Reactogenicity and immunogenicity of adult versus paediatric diphtheria and tetanus booster dose at 6 years of age

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Abstract

We evaluated the reactogenicity and immunogenicity of a booster dose of diphtheria–tetanus vaccine administered at the age of school-entry, comparing a low-dose vaccine (dT) to the standard paediatric dose (DT). Participants were randomly assigned to receive one of the two vaccines; the study was evaluator-blinded. The frequency of side-reactions was similar when comparing the two groups, except when considering local redness and swelling, which were significantly more frequent among the DT group. The post-booster geometric mean titre of diphtheria antibodies in the DT group was twice as high as that in the dT group (14.1 IU/ml versus 7.7 IU/ml; \(P<0.001\)). The higher antibody response and the comparable reactogenicity indicate that DT should be used as booster at school-entry, particularly if additional booster doses during adolescence or adulthood are not administered. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Diphtheria; Booster; Vaccination

1. Introduction

The high levels of anti-diphtheria vaccination coverage achieved in Italy [1] have resulted in a dramatic reduction in the number of diphtheria cases, from 20–30,000 cases reported each year in the 1940s to a total of four cases reported in the 1990s. The recommended vaccination schedule in Italy consists of three doses of diphtheria–tetanus–pertussis vaccine (DTP) in the first year of life, followed by a booster dose at 5–6 years of age. In 1996, in response to the huge epidemic that hit the countries of the Russian confederation, the Italian Ministry of Health recommended that an additional diphtheria–tetanus booster dose be provided every subsequent 10 years [2]. The vaccines used for primary vaccination and the first booster dose contain 25–30 Lf of diphtheria toxoid and 10 Lf of tetanus toxoid (DT), but from 7 years of age vaccines containing a reduced quantity of diphtheria is 2 Lf (flocculating unit) in dT vaccine.

The use of dT has been shown to be associated with fewer side-reactions compared to DT, and it induces a good response when administered as a booster in school-age children and adults who have already undergone primary vaccination and when used for the primary vaccination of adults [3]. In the European Union, the recommended age for shifting from DT to dT varies by country, ranging from 4 years in The Netherlands, where it is administered as a fifth dose, to 15 years in Great Britain [4].

We conducted a prospective cohort study to determine whether or not using dT instead of DT as the booster dose in 6-year-old children provides a benefit in terms of reducing reactogenicity without significantly modifying immunogenicity.

2. Methods

2.1. Study population and enrolment of participants

We conducted a prospective, randomised, multicentre study in the vaccination facilities of seven Local Health Units (LHUs) of the Veneto Region of Italy in the period from January through March 2000. All of the resident 6-year-old children who had not yet received a booster dose for diphtheria and tetanus were considered eligible for

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participation. Inclusion criteria were: documented primary DT vaccination, no fever record in the previous 4 years, and no permanent or temporary contraindication to vaccination (i.e. anaphylactic reactions to previous DT doses or ongoing illnesses). Parents were invited to enter their child in the study, and those who agreed provided written informed consent. At enrolment, information was collected on all other previous vaccinations. The study was approved by the local ethics committee.

2.2. Vaccines

The DT and dT vaccines used in the study were the commercial products already in use at the LHUs (manufactured by Chiron, Berna and SmithKline Beecham). The DT vaccines contain 25Lf of diphtheria toxoid per dose; the dT vaccines contain 21Lf of diphtheria toxoid. Both the DT and the dT vaccines contain 10Lf of tetanus toxoid, 0.05 mg of NaCl, ≤1.25 mg of aluminium hydroxide, and ≤0.05 mg of thimerosal, per dose.

2.3. Sample size, randomisation and blinding

Assuming a 15% incidence of local side-reactions in the dT group, we calculated that 380 children in each vaccine group would provide an 80% power of detecting a relative risk of 1.55, with a confidence interval of 95%.

At enrolment, each child was randomly assigned to receive either the DT or the dT vaccine; the specific vaccine used was known only to the LHU personnel who actually administered the vaccine and not to the parents or the study personnel responsible for data collection. Vaccines were injected intramuscularly in the routine site (i.e. buttock, thigh, or arm) using a 22–24-gauge, 2.5 cm long needle. Injection was performed by the LHU personnel, in accordance with standard techniques recommended by the Veneto Regional Health Authority.

2.4. Data collection and analysis

2.4.1. Reactogenicity

We instructed parents to record any local or systemic symptoms occurring within 8 days of receiving the booster dose on a standardised daily diary and to record the child’s external temperature (axillary or inguinal) in the three evenings following booster administration; if the child was afebrile, the parents were not required to record the temperature after the third evening. Parents were also asked to immediately notify the study coordinators of severe adverse events (i.e. anaphylaxis within 24 h of receiving the booster; fever ≥39.5 °C within 48 h, hypotonic–hyporesponsive episodes within 48 h, seizures within 72 h, and generalised cyanosis within 48 h).

2.4.2. Immunogenicity

Parents of the children enrolled in the study were offered the possibility to enter their child in the immunogenicity study; participation was voluntary. The sample size was established arbitrarily at 150, and each LHU was required to enrol at least 10% of the study children. After having obtained informed consent from parents, we collected paired capillary blood samples. The first sample was taken prior to booster administration; the second sample was taken 1 month later.

Diphtheria antitoxin antibodies (IgG) were measured by a time-resolved fluorometric immunoassay system known as “DA-DELFLIA” [5], whose sensitivity and specificity are comparable to those of the in vitro neutralisation test [6]. DA-DELFLIA is a sandwich-type assay in which microtiter plates are coated with diphtheria toxoid, and after incubation with a serum sample, the diphtheria toxoid labelled with europium is added. When the enhancement solution is added, the lanthanide is dissociated from the antigen forming a fluorescent chelate, which is read in a time-resolved fluorimeter. The concentration of fluorescent chelate is related to the amount of diphtheria antibodies on each plate, a three-fold dilution series of a reference serum (World Health Organisation First International Standard for diphtheria antitoxin, 10 IU/ml Statens Serum Institute, Copenhagen, Denmark) was performed to obtain a calibration curve. The positive control was represented by a single human serum. The serum samples were titrated in three-fold dilution series (six dilutions, starting from 1:3 to 1:729) to calculate antibody concentrations, which were expressed as International Units (IU) per millilitre.

Tetanus antitoxin specific IgG antibodies were determined by a commercial ELISA kit (DiaSorin s.r.l), following the manufacturer’s instructions. The antibody concentration of each sample (two dilutions, 1:100 and 1:300) was calculated in IU/ml according to a human reference serum (included in the kit) calibrated against the WHO standard for tetanus antitoxin (NIBSC Reagent 76/589).

The pre- and post-booster levels of diphtheria antibodies were categorised into four groups: <0.01 IU/ml (child considered as “unprotected”); 0.01–0.09 IU/ml (basic protection); 0.1–0.9 IU/ml (protected); ≥1 IU/ml (long-term protection), according to internationally accepted criteria [7,8].
The pre- and post-booster levels of tetanus antibodies were categorised into three groups: <0.1 IU/ml (unprotected); 0.1–0.9 IU/ml (protected); ≥1 IU/ml (long-term protection) [7,9].

The geometric mean titres (GMTs) of antibodies to diphtheria and tetanus were also calculated before and after booster administration.

2.4.3. Statistical analysis

Statistical analyses were performed using Epi-info version 6.04 (CDC, USA, and WHO, Switzerland, January 1997). In performing statistical analyses, both the DT and the dT group were analysed as a whole, without considering the specific brand used or the specific LHU where the child was enrolled. The frequency of each symptom with onset within the first 3 days of the booster administration, the day of onset and the duration of the symptom were calculated by vaccine group. The frequency of symptoms was also calculated by pre-vaccination IgG titre against diphtheria (<0.09 and ≥0.09 IU/ml) and tetanus (<0.9 and ≥0.9 IU/ml). Differences between proportions were assessed by the chi-square test or the Fisher’s exact test. Mean values for continuous variables were compared using the Kruskal–Wallis test.

3. Results

A total of 752 children participated in the study. They had been vaccinated with three doses of DTP/DT, oral polio vaccine (OPV) and hepatitis B vaccine in the first year of life, and with one booster dose of OPV in the third year of life. The type of DTP/DT vaccine used for primary vaccination was known for 678/752 of the children (90.1%); 570 of them (84.1%) had received whole-cell DTP, 5 (0.7%) acellular DTP, and 103 (15.2%) DT vaccine. A total of 665 children (88.4%) had been vaccinated against measles, mumps and rubella, and 52 children (6.9%) received at least one dose of anti-Haemophilus influenzae type b vaccine. A total of 371 children were randomised to the DT group and 381 to the dT group. The distribution of these children by vaccine manufacturer is reported in Table 1.

Table 1

| Distribution of vaccines used as booster dose by manufacturer |
|-----------------|-----------------|
|                | DT (%)          | dT (%)          |
| Chiron SpA      | 36 (9.7)        | 330 (86.6)      |
| Berna           | 171 (46.1)      |                  |
| SmithKline Beecham | 164 (44.2)  | 51 (13.4)       |
| Total           | 371             | 381             |

No significant differences were detected between the two study groups in terms of gender, previous vaccination history, age at vaccination or site of booster injection, which was the arm in 98.5% of total children (Table 2).

3.1. Reactogenicity

The rate of symptoms within 3 days of booster administration is reported by vaccine group in Table 3. The most frequently reported symptom was pain at the injection site, which was experienced by 55.0% of the DT children and 52.0% of the dT children. Fever (external temperature ≥37.5°C) was reported by 8.4% of the DT children and 7.7% of the dT children.

The rate of symptoms was similar in the two groups, except for local redness and swelling, which were significantly more common in the DT children (redness: 31.3% in the DT group versus 15.7% in the dT group, P = 0.0001; swelling: 36.4% in the DT group versus 26.2% in the dT group, P = 0.002).
Table 3
Number and rate of side effects within 3 days of vaccination by vaccine group

<table>
<thead>
<tr>
<th></th>
<th>DT, n = 371 (%)</th>
<th>dT, n = 381 (%)</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever ≥37.5°C</td>
<td>31 (8.4)</td>
<td>29 (7.7)</td>
<td>1.01 (0.97–1.05)</td>
</tr>
<tr>
<td>Fever &gt;40°C</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Irritability</td>
<td>41 (11.1)</td>
<td>49 (12.9)</td>
<td>0.98 (0.93–1.03)</td>
</tr>
<tr>
<td>Itchiness, any</td>
<td>42 (11.3)</td>
<td>42 (11.0)</td>
<td>1 (0.95–1.06)</td>
</tr>
<tr>
<td>Redness, &gt;5 cm</td>
<td>22 (5.9)</td>
<td>6 (1.6)</td>
<td>1.09 (1.02–1.08)</td>
</tr>
<tr>
<td>Swelling, any</td>
<td>135 (36.4)</td>
<td>100 (26.2)</td>
<td>1.16 (1.05–1.28)</td>
</tr>
<tr>
<td>Swelling, extensive</td>
<td>20 (5.4)</td>
<td>10 (2.6)</td>
<td>1.03 (1.00–1.06)</td>
</tr>
<tr>
<td>Pain</td>
<td>204 (55.0)</td>
<td>198 (51.7)</td>
<td>1.07 (0.92–1.24)</td>
</tr>
<tr>
<td>Pain, severe</td>
<td>64 (17.3)</td>
<td>43 (11.3)</td>
<td>1.07 (1.01–1.14)</td>
</tr>
</tbody>
</table>

A statistically significant difference was also observed for extensive local redness (>5 cm diameter) (P = 0.002) and severe pain at the injection site (P = 0.02).

The mean duration of local symptoms ranged from 2.6 (redness, dT group) to 3.6 days (swelling, DT group). No statistically significant differences between the two groups were observed, except for the mean duration of local redness, which was 3.3 days in the DT group and 2.6 days in the dT group (P = 0.01).

No severe events were reported. Seven children (four in the DT group and three in the dT group) experienced extensive swelling at the injection site. All seven also reported local pain and redness, and three had fever on at least 1 of the first 3 days after booster administration. Six of the seven children were vaccinated in the same LHU (P < 0.0001).

The duration of the extensive swelling was 2 days in four of the children, and 3–5 days in the other three children.

Ichiness and fever were statistically more frequent in children with higher pre-vaccination diphtheria antibody levels (itchiness: 13/70 in children with levels >0.09 IU/ml versus 4/73 in children with levels ≤0.09 IU/ml; P = 0.03; fever: 17/70 in children with levels >0.09 IU/ml versus 6/73 in children with levels ≤0.09 IU/ml; P = 0.02). No differences in the rate of symptoms by pre-vaccination tetanus antibody titres were observed.

3.2. Immunogenicity

We collected 143 pairs of capillary blood samples (76 from the DT group and 67 from the dT group). This sample size provided a statistical power of 80% to detect a 50% difference in GMTs, with a confidence interval (CI) of 95%.

The mean interval between vaccination and the collection of the second capillary blood sample was 29 days in both vaccine groups (range: 25–37 days in the dT group and 26–35 days in the DT group). All of the samples were tested for diphtheria antibody titres, but the quantity of serum was sufficient for tetanus testing in 130/143 samples (70 in the DT group and 60 in the dT group).

3.2.1. Diphtheria

Prior to the booster administration, only three children (2.1% of the total) were found to have been unprotected (antibody level <0.01 IU/ml). After the booster administration, all of the children had antibody levels of at least 0.01 IU/ml (Table 4). Long-term protection (antibody levels

Table 4
Diphtheria antibody titres (IU/ml) before booster administration and 30 days later by vaccination group

<table>
<thead>
<tr>
<th>Vaccination group</th>
<th>Number (%) of individuals with diphtheria antitoxin (IU/ml)</th>
<th>GMT (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.01</td>
<td>0.01–0.09</td>
</tr>
<tr>
<td>DT (n = 76)</td>
<td>2 (2.6)</td>
<td>37 (48.7)</td>
</tr>
<tr>
<td>Post-booster</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>dT (n = 67)</td>
<td>1 (1.5)</td>
<td>32 (47.8)</td>
</tr>
<tr>
<td>Post-booster</td>
<td>0</td>
<td>1 (1.5)</td>
</tr>
</tbody>
</table>

Table 5
Tetanus antibody titres (IU/ml) before booster administration and 30 days later by vaccination group

<table>
<thead>
<tr>
<th>Vaccination group</th>
<th>Number (%) of individuals with tetanus antitoxin (IU/ml)</th>
<th>GMT (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.1</td>
<td>0.1–0.9</td>
</tr>
<tr>
<td>DT (n = 70)</td>
<td>13 (18.6)</td>
<td>42 (60.0)</td>
</tr>
<tr>
<td>Post-booster</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>dT (n = 60)</td>
<td>5 (8.3)</td>
<td>45 (75.0)</td>
</tr>
<tr>
<td>Post-booster</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
as were the GMTs of the post-booster samples (14.08 and 0.10 IU/ml in the DT group and 0.30 and 0.31 IU/ml, respectively). Pre-booster antibody titres were practically identical between the DT and dT groups (7.7 IU/ml in the DT group and 7.1 IU/ml in the dT group (P < 0.001).

3.2.2. Tetanus

Prior to the booster administration, 18/130 children (13.8%) were found to have been unprotected (antibody levels < 0.1 IU/ml), whereas after the booster administration, all of the children had antibody levels indicative of long-term protection (≥ 1 IU/ml) (Table 5). The GMTs of the pre-booster samples were practically identical between the DT and the dT groups (0.30 and 0.31 IU/ml, respectively), as were the GMTs of the post-booster samples (14.08 and 14.09 IU/ml for the DT and dT groups, respectively).

4. Discussion

A higher standard of safety is expected for vaccines than for other medical interventions, given that vaccines are administered to healthy children to prevent diseases rather than to treat illnesses. The experience of many countries has shown that even the occurrence of minor side-reactions can affect public confidence in vaccination programmes, especially for vaccines that must be administered in multiple doses. For example, in Russia, one of the factors involved in the recent diphtheria epidemic was that the DT vaccine had been used for the primary vaccination of children because there had been concern over the presumptive side-reactions associated with the DT vaccine [10].

The results of the present study show that the frequency of symptoms is similar when comparing DT and dT administered as a fourth dose in children at the age of school-entry. The only difference in terms of the onset of symptoms was that local redness and swelling were more common following DT administration, yet the relative risk of these symptoms for the DT children compared to the dT children was small, ranging from 1.16 to 1.23. Thus, the quantity of diphtheria toxoid plays only a minor role in the onset of this symptom. It is known that local reactions are more frequent and severe if adsorbed vaccines are administered subcutaneously rather than intramuscularly [14, 16]. In our study, all vaccines were administered intramuscularly by the LHU personnel, in accordance with standard recommendations. Since the LHU personnel administer a high number of vaccines, their expertise is considered to be quite high. Nevertheless, even among well-trained personnel, differences in injection technique can occur. It is, therefore, possible that the depth of intramuscular administration differed according to the vaccinator, possibly explaining the finding that six of the children with extensive swelling had received the booster dose in the same LHU.

The immunogenicity analysis showed that 98% of the study children had a protective diphtheria antibody titre before the booster dose, which was administered 5 years after the third dose of primary vaccination. This finding is consistent with data on the seroprevalence of diphtheria in Italy, which indicate that less than 5% of children between 4 and 6 years of age are susceptible [5]. For the tetanus antitoxin titres, the cut-off of 0.1 IU/ml is generally accepted as the protective threshold when titre is measured using ELISA [9, 17]. According to this definition, 14% of the children in our study were unprotected prior to booster administration. This rate is lower than that observed in the United Kingdom, where more than 20% of children prior to pre-school booster dose had titres < 0.1 IU/ml [17]. Clinical cases of tetanus are rare in vaccinated individuals, and in Italy no cases of tetanus in children under 5 years of age have been reported since 1989, so it is possible that children with titres < 0.1 IU/ml are also clinically protected.
After administration of the booster dose, more than 90% of children in both groups had diphtheria antibody titres indicative of long-term protection; for tetanus, antibody titres were indicative of long-term protection for all study children. Nevertheless, the diphtheria GMT for the DT group was twice as high as that for the DT group.

In many industrialised countries, serological data have demonstrated that a large proportion of adults are now susceptible to diphtheria because vaccine-induced immunity wanes over time if periodic boosters are not given and if exposure to toxigenic Corynebacterium diphtheriae does not occur [3]. Among countries of the European Union, it has been recently shown that there are very large differences exist in diphtheria antitoxin levels and in the percentages of protected persons [18]. Vaccination schedules, number of doses, and diphtheria-toxoid content greatly influence the levels of protective antibodies and their duration [18–21].

In Italy, the prevalence of persons who are immune to diphtheria decreases gradually with increasing age, reaching the lowest level in persons between 40 and 59 years of age [5]. Booster doses have been recommended every 10 years in adults in order to maintain adequate immunity levels. Nevertheless, data on vaccination coverage among adults are not routinely collected, and coverage is suspected to be low. The diphtheria antitoxin GMT has been widely used to evaluate the response to diphtheria vaccination [8], and it has been shown that the duration of immunity is related to the level of post-vaccination antibody titres [22].

A larger study conducted in Sweden demonstrated that a higher post-vaccination GMT is associated with a longer persistence of protection [19]. In our study, children who received a DT booster at school entry had significantly higher GMT than children who received DT; we can thus, assume that the DT group will have a shorter duration of protection. The higher antibody response and the comparable reactogenicity indicate that DT should be used as booster at school-entry, particularly if additional booster doses during adolescence or adulthood are not administered.

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