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ORIGINAL ARTICLE

Immunogenicity and safety of the new reduced-dose tetanus–diphtheria vaccine in healthy Korean adolescents: A comparative active control, double-blind, randomized, multicenter phase III study

Seung Beom Han [a], Jung-Woo Rhim [a], Hye Jo Shin [a], Sang Yong Kim [a], Jong-Hyun Kim [a], Hyun-Hee Kim [a], Kyung-Yil Lee [a], Hwang Min Kim [b], Young Youn Choi [c], Sang Hyuk Ma [d], Chun Soo Kim [e], Dong Ho Kim [f], Dong Ho Ahn [g], Jin Han Kang [a,*]

[a] *Department of Pediatrics, College of Medicine, Catholic University of Korea, Seoul, Republic of Korea*
[b] *Department of Pediatrics, Wonju College of Medicine, Yonsei University, Wonju, Republic of Korea*
[c] *Department of Pediatrics, Medical School, Chonnam National University, Gwangju, Republic of Korea*
[d] *Department of Pediatrics, Changwon Fatima Hospital, Changwon, Republic of Korea*
[e] *Department of Pediatrics, College of Medicine, Keimyung University, Daegu, Republic of Korea*
[f] *Department of Pediatrics, Korea Cancer Center Hospital, Seoul, Republic of Korea*
[g] *Research Center, Green Cross Corporation, Yongin, Republic of Korea*

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Background/Purpose: A new reduced-dose tetanus–diphtheria (Td) vaccine was developed in Korea, and phase I and II clinical trials were successfully undertaken. We conducted this double-blind, randomized, multicenter phase III clinical trial to assess the immunogenicity and safety of the new Td vaccine.
Methods: Healthy adolescents 11–12 years of age were enrolled and randomized to receive the new Td vaccine (study group) or a commercially available Td vaccine (control group). Blood samples were collected prior to and 4 weeks after the vaccination. Between the study and control groups, seroprotection rate, booster response, and geometric mean titer of antibodies

* Corresponding author. Department of Pediatrics, Seoul St. Mary's Hospital, College of Medicine, Catholic University of Korea, 222, Banpo-daero, Seocho-gu, Seoul 137-701, Republic of Korea.
E-mail address: kjhan@catholic.ac.kr (J.H. Kang).

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against diphtheria and tetanus toxoids were compared after the vaccination. All solicited and unsolicited adverse events and serious adverse events during the 6-week study period were monitored.
Results: A total of 164 adolescents received vaccination, and 156 of them were evaluated to assess immunogenicity. The seroprotection rate and geometric mean titer for antibodies against diphtheria were significantly higher in the study group, whereas those against tetanus were significantly higher in the control group. However, all seroprotection rates against diphtheria and tetanus in the study and control groups were high: 100% against diphtheria and tetanus in the study group, and 98.7% against diphtheria and 100% against tetanus in the control group. No significant differences in the frequency of solicited and unsolicited adverse events were observed between the two vaccine groups.
Conclusion: The new Td vaccine is highly immunogenic and safe, and this new Td vaccine can be effectively used for preventing diphtheria and tetanus.

Introduction

Although the incidence of diphtheria and tetanus has been markedly reduced owing to the use of diphtheria—tetanus—pertussis (DTP) vaccines, intermittent diphtheria outbreaks have continued to be reported in countries where the DTP immunization program in children has not been successful or where reduced-dose tetanus—diphtheria (Td) booster immunization in adolescents and adults has been ineffective.[1—3] Furthermore, tetanus can only be prevented by vaccination because protective immunity is not acquired after natural infections or exposures.[4,5] In this aspect, booster immunization against diphtheria and tetanus has been strongly recommended for maintaining long-term protective immunity.[6—10]

Td vaccines, which can elicit protective immunity and are less reactogenic, have been developed since the late 1990s through many manufacturing trials,[11] and they are currently available in many countries. Sequentially, the Td and inactivated poliovirus (Td-IPV) vaccine and the reduced-dose tetanus—diphtheria—acellular pertussis (Tdap) vaccines were also developed. In most countries, decennial booster immunization for diphtheria and tetanus is recommended for people who completed four DTP vaccinations prior to 6 years of age with the booster Td vaccine recommended to start at 11—12 years of age.[12] However, the shortage of Td or Td-combined vaccines is a concern because of a limited Td vaccine supply, which is particularly problematic for Korea because Korea depends entirely on Td vaccine imports. Because of this, a novel Td vaccine was developed in Korea, and we attempted to confirm the immunogenicity and safety of this new Td vaccine in adolescents through a noninferiority comparison study.

Materials and methods

Participants and study design

Healthy adolescents aged 11—12 years, who had received four or five doses of diphtheria—tetanus—acellular pertussis (DTaP) vaccine prior to 6 years of age and had no history of Td or Tdap vaccination, were enrolled in this study. This study was an active controlled, double-blind, randomized, multicenter phase III study, that was conducted at outpatient clinics in the pediatric departments of 10 hospitals in Korea. Participants with no proven vaccination history, acute febrile illness within 3 days, and underlying acute or chronic diseases were excluded. All participants visited the hospital three times for vaccination and prevaccination blood sampling, postvaccination blood sampling, and adverse diary card confirmation. Based on the premise of a 98% production rate of diphtheria and tetanus protective antibody, a 5% allowable error for the trial, and a 5% level of significance, the necessary number of participants was 73 per group; 82 per group considering a 10% wastage rate. This study was approved by the Institutional Review Board of each investigator hospital (Approval No. XC11MDMS0089K), and written informed consent was obtained from all participants and their parents. The study was registered in ClinicalTrials.gov (NCT01402713).

Vaccines and immunization

The GC1107-T5.0 Td vaccine (Green Cross Corporation, Yongin, Korea) was selected as the phase III study vaccine based on the previous phase II study,[13] and used as the study vaccine. The study Td vaccine contained 5.0 Lf (20 IU/0.5 mL) tetanus toxoid and 2.5 Lf (2 IU/0.5 mL) diphtheria toxoid. The control vaccine was Td pure (Novartis Korea, Seoul, Korea), which contains 20 IU tetanus toxoid and 2 IU diphtheria toxoid in a 0.5-mL suspension. The diphtheria seed bacteria were the *Corynebacterium diphtheriae* Park-Williams #8 strain in both the study and control groups; the tetanus seed bacteria were the *Clostridium tetani* Harvard strain in the study group and the *C. tetani* Massachusetts F1 strain in the control group. Both the study and control Td vaccines included alum salt and were prefilled vaccines with a dose of 0.5 mL. Their color was light yellow or white, which could not be visually differentiated, and all the vaccines were preserved and monitored in a refrigerator at 2—8°C. The vaccine was

intramuscularly injected in the deltoid muscle of all participants.

Immunogenicity assessment

Blood samples (5.0 mL) were collected prior to and 4 weeks after the vaccination. All serum samples were kept at −70°C until analysis. Two different enzyme-linked immunosorbent assay kits (RE56191 for diphtheria, RE56901 for tetanus) from the same company (IBL, Hamburg, Germany) were used to determine serum antibody levels against diphtheria and tetanus toxoids according to the manufacturer's instructions. An antibody level of ≥0.1 IU/mL against diphtheria or tetanus toxoid was considered indicative of seroprotection. A group comparison was conducted by calculating the geometric mean titer (GMT) and geometric mean titer ratio (GMR) of antibodies against diphtheria and tetanus toxoids between participants who received the study vaccine (study group) and the control vaccine (control group). The GMR was defined as the ratio of postvaccination antibody titer to prevaccination antibody titer. In addition, boosting responses for diphtheria and tetanus were analyzed by the following definition previously described.[14] A positive boosting response was defined as a postvaccination antibody titer of ≥0.4 IU/mL in participants with a prevaccination antibody titer <0.1 IU/mL, or a ≥4-fold increase of the postvaccination antibody titer in participants with a prevaccination antibody titer of ≥0.1 IU/mL.

Safety assessment

The study physicians observed any immediate adverse reactions within 30 minutes after vaccination. Daily telephone monitoring was conducted for 7 days after vaccination, and any solicited local or systemic adverse events (AEs) that occurred within 4 weeks after the vaccination were recorded on a diary card by the participants' parents. All participants were monitored for an additional 2-week safety follow-up period for unscheduled hospital visits and serious unsolicited AEs. The symptom intensity of AEs was graded on a scale of 0 to 3 with "grade 0" representing an absence of symptoms and "grade 3" representing a symptom that prevented normal activity, redness or swelling with a diameter ≥50 mm, or an axillary temperature ≥39.5°C. All unsolicited AEs and serious AEs that occurred during the study period were monitored, and the causal relationship between the AEs and vaccination was evaluated. All AEs were followed up until they were resolved.

Statistical analysis

The sex distribution, age, height, and weight were compared between the study and control groups. For immunogenicity analysis, the seroprotection rates against diphtheria and tetanus were evaluated with a 95% confidence interval. GMTs, GMRs, and boosting responses prior to and after the vaccination were also calculated using a 95% confidence interval. In safety assessments, all solicited local and systemic AEs were actively observed up to 28 days after vaccination, and all unsolicited AEs were observed up to 42 days after vaccination. Categorical factors were compared using a Chi-square test, and numerical factors were compared using Student *t* test between the study and control groups. A two-tailed $p < 0.05$ was considered significant. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Study participants

A total of 164 participants (study group, 83; control group, 81) were enrolled in and completed this clinical study. Among them, three in each group were eliminated owing to violations of the selection criteria and one in each group was eliminated owing to a coadministration drug violation. Overall, a total of 156 participants (study group, 79; control group, 77) were included in a per-protocol set in this study. The sex ratio, age, height, and weight were not significantly different between the two vaccine groups (Table 1).

Immunogenicity

Immunogenicity was assessed in a per-protocol set. The seroprotection rate against diphtheria after vaccination was 100.0% in the study group and 98.7% in the control group (Table 2). The seroprotection rate against tetanus after vaccination was 100.0% in both groups (Table 2). The boosting response against diphtheria was 87.3% (69/79) in the study group and 77.9% (60/77) in the control group (Table 2). The boosting response against tetanus was 92.4% (73/79) in the study group and 97.4% (75/77) in the control group (Table 2). There were no significant differences in boosting responses against diphtheria and tetanus between the two groups (Table 2). The GMTs for antibodies against diphtheria prior to the vaccination were 0.31 IU/mL and 0.32 IU/mL in the study and control groups, respectively (Table 3). The GMTs for antibodies against diphtheria after vaccination were 3.56 IU/mL in the study group and 2.73 IU/mL in the control group (Table 3). The GMRs for diphtheria were 11.45 in the study group and 8.54 in the control group (Table 3). The GMT and GMR for anti-diphtheria antibodies in the study group were significantly higher than those in the control group after vaccination

Table 1 Demographic characteristics of safety-analyzed study participants and control groups.

Characteristics	Study group ($n = 83$)	Control group ($n = 81$)
Sex		
Male	40 (48.2)	43(53.1)
Female	43 (51.8)	38 (46.9)
Age (y)	11.7 ± 1.6	11.5 ± 1.7
Height (cm)	152.7 ± 10.5	151.6 ± 11.1
Weight (kg)	47.1 ± 12.4	46.4 ± 13.2

Data are presented as *n* (%) or mean ± SD.

Table 2 Seroprotection rate and boosting response after reduced-dose tetanus–diphtheria vaccination.

	Study group	Control group	*p*
Seroprotection rate against diphtheria	100.0 (79/79)	98.7 (76/77)	0.494
Boosting response against diphtheria	87.3 (80.0–94.7)	77.9 (68.7–87.2)	0.120
Seroprotection rate against tetanus	100.0 (79/79)	100.0 (77/77)	NA
Boosting response against tetanus	92.4 (86.6–98.3)	97.4 (93.9–100.0)	0.277

Data are presented as %, *n*/*N* or % (95% CI).
CI = confidence interval; NA = not available.

(Table 3). The GMTs for antibodies against tetanus prior to the vaccination were 0.44 IU/mL in the study group and 0.41 IU/mL in the control group (Table 4). The GMTs for antibodies against tetanus after vaccination were 15.04 IU/mL in the study group and 16.34 IU/mL in the control group (Table 4). The GMRs for tetanus were 34.09 in the study group and 39.47 in the control group (Table 4). The GMT and GMR for antitetanus antibodies in the control group were significantly higher than those in the study group after vaccination (Table 4). Although significant differences were found in the immunogenicity analyses, both vaccines produced very strong immunogenicity against diphtheria and tetanus after Td booster vaccination.

Safety

Safety was assessed in all of the 164 vaccinated participants. A total of 172 AEs were observed in 62 (74.1%) of the study group participants and 175 AEs were observed in 65 (80.3%) of the control group participants, and AEs due to the vaccine were observed in 165 episodes in 61 (73.5%) study group participants and 169 episodes in 63 (77.8%) control group participants (Table 5). The frequencies of AEs in both groups were not significantly different. Unsolicited AEs included seven episodes in six (7.2%) participants in the study group and six episodes in four (4.9%) children in the control group. No significant differences in the frequency of

Table 3 Antidiphtheria antibody responses following reduced-dose tetanus–diphtheria vaccination.

	Study group (*n* = 79)		Control group (*n* = 77)	
	Prevaccination	Postvaccination	Prevaccination	Postvaccination
Antibody titer (IU/mL)				
0.01–0.1	14 (17.7)	0 (0.0)	9 (11.7)	1 (1.3)
0.1–1.0	55 (69.6)	2 (2.5)	53 (68.8)	4 (5.2)
1.0–2.0	4 (5.1)	12 (15.2)	12 (15.6)	6 (7.8)
≥2.0	6 (7.6)	65 (82.3)	3 (3.9)	66 (85.7)
GMT (IU/mL)	0.31 (0.24–0.41)	3.56 (2.95–4.30)*	0.32 (0.25–0.41)	2.73 (2.29–3.27)*
GMR		11.45 (9.26–14.15)**		8.54 (6.87–10.62)**

Data are presented as *n* (%) or *n* (95% CI).
* The *p* value for postvaccination GMTs between the study and control groups was 0.024.
** The *p* value for GMRs between the study and control groups was <0.001.
CI = confidence interval; GMR = geometric mean titer ratio; GMT = geometric mean titer.

Table 4 Antitetanus antibody responses following reduced-dose tetanus–diphtheria vaccination.

	Study group (*n* = 79)		Control group (*n* = 77)	
	Prevaccination	Postvaccination	Prevaccination	Postvaccination
Antibody titer (IU/mL)				
0.01–0.1	6 (7.6)	0 (0.0)	9 (11.7)	0 (0.0)
0.1–1.0	58 (73.4)	0 (0.0)	53 (68.8)	0 (0.0)
1.0–2.0	7 (8.9)	0 (0.0)	12 (15.6)	0 (0.0)
≥2.0	8 (10.1)	79 (100.0)	3 (3.9)	77 (100.0)
GMT (IU/mL)	0.44 (0.34–0.57)	15.04 (12.86–17.59)*	0.41 (0.32–0.53)	16.34 (13.85–19.28)*
GMR		34.09 (25.69–45.24)**		39.47 (30.81–50.56)**

Data are presented as *n* (%) or *n* (95% CI).
* The *p* value for postvaccination GMTs between the study and control groups was <0.001.
** The *p* value for GMRs between the study and control groups was <0.001.
CI = confidence interval; GMR = geometric mean titer ratio; GMT = geometric mean titer.

Table 5 Incidence of solicited local and systemic adverse events due to vaccination during a 4-week follow-up period after reduced-dose tetanus–diphtheria vaccination.

	Study group (n = 83)				Control group (n = 81)				p
	Grade 1	Grade 2	Grade 3	Total	Grade 1	Grade 2	Grade 3	Total	
Local adverse events	139 episodes in 60 (72.3%) participants				138 episodes in 63 (77.8%) participants				0.533
Pain	45 (54.2)	7 (8.4)	1 (1.2)	53 (63.9)	57 (70.4)	3 (3.7)	0 (0.0)	60 (74.1)	0.158
Induration	32 (38.6)	5 (6.0)	1 (1.2)	38 (45.8)	35 (43.2)	4 (4.9)	0 (0.0)	39 (48.1)	0.762
Swelling	23 (27.7)	1 (1.2)	1 (1.2)	25 (30.1)	18 (22.2)	0 (0.0)	0 (0.0)	18 (22.2)	0.250
Erythema	14 (16.9)	5 (6.0)	4 (4.8)	23 (27.7)	14 (17.3)	5 (6.2)	2 (2.5)	21 (25.9)	0.797
Systemic adverse events	26 episodes in 17 (20.5%) participants				31 episodes in 21 (25.9%) participants				0.409
Malaise	11 (13.3)	3 (3.6)	0 (0.0)	14 (16.9)	11 (13.6)	0 (0.0)	0 (0.0)	11 (13.6)	0.558
Headache	4 (4.8)	0 (0.0)	0 (0.0)	4 (4.8)	7 (8.6)	1 (1.2)	0 (0.0)	8 (9.9)	0.214
Arthralgia	3 (3.6)	0 (0.0)	0 (0.0)	3 (3.6)	2 (2.5)	0 (0.0)	0 (0.0)	2 (2.5)	1.000
Fever	1 (1.2)	0 (0.0)	0 (0.0)	1 (1.2)	2 (2.5)	0 (0.0)	0 (0.0)	2 (2.5)	0.494
Skin rash or itching	3 (3.6)	0 (0.0)	0 (0.0)	3 (3.6)	6 (7.4)	0 (0.0)	0 (0.0)	6 (7.4)	0.440
Gastrointestinal symptoms	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.2)	2 (2.5)	0 (0.0)	0 (0.0)	2 (2.5)	0.618
Total adverse events	165 episodes in 61 (73.5%) participants				169 episodes in 63(77.8%) participants				0.532

Data are presented as n (%).

solicited and unsolicited AEs were observed between the two groups. In addition, no cases with serious AEs were reported in either the study or control group. Pain was most frequent among solicited local AEs in both vaccine groups (Table 5). For the solicited local AEs of grade 3, there were seven (8.4%) participants in the study group and two (2.5%) participants in the control group. An analysis of the solicited systemic AEs revealed that malaise was most frequent in both groups and no solicited systemic AEs of grade 3 were reported in either group (Table 5). All unsolicited AEs revealed no causalities with the vaccines in either group. No significant differences in the number of AEs, or the prevalence and degree of severity of the solicited and unsolicited AEs, were observed in either group. In addition, no statistical significance in the rate of solicited AEs of grade 3 was detected between the two groups. All solicited AEs spontaneously resolved within 3–5 days without any medical management.

Discussion

The results of this study, which evaluated the immunogenicity and safety of a newly developed Td vaccine in Korean adolescents, showed satisfactory immunogenicity and safety. There have been a few comparative studies concerning the immunogenicity and safety of available Td vaccines from different manufactures and a few clinical studies concerned on a single Td vaccine. In this respect, the results of this comparative study of Td vaccines may be valuable.

In Korea, the diphtheria–tetanus–whole cell pertussis vaccine was introduced in 1958, and the DTaP vaccine has been used for primary and booster immunizations since 1982.[15–17] The DTaP immunization coverage rate of >95% resulted in a decrease in the prevalence of diphtheria and neonatal tetanus, and no cases of diphtheria have been reported since 1987 in Korea.[15] About 10 cases of tetanus have been reported annually in Korea,[18] and one case of polyneuropathy that was assumed to be caused by a diphtheria infection was reported.[19] However, previous seroepidemiological studies in Korea reported that most of the studied participants older than 40 years had antibodies against diphtheria and tetanus at < 0.1 IU/mL,[16,17,20] suggesting that Td booster vaccination is necessary for adolescents and adults to maintain long-term protective immunity against diphtheria and tetanus.

In the earlier stages of adult-type Td vaccine development, the goals were to overcome the poorly immunogenic and reactogenic low-dose diphtheria toxoid preparations.[21] Now, Td vaccines containing reduced-dose diphtheria and tetanus toxoids are well known to be immunogenic in adolescents and adults with previous DTP vaccinations, and the antibody response might be dependent on the history of DTP vaccination.[22–24] Also, the dose of diphtheria and tetanus toxoids contained in the vaccines may influence the immunogenicity against diphtheria and tetanus. Nevertheless, many studies reported that available Td or Td-combined vaccines could produce remarkable immunogenicity in adolescents and adults.[25–29] Typically, the GMTs of antibodies against diphtheria and tetanus are strongly protective (exceeding 1.0 IU/mL) and the GMT of antibodies against tetanus is higher than that of diphtheria after Td booster immunization. Both study and control Td vaccines in the present study revealed strong immunogenicity against diphtheria and tetanus with 100.0% seroprotection rates against diphtheria and tetanus after vaccination. Antidiphtheria antibody levels after vaccination of the study group were raised to >1.0 IU/mL in almost all participants, and the antitetanus antibody levels after boosting immunization were raised to >2.0 IU/mL. These results were not significantly different from those of the control group. The GMT and GMR levels of antidiphtheria antibodies in the study group were significantly higher than those of the control group; however, the GMT and GMR levels of antitetanus antibodies in the control group were significantly higher than those of the study group. Based on our results, we speculated that the differences in the extent of titer increase were caused by interindividual variability of the immune response against diphtheria and tetanus

toxoids. Moreover, we suspected that the different toxoid purification processes and different seed bacteria might also influence these results. The study vaccine was developed with an enhanced manufacturing method that consists of fermentation followed by a gel filtration purification process. Variable immune responses to Td vaccines have also been reported in previous studies.[30,31]

By contrast, the boosting responses against diphtheria and tetanus in the study group were 87.3% and 92.4%, respectively. The concept of the boosting response after Td vaccination was introduced in the early 21st century, and this response may be dependent on the definition of boosting response. In this context, the results of boosting responses in the present study may not definitively indicate a good response. However, the boosting responses against diphtheria and tetanus in the study group showed non-inferiority compared with those of the control group.

Generally, it is well known that adverse reactions can occur after immunization with diphtheria and tetanus toxoids-containing vaccines. The greater number of AEs occurring after Td vaccination may be related to previous frequent DTP vaccinations with alum salts, young age, female sex, the amount of antidiphtheria and antitetanus toxoid level, and high immunoglobulin E response rates to diphtheria and tetanus toxoids.[32–34] There have been attempts to minimize these adverse reactions, including the use of adjuvants not eliciting an immunoglobulin E response. However, many studies have reported that most AEs were mild and resolved within a few days.[27,29] Previous studies reported that the most common solicited local AE after Td vaccination was injection site pain (60–80%), followed by swelling and erythema (13–19%).[26–28] In the present study, injection site pain was also the most common local event in both groups. The number of participants with grade 3 local reactions was seven in the study group and two in the control group. Erythema, which occurs when antigen–antibody complexes are formed in the presence of high antibody levels that deposit in blood vessel walls and initiate a local inflammatory response,[22] was the most common severe local adverse reaction in both groups. However, the rates of local AEs of the two groups were not significantly different, and severe local AEs resolved spontaneously without treatment within 3–5 days. Previous studies reported that headache was the most common systemic AE (30–40%), followed by malaise, then skin rash with itching. In addition, systemic serious AEs were rare after Td booster vaccinations. In the present study, the most common systemic AE was malaise rather than headache. Malaise as the most common systemic AE was similar to the results of a previous study in Korea,[24] suggesting that malaise might be a race-specific AE. The rates of systemic AEs of the two groups were not significantly different. All unsolicited AEs were not related to Td vaccination, and no significant differences in the unsolicited AEs were observed in the two vaccine groups.

In conclusion, we found that the new Td vaccine was successfully immunogenic against diphtheria and tetanus after the boosting vaccination, and was not inferior to the control vaccine. The study vaccine was safe and tolerable. Based on these results, we hope that this new Td vaccine will be available in the future and contribute to the control of diphtheria and tetanus.

Conflicts of interest

D.H.A. is an employee of the Green Cross Corporation, but he was not involved in the data acquisition and analysis. All of the other authors declare no conflict of interest.

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