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Antigenicity testing of plain and aluminium hydroxide-adsorbed whole-cell cholera vaccines

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In memoriam Oscar Felsenfeld

Summary

The antigenicity of a plain and of an aluminium hydroxide-adsorbed whole-cell cholera vaccine was investigated by the active mouse protection test and the vibriocidal antibody production assay in mice. In the active mouse protection test, between the antigenicity of the Inaba and Ogawa component of the two vaccines was no significant difference. The antibody production test, however, revealed that the adsorbed vaccine elicited higher and longer lasting immune response than the plain one. The antibody response to a two-dose immunization schedule was substantially superior to that after a one-dose schedule.

Key words: cholera vaccine; plain; adsorbed; protection; antibody response.

Introduction

Controlled field trials carried out in Bangladesh, India and the Philippines have shown that the protection afforded by the conventional whole-cell vaccines in present use does not exceed 50–60%; the period of protection is short, generally not exceeding 3 to 6 months [7, 11, 13, 14]. Recent investigations aimed to improve the efficacy of vaccines by using adjuvants. As aluminium hydroxide-adsorbed vaccines were reported to induce higher and longer lasting antibody response in several animal models [5, 6, 19], we decided for further comparative experiments according to these lines.

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Table 1. Antigenicity testing of cholera vaccines by active mouse protection test

Challenge strain	Vaccines	RP[a]	95% confidence limits	Level of significance
Inaba	International Reference plain	1.01	0.72–1.42 (71.3–140.6%)	>0.1
	International Reference adsorbed	1.14	0.85–1.56 (74.6–136.8%)	>0.1
	plain adsorbed	1.16	0.84–1.39 (72.2–119.4%)	>0.1
Ogawa	International Reference plain	1.63	0.78–3.81 (47.8–233.7%)	>0.05
	International Reference adsorbed	1.28	0.71–2.44 (55.5–190.6%)	>0.1
	plain adsorbed	0.81	0.55–1.20 (67.9–147.8%)	>0.1

RP[a] = relative potency

Material and methods

Vaccines. For the preparation of vaccines the NIH 35A–3 Inaba and NIH 41 Ogawa strains of *V. cholerae* were used. The bacteria were cultivated on agar medium. After an 18-hour incubation period at 36° C, the harvested suspensions were inactivated by 0.02% Thiomersal (sodium ethylmercuri thiosalicylate) and heating in a water bath at 56° C for 1 hour. The finished vaccine contained 8×10^9 Inaba and 8×10^9 Ogawa organisms per ml. The adsorbed vaccine was prepared by adding to the suspension preformed aluminium hydroxide gel to a final concentration of 1.5 mg per ml; the pH of the vaccine was adjusted to 6.8. As preservative 0.01% Thiomersal was applied.

Antigenicity testing of vaccines. 1. *Active mouse protection test.* This test was carried out as outlined in the WHO Requirements for Cholera Vaccine [15, 16] in comparison with the second International Reference Preparations of Cholera Vaccine (Inaba) and of Cholera Vaccine (Ogawa) [20]. As challenge strains, *V. cholerae* Inaba NIH 35A-3 and Ogawa NIH 41 were applied. With both Inaba and Ogawa challenge 4 protection tests were carried out. The summarized data obtained in the protection tests were evaluated by variance analysis after preceding probit transformation using the parallel line assay proposed by Finney [2]. The computation of the 95% fiducial limits was made using Filler's theorem. 2. *Antibody production test.* Anti-Inaba and anti-Ogawa vibriocidal antibodies in the sera of immunized mice were determined by the method of McIntyre and Feeley [9] as modified by Verwey et al. [18]. Groups of mice (20 CFLP mice per group, of 18–20 g body weight, of both sexes in equal ratio) were inoculated intraperitoneally with 1.6×10^9 vibrios of the plain vaccine and other groups with the same amount of the adsorbed vaccine. In both the plain and the adsorbed vaccine groups one- and two-dose schedules were applied; the 2nd dose was given 30 days after the first dose. Animals were exsanguinated 30 (in the 2-dose groups just prior to the 2nd vaccine dose), 44, 60, 90, 120, 150 and 180 days after the 1st inoculation. The sera of each day-group were mixed in equal parts (from the sera of males and females separate pools were prepared) and the serum pools were stored at –20° C till use. Serum pools were tested for the presence of vibriocidal antibodies also after mercaptoethanol (2–ME) treatment of the sera.

Table 2. Antigenicity testing of cholera vaccines by antibody production test. Anti-Inaba vibriocidal antibodies

Serum sample at day	Plain vaccine				Adsorbed vaccine			
	1 dose		2 doses		1 dose		2 doses	
	native	after 2-ME treatment	native	after 2-ME treatment	native	after 2-ME treatment	native	after 2-ME treatment
30	1:1280	1:200	1:1280	1:180	1:20,480	1:480	1:15,360	1:320
44	480	100	25,600	3680	7680	1920	51,200	5120
60	480	80	10,240	1440	5120	1440	30,720	15,360
90	320	80	5120	960	3200	1280	30,720	10,240
120	240	40	3840	640	1920	480	15,360	5120
150	240	<20	1920	400	1280	320	7680	960
180	160	<20	1920	360	1280	320	2560	960

Table 3. Antigenicity testing of cholera vaccines by antibody production test. Anti-Ogawa vibriocidal antibodies

Serum sample at day	Plain vaccine				Adsorbed vaccine			
	1 dose		2 doses		1 dose		2 doses	
	native	after 2-ME treatment	native	after 2-ME treatment	native	after 2-ME treatment	native	after 2-ME treatment
30	1:7680	1:640	1:10,240	1:800	1:81,920	1:1600	1:81,920	1:1600
44	5120	960	102,400	20,480	81,960	3840	245,760	51,200
60	5120	1280	81,920	15,360	40,960	15,360	122,880	51,200
90	3200	1600	51,200	7680	30,720	7680	122,880	30,720
120	3200	2560	40,960	7680	20,480	3840	61,440	10,240
150	1920	1280	40,960	3840	10,240	2560	61,440	7680
180	800	960	30,720	3840	10,240	2560	40,960	3840

Results

Active mouse protection test. The results are shown in Table 1. From the data it is obvious that between the antigenicity (both Inaba and Ogawa) of the reference preparations and of the plain and the adsorbed vaccine there is no significant difference. Similarly, the difference between the antigenicity (both Inaba and Ogawa) of the plain and the adsorbed vaccine is not significant.

Antibody production test. The results are presented in Tables 2 and 3. The titres represent geometric mean values of the male and female serum pools tested twice. From the data the following conclusions may be drawn: a) the adsorbed vaccine elicited a higher and longer lasting immune response than the plain one; b) with both plain and adsorbed vaccine the immune response after the two-dose immunization schedule exceeds substantially that after the one-dose schedule; c) the effect of the 2-ME treatment proved that after priming mainly antibodies belonging to the IgM immunoglobulin class are produced.

Discussion

The exposed results brought evidence that the mouse protection test does not reveal a superiority of the adjuvant-adsorbed cholera vaccine over the plain one. On the other hand, the antibody production test showed distinctly superior value of the adsorbed vaccine. Recently a plain and an aluminium hydroxide-adsorbed whole-cell cholera vaccine – both prepared from the same bulk suspension – were tested (one-dose schedule) in a controlled field trial organized by the WHO in Indonesia [17]. The results have shown that the adsorbed vaccine gave greater and longer lasting protection in the age group 1 to 4 years than the plain vaccine. Similarly, an aluminium phosphate-adsorbed whole-cell vaccine [12] afforded greater and more durable protection in both children and adults [1, 3, 4].

WHO included in the Requirements for Cholera Vaccine as antigenicity test the active mouse protection test [15, 16]. But, as shown by us above and elsewhere [8], the higher value of the aluminium hydroxide-adsorbed whole-cell cholera vaccine can not be demonstrated by this test. This is in agreement with others [19]. Thus it seems that the active mouse protection test is not suitable for the antigenicity testing of aluminium hydroxide- or aluminium phosphate-adsorbed cholera vaccines. The results of previous [6, 8, 19] and of the present investigations have shown unequivocally that the vibriocidal antibody production test may be applied for the antigenicity testing of adsorbed cholera vaccines, because the results correlate with the efficacy of vaccines in man.

We have found that with both plain and adsorbed vaccines the two-dose immunization schedule elicits higher and longer lasting antibody response than the one-dose schedule. Similar observations were reported on the basis of their

investigations in volunteers by Verwey et al. [18]. The investigations of Mosley et al. [10] during the third Bangladesh field trial have shown that a single dose was virtually ineffective in children less than 5 years old. Children of this age group showed a degree of protection of 31.8% for 3 months after a single vaccination dose, while among children inoculated on the two-dose schedule, the degree of protection was 90.8%; the first dose had obviously compensated for the lack of basic immunity. On the basis of these observations, the comparative investigation in a controlled field trial of an adsorbed vaccine by applying one- and two-dose schedules, especially in 1 to 5-year-old children, seems to be indicated.

1 Cholera Research Centre, Calcutta: Controlled field trial on the efficacy of aluminium phosphate adjuvant cholera vaccine in Calcutta. Annual Report, p. 5–6 (1977).
2 Finney D. J.: Statistical methods in biological assay, 2nd ed. Griffin, London 1964.
3 Indian Council of Medical Research: Annual Report of the Director General, New Delhi, p. 13–15 (1975).
4 Indian Council of Medical Research: Annual Report of the Director General, New Delhi, p. 22 (1976).
5 Joó I., Pusztai Zs., P. Juhász V.: Studies on vaccination against cholera. IV. Experiments with adsorbed vaccines. Z. Immun.-Forsch. *133*, 317–325 (1967).
6 Joó I., Pusztai Zs., Csizér Z., P. Juhász V.: Immune response to plain and adsorbed cholera vaccines. Progr. immunobiol. Standard. *5*, 350–354 (1972).
7 Joó I.: Cholera vaccines. In: Cholera (ed. by D. Barua and W. Burrows), p. 333–355. W. B. Saunders Company, Philadelphia 1974.
8 Joó I., Csizér Z.: Preparation and laboratory testing of plain and aluminium hydroxide-adsorbed cholera vaccines used in a field trial in Indonesia. Bull. Wld Hlth Org. *56*, 615–618 (1978).
9 McIntyre O. R., Feeley J. C.: Passive serum protection of the infant rabbit against experimental cholera. J. infect. Dis. *114*, 468–475 (1964).
10 Mosley W. H., McCormack W. M., Ahmed A., Chowdhury A. K. M. A., Barui R. K.: Report of the 1966–67 cholera vaccine field trial in rural East Pakistan. 2. Results of the serological surveys in the study population – the relationship of case rate to antibody titre and an estimate of the inapparent infection rate with *Vibrio cholerae*. Bull. Wld Hlth Org. *40*, 187–197 (1969).
11 Mosley W. H., Aziz K. M. A., Rahman A. S. M. M., Chowdhury A. K. M. A., Ahmed A.: Field trial of monovalent Ogawa and Inaba cholera vaccines in rural Bangladesh – three years of observation. Bull. Wld Hlth Org. *49*, 381–387 (1973).
12 Pal S. C.: Cholera vaccines and their prospects. J. commun. Dis. *7*, 135–141 (1975).
13 Philippines Cholera Committee: A controlled field trial of the effectiveness of monovalent classical and El Tor cholera vaccines in the Philippines. Bull. Wld Hlth Org. *49*, 13–19 (1973).
14 Philippines Cholera Committee: A controlled field trial on the effectiveness of the intradermal and subcutaneous administration of cholera vaccine in the Philippines. Bull. Wld Hlth Org. *49*, 389–394 (1973).
15 Requirements for Cholera Vaccine. Wld Hlth Org. techn. Rep. Ser. No. 413, 27–44 (1969).
16 Requirements for Cholera Vaccine. Addendum 1973. Wld Hlth Org. techn. Rep. Ser. No. 530, 18–21 (1973).
17 Saroso J. S., Bahrawi W., Witjaksono H., Budiarso R. L. P., Brotowasisto, Benčić Z., Dewitt W. E., Gomez C. Z.: Controlled field trial of plain and aluminium hydroxide adsorbed cholera vaccines in Surabaja, Indonesia, during 1973–1975. Bull. Wld Hlth Org. *56*, 619–627 (1978).
18 Verwey W. F., Watanabe Y., Guckian J. C., Williams H. R. jr., Phillips P. E., Rocha S. S. jr.,

Bridgeforth E. B.: Serologic responses of human volunteers to cholera vaccine. Texas Reps. Biol. Med. *27*, Suppl. 1, 243–274 (1969).
19 Verwey W. F., Watanabe Y.: Vaccine development. In: Symposium on cholera. Proceedings of the 6th Joint Conference, US-Japan Cooperative Medical Science Program, p. 97–105. National Institute of Health, Tokyo 1971.
20 Wld Hlth Org. techn. Rep. Ser. No. 486, 13 (1972).