Budget # 01 to 02 RKC for information

Attachment 1. 36 Date 5-2-85

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Zia U. Ahmed

Application No. 85-007(P)

Title of Study Adaptation of an adult rabbit model to Shigella dysenteriae 1 for assessing virulence colonization and immunogenecity of strains.

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) _____

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: N/A
   (a) Ill subjects Yes No
   (b) Non-ill subjects Yes No
   (c) Minors or persons under guardianship Yes No
2. Does the study involve: N/A
   (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: No
   (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: N/A
   (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: N/A
   (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 N/A 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.

- THIS PROTOCOL DOES NOT INVOLVE HUMAN SUBJECTS.

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

Zia U. Ahmed

Principal Investigator

Trainee

85-007(P)

SECTION I - RESEARCH PROTOCOL 6/2/85

1. Title : Adaptation of an Adult Rabbit Model to Shigella dysenteriae I for assessing virulence, colonization and immunogenecity of strains

2. Principal Investigator : Dr. Zia Uddin Ahmed

   Co-investigators : Mrs. Khaleda Haider
   Dr. A.S.M. Hamidur Rahman
   Mr. Khorshed Alam

   Consultant : Dr. David A Sack

3. Starting Date : January 1985

4. Completion Date : Three months from starting date

5. Total Direct Cost : $ 3,514.00

6. Scientific Program Head : Dr. David A Sack

This protocol has been approved by the Diarrhoeal Transmission Working Group.

Signature of Scientific Program Head : [signature]

Date : 8 Jan 1985

7. Abstract Summary

A suitable animal model is essential to assess bacterial colonization, virulence and development of immune protection. An adult rabbit model developed for V. cholerae and enterotoxigenic E. coli (Spira et al. 1981) has been successfully adapted to Shigella flexneri 6 (D.A. Sack, unpublished data) following the modifications that have been used by Cray et al. (1983) for oral inoculation of the bacteria.

The present protocol would establish the parameters necessary for successful colonization of rabbit intestine by Shigella dysenteriae I. This strain is presently in focus of the centre for major genetic and immunological studies relating to vaccine strain construction.

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8. Reviews

a. Research Involving Human Subject _____

b. Research Review Committee _____

c. Director _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective :

To adapt an adult rabbit model (that has been worked out with Shigella flexneri 6) to Shigella dysenteriae 1.

2. Background :

The DTWG is planning to initiate work on the isolation of potential vaccine strains of Shigella dysenteriae I. A suitable animal model to assess virulence and protective properties of strains is an essential pre-requisite for such studies. Fortunately a simple and reliable rabbit model is now available that has been developed following the RITARD (removable intestinal tie-adult rabbit diarrhoea) model (Spira et al. 1981) for V. cholerae and enterotoxigenic E. coli, and adapted to Shigella flexneri 6 (D.A. Sack, personal communication). Briefly, the procedure is as follows. Rabbits are fed with at least 25 mg of tetracycline over a 36h period prior to administration of inoculum. Then, at time 0, cimetidine (50 mg/kg body wt) is administered intravenously. At 15 and 30 min, 15 ml of a solution of 5% $NaHCO_3$ is administered by gastric tube. Immediately after the second $NaHCO_3$ dose, 15 ml of bacterial inoculum is given by gastric tube followed by i.p. administration of 2 ml of tincture of opium ~~containing 10 mg of morphine~~. The animals are sacrificed later and the number of washed bacteria in the intestine is determined.

see next page

3. Rationale :

Genetic and immunological studies of Shigella dysenteriae I aimed at constructing a vaccine strain require a suitable animal model. The

present work will meet this essential pre-requisite.

B. SPECIFIC AIMS

To adapt an adult rabbit model to Shigella dysenteriae I.

C. METHODS AND PROCEDURES

a. Administration of inoculumn

The procedure outlined in Introduction will be followed to prepare rabbits and administer inoculum through the gastric route.

b. Determination of LD_{50}

LD_{50} will be determined as follows. Inocula of seven different sizes (10^4, 10^5, 10^6, 10^7, 10^8, 10^9 and 10^{10}) will be given to groups of five rabbits and death within seven days will be recorded.

c. Determination of colonization

Three inocula of different sizes will be chosen from the LD_{50} data. Each inoculum will be given to groups of two rabbits. Eithteen hours after inoculation the animals will be sacrificed. A 10cm segment of the intestine will be isolated by ties and removed. The segment will be opened by a longitudinal cut and immersed ten times in a measured volume of phosphate buffered saline. The wet weight of the segment will then be determined. Serial dilutions of the PBS will be plated and from the colony count the number of bacteria in the wash fluid will be determined.

Colonization will be expressed as mean log of bacteria recovered in the wash fluid ± S.E. per gram of tissue.

(d) Challenging immunized rabbits : Future projection.

How ?

Challenge by 1 LD_{50} second is 1 colony'g dose ?

Immunized rabbits will be challenged with two hamologous inocula of different sizes, separated by a time interval of 21 days. One inoculum will be of the size of LD_{50} administered to a group of 5 rabbits and death recorded within 7 days of each inoculation. The other dose will be the one which gives "good" colonization in an unimmunized rabbit. These animals will be sacrificed 18h after each inoculum and colonization will be determined.

How many experiments can be done ?

D. SIGNIFICANCE

See the heading 'Abstract Summary'

E. FACILITIES REQUIRED

Animals and the laboratory facilities that will be needed for the work are available of the centre. Chemicals that will be necessary are; Cimetidine & tincture of opium.

OUT PATIENT CARE

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% time used	Project Requirement Taka	US Dollar
Dr. Zia Uddin Ahmed	Associate Scientist	30%	9,000/-	-
Mrs. Khaleda Haider	Sr.Res.Officer	25%	3,817/-	-
Mr. Hamidur Rahman		10%	2,500/-	-
Mr. Kohrshed Alam		10%	2,291/-	-
Mr. Rezaur Rahman	Technician	30%	1,281/-	-
	Sub total		Tk. 18,889/-	-

2. SUPPLIES

Bacteriologic medium) Disposable Petri plates)		$ 1,500/-
Rabbit (100 @ Tk. 200/-	Tk. 20,000/-	-
Supplies and chemicals	Tk. 5,000/-	500/-
Sub total	Tk. 25,000/-	$ 2,000/-

3. EQUIPMENT - None

4. PATIENT HOSPITALIZATION - None

5. OUTPATIENT CARE - None

6. TRANSPORTATION OF PERSONS - None

7. TRAVEL AND TRANSPORTATION OF PERSONS - None

8. TRANSPORTATION OF THINGS - None

9. RENT, COMMUNICATION, UTILITIES - None

10. PRINTING AND PUBLICATION - None

11. OTHER CONTRACTUAL SERVICE - None

12. CONSTRUCTION, RENOVATION, ALTERATION - None

B. BUDGET SUMMARY

CATEGORY	TAKA	US DOLLAR
1. Personnel	18,889.00	-
2. Supplies	25,000.00	2,000.00
3 - 12 None	-	-
Total	Tk..43,889.00	$ 2,000.00
	= $ 1,514.00 +	$ 2,000.00

Grand total $ 3,514.00

(Conversion rate 1 US $ = Tk. 29.00)

REFERENCES

Cray, W.C. Jr., Tokunaga, E. and Pierce, N.F. 1983.
Successful colonization and immunization of adult rabbits by oral inoculation with Vibrio cholerae 01.
Infect. Immun. 41, 735-741.

Spira, W.M., Fedroka-Cray, P.J. and Pettebone, P. 1981.
Colonization of rabbit small intestine by clinical and environmental isolates of non-01 Vibrio cholerae and Vibrio mimicus.
Infect. Immun. 41, 1175-1183.