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Short Communication

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

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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 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 (LD_{50}) 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 m1, and observed daily during 2 weeks for the development of rabies symptoms. The mice were observed for 14 days and the effective immunizing dose (ED_{50}) of the reference and test vaccines were calculated based on the number of survivors and by Reed and Menche, 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).

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

	First month	Sixth month	Tenth month	Total row (mean ± SD)
Vaccine- without Thimerosal	1.43	1.49	1.56	1.50±0.07
Vaccine- with Thimerosal	1.39	1.37	1.27	1.34±0.06
Total column (mean ± SD)	1.41±0.03	1.43±0.08	1.42±0.21	

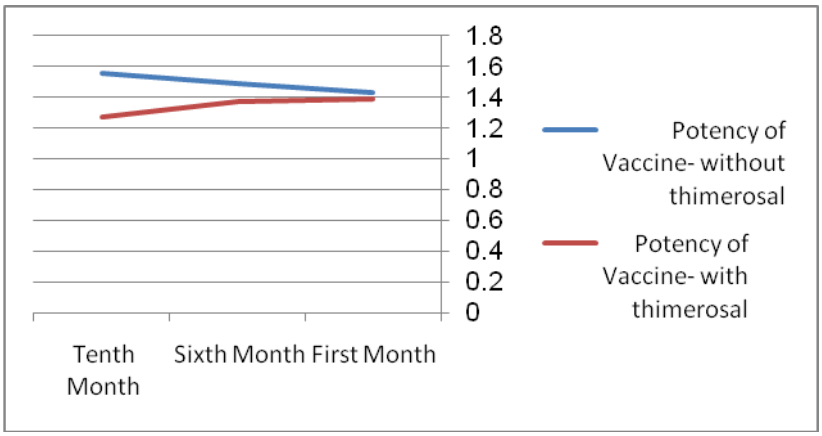

Figure 1. Trend of potency of Rabies vaccine over ten months between two study groups (absence and presence of Thimerosal)

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).

There are differnt opinions on thimerosal and its effects: some studies have reported the harmful effects on organisms (human/ animals) and biological products. In addition to allergic reactions, several cases of mercury poisoning and autism have been reported from thimerosal- containing products. It seems that ethylmercury and inorganic Hg produced from Thimerosal metabolism accumulate in different organs such as brain [4, 6], while some studies did not demonstrate an association between thimerosal in vaccines and autism or neuropsychological effects [7, 8].

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.

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Conflict of interest

The authors declare no conflict of interest.

References

1.Meslin F-X, Kaplan MM, Koprowski H. Laboratory techniques in rabies. 4th ed ed. Geneva: World Health Organization; 1996.

2.Food and Drug Administration (FDA). Thimerosal and Vaccines [(Accessed 2017 Sep 10)]. Available from: https://www.fda.gov/biologicsbloodvaccines/safetyavailability/vaccinesafety/ucm096228.htm

3.Geier DA, Jordan SK, Geier MR. The relative toxicity of compounds used as preservatives in vaccines and biologics. Medical science monitor : international medical journal of experimental and clinical research. 2010 May;16(5):SR21-7. PubMed PMID: 20424565.

4.Burbacher TM, Shen DD, Liberato N, Grant KS, Cernichiari E, Clarkson T. Comparison of blood and brain mercury levels in infant monkeys exposed to methylmercury or vaccines containing thimerosal. Environmental health perspectives. 2005;113(8):1015-21.

5.Products CfPM. European medicines agency. CPMP/BwP/2517/00: Points to consider on the reduction, elimination or substitution of thiomersal in vaccines. 2001.

6.Olczak M, Duszczyk M, Mierzejewski P, Majewska MD. Neonatal administration of a vaccine preservative, thimerosal, produces lasting impairment of nociception and apparent activation of opioid system in rats. Brain research. 2009 Dec 8;1301:143-51. PubMed PMID: 19747466.

7.Fombonne E, Zakarian R, Bennett A, Meng L, McLean-Heywood D. Pervasive developmental disorders in Montreal, Quebec, Canada: prevalence and links with immunizations.

Pediatrics. 2006 Jul;118(1):e139-50. PubMed PMID: 16818529.
8.Thompson WW, Price C, Goodson B, Shay DK, Benson P, Hinrichsen VL, et al. Early thimerosal exposure and neuropsychological outcomes at 7 to 10 years. The New England journal of medicine. 2007 Sep 27;357(13):1281-92. PubMed PMID: 17898097.
9.Baskin DS, Ngo H, Didenko VV. Thimerosal induces DNA breaks, caspase-3 activation, membrane damage, and cell death in cultured human neurons and fibroblasts. Toxicological sciences : an official journal of the Society of Toxicology. 2003 Aug;74(2):361-8. PubMed PMID: 12773768. Pubmed Central PMCID: 1892749.
10.Dórea JG. Integrating experimental (in vitro and in vivo) neurotoxicity studies of low-dose thimerosal relevant to vaccines. Neurochemical research. 2011;36(6):927-38.
11.Olczak M, Duszczyk M, Mierzejewski P, Wierzba-Bobrowicz T, Majewska MD. Lasting neuropathological changes in rat brain after intermittent neonatal administration of thimerosal. Folia neuropathologica. 2010;48(4):258-69. PubMed PMID: 21225508.
12.Kravchenko AT, Dzagurov SG, Chervonskaia GP. [Evaluation of the toxic action of prophylactic and therapeutic preparations on cell cultures. III. The detection of toxic properties in medical biological preparations by the degree of cell damage in the L132 continuous cell line]. Zhurnal mikrobiologii, epidemiologii, i immunobiologii. 1983 Mar(3):87-92. PubMed PMID: 6845931. Otsenka toksicheskogo deistviia profilakticheskikh i lechebnykh preparatov na kul'turakh kletok. Soobshchenie III. Vyiavlenie toksicheskikh svoistv v meditsinskikh biologicheskikh preparatakh po stepeni povrezhrdeniia kletk v perebvivaemoi linii L132.
13.Ni J, Bi S, Xu W, Zhang C, Lu Y, Zhai L, et al. Improved immune response to an attenuated pseudorabies virus vaccine by ginseng stem-leaf saponins (GSLS) in combination with thimerosal (TS). Antiviral research. 2016 Aug;132:92-8. PubMed PMID: 27241688.
14.Jamieson W, Powell H. Merthiolate as a preservative for biological products. American journal of epidemiology. 1931;14(1):218-24.
15.Blair A, Clark B, Clarke AJ, Wood P. Tissue concentrations of mercury after chronic dosing of squirrel monkeys with thiomersal. Toxicology. 1975;3(2):171-6. PubMed PMID: 804725.
16.Geier DA, Sykes LK, Geier MR. A review of Thimerosal (Merthiolate) and its ethylmercury breakdown product: specific historical considerations regarding safety and effectiveness. Journal of toxicology and environmental health Part B, Critical reviews. 2007 Dec;10(8):575-96. PubMed PMID: 18049924.
17.Van Damme P, von Sonnenburg F, Hatz C, Hoet B, Lefevre I, Leyssen M. Long- term immunogenicity of preservative- free hepatitis B vaccine formulations in adults. Journal of medical virology. 2009;81(10):1710-5.
18.Sawyer LA, McInnis J, Patel A, Horne AD, Albrecht P. Deleterious effect of thimerosal on the potency of inactivated poliovirus vaccine. Vaccine. 1994 Jul;12(9):851-6. PubMed PMID: 7526574.
19.Gama Ker H, Dian de Oliveira Aguiar-Soares R, Mendes Roatt B, das Dores Moreira N, Coura-Vital W, Martins Carneiro C, et al. Effect of the preservative and temperature conditions on the stability of Leishmania infantum promastigotes antigens applied in a flow cytometry diagnostic method for canine visceral leishmaniasis. Diagnostic microbiology and infectious disease. 2013 Aug;76(4):470-6. PubMed PMID: 23726649.