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Vaccine 19 (2001) 213–216

Vaccine

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Anthrax vaccine: increasing intervals between the first two doses enhances antibody response in humans

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Received 27 April 2000; received in revised form 8 May 2000; accepted 8 May 2000

Abstract

The influence of dosing interval on the human antibody response to anthrax vaccine adsorbed (AVA) was evaluated in two retrospective serological studies. In both studies, the interval between the first two doses was 2, 3 or 4 weeks. In the first study, banked sera were selected from 89 at-risk individuals at a mean time of 13 days after the second dose of vaccine. In the second study, banked sera were selected from 51 at-risk individuals at a mean time of 48 days following the first dose of AVA. In both studies, the geometric mean anti-protective antigen IgG antibody titer increased significantly as the interval between the two doses increased from 2 to 4 weeks ($p = 0.0005$–0.029). In the first study, the seroconversion rate also increased as the interval between the first two doses increased ($p = 0.0034$). A prospective, randomized study has been completed and is being analyzed to confirm these findings. Published by Elsevier Science Ltd.

Keywords: Anthrax vaccine; Protective antigen ELISA titers

1. Introduction

The Anthrax Vaccine, Adsorbed (AVA, BioPort Corporation, Lansing, MI) licensed vaccination schedule of 0, 2, and 4 weeks; and 6, 12, and 18 months with subsequent yearly boosters was established in the 1950s [1].

Protective antigen (PA) is the protective component of AVA which stimulates a protective immune response against infection with *Bacillus anthracis*. A predecessor of AVA, an alum-precipitated vaccine, was first administered to 55 humans as two 0.5 ml subcutaneous doses given 2 weeks apart [2]. This vaccination regimen was acceptably safe in humans. The next group of 660 individuals received three 0.5 ml doses 2 weeks apart and a boost at 6 months. Brachman et al., who used this earlier vaccine in a human anthrax vaccine field trial during the 1950s, indicated that the vaccination schedule was based on results of animal vaccination studies [3]. Later, investigators developed a more potent anthrax vaccine adsorbed onto aluminum hydroxide using the same vaccination schedule [4–6].

The dose interval is an important determinant of a vaccine's immunogenicity. After an initial 'priming'

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0264-410X/00/$ - see front matter Published by Elsevier Science Ltd.
PII: S0264-410X(00)00174-2

dose, there is generally a time at which an additional one or more doses can be administered to generate an optimal anamnestic response. This booster dose of antigen is usually given after the antibody response to the priming dose begins to wane. In most cases 3 or more weeks between two of the early doses are required to achieve a meaningful booster response. It is possible that the 2-week dose of AVA may offer little or no immune enhancement.

We sought to determine if an increased interval between the two initial doses in the AVA vaccination schedule was associated with an enhanced humoral response. To assess this, the serum data bank of the special immunizations program for at-risk laboratory workers at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) was searched for serum specimens from subjects who had received a second dose of AVA beyond the 2-week interval specified for the product. These sera were tested for anti-PA IgG antibodies with an ELISA that employed a recombinant PA (rPA) antigen.

2. Materials and methods

2.1. Source of human serum samples for rPA antibody determination

Blood samples are routinely drawn from at-risk laboratory personnel enrolled in the USAMRIID special immunizations program. These serum samples are stored at −20°C in controlled freezers, and information on each serum sample is stored in a data bank [7,8]. The data bank was scanned to identify sera from subjects who had received AVA.

Two retrospective analyses were conducted that could provide antibody response data on the first two doses given at 2-, 3-, and 4-week intervals. In the first study, serum samples were analyzed at a mean of 13 days (ranging from 7 to 19 days) after the second dose was administered, regardless of the interval between the two doses of AVA. There was no significant difference ($p = 0.86$) among the 2-, 3-, or 4-week groups in the mean time the sera were drawn after the second dose (Table 1).

In the second study, the data bank was scanned for sera that were drawn 7 weeks (40–62 days, mean of 48 days) after administration of the first dose and that had an interval of 2, 3, or 4 weeks between the first and second doses. There was no significant difference ($p = 0.47$) in the mean time the sera were drawn among the 2-, 3-, or 4-week groups (Table 2). The titers, therefore, represent time points at 5, 4, and 3 weeks after administration of the second dose for the 2-, 3-, and 4-week interval groups, respectively.

2.2. Capture ELISA for detection of human anti-PA antibodies

The capture ELISA using rPA from baculovirus was described in detail by Iacono-Connors et al. [9]. This rPA when used as a vaccine protected guinea pigs against a parenteral challenge with *B. anthracis* [10]. ELISA results were expressed as antibody titer, defined as the reciprocal of the last dilution equal to or greater than an OD value of 0.2. Sera were coded and tested beginning at a 1:100 dilution.

2.3. Statistical analyses

Response rates were compared using Fisher's exact test, two-tailed. Geometric mean titers were compared by using analysis of variance (ANOVA) on the log-transformed titers followed by multiple comparisons with the Tukey–Kramer method. The Jonckheere–Terpstra trend test, a method for analyzing trends in increasing response rate with increasing interval between doses of vaccine was applied as well. Analyses were performed using SAS version 6.12 and exact methods for trends by using StatXact Turbo [11]. All tests were at the 95% confidence (two-tailed) level.

Table 1
Anti-PA IgG ELISA titers in human sera obtained within 3 weeks of the second dose of AVA when this second dose was administered at 2, 3 or 4 weeks after the first dose

Interval between doses (weeks)	Number of subjects	Mean time (days) from second dose (SD[a])	Geometric mean titer (GMT)	GMT p-value by ANOVA[b]	% Subjects with titers ≥1:100
2	74	13.3 (2.7)	81	–	46
3	9	13.2 (2.3)	252	0.0005	78
4	6	12.7 (2.9)	317	0.0005	100
Trend test[c]					0.0034

[a] SD = standard deviation.
[b] ANOVA = analysis of variance; 2-week interval as referent.
[c] Trend test p-value by Jonckheere–Terpstra method.

3. Results

3.1. Antibodies to rPA in human sera obtained 2 weeks after administration of two doses of AVA with varying intervals between the two doses

Antibody titers were obtained on sera drawn within 2 weeks after the second dose of AVA, when the second dose was administered at weeks 2, 3, or 4 after the first dose. Baseline serum titers before the first inoculation were < 1:100 in all cases except one subject who had a missing baseline serum sample. Table 1 shows the rate of seroconversion (titer ≥1:100) increased as the interval between the two doses increased: from 46% to 78% to 100%. The test for trend shows that the increasing seroconversion rate with increasing interval between doses is statistically significant ($p = 0.0034$). The geometric mean titer of the sera increased three- to four-fold as the interval between the two doses of AVA increased from 2, to 3, to 4 weeks. ANOVA shows these differences to be statistically significant when the geometric mean titer of the two week interval group is compared to the 3 or 4 week interval group ($p = 0.0005$, for both groups).

3.2. Antibodies to rPA in human sera obtained 7 weeks after administration of the first dose when the second dose was administered at 2, 3, or 4 weeks after the first dose

Higher antibody titers in the above groups with an increased interval between the first two doses may have been due to the increased length of time from the first dose to the time the sera was assayed. This is also supported by the observation that in subjects given doses of AVA at 0 and 2 weeks, there was an increase in seroconversion rate from 46% (Table 1) to 95% (Table 2) when sera were analyzed at 4 and 7 weeks from day 0, respectively. Sera from subjects who received a single dose of AVA would be helpful in evaluating whether the increase in titer was due to the increased time after the first dose when the sera were assayed. Because there were not adequate numbers of single-dose sera recorded in the data bank, we decided to examine two-dose sera obtained at a constant time after the first dose, recognizing the complication of increasing intervals of 2, 3, or 4 weeks for the second dose. There was a three- to four-fold increase in geometric mean titers (GMT) at week 7 as the interval between doses increased from 2, to 3, to 4 weeks. Compared to the 2-week interval group GMT, the differences (ANOVA) in GMTs for the 3 and 4 week groups were statistically significant, $p = 0.029$ and 0.013, respectively (Table 2).

4. Discussion

Although retrospective, this is the first study evaluating the human antibody response to AVA after different vaccination schedules. In the present study we found that the anti-PA IgG antibody titers increased as the interval between the first two doses increased from 2 to 4 weeks. This was true whether the antibody response was evaluated at a standard interval (mean of 48 days) from the first dose or 2 weeks (mean of 13 days) after the second dose. Both studies showed a three- to four-fold increase in geometric mean anti-PA IgG antibody titer as the interval between the first and second doses of AVA increases from 2 to 4 weeks. The difference in the level of anti-PA IgG titer detected in the two studies may be related to the interval between the two doses as well as the timing of the titer in relation to the second dose. The intervals between the second dose and the timing of the blood draw for the determination of anti-PA IgG titer is a constant 2 weeks in study 1 but is 5, 4 or 3 weeks in study 2. The difference in seroconversion rates between the two studies appears to be related to the total length of time following the initial dose. Thus, seroconversion rates range from 46% to 78% to 100% as determined by blood samples taken 2 weeks after dose 2 in the first

Table 2
Anti-PA IgG ELISA titers in human sera obtained at a fixed time (7 weeks) after the first dose of AVA at day 0 with the second dose administered 2, 3 or 4 weeks after the first dose

Interval between doses (weeks)	Number of subjects	Mean time (days) from first dose (SD[a])	GMT[b]	*P*-Value[c] for GMT	% Subjects with titers ≥ 1:100 (*p*-value[d])
2	21	49 (6.8)	450	–	95
3	19	47 (5.8)	1225	0.029	100 (1.00)
4	11	49 (7.0)	1666	0.013	100 (1.00)

[a] SD = standard deviation.
[b] GMT = geometric mean titer.
[c] ANOVA = analysis of variance; 2 weeks is referent.
[d] Fisher's exact test (two-tailed); 2 weeks is referent.

study but increased from 95% to 100% in the second study.

In summary, this retrospective, serological analysis demonstrates that increasing the interval between the first two doses of AVA resulted in an increase in the titer of anti-PA IgG antibodies. Based on these observations, and because of the limitations of a retrospective study, a prospective, randomized clinical trial was conducted to evaluate the effect of a single dose and various intervals between the first two vaccine doses on antibody response to AVA. The analysis of the results of this clinical trial is nearing completion and will be presented in a future publication.

References

[1] Anthrax vaccine adsorbed [package insert]. Lansing, Michigan, Department of Public Health; 1978.
[2] Wright GG, Green TW, Kanode Jr. RG. Studies of Immunity in Anthrax. V. Immunizing activity of alum-precipitated protective antigen. J Immunol 1954;73:387–91.
[3] Brachman PS, Herman G, Plotkin SA, Fekety R, Werrin M, Ingraham NR. Field evaluation of a human anthrax vaccine. Am J Pub Hlth 1962;52:632–45.
[4] Wright GG, Puziss M, Neely WB. Studies on immunity in anthrax. IX. Effect of variations in cultural conditions on elaboration of protective antigen by strains of *Bacillus anthracis*. J Bacteriol 1962;83:515–22.
[5] Puziss M, Wright GG. Studies on immunity in anthrax. X. Gel-Adsorbed protective antigen for immunization of man. J Bacteriol 1963;85:230–6.
[6] Puziss M, Manning LC, Lynch JW, Barclay E, Abelow I, Wright GG. Large-scale production protective antigen of *Bacillus anthracis* in anaerobic cultures. Appl Microbiol 1963;11:330–4.
[7] Pittman PR, Makuch RS, Mangiafico J, Cannon TL, Gibbs PH, Peters CJ. Long-term duration of detectable neutralizing antibodies after administration of live-attenuated VEE vaccine and following booster vaccination with inactivated VEE vaccine. Vaccine 1996;14:337–43.
[8] Pittman PR, Liu CT, Cannon T, Makuch RS, Mangiafico JA, Gibbs PH, Peters CJ. Safety and immunogenicity of an inactivated Rift Valley fever vaccine in humans over a 12-year period. Vaccine 1999;18:181–9.
[9] Iacono-Connors LC, Novak J, Rossi C, Mangiafico J, Ksiazek T. Enzyme-linked immunosorbent assay using a recombinant baculovirus-expressed *Bacillus anthracis* protective antigen (PA): measurement of human anti-PA antibodies. Clin Diag Lab Immunol 1994;1:78–82.
[10] Iacono-Connors LC, Welkos SL, Ivins BE, Dalrymple JM. Protection against anthrax with recombinant virus-expressed protective antigen in experimental animals. Infect Immun 1991;59:1961–5.
[11] StatXact Turbo User Manual, "Statistical Software for Exact Non-parametric Inference," Cytel Corp., 1992. pp. 547–554.