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Immunogenicity and safety of a novel recombinant protective antigen anthrax vaccine (GC1109), a randomized, single-blind, placebo controlled phase II clinical study

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ABSTRACT

Background: The demand on effective and safe anthrax vaccine is increasing as a part of the preparedness for possible bioterrorism in the future. We performed a randomized, single-blind, placebo controlled phase II clinical study to evaluate the immunogenicity and safety of a novel recombinant protective antigen (rPA) anthrax vaccine, GC1109, in healthy adult volunteers.

Methods: Participants were randomized to experiment groups (0.3 mL, 0.5 mL, and 1.0 mL of GC1109) or placebo group (normal saline 0.5 mL) in 2:2:2:1 ratio. They received respective vaccines intramuscularly at 0, 4 and 8 weeks. Immunogenicity was evaluated by seroconversion rate and geometric mean titer (GMT) of lethal toxin neutralizing assay (TNA) and anti-PA IgG by ELISA. Safety was assessed by laboratory tests, and solicited and unsolicited adverse events on diary cards.

Results: 30, 29, 30 participants were randomized to 0.3, 0.5, and 1.0 mL of GC1109 groups, respectively, while 15 to placebo group. 92 participants received all three doses. In per-protocol analysis, TNA GMTs at week 12 were 296.5, 285.2, and 433.2 in the three groups, respectively. Seroconversion rates measured by ELISA were 100% at week 12 in the three groups. Local and systemic vaccine-related adverse events were frequent; however, most of them were mild, and no serious events were observed.

Conclusions: A new rPA anthrax vaccine GC1109 was immunogenic after three doses of intramuscular administration, and was well-tolerated.

1. Introduction

Bacillus anthracis is a zoonotic organism which can cause distinctive three forms of infection in human, namely, cutaneous, gastrointestinal, and inhalation anthrax [1]. Although anthrax is uncommon, the microorganism has the potential to be used in bioterrorism because it produces endospores which is able to withstand harsh environments [2]. The United States already had experienced a deliberate use of *B. anthracis* through the postal system in 2001 which resulted in five deaths out of 11 patients by inhalation anthrax [3].

Since its significant potential for bio-weapon was recognized, efforts have been made to develop an effective and safe anthrax vaccine [4]. However, to date, Anthrax Vaccine Adsorbed (AVA, BioThrax™, Lansing, Michigan) is the only US FDA-approved anthrax vaccine which has been studied for decades. Nevertheless, the optimal administration schedule and route of AVA are still unknown [4], and several concerns has been raised on its pharmaceutical quality and consistency from lot to lot [5,6], which all necessitate a novel anthrax vaccine [7,8]. Moreover, developing anthrax vaccine of a nation's own is an important task to prepare for a possible bioterrorism in the future.

We performed a randomized, single-blind, placebo controlled phase II clinical trial to evaluate the immunogenicity and safety of three different doses of GC1109, a novel purified recombinant protective antigen (rPA) anthrax vaccine developed by GC Pharma (Yongin, Republic of Korea), in healthy adult volunteers.

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2. Methods

2.1. The vaccine and placebo

GC1109 was derived from supernatant of mass-cultured *B. brevis* 47-5Q which was transformed with avirulent PA gene originated from *B. anthracis* Sterne strain. Each GC1109 used in the present study contained 100 μg/mL of purified rPA and 1 mg/mL of aluminum hydroxide. Three doses of GC1109 (0.3 mL, 0.5 mL, or 1.0 mL) or placebo (normal saline 0.5 mL) was administered three times intramuscularly at weeks 0, 4, and 8.

2.2. Participants and enrollment

Healthy volunteers aged from 18 to 55 years were enrolled from November 29th 2011 through December 3rd 2012. All volunteers provided written informed consent before screening.

Volunteers with contraindications for anthrax vaccination specified in the guidelines published by the US Centers for Disease Control and Prevention were excluded from the study [9]. Additionally excluded were volunteers who had been occupationally exposed to a ruminant, or had HIV-1, active hepatitis B or hepatitis C infection, history of immunodeficiency or malignant disease, and pregnancy. Concurrent administration of immunosuppressant (not including inhaled or topical steroid), blood product, and other vaccine was not allowed in this study. Medical histories, physical examinations, and laboratory tests were used to ensure that the participants were healthy. Screening laboratory tests included complete blood cell count with differential count, sodium, potassium, calcium, magnesium, phosphorus, glucose, blood urea nitrogen, creatinine, creatine kinase, albumin, total protein, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin, cholesterol, γ-glutamyl transferase, uric acid, antibody to the hepatitis A virus, hepatitis C virus, and human immunodeficiency virus, surface antigen of the hepatitis B virus, rapid plasma regain or fluorescent treponemal antibody absorption, urinalysis, prothrombin time, activated partial thromboplastin time, chest radiograph, electrocardiogram, and urine human chorionic gonadotrophin for woman.

The study was approved by the Institutional Review Board of Seoul National University Hospital (No. 1109-024-377), and was registered at ClinicalTrials.gov (identifier NCT01624532).

2.3. Study design

The participants were randomized to experimental groups to receive 0.3 mL, 0.5 mL, or 1.0 mL of GC1109 or placebo group to receive 0.5 mL of 0.9% NaCl in a 2:2:2:1 ratio, respectively. The block randomization was performed using Proc Plan using SAS version 9.1 (SAS Institute Inc. Cary, NC). The participants were received GC1109 or placebo at weeks 0, 4, and 8. They were followed-up at four weeks after each vaccination. Samples for toxin neutralization assay (TNA) and anti-PA antibody measurement by enzyme-linked immunosorbent assay (ELISA) were taken at each visit. Immunogenicity was evaluated by seroconversion rate and geometric mean titer (GMT) of TNA and anti-PA IgG by ELISA at those time points. Seroconversion was defined as a more than four-fold rise in the TNA activity or anti-PA IgG by ELISA [9].

Participants who received at least one dose of GC1109 or placebo were included in safety analysis. Those who received at least two doses of GC1109 were included in the immunogenicity analyses.

2.4. TNA

The J774A.1 cell-based TNA was performed as previously described with some modifications [10]. Briefly, cells cultured in flasks for 4 to 7 days were harvested and seeded in 96-well tissue culture plates at 4×10^5 cells/well followed by 17 to 19 h of incubation. Serum samples were prepared in a separate 96-well microtiter plate at 2-fold dilutions following an initial 1:50 dilution for a total of 11 dilutions per sample. The samples were then incubated with a constant concentration of lethal toxin (LT, 30 ng/mLPA and 40 ng/mL lethal factor), unless otherwise stated, for 30 min prior to addition to the cells. These concentrations of LT, chosen after toxin titration, result in approximately 90% killing of the corresponding cell line in the absence of neutralizing antibodies. The cell-serum-toxin mixture was incubated for 4 h, after which 25 μl/well of 5 mg/ml of a tetrazolium salt, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, was added. After a 1-hour incubation, the cells were lysed by using 100 μl/well of acidified isopropanol (90% isopropanol, 0.5% SDS [wt/vol], 25 mM HCl), and the optical density (OD) at 570 nm was determined. All TNA assay analyses and ED50 (effective dilution 50%) calculations were done in Softmax program. This program used a 4 –parameter logistic model to fit a dose–response curve and calculated ED50. If the ED50 was less than the lower limit of quantification (LLOQ) of the assay, then the TNA ED50 was replaced with 79, the half of the LLOQ.

2.5. ELISA for anti-rPA antibody

Anti-PA IgG titer was measured by coating microplates with purified recombinant protective antigen (rPA). Antibodies to PA were measured in 96-well format plates (NUNC flat-bottomed wells; ThermoFisher, Waltham, MA) coated with 1 μg/mL of rPA diluted in Phosphate Buffered Saline (PBS) in a volume of 100 μl per well, sealed and incubated at 4 °C for 12–18 h. After incubation, plates were washed five times with wash buffer (0.1% Tween 20 in PBS). Serum samples were two-fold serially diluted in assay buffer (5% non-fat dry milk, 0.1% Tween 20; PBS) following an initial 1:10 dilution in assay buffer. Single samples were added to the plate at a volume of 100 μl per well and incubated for one hour at RT. Following five washes, 100 μl of biotin-labeled PA diluted 1:100 in assay buffer was added to each of the wells and incubated for 60 min at RT. Following five washes, Streptavidin labeled-horseradish peroxidase diluted 1:100 in assay buffer was added to each of the wells and incubated for 45 min at RT. After five washes, 100 μl of 3,3′,5,5′-tetramethylbenzidine solution was added to each of the wells and incubated for 15 min at RT and the reaction was stopped by adding the 100 μl of stop solution. Plates were read at 450 nm using a microplate reader (SpectraMax 190, Molecular Devices, Sunnyvale, CA). Cut off value was calculated that the absorbance of negative sera of each plate and the normalization factor (determined during assay validation). The anti-PA antibody titer was determined by the highest dilution factor that over cut off values. If the anti-PA IgG value was less than the minimum dilution factor, then the anti-PA IgG value was replaced with 5.

2.6. Safety evaluation

At every visit, the participants returned a completed diary containing a questionnaire about adverse events (AEs) within 14 days after each vaccination and received a new diary. Causality was assessed according to Tozzi et al. [11], and vaccine-related adverse drug reaction (ADR) was defined as an event with possible or stronger causality with the immunization. Severity of those reactions were assessed according to FDA guideline [12]. Laboratory tests mentioned above were repeated at week 12 (four weeks after the third vaccination).

3. Results

3.1. Flow diagram and baseline characteristics of volunteers

Of the 133 volunteers assessed for eligibility, 17 did not meet the inclusion criteria, and 11 refused to participate, and 1 withdrew consent after randomization (Fig. 1). Of the 104 participants, 104 received at least one dose of GC1109, 92 received all 3 doses of GC1109 per protocol. The mean (±SD) age of the 92 participants (per-protocol set) were 32.7 (±9.5) years and 55.4% (51/92) were male.

3.2. Immunogenicity

Seroconversion rates measured by TNA and anti-PA IgG by ELISA are shown in Table 1.

In per-protocol analysis, the seroconversion rates by TNA at week 8 were 16.7%, 28.0%, and 39.3% in GC1109 0.3 mL, 0.5 mL, and 1.0 mL group, respectively, and those at week 12 were 54.2%, 52.0%, and 78.6%, respectively. The seroconversion rates by anti-PA IgG ELISA at week 8 were 100%, 88.0%, and 100% in GC1109 0.3 mL, 0.5 mL, and 1.0 mL group, respectively, and those at week 12 were 100% in all three groups. The results were similar in participants who had received more than one dose (Supplementary Table 1).

Geometric mean titer (GMT) of TNA and anti-PA IgG by ELISA in per protocol set are shown in Table 2. Those results were similar in those who had received more than one dose (Supplementary Table 2).

3.3. Safety

All solicited AEs and vaccine-related ADRs are shown in Table 3 and Supplementary Table 3, respectively. Although majority of

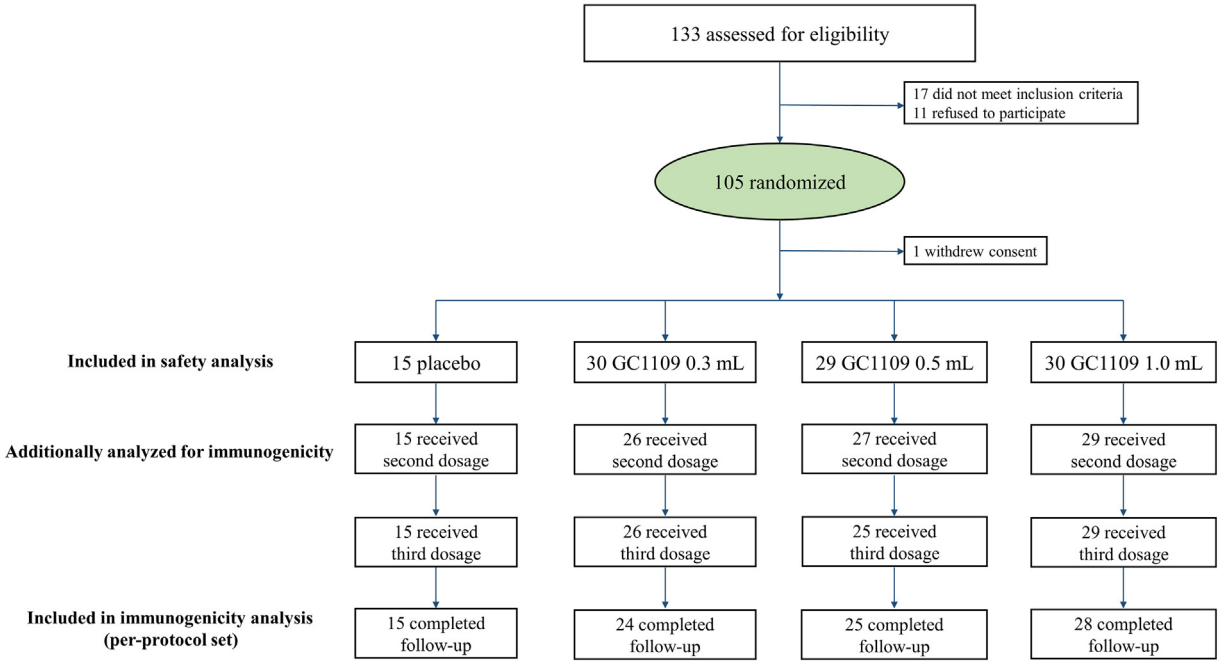

Fig. 1. Flow diagram of this study.

Table 1
Seroconversion rates in measured (A) by toxin neutralizing assay (TNA) and (B) by ELISA (per-protocol analysis).

Groups	Seroconversion rate (%) by TNA (95% CI)				Seroconversion rate (%) by ELISA (95% CI)			
	Placebo (n = 15)	GC1109 0.3 mL (n = 24)	GC1109 0.5 mL (n = 25)	GC1109 1.0 mL (n = 28)	Placebo (n = 15)	GC1109 0.3 mL (n = 24)	GC1109 0.5 mL (n = 25)	GC1109 1.0 mL (n = 28)
Week 4	0 (0.0–21.8)	4.2 (0.1–21.1)	4.0 (0.1–20.4)	7.1 (0.9–23.5)	0 (0.0–21.8)	0 (0.0–14.2)	16.0 (4.5–36.1)	42.9 (24.5–62.8)
Week 8	0 (0.0–21.8)	16.7 (4.7–37.4)	28.0 (12.1–49.4)	39.3 (21.5–59.4)	0 (0.0–21.8)	100.0 (84.8–100.0)	88.0 (68.8–97.5)	100.0 (87.7–100.0)
Week 12	0 (0.0–21.8)	54.2 (32.8–74.4)	52.0 (31.3–72.2)	78.6 (59.0–91.7)	0 (0.0–21.8)	100.0 (85.8–100.0)	100.0 (86.3–100.0)	100.0 (87.7–100.0)

Table 2
Geometric mean titer (GMT) of (A) toxin neutralizing assay (TNA) and (B) anti-PA IgG by ELISA (per-protocol analysis).

Groups	GMT by TNA (95% CI)				GMT of anti-PA IgG by ELISA (95% CI)			
	Placebo (n = 15)	GC1109 0.3 mL (n = 24)	GC1109 0.5 mL (n = 25)	GC1109 1.0 mL (n = 28)	Placebo (n = 15)	GC1109 0.3 mL (n = 24)	GC1109 0.5 mL (n = 25)	GC1109 1.0 mL (n = 28)
Pre-vaccination	79.0[a]	79.0[a]	79.0[a]	79.0[a]	5.0[a]	5.0[a]	5.0[a]	5.0[a]
Week 4	79.0[a]	89.9 (76.5–105.8)	86.4 (75.6–98.8)	93.4 (73.5–118.7)	5.0[a]	5.1 (4.8–5.5)	8.7 (5.0–15.1)	13.8 (7.9–24.0)
Week 8	79.0[a]	134.4 (100.9–178.9)	168.0 (108.8–259.3)	224.0 (164.8–304.5)	5.0[a]	113.1 (68.4–187.1)	128.2 (65.6–250.4)	262.5 (172.6–399.3)
Week 12	79.0[a]	296.5 (214.9–409.1)	285.2 (189.5–429.5)	443.2 (356.1–551.5)	5.2 (4.7–5.8)	987.0 (692.8–1406.1)	798.9 (471.6–1353.3)	1485.0 (1005.9–2192.3)

[a] Values below the detection limits were replaced to 79.0 and 5.0 in TNA and ELISA, respectively.

Table 3
Solicited adverse events in this study.

Characteristics	Placebo (n = 15)	GC1109 0.3 mL (n = 30)	GC1109 0.5 mL (n = 29)	GC1109 1.0 mL (n = 30)
Overall adverse events (%)	6 (40.0)	28 (93.3)	26 (89.7)	30 (100.0)
Local adverse events (%)	1 (6.7)	26 (86.7)	26 (89.7)	29 (96.7)
Pain	1 (6.7)	23 (76.7)	25 (86.2)	28 (93.3)
Tenderness	1 (6.7)	23 (76.7)	25 (86.2)	29 (96.7)
Erythema	0 (0)	2 (6.7)	5 (17.2)	3 (10.0)
Swelling	0 (0)	4 (13.3)	6 (20.7)	3 (10.0)
Systemic adverse events (%)	6 (40.0)	22 (73.3)	19 (65.5)	25 (83.3)
Fever	0 (0)	0 (0)	1 (3.4)	1 (3.3)
Nausea, vomiting	1 (6.7)	3 (10.0)	1 (3.4)	4 (13.3)
Diarrhea	2 (13.3)	3 (10.0)	8 (27.6)	5 (16.7)
Headache	5 (33.3)	12 (40.0)	7 (24.1)	12 (40.0)
Fatigue	4 (26.7)	14 (46.7)	14 (48.3)	16 (53.3)
Myalgia	4 (26.7)	13 (43.3)	11 (37.9)	18 (60.0)

participants reported local or systemic AEs, most of them were mild (Supplementary Table 4). Although three serious AEs were reported only in GC1109 0.3 mL group, all of them occurred in one patient related to her knee injury, apparently not attributable to the immunization.

Blood and urine tests, and electrocardiography at week 12 revealed no clinically significant change from those at the baseline.

4. Discussions

Here we demonstrated immunogenicity and safety of three different doses of GC1109. Seroconversion rate by anti-rPA antibody ELISA was 100% in every dosage groups at week 12. Although local and systemic vaccine-related ADRs were frequent, most of them were mild with no serious one.

The most accurate immunological correlates of protection (COP) to predict probability of survival in human is unknown since clinical trials to assess the efficacy of anthrax vaccine is not ethical nor feasible [13]. However, recent studies suggested that anti-PA IgG levels at the time of exposure might be more accurate than TNA as a vaccine-induced immunological COP against inhalation anthrax [14,15]. In a nonhuman primate model, the probability of survival was over 90% when the anti-PA IgG levels were greater than 10.0 μg/mL [14]. In the current study, GMTs of anti-PA IgG at week 8 were over 100 in all three dosage groups, and the anti-PA IgG titers persisted until week 12, suggesting three doses of GC1109 may provide protection against inhalation anthrax from weeks 8 to week 12.

The majority of AEs after AVA immunization were non-serious, local reactions [5,16]. Similar to the occasion of AVA, no serious vaccine-related ADR was noted in this study. Although AEs were tended to be more frequent in higher doses of GC1109, it was not statistically significant nor more severe (Supplementary Table 4). However, more frequent systemic AEs than AVA or other rPA anthrax vaccine [7,16], even though they were rarely severe, needs to be further evaluated in a larger clinical studies.

Anthrax vaccine may be used as a post-exposure prophylaxis as well as a pre-exposure prophylaxis, and dosing schedule and route of administration are different for the two strategies of prophylaxis. For example, for pre-exposure prophylaxis, AVA was licensed to administer 3-dose priming series at 0, 1, 6-months and boosting dose at 12 and 18 months, and then annually, by intramuscular route. Whereas for post-exposure prophylaxis, subcutaneous administration of 3-dose series at 0, 2, and 4 week was recommended, along with co-administration of antibiotics for 60 days [9]. In our study, we evaluated the immunological response only up to week 12, therefore further studies are needed for dosing schedule and route of administration for pre-exposure prophylaxis.

In conclusion, a new rPA anthrax vaccine GC1109 showed promising anti-PA IgG response and was generally well-tolerated without any serious AEs. Further studies on the vaccine is warranted.

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Declaration of Competing Interest

The authors declare no competing interests.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.05.057.

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