-SUBUNIT/WHOLE-CELL CHOLERA VACCINE

Trainee Investigator (if any) ____

Supporting Agency (if Non-ICDDR,B) ____

Project status:
(x) 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).

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

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 Ctteec. for review.

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

Ivan Cienar

Principal Investigator

Trainee

85-017.
7-7-85.

SECTION-I - RESEARCH PROTOCOL

1. Title : Immunogenicity of oral B-subunit/whole-cell cholera vaccine

2. Principal Investigator: Dr. Ivan Ciznar

   Coinvestigators : Dr. Asma Khanam
   Dr. Amar Bin Ashraf

   Consultants : Dr. D. A. Sack
   Dr. J. D. Clemens

3. Starting date : September 01, 1985

4. Completion date : August 31, 1986

5. Total direct cost : US$ 85,910

6. Scientific Program Head: Dr. Ivan Ciznar

This protocol has been approved by the Host Defense Working Group

Signature of Scientific Program Head : Ivan Ciznar

Date : 25/06/85

7. Abstract Summary

Antigenic components of oral B-subunit/whole-cell cholera vaccine will be analyzed by crossed-immunoelectrophoresis and immunoblotting technique using rabbit antisera and sera of vaccinated persons. These techniques are known for high resolution sensitivity and capacity to distinguish at least 21 antigens in V. cholerae and corresponding antibodies. Rabbits will be immunized intravenously with the B-subunit/whole-cell in order to stimulate antibody response against maximal number of antigens present in the vaccine. Comparison will be made between

spectrum of antigens reacting with antibodies in animal sera and in sera of vaccinated persons. Antigen additional to B-subunit and LPS will be identified and isolated. Such antigens may be later applied for largescale serosurvey by ELISA method in vaccinated population.

8. Reviewers

a. Ethical Review Committee _____

b. Research Review Committee _____

c. Director _____

SECTION-II - RESEARCH PLAN

A. INTRODUCTION

1. Objective

To analyse antigenic spectrum of oral B-subunit/whole-cell cholera vaccine and to compare immune response in terms of specific antibodies in vaccinated persons and convalescents from cholera.

2. Background

Oral candidate cholera vaccine consisting of B-subunit toxoid and killed whole-cells stimulates both local and systemic immune response (A. M. Svennerholm, et al, 1984). Major antigenic determinants stimulating antibody production in vaccinated persons are located in B-subunit of toxin and on lipopolysaccharide of the whole-cells. It has been known that in Vibrio cholerae, other antigens are located in various parts of the cells. Thus, Rowley and Attridge (1983) described glycoprotein in a flagellar sheet of Vibrio cholerae. Soluble hemagglutinin originally referred to by Finkelstein, et al (1978) and described as "cholera lectin" represents another component. Non-soluble or cell-bound hemagglutinin have been found in Vibrio cholerae 01 by Hanne and Finkelstein (1983). The outer-membrane of Vibrio cholerae contains several proteins which are potential candidates for protective antigens. Kabir (1980) described a major protein with a molecular weight of 45.000 to 48.000 within both bio- and sero-type. Similarly, Kelley and Parker (1981) using a mouse virulent strain found major outer-membrane protein of the same size. Sears, et al (1984) found significant rise in anti-outer-membrane protein antibodies in sera of volunteers with diarrhoea

after experimental challange with four strains of Vibrio cholerae O1. Results obtained in ICDDR,B laboratory showed that live Vibrio cholerae when injected into rabbits can stimulate production of antibodies against at least 21 antigens (Ciznar, et al, unpublished results). Consequently, the questions arised: How many antigens are present in oral B-subunit/whole-cell vaccine presently under the field trial? Do all these antigens stimulate antibody production in vaccinated persons? What is the difference in a spectrum of antibodies between vaccinated persons and convalescents as far as antigenic specificity is concerned? Could we assess the relation between clinical protection and antigenic spectrum of the vaccine? Answers are likely to advance our knowledge of immunity in cholera with impact on development of more effective vaccine.

Such study is feasible in ICDDR,B facilities and represents a new approach based on an utilization of immunochemical techniques as cross-immunoelectrophoresis and Western-blotting known for their sensitivity, resolution potential and capacity to distinguish large number of antigens.

3. Rationale

New oral B-subunit/whole-cell cholera vaccine represents a significant step towards the achievement of efficient protective immunity. While the efficacy is expected to stem from toxin-derived antigen and stimulation of immune response locally in intestine, there has been a feeling that more research is needed, particularly on the role of other antigens present in the whole-cells inactivated by heat and formaldelyde. The proposed study intends to analyse

these antigens and antibodies found in vaccinated persons and to compare them with antibodies in convalescent sera. It is expected that such analysis could contribute to upgrading the efficiency of vaccination against cholera.

B. SPECIFIC AIMS

1. Estimation of maximal number of antigens present in the B-subunit/whole-cell oral cholera vaccine by means of crossed-immunoelectrophoresis and Western-bloting technique with rabbit antisera.

2. To determine the additional antigenic components to B-subunit and LPS which stimulate antibody production in sera and intestinal content of vaccinated persons.

3. To isolate the additional antigenic components with intention of utilizing them for a large scale serosurvey in the vaccinated population.

C. EXPERIMENTAL

1. Vaccine

The vaccine used will be the whole-cell and B-subunit/whole-cell vaccine being used in the 1985 Cholera Vaccine Field Trial and will be from the same lot.

2. Animal sera

Rabbits will be immunized in vitro by B-subunit/whole-cell vaccine according to this schedule. First dose corresponding to 10^5 of cells per rabbit of 2 kg weight on day 0. After one week, each third day, increased dose of vaccine will be injected starting with 10^6 further $5x10^6$, 10^7, $5x10^7$ and $5x10^8$ cells. One week after the

last dose, the rabbits will be bled and serum recovered. Totally, 4 rabbits will be immunized with B-subunit/whole-cell and 4 rabbits with whole-cell component only.

3. Human sera

Six adult healthy male volunteers will receive 3 oral doses of B-subunit/whole-cell at 6-week intervals with selected acid neutralizing regimen. Before vaccination, 2 ml and one week after the last dose, 5 ml of blood will be taken by venepuncture. Serum will be separated and kept at -20°C till further analysis. Sera of healthy blood donors from a non-endemic country (Czechoslovakia) will be used as a control.

4. Lavage specimen

The lavage specimens will be collected from the volunteers by the procedure which has been used in ICDDR,B since 1979. The volume 1000 ml of watery stool will be heated at 56°C for 15 minutes to inactivate proteolytic enzymes, then sterile-filtered and concentrated on ultramembrane to 20 ml. The concentrated material will be used for further analysis.

The six healthy adult volunteers will be recruited from the employee staff of ICDDR,B.

5. Antigen preparation

For analysis of sera and intestinal contents, the B-subunit/whole-cell vaccine will be lyophilized and extracted with veronal buffer pH 8.2. After dialysis, the extract will be adjusted to concentration of proteins (10 mg 1 ml) by standard Lowry's (1951) procedures and tested against animal sera.

6. Crossed-immunoelectrophoresis and immunoblotting

A modification described by Kroll (1973) will be used, with Tricine buffer and Sigma agarose. Western-blot procedure will be employed as described by Towbin, et al (1979) and modified by Burnette (1981).

7. Outer-membrane proteins will be isolated from the method of Kelley and Parker (1981) and flagella by the method of Resnich, et al (1980). LPS will be isolated by the procedure of Westphal and Jann (1965). All these preparations will be used for identification of reactive components from B-subunit/whole-cell vaccine with animal and human sera.

In the case of detection of antigenic components different of LPS and B-subunit, these will be isolated using FPLC system or gel chromatography and apply for serosurvey in vaccinated population.

D. SIGNIFICANCE

While the vaccine trial with B-subunit/whole-cell oral vaccine is going on and evaluation of its impact on diarrhoeal diseases morbidity in the population is prepared, it is justified to utilize all available techniques for extraction of maximum informat-on related to the nature of immunity status in vaccinated persons. This study is intended to obtain data about antigens involved in stimulation of antibody production after administration of the vaccine. Such information may have an impact on improvement of future vaccines.

E. FACILITIES REQUIRED

No extra laboratory space will be needed. Planned two laboratories for HDWG will offer sufficient space for experimental work. Most of the equipments for analysis and separation of antigens are available. Additional instrument and parts , such as Pharmacia columns, UVICORD and Recorder, may be purchased.

REFERENCE

1. Attridge, S.R., Rowley, D. The role of the Flaggellum in the Adherence of Vibrio cholerae. J Inf Dis, 147, 5:864-72, 1983

2. Burnette, W.N. Western blotting: electrophoretic transfer of proteins from SDS-PAGE to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein. A Anal Biochem, 112:195-203, 1981

3. Finkelstein, R.A., Arita, M., Clemens, J.D., Nelson, E.T. Isolation and purification of an adhesion factor (cholera lectin) from Vibrio cholerae. Proceedings of the 13th Joint Conference on Cholera, U.S.-Japan Cooperative Medical Science Program. NIH Publication No. 78-1590, Bethesda Md., 139-51. 1978

4. Finkelstein, R.A., Boesman-Finkelstein, Holt, P. Vibrio cholerae hemagglutinin (lectin) protease hydrolyses fibronectin and ovomucin. Proc Natl Acad Sci, U.S.A. 80 1092-5, 1983

5. Kabir, S. Composition and immunochemical properties of outer membrane proteins of Vibrio cholerae. J Bacteriol, 144:382-9, 1980

6. Kelly, J.T., Parker, C.D. Identification and preliminary characterization of Vibrio cholerae outer membrane proteins. J Bacteriol, 145:1018-23, 1981

7. Kroll, J. Crossed-immunoelectrophoresis, p79-81. In H.H. Axelsen, J. Kroll, B. Wecke, ed. A manual of quantitative immunoelectrophoresis. Blackwell Scientific Publications, Oxford, 1983

8. Lowry, C.H. Protein measurement with Folin phenol reagent. J. B. C. Chem, 193:265-75, 1951

9. Resnick, I.G., et al. Improved protection against cholera in adult rabbits with combine Flagella toxoid vaccine. Infect Immun, 30:375-80, 1980

10. Sears, S.D., Richardson, K., Young, Ch., Parker, Ch., Levine, M.M. Evaluation of the human immune response to outer membrane proteins of Vibrio cholerae. Inf Immun, 44(2):430-44, 1984

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12. Towbin, H., Stachelin, T., Gordon, J. Electrophoretic transfer of proteins from polyacrylamid gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci, U.S.A. 76:4350-4, 1979

13. Westphal, O., Jann, K. Bacterial lipopolysaccharides. Methods in carbohydrate chemistry. 5:83-96, 1965

SECTION-III - BUDGET

A. DETAILED BUDGET

STAFF SALARIEIS & BENEFITS

Name	Position	Time Effort	Project Requirement Taka	Dollar
INTERNATIONAL (03 32 00)				
Dr. I. Ciznar	Principal Investigator	40%	-	35,000
LOCAL (03 31 00)				
Dr. Asma Khanam	Coinvestigator	10%	20,000	-
Dr. Amar Bin Ashraf	Coinvestigator	20%	-	-
Mrs. Taharat Yasmin	Research Officer	100%	76,000	-
Mr. C. R. Ahsan	Research Officer	100%	76,000	-
Laboratory Technician (Animal)		10%	5,000	-
Laboratory Attendant		15%	5,000	-
CONSULTANTS				
Dr. D. A. Sack			-	-
Dr. J. D. Clemens			-	-

SUPPLIES AND MATERIALS (03 37 00)

	Unit	Rate	Project Requirement Taka	Dollar
- Plastic, glassware (plates, dialysis bags, tubes, flasks, pipetts, nitrocellulose membranes)			-	4,000
- Reagents, chemicals, drugs, media			-	7,000
- New Zealand Albino rabbits	270	35	8,400	-

CAPITAL EQUIPMENT (03 03 00)

Pharmacia Columns SK-25, SK-10, UVICORD and RECORDER			-	5,000

PATIENT HOSPITALISATION (STUDY) (03 48 13)			3,000	-

		Tak	Dollar
	OUTPATIENT COST		-
6.	ICDDR,B TRANSPORT		-
7.	TRAVEL		
	Local	-	
	International (03 36 00)		
	Participation in Scientific Conferences, U.S.A.	-	
8.	TRANSPORTATION OF THINGS	-	
9.	RENT, COMMUNICATION AND UTILITIES (03 39 00)	-	200
10.	INTERDEPARTMENTAL SERVICES COST (03 48 00)	2,000	-
11.	REPRODUCTION (03 48 06)	15,000	-
12.	CONSTRUCTION	-	-

B. BUDGET SUMMARY

	Project Requirement	
	Taka	Dollar
STAFF SALARIES & BENEFITS		
Local (03 31 00)	182,000	
International (03 32 00)	-	35,000
SUPPLIES AND MATERIALS (03 37 00)	8,400	11,000
CAPITAL EQUIPMENT (03 03 00)	-	5,000
PATIENT HOSPITALISATION (STUDY) (03 48 13)	3,000	-
OUTPATIENT COST	-	-
ICDDR,B TRANSPORT	-	-
TRAVEL		
Local	-	-
International (03 36 00)	-	3,000
TRANSPORTATION OF THINGS	-	500
RENT, COMMUNICATION AND UTILITIES (03 39 00)	-	200
INTERDEPARTMENTAL SERVICES COST (03 48 00)	2,000	-
REPRODUCTION (03 48 06)	15,000	-
CONSTRUCTION	-	-
TOTAL	210,400	54,700
Dollar equivalent US$ 1 = Tk.26	210,400	8,092
TOTAL DIRECT OPERATING COST		62,792
Overheads		17,338
Incremental cost 10%		5,780
GRAND TOTAL		85,910

CONSENT FORM

The International Centre for Diarrhoeal Disease Research, Bangladesh is carrying out research to better understand how to protect people from cholera and other diarrhoeal diseases. We would like you to participate in a study to determine the immune (protective) response which occurs when you are vaccinated with the new oral B-subunit/whole-cell cholera vaccine. We hope that the information we gain will be helpful in improvement of present oral cholera vaccine. If you agree to participate in this study, you can expect the following:

1. You will drink 3 oral doses of B-subunit/whole-cell at 6-week intervals. Before taking the vaccine, 2 ml of blood and one week after the last dose, 5 ml of blood will be taken.

2. Also we will have you do the intestinal lavage procedure one week after the last dose. This is a procedure in which you will drink a large volume (up to 5 liters) of salty water, and this will cause a temporary diarrhoea. The diarrhoea stops shortly after you stop drinking the salty water. During the lavage, you will have a full feeling in the abdomen, you will gain 1-3 kg in weight, but you will not have pain or any serious side-effects.

3. None of the tests are harmful to your health. Drawing blood and drinking a large volume of salty water are somewhat uncomfortable; they do not have any serious side-effects.

4. You will have a medical check-up before entering the study.

5. We will answer any question you have concerning the study.

If you agree to participate in this study, please sign your name here.

_____
Signature/Right-hand thumb print

Date: _____

_____
Investigator's Signature

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ, ঢাকা

সম্মতি পত্র

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ, কলেরা এবং অন্যান্য উদরাময় রোগের প্রতিরোধ সম্পর্কে ভালভাবে অবগত হইবার জন্য একটি গবেষণা করিতেছে।

নতুন এক প্রকারের বি-সাবইউনিট/পূর্ণ-কোষ কলেরার খাইবার টিকা দ্বারা আপনার শরীরে যে প্রতিরোধ ক্ষমতার সৃষ্টি হয় উহা পরীক্ষার জন্য আমরা আপনাকে গবেষণায় অংশ গ্রহন করিতে আহ্বান জানাইতেছি। আমরা আশাকরি এই গবেষণায় যে তথ্য পাওয়া যাইবে উহা বর্তমানের খাইবার কলেরার টিকার উন্নতির সহায়ক হইবে। আপনি যদি এই গবেষণায় সহায়তা করিতে ইচ্ছুক হন, তাহা হইলে আপনি নিম্নলিখিত ব্যাবস্থাদিতে অংশ গ্রহন করিতে পারেন:

১/ আপনাকে প্রতি ছয় সপ্তাহ অন্তর তিন মাত্রায় বি-সাবইউনিট/পূর্ণ-কোষ কলেরার টিকা খাওয়ান হইবে। টিকা খাইবার পূর্বে ২ সিসি এবং শেষ মাত্রা খাইবার এক সপ্তাহ পর ৫ সিসি রক্ত আপনার শরীর হইতে লওয়া হইবে।

২/ শেষ মাত্রা টিকা খাইবার এক সপ্তাহ পর, আমরা আপনার অন্ত্রের যোষিন-পদ্ধতি পরীক্ষা করিব। এই পরীক্ষার জন্য আপনাকে বেশ কিছু পরিমান পেয়ন-পানি (৫ লিটার পর্যন্ত) পান করিতে হইবে, ইহার ফলে আপনার

আপনার ওজন বাড়াও হইতে পারে। লবন-পানি পান বন্ধ করা মাত্র ওজন বাড়া বন্ধ হইয়া যাইবে। এই পরীক্ষার সময় আপনার উদর সম্পূর্ণ ভরাট মনে হইবে, এবং আপনার ওজন ১-৩ কিলো বৃদ্ধি পাইবে কিন্তু আপনি কোন প্রকার বেদনা অথবা কোন পার্শ্ব প্রতিক্রিয়া অনুভব করিবেন না।

উপরের কোন পরীক্ষা আপনার শরীরের জন্য ক্ষতিকর নয়, রক্ত গ্রহন এবং অধিক পরিমাণে লবন-পানি পান করিবার সময়ে আপনি সামান্য অস্বস্তি বোধ করিতে পারেন। ইহাতে কোন মারাত্মক পার্শ্বপ্রতিক্রিয়া নাই।

৩/ এই গবেষণায় অংশগ্রহনের পূর্বে আপনার স্বাস্থ্য পরীক্ষা করা হইবে।

৪/ এই গবেষণা সম্পর্কিত আপনার যাবতীয় প্রশ্নের জবাব দিতে আমরা সচেষ্ট থাকিব।

আপনি যদি এই গবেষণায় স্বেচ্ছায় অংশগ্রহন করিতে সম্মত হন, তাহা হইলে অনুগ্রহপূর্বক নিম্নে আপনার দস্তখত অথবা আপনার বামহস্তের বৃদ্ধাঙ্গুলির ছাপ দিন।

গবেষকের স্বাক্ষর

তারিখ

স্বাক্ষর/বামহস্তের বৃদ্ধাঙ্গুলির ছাপ

তারিখ