Short Communication

Evaluation of Thimerosal on the Potency and Stability of Inactivated Animal Rabies Vaccine


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Article Information
Received: 2019-07-13
Revised: 2019-07-17
Accepted: 2019-07-21

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Abstract
Thimerosal is used as a preservative in Rabies vaccine. This study aimed at comparing potency and stability of inactivated animal rabies vaccine with and without thimerosal. Therefore, two groups of rabies vaccine were produced. The results indicated that thimerosal has no deleterious effect on the vaccine; furthermore, its elimination did not influence the potency and stability of the vaccine.

Keywords: Thimerosal, Potency, Rabies vaccine

1. Introduction
The first attempts to the production of Rabies vaccine occurred in the 1800s through a direct injecting of avirulent virus derived from animal sources. Nowadays, cell culture and recombinant techniques are mostly used for the production of the vaccine [1]. By and large, most of vaccines need additives such as adjuvant, stabilizer and preservatives in their formulation process. Preservatives are added to prevent accidental microbial contamination [2]. Thimerosal, also called Thiomersal or Merthiolate, is an ethylmercury-containing pharmaceutical compound which has been used as a preservative or inactivating agent in some vaccines and other products since 1930s. Due to its deleterious effect on live vaccines, it is used only in inactivated vaccines. While thimerosal has been taken out of childhood vaccines in the United States, its deleterious effect is in doubt. However, the problem with thimerosal is that it contains 49.6% mercury by weight, which may cause neurotoxicity in humans and animals [2-4]. Vaccine additives may affect the antigens and consequently influence the quality of the vaccine. They may affect vaccine quality assessment by impacting on the antigens (directly), and on the organism and its cells, subsequently on the immune response to the vaccine (indirectly). Since the compounds in the vaccine manufacturing process have a significant impact on vaccine quality, the potential impact of the used substances should be evaluated. Correspondingly, elimination and reduction of some materials such as
Thimerosal effect on the animal rabies vaccine, Ahmadnejad F et al.

Thimerosal from a product can have some unexpected effects on vaccine quality, safety and efficacy [5]. Although some studies have evaluated the safety and effectiveness of some preservatives in vaccines, there are few reports about thimerosal effect on the vaccine quality.

In Iran, rabies is endemic and a major public health problem. Vaccination of animals has an important role in protecting animal health and public health against rabies. Rabies vaccine is produced for veterinary use in Iran and Thimerosal is used as a preservative in the vaccine. The study objective was to assess the effect of thimerosal on the vaccine potency, as well as its elimination effect, and ensuring the vaccine stability in these conditions.

2. Methods

The study was carried out on Viral Vaccines Production Department, Production and Research Complex, Pasteur Institute of Iran and was approved by the Pasteur Institute’s ethics committee (No: 94/0201/10267).

The rabies virus strain PV-Paris was harvested according the manufacture process. Two groups of rabies vaccine were produced. One group was formulated with Thimerosal (Sigma, USA) and aluminum hydroxide (Alhydrogel ®) as adjuvant. Another group was formulated at the same time only with the adjuvant.

The immunogenic activity of rabies vaccine was evaluated by NIH method [1]. Briefly, at first, the median lethal dose (LD50) of CVS (Challenge virus standard) was determined in control mice and calculated according to the procedure of Reed and Muench. All mice under reference and test vaccine were immunized two times intraperitoneally with 0.5 ml of national reference and test vaccines in dilutions of 1:5, 1:25, 1:125, 1:625 within an interval of 7 days.

Then, on day 14, all mice were challenged intracerebrally with CVS strain at a dose of 30 LD50 of virus per 0.03 ml, and observed daily during 2 weeks for the development of rabies symptoms. The mice were observed for 14 days and the effective immunizing dose (ED50) of the reference and test vaccines were calculated based on the number of survivors and by Reed and Muench, which are expressed, relative to the reference vaccine in IU.

Potency test was carried out at three interval time, at the beginning of the vaccines production, 6 and 10 months after. Repeated measures ANOVA was performed for multiple comparisons between groups.

3. A combined Results and Discussion section

Table 1 presents the potency of Rabies Vaccine between two study groups: vaccine with and without Thimerosal over ten months. The trend of the potency of Rabies vaccine between the two study groups over the time period was depicted as figure 1. At the first month, the potency of the vaccine with and without Thimerosal was approximately identical. Despite the fact that the potency was higher in the group of Thimerosal-free in comparing with the other one, there was no statistically significant difference between them (p=0.43).
Our results showed that there was no significant difference between Thimerosal-containing and Thimerosal-free vaccines at 1, 6 and 10 months when held at 4°C (p=0.18). On the other hand, the time trend and multiple comparisons within each intervention group demonstrated that the potency of Rabies vaccine was not significantly affected over the time period (stability, p=0.97).

Table 1. Comparison of Relative potency of Thimerosal-containing and Thimerosal-free vaccines at months 1, 6 and 10 (between and within group comparison)

<table>
<thead>
<tr>
<th></th>
<th>First month</th>
<th>Sixth month</th>
<th>Tenth month</th>
<th>Total row (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine- without Thimerosal</td>
<td>1.43</td>
<td>1.49</td>
<td>1.56</td>
<td>1.50±0.07</td>
</tr>
<tr>
<td>Vaccine- with Thimerosal</td>
<td>1.39</td>
<td>1.37</td>
<td>1.27</td>
<td>1.34±0.06</td>
</tr>
<tr>
<td>Total column (mean ± SD)</td>
<td>1.41±0.03</td>
<td>1.43±0.08</td>
<td>1.42±0.21</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Trend of potency of Rabies vaccine over ten months between two study groups (absence and presence of Thimerosal)

Beside thimerosal effects on man, it was revealed that it is toxic to cultured cells of human and animals. Thimerosal has shown to induce membrane and DNA damage and initiate apoptosis in neurons and fibroblasts [9].

The studies in animals detected toxicity effects of low doses of Thimerosal in vaccines [10]. The neurotoxic effects of thimerosal were also reported in developing rat brain, suggesting its involvement in neurodevelopmental disorders [11].

Kravchenko et al suggested that the toxic effect of thimerosal could damage cells at the injection site and induce the formation of autoantigens affecting the body [12]. However, Ni et al suggested that thimerosal significantly increases antibody response in a mouse model [13].

To evaluate the potency of Rabies vaccine, mice were injected with the vaccine; however the injection site, dose and effect duration should be considered in assessing the thimerosal effect [14, 15].
Some studies indicated that the bacterial cultures devitalized with thimerosal had high antigenic values and found to incite antibody of higher titer than the heat killed vaccine [14]. On the other hand, another study indicated that Pertussis vaccine preserved with thimerosal was more toxic to mice than the unpreserved vaccine, and that increasing Thimerosal concentration increased the toxicity of the vaccine [16]. Among viral vaccines, thimerosal-free hepatitis B vaccine had similar seroprotection rates compared with thimerosal-containing vaccine [17]. This finding is similar to the present study’s results in that there was no significant difference between Rabies vaccines with and without Thimerosal. Since both vaccine potency and its stability were significant, the effect of thimerosal on Rabies vaccine potency under conditions of long term storage at + 4° was investigated. The study indicated that the stability of the vaccine was not affected by thimerosal, while studying for poliovirus vaccine indicated a different result. Sawyer et al. reported that the potency of enhanced inactivated poliovirus vaccine (eIPV) was significantly affected by the presence of thimerosal when held at 4°C for up to 8 months. Deleterious effect of thimerosal has been observed in eIPV, both with thimerosal, and in combination with diphtheria-tetanus-pertussis (DTP) vaccine and thimerosal. Likewise, they reported that thimerosal enhanced sensitivity of poliovirus antigens to elevated temperature [18]. Thimerosal had intermediate stability among different preservatives on Leishmania antigens, during 12 months of follow-up, while not showing long-term stability [19].

Although a vaccine without an additive such as thimerosal or other unwanted materials would be more helpful without any safety issues for use in both human and animals and although nowadays rabies vaccines with and without thimerosal are produced in some countries, other studies seem necessary due to the different manufacturing process in each country.

**Acknowledgement**

The authors thank Dr. Aghasadeghi, Dr. Janani, Dr. Nouri Inanlou and Dr. Ghorbani for their good advice and English editing in some parts of the manuscript. We also thank Hasan Amiri for his technical assistance. This work was supported by a grant from Pasteur Institute of Iran.

**Conflict of interest**

The authors declare no conflict of interest.

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