Diphtheria booster vaccination: one or two injections?

U. Nicolay, O.-E. Girgsdies, A. Banzhoff, E. Hundt, W. Jilg

Abstract

In a prospective, controlled, randomized, multicenter study the immunogenicity and tolerance of a single vaccination (day 0) and two (day 0, 28) booster vaccinations against diphtheria were compared in subjects who had received their last diphtheria vaccination more than 10 years ago. 415 subjects received the first booster vaccination, and 203 were vaccinated twice. The geometric mean diphtheria antitoxin concentration after the first booster (day 28) was 2.354 I.U./ml, and after the second booster (day 56) 2.238 I.U./ml. Prior to the first vaccination 48.9% of the subjects had a diphtheria antitoxin level below 0.1 I.U./ml; after the first and second boosters 95.4% and 97.5% of subjects, respectively, showed a level of at least 0.1 I.U./ml. A clear serological effect of a second booster 4 weeks after the first one could not be demonstrated.

Keywords: Diphtheria; Booster; Vaccination

1. Introduction

Diphtheria continues to be a problem in Europe, despite efforts to eliminate it. Within the successor states of the former Soviet Union, current health problems caused by diphtheria have again become an issue of public interest. In 1994, the World Health Organisation (WHO) registered 47808 cases of the disease within the region, and among these, approximately 1746 proved fatal [1]. Adults were affected in 70–80% of the new cases. Considerable differences in immunity in large sections of the population, large migrations of the population, and the predominance of a pathogenic organism with exceptionally strong toxin formation capability contributed to the distribution and seriousness of this epidemic disease [2].

Although the prevalence of diphtheria appears to be declining in Eastern Europe, it cannot yet be deduced that the disease is no longer a problem [3]. In recent decades, diphtheria has been controlled in Germany by specific vaccination campaigns. In particular, primary immunization and booster vaccinations administered to individuals at an early age have resulted in a clear reduction of cases of the disease. It may be postulated that an immune barrier against the epidemic spread of diphtheria is possible in Germany where more than 70% of the population is inoculated [2]. Studies have shown, however, that gaps in vaccination have occurred in Germany, predominantly in adults [4–7]. Therefore the booster vaccination of adults is of great importance.

The German ‘permanent vaccination committee’ (STIKO) recommends a booster vaccination against diphtheria every 10 years [8], preferably with a diphtheria tetanus vaccine. Some authors [6, 9], have questioned whether a single boosting of a vaccination after so many years is sufficient to provide protection for another 10 years. The objective of the current study was to compare a single diphtheria booster vaccination with two boosting injections when given 4 weeks apart.
2. Materials and methods

2.1. Design

This was a prospective, controlled, randomized, multicenter study conducted in Germany, Austria, and Slovenia. A total of 422 healthy subjects participated in the study between November 1995 and June 1996.

The following inclusion criteria were to be met:

- Men and women between 16 and 60 years of age
- Last diphtheria vaccination at least 10 years prior to study entry
- Complete documented primary diphtheria immunization (defined as at least three vaccinations within 2 years)
- Written informed consent of the subject

On day 0, all subjects were vaccinated against either diphtheria (Diphtheria Adsorbed Vaccine Behring for adults) or diphtheria–tetanus (Td Vaccine Behring), depending on whether a tetanus booster was indicated by the participant's vaccination history. Both vaccines contained at least 2 I.U./ml purified diphtheria toxoid according to the European Pharmacopoeia. On day 28, half of the subjects were randomized to receive a second booster vaccination.

2.2. Efficacy variables

To assess diphtheria antitoxin levels, blood was taken from each subject prior to the first vaccination (day 0), 4 weeks after the first vaccination (day 28), and additionally after another 4 weeks (day 56) for those participants who received a second booster vaccination. To evaluate the duration of immunity, it is planned that the diphtheria antitoxin levels of each subject will be monitored after 1, 2 and 5 years, to examine whether the second booster vaccination causes a longer-lasting effect than a single booster only.

The diphtheria antitoxin levels were determined by the VERO cell culture neutralization test essentially as described by Miyamura [10] and were divided into the following categories [11] for the purpose of assessment:

- $< 0.01$ I.U./ml susceptible to diphtheria
- $\geq 0.01$ to $< 0.1$ I.U./ml minimal protection
- $\geq 0.1$ to $< 1.0$ I.U./ml protection
- $\geq 1.0$ I.U./ml long-term protection

The main objective of the study was to compare diphtheria antitoxin categories after one or two diphtheria booster vaccinations. Secondary objectives were to determine diphtheria antitoxin levels and the antitoxin booster capability.

2.3. Safety variables

Subjects recorded adverse reactions to the vaccination in a diary for 2 days after the vaccination. Adverse events were classified as either local reactions (e.g. redness, swelling, pain, itching) or systemic reactions (e.g. fever, tiredness, joint pains, nausea, vomiting). Other adverse events that occurred were also recorded in the appropriate case report forms.

2.4. Statistical methods

Allowing for nonanalyzable subjects, 195 subjects per treatment arm were required to show that the one-sided 95% confidence limit for the proportion of seroprotected subjects (those with an antitoxin of at least 0.1 I.U./ml after vaccination) was greater than or equal to 80% on the condition that the real portion was 90%.

The difference between single and double booster vaccinations with respect to the diphtheria antitoxin category was tested using the stratified Wilcoxon's test for which the category prior to the first vaccination was used as a stratification variable [12]. Diphtheria antitoxin levels were assumed to be log-normally distributed [13]. For further exploratory analyses, analysis of covariance techniques were used. Study results were analyzed using SAS [14] and StatXact [15].

3. Results

3.1. Demographic and background characteristics

A total of 422 subjects at six centers in Germany, Austria, and Slovenia were included in the study. On day 0 all subjects received a first booster vaccination and 28 days later, 203 subjects received a second booster vaccination. The demographic characteristics of the participants are summarized in Table 1.

More female volunteers were in the single booster vaccination group than in the group receiving a double diphtheria booster vaccination. There were no other conspicuous differences between vaccination groups. A complete primary immunization against diphtheria could be verified for 326 (77.3%) subjects. The remaining 96 subjects either received fewer than three vaccinations within 2 years or received the three required vaccinations during a period that exceeded 2 years.
3.2. Efficacy

A total of 256 (60.7%) subjects received the first booster vaccination with the diphtheria vaccine and 166 (39.3%) received the diphtheria tetanus vaccine. Included in the analysis of the primary study objective were all subjects for whom diphtheria antitoxin levels had been measured following the first or second vaccinations. Subjects who did not meet the inclusion criteria were also included in the analysis (intention-to-treat analysis). Therefore, diphtheria antitoxin levels for 415 of the 422 subjects who received the first vaccination, and 199 of the 203 subjects who received the second booster vaccination could be included in the analysis. There was no diphtheria antitoxin measurement after the first or second vaccinations for 7 and 4 subjects, respectively.

Prior to the first vaccination, 97 (23.4%) subjects were susceptible to diphtheria (<0.01 I.U./ml), 106 (25.5%) had a minimum of protection (0.01 to <0.1 I.U./ml), and 212 (51.1%) could be considered serologically protected (≥0.1 I.U./ml). The mean geometric diphtheria antitoxin level of the 415 subjects was 0.077 I.U./ml.

After the first booster vaccination, 11 subjects were still susceptible to diphtheria. Eight subjects showed a minimum protection, and 396 (95.4%) (95% confidence limit: ≥93.4%) of the 415 subjects had a diphtheria antitoxin level of at least 0.1 I.U./ml 4 weeks after the first vaccination. One hundred eighty-four (90.6%) of the 203 subjects who at prevaccination were serologically unprotected or had only a minimum of protection, had a diphtheria antitoxin level of 0.1 I.U./ml or higher after the first booster vaccination (Table 2).

The mean geometric diphtheria antitoxin level for the total population of 415 subjects was 2.354 I.U./ml. On average, antitoxin levels increased between prevaccination (day 0) and day 28 by about 30.7 times, and 379 (91.3%) of the subjects had at least a fourfold diphtheria antitoxin increase.

The higher the diphtheria antitoxin level was before vaccination, the less booster capability was observed after the first booster vaccination (Table 3).

Table 1
Demographic characteristics and vaccination history

<table>
<thead>
<tr>
<th>Vaccination group (N = 422)</th>
<th>1 vaccination (N = 219)</th>
<th>2 vaccinations (N = 203)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>86 (39.3%)</td>
<td>104 (51.2%)</td>
</tr>
<tr>
<td>female</td>
<td>133 (60.7%)</td>
<td>99 (48.8%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (standard deviation)</td>
<td>28.6 (7.6)</td>
<td>28.5 (6.6)</td>
</tr>
<tr>
<td>Complete primary diphtheria immunization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>46 (21.0%)</td>
<td>50 (24.6%)</td>
</tr>
<tr>
<td>yes</td>
<td>173 (79.0%)</td>
<td>153 (75.4%)</td>
</tr>
<tr>
<td>Time since last diphtheria vaccination (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (standard deviation)</td>
<td>22.3 (6.8)</td>
<td>22.4 (6.0)</td>
</tr>
<tr>
<td>Number of previous diphtheria vaccinations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean (standard deviation)</td>
<td>3.9 (1.3)</td>
<td>3.8 (1.4)</td>
</tr>
</tbody>
</table>

Table 2
Comparison of diphtheria antitoxin categories prevaccinal and after first booster

<table>
<thead>
<tr>
<th>Prevaccinal (day 0) antitoxin category (I.U./ml)</th>
<th>Antitoxin category after first booster (day 28) (I.U./ml)</th>
<th>&lt;0.01</th>
<th>≥0.01− &lt; 0.1</th>
<th>≥0.1− &lt; 1.0</th>
<th>≥1.0</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.01</td>
<td></td>
<td>9</td>
<td>8</td>
<td>38</td>
<td>42</td>
<td>97 (23.4%)</td>
</tr>
<tr>
<td>≥0.01− &lt; 0.1</td>
<td></td>
<td>2</td>
<td>0</td>
<td>20</td>
<td>84</td>
<td>106 (25.5%)</td>
</tr>
<tr>
<td>≥0.1− &lt; 1.0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>149</td>
<td>155 (37.3%)</td>
</tr>
<tr>
<td>≥1.0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>56</td>
<td>57 (13.7%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>11 (2.7%)</td>
<td>8 (1.9%)</td>
<td>65 (15.7%)</td>
<td>331 (79.8%)</td>
<td>415 (100%)</td>
</tr>
</tbody>
</table>
On day 28, 203 subjects received a second booster vaccination against diphtheria. For 199 of the subjects, a diphtheria antitoxin value was available. Six of nine (66.7%) subjects who were either susceptible to diphtheria (<0.01 I.U./ml) or who had only a minimum protection (≥0.01 to <0.1 I.U./ml) four weeks after the first booster profited from the second booster by an increase of the diphtheria antitoxin category. A meaningful decrease was seen in one subject (Table 4). The mean geometric antitoxin level of the 199 subjects increased from 2.151 I.U./ml on day 28 to 2.238 I.U./ml on day 56, representing a mean increase of 1.04 times. A total of 19 (9.5%) subjects showed at least a fourfold increase of the antitoxin level.

As was seen after the first booster, the increase in diphtheria antitoxin following the second vaccination was dependent on the antitoxin level prior to vaccination (day 28). However, the observed increases were clearly lower compared with the first booster.

Taking into account the prevaccination antitoxin category, a statistical difference between the single and the repeated vaccination with regard to the diphtheria antitoxin categories could not be demonstrated (P = 0.25 stratified, two-sided Wilcoxon’s test). The analysis of only those subjects who strictly adhered to the inclusion criteria revealed a similar result (P = 0.53) suggesting that the effect of the second booster did not depend on whether the primary immunization against diphtheria had been complete.

### 3.3. Non-responder

After the first or second diphtheria booster vaccination, a total of 12 subjects (seven women, five men) were serologically unprotected (<0.01 I.U./ml). Analysis of relevant background factors (i.e. age, sex, complete primary immunization [yes/no], number of previous diphtheria vaccinations, time since the last diphtheria vaccination) did not reveal a common reason for the lack of vaccine booster ability. Eleven of the 12 subjects had a complete primary immunization documented with an average of 3.4 vaccinations. The last diphtheria vaccination was given, on average, 28.3 years before study start.

### 3.4. Exploratory analysis

If the influence of background factors on the diphtheria antitoxin level after the first booster vaccination was analyzed using analysis of covariance techniques, the prevaccinal antitoxin level was by far the most influential. The higher the prevaccinal antitoxin level, the lower the increase of the antitoxin level after the booster. Prevaccinal antitoxin levels contributed to this explanation to such a large extent as to rule out other possible prognostic factors such as sex, age, time since the last diphtheria vaccination, number of previous diphtheria vaccinations, complete primary immunization, and type of vaccines administered (e.g., diphtheria, diphtheria–tetanus vaccine).

### 3.5. Tolerance

A total of 215 (50.9%) of the 422 subjects experienced adverse events after the first booster vaccination, and 56 of the subjects (27.6%) after the second vaccination. No serious adverse events were observed. The majority of the events were of mild or moderate severity, appeared within the first 2 days after vaccination, and were limited in most cases to local reactions such as swelling, redness, pain, and itching. More local reac-

### Table 3

<table>
<thead>
<tr>
<th>Prevaccinal (day 0) antitoxin category (I.U./ml)</th>
<th>N</th>
<th>Mean increase (day 28/day 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.01</td>
<td>97</td>
<td>89.5</td>
</tr>
<tr>
<td>≥0.01–&lt;0.1</td>
<td>106</td>
<td>55.5</td>
</tr>
<tr>
<td>≥0.1–&lt;1.0</td>
<td>155</td>
<td>20.6</td>
</tr>
<tr>
<td>≥1.0</td>
<td>57</td>
<td>4.9</td>
</tr>
</tbody>
</table>

### Table 4

<table>
<thead>
<tr>
<th>Day 28 antitoxin category (I.U./ml)</th>
<th>Antitoxin category after second booster vaccination (day 56) (I.U./ml)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.01</td>
<td>≥0.01–&lt;0.1</td>
</tr>
<tr>
<td>&lt;0.01</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>≥0.01–&lt;0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥0.1–&lt;1.0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>≥1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4 (2.0%)</td>
<td>1 (0.5%)</td>
</tr>
</tbody>
</table>
tions occurred after the diphtheria tetanus vaccination than after the diphtheria vaccination.

4. Discussion

The primary objective of this prospective, controlled, randomized, multicenter study was to determine whether a single booster vaccination is sufficient for individuals whose last diphtheria vaccination was received more than 10 years ago, or whether a second vaccination is necessary 4 weeks later. The primary criterion for assessment were the diphtheria antitoxin levels 4 weeks after the first or second vaccination. Additionally, antitoxin levels after 1, 2 and 5 years for both vaccination schemes are planned to be compared in a longitudinal examination. A total of 219 subjects received a single booster vaccination only and 203 subjects received two vaccinations. There were no marked differences in the documented demographic data or other relevant background variables between the treatment groups.

On average the subjects were relatively young, with a mean age of 28.5 years, and the last documented diphtheria vaccination was received 22.3 years prior to the study.

Defining the complete primary immunization was problematic because there were different vaccination recommendations within the countries involved in the study. Therefore a complete primary immunization was defined in the test schedule as three vaccinations within 2 years, independent of the types of vaccine received and the age at which the primary immunization was performed. For the 422 subjects treated, a total of 396 different diphtheria vaccination schemes were documented, and for 326 (72.3%), a complete diphtheria primary immunization could be verified. As a separate analysis revealed, the effect of the booster vaccination was not dependent on whether the primary immunization was complete, which justified the inclusion of all subjects in the evaluations (i.e. intention-to-treat analysis). The immunity status of the subjects was clearly better than expected according to the vaccination documentation.

4.1. Efficacy

A total of 415 subjects were evaluable. Prior to the first vaccination, 23.4% of the study participants were serologically completely unprotected (diphtheria antitoxin level < 0.01 I.U./ml) and 51.1% could be considered protected against diphtheria (antitoxin level ≥ 0.1 I.U./ml). Similar values were observed in other investigations [16]. Hasselhorn [6] reported in a similar study a percentage of serologically unprotected subjects nearly twice as high (46%).

After the first diphtheria booster vaccination, the percentage of subjects protected against diphtheria increased from 51.1 to 95.4%. Mean antitoxin level increased by 30.7 times. The diphtheria antitoxin level increased less the higher the antitoxin level was prior to the first vaccination. A comparable result was observed in young school children by Naumann [17]. After a second vaccination (4 weeks after the first vaccination), on the whole, only those subjects benefitted who had had antitoxin levels below 1.0 I.U./ml after the first booster. In particular those subjects with an inadequate response after the first booster (< 0.1 I.U./ml) tended to show more favorable antitoxin levels after the second booster.

In other words, the expected decrease of diphtheria antitoxin levels after the first vaccination could be delayed by the second vaccination, eliciting in those subjects vaccinated twice nearly the same diphtheria antitoxin levels on day 56 as on day 28 for subjects who only received a single vaccination. The planned longitudinal examination will show to what extent a long-term effect was induced.

A total of 11 study participants did not show a minimum serologic protection (≥ 0.01 I.U./ml) after the first booster and were thus classified as ‘vaccination failures’. Six of these 11 received a second booster vaccination which triggered in three of them a diphtheria antitoxin rise to a value of at least 0.01 I.U./ml. One participant who boasted a clear response after the first booster dropped again after the second booster to an inappropriate level below 0.01 I.U./ml. Our data could not confirm a connection between vaccination failure and sex or interval from last diphtheria vaccination, as stated by Hasselhorn [6].

The variable with the greatest effect on diphtheria antitoxin levels after the first booster vaccination was the antitoxin level prior to the booster, independent of other factors such as sex, age, or other background characteristics. The influence of the prevaccinal antitoxin levels was so prominent that the other factors were not considered relevant in the statistical model.

4.2. Tolerance

The types of adverse events observed, as well as the frequency and severity of the events were similar to those reported in other investigations using comparable methods of collection and documentation. The proportion of subjects reporting adverse events after vaccination with Td vaccine was higher than the pro-
portion after diphtheria vaccination. In 98.2% of subjects who received the Td vaccine, a tetanus antitoxin level of at least 1.0 I.U./ml was seen prior to vaccination, indicating that these subjects had sufficient serologic protection and actually were not in need of a tetanus booster vaccination. As discussed in the literature, local and systemic reactions following tetanus vaccination appear to be more frequent and severe in persons who already have a high tetanus antitoxin titer [18].

In summary, this study shows that in 91% of adults whose last diphtheria vaccination was received 10 or more years ago and who had diphtheria antitoxin concentrations below 0.1 I.U./ml a single booster vaccination induced safe protection against diphtheria according to serological criteria. For a second booster four weeks after the first vaccination a significant serologic difference could not be demonstrated. In fact, six of nine low/non-responders (comprising 2.5% of the study population) did respond to the second booster. In our opinion this is not enough to justify two booster injections on a routine basis for the whole population. However, a second booster given later (e.g. after one year) might have a more favorable effect on both low-responders and non-responders.

4.3. Members of the study group


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References