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Immunogenicity and Safety of an Inactivated Trivalent Split Influenza Virus Vaccine in Young Children with Recurrent Wheezing

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Influenza virus vaccination is recommended for children, but so far, active vaccination has not been achieved because most parents lack knowledge of vaccine safety and many doctors are reluctant to administer vaccine due to concerns that steroids might alter immunogenicity. The aim of this study was to compare the immunogenicity and safety of inactivated trivalent split influenza virus vaccine between children with recurrent wheezing and healthy children of the same age group. Sixty-eight healthy children and 62 children with recurrent wheezing took part in this study. Seroconversion rates, seroprotection rates, geometric mean titers (GMTs), and geometric mean titer ratios (GMTRs) were measured by a hemagglutination inhibition assay for the assessment of immunogenicity. Solicited and unsolicited local and systemic adverse events were measured for the assessment of safety. Regarding immunogenicity, the seroconversion and seroprotection rates showed no difference overall between healthy children and children with recurrent wheezing. Also, no difference was observed between steroid-treated and nontreated groups with recurrent wheezing. Generally, the GMTs after vaccination were higher in the one-dose vaccination groups for healthy children and children with recurrent wheezing, but the GMTRs revealed different results according to strain in the two groups. Regarding safety, solicited local and systemic adverse events showed no differences between healthy children and children with recurrent wheezing. This study demonstrates that inactivated split influenza virus vaccine is able to induce protective immune responses in healthy children, as observed in previous studies, as well as in children with recurrent wheezing who require frequent steroid treatment.

Influenza activity peaks from early winter to the following spring season, due to dry climates with wide temperature ranges. Therefore, influenza virus vaccination in early autumn is recommended for the development of protective immunity before the start of the winter influenza season. Young children under 3 years of age are at increased risk for acute viral respiratory infections and high influenza morbidity levels during the influenza season. Especially in infancy, wheezing frequently is associated with respiratory viral infections and allergy; about 20% of these children have recurrent wheezing episodes and might become asthmatic patients (1–3). Therefore, influenza virus vaccination is strongly recommended for children under 3 years of age during the peak influenza season (4–7). However, vaccination rates are low among children with recurrent wheezing, due to frequent hospital visits and hospitalizations. Some parents and physicians have the misconception that vaccination might aggravate wheezing illnesses. Also, doctors are reluctant to recommend influenza virus vaccination for children taking steroids, due to the lack of knowledge about influenza virus vaccine effectiveness and prescription information for the influenza virus vaccine that states that immunosuppressive agents might decrease immune responses (8). Such perceptions might cause incomplete or delayed vaccination among children with recurrent wheezing.

Therefore, the aim of this study was to compare the immunogenicity and safety of inactivated trivalent split influenza virus vaccine (TIV) between young children with recurrent wheezing and healthy children of the same age group. This study was designed to improve vaccination coverage and to recommend vaccination actively and at appropriate times for these infants.

MATERIALS AND METHODS

Study design and subjects. This study was a phase 4, multicenter, open-label study to evaluate the immunogenicity and safety of inactivated trivalent split influenza virus vaccine in 62 children with recurrent wheezing and in 68 healthy children from 6 months to 3 years of age. Children who were born by normal delivery with a birth history of gestational age of more than 37 weeks were eligible for enrollment. Recurrent wheezing was defined as ≥2 reports in the first year of life. The duration of enrollment was a total of 5 months between September 2011 and January 2012. Vaccine-naive children received two doses of TIV, and previously vaccinated children received 1 dose. Children who received two doses had 2 visits for influenza virus vaccination followed by 1 visit for blood sampling 28 days after the second dose. To evaluate reactogenicity, parents were contacted twice by telephone, between the first and second visits and between the second and third visits. Children who received 1 dose had 1 visit for influenza virus vaccination followed by 1 visit for blood sampling 28 days after vaccination. Parents also were contacted once by telephone between the first and second visits. Before participation in the study, we obtained written informed consent from parents or legally acceptable representatives, after informing them of the aim and background of this study. The study was conducted according to the Helsinki Declaration. This study protocol was approved by the institutional review boards of the United Catholic Medical Center, Seoul, South Korea, and each study center. Chil-

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dren who had received influenza virus vaccine within the preceding 6 months, who had acute, chronic, or congenital disease, or who had a history of hypersensitivity to eggs or egg proteins were excluded. We also excluded children who had received prednisolone at more than 20 mg per day or more than 2 mg/kg for 2 weeks or more continually. However, children who had received less than 20 mg prednisolone per day or 0.5 to 1.0 mg/kg for less than 2 weeks continually and those who were being treated with topical or inhalation treatment were included.

Vaccine. The study vaccine was prepared in embryonated chicken eggs with standard inactivated vaccine techniques that are used for the production of seasonal trivalent inactivated vaccine. It was a purified split vaccine (GC Flu; manufactured by Green Cross Company in South Korea), including 7.5 μg each of hemagglutinin antigens against A/California(H1N1), A/Perth(H3N2), and B/Brisbane influenza virus strains, in a 0.25-ml prefilled syringe, as recommended for the northern hemisphere by WHO in 2011 to 2012. It was administered by intramuscular injection on the anterolateral border of the thigh or deltoid site, depending on the patient's age.

Immunogenicity assessment. Blood samples were obtained before the first dose of vaccine and 28 days after the last dose. Approximately 3- to 5-ml blood samples were collected aseptically. These samples were centrifuged, and supernatant serum was apportioned into 0.5-ml aliquots for storage. Antibody responses were measured by hemagglutination inhibition (HI) assays, according to established procedures (9, 10) and with the use of guinea pig erythrocytes, at the Vaccine-Bio Institute of the Catholic University of Korea in Seoul. Before the HI assay, we performed validated screening HI assays of serum samples from 35 healthy children using fowl (chicken and turkey) red blood cells (RBCs) and guinea pig RBCs. The HI assay with guinea pig RBCs showed more-positive results for the H3N2 strain and similar results for the H1N1 and B strains. As the result, we conducted the HI assay of our study with guinea pig RBCs (the percentage of guinea pig RBCs was 0.75%). The primary immunogenicity endpoint was the percentage of subjects with antibody titers of 1:40 or more in HI assays (seroprotection rate). The secondary immunogenicity endpoints were the percentage of subjects with seroconversion (a prevaccination titer of less than 1:10 with a postvaccination antibody titer of 1:40 or more or a prevaccination titer of 1:10 or more with a ≥4-fold increase in antibody titer), the geometric mean titer (GMT), and the geometric mean titer ratio (GMTR) (i.e., the geometric mean of the postvaccination/prevaccination titer ratio).

Safety assessment. Solicited local and systemic adverse events up to 6 days and unsolicited adverse events up to 28 days were reported using diary cards recorded by legal representatives. The severity of adverse events was measured on a scale of 0 to 4. Pain at the site of injection was graded as follows: grade 0, no pain or tenderness; grade 1, no limitation of activities; grade 2, limitation of activities or need for nonnarcotic analgesics; grade 3, limitation of daily life or need for narcotic analgesics; grade 4, hospitalization required. The severity of erythema and swelling was graded as follows: grade 0, no reaction; grade 1, <2.5 cm; grade 2, <5.0 cm; grade 3, ≥5.0 cm; grade 4, necrosis. The severity of fever was graded as follows: grade 0, <38.0°C; grade 1, 38.0 to 38.4°C; grade 2, 38.5 to 38.9°C; grade 3, 39.0 to 40°C; grade 4, >40°C. The severity of other adverse events was graded as follows: grade 1, no limitation of activities; grade 2, limitation of activities; grade 3, unable to carry out daily life; grade 4, hospitalization required. Unsolicited adverse events were graded into 3 groups, i.e., mild, moderate, and severe.

Statistical analysis. The primary objective of this study was to demonstrate that the lower boundary of the two-sided 95% confidence interval (CI) for the percentage of subjects achieving seroconversion for HI antibody met or exceeded 40% and the lower boundary of the two-sided 95% CI for the percentage of subjects achieving HI antibody titers of ≥1:40 met or exceeded 70% in both groups. The immunogenicity and safety endpoint analyses were descriptive, with calculation of two-sided 95% CIs. For dichotomous variables, 95% CIs were calculated with the exact method for proportions. GMTs and 95% CIs were calculated using the means and lower and upper limits of the 95% CIs of log-transformed titers. Significant differences in the distribution of variables between healthy children and children with recurrent wheezing were estimated with the chi-square test or Fisher's exact test for categorical variables and Student's *t* test for quantitative variables. A bilateral *P* value of <0.05 was considered a significant result. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL).

TABLE 1 Demographic and baseline characteristics of the healthy children and the children with recurrent wheezing

Characteristic	Healthy children ($N = 68$)	Children with recurrent wheezing ($N = 62$)
Gender (*n* [%[)		
Male	33 (48.53)	39 (62.90)
Female	35 (51.47)	23 (37.10)
Age (mean ± SD) (yr)	0.91 ± 0.71	1.21 ± 0.79[a]
Age group (*n* [%])		
6 mo to <1 yr	20 (29.41)	14 (22.58)
1 yr to <2 yr	34 (50.00)	21 (33.87)
2 yr to <3 yr	14 (20.51)	27 (43.55)[b]
Height (mean ± SD) (cm)	81.50 ± 7.42	84.59 ± 7.38
Weight (mean ± SD) (kg)	11.14 ± 2.07	11.91 ± 2.06
One-dose vaccination (*n* [%])	28 (41.2)	24 (38.7)
Two-dose vaccination (*n* [%])	40 (58.8)	38 (61.3)
Steroid-treated cases (*n* [%])	0 (0.00)	33 (53.2)

[a] $P = 0.0251$.
[b] $P = 0.0184$ (calculated with the chi-square test).

RESULTS

Study subjects. Overall, 68 healthy children and 62 children with recurrent wheezing were enrolled and included in the final analysis. Of all healthy children, 33 (48.53%) were male and 35 (51.47%) were female. Of all children with recurrent wheezing, 39 (62.90%) were male and 23 (37.10%) were female. The mean age of the healthy children was 0.91 ± 0.71 years and that of the children with recurrent wheezing was 1.21 ± 0.79 years. Children with recurrent wheezing had a greater proportion in the 2- to 3-year age group and greater mean age, height, and weight (Table 1). Influenza virus vaccination rates in the previous influenza season were similar for the two groups, as follows: healthy children, 28 children (41.2%); children with recurrent wheezing, 24 children (38.7%). The number of children who had received steroid treatment was 33 (53.2%) (Table 1).

Immunogenicity. The rates of seroconversion against the A/California(H1N1) strain were 69.12% for healthy children and 66.13% for children with recurrent wheezing. Both groups met the immunogenicity criterion of a seroconversion rate above 40%, and there was no difference between the two groups. The overall seroprotection rates were 75.53% for healthy children and 74.19% for children with recurrent wheezing, which were sufficient. The GMTs for preimmunization and postimmunization sera of children with recurrent wheezing were higher than those for the sera of healthy children, but the GMTR for healthy children was higher than that for children with recurrent wheezing. The rates of seroconversion against the A/Perth(H3N2) strain were 80.88% for healthy children and 74.19% for children with recurrent wheezing. The seroprotection rates were 83.82% for healthy children and 77.42% for children with recurrent wheezing. The GMTs be-

TABLE 2 Immunogenicity in both groups

Influenza strain and immunogenicity endpoint[a]	Healthy children ($N = 68$)	Children with recurrent wheezing ($N = 62$)	P
A/California(H1N1)			
Seroconversion (n [% (95% CI)])	47 (69.12 [58.14–80.10])	41 (66.13 [54.35–77.91])	0.7159
Seroprotection (n [% (95% CI)]), day 28	50 (75.53 [63.04–84.02])	46 (74.19 [63.30–85.09])	0.9314
GMT (95% CI), day 0	2.94 (1.80–4.54)	5.93 (3.56–9.53)	
GMT (95% CI), day 28	52.91 (40.45–69.12)	56.37 (38.89–81.31)	
GMTR	12.69	7.28	<0.0001
A/Perth(H3N2)			
Seroconversion (n [% (95% CI)])	55 (80.88 [71.54–90.23])	46 (74.19 [63.30–85.09])	0.3602
Seroprotection (n [% (95% CI)]), day 28	57 (83.82 [75.07–92.58])	48 (77.42 [67.01–87.83])	0.3548
GMT (95% CI), day 0	3.96 (2.43–6.15)	1.95 (1.01–3.32)	
GMT (95% CI), day 28	62.29 (46.30–83.68)	58.84 (42.40–81.51)	
GMTR	11.77	19.29	<0.0001
B/Brisbane			
Seroconversion (n [% (95% CI)])	42 (61.76 [50.21–73.32])	38 (61.29 [49.17–73.41])	0.7443
Seroprotection (n [% (95% CI)]), day 28	50 (75.53 [63.04–84.02])	44 (70.97 [59.67–82.27])	0.7444
GMT (95% CI), day 0	11.78 (9.38–14.73)	9.12 (6.66–12.38)	
GMT (95% CI), day 28	49.35 (40.17–60.59)	43.88 (35.76–53.79)	
GMTR	2.94	3.43	0.1777

[a] Seroconversion was defined as a prevaccination antibody titer of ≤1:10 and a postvaccination titer of ≥1:40. Seroprotection was defined as a postvaccination antibody titer of ≥1:40. CI, confidence interval; GMTR, geometric mean of post- to prevaccination titer ratios; GMT, geometric mean titer.

fore and after vaccination were higher for healthy children, but overall the GMTR was higher for children with recurrent wheezing than for healthy children. In addition, the rates of seroconversion against the B/Brisbane strain were 61.76% for healthy children and 61.29% for children with recurrent wheezing. The seroprotection rates were 75.53% for healthy children and 70.97% for children with recurrent wheezing. The GMTRs were 2.94 for healthy children and 3.43 for children with recurrent wheezing (Table 2).

Among children with recurrent wheezing, 33 children had received low-dose steroid treatment and 29 children had not been exposed to steroids. In a comparison of the two groups, the rate of seroconversion against the A/California(H1N1) strain for children receiving steroids was 69.70% and that for children who had not received steroids was 62.07%. The seroprotection rates were 75.76% and 72.41% and the GMTRs were 6.91 and 7.74, respectively. The rates of seroconversion against the A/Perth(H3N2) strain were 78.99% for the children receiving steroids and 68.97% for the other children. The seroprotection rates were 78.79% and 75.86% and the GMTRs were 19.72 and 18.81, respectively. In addition, the rates of seroconversion against the B/Brisbane strain were 54.55% for children receiving steroids and 68.97% for the remaining children. The seroprotection rates were 63.64% and 79.31%, respectively. Children who had not received steroids seemed to show greater immune responses, but no significant difference was observed. The GMTRs were similar for the two groups (Table 3).

Subgroup analysis according to previous influenza virus vaccination history was performed. Twenty-eight healthy children and 24 children with recurrent wheezing received one dose, and 40 healthy children and 38 children with recurrent wheezing received two doses. For healthy children, the rate of seroprotection against the A/California(H1N1) strain was significantly higher for the one-dose subgroup. In addition, preimmunization and postimmunization GMTs were higher for the one-dose subgroup but the GMTR was higher for the two-dose group. Similarly, for children with recurrent wheezing, the GMT was higher but the GMTR was lower for the one-dose subgroup. Preimmunization and postimmunization GMTs against the A/Perth(H3N2) strain were significantly higher for the one-dose subgroup of healthy children. However, preimmunization and postimmunization GMTs were higher for the two-dose subgroup of children with recurrent wheezing. The rates of seroprotection against the B/Brisbane strain were higher for the one-dose subgroups of healthy children and children with recurrent wheezing. In addition, preimmunization and postimmunization GMTs were higher for the one-dose subgroups of healthy children and children with recurrent wheezing, but no difference in GMTRs was observed between the subgroups (Table 4).

A comparison of antibody responses between healthy children and steroid-treated children with recurrent wheezing, according to previous influenza virus vaccination history, showed the following: in the group with previous influenza virus vaccinations, there were no differences in the rates of seroconversion and seroprotection against all strains. The GMTR against the A/California(H1N1) strain was higher in healthy children, but the GMTR against the A/Perth(H3N2) strain was higher in steroid-treated children. In the group without prior influenza virus vaccinations, there were no differences in the rates of seroconversion and seroprotection against all strains but the rate of seroprotection against the B/Brisbane strain was below 70% in both subgroups (67.50% for healthy children and 54.55% for steroid-treated children). The GMTR against the A/California(H1N1) strain was higher in healthy children, but the GMTR against the A/Perth(H3N2) strain was higher in steroid-treated children (Table 5).

Safety. Of the healthy children, 29.41% were reported to have solicited local adverse events, including tenderness, pain, redness,

TABLE 3 Comparison of immunogenicity between steroid-treated and nontreated groups of young children with recurrent wheezing

Influenza strain and immunogenicity endpoint[a]	Steroid-treated group ($N = 33$)	Non-steroid-treated group ($N = 29$)	P
A/California(H1N1)			
Seroconversion (n [% (95% CI)])	23 (69.70 [54.02–85.38])	18 (62.07 [44.31–79.73])	0.5266
Seroprotection (n [% (95% CI)]), day 28	25 (75.76 [61.14–90.38])	21 (72.41 [56.15–88.68])	0.7640
GMT (95% CI), day 0	6.29 (3.02–12.24)	5.53 (2.50–11.18)	0.4870
GMT (95% CI), day 28	56.67 (33.05–96.67)	56.04 (32.88–95.05)	0.4430
GMTR	6.91	7.74	0.3901
A/Perth(H3N2)			
Seroconversion (n [% (95% CI)])	26 (78.99 [64.84–92.74])	20 (68.97 [52.13–85.80])	0.3378
Seroprotection (n [% (95% CI)]), day 28	26 (78.79 [64.84–92.74])	22 (75.86 [60.29–91.44])	0.7834
GMT (95% CI), day 0	1.70 (0.65–3.41)	2.27 (0.75–5.11)	0.5455
GMT (95% CI), day 28	54.87 (34.51–86.91)	63.71 (38.96–103.79)	<0.0001
GMTR	19.72	18.81	0.4671
B/Brisbane			
Seroconversion (n [% (95% CI)])	18 (54.55 [37.56–71.53])	20 (68.97 [52.13–85.80])	0.2448
Seroprotection (n [% (95% CI)]), day 28	21 (63.64 [47.72–80.05])	23 (79.31 [64.57–94.05])	0.1749
GMT (95% CI), day 0	7.69 (4.75–12.12)	11.04 (7.19–16.70)	<0.0001
GMT (95% CI), day 28	37.85 (28.88–49.50)	51.89 (37.77–71.15)	<0.0001
GMTR	3.47	3.39	0.8766

[a] Seroconversion was defined as a prevaccination antibody titer of ≤1:10 and a postvaccination titer of ≥1:40. Seroprotection was defined as a postvaccination antibody titer of ≥1:40. CI, confidence interval; GMTR, geometric mean of post- to prevaccination titer ratios; GMT, geometric mean titer.

and swelling (in order of frequency). Solicited local adverse events were reported by 20.97% of the children with recurrent wheezing (in the same order as described above). The healthy children were frequently reported to have local adverse events, but no difference was observed between the two groups. There also was no difference in grade 2 adverse events between the two groups. Of the healthy children, 26.47% were reported to have solicited systemic adverse events, including fatigue, drowsiness, fever, myalgia, headache, shivering, and arthralgia (in order of frequency). Of the children with recurrent wheezing, 20.97% were also reported to have solicited systemic adverse events, including fever, fatigue, headache, drowsiness, sweating, shivering, and myalgia (in order of frequency), while arthralgia was not reported. In healthy children, fatigue and drowsiness were more common. The drowsiness incidence was higher for healthy children. However, no differences were observed for drowsiness over grade 2 (Table 6). All local or systemic adverse events spontaneously resolved within 3 days, and no cases of aggravation of wheezing or respiratory symptoms after influenza virus vaccination were confirmed in children with recurrent wheezing.

DISCUSSION

Nonatopic wheezing is a transient symptom that might be related to respiratory viral infections in young infants and might spontaneously disappear after they grow up. Atopic wheezing is a recurrent symptom with a greater risk of becoming asthma

TABLE 4 Comparison of antibody responses with one-dose vaccination and two-dose vaccination in both groups

	Healthy children			Children with recurrent wheezing		
Influenza strain and antibody response[a]	One-dose group ($N = 28$)	Two-dose group ($N = 40$)	P	One-dose group ($N = 24$)	Two-dose group ($N = 38$)	P
A/California(H1N1)						
Seroconversion (n [% (95% CI)])	22 (78.57 [63.37–93.77])	25 (62.50 [47.50–77.50])	0.1580	16 (66.67 [47.81–85.23])	25 (65.79 [50.71–80.87])	0.9433
Seroprotection (n [% (95% CI)]), day 28	25 (89.29 [77.83–100])	25 (62.50 [47.50–77.50])	0.0137	21 (87.50 [74.27–100])	25 (65.79 [50.71–80.87])	0.0570
GMT (95% CI), day 0	7.01 (3.71–11.63)	1.39 (0.62–2.55)	<0.0001	13.31 (6.61–25.92)	3.38 (1.60–6.39)	<0.0001
GMT (95% CI), day 28	78.50 (56.01–109.87)	40.07 (27.39–58.42)	<0.0001	98.72 (64.12–151.71)	39.47 (23.39–66.15)	<0.0001
GMTR (95% CI)	8.92 (4.35–17.4)	16.15 (9.51–27.00)	<0.0001	5.97 (2.73–12.03)	8.24 (4.61–14.21)	0.0155
A/Perth(H3N2)						
Seroconversion (n [% (95% CI)])	23 (82.14 [67.96–96.33])	32 (80.00 [67.60–92.40])	0.8250	16 (66.67 [47.81–85.23])	30 (78.95 [65.98–91.91])	0.2817
Seroprotection (n [% (95% CI)]), day 28	24 (85.71 [72.75–98.68])	33 (82.50 [70.72–94.28])	1.0000	17 (70.83 [52.65–89.02])	31 (81.58 [69.25–93.90])	0.3243
GMT (95% CI), day 0	10.16 (5.92–17.02)	1.81 (0.77–3.46)	<0.0001	3.64 (1.60–7.30)	1.22 (0.34–2.66)	0.0079
GMT (95% CI), day 28	75.17 (44.75–125.79)	54.60 (37.90–78.48)	<0.0001	41.64 (27.64–62.47)	73.13 (45.77–116.51)	<0.0001
GMTR (95% CI)	5.82 (3.90–8.51)	18.81 (12.00–29.17)	<0.0001	8.18 (4.45–14.48)	32.47 (18.37–56.82)	<0.0001
B/Brisbane						
Seroconversion (n [% (95% CI)])	19 (67.86 [50.56–85.16])	23 (57.50 [42.18–77.82])	0.3871	16 (66.67 [47.81–85.23])	22 (57.89 [42.20–73.59])	0.4898
Seroprotection (n [% (95% CI)]), day 28	23 (82.14 [67.96–96.33])	27 (67.50 [59.28–82.02])	0.1780	21 (87.50 [74.27–100])	23 (60.53 [44.98–76.07])	0.0227
GMT (95% CI), day 0	15.08 (11.20–20.18)	9.88 (7.09–13.63)	<0.0001	14.87 (9.85–22.22)	6.62 (4.26–10.03)	<0.0001
GMT (95% CI), day 28	59.73 (43.97–80.99)	43.16 (32.59–57.06)	<0.0001	61.31 (43.48–86.28)	35.48 (27.86–45.10)	<0.0001
GMTR (95% CI)	2.78 (1.85–4.01)	3.06 (1.97–4.54)	0.3992	2.93 (1.86–4.39)	3.79 (2.15–6.28)	0.0769

[a] CI, confidence interval; GMTR, geometric mean of post- to prevaccination titer ratios; GMT, geometric mean titer.

TABLE 5 Comparison of antibody responses according to previous influenza virus vaccination history for healthy children and steroid-treated children with recurrent wheezing

Influenza strain and antibody response[a]	Group with previous vaccination (one-dose group)			Group without previous vaccination (two-dose group)		
	Healthy children ($N = 28$)	Steroid-treated children ($N = 11$)	P	Healthy children ($N = 40$)	Steroid-treated children ($N = 22$)	P
A/California (H1N1)						
Seroconversion (n [% (95% CI)])	22 (78.57 [63.37–93.77])	8 (72.73 [46.41–99.05])	0.6927	25 (62.50 [47.50–77.50])	15 (68.18 [48.72–87.65])	0.6546
Seroprotection (n [% (95% CI)]), day 28	25 (89.29 [77.83–100])	10 (90.91 [73.92–100])	1.0000	25 (62.50 [47.50–77.50])	15 (68.18 [48.72–87.65])	0.6546
GMT (95% CI), day 0	7.01 (3.71–12.63)	18.03 (7.07–45.18)	<0.0001	1.39 (0.62–2.55)	3.48 (1.15–8.36)	0.0427
GMT (95% CI), day 28	78.50 (56.01–109.87)	110.49 (55.14–220.42)	<0.0001	40.07 (27.39–58.42)	40.47 (19.50–82.92)	0.6599
GMTR (95% CI)	8.92 (4.35–17.40)	4.78 (2.27–9.20)	<0.0001	16.15 (9.51–27.00)	8.25 (3.76–16.97)	<0.0001
A/Perth (H3N2)						
Seroconversion (n [% (95% CI)])	23 (82.14 [67.96–96.33])	8 (72.73 [46.41–99.05])	0.6632	32 (80.00 [67.60–92.40])	18 (81.82 [65.70–97.94])	1.0000
Seroprotection (n [% (95% CI)]), day 28	24 (85.71 [72.75–98.68])	8 (72.73 [46.41–99.05])	0.3791	33 (82.50 [70.72–94.28])	18 (81.82 [65.70–97.94])	1.0000
GMT (95% CI), day 0	10.16 (5.92–17.02)	3.88 (1.08–10.45)	<0.0001	1.81 (0.77–3.46)	1.00 (0.09–2.69)	0.3368
GMT (95% CI), day 28	75.17 (44.75–125.79)	45.92 (23.14–90.20)	<0.0001	54.56 (37.90–78.48)	59.96 (31.56–113.12)	<0.0001
GMTR (95% CI)	5.82 (3.90–8.51)	8.62 (4.74–15.13)	<0.0001	18.81 (12.00–29.17)	29.41 (15.82–53.95)	<0.0001
B/Brisbane						
Seroconversion (n [% (95% CI)])	19 (67.86 [50.56–85.16])	7 (63.64 [35.21–92.06])	1.0000	23 (57.50 [42.18–77.82])	11 (50.00 [29.11–70.89])	0.5702
Seroprotection (n [% (95% CI)]), day 28	23 (82.14 [67.96–96.33])	9 (81.82 [59.03–100])	1.0000	27 (67.50 [59.28–82.02])	12 (54.55 [33.74–75.35])	0.3123
GMT (95% CI), day 0	15.08 (11.20–20.18)	11.68 (5.14–25.19)	0.0001	9.88 (7.09–13.63)	6.19 (3.26–11.13)	<0.0001
GMT (95% CI), day 28	59.73 (43.99–80.99)	51.85 (29.62–90.23)	<0.0001	43.16 (32.59–57.06)	32.30 (23.77–43.77)	<0.0001
GMTR (95% CI)	2.78 (1.85–4.01)	3.17 (1.40–6.25)	0.4618	3.06 (1.97–4.54)	3.63 (1.62–7.19)	0.3605

[a] CI, confidence interval; GMTR, geometric mean of post- to prevaccination titer ratios; GMT, geometric mean titer.

(11) because of bronchial hyper-responsiveness. As children with recurrent wheezing frequently receive steroid treatment and therefore are at risk to acquire viral respiratory infections, it is highly recommended that they be vaccinated with influenza virus vaccine (12). In reality, however, these children frequently receive delayed vaccination or even miss vaccination, and some studies reported that these populations should be closely monitored for timely vaccination (13).

TABLE 6 Solicited adverse events within 7 days after vaccination in both groups

Solicited adverse reaction	No. (%) experiencing an adverse event			
	Healthy children ($N = 68$)		Children with recurrent wheezing ($N = 62$)	
	Any event	Grade ≥2 event	Any event	Grade ≥2 event
Local				
Tenderness	16 (23.53)	0	10 (16.13)	0
Pain	15 (22.06)	0	10 (16.13)	1
Redness	4 (5.88)	0	3 (4.84)	1
Swelling	2 (2.94)	0	1 (1.61)	0
Subtotal	20 (29.41)	0	13 (20.97)	2
Systemic				
Fever	9 (13.24)	9 (13.24)	8 (12.90)	8 (12.90)
Fatigue	11 (16.18)	1 (1.47)	5 (8.06)	0 (0.00)
Drowsiness	10 (14.71)[a]	1 (1.47)	2 (3.23)	0 (0.00)
Sweating	5 (7.35)	2 (2.94)	2 (3.23)	1 (1.61)
Headache	3 (4.41)	0 (0.00)	3 (4.84)	0 (0.00)
Myalgia	4 (5.88)	0 (0.00)	1 (1.61)	0 (0.00)
Shivering	1 (1.47)	1 (1.47)	2 (3.23)	1 (1.61)
Arthralgia	1 (1.47)	0 (0.00)	0 (0.00)	0 (0.00)
Subtotal	18 (26.47)	9 (13.24)	13 (20.97)	8 (12.90)

[a] $P = 0.0239$ (calculated with the chi-square test).

The average age of children with recurrent wheezing was older than that of healthy children, and the average age of vaccine-naive children with recurrent wheezing was older than that of vaccine-naive healthy children (Table 1). We can assume a tendency for delayed influenza virus vaccination in children with recurrent wheezing.

There is no absolute standardized criterion to evaluate the immunogenicity of influenza virus vaccines. However, the HI assay commonly is used to assess vaccine immunogenicity by measuring serum HI antibody titers against the three influenza virus vaccine strains and to evaluate postvaccination seroconversion rates, seroprotection rates, and GMTs, including GMTRs (14–16). Specific antibodies against hemagglutinin of influenza virus offer protective immunity, and those antibodies produced by B cells are T cell dependent (17). Therefore, antibody responses to influenza virus vaccines decrease in cases of alterations in the interactions between T cell and B cell immunity. Decreased helper T cell function alters overall immune responses (18, 19) and, as steroids are considered to affect T cell immunity, some experts assume that steroid treatment decreases vaccine immunogenicity (20). In that context, this study was carried out. There are reports that temporary treatment with high-dose steroids for asthmatic patients does not influence immunogenicity (21, 22), as reported for long-term steroid therapy (23, 24). Moreover, another study demonstrated that steroids did not influence the immunogenicity of influenza virus vaccination, and aggravation of asthmatic symptoms was not noted after vaccination among asthmatic children (8, 25, 26). According to these study results, the Committee on Infectious Diseases of the American Academy of Pediatrics strongly recommends timely vaccination for these children before the winter influenza season, with the exception of allowing delayed vaccination during the time of receipt of high-dose corticosteroids as long as

the likelihood of immunization before the start of the influenza season is not compromised (27).

Our study participants who were children with recurrent wheezing had more than two wheezing episodes during their first year of life. The average number was more than four. During this period, the children received inhaled or systemic steroid treatment more than 3 times. According to the study design, we excluded only children receiving long-term high-dose steroid treatment and those with acute fever; other children receiving steroids and those with wheezing symptoms were vaccinated. Generally for the pediatric population, the requirements are that the lower boundary of the two-sided 95% CI for the percentage of subjects achieving seroconversion for the HI antibody should meet or exceed 40% and the lower boundary of the two-sided 95% CI for the percentage of subjects achieving protective levels should meet or exceed 70% (28, 29). Both healthy children and children with recurrent wheezing sufficiently met these immunogenicity criteria (Table 2). The results showed that there was no difference between the two groups. Also, no difference in vaccine immunogenicity against vaccine strains between the steroid-treated and nontreated groups of children with recurrent wheezing was observed (Table 3). Previous studies have shown that young children tend to show lower immunogenicity for B strains than for A strains after influenza virus vaccination (30, 31). In our study, lower immunogenicity for the B strain (lower GMTR) also was demonstrated, similar to the aforementioned results (Tables 2 and 3). The rate of seroprotection against the B strain in the steroid-treated group was below 70%, but there was no statistically significant difference (Table 3). This result is similar to reports that the antibody response against the B strain in the steroid-treated group is lower than that in the non-steroid-treated group (32–34) but is in contrast to the research of Park et al. indicating that the antibody response against the B strain in asthmatic patients who receive steroid treatment is higher (25).

The immunogenicity of one-dose versus two-dose vaccination was evaluated in both groups. For healthy children, rates of seroprotection against the H1N1 and B strains were higher in the one-dose subgroup, and preimmunization and postimmunization GMTs against all vaccine strains were higher in the one-dose subgroup. The results were similar for children with recurrent wheezing except that the two-dose subgroup showed higher postimmunization GMTs against A/Perth(H3N2), like healthy children (Table 4), a trend that was not observed in an overall comparison of healthy children and children with recurrent wheezing (Table 2). The aforementioned results suggest that immune responses after influenza virus vaccination might be more active in the one-dose subgroup, because of older age, previous vaccination, and greater possibility of natural exposure. In a subanalysis according to previous influenza virus vaccination history, seroconversion and seroprotection rates and GMTRs showed no difference between healthy children and steroid-treated children with recurrent wheezing, except that rates of seroprotection against the B strain were below 70% for the two-dose subgroups of healthy and steroid-treated children (Table 5). These results suggest that short-term low-dose steroid treatment might not influence influenza virus vaccine immunogenicity. We need to consider that influenza virus vaccine immunogenicity might be affected by exposure to natural infection sources. This study was performed from autumn to the following early spring, during the annual influenza season in the northern hemisphere. During the study period, the first case of A(H3N2) influenza infection in South Korea was reported in December 2011. Since then, A(H1N1) influenza infection cases were continuously reported but with a lower incidence than in the previous year. B strain influenza spread widely across the nation in January and thereafter. Fortunately, the study closed before widespread infection caused by B strains. Among the study participants, only 1 subject fulfilled the clinical criteria of influenza-like illness. The patient was confirmed to have B strain infection, received conservative upper respiratory infection treatment in the outpatient clinic, and recovered fully without complications. In conclusion, our study demonstrates that inactivated TIV is able to induce protective immune responses in healthy children, as observed in previous studies (35–37), as well as in children with recurrent wheezing.

Previous studies (36–38) reported that the split inactivated influenza virus vaccine appeared to be safe and well tolerated, and adverse events generally were mild. There were no moderate-to-severe adverse events in either group in the study. The incidences of solicited local and systemic reactions and the incidences of adverse events of grade ≥ 2 showed no differences between the two groups.

This study has several limitations. First, the number of patients who participated in each cohort was small. A key characteristic of influenza virus vaccination is that it is mostly performed within a short period preceding seasonal outbreaks, unlike other vaccinations that are performed perennially, which limits the overall accrual of patients. Another concern is the lack of a placebo-treated patient group in our study design. However, the efficacy of influenza virus vaccination in healthy children is well established, precluding the need for additional placebo trials in our study. Our main objective in this study was to confirm comparable vaccine efficacy and safety between children with recurrent wheezing and healthy children. The two cohorts in our study were chosen with this objective in mind.

This study demonstrated that the immunogenicity and safety of inactivated trivalent split influenza virus vaccine are promising even among young children (under 3 years of age) with recurrent wheezing who have frequent wheezy respiratory symptoms or receive short-term low-dose steroid treatment, with immunogenicity similar to that found in healthy children of the same age. We hope that our study results are helpful for parents and physicians with regard to influenza virus vaccination.

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