SHORT COMMUNICATION

Gender-selective toxicity of thimerosal

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Abstract

A recent report shows a correlation of the historical use of thimerosal in therapeutic immunizations with the subsequent development of autism; however, this association remains controversial. Autism occurs approximately four times more frequently in males compared to females; thus, studies of thimerosal toxicity should take into consideration gender-selective effects. The present study was originally undertaken to determine the maximum tolerated dose (MTD) of thimerosal in male and female CD1 mice. However, during the limited MTD studies, it became apparent that thimerosal has a differential MTD that depends on whether the mouse is male or female. At doses of 38.4–76.8 mg/kg using 10% DMSO as diluent, seven of seven male mice compared to zero of seven female mice tested succumbed to thimerosal. Although the thimerosal levels used were very high, as we were originally only trying to determine MTD, it was completely unexpected to observe a difference of the MTD between male and female mice. Thus, our studies, although not directly addressing the controversy surrounding thimerosal and autism, and still preliminary due to small numbers of mice examined, provide, nevertheless, the first report of gender-selective toxicity of thimerosal and indicate that any future studies of thimerosal toxicity should take into consideration gender-specific differences.

Keywords: Thimerosal; Thimerosal toxicity; Gender-selective toxicity; Maximum tolerated dose; Autism

Introduction

Thimerosal is an organic compound that contains mercury and has been used historically as a preservative in vaccines and pharmaceutical products. The breakdown product, ethylmercury, in thimerosal-preserved childhood vaccines has been suggested to be neurotoxic and to contribute to the etiology of neurodevelopmental disorders, including autism; however, this supposition is highly controversial (Mutter et al., 2005; Geier et al., 2007; Ng et al., 2007; Zareba et al., 2007; Thompson et al., 2007, Schechter and Grether, 2008). It has, however, been shown that mercury and thimerosal administration results in the decreased production of...
the proinflammatory cytokines, TNFz, IL-6 and IL-12p70 from LPS-stimulated human monocyte-derived dendritic cells (Agrawal et al., 2007). These investigators also showed that thimerosal could differentially affect cytokine production from T lymphocytes through depletion of intracellular glutathione. However, a relationship to neurodevelopmental disorders has not been shown (Berman et al., 2008).

In humans, a relationship between thimerosal administration and autism has recently been reported where it was found that the incidence of autism was significantly higher (2.35-fold) in children born of Rh-negative compared to Rh-positive women, where the administration of immunoglobulins containing thimerosal had been common practice to prevent a disease known as hemolytic disease of the newborn (Geier and Geier, 2007). However, this conclusion is disputed by another recent study (Miles and Takahashi, 2007). Nevertheless, whether or not pre- or postnatal exposure to mercury-containing thimerosal had a role in some children who subsequently developed autism remains unresolved.

Our studies were not trying to determine whether thimerosal has any neurotoxicity or whether there is any connection of thimerosal to autism. Instead, we were doing an unrelated study that was testing only for the maximum tolerated dose (MTD) levels for thimerosal in a mouse model to be subsequently used in a study of reversal of immune-mediated platelet destruction (Rampersad et al., 2005). However, during our MTD studies, we serendipitously discovered that thimerosal shows a differential toxicity that depends upon whether the test animals are male or female.

Materials and methods

Animals

Female or male CD1 mice (Mus musculus) approximately 5 weeks old and 18–20 g were purchased from Charles River Canada Inc., Montreal, PQ. All MTD testing was performed by Nucro-Technics, Scarborough, Ontario, after an approved animal protocol. Nucro-Technics is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and the Canadian Council on Animal Care. The study was conducted under the direction of Dr. Albert Licollari, DVM, PhD.

Protocol

Mice were individually weighed and marked prior to treatment and a single dose of thimerosal was administered intravenously via the tail vein. A dose escalation study was initiated in accordance with standard testing to determine the MTD. Two or three male and female mice were used for each dose level and animals were observed for 7–14 days with necropsies performed at the end of the study. The escalating doses were not applied in parallel but were done subsequently with 2–5 days between each dose level. Stock solutions of thimerosal (BioShop Canada Inc., Burlington, Ontario) were prepared in 0.5-mL dimethyl sulfoxide (DMSO) and were stored at 2–8 °C for not more than 1 week. Dosing solutions were freshly prepared in phosphate buffered saline (PBS) on the day of dosing. 5% DMSO was sufficient to solubilize thimerosal up to a dose of 19.2 mg/kg. When doses were being prepared at 38.4 mg/kg, it was noticed that not all the thimerosal was being solubilized and a concentration of 10% DMSO was used for this and higher dosing. We had previously determined that CD1 mice can tolerate 10% DMSO.

Results

Initially, using a dose escalating approach, all mice tolerated thimerosal in 5% DMSO well up to doses of 38.4 mg/kg (Table 1). Although there was some initial weight loss during 2–3 days after dosing, animals appeared normal and regained pre-treatment body weights. When using 38.4 mg/kg thimerosal, however, it was noticed that the thimerosal was not going completely into solution; hence subsequent dosing with thimerosal was in 10% DMSO, starting with the next escalated dose of 115.2-mg/kg thimerosal. However, at this dose, two male and two female mice died (Tables 1 and 2). Thus, a ‘step-down’ approach was initiated as indicated in Table 1. During this step-down series of experiments, gender-related differential toxic effects were seen starting at 76.8 mg/kg. Even at 38.4 mg/kg when using 10% DMSO, all male mice died but no female mice. Indeed, within this dose range of 38.4–76.8 mg/kg thimerosal, seven of seven male mice died or had to be sacrificed in moribund condition (substantial weight loss, lethargy, piloerection) (Tables 1 and 2). In contrast, within the same dose range, no females showed any significant toxic effects of thimerosal, although there was some transient weight loss; however, there were no gross findings at necropsy. A summary of the gender-selective toxicity of thimerosal is provided in Table 2.

Discussion

The organomercurial preservative thimerosal has been used in various vaccine and immunoglobulin preparations for use in humans since the 1930s to help prevent potentially life-threatening contamination with
harmful microbes. Recent attention due to the mercury contained within the thimerosal molecule has focused on the potential impact of thimerosal use on the development of autism (Berman et al., 2008; Geier and Geier, 2007; Geier et al., 2007; Miles and Takahashi, 2007; MMWR, 2007; Ng et al., 2007).

An important consideration when trying to evaluate the possible role of thimerosal toxicity is that autism occurs approximately four times more frequently in males compared to females (MMWR, 2007). Thus, if males have an increased sensitivity to thimerosal, this would have to be taken into account with analyses of any outcomes. Our results that demonstrate a significant difference in the MTD of thimerosal depending upon whether the test animal is male or female is, thus, an important finding. Indeed, in mice, we found when using 10% DMSO to completely dissolve the thimerosal prior to injection that the MTD of thimerosal in males was only 25.6 mg/kg; whereas, in females, the MTD was 76.8 mg/kg. Thus, thimerosal has a 3-fold increased toxicity in males compared to females. Use of 10% DMSO was necessary to completely solubilize the thimerosal. Thus, results using 5% DMSO may be somewhat misleading as tolerated results obtained at the higher doses, particularly when using 38.4 mg/kg in 5% DMSO, may be due to incomplete solubilization of the thimerosal. Regardless of the concentration of DMSO, only male mice were susceptible to severe thimerosal toxicity between the dose range of 38.4–76.8 mg/kg. This is the first report of gender-selective toxicity for thimerosal.

Although the in vivo levels of thimerosal used in our studies are far higher than what would be expected in humans, this may not preclude the impact of our findings as we were using mortality as our outcome. Although our study is not extensive as the group sizes are small, nevertheless, we clearly found that male mice were more susceptible to the toxic effects of thimerosal than were female mice. Indeed, this is the first report of gender-selective toxicity for thimerosal and it is intriguing as to why this compound would show this differential effect.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>DMSO (%)</th>
<th>Date dosed</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>5</td>
<td>03/02/05</td>
<td>Animals appeared normal</td>
</tr>
<tr>
<td>9.6</td>
<td>5</td>
<td>03/04/05</td>
<td>Animals appeared normal</td>
</tr>
<tr>
<td>19.2</td>
<td>5</td>
<td>03/08/05</td>
<td>Animals appeared normal</td>
</tr>
<tr>
<td>38.4</td>
<td>5b</td>
<td>03/09/05</td>
<td>Animals appeared normal</td>
</tr>
<tr>
<td>115.2</td>
<td>10c</td>
<td>03/14/05</td>
<td>Animals died or were sacrificed</td>
</tr>
<tr>
<td>76.8</td>
<td>10c</td>
<td>03/22/05</td>
<td>Animals died or were sacrificed</td>
</tr>
<tr>
<td>51.2</td>
<td>10c</td>
<td>03/28/05</td>
<td>Animals died or were sacrificed</td>
</tr>
<tr>
<td>38.4</td>
<td>10b</td>
<td>03/30/05</td>
<td>Animals died or were sacrificed</td>
</tr>
<tr>
<td>38.4</td>
<td>5b</td>
<td>04/21/05</td>
<td>Animals appeared normal</td>
</tr>
</tbody>
</table>

aNo female mice showed significant toxicities except when using 115.2 mg/kg where two of two female mice died or were sacrificed.
bTwo mice of each sex dosed at 38.4 mg/kg with 5% DMSO survived and appeared healthy after being dosed. This was the maximum tolerated dose level and in order to have at least 5 mice/sex an additional 3 mice/sex were added (DMSO was 10%) and then mortalities were observed, thus a third group of 3 mice/sex was added (38.4 mg/kg at 5% DMSO).

cDue to solubility issues the amount of DMSO was increased from 5% to 10%.

Table 1. Step-up/step-down dosing results using thimerosal in CD1 male mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose level (mg/kg)</th>
<th>DMSO(%)a</th>
<th>Number of males</th>
<th>Number of females</th>
<th>Number of deaths/moribund</th>
</tr>
</thead>
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<tr>
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<td></td>
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<td></td>
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<tr>
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<td>2</td>
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</tr>
<tr>
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<tr>
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<td>38.4b</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>3</td>
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<tr>
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<td>2</td>
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<tr>
<td>7</td>
<td>76.8b</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>115.2b</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

aDue to decreased solubility, the concentration of DMSO was increased from 5% to 10%.
bThere was no toxicity due to DMSO alone and this was so even in female mice administered thimerosal in 10% DMSO except when given a dose of 115.2 mg/kg.

Table 2. Summary of thimerosal toxicity in male and female CD1 mice

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It is believed that autism has a genetic basis (Zhao et al., 2007), although the specific genes involved have not, as yet, been clearly delineated. Recently, it has been argued that prenatal and neonatal testosterone exposures may be risk factors for autism (Knickmeyer and Baron-Cohen, 2006). Furthermore, it has been reported that estrogens are protective against neurotoxic effects (Wang et al., 2006) and may protect against certain neurodegenerative diseases, such as Parkinson’s disease (Benedetti et al., 2001; Dean and McCarthy, 2007). Thus, our findings that thimerosal has increased toxicity in males compared to female mice suggest that estrogens produced in female mice may have some protective effect on the toxicity of thimerosal, while testosterone may have no effect or, perhaps, accelerate these toxic effects. This hypothesis requires future studies when using animal models to distinguish between male and female outcomes.

In summary, this is the first report of gender-selective toxicity of thimerosal. As autism occurs much more frequently in males than in females (MMWR, 2007), our findings may relate to a potential selectivity of thimerosal for toxic effects in some male children, particularly in utero. Thus, our results suggest that any future studies of thimerosal toxicity, as it may relate to childhood autism, need to take into account a potential for gender-selectivity of the effects of thimerosal.

References


MMWR, Morbidity and Mortality Weekly Report, Department of Health and Human Services, Centers for Disease Control and Prevention, 2007;56:No.SS-1.


