Immunogenicity and reactogenicity of a reduced-antigen-content diphtheria-tetanus-acellular pertussis vaccine as a single-dose booster in Singaporean adults

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ABSTRACT

Introduction: Older children and adults, susceptible to pertussis because of waning immunity, may serve as a reservoir of infection, leading to severe disease among young unvaccinated infants. Booster diphtheria-tetanus-acellular pertussis (dTpa) vaccination in older age groups is rare in Singapore, one reason being the increase in reactogenicity with each successive dose. The aim of this study was to assess the immunogenicity, safety and reactogenicity of a reduced antigen, combined dTpa vaccine as a single booster dose in healthy adults aged 18 years or older.

Methods: A total of 150 healthy adults, 18 to 60 years of age, received a single dose of GlaxoSmithKline Biologicals' dTpa vaccine with reduced content for diphtheria and pertussis, with measurement of pre- and post-vaccination antibody titres.

Results: Prior to vaccination, 71.6 percent and 92.6 percent of the subjects had anti-diphtheria and anti-tetanus antibody levels greater than or equal to 0.1 IU/mL, respectively. 46.7 percent, 98.5 percent and 44.4 percent of subjects were seropositive for pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) antibodies, respectively. One month after vaccination, there was an increase in geometric mean titres from pre-vaccination to post-vaccination blood samples for anti-diphtheria (greater than seven-fold), anti-tetanus (greater than five-fold), anti-PT (greater than 11-fold), anti-FHA (greater than 25-fold) and anti-PRN (greater than 31-fold) antibodies. Solicited grade three local symptoms (pain, redness and swelling) were reported in 14.1 percent, 8.1 percent and 10.4 percent of subjects, respectively. No serious adverse events were reported.

Conclusion: In summary, the dTpa vaccine is immunogenic, safe and well-tolerated in Singaporean adults.

Keywords: diphtheria, diphtheria-tetanus-acellular pertussis vaccines, immunisation, pertussis, tetanus, whooping cough

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INTRODUCTION

Bordetella pertussis is transmitted from person to person in airborne droplets. The bacterium is highly infectious and unprotected close contacts are liable to become infected. Neither vaccination-acquired nor natural immunity from pertussis is life-long and protection is thought to be minimal after ten years without boosting[1-3]. There has been an increase in the incidence of pertussis in older age groups in recent years. One of the major concerns is that these infected individuals will serve as a reservoir of infection to younger unvaccinated infants[4-7] in whom the disease is most severe[8,9]. One of the problems of repeated vaccination is the increase in reactogenicity with each successive dose. However, it has been demonstrated that reducing the antigen content can reduce the reactogenicity[10,11].

Current reduced antigen content diphtheria-tetanus boosters are widely recommended in adult life. GlaxoSmithKline Biologicals has developed a reduced combined diphtheria-tetanus-acellular pertussis (dTpa) vaccine which will meet the need to vaccinate older age groups against all three diseases in a single injection. The dTpa vaccine is recommended for booster vaccination of children from four years of age, adolescents and adults who have been previously primed with diphtheria-tetanus-pertussis (DTP) vaccine[10,11]. The aim of this study was to assess the immunogenicity, safety and reactogenicity of the dTpa vaccine as a single booster dose in healthy adults aged ≥18 years.

METHODS

This was an open study conducted at University Health Services, National University of Singapore. Written informed consent was obtained from the subjects prior to entry into the study. This study was conducted according to Good Clinical Practice and in accordance with the Declaration of Helsinki as amended in Somerset West, Republic of South Africa (October 1996), and received approval from Research and Ethics Committee, National University Hospital.

A total of 150 healthy male and female volunteers (≥18 years) were enrolled at a single study centre. Each subject's medical history was recorded (including axillary body temperature and record of any medication). Exclusion criteria include: history of diphtheria and/or tetanus, known history of pertussis within the previous five years, known exposure to diphtheria or pertussis within the previous five years, vaccination against tetanus within the previous five years, or administration of more than seven doses of licensed diphtheria-tetanus toxoid (Td) since birth.

The vaccine used in this study was developed and manufactured by GlaxoSmithKline Biologicals, and was licensed for use in Singapore in March 2002. The reduced antigen content combined diphtheria, tetanus, acellular pertussis (dTpa) vaccine of Lot No. 37719A2 was supplied as a liquid in monodose vials. One dose (0.5 ml) contained the following components: 8 mcg pertussis toxin (PT), 8 mcg filamentous haemagglutinin (FHA), 2.5 mcg pertactin (PRN), 2.5 Lf diphtheria toxoid, 5 Lf tetanus toxoid, 0.5 mg aluminium as salts and 2.5 mg phenoxyethanol as preservative. The single dose dTpa vaccine was administered by deep intramuscular injection in the left deltoid region.

Subjects used diary cards to record solicited local (redness, swelling and pain at injection site) or general (fever, headache, fatigue and gastrointestinal symptoms) signs and symptoms, occurring on the day of vaccination and during the 14 subsequent days. Redness and swelling with a diameter >20 mm and fever >39.0ºC (oral route) were considered as an adverse event of intensity grade three, that is, an adverse event which prevents normal, everyday activities, except for pain. The follow-up period for unsolicited symptoms and serious adverse events was 0-30 days. The investigator assessed the relationship of all general symptoms to vaccination. It was assumed that all local (injection site) symptoms reported were related to vaccination.

Blood was drawn immediately prior to and approximately one month (30-38 days) after vaccination. Pre- and post-vaccination serum samples from all subjects were tested for antibodies against all vaccine antigens (three pertussis antigens [PT, FHA, and PRN], diphtheria and tetanus toxoids). Anti-diphtheria and anti-tetanus antibody titres were measured by enzyme-linked immunosorbent assay (ELISA) and expressed in International Units per ml (IU/ml). It is generally accepted that for both diphtheria and tetanus, titres ≥0.1 IU/ml, as measured by *in-vivo* neutralisation tests are protective. Antibodies against the pertussis components PT, FHA and PRN were measured by ELISA techniques, and the cut-off for all three pertussis antibodies was 5 EL.U./ml[12].

Geometric mean titres (GMTs) with their 95% CI were calculated for all antibodies at each blood sampling time point (i.e., before and one month after the vaccine dose) by taking the anti-log of the mean of the log titre transformations. Antibody titres below cut-off level were given an arbitrary value of half the cut-off value for the purpose of GMT calculation. Statistical analyses were performed using Statistical Analysis System (SAS) version 6.12.

RESULTS

Of the 150 subjects enrolled, 138 subjects completed the study. A total of 12 subjects dropped out after receiving the vaccine dose. All cases of drop-outs, except one, were due to lost to follow-up after visit one. One subject withdrew consent after visit one. There were also three subjects who came back for visit two, but did not return their diary cards, hence only 135 subjects were included in the according-to-protocol (ATP) reactogenicity and immunogenicity analyses. The mean age of the subjects included in the ATP cohort was 25.5 years, with a standard deviation of 9.22 years. The male: female ratio was approximately 7:10.

Data from 135 subjects were included in the ATP analysis of immunogenicity. Table I shows the pre- and post-vaccination seropositivity rates and GMC data. The majority of subjects were seropositive for anti-diphtheria, anti-tetanus and anti-FHA antibodies at the time of vaccination, at 71.6%, 92.6% and 98.5%, respectively. In comparison, the percentages of anti-PRN and anti-PT seropositive subjects were lower at 44.4% and 46.7%, respectively. The vaccine response to all the three pertussis antigens, namely anti-PT, anti-FHA and anti-PRN, was 92.6%, 98.5% and 94.8%, respectively. Anti-PT, anti-FHA and anti-

PRN ranged between 96.3% and 99.3%. There was an increase in GMTs from pre-vaccination to post-vaccination blood sampling time points for anti-PT (>11-fold), anti-FHA (>25-fold) and anti-PRN (>31-fold) antibodies.

The percentage of subjects with anti-diphtheria antibody titres ≥0.1 IU/ml (antibody titre levels required for seroprotection) before vaccination was 71.6%. One month after booster vaccination, a total of 94.8% of subjects were seroprotected for anti-diphtheria antibodies. There was a greater than seven-fold increase, from 0.294 IU/ml to 2.310 IU/ml, in GMTs of anti-diphtheria antibodies from pre-vaccination to post-vaccination blood sampling time points. The percentages of subjects with anti-tetanus antibody titres ≥0.1 IU/ml (antibody titre levels required for seroprotection) before vaccination was 92.6%. One month after booster vaccination, a total of 99.3% of subjects were seroprotected for anti-tetanus antibodies. There was a greater than five-fold increase, from 1.093 IU/ml to 6.429 IU/ml, in GMTs of anti-tetanus antibodies from pre-vaccination to post-vaccination blood sampling time points.

All vaccine doses were well tolerated and no serious adverse events were recorded. The incidence of solicited local injection symptoms reported during the 15-day follow-up period (day 0-14) after the vaccine dose was considered causally related to vaccination. Pain was the most prevalent solicited local symptom reported following 83.7% (113/135) of doses (Table II). All patients except two cases with grade three pain resolved within two days after the administration of the vaccine dose. The two cases of pain that persisted beyond the two-day follow-up resolved on the third and seventh days after vaccination. Solicited grade three local symptoms (pain, redness and swelling) were reported in 14.1%, 8.1% and 10.4% of subjects, respectively. All grade three solicited local symptoms resolved within the 15-day follow-up period.

Fatigue was the most prevalent solicited general symptom reported following 37.3% (50/134) of doses. Only one solicited general symptom (fatigue) was determined by the investigator to be related to the study vaccine. Only one grade three solicited general symptom (headache) was reported. This symptom resolved within three days of onset. A total of 16 unsolicited symptoms classified by WHO preferred terms were reported by 14 subjects during the 30-day follow-up period after vaccination, but none of the symptoms were of grade three intensity, i.e., prevented normal daily

Table I. Seropositivity rates and geometric mean titres of anti-diphtheria, anti-tetanus and anti-pertussis antibodies (ATP immunogenicity cohort).

Antibodies	Timing	% S+	GMT (EL.U/ml)
Anti-D	Pre-	71.6	0.294
	Post-	94.8	2.310
Anti-T	Pre-	92.6	1.093
	Post-	99.3	6.429
Anti-PT	Pre-	46.7	5.9
	Post-	96.3	66.7
Anti-FHA	Pre-	98.5	30.3
	Post-	99.3	762.1
Anti-PRN	Pre-	44.4	5.6
	Post-	96.3	175.6

Key: S+ (%): Seropositivity; GMT: Geometric mean titre (calculated on all subjects); Pre: Pre-vaccination blood sample obtained before the vaccine dose; Post: Post-vaccination blood sample obtained one month after the vaccine dose; Anti-D: Anti-diphtheria; Anti-T: Anti-tetanus; Anti-PT/FHA/PRN: Anti-pertussis toxin/filamentous haemagglutinin/pertactin.

Table II. Incidence of solicited local and general symptoms reported during the 15-day follow-up period after the vaccine dose.

Local symptom	Parameters	Percentage of subjects
Pain	Any	83.7
	Grade three	14.1
Redness	Any	51.1
	Grade three	8.1
Swelling	Any	41.5
	Grade three	10.4
General symptom	**Parameters**	**Percentage of subjects**
Fatigue	Any	37.3
	Grade three	0.7
Gastrointestinal	Any	12.7
	Grade three	0.0
Headache	Any	26.9
	Grade three	0.7
	Related	0.0
Fever	Any	8.2
	Related	0.0

Key: Any: Any symptom regardless of intensity and relationship to vaccination; Grade three pain: Spontaneously painful; Grade three redness/swelling: Largest diameter >20 mm; Fever defined as oral/axillary temperature ≥37.5°C; Related: Symptoms with a causal relationship to vaccination; Grade three headache: Prevented normal activity.

activity. All these 16 symptoms were general symptoms and were determined by the investigator to have no relationship to vaccination.

DISCUSSION

Since the introduction of routine vaccination against diphtheria, tetanus and pertussis, the epidemiology of pertussis has shifted: as the number of cases in children has dropped, cases in older individuals have increased. This is of concern not only because these individuals are susceptible to the deleterious effects of pertussis, but also because they serve as the main reservoir of infection for non-immunised neonates and infants at high risk for the severe effects of pertussis[13]. From the pre-vaccination serology results of this study, it was observed that only 46.7% and 44.4% of the study cohort upon enrollment were seropositive against PT and PRN pertussis components, respectively, whereas the seropositivity percentage was much higher against diphtheria (71.6%) and tetanus (92.6%).

Although there is no generally accepted serological correlate of protection against pertussis, antibodies to the pertussis antigens that form the components of acellular pertussis vaccines have been shown to correlate with protection against pertussis disease[14,15]. The titres achieved for pertussis antibodies by vaccination with the reduced antigen content combined diphtheria, tetanus, acellular pertussis or dTpa vaccine are at least equivalent to those achieved after primary vaccination with DTP which has been shown in several large, controlled clinical trials to have protective efficacy against pertussis disease in infants of 84%-89%[16,17]. This data supports the need for booster vaccination in adults above the age of 18 years in Singapore.

The use of additional booster vaccination is a feasible strategy, which could improve levels of herd-immunity, and reduce the circulation of the infection[18]. This study showed that, after a booster dTpa vaccination for study subjects aged ≥18 years old, the seropositivity rates for all three pertussis antigens (anti-PT, anti-FHA and anti-PRN) increased to 96.3%-99.3%. In addition, the anti-diphtheria seropositivity percentage was also boosted from 71.6% to 94.8% after vaccination, while anti-tetanus seropositivity, already high in this study group, increased from 92.6% to 99.3%. This immunogenic response to the dTpa vaccine antigens was comparable to other clinical trials conducted in Belgium and Australia[10,11], eliciting a satisfactory immune response to all the vaccine antigens. The boosted immunity against pertussis will not only protect a higher percentage of adults, but also reduce the reservoir of pertussis infection amongst the adult population.

Although combined diphtheria-tetanus-pertussis vaccines have become routine paediatric vaccines in many countries, their reactogenicity increases with successive doses, making the paediatric formulation less suitable for older individuals. However, reducing the content of acellular pertussis antigens and formulating it with an established adult Td booster vaccine could result in a new candidate combination vaccine with improved tolerability and the potential to boost titres against all three diseases[10]. In this study, the dTpa vaccine was well tolerated. There were no serious adverse events recorded. The percentage of local and general reaction was comparable with published data[19]. Hence, the adult dTpa booster vaccine (with reduced content for diphtheria and pertussis) was safe and well tolerated in this study report.

Pertussis immunisation in adults and adolescents has the potential not only to prevent severe illness, but also to interrupt transmission from adults to unprotected infants[11]. Hence, there is a need for harmonised, universal primary vaccination followed by a booster vaccine. A dTpa vaccine for adolescents and adults could serve an important place in a revised booster policy[10]. GlaxoSmithKline Biologicals' reduced antigen content diphtheria-tetanus-acellular pertussis vaccine elicited a satisfactory immune response to all vaccine antigens, and was safe and well tolerated, when administered as a single vaccination to healthy adults aged ≥18 years in Singapore, who were previously primed with four doses of DTP vaccine and at least one dose of DT.

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