Reactogenicity and immunogenicity of reduced antigen content diphtheria–tetanus–acellular pertussis vaccine (dTpa) administered as a booster to 4–6 year-old children primed with four doses of whole-cell pertussis vaccine

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Abstract

A trial to compare the reactogenicity and immunogenicity of a reduced antigen content diphtheria–tetanus–acellular pertussis (dTpa) vaccine with diphtheria–tetanus–whole-cell pertussis (DTPw) vaccine was conducted in Thailand. Three hundred and thirty children aged 4–6 years, primed with four doses of DTPw, received a single injection of either dTpa or DTPw. There was a significantly lower incidence of local and general reactions following dTpa than DTPw (P < 0.001). One month after vaccination, 99.4 and 100% of all subjects had protective anti-diphtheria and -tetanus titers, respectively. The vaccine response rate to pertussis antigens was similar in both groups, with 96.9% versus 92.5% for anti-pertussis toxin (PT), 96.9% versus 97.5% for anti-filamentous hemagglutinin (FHA) and 95.1% versus 90.8% for anti-pertactin (PRN) in the dTpa and DTPw groups, respectively. For anti-BPT, the vaccine response in the dTpa group was 29.6% versus 94.4% for DTPw. In conclusion, the dTpa vaccine was as immunogenic and significantly better tolerated than DTPw. The new dTpa vaccine could improve coverage for routine booster vaccination in children and provide a good replacement for DTP vaccines at 4–6 years of age.

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1. Introduction

The World Health Organization (WHO) has implemented childhood immunization programs against diphtheria, tetanus and pertussis world-wide since 1974 [1]. However, concerns have been raised about the frequency of local and systemic reactions, as well as the potential relationship between the whole-cell pertussis vaccine and encephalopathy in countries such as Japan, Germany, Italy, Sweden and UK [2–8]. This has led to the development of acellular pertussis vaccines that have been demonstrated to be less reactogenic than whole-cell vaccines [10–12]. Rare and serious adverse events associated with pertussis vaccination are also less frequent after administration of diphtheria–tetanus–acellular pertussis (DTPa). Furthermore, a number of DTPa vaccines have shown to be highly efficacious against the disease [13–15]. Presently, acellular pertussis vaccines are licensed for infant immunization in many countries such as the United States, most European countries, Australia, Latin America and the Asia–Pacific region [16–18]. DTPa vaccines display a somewhat increasing reactogenicity with subsequent doses and age [13,19–21]. Therefore, a vaccine with reduced antigen content (dTpa) has been investigated to enable booster vaccination against diphtheria, tetanus and pertussis in one combination for children older than 10 years, adolescents and adults rather than using DT boosters alone. The purpose of this study was to compare the reactogenicity and immunogenicity of reduced antigen content vaccine (dTPa) with a dose of...
diphtheria–tetanus–whole-cell pertussis (DTPw) vaccine, both administered as a booster to healthy children at 4–6 years of age who were immunized with four doses of DTPw before 2 years of age, according to the local Thai vaccination schedule.

2. Methods

2.1. Subjects

Healthy 4–6 year-old children who had received four doses of diphtheria–tetanus–whole-cell pertussis vaccine at 2, 4, 6 and 18 months of age according to the locally recommended vaccination schedule, were included in this study. The subjects were excluded if they had a history of diphtheria or tetanus at any time or confirmed pertussis in the previous 5 years; if they had received vaccines not foreseen in the study protocol within 30 days prior to study start or after receiving a study vaccine. Additional exclusion criteria were a history of allergic disease or reaction likely to be exacerbated by any component of the vaccine or previously recorded following DTP vaccination, or a history of any serious adverse reaction following previous DTP vaccination. Children were excluded if they had a history of administration of immunosuppressive agents, immunoglobulin or blood products within the previous 3 months or during the trial. Also excluded if they had any underlying condition such as major congenital defects, neurological including seizure disorders or acute febrile illness at the time of enrollment.

This blinded randomized trial was conducted at Chulalongkorn University Hospital, Bangkok, Khon Kaen and Hat Yai Regional Hospital, Song Kla, in Thailand. Prior to enrollment, written informed consent was obtained from parents or guardians of all children. The study protocol was approved by National and Institutional Ethics Committees, and was conducted according to the Declaration of Helsinki and Good Clinical Practice. After physical examination and recording of the axillary temperature, note the presence or absence and measure the greatest diameter of local reactions and record local and general solicited signs and symptoms on the vaccination day and the 14 subsequent days. Unsolicited symptoms were coded according to the World Health Organization dictionary for adverse events and were recorded over a 30 day period post-vaccination. Parents or guardians of the subjects were instructed to return the completed diary card at the next visit but to contact the investigator immediately if the subject manifested any signs or symptoms they perceived as serious. All general solicited symptoms and unsolicited adverse events following vaccination were assessed for their relationship to the vaccine and were classified by the investigators as not related, unlikely, suspected or probably related.

2.2. Assessment of immunogenicity

Antibody measurements were performed on blood samples taken immediately before and 1 month post-vaccination. All antibody levels were measured by an ELISA assay in GlaxoSmithKline Biologicals’ central laboratory. Based on the Expanded Program on Immunization in the European Region of WHO, 1994 [22] the assay cut-off for diphtheria and tetanus antibodies was used for protective level at 0.1 IU/ml. IgG antibodies were assayed against the pertussis vaccine components pertussis toxin, filamentous hemagglutinin and pertactin with a cut-off assay of 5 EL.U/ml; and anti-B. pertussis antibody titers with an assay cut-off of 15 EL.U/ml. A vaccine response was defined as the appearance of antibodies in initially seronegative subjects, or a two-fold increase in concentration in initially seropositive subjects.

2.3. Assessment of reactogenicity

The vaccines were observed closely for at least 15 min after vaccination with appropriate medical treatment readily available in case of rare anaphylactic reaction following the administration of vaccine. On the day of vaccination, diary cards and digital thermometers were distributed to the parents or guardians of the subject. They were asked to take and record the evening axillary temperature, note the presence or absence and measure the greatest diameter of local reactions and record local and general solicited signs and symptoms following vaccination and the 14 subsequent days. Unsolicited symptoms were coded according to the World Health Organization dictionary for adverse events and were recorded over a 30 day period post-vaccination. Parents or guardians of the subjects were instructed to return the completed diary card at the next visit but to contact the investigator immediately if the subject manifested any signs or symptoms they perceived as serious. All general solicited symptoms and unsolicited adverse events following vaccination were assessed for their relationship to the vaccine and were classified by the investigators as not related, unlikely, suspected or probably related.

2.4. Statistical analysis

For immunogenicity and reactogenicity, two sets of analyses were performed, one for those who conformed to the protocol (according to the protocol (ATP)) and one for all subjects enrolled and vaccinated (intention to treat (ITT)). In this report, ATP analysis is presented. Reactogenicity data were compared, using one-sided Fisher’s exact test. Geometric mean titres (GMT) with their 95% confidence interval were calculated for all antibodies. Differences in seropositivity rates for antibodies to all vaccine antigens and vaccine response rates for pertussis antigens with their 95% confidence interval were calculated. A lower limit of the 95% confidence interval for the difference between the two values of higher than −5% was taken to indicate non-inferiority.
of dTpa vaccine compared to DTPw vaccine. An exploratory analysis was done for anti-B. pertussis antibody response.

3. Results

Of 330 subjects enrolled, all but three, who migrated from the study area, completed the study. All 330 subjects enrolled were eligible for inclusion in the ATP analysis of safety. Five subjects were eliminated from ATP analysis of immunogenicity: one subject in group II was younger than the protocol specified and for two subjects from each group, post-booster blood sampling could not be taken. In the study cohort, the male/female ratio was 1:1. The mean age of the total cohort was 4.6 years. There were no significant differences between groups with regard to these parameters.

3.1. Immunogenicity analysis

Prior to vaccination, 71.0% of the dTpa group and 85.2% of the DTPw group had anti-diphtheria antibody titers >0.1 IU/ml and 95% of the children in both groups were seroprotected against tetanus. One month post-vaccination, 99.4% and 100% of subjects were seroprotected against diphtheria and tetanus. GMTs pre- and post-vaccination for anti-diphtheria and -tetanus antibodies are presented in Fig. 1.

For pertussis, prior to booster immunization, levels of all four pertussis antibodies were similar in both groups. One month after the booster dose, a vaccine response to PT, FHA and PRN was observed in 96.9, 96.9 and 95.1% of subjects in the dTpa, with comparable rates in the DTPw group. For anti-BPT, the vaccine response in the dTpa group was 29.6% versus 94.4% in the DTPw group. GMT levels are shown in Fig. 2.
3.2. Reactogenicity

The analysis of reactogenicity showed a significantly lower incidence of overall local symptoms in subjects receiving dTpa compared to DTPw administration (50.3% versus 72.1%; \( P < 0.001 \)). Pain at the injection site was the most frequently reported solicited local event, occurring in a significantly lower percentage of subjects receiving dTpa than DTPw (41.8% versus 67.3%; \( P < 0.001 \)). Additionally, severe pain was reported in only 0.6% of subjects receiving dTpa compared to 11.5% of DTPw recipients. Redness was reported by 23.6 and 33.9% in the dTpa and DTPw groups, respectively (\( P = 0.026 \)). The study also showed a significantly lower incidence of swelling following the dTpa group when compared to the DTPw group (21.2% versus 44.2%; \( P < 0.001 \)), see Fig. 3. All solicited local symptoms reported resolved spontaneously within the 15 day follow-up period after vaccination.

Solicited general symptoms were reported in 50.3% of the dTpa group and 70.3% of the DTPw group, which was also significant on \( P < 0.001 \) level. The findings for general solicited symptoms of drowsiness, irritability, loss of appetite and fever are also shown in Fig. 3. In our study, fever was found in 13.9% of the dTpa group and 30.9% of the DTPw group. Grade 3 fever (fever >39 \(^\circ\)C) was found in 1.8% compared to 4.8% of the dTpa and DTPw groups, respectively. A total of 23 unsolicited symptoms, 12 in the dTpa group and 11 in the DTPw group, were reported. Twenty-one were general symptoms and two were local symptoms. Only two local symptoms were determined by the investigator to have a suspected relationship to the vaccination. All other symptoms were determined by the investigator to be not related or unlikely to be related to vaccination. No serious adverse events were reported during the study.

There was also a significant difference in the overall incidence of any symptoms observed between dTpa and DTPw groups, 72.7 and 85.5%, respectively (\( P = 0.003 \)).

4. Discussion

Waning of pertussis immunity after primary immunization suggests the need for booster immunization of 4–6 year-old children to ensure continuing immunity and prevent the development of a reservoir of infection which could affect non-immune infants [23]. Therefore, pre-school fifth dose booster immunization at 4–6 years of age has been established in many countries around the world, including Thailand where DTPw vaccine has been given to children aged 2, 4, 6, 18 months and 4–6 years. However, a notable increase in reactogenicity with subsequent doses DTPw vaccines has led to either a decrease in coverage rate of the fifth dose compared to the previous doses, or abandonment or omission of this vaccination in the recommendations in some countries [6–9]. Previous studies have demonstrated that acellular pertussis vaccine was safe and immunogenic as a fourth and fifth dose in children primed with DTPw [24–26]. In the United States, DTPa vaccine has been used as fourth and fifth dose boosters for children 15–20 months of age and 4–6 years of age, respectively, for several years [27], and more recently, was introduced for a primary vaccination of infants as well [16]. Also, the reactogenicity of DTPa vaccine has been shown to increase with subsequent doses such that higher rates of reactions were observed when
the vaccine was administered as a booster in older children [13,19–21]. Therefore, a reduced antigen formulation of acellular pertussis vaccine has been developed, which offers the possibility of booster doses with acceptable reactogenicity in older children and adults.

In seeking to improve the reactogenicity profile of DTPa vaccination in pre-school children, an assessment of a low dose booster vaccine as a fifth dose in children aged 4–7 years was conducted in Israel [28]. This earlier study showed that both full dose and reduced antigen content DTPa vaccines were successful in boosting immunity against the three diseases in 4–7 year-old children, originally immunized with four doses of DTPw vaccine. Our study is the first report on a DTPw primed cohort of children who received a reduced antigen content combined diphtheria–tetanus–acellular pertussis vaccine in comparison to DTPw as a fifth dose in children 4–6 years, being the current recommendation in Thailand and many other countries around the world. The study demonstrated that DTPa vaccine was safe, well tolerated and highly immunogenic, indicating that vigorous booster responses can be elicited by reduced quantities of vaccine antigen in children primed with four doses of DTPw.

The serological data demonstrate that antibody levels 1 month after the dTpa dose were generally equivalent or exceeded the level achieved with DTPw, in line with previous studies with full dose acellular pertussis vaccine following DTPw in the same age group priming [29–31]. One month after vaccination, the dTpa vaccines' immunity for both diphtheria and tetanus components was comparable to that of the DTPw vaccines. GMTs for antibodies to diphtheria and tetanus toxoids increased 10–15-fold in both groups. The dTpa formulation elicited somewhat lower, but not statistically significantly different anti-diphtheria levels, while anti-tetanus antibody levels were higher when compared to the DTPw group. The finding of the relatively low levels of diphtheria antibody before the fifth dose, supports the use of a diphtheria toxoid booster for children in this age group. In the absence of the 4–6 year-old booster dose, a large proportion of children could fall below the presumed protective titters during the 10 years interval until the next recommended vaccination at adolescent age.

A multivariate analysis of protective titters, derived from acellular pertussis vaccine efficacy trials in infants, have indicated the importance only of pertactin, and pertussis toxin in protection against pertussis [32,33]. PT appears to be one of the biologically active toxins important in disease pathogenesis, causing some of the clinical symptoms of pertussis [34,35]. In our study, we found the fifth dose of dTpa vaccine to be highly immunogenic, inducing seropositivity values of 98.1, 100 and 99.4% for anti-PT, FHA and PRN in the dTpa group compared to 93.1, 100 and 95.1%, respectively in the DTPw group. Titters achieved for antibodies against pertussis antigens PT, FHA and PRN were shown to be 10-20 times higher than pre-vaccination titers in both groups. This is in line with the previous study of dTpa vaccine in pre-school age children [28]. Thus, our study supports that the reduced antigen content diphtheria–tetanus–acellular pertussis did not affect the clinically important parameters of the immune response in children primed with four doses of DTPw.

Circulating antibodies may play a role in toxin neutralization and prevent bacterial attachment to respiratory epithelial cells but cell-mediated immunity may be required for sufficient and long-lasting protection against B. pertussis [36,37]. It has been shown that recovery from pertussis and immunization of children with whole-cell pertussis vaccine both induce a Th1 response while acellular pertussis vaccines induce T-cells that secrete Th1 and Th2 cytokines [38,39]. In our study, the percentage of subjects who developed protective antibodies against pertussis were comparable in both groups. However, long-term evaluation of antibodies and cell-mediated immunity may provide important information pertaining to the appropriate vaccination schedule.

A striking finding was the significant reduction in local and general reactogenicity following dTpa vaccination. Also the severity of local reactions in this study was much less in dTpa vaccine recipients. Not only were the percentages of pain, redness and swelling markedly less in the dTpa group, but the degree of pain was also much lower. In our study, parents assessed painful events of varying severity using the following criteria: grade 1—minor reaction to touch; grade 2—cries/protests on touch; grade 3—cries when limb is moved/spontaneous pain. Only 0.6% of dTpa recipients developed severe pain, preventing normal daily activity, which was only 1.4% of total reports of pain while severe pain was reported in 11.5% of DTPw vaccine recipients, representing 17% of total subjects with pain reported in the DTPw group. A similar pattern was found for severe redness and swelling. Among common general symptoms, fever is generally of the most concern adverse event in relation to DTP vaccination. In our study, fever was reported less frequently in the dTpa group when compared to the DTPw group (13.9% versus 30.9%), and 1.4% versus 4.8% of vaccinees developed fever higher than 39.0°C in the dTpa and DTPw groups, respectively, in line with the finding of a previous study of acellular pertussis vaccine as a booster in the same age group [28]. It was observed that adverse reactions were common in individuals with higher pre-vaccination diphtheria and tetanus antibody titers [40,41]. Unfortunately, we did not analyze the correlation of the pre- and post-vaccination reactogenicity.

Reduced antigen content diphtheria–tetanus–acellular pertussis vaccine when given as a booster in children aged 4–6 years primed with DTPw vaccine demonstrates comparable immunogenicity and significantly improved reactogenicity as compared to DTPw. The vast majority of local and systemic reactions are considered mild to moderate, with no vaccine-related serious adverse event reported. Thus, in developing or intermediate countries where the primary vaccination consist of use four doses of DTPw vaccine, dTpa vaccination may potentially replace DTPw as a fifth dose in 4–6 years children and contribute to...
improved coverage of booster immunization against diphtheria, tetanus and pertussis in this age group.

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