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Effects of lipopolysaccharide and chelator on mercury content in the cerebrum of thimerosal-administered mice

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Abstract

Thimerosal is one of the best-known preservative agents for vaccines in the world but a relationship between its use and autism has long been suspected so that its effects on the brain need more detailed research. We here examined the influence of lipopolysaccharide injury to the blood–brain barrier on the penetration of mercury from thimerosal into mouse cerebrums, as well as the effect of chelator of heavy metals on cerebrum mercury content. Mercury can be expected to be detected in the cerebrum of normal mice, because the metal is present in standard mouse chow. When 60 μg/kg of thimerosal was subcutaneously injected into the mouse, the mercury content in the cerebrum was significantly higher 48 h after the thimerosal injection with a maximum peak after 72 h. In addition, mercury content in the cerebrum was still higher on day 7 than in the control group. When lipopolysaccharide was pre-injected into mice to induce damage on blood–brain barrier, the mercury content in the cerebrum was significantly higher at 24 and 72 h after the injection of 12 μg/kg of thimerosal compared to the control group, this dose alone does not cause any increase. The mercury content in the cerebrums of mice was decreased to the control group level on day 7 when a chelator, dimercaprol, was administered once a day from days 3 to 6 after a 60 μg/kg, s.c. injection. In addition, D-penicillamine as a chelator decreased the mercury contents in the cerebrum after the high dose administration. In conclusion, a physiological dose of thimerosal did not increase the content of mercury in the cerebrum, but levels were increased when damage to the blood–brain barrier occurred in mice injected with thimerosal. In addition, a chelator of heavy metals may be useful to remove mercury from the cerebrum.

Keywords: Mercury; Cerebrum; Thimerosal; Lipopolysaccharide; Dimercaprol; D-Penicillamine

1. Introduction

Although thimerosal is the most well established preservative reagent for vaccines, it is a mercuric compound and ethyl and inorganic mercury can be produced from thimerosal in organs (Clarkson, 2002; Burbacher et al., 2005). Organic mercury, including ethyl mercury, can damage cells and tissues (Ueda-Ishibashi et al., 2004; Yel et al., 2005; Slodownik and Ingber, 2005; Havarinasab and Hultman, 2006; Zarini et al., 2006). Furthermore, methyl mercury can cause severe damage in the central nervous system and a relationship between thimerosal use and autism has long been suspected (Stajich et al., 2000; Bernard et al., 2002; Pichichero et al., 2002; François et al., 2005; Mutter et al., 2005). However, the concentration of mercury in the blood of infants and children receiving vaccines with thimerosal has been reported to be very low, without any toxic effects (Madsen et al., 2003; Bigham and Copes, 2005; Clements and McIntyre, 2006), and the agent is still recommended as a cheap and stable preservative for vaccines. Countries such as Japan and United States are now tending to reduce application of thimerosal as much as possible, but it continues to be employed for influenza, tetanus, hepatitis B, poliomyelitis, and measles vaccines in Japan. Clearly, there is a need for more detailed research on the effects of thimerosal in the body, especially in central nervous system.

While the content of mercury in the brain of experimental animals was not found to increase after thimerosal injection (Burbacher et al., 2005), it is not clear whether adverse effects may occur under different circumstances. We previously observed that lipopolysaccharide (LPS) induction of inflammation is associated with an increase in the permeability of the blood–brain barrier (Minami et al., 1996, 1998a,b, 2002). The aim of the present study was to determine whether injection of LPS into mice exerts any effect on mercury content in

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the cerebrum after thimerosal treatment. In addition, we examined the effects of chelator of heavy metals.

2. Materials and methods

2.1. Animals

Five-week-old ddY strain male mice were purchased from Japan SLC Co. (Shizuoka, Japan), and housed for 1 week before use in the experiments at a controlled room temperature (24 ± 1 °C, 60–65% relative humidity) on a 12:12 light/dark cycle. The mice were provided with standard diet (MF; Oriental Yeast Co., Tokyo, Japan) and tap water *ad libitum*.

2.2. Chemicals

Thimerosal was purchased from Nacalai Tesque Co. (Kyoto, Japan), mercury standard solution from Wako Pure Chemicals Co. Ltd. (Osaka, Japan), and saline from Otsuka Pharmaceuticals Co. (Tokyo). Lipopolysaccharide (LPS) was obtained from Sigma Co. Ltd. (MO, USA), dimercaprol from Daiichi Pharmaceutical Co. Ltd. (Tokyo) and D-penicillamine from Taisho Pharmaceutical Co. Ltd. (Tokyo). The other reagents were ultra-pure grade from Wako Pure Chemicals Co. Ltd. Ultrapure water (Direct-Q, Japan Millipore Ltd., Tokyo) was used for the preparation of reagent solutions.

2.3. Animal treatment

For observation of dose-dependent effects of thimerosal on mouse cerebrum, mice were injected with 0, 3, 6, 12, 24, or 60 μg/kg in saline, subcutaneously. There have been reports of thimerosal administration by intramuscular injection or in the drinking water (Harry et al., 2004; Burbacher et al., 2005). But vaccines including thimerosal have mainly been injected subcutaneously in Japan. Therefore, in the present study, we administered thimerosal to mice subcutaneously and observed the concentration of mercury in organs. Three days after injection, the mice were decapitated, and their cerebrums were removed and frozen at −20 °C until measurement.

For observation of time-dependent effects of thimerosal on mouse plasma, liver, kidney, cerebrum, and cerebellum, a 60 μg/kg dose was subcutaneously injected and animals were anesthetized by pentobarbital after 2 h, 6 h, 10 h, 1 day, 2 days, 3 days, or 7 days and plasma was collected. The cerebrum, cerebellum, liver, and kidneys were removed and frozen at −20 °C.

For observation of effects of LPS on penetration of mercury into the cerebrum, 4 mg/kg of LPS was intraperitoneally injected 3 h before 12 μg/kg of thimerosal was subcutaneously injected. After 1 or 3 days, the cerebrums of decapitated mice were removed for measurement.

To observe the effects of chelator of heavy metals cerebrum, mice were injected with 60 μg/kg of thimerosal, and D-penicillamine (500, 1000, or 2000 mg/kg, p.o.) or dimercaprol (50 mg/kg, s.c.) were administered once a day from days 3 to 6, then the cerebrums were removed on day 7.

2.4. Measurement of mercury

Samples of known wet weight were placed in combustion tubes made of Quartz, and burned (Barnstead Thermolyne Furnace, 1300) to make gold amalgam using vaporization equipment (Mercury Evaporator ME-300: Hiranuma Industrial). Then, the mercury vapor obtained from the heat treatment of gold amalgam was measured using an atomic absorption photometer (Mercury Analyzer HG-300; Hiranuma Industrial). A linear standard curve was generated using standard mercury in the range of 0–4 ng and the detection limit determined to be 0.087 ng.

2.5. Statistical analysis

All results are expressed as means with S.D.s, and differences among groups were determined by one-way analysis of variance. Means were compared with Student–Newman–Keuls' multiple comparison test. Significance was set at $p < 0.05$ and <0.01 levels.

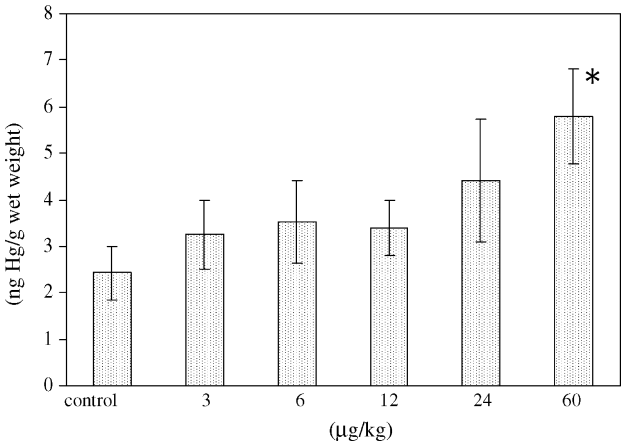

Fig. 1. Contents of mercury in cerebrums of thimerosal-injected mice. Tissues were removed 3 days after the injection of thimerosal. Each group consisted of six mice. Mean ± S.D., $*p < 0.05$ vs. control group.

3. Results

Cerebrum after decapitation without perfusion was used for the measurement of mercury in the present study. Because, there was no difference of mercury contents between perfused (19.5 ± 14.8 ng/g tissue, $n = 5$) and non-perfused (21.3 ± 6.4 ng/g tissue, $n = 5$) cerebrums when the cerebrum was removed 72 h after 600 μg/kg of thymerosal was injected. And a similar result was obtained in our previous report using fluorescein (Minami et al., 1998b). Mercury is included at 6.2 ± 0.8 ng/g in standard mouse feed which uses fish as a raw material and could be detected in all organs of the control group, including the cerebrum. Fig. 1 shows contents of mercury in the cerebrum 3 days after thimerosal injection. There were no differences between the control and treated groups with 24 μg/kg of thimerosal or less. However, mercury content was elevated with the 60 μg/kg dose.

When 60 μg/kg of thimerosal was injected, mercury content in the cerebrum did not rise until 48 h after the injection and maximum levels were detected after 72 h. In addition, mercury content was significantly higher at day 7 than in the control group (Fig. 2).

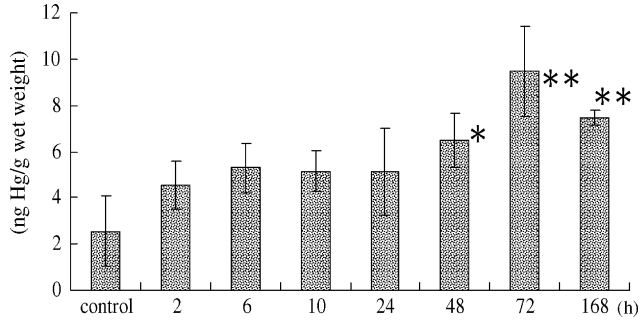

Fig. 2. Mercury contents in cerebrum of thimerosal-injected mice. Tissues were removed from pentobarbital-anesthetized mice at different time-points after injection of thimerosal (60 μg/kg). In control group, cerebrums were sampled 3 days after saline injection. Each group consisted of six mice. Mean ± S.D., $*p < 0.05$, $**p < 0.01$ vs. control group.