DOSE FINDING STUDIES OF PROCHOLERAGENOID IN THAI VOLUNTEERS

SRICHAROEN MIGASENA, WICHAI SUPANARANOND, PUNNEE PITISUTTITHAM, PRAVAN SUNTHARASAMAI and LADAWAN SUPEERANUN

Vaccine Trial Centre, Faculty of Tropical Medicine Mahidol University, Bangkok, Thailand.

INTRODUCTION

Procholeragenoid is an aggregated intermediate in the heatmediated conversion of choleragen to choleragenoid. Germanier *et al.*, in 1976 prepared procholeragenoid by heating cholera toxin at 65 ° C for 25 minutes. This preparation exhibited less than 1% of the biologically active toxic activity of the parent toxin. The minimal residual toxic activity could be abolished by further treatment with formaldehyde. The formaldehyde-treated procholeragenoid was shown to be at least as potent as the parent toxin in stimulating serum antitoxin after immunization of rabbits (Germanier *et al.*, 1976) and could protect against intravenous challenge with toxin in mice (Finkelstein *et al.*, 1971). Further studies have shown that procholeragenoid fed to mice could protect against subsequent challenge of a ligated intestinal loop with living *V. cholerae* (Fujita and Finkelstein, 1972) and similar results were obtained in rabbits (Peterson, 1979). This preparation was found to be immunogenic when given parenterally (Germanier *et al.*, 1977), but as an oral antigen procholeragenoid without formaldehyde treatment was more immunogenic (Pierce *et al.*, 1983).

In human volunteers, procholeragenoid in doses of 50 and 200 µg with $NaHCO_3$ has been given to groups of six and four North American adults respectively without eliciting adverse reactions (Levine *et al.*, 1983). Three doses of procholeragenoid (50 µg, 50 µg and 200 µg) in combination with 2×10^{11} killed *V. cholerae* were given approximately a fortnight apart to immunize volunteers (Black *et al.*, 1986). No adverse effects were recorded, and 13 out of 17 volunteers had serum antitoxin responses after vaccination. Vaccinees subsequently challenged were found to have a significant reduction in the severity of cholera. However, the attack rate was similar to that of controls.

To determine if a larger dose of procholeragenoid could result in a better immune response and greater protection in humans, the response to different doses of procholeragenoid was studied in order to define the maximum safe dose which can be given without causing any diarrhoea in Thai adults.

MATERIALS AND METHODS

Volunteers 14 healthy adult males aged

This study was supported by the Diarrhoeal Diseases Control Programme of the World Health Organization.

20 to 30 years with no history of cholera vaccination for the past 5 years gave informed consent to participate in the study. Volunteers were randomly allocated into groups to receive sequentially increasing doses of the vaccine. It was intended that 3 volunteers would receive 50 μg and that sequential groups of 3 would then be given 100 μg and 250 μg etc., until diarrhoea occurred.

Vaccine Procholeragenoid Lot No. 118-1 was prepared by Swiss Serum and Vaccine Institute, Berne. The product was supplied in lyophilized form with each vial containing 1 mg of procholeragenoid; 1 ml of distilled water was added to rehydrate the sample before use.

Administration of vaccine Groups of volunteers were sequentially admitted into the isolation ward of the Vaccine Trial Centre, Mahidol University, Bangkok, Thailand. In the first group, 3 volunteers received 50 μg and 3 received 100 μg of procholeragenoid. Volunteers were admitted one day before vaccination and were observed closely for 48 hours thereafter. If neither diarrhoea nor other symptoms occurred, they were discharged. If any clinical symptoms developed, they were treated and discharged 24 hours after all symptoms disappeared.

Stool collection and grading All stools were collected and their amounts were measured and recorded. The consistency of stools was graded on a five-point scale as described below:

Grade 1 – firm
Grade 2 – soft
Grade 3 – thick liquid
Grade 4 – opaque watery
Grade 5 – rice water

Grade 1 and 2 were classified as normal while grades 3-5 were considered abnormal.

Definition of diarrhoeal illness Diarrhoea was defined as the passage of two or more unformed (grade 3-5) stools over a 48 hour period with a total weight of at least 200 g or a single loose stool of 300 g or greater.

RESULTS

The volunteers receiving 50, 100 and 250 μg vaccine doses were comparable in terms of age, sex, weight, height and history of cholera vaccination (Table 1 and 2). No volunteers had received cholera vaccine during the previous five years.

The clinical responses to a single dose of procholeragenoid are shown in Table 3. All of the volunteers who received 50 μg of procholeragenoid passed only normal stools. Three of 8 volunteers who received the 100 μg dose had loose stools; but none of them had stools which could be classified as a diarrhoeal illness. The details are as follows: one of the first group of three volunteers passed a single grade 3 stool weighing 225 g. Of two other volunteers who were retested, one had a grade 3 stool of 115 g during the 48 hour follow-up period. Another three volunteers were retested again, of whom one had a single grade 3 stool weighing 95 g.

With the higher dose of 250 μg of procholeragenoid, 2 of 3 volunteers had a definite diarrhoeal illness. One of them had one grade 3 stool of 330 g. The other volunteer passed two grade 3 stools in a 48 hour period with the total weight of 295 g.

Since the maximum safe dose of procholeragenoid was defined as the highest dose which did not produce any diarrhoeal illness in volunteers, 100 μg of procholeragenoid was therefore the maximum safe dose in Thai volunteers.

Table 1

Characteristics of Volunteers

		Procholeragenoid		
		50 μg (n = 3) mean ± SE	100 μg (n = 8) mean ± SE	250 μg (n = 3) mean ± SE
Age	(Years)	23.0 ± 0.6	22.5 ± 0.27	22.7 ± 0.3
Weight	(Kilograms)	62.2 ± 2.2	59.4 ± 1.8	60.6 ± 1.8
Height	(Centimetres)	171.1 ± 1.2	167.6 ± 0.8	166.0 ± 1.5

Table 2

Number of volunteers by history of previous cholera vaccination.

History of previous cholera vaccination (years)	Procholeragenoid		
	50 μg	100 μg	250 μg
< 5	0	0	0
5 – 10	0	1	0
> 10	3	7	3
Total	3	8	3

Table 3

Reactogenicity to a single dose of procholeragenoid

	50 μg (n=3)	100 μg (n=8)	250 μg (n=3)
Abdominal gurgling	1	4	2
Abdominal cramps	0	2	0
Abnormal stool			
– grade 3	0	3	2
– grade 4	0	0	0
– grade 5	0	0	0
Diarrhoeal illness	0	0	2

DISCUSSION

Procholeragenoid retains less than 1% of the residual biological activity of the parent cholera toxin. An appropriate dose in man should stimulate an antitoxin response without causing any adverse reactions. Pierce *et al.*, in 1983 showed that 500 μg of procholeragenoid did not result in diarrhoea in dogs. At the Centre for Vaccine Development at the University of Maryland, Baltimore, 50 μg was arbitrarily chosen for the first two doses and 200 μg for the third dose of vaccination (Black *et al.*, 1986). The result of this study indicates that adult Thai volunteers can tolerate up to 100 μg for the first dose of vaccination. This dosage will be used in further studies of procholeragenoid in Thai volunteers.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Professors Natth Bhamarapravati, Santasiri Sornmani, Danai Bunnag, N.F. Pierce and Dr. May Ho and to the staff members of the Vaccine Trial Centre.

REFERENCES

BLACK, R.E., LEVINE, M.M., CLEMENTS, M.L., YOUNG, C.R., SVENNERHOLM,

A-M., HOLMGREN, S. and GERMANIER, R., (1986). Oral immunization with killed whole vibrio and B subunit or procholeragenoid combination cholera vaccines: immune response and protection from *V. cholerae* challenge, *In* : K. Kuwahara and N.F. Pierce (ed.), Advances in research on cholera and related diarrheas, 3rd ed. KTK Scientific Publishers, Tokyo. pp. 271-275.

FINKELSTEIN, R.A., FUJITA, K. and LOSPALLUTTO, J.J., (1971). Procholeragenoid : and aggreagated intermediate in the formation of choleragenoid. *J. Immunol., 107* : 1043.

FUJITA, K., FINKELSTEIN, R.A., (1972). Antitoxic immunity in experimental cholera : comparison of immunity induced perorally and parenterally in mice. *J. Infect. Dis., 125* : 647.

GERMANIER, R., FURER, E., VARALLYAY, S., and INDERBITZIN, T.M., (1976). Preparation of a purified antigenic cholera toxoid. *Infect. Immun., 13* : 1692.

GERMANIER, R., FURER, E., VARALLYAY, S. and INDERBITZIN, T.M., (1977). Antigenicity of cholera toxoid in humans. *J. Infect. Dis., 135* : 512.

LEVINE, M.M., KAPER, J.B., BLACK, R.E., and CLEMENTS, M.L., (1983). New knowledge on pathogenesis of bacterial enteric infections as applied to vaccine development. *Microbiol Rev., 47* : 510.

PETERSON, J.W., (1979). Protection against experimental cholera by oral or parenteral immunization. *Infect. Immun., 26* : 594.

PIERCE, N.F., GRAY, W.C., JR., SACCI, J.B., JR., CRAIG, J.P., GERMANIER, R. and FURER, E., (1983). Procholeragenoid : a safe and effective antigen for oral immunization against experimental cholera. *Infect. Immun., 40* : 1112.