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Anthrax vaccine: immunogenicity and safety of a dose-reduction, route-change comparison study in humans☆

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Abstract

Anthrax vaccine adsorbed (AVA), an effective countermeasure against anthrax, is administered as six subcutaneous (SQ) doses over 18 months. To optimize the vaccination schedule and route of administration, we performed a prospective pilot study comparing the use of fewer AVA doses administered intramuscularly (IM) or SQ with the current schedule and route. We enrolled 173 volunteers, randomized to seven groups, who were given AVA once IM or SQ; two doses, 2 or 4 weeks apart, IM or SQ; or six doses at 0, 2, 4 weeks and 6, 12, and 18 months (control group, licensed schedule and route). IM administration of AVA was associated with fewer injection site reactions than SQ administration. Following the first SQ dose of AVA, compared to males, females had a significantly higher rate of injection site reactions such as erythema, induration and subcutaneous nodules ($P < 0.001$). Reaction rates decreased with a longer dose interval between the first two doses. The peak anti-PA IgG antibody response of subjects given two doses of AVA 4 weeks apart IM or SQ was comparable to that seen among subjects who received three doses of AVA at 2-week intervals. The IM route of administering this aluminum hydroxide adsorbed vaccine is safe and has comparable peak anti-PA IgG antibody levels when two doses are administered 4 weeks apart compared to the licensed initial dose schedule of three doses administered 2 weeks apart. A large pivotal study is being planned by the Centers for Disease Control and Prevention to confirm these results. Published by Elsevier Science Ltd.

Keywords: Anthrax vaccine adsorbed; Immune response to protective antigen; Randomized clinical trial; AVA gender safety

1. Introduction

Anthrax, the disease caused by *Bacillus anthracis* spores, is the pre-eminent bioterrorist and biological warfare threat to civilian and military populations [1–3]. Inhalation anthrax, the form of anthrax likely to result from the intentional release of spores, is extraordinarily rare in nature with only approximately 20 cases reported in the US in the last century [4]. The onset of fever, malaise, and fatigue, sometimes with a non-productive cough and mild chest discomfort, is usually gradual and non-specific. The initial symptoms are followed in 2–3 days by the abrupt development of severe respiratory distress with dyspnea, diaphoresis, stridor, and cyanosis. Hypotension and death usually follow within 24–36 h of the onset of respiratory distress [4–8]. Survival has rarely been reported [4,8–11].

Anthrax vaccine adsorbed (AVA) has been a highly effective prophylactic agent against inhalational anthrax in laboratory animals, including rhesus monkeys [12,13]. AVA was licensed for use in the US in 1970. The product label recommends 0.5 ml subcutaneous (SQ) injections at 0, 2, and 4 weeks and 6, 12, and 18 months with annual revaccination as long as the individual is at risk of infection with anthrax [14]. In a previous serological analysis, we found that as the intervals between the first two doses of AVA increased from 2 to 4 weeks, the magnitude and rate of antibody response also increased [15]. Based upon passively

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collected data, we have known since the mid-1990s that the rate of SQ injection site reactions was higher among women compared to men [16]. AVA is the only vaccine containing an aluminum compound that is licensed by the US Food and Drug Administration (FDA) that is administered SQ. All other FDA-licensed vaccines containing aluminum compounds (DTaP, DT, DTP-HbOC, HepA, HepB, etc.) are administered IM.

The elimination of one dose in the initial vaccination series of AVA from three to two doses without a reduction in antibody response to *B. anthracis* would offer significant advantages for mass prophylaxis in the face of exposure to aerosolized anthrax. The logistical problems of vaccinating large numbers of individuals would be reduced, and the potential for adverse events would decrease.

The primary objective of this randomized, open-label study was to select an optimal two-dose initial vaccination schedule and route of administration based on antibody response and reactogenicity. We compared the responses of subjects receiving the abbreviated schedule with those of subjects after the standard three SQ doses given 2 weeks apart over 4 weeks. The primary endpoints for this study were anti-PA IgG concentration and antibody response rates. The secondary endpoints were systemic and local reactions observed after IM and SQ administration of AVA. Finally, to the extent possible, in a pilot study, we aimed to discern if indeed females had a higher reaction rate than men.

2. Methods

The protocol for this prospective, randomized, open-label study was approved by the institutional review boards at USAMRIID and the Office of the Surgeon General of the Army and was submitted to the FDA. Personnel conducting antibody assays were blinded to subjects, treatments and weeks of blood draw. All subjects provided written informed consent. This clinical research was conducted in accordance with ethical principles that have their origins in the Declaration of Helsinki and in accordance with federal regulations and guidelines.

2.1. Study subjects

One hundred seventy-one men and women, ages 18 through 64 years, were assigned randomly to one to seven study groups (22–28 subjects per group), without regard to demographics. Two additional military subjects who required the licensed schedule and route were added to the control group, yielding a total enrollment of 173 subjects. Subjects were not enrolled in the study if they were pregnant, HIV positive, or acutely ill with an oral temperature $\geq$38.3 °C.

Subjects were given AVA (0.5 ml per dose) according to one of seven regimens (Table 1). Six groups received the anthrax vaccine SQ or IM at week 0, weeks 0 and 2, or weeks 0 and 4. The control group received SQ doses at 0, 2, and 4 weeks and 6, 12, and 18 months (licensed schedule). A single lot (FAV032) was used throughout the study. Two subjects did not complete the initial series (subject no. 118 in the 0–4 IM group was incarcerated for alleged auto theft, and subject no. 145 in the control group developed urticaria after the second dose of AVA).

2.2. Test article

AVA, an FDA-licensed product in the United States, is prepared from a sterile culture filtrate containing the protective antigen of an avirulent strain of *B. anthracis*, V770-NP1-R, which was adsorbed onto an aluminum hydroxide adjuvant. The vaccine was provided by BioPort Corporation (Lansing, MI) [14].

2.3. Safety evaluation

Volunteers were evaluated clinically for local and systemic reactions at 30 min, 1–3 days, 1 week, and 1 month after each vaccination. Reactions were determined to be

Table 1
Anti-PA IgG antibody geometric mean concentration (GMC) at peak (week 6) and seroconversion rate for all seven study groups

Schedule	Women	Men	Mean age (range)	GMC in μg/ml (95% CI)	*P*-value[a]	Seroconversion rate[b]	*P*-value
0–2–4 SQ[c]	7	21	32 (19–52)	478 (313–728)	–	28/28 (100)	–
0 SQ	8	17	34 (21–60)	36 (22–58)	0.0001	14/25 (56)	<0.0001
0 IM	10	15	34 (20–61)	18 (15–22)	0.0001	7/24 (29)	<0.0001
0–2 SQ	11	14	35 (19–56)	205 (132–320)	0.005	25/25 (100)	1.000
0–2 IM	8	17	32 (19–57)	147 (89–245)	0.0001	24/25 (96)	0.47
0–4 SQ	10	13	32 (20–60)	625 (404–971)	0.65	23/23 (100)	1.000
0–4 IM	10	12	33 (19–64)	482 (304–764)	1.000	20/21 (96)	0.43

[a] *P*-values for GMC comparisons were from Student *t*-tests, and for rate comparisons were from Fisher exact tests, in all cases compared to the referent group. The *P*-values for the 4-week interval groups were adjusted for multiple comparisons. The 0–4 SQ versus IM comparison had a *P*-value of 0.691; the 0–4 SQ, 0–4 IM and the referent group had an overall *P*-value of 0.615.

[b] Seroconversion rate = the percentage of volunteers in a group exceeding minimum detectable anti-PA IgG concentration (>25 μg/ml) at least once during 0–8 weeks after first AVA dose.

[c] The licensed schedule group is the referent group.

present or absent, measured for maximum dimension, or graded for severity. Local reactions are defined as signs and symptoms at the injection site, such as erythema, induration, swelling, pain, pruritis, etc. Systemic reactions are all reactions not at the injection site itself.

2.4. Anti-PA IgG concentration

Blood (15 ml) was drawn at scheduled intervals (weekly for the first 12 weeks, then at weeks 14, 16, 18, 20, and 24) from each subject. Serum sample tubes were identified by tube number and date of blood collection. Immune response to AVA was determined by assay of antibody specific for *B. anthracis* PA by using a validated ELISA developed for this study. The peak PA-specific IgG concentration was chosen as an endpoint because it correlated with protection against inhalation anthrax in rabbits [17]. The overall cumulative rates of antibody response were determined based on a positive response at least once up to 8 weeks after the first dose. The limit of detection of the assay was 25 μg/ml; an IgG concentration >25 μg was considered a positive response.

2.5. Toxin neutralization

A validated, in vitro cytotoxicity assay, the toxin-neutralizing antibody (TNA) assay developed for this study was used to assess a random subset of serum samples for their ability to neutralize anthrax lethal toxin. The assay was a modification of the procedure described by Moseman [18] and Hansen et al. [19]. All samples were performed in triplicates. The final concentrations of *B. anthracis* PA and LF were 50 ng/ml PA and 40 ng/ml LF.

Absorbance was read at dual wavelengths (570 nm and a reference wavelength at 690 nm) using a microplate reader, and calculated the mean, standard deviation, and coefficient of variation for each triplicate sample dilution of all the standards, controls and test samples. Using the linear regression curve, the dilution of the positive anti-AVA standard and the test sample that resulted in 50% neutralization of the anthrax lethal toxin (effective dilution 50 or ED50) was determined. Finally, using this value as a standard, the neutralization capacity of test samples was calculated by dividing the ED50 of the test sample by the ED50 of the standard (neutralization factor 50 or NF50) [18,19].

2.6. Statistical analysis

Based on a previous study, a minimum sample size of 20 per group was required to detect a difference by analysis of variance (ANOVA) in peak titers after two doses with 80% power when testing at the 95% confidence level (two-tailed) [15].

Peak mean $\log_{10}$ anti-PA IgG concentrations were tested for differences by ANOVA with Tukey's adjustment for multiple comparisons. The reverse cumulative distributions of peak IgG concentration were compared by using a non-parametric test for equality of distributions and linear trends in decay from peak by regression analysis. Treatments were compared at each week after the first dose by a bootstrap re-sampling technique to control for multiple testing. The results of the TNA assay were compared with the ELISA results on a subset of samples by correlation analysis. All statistical analyses were performed using SAS version 6.12 [20].

Systemic and local reaction rates were compared by using Fisher exact tests and logistic regression analyses. An intent-to-treat analysis was performed by using all available data from all enrolled subjects.

3. Results

The mean age among the groups ranged from 32 to 35. 37% of the volunteers (64/173) were females. 58% (101/173) of the volunteers received the vaccine SQ. Of those, 21% (36/101) were females. No statistical difference was observed among the study groups for gender ($P = 0.678$) or age ($P = 0.965$). Women received 46 doses IM and 71 doses SQ; men received 72 doses IM and 132 doses SQ.

3.1. Antibody response

3.1.1. Anti-PA IgG concentration

Between weeks 4 and 24, anti-PA IgG concentrations were significantly lower in groups given only one dose or in groups given two doses of AVA 2 weeks apart as compared with the control group (the P-value for the study groups compared to the control group ranged between $P = 0.0001$ and 0.047) (Fig. 1A and B).

No difference was observed from week 6 to 24 in the antibody response of subjects given the vaccine SQ or IM 4 weeks apart (Fig. 1C). Both of these reduced-schedule vaccination groups had statistically lower anti-PA IgG responses at weeks 4 and 5 compared with the control group ($P < 0.001$–0.004). Thereafter, no difference was detected between the 4-week interval reduced schedule and the licensed schedule to week 24, including the peak of IgG concentration at week 6, when the three groups were compared overall ($P = 0.615$). The rate of linear decay from peak to week 24 was not different among the three competing groups ($P = 0.317$).

Table 1 shows the geometric mean antibody concentration (GMC) at peak for all groups (the peak for the 0 group was at 3–4 weeks; for the 0–2 group at 4–5 weeks; and for the 0–4 and 0, 2, 4 groups at 6 weeks). The single-dose groups IM or SQ had low seroconversion rates (29 and 60%, respectively) and achieved a low GMC that peaked between weeks 3 and 4. The antibody concentrations for the 0–2 week groups IM or SQ peaked at week 4–5 and were inferior to those seen with the standard schedule ($P = 0.0001$ and 0.005, respectively). The antibody concentrations for the IM or SQ groups given AVA 4 weeks apart peaked at week 6 and were comparable

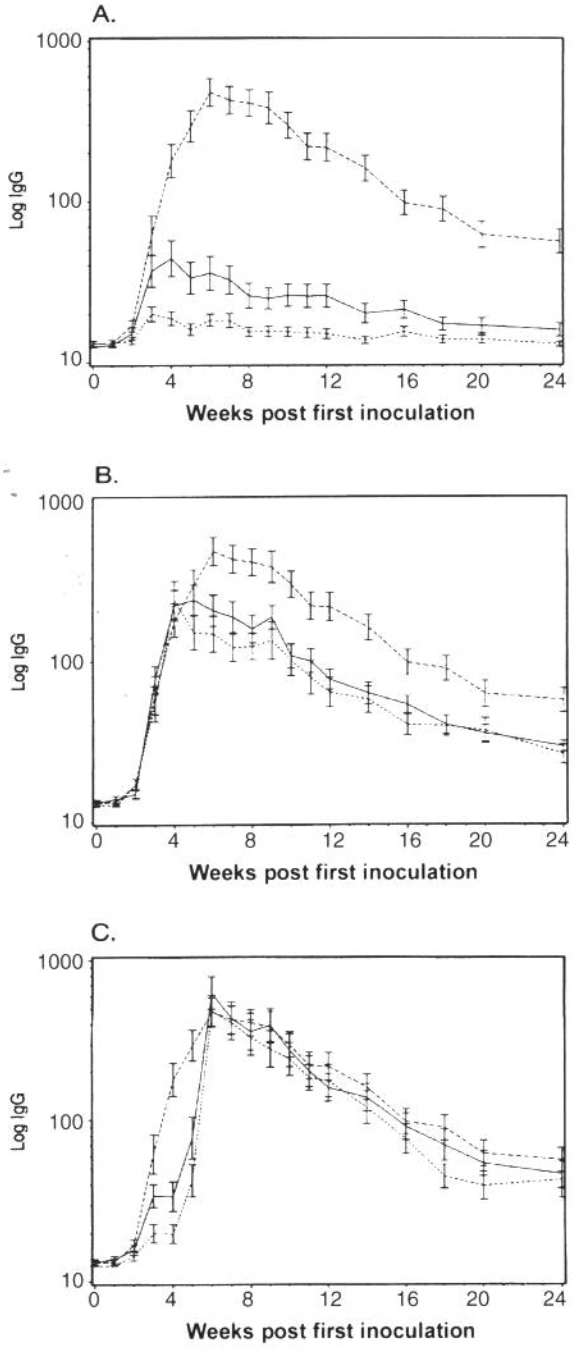

Fig. 1. Antibody response to priming doses of AVA. (A) Comparison of anti-PA IgG concentrations: licensed vaccination schedule vs. week 0. Group: 0–2–4 SQ (- - -); 0 IM (· · ·); 0 SQ (—). (B) Comparison of anti-PA IgG concentrations: licensed vaccination schedule vs. weeks 0–2. Group: 0–2–4 SQ (- - -); 0–2 IM (· · ·); 0–2 SQ (—). (C) Comparison of anti-PA IgG concentrations: licensed vaccination schedule vs. weeks 0–4. Group: 0–2–4 SQ (- - -); 0–4 IM (· · ·); 0–4 SQ (—). Bars represent 1 standard error of the geometric mean.

to those seen with the standard schedule ($P = 1.0$ and 0.65, respectively). Seroconversion rates were 96–100% for all 0–2 and 0–4 groups. Same-route comparison of the 0–2 and 0–4 week schedules showed that the groups given AVA 4 weeks apart had approximately three-fold or higher anti-PA IgG concentration at peak compared to the groups given the vaccine 2 weeks apart.

3.1.2. Peak antibody response distribution

The cumulative distribution of peak (week 6) anti-PA IgG concentrations did not differ between the 0–4 IM, 0–4 SQ, and control groups ($P = 0.996$, Kolmogorov–Smirnov test) (Fig. 2). When tested for non-inferiority in GMC compared with the licensed schedule based on peak IgG concentration (week 6) by using two-tailed confidence intervals, the anti-PA IgG response of the 0–4 IM group was 100.9% (95% confidence limits of 48–213%) and that of the 0–4 SQ group was 131.2% (63–272%) of the GMC response of the control group. The reduced (0–4) vaccination schedule given by either route was comparable to the 0–2–4 SQ schedule at peak (week 6).

3.1.3. Toxin neutralization assay

Toxin neutralizing antibody titers were correlated with ELISA anti-PA IgG antibody data on a randomly selected subset of 10 subjects in all groups except in the single-dose group. Consistent with the ELISA results, no difference in geometric mean or TNA titers was detected at peak (week 6) between groups 0–2–4 SQ, 0–4 SQ, and 0–4 IM ($P = 0.384$) (data not shown). For samples with detectable neutralizing antibody titers, the correlation between anti-PA IgG concentrations and the neutralization ratio was 0.75. With the criteria of ELISA $> 25\ \mu g/ml$ and neutralization ratio > 0 as positive assays, the assays agreed in 211/239 samples (88%). In 25 of the 154 samples (16%) which were ELISA positive, neutralization was not detected and in 3 of the 85 samples that were ELISA negative, neutralization was detected. A high association was detected in positive rates between the two assays with the Fisher exact test ($P < 0.0001$). Also, time profiles of the two assays (rise, peak, and decline) had similar patterns over 26 weeks regardless of the level of activity or concentration measured.

3.2. Safety

3.2.1. Safety evaluation

Ten acute adverse events were reported within the first 30 min after a total of 372 vaccine injections (2.7%). Local pain, erythema, and headache were the most common reactions. In addition, one case of muscle aches, and one individual with a temperature increase above 38 °C were seen. All resolved and did not occur after a subsequent dose.

Serious adverse events (SAEs) unrelated to administration of AVA were reported for four hospitalized volunteers: motor vehicle collision, herniated disc, cholecystectomy, and syncopal episode. The time between the last dose of AVA and SAEs were 45 days for the syncopal episode and approximately 6 months for all other SAEs. Seven women became pregnant after AVA doses were completed and gave birth to 8 healthy babies (one volunteer had two separate pregnancies).

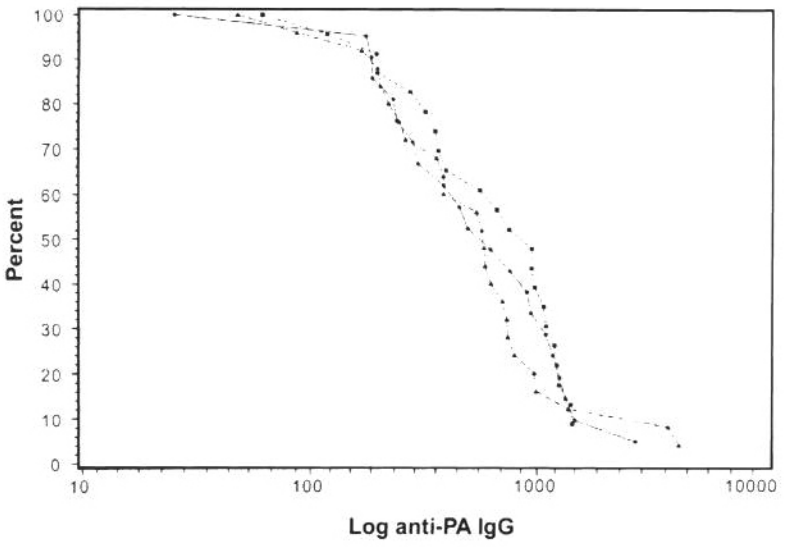

Fig. 2. Reverse cumulative anti-PA IgG distributions at week 6. The reverse cumulative anti-PA IgG distributions at week 6 for the control group, 0–4 IM, and 0–4 SQ groups. This plot displays the cumulative percent of anti-PA IgG concentrations on the $\log_{10}$ scale which exceeds each anti-PA IgG value. No difference ($P = 0.996$) in the distribution was detected by the Kolmogorov–Smirnov test. Group: 0–4 IM (●); 1–4 SQ (■); 0–2–4 SQ (▲).

3.2.2. Systemic reactions

Systemic vaccine-related adverse events recorded after each dose of AVA were independent of route, gender, or dose interval (Table 2). No serious reaction attributable to administration of the vaccine was observed. Headache, malaise, anorexia, and nausea were the most frequent complaints. One control subject experienced a delayed systemic rash characteristic of urticaria after the second dose of AVA.

Table 2
Systemic AVA-related adverse events for each gender after 1–3 doses of AVA[a]

Reaction	Gender	IM incidence (%)	SQ incidence (%)
Headache	Female	8/46 (17.4)	8/71 (11.3)
	Male	5/72 (6.9)	12/132 (9.1)
Anorexia	Female	4/46 (8.7)	0/71 (0)
	Male	2/72 (2.8)	3 (2.3)
Malaise	Female	3/46 (6.5)	6/71 (8.5)
	Male	3/72 (4.2)	13/132 (9.8)
Myalgia	Female	1 (2.2)	5/71 (7.0)
	Male	4/72 (5.6)	4/132 (3.0)
Nausea	Female	3/46 (6.5)	2/71 (2.8)
	Male	2/72 (2.8)	3/132 (2.3)
Respiratory difficulty	Female	1/46 (2.2)	1/71 (1.4)
	Male	3/72 (4.2)	4/132 (3.0)
General pruritus	Female	0/46 (0)	2/71 (2.8)
	Male	0/72 (0)	3/132 (2.3)
Fever	Female	0/46 (0)	1/71 (1.4)
	Male	1/72 (1.4)	4/132 (3.0)

[a] Total number of first three doses administered: IM = 118; SQ = 203.

3.2.3. Local reactions

The occurrence of certain local, vaccine-related adverse events was related to both gender and route of administration of AVA (Table 3). The most common local adverse event in dose series 1–3 was tenderness at the injection site (49–85%), followed by SQ nodules (0–63%) and erythema (5.6–63%). Local adverse events such as SQ nodules, erythema, induration, and edema were more common in women and associated with the SQ route of administration. SQ nodules did not occur when AVA was given by the IM route. Erythema, induration, and edema were uncommon adverse events after IM administration of AVA and were less common among men compared to women when given by the SQ route.

Fig. 3 shows the proportion of subjects with clinician-observed injection site erythema/induration by time. Injection site erythema/induration peaked on day 2 following SQ AVA vaccination. For IM injections, the proportion of subjects who developed injection site reactions was low and peaked during the first day following AVA administration. No subject had injection site erythema or induration still present at 1 month after an initial series dose of AVA.

Table 3 displays the odds ratio of having a given injection site reaction for females relative to males controlling for the effect of route, and of SQ versus IM route controlling for gender. For each comparison, the largest odds ratios are for SQ nodules, erythema and induration during the first three doses of AVA.

Changing the dose interval between the first two doses from 2 to 4 weeks and the route from SQ to IM significantly decreased the rate of local reactions for women (Table 4). An increased interval between SQ doses from 2 to 4 weeks significantly decreased the rates of SQ nodules ($P = 0.035$), and induration ($P = 0.041$) in women after the first two

Table 3
Local AVA-related adverse events for each gender after 1–3 doses of AVA[a]

Reaction	Gender	IM incidence (% of doses)	SQ incidence (% of doses)	Comparison	*P*-value[b]	Odds ratio (95% CL)[c]
Tenderness	Female	31/46 (67.4)	60/71 (84.5)	F vs. M	0.0002	2.7 (1.6, 4.6)
	Male	35/72 (48.6)	83/132 (62.9)	SQ vs. IM	0.0048	2.0 (1.2, 3.3)
SQ nodule	Female	0/46 (0.0)	45/71 (63.4)	F vs. M SQ	<0.0001	5.4 (2.9, 10.1)
	Male	0/72 (0.0)	32/132 (24.2)	SQ vs. IM (F)	<0.0001	–[d]
				SQ vs. IM (M)	<0.0001	–[d]
Erythema	Female	3/46 (6.5)	45/71 (63.4)	F vs. M	0.0001	4.9 (2.8, 8.9)
	Male	4/72 (5.6)	29/132 (22.0)	SQ vs. IM	0.0001	12.0 (5.4, 30.5)
Warmth	Female	3/46 (6.5)	25/71 (35.2)	F vs. M	0.0001	6.2 (3.0, 13.7)
	Male	3/72 (4.2)	8/132 (6.1)	SQ vs. IM	0.0021	4.3 (1.8, 12.0)
Induration	Female	1/46 (2.2)	27/71 (38.0)	F vs. M	0.0001	15.5 (6.1, 47.6)
	Male	1/72 (1.4)	4/132 (3.0)	SQ vs. IM	0.0004	14.3 (4.0, 90.9)
Pruritus	Female	3/46 (6.5)	21/71 (29.6)	F vs. M	0.0001	5.5 (2.6, 12.7)
	Male	2/72 (2.8)	8/132 (6.1)	SQ vs. IM	0.0039	4.4 (1.7, 13.4)
Arm motion limitation	Female	8/46 (17.4)	7/71 (9.9)	F vs. M	0.1126	1.8 (0.9, 4.0)
	Male	4/72 (5.6)	11/132 (8.3)	SQ vs. IM	0.7474	0.9 (0.4, 2.0)
Edema	Female	1/46 (2.2)	7/71 (9.9)	F vs. M	0.0160	5.3 (1.5, 24.7)
	Male	0/72 (0.0)	3/132 (2.3)	SQ vs. IM	0.0736	6.7 (1.2, 123.7)

[a] Total number of first three doses administered: IM = 118; SQ = 203.

[b] *P*-value calculated by logistic regression.

[c] The odds ratio is an estimate of the relative risk of a reaction in females relative to males, and by the SQ route relative to the IM route. The odds ratios were adjusted so that the confounding effect of gender was removed from the effect of route and, similarly, the effect of route was removed from gender, by logistic regression.

[d] The value cannot be calculated.

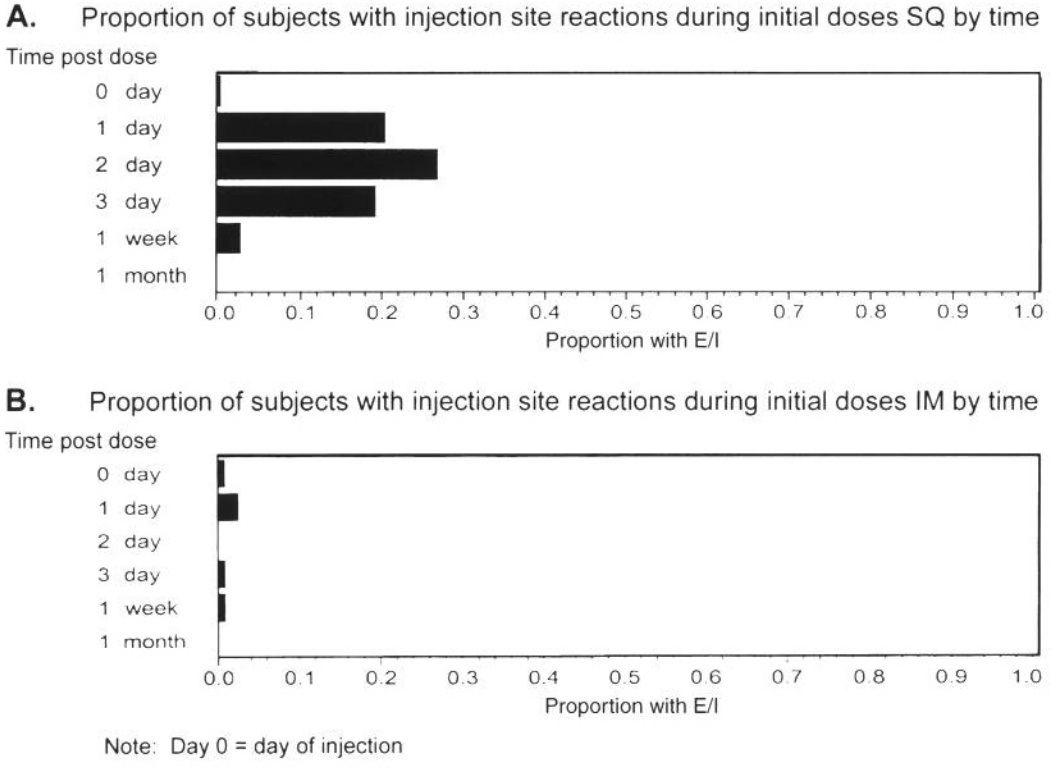

Fig. 3. Injection site erythema/induration by time based on SQ (A) or IM (B) administration of AVA. Injection site erythema/induration peaked at day 2 when AVA was administered SQ but had an earlier peak at day 1 when administered IM. The overall rate of erythema/induration is statistically lower during IM administration of AVA ($P < 0.001$).

Table 4
Incidence of local AVA-related adverse events after dose 2 of AVA for women[a]

Reaction	Incidence (%)	*P*-value[b] vs. 0–2 SQ	*P*-value[b] 0–4 IM vs. 0–4 SQ
SQ nodule			
0–2 SQ	15/18 (83)		
0–4 SQ	4/10 (40)	0.035	0.087
0–2 IM	0/8 (0)	<0.001	
0–4 IM	0/10 (0)	<0.001	
Erythema			
0–2 SQ	13/18 (72)		
0–4 SQ	6/10 (60)	0.677	0.057
0–2 IM	0/8 (0)	<0.001	
0–4 IM	1/10 (10)	0.000	
Induration			
0–2 SQ	10/18 (56)		
0–4 SQ	1/10 (10)	0.041	1.000
0–2 IM	0/8 (0)	0.010	
0–4 IM	0/10 (0)	0.004	
Edema			
0–2 SQ	0/18 (0)		
0–4 SQ	4/10 (40)	0.010	0.303
0–2 IM	0/8 (0)	1.000	
0–4 IM	1/10 (10)	0.357	

[a] An analysis of similar data for men revealed no significant difference except for incidence of SQ nodules, 0–2 SQ vs. 0–2 IM ($P = 0.023$).
[b] *P*-values by Fisher exact test ($P < 0.05$).

doses, but not edema (which increased) or erythema. In the groups given doses at 0 and 2 weeks, a change from the SQ to the IM route significantly reduced the incidences of SQ nodules ($P < 0.001$), erythema ($P < 0.001$) and induration ($P = 0.004$) for women. These differences between SQ and IM were not detected with the 0–4 week schedule (SQ nodule, $P = 0.087$; erythema, $P = 0.057$; induration, $P = 1.0$) although the IM groups had lower reaction rates than the SQ groups. The only significant difference observed in men was a reduction in SQ nodules in the IM versus SQ groups given AVA at 0 and 2 weeks ($P = 0.023$).

Severity of local reaction, as determined by the dimensions of erythema/induration occurring after injection, varied by both gender and route of administration (Table 5). Erythema and/or induration did not exceed 50 mm for any members of the IM group. Females developed increased erythema and/or induration lesions of increased severity in the SQ groups, relative to the males ($P < 0.0001$ for doses 1–3).

4. Discussion

4.1. Vaccine safety

Vaccine safety is a topic of concern to health care professionals, parental groups and others involved in vaccination programs as providers or recipients. Vaccines are administered to protect the public from the ravages of certain devastating infectious diseases. Some biologicals have characteristics that allow them to be desirable as agents for mass destruction in biological warfare or bioterrorist attacks. The success of vaccines has resulted in the control of many infectious diseases, which exist at low frequencies today, if at all, in developed countries. A focus of this study was short-term safety and immunogenicity of the anthrax vaccine when given IM or SQ.

The short-term reactions associated with AVA must be compared to other vaccines in common use today. Diphtheria and tetanus toxoids and pertussis vaccine adsorbed and haemophilus b conjugate vaccine (diphtheria CRM_{197} protein conjugate) TETRAMUNE (DTP-HbOC), show the following incidence of reactions for infants ($N = 7269$): erythema 19%, pain/tenderness 30%, fever 40%, irritability 54%; for toddlers ($N = 107$): erythema 40%, pain/tenderness 65%, fever 33%, and irritability 49% [21]. In addition, diphtheria and tetanus toxoids and acellualar pertussis vaccine adsorbed (ACEL-IMUNE) (DTaP), an aluminum adjuvant vaccine, has an erythema incidence of 35% ($N = 357$) and an induration incidence of 30% for dose 4 in infants and children (not stratified by gender). The rates are comparable to the SQ local reaction rates of AVA and are higher than the IM rates observed in this study for AVA [22,23]. Both TETRAMUNE and ACEL-IMUNE are for intramuscular use only. Local reaction rates are significantly higher than the IM rates observed for AVA. Whether this difference in reaction rates reflects a difference between reactions in children and adults requires further study.

Personal experience over many years and several recent publications [12,13,16] have shown AVA to be a safe and

Table 5
Maximum dimension of erythema and induration per dose

	No reaction (%)	0–50 mm (%)	50–120 mm (%)	>120 mm (%)	Total	*P*-value
Primary IM						
Female	45 (97.8)	1 (2.2)	0	0	46	0.5664[a]
Male	69 (95.8)	3 (4.2)	0	0	72	
Primary SQ						
Female	27 (38.0)	23 (32.4)	18 (25.4)	3 (4.2)	71	<0.0001
Male	103 (78.0)	20 (15.2)	9 (6.8)	0	132	

[a] Test for gender effect on the distribution of erythema and/or induration severity (testing effect of females relative to males on the severity score for erythema and/or induration using logistic regression analysis).

effective vaccine for preventing anthrax. However, a relatively high frequency of local reactions and an unwieldy vaccination schedule present obstacles to the efficient use of this product on a wide scale. The current study was undertaken to determine whether a reduction in the number of doses might be possible without reducing the immune response and whether local reactions could be reduced by changing from the SQ to the IM route. In the current study, systemic adverse events, infrequent and uniformly transient, were not associated with either gender or route of AVA administration. Headache, the most common systemic adverse event, occurred in males and females and in both routes with equal frequency.

Local reactions such as tenderness after SQ administration were significantly higher in females compared to males. In females, the incidences of erythema, induration and SQ nodules were significantly reduced when AVA was given by the IM route compared to the standard SQ route. Furthermore, the incidences of local reactions decreased significantly for women when the second AVA dose interval was increased from 2 to 4 weeks. Notably, SQ nodules, which resolved spontaneously, did not limit arm motion nor were they otherwise incapacitating. Tenderness at the injection site occurred more often in women and after SQ administration.

Overall injection site reactions occurred significantly more often in females than in males and in those receiving AVA by the SQ route relative to the IM route. However, the vast majority of these reactions did not require medical attention and none resulted in lost work time. With the objective measurement of erythema and/or induration, few substantive reactions occurred in those vaccinated by the IM route, while among those receiving the vaccine by the SQ route, there were few reactions >120 mm. All reactions resolved completely without residua.

Whether the subcutaneous nodule is a transient finding related to aluminum hydroxide injection into the subcutaneous space will require investigation. An emerging entity, macrophagic myofasciitis (MMF), apparently related to the intramuscular injection of aluminum compound adjuvanted vaccines has been described in France [24]. It is not known if this microscopic finding represents a pathologic entity or an epiphenomenon. MMF has not been described in the United States despite the use of aluminum compound-containing vaccines since the early part of the 20th century.

AVA is the only vaccine containing aluminum hydroxide that is licensed for SQ administration. Our pilot study, though limited in scope and containing a relatively small number of volunteers per group, provides compelling evidence that the IM route of vaccine administration is associated with fewer short-term adverse events than the SQ route. However, the vast majority of reactions by the SQ route were not severe. The reason for the gender difference in the rate of injection site reactions when AVA is administered SQ is not known. Similarly, the reason for the decrease in the rate of injection site reactions when AVA is administered 4 weeks rather than 2 weeks apart SQ is not known. These findings require further investigation.

4.2. Antibody response

A single dose of AVA given IM or SQ was not sufficient to elicit acceptable peak anti-PA concentrations or seroconversion rates. Two doses given 2 weeks apart elicited anti-PA IgG antibody in 96–100% of subjects; however, vaccination by either SQ or IM routes elicited sub-optimal antibody levels compared with the licensed schedule. In contrast, a two-dose initial vaccination series given 4 weeks apart was comparable to the initial three-dose licensed vaccination series of AVA based on geometric mean anti-PA IgG concentration at peak and seroconversion rate.

The geometric mean anti-PA IgG concentration at peak (week 6) was not statistically different for the licensed schedule (0–2–4 SQ) and 0–4 SQ and 0–4 IM schedules (overall $P = 0.615$). While the geometric mean peak concentration was higher in the 0–4 SQ group compared to the 0–4 IM group, this difference was not statistically significant ($P = 0.65$). Based on antibody response data 6–24 weeks after the initial dose of AVA, the responses of the 0–4 SQ and 0–4 IM groups were not statistically different from those seen in the recipients of the licensed schedule. The TNA results corroborated with the ELISA results. No significant differences were detected in neutralization ratios at peak (week 6) between 0–2–4 SQ, 0–4 SQ, and 0–4 IM treatments ($P = 0.384$) or in linear rates of decline from peak ($P = 0.13$) at weeks 6, 12, and 24.

At weeks 4 and 5, the antibody levels were higher among groups administered AVA at weeks 0–2 compared to weeks 0–4. For the prophylactic use of AVA, this does not represent a major concern. A delay in achieving protective antibody concentration could, however, be important in the setting of emergency pre- or post-exposure vaccination. Further study of this point should be conducted.

This pilot study for proof of concept evaluation of the AVA dose-reduction route-change and safety comparison was relatively small, unblinded, and did not have a placebo arm. The study plan required follow-up with a large pivotal study once proof of concept was established. This pilot study did prove the concepts being tested. Objective clinicians who were unaware of the route of AVA administration performed the physical examinations to determine injection site reactions. The possible effect of unblindedness on systemic symptoms cannot be disregarded, but there were no significant differences between genders or routes for these symptoms. The laboratory technicians who performed the validated anti-PA IgG ELISA and the TNA were blinded to specimen donor (specimens numbered) and study group.

This study supports the non-inferiority of a reduced schedule (0 and 4 weeks) of AVA administration when compared with three-dose (0, 2, 4 weeks) initial vaccination regimens. Moreover, we found that subjects had a lower incidence of adverse events after IM administration compared to SQ.

A larger, randomized, placebo-controlled, double-blinded, multi-center study is planned by the Centers for Disease Control and Prevention in collaboration with the National Institutes of Health and the DoD to confirm and expand these findings.

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