Vaccine 32 (2014) 6284–6293

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Immunogenicity and safety of four different dosing regimens of anthrax vaccine adsorbed for post-exposure prophylaxis for anthrax in adults

David I. Bernstein[a,*], Lisa Jackson[b], Shital M. Patel[c], Hana M. El Sahly[c], Paul Spearman[d], Nadine Rouphael[e], Thomas L. Rudge Jr.[f], Heather Hill[g], Johannes B. Goll[g]

[a] Cincinnati Children's Hospital Medical Center, University of Cincinnati, 3333 Burnet Ave., Cincinnati, OH 45229, USA
[b] Group Health Research Institute, Group Health Cooperative, Seattle, WA, USA
[c] Baylor College of Medicine, Houston, TX, USA
[d] Emory University School of Medicine, Atlanta, GA, USA
[e] Hope Clinic of the Emory Vaccine Center, Atlanta, GA, USA
[f] Battelle Memorial Institute, Columbus, OH, USA
[g] The EMMES Corporation, Rockville, MD, USA

ARTICLE INFO

Article history:
Received 10 June 2014
Received in revised form 13 August 2014
Accepted 27 August 2014
Available online 17 September 2014

Keywords:
Anthrax
Vaccine
Post exposure prophylaxis

ABSTRACT

Background: Strategies to implement post exposure prophylaxis (PEP) in case of an anthrax bioterror event are needed. To increase the number of doses of vaccine available we evaluated reducing the amount of vaccine administered at each of the vaccinations, and reducing the number of doses administered.
Methods: Healthy male and non-pregnant female subjects between the ages of 18 and 65 were enrolled and randomized 1:1:1:1 to one of four study arms to receive 0.5 mL (standard dose) of vaccine subcutaneously (SQ) at: (A) days 0, 14; (B) days 0 and 28; (C) days 0, 14, and 28; or (D) 0.25 mL at days 0, 14, and 28. A booster was provided on day 180. Safety was assessed after each dose. Blood was obtained on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 84, 100, 180, and 201 and both Toxin Neutralizing antibody and anti-PA IgG antibody measured.
Results: Almost all subjects developed some local reactions with 46–64% reported to be of moderate severity and 3.3% severe during the primary series. Vaccine groups that included a day 14 dose induced a ≥4 fold antibody rise in more subjects on days 21, 28, and 35 than the arm without a day 14 dose. However, schedules with a full day 28 dose induced higher peak levels of antibody that persisted longer. The half dose regimen did not induce antibody as well as the full dose study arms.
Conclusion: Depending on the extent of the outbreak, effectiveness of antibiotics and availability of vaccine, the full dose 0, 28 or 0, 14, 28 schedules may have advantages.

1. Introduction

Anthrax is an acute infectious disease caused by the spore-forming bacterium *Bacillus anthracis* (*B. anthracis*). Cutaneous anthrax is the most commonly reported form in humans (greater than 95% of all cases) and occurs when the bacterium enters a cut or abrasion on the skin. Gastrointestinal anthrax usually occurs after ingestion of meat contaminated with anthrax spores. Inhalational anthrax is the most lethal form of anthrax [1] and occurs after exposure to aerosolized spores [1]. A major concern is the potential to weaponize the spores and use it as an agent of bioterrorism.

Since the majority of individuals are not vaccinated against anthrax, strategies to implement post exposure prophylaxis (PEP) in case of a bioterror event are needed. Although anthrax vaccine adsorbed (AVA, BioThrax®) is currently only approved for pre-exposure prophylaxis, a 60-day course of antibiotics in conjunction with three doses of BioThrax® 2 weeks apart are recommended by the Advisory Committee on Immunization Practices (ACIP) for PEP [2]. Although antibiotics are effective when administered prior to or immediately after spore exposure, residual spores can germinate and release toxin after discontinuation of antibiotics, causing disease and death [3–5]. To be highly effective, antimicrobial therapy must be initiated as soon as possible after infection since

* Corresponding author. Tel.: +1 513 636 7625; fax: +1 513 636 7682.
E-mail address: david.bernstein@cchmc.org (D.I. Bernstein).

http://dx.doi.org/10.1016/j.vaccine.2014.08.076
0264-410X/

efficacy diminishes rapidly as toxemia progresses. However, even if mass distribution of antimicrobials can be aggressively completed within 6 days of the initial exposure, it is estimated that only approximately 70% of cases can be prevented [6]. For these reasons, it is critical to combine antibiotics with vaccination in the post-exposure setting.

The current target dosing schedule for PEP is a full dose (0.5 mL) administered on day 0, 14, and 28. In the event of a large anthrax outbreak, it is possible that there will be an insufficient number of doses available to vaccinate the entire exposed population. Strategies to increase the number of doses available include reducing the amount of vaccine administered at each of the vaccinations, or reduce the number of doses administered. Policy makers need data on the immunogenicity of alternative dosing regimens. The purpose of this protocol was to generate these data. We compared the antibody response to the standard three-dose schedule to the antibody response of two-dose vaccination regimens (days 0 and 14, and days 0 and 28) using full dose (0.5 mL) BioThrax® and to the antibody response of a three-dose regimen (days 0, 14, and 28) using half the dose (0.25 mL) of BioThrax® in adults. Since it may become necessary to ensure longer term protection for individuals who received a PEP regimen, a 6-month boost was also administered to explore the effect of a booster dose when administered following the different PEP regimens.

2. Methods

Subjects: Healthy male and non-pregnant female subjects between the ages of 18 and 65 were recruited at four sites within the USA. Subjects were excluded if they had a prior anthrax immunization, were allergic to any vaccine component, had received immunosuppressive therapy, blood products or immunoglobulin within 3 months, had a history of Guillain–Barré Syndrome, a malignancy, diabetes requiring insulin, significant cardiovascular, pulmonary, renal, autoimmune, inflammatory, vasculitic, rheumatic, neurologic, or liver disease. Those with significant psychiatric disease or taking more than one antidepressant or selected psychiatric drugs were also excluded. Subjects were screened for HIV, hepatitis B surface antigen, and antibody to hepatitis C virus. For females of childbearing potential, a serum pregnancy test was performed. These screening tests had to be negative for subjects to be eligible for participation in the study. Serum chemistries were also obtained prior to enrollment and were required to be normal or near normal. Tattoos that obscured the vaccination site were not allowed. Full inclusion and exclusion criteria are available at Clinicaltrials.gov NCT01641991.

Vaccine: Anthrax vaccine adsorbed (AVA, BioThrax®) was supplied by the Centers for Disease Control and Prevention in 5 mL multi-dose vials. It was made from cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *B. anthracis*. The final product contains culture fluid proteins including the *B. anthracis* protective antigen (PA) and 1.2 mg/mL aluminum, added as aluminum hydroxide in 0.85% sodium chloride.

2.1. Study design

This was a randomized, open-label immunogenicity and safety study to evaluate four dosing regimens of BioThrax® for PEP for anthrax. Subjects were enrolled and randomized 1:1:1:1 to one of four study arms to receive 0.5 mL (standard dose) of vaccine subcutaneously (SQ) at: (A) days 0, 14; (B) days 0 and 28; (C) days 0,14, and 28; or (D) 0.25 mL at days 0,14, and 28. These vaccinations are referred to as the primary series. Enrollment was stratified by gender, with approximately equal numbers of males and females enrolled into each dosing regimen. Subjects were followed for approximately 201 days. Blood was obtained on days 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 84, 100, 180, and 201 and anthrax antibody measured. All subjects received a 0.5 mL dose intramuscularly (IM) at approximately 6 months (booster dose). Systemic and local reactions were collected with the use of a memory aid for at least 8 days (days 0–7) following each vaccination. Unsolicited adverse events were collected at every visit up to 28 days post last vaccination with the primary series and then again after the 6-month boost until the day 201 visit. Serious adverse events were collected throughout the study period

2.2. Antibody assays

Serum samples were evaluated for levels of anti-anthrax antibodies in both the Toxin Neutralization Activity (TNA) Assay and the anti-PA IgG Enzyme Linked Immunosorbent Assay (ELISA).

TNA assay—The TNA assay measures the levels of anthrax lethal toxin neutralizing antibody using an in vitro cytotoxicity assay. The assay was originally validated at the CDC, but was then transferred and validated at Battelle, where the testing of these serum samples occurred [7,8]. Briefly, microtiter cell plates were seeded with J774A.1 cells and allowed to adhere. In separate microplates a mixture of recombinant protective antigen (rPA, List Biological Laboratories, Inc., Campbell, California, Cat. no. 171B) and recombinant lethal factor (rLF, List Biological Laboratories, Inc., Campbell, California Cat. no. 172B) was added to serial dilutions of the test samples and controls and incubated prior to transfer to the cell plate. The final concentration of rPA was 0.05 μg/mL and the final concentration of rLF was 0.04 μg/mL. MTT was then added to the cell plates to allow viable cells to reduce the MTT dye. The OD values for each plate were read on a BioTek microplate reader at a wavelength of 570 nm using a 690 nm reference wavelength. The TNA SAS program [7] was used to fit the seven-point serial dilutions of the reference serum standard and test sample serum OD values to a four parameter logistic-log (4PL) function, which is in turn was used to calculate the reportable values (ED_{50} and NF_{50}). The assay endpoints are the Effective Dilution 50 (ED_{50}) and the Neutralization Factor 50 (NF50). The ED_{50} is the reciprocal of the dilution of a serum sample that results in 50% neutralization of anthrax lethal toxin. The ED_{50} is determined as the reciprocal of the dilution corresponding to the inflection point ('*c*' parameter) of the four-parameter logistic log fit of the curve. The NF_{50} is the ED_{50} of the test sample divided by the ED_{50} of the reference standard. The NF50 Lower Limit of Quantification (LLOQ) was 0.064 [9]. The TNA reference standard was the human serum AVR801 (BEI Resources).

ELISA assay—The anti-PA IgG ELISA measures the quantity of serum anti-PA IgG antibodies. The ELISA was originally validated at the CDC, but was transferred and validated at Battelle, where the testing of these serum samples occurred [10]. Briefly, microtiter plates were coated with 1 μg/mL rPA (List Biological Laboratories, Campbell, California, Catalog number 171B). Test samples, anti-PA IgG reference standard serum, and positive control sera were then added to the microtiter plate. After washing, the bound anti-PA antibodies were detected by a species-specific anti-gamma chain IgG–horseradish peroxidase (HRPO) conjugate followed by addition of a peroxidase substrate. The optical density (OD) values for each plate were read on a microplate reader at a wavelength of 405 nm using a 490 nm reference wavelength. The anti-PA IgG concentration was determined by taking the average of the acceptable concentrations from the eight-point dilution of the test sample back-calculated from the standard curve. Results are reported in μg/mL of anti-PA IgG. The ELISA LLOQ was 9.27 μg/mL.