Immunogenicity and safety of a single intramuscular dose of a diphtheria–tetanus toxoid (Td) vaccine (GC1107) in Korean adults

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A B S T R A C T

The current study aimed to evaluate immunogenicity and safety of a newly developed diphtheria–tetanus toxoid (Td) vaccine, GC1107 (Green Cross Corporation, Yongin, Korea), in comparison with placebo and active comparator (licensed Td vaccine) in healthy Korean adults. A randomized, double-blind, placebo and active comparator-controlled study was conducted. Forty subjects were randomly administered a single intramuscular dose of GC1107, active comparator or placebo in a ratio of 2:1:1. At 2 and 4 weeks after vaccination, anti-diphtheria antibody levels in the GC1107 group increased 9.2 and 9.3 times, respectively, compared to pre-dose titers. The corresponding values were 9.3 and 8.3 times for the active comparator group. Anti-tetanus antibody levels increased 39.0 and 37.9 fold at 2 and 4 weeks, respectively, after GC1107 administration, and 12.2 and 14.7 fold after active comparator administration. No increases in tetanus or diphtheria antibody were observed for the placebo group. Adverse events in the GC1107 and active comparator groups were more frequent than for the placebo group, but there were no significant differences between the two active treatments. In conclusion, GC1107 was well tolerated and provided significant boosts of anti-tetanus and anti-diphtheria antibodies.

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1. Introduction

Diphtheria is an acute upper respiratory tract illness caused by Corynebacterium diphtheria that is characterized by sore throat, low grade fever and adherent pseudomembrane of the tonsil and pharynx. The mortality rate is 5–10%, with higher (up to 20%) rates among children under 5 years and adults over 40 years [1,2]. Tetanus is an acute, often fatal, disease caused by Clostridium tetani infection. C. tetani produces a neurotoxin, tetanospassmin, which blocks neurotransmission and causes rigidity and convulsive spasms of skeletal muscles. In recent years the mortality rate for tetanus has been reported to be about 11% [3,4].

Immunity against diphtheria and tetanus is obtained only by vaccination, because immunity against these pathogens is not acquired naturally. After the 1940s, the world-wide incidences of diphtheria and tetanus were significantly reduced by the broad use of DTP vaccines. Outbreaks of diphtheria and tetanus also have been markedly reduced in Korea since the 1980s [5,6]. However, occasional outbreaks of diphtheria and tetanus have been reported for developing and developed countries [7–9].

Several previous studies suggested that anti-diphtheria and anti-tetanus antibody levels over 0.1 IU/ml are protective [1–4,10]. The US Centers for Disease Control (CDC) recommends that adults be given a booster dose of anti-diphtheria and tetanus vaccine every 10 years for purposes of prevention [11]. In Korea, the risk and importance of diphtheria and tetanus are growing due to the increase in outdoor activities that accompany with social economic growth. However, the Korean population’s immunity against diphtheria and tetanus expressed in antibody titers significantly decreased in age groups that are 20 years and older [5,12]. For those reasons, booster immunization should be considered for adolescents and adults to maintain long-term immunity [5,12–14]. A Td vaccine has been designated as a mandatory vaccine for adolescents by the Korea Centers for Disease Control and Prevention (KCDC) since 2005.

Considering the increased demands for booster immunization against diphtheria and tetanus, stable Td vaccine supplies are essential for meeting the public immunization goal. A new diphtheria–tetanus toxoid (Td) vaccine, GC1107 (Green Cross Corporation, Yongin, Korea), was developed with an enhanced manufacturing method that consists of fermentation followed by
purification process containing gel filtration to meet the increased need for a Td vaccine.

The current study aimed to evaluate the immunogenicity and safety of this new adult Td vaccine in comparison with placebo and active comparator, a licensed Td vaccine (SK Td Vaccine Intr. (pre-filled)® (SK Chemicals, Seongnam, Korea)), in healthy Korean adults, and to explore the possibility of market authorization for the new Td vaccine.

2. Material and methods

2.1. Subjects

Korean male adults aged 20 years or older were enrolled in the study if they were determined to be healthy through screening tests that included a medical interview, physical examination, clinical laboratory tests, 12-lead ECG, chest radiograph and measurement of vital signs. Subjects who were vaccinated against diphtheria, tetanus or pertussis within the last 10 years, experienced complications after anti-diphtheria or anti-tetanus vaccination, had a history of tetanus immunoglobulin administration, had an infection history of diphtheria, tetanus or pertussis, or those who had a medical history of Guillain-Barré syndrome were excluded from the study. Written informed consent was obtained from all subjects prior to study participation.

2.2. Study design

The current study used a randomized, double-blind, placebo and active comparator controlled, parallel design. The study protocol was reviewed and approved by the institutional review board (IRB) of Seoul National University Hospital (SNUH), Seoul, Korea and Korea Food and Drug Administration (KFDA) (Approval number: Biopharmaceutical Policy Division-219). The study was conducted according to the declaration of Helsinki [15] and Korean Good Clinical Practice [16].

Subjects were randomly assigned to one of three treatment groups (GC1107, active comparator or placebo) at a ratio of 2:1:1. Subjects were monitored in at the SNUH Clinical Trials Center (CTC) for 30 min after vaccine administration, and they were then discharged if they experienced no acute adverse events. Subjects visited the CTC on the subsequent 3 days for safety evaluation and on the 14th and 28th day after vaccine administration for safety and immunogenicity evaluation.

2.3. Vaccination

The GC1107 vaccine was manufactured according to good manufacturing practice (GMP). The diphtheria and tetanus toxoid were produced by growing C. diphtheria and C. tetani in NZ-case medium, respectively. The supernatants were detoxified with formaldehyde and the toxoids were purified by serial ammonium sulfate fractionation and gel column chromatography. The purified toxoids were then adsorbed to aluminum hydroxide and diluted using phosphate-buffered physiologic saline. The manufacturing method was different than the widely used method for previous Td vaccines, consisted of a stationary culture followed by salting out fractionation. Based on the advances of manufacturing processes, the titer of the final product was approximately 2500 LF/mg, which was higher than the previous method (not less than 1500 LF/mg).

Each 0.5 mL dose of GC1107 contained ≥2 IU of diphtheria toxoid and ≥20 IU of tetanus toxoid. Active comparator vaccine had the same composition. Normal saline (0.5 mL) was used for the placebo. A single dose of study vaccine was administered into the deltoid muscle of the non-dominant arm.

2.4. Immunogenicity

Blood samples (5 mL each) for determining plasma anti-diphtheria and anti-tetanus antibody levels were collected 2 and 4 weeks after vaccination, in addition to a pre-vaccination sample. The blood samples collected in EDTA tubes were centrifuged at 1800 × g for 10 min, and the supernatants (plasma) were separated and stored at −70 °C until use.

Plasma anti-diphtheria and anti-tetanus antibody titers were determined using a commercial Diphtheria IgG enzyme-linked immunosorbent assay (ELISA) kit and Tetanus IgG ELISA kit (IBL International GmbH, Hamburg, Germany), respectively. The lower limit of quantification (LLOQ) of antibodies against diphtheria and tetanus was 0.01 IU/mL and 0.1 IU/mL, respectively. The precision of each analytic method was below 12.8% and 4.2%, and the accuracy ranged 90.0–103.9% and 99.5–101.7%, respectively.

The geometric mean concentrations of anti-diphtheria and anti-tetanus antibody before and after vaccination (2- and 4-weeks post-vaccination) were calculated. Antibody titers below LLOQ were considered to be the value of half the LLOQ. The percentage of subjects whose antibody concentrations were ≥0.1 IU/mL or ≥1.0 IU/mL were calculated.

2.5. Safety

Subjects were monitored until 4 weeks after vaccination through medical interview, physical examination, clinical laboratory tests, 12-lead ECG and measurement of vital signs. Patient diary cards were given to all subjects who were asked to record any solicited local and systemic adverse events (AEs) for 7 days after vaccination. All of the observed and self-reported AEs were recorded regardless of any suspected relation to the study vaccines, and relationships were assessed as unassessable/unclassifiable, conditional/unclassified, none, unlikely, possible, probable/likely, or certain by investigators.

2.6. Statistical analysis

We choose an arbitrary sample size of 40 subjects based on the subject numbers used for previous phase 1 vaccine studies [17–19] and regulatory guidelines for clinical evaluation of vaccines by the World Health Organization (WHO) [20] and KFDA [21]. The frequency of AEs and AEs considered to be related to the study vaccines were compared among three treatment groups using Fisher’s exact test. An analysis of variance (ANOVA) was performed to evaluate the differences of anti-diphtheria and anti-tetanus antibody titers between pre-vaccination and post-vaccination. The differences were deemed statistically significant at the level of 0.05. The geometric mean ratios and 95% confidence intervals (CI) for anti-diphtheria and anti-tetanus antibody titers of post-vaccination to pre-vaccination were calculated. Statistical analyses were performed using SPSS® 12.0 (SPSS Korea, Seoul, Republic of Korea).

3. Results

3.1. Subjects

Forty-two Korean male adults were enrolled in this study. Two subjects received no treatment due to their withdrawal from the study. Twenty subjects were vaccinated with GC1107, 10 subjects with active comparator and the remaining 10 subjects with placebo (Fig. 1). The mean (SD) age, height and weight were 28.1 (7.3) years, 172.1 (5.1) cm and 68.5 (7.6) kg, respectively.
3.2. Immunogenicity

Prior to vaccination, the geometric mean anti-diphtheria antibody concentration in GC1107, active comparator or placebo group was 0.12 IU/mL, 0.08 IU/mL and 0.17 IU/mL, respectively. At 2 and 4 weeks after vaccination, the antibody levels of the GC1107 group increased 9.2 fold (p < 0.001) and 9.3 fold (p < 0.001), respectively, compared to pre-vaccination titers, and the corresponding values of the active comparator group increased 9.3 fold (p = 0.003) and 8.3 fold (p = 0.004), respectively. The anti-diphtheria antibody titers were not significantly changed in the placebo group (2-week and 4-week post-vaccination; p = 0.996, p = 0.967).

The geometric mean titer of anti-tetanus antibody at baseline was 0.23 IU/mL in the GC1107 group, 0.19 IU/mL in the active comparator group and 0.30 IU/mL in the placebo group. Anti-tetanus titers increased 39.0 times (p < 0.001) and 37.9 times (p < 0.001) at 2 and 4 weeks, respectively, after GC1107 administration, and 12.2 times (p < 0.001) and 14.7 times (p < 0.001) after active comparator administration. No increases in anti-tetanus antibody were observed for the placebo group (2-week and 4-week post-vaccination; p = 0.964, p = 0.998) (Fig. 2, Tables 1 and 2).

After vaccination, 95% of the GC1107 group subjects and 90% of the active comparator group subjects reached the protective anti-diphtheria antibody titer (≥0.1 IU/mL) [1,2]. The anti-tetanus levels of all subjects who were administered GC1107 or active comparator were ≥0.1 IU/mL [3,4]. The percentage of subjects whose antibody titer against diphtheria or tetanus was ≥0.1 IU/mL did not increase in the placebo group (Tables 1 and 2).

3.3. Safety

No serious AEs were reported or observed, and none of the subjects withdrew from the study due to AEs. Thirty-three AEs (both local and systemic AEs) in 14 subjects with GC1107, 22 AEs in 8 active comparator subjects and 9 AEs in 4 placebo subjects were reported. Among them, 27 AEs with GC1107, 21 AEs with active comparator and 3 AEs with placebo were considered to be related to the study vaccines (Table 3). All AEs except for two (fatigue, neck stiffness) reported in the placebo group were mild.

Fig. 1. Flow diagram of subject participation.

Fig. 2. (a) Anti-diphtheria and (b) anti-tetanus antibody titer 14 and 28 days after a single intramuscular administration of GC1107, active comparator or placebo. Boxes represent arithmetic mean and bars show standard deviations.
Table 1
Anti-diphtheria antibody responses 14 and 28 days after a single intramuscular administration of GC1107, active comparator or placebo in Korean adults.

<table>
<thead>
<tr>
<th></th>
<th>GC1107 (N=20)</th>
<th></th>
<th>Active comparator (N=10)</th>
<th></th>
<th>Placebo (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-dose</td>
<td>14 days post-dose</td>
<td>28 days post-dose</td>
<td>Pre-dose</td>
<td>14 days post-dose</td>
</tr>
<tr>
<td>Titer (IU/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.256</td>
<td>2.768</td>
<td>2.514</td>
<td>0.166</td>
<td>1.649</td>
</tr>
<tr>
<td>Range</td>
<td>&lt;LLOQ–0.849</td>
<td>&lt;LLOQ–9.414</td>
<td>0.014–9.113</td>
<td>0.022–0.636</td>
<td>0.024–4.413</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>0.124</td>
<td>1.134</td>
<td>1.149</td>
<td>0.081</td>
<td>0.752</td>
</tr>
<tr>
<td>GMR to pre-dose</td>
<td>–</td>
<td>9.2</td>
<td>9.3</td>
<td>–</td>
<td>9.3</td>
</tr>
<tr>
<td>95% CI of GMR</td>
<td>–</td>
<td>3.3–25.9</td>
<td>3.3–26.2</td>
<td>–</td>
<td>2.3–37.0</td>
</tr>
<tr>
<td>Percentage of subjects (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody titer ≥ 0.1 IU/mL</td>
<td>55.0</td>
<td>95.0</td>
<td>95.0</td>
<td>40.0</td>
<td>90.0</td>
</tr>
<tr>
<td>Antibody titer ≥ 1.0 IU/mL</td>
<td>0.0</td>
<td>60.0</td>
<td>60.0</td>
<td>0.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

LLOQ (lower limit of quantification); 0.01 IU/mL; GMR (geometric mean ratio); CI (confidence interval).

Table 2
Anti-Tetanus antibody responses 14 and 28 days after a single intramuscular administration of GC1107, active comparator or placebo in Korean adults.

<table>
<thead>
<tr>
<th></th>
<th>GC1107 (N=20)</th>
<th></th>
<th>Active comparator (N=10)</th>
<th></th>
<th>Placebo (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-dose</td>
<td>14 days post-dose</td>
<td>28 days post-dose</td>
<td>Pre-dose</td>
<td>14 days post-dose</td>
</tr>
<tr>
<td>Titer (IU/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.592</td>
<td>13.60</td>
<td>13.06</td>
<td>0.307</td>
<td>5.158</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>0.230</td>
<td>8.972</td>
<td>8.700</td>
<td>0.191</td>
<td>2.332</td>
</tr>
<tr>
<td>GMR to pre-dose</td>
<td>–</td>
<td>39.0</td>
<td>37.9</td>
<td>–</td>
<td>12.2</td>
</tr>
<tr>
<td>95% CI of GMR</td>
<td>–</td>
<td>18.8–80.9</td>
<td>18.3–78.4</td>
<td>–</td>
<td>3.5–42.7</td>
</tr>
<tr>
<td>Percentage of subjects (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody titer ≥ 0.1 IU/mL</td>
<td>65.0</td>
<td>100.0</td>
<td>100.0</td>
<td>70.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Antibody titer ≥ 1.0 IU/mL</td>
<td>20.0</td>
<td>100.0</td>
<td>100.0</td>
<td>10.0</td>
<td>70.0</td>
</tr>
</tbody>
</table>

LLOQ (lower limit of quantification); 0.1 IU/mL; GMR (geometric mean ratio); CI (confidence interval).
and resolved without any treatment. The frequency of AEs considered to be related to the study vaccines in the GC1107 and active comparator groups were higher compared to placebo group, but statistical significance was not observed for the GC1107 group (GC1107 group: p = 0.088, active comparator group: p = 0.035). No significant difference in the AE frequency between the GC1107 and active comparator groups was observed (p = 0.293). Other safety assessments such as vital signs, electrocardiograms, physical examinations and clinical laboratory tests did not indicate any clinically significant abnormality.

4. Discussion

The purpose of the current study was to evaluate the immunogenicity and safety of a newly developed Td vaccine, GC1107, in comparison with placebo and active comparator.

As a main finding of this study, GC1107 provided significant boosts of anti-diphtheria and anti-tetanus antibodies compared to placebo, and the increases of the antibodies were sufficient to attain the protective antibody levels against diphtheria and tetanus ($\geq 0.11$ IU/mL) [1–4,10].

Although the AEs considered to be related to the vaccine in the GC1107 group were more frequent than for the placebo group, their frequency and nature were similar to the active comparator group. Based on these observations, we suggest that GC1107 is safe and tolerable compared to the licensed Td vaccine.

The extent of the increase of anti-diphtheria antibody titer after GC1107 vaccination was similar to that after active comparator vaccination, but the extent of the increase of anti-tetanus antibody titer in the GC1107 group was higher than that in the active comparator group. The changes in antibody titers from the current study were highly variable in the same active treatment group. Variable immune responses to Td vaccines have also been reported in previous studies [22,23]. Based on our observations, we suggest that the differences in the extent of the titer increase were caused by individual variability of the immune response against tetanus toxoid. However, the number of subjects in the current study was insufficient to confirm the difference in the observed titer increase and elucidate the causes of the difference. Therefore, an additional clinical study with a larger number of subjects should be considered in order to evaluate the difference in tetanus antibody titer increase between the two active vaccines as well as the causes of the difference.

Although the relationship between the extent of antibody increases and safety has not been reported, we suggest that the higher increase is not correlated with the safety of the vaccine because the frequency and the nature of the AEs for the two active vaccine groups were similar. However, we could not draw conclusions concerning this relationship from this study due to the limitation of small sample size. If the higher antibody titer provides longer periods of immunity against tetanus, higher boosts of antibody titers might be an advantage of this newly developed vaccine. However, the relationship between the extent of antibody increase and long-term immunity has not been reported and the long-term immunogenicity was not evaluated in the current study. For these reasons, a larger clinical study with long-term follow-up should be considered to evaluate the safety and immunogenicity of GC1107.

5. Conclusion

GC1107 was well tolerated and provided significant boosts of anti-diphtheria and anti-tetanus antibodies.

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Conflict of interest: The authors declare no conflict of interest.

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