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Anthrax vaccine adsorbed: Further evidence supporting continuing the vaccination series rather than restarting the series when doses are delayed

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ABSTRACT

Whether to restart or continue the series when anthrax vaccine doses are missed is a frequent medical management problem. We applied the noninferiority analysis model to this prospective study comparing the *Bacillus anthracis* protective antigen IgG antibody response and lethal toxin neutralization activity at day 28 to the anthrax vaccine adsorbed (AVA) (Biothrax®) administered on schedule or delayed. A total of 600 volunteers were enrolled: 354 in the on-schedule cohort; 246 in the delayed cohort. Differences were noted in immune responses between cohorts ($p < 0.0001$and among the racial categories ($p < 0.0001$). Controlling for covariates, the delayed cohort was non-inferior to the on-schedule cohort for the rate of 4-fold rise in both anti-PA IgG concentration ($p < 0.0001$) and TNA ED_{50} titers (($p \leq 0.0001$); as well as the mean log_{10}-transformed anti-PA IgG concentration ($p < 0.0001$) and the mean log_{10}-transformed TNA ED_{50} titers ($p < 0.0001$). Providing a missed AVA dose after a delay as long as 5–7 years, elicits anti-PA IgG antibody and TNA ED_{50} responses that are robust and non-inferior to the responses observed when the 6-month dose is given on-schedule. These important data suggest it is not necessary to restart the series when doses of the anthrax vaccine are delayed as long as 5 or more years.

1. Introduction

The aim of this study was to compare the level of persistence of anti-PA IgG concentration and *Bacillus anthracis* lethal toxin neutralization (TNA) response of a cohort of vaccinees delayed as long as 7 years in receiving the 6-month dose of AVA versus a control cohort that received the vaccine on schedule. Both cohorts received a single dose and had blood drawn and immune assays performed pre-vaccination and post-vaccination days 28 and 180.

In 1998, Secretary of Defense William Cohen announced plans to vaccinate U.S. military service members with Anthrax Vaccine Adsorbed to protect them from *B. anthracis* spores used as a biological warfare weapon under the Anthrax Vaccine Immunization Program [1,2]. The current policy requires vaccination when deploying to a high threat area and volunteer continuation upon return from the high threat area [3].

The anthrax vaccine adsorbed is the only vaccine licensed by the U. S. Food and Drug Administration to protect against anthrax disease [4]. The continuing use of anthrax vaccine among military personnel, veterinarians and laboratorians because of the looming threat posed by anthrax spores makes it imperative that biomedical scientists continue to improve medical countermeasures against this agent [5].

We and others have previously reported on the safety and/or immunogenicity of the anthrax vaccine adsorbed [6–16]. As with other vaccines that require multiple-dose priming schedules and frequent boosts, doses of the anthrax vaccine are frequently delayed. Whether to restart or continue with the series is a frequent clinical decision point. Though little data exist to support the practice, it is general practice in medicine to continue the vaccine series when a patient returns after missing one or more scheduled doses rather than restart the series [17].

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We previously reported on the prevalence of anti-PA IgG antibody 18–24 months after an incomplete priming series and determined the immune response to a delayed dose [18,19]. Following this delayed dose, robust IgG antibody levels against PA were noted. The study provided evidence that individuals delayed in receiving a priming dose of anthrax vaccine as long as 24 months could continue with the series rather than restart the series without sacrificing immune readiness against anthrax spores. Although the finding of high antibody levels following a delayed dose was reassuring, the study did not have a control group who had received the anthrax vaccine on schedule. Therefore, it was not known how antibody levels of the two groups might compare. Thus, the current study was conceived and executed. This study determines the *B. anthracis* protective antigen IgG antibody response and *B. anthracis* lethal toxin neutralization activity in individuals whose 6-month dose was delayed 2 to 7 years and compares their responses to a group who received the 6-month dose on time. The sample size allowed stratification by gender, age and race.

2. Methods

2.1. Vaccine

The anthrax vaccine adsorbed is derived from an attenuated, toxigenic, non-encapsulated strain of *B. anthracis* (V770-NPI strain) [20]. At the time of this study, the vaccine was administered in a dose of 0.5 mL *subcutaneously* at weeks 0–2–4 and months 6–12–18; annual boosts were recommended for individuals who remained at risk [20]. In 2008, the U.S. Food and Drug Administration changed the route of administration from subcutaneous to intramuscular and reduced the number of priming doses [21]. Biothrax® (as the vaccine is currently known) is currently manufactured by Emergent BioDefense Operations, Lansing Inc., Lansing, MI 48906, US License No. 1755 [22]. The vaccine is currently administered at months 0–1–6 and boosts at months 12 and 18 as 0.5 mL IM with annual boosts as long as the individual remains at risk for anthrax infection.

2.2. Design

Volunteers who were delayed or on-schedule for the anthrax vaccine adsorbed, AVA, willing to receive a dose of anthrax vaccine, and agreed to have blood drawn pre-vaccination, and days 28 and 180 after Study Vaccine Dose, were recruited for participation in the study. In order to minimize confusion due to the schedule change from weeks 0–2–4 and months 6–12–18 to months 0–1–6 with boosters at 12 and 18 months the terms month 1 dose and month 6 dose will be used in this report. A total of 600 individuals were enrolled: on-schedule cohort = 354; delayed cohort = 246. Of the 600, 405 completed the study: on-schedule = 224; delayed schedule group = 181. The one-third drop-out rate was due to the rapid operational tempo at the military study site (Fort Bragg, NC) due to wars in Iraq and Afghanistan during the enrollment and execution phases of the study. The first volunteer was enrolled August 05, 2004; the last completed August 11, 2009. Again, during this study period, AVA was administered subcutaneously and had a 6-dose priming series.

2.3. Immunogenicity assessment

Both assays used in this report have been reported extensively previously. The enzyme-linked immunosorbent assay (ELISA) is a validated quantitative assay that determines the level of anti-PA IgG antibodies and reports the level as μg/mL [15,23]. The lower limit of quantification (LLOQ) for anti-PA IgG concentration was 3 μg/mL. ELISA anti-PA IgG concentrations below the LLOQ were set to 1/2LLOQ or 1.5 μg/mL.

The *B. anthracis* lethal toxin neutralization activity was measured using a validated colorimetric toxin neutralization assay (TNA). The TNA is an *in vitro* cytotoxicity assay that measures the functional ability of serum to neutralize anthrax lethal toxin cytotoxic activity [15,24]. Results were reported as the reciprocal of the serum dilution that neutralized 50% of the in vitro lethal toxin cytotoxicity (TNA ED_{50} titer). The lower limit of detection (LLOD) for TNA ED_{50} titer = 12; the LLOQ = 36. TNA ED_{50} titers below the LLOQ were set at 1/2LLOQ or 18.

The main immunologic endpoints for the study are [1] the geometric mean *B. anthracis* protective antigen (PA) IgG antibody concentration [2], proportion of volunteers with 4-fold rise in PA IgG antibody concentration [3], the geometric mean *B. anthracis* lethal toxin neutralization activity ED_{50} titer, and proportion [4] of volunteers with 4-fold increase in TNA ED_{50} titers.

2.4. Statistical analysis

Descriptive statistics were calculated for demographic variables, antibodies measured by ELISA anti-PA IgG concentration and TNA ED_{50} titer. Fisher exact test was used to compare the rates of antibody response, gender, and race between cohorts. Student's *t*-test was utilized for comparison of mean age between cohorts. Logistic regression model (two-tailed, 97.5% confidence level) was used to examine the effect of demographic variables (gender, age and race) and interval between day 28 dose of AVA and study dose on the rate of fourfold rise from baseline in ELISA anti-PA IgG concentration and TNA ED_{50} titer at day 28 or day 180. The general linear model (two-tailed, 97.5% confidence level) was used to examine the effect of gender, age, race and interval between day 28 dose and study dose of AVA on mean $\log_{10}$-transformed anti-PA IgG concentration and mean $\log_{10}$-transformed TNA ED_{50} titer along with the cohort factor to select a set of statistically significant covariates by backward elimination and subsequently decided their inclusion and exclusion from the models, with Tukey–Kramer test for pairwise comparisons. Racial categories "American Indian or Alaskan Native" and "Other" were excluded from statistical analyses due to small numbers and complete absence from the delayed cohort. Age <40 or ≥40 were used in the analysis rather than age by decade due to small numbers in the 50–59 age group when decades were assessed.

Exact binomial method was used to test the non-inferiority of delayed cohort to on-schedule cohort for the rate of 4-fold rise from baseline in ELISA anti-PA IgG concentration and TNA ED_{50} titer. General linear model was used to test non-inferiority of the difference (delayed–on-schedule) in mean $\log_{10}$ anti-PA IgG concentrations or mean $\log_{10}$ TNA ED_{50} titer. The lower limit of a one-tailed 97.5% confidence level (equivalent to the lower limit of the two-sided 95% confidence interval) on the difference in the rates or the means was used for testing non-inferiority.

The antibody decay rate (K) was derived from the negative slope of the linear regression line of $\log_{10}$ anti-PA IgG concentration versus time in days after receiving the AVA study dose. The decay rate was expressed as $\log_{10}$ μg/mL/d. Repeat measures ANOVA of $\log_{10}$ ELISA concentrations/$\log_{10}$ TNA ED_{50} titers were used to compare antibody decay rates between two cohorts. The half-life ($t_{1/2}$) was calculated as the time required for anti-PA IgG concentrations or TNA ED_{50} titers to decrease by 50% from the initial value. The $t_{1/2}$ was calculated as $t_{1/2} = \log_{10}(2)/K$.

All ELISA and TNA values were $\log_{10}$-transformed for analysis. After transformations, variables met assumptions of normality and homogeneity of variance. Results of ELISA anti-PA IgG concentrations below the LLOQ were set to 1/2LLOQ or 1.5 μg/mL. TNA

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