

Transcriptomic Analyses of Neurotoxic Effects in Mouse Brain After Intermittent Neonatal Administration of Thimerosal

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Thimerosal is a vaccine antimicrobial preservative which has long been suspected an iatrogenic factor possibly contributing to neurodevelopmental disorders including autism. The association between infant vaccine thimerosal exposure and autism remains an open question. Although thimerosal has been removed from mandatory childhood vaccines in the United States, thimerosal-preserved vaccines are still widely used outside of the United States especially in developing countries. Notably, thimerosal-containing vaccines are being given to the newborns within the first 12–24 h after birth in some countries. To examine the possible neurotoxic effects of early neonatal exposure to a higher level of thimerosal, FVB mice were subcutaneously injected with thimerosal-mercury at a dose which is 20× higher than that used for regular Chinese infant immunization during the first 4 months of life. Thimerosal-treated mice exhibited neural development delay, social interaction deficiency, and inclination of depression. Apparent neuropathological changes were also observed in adult mice neonatally treated with thimerosal. High-throughput RNA sequencing of autistic-behaved mice brains revealed the alternation of a number of canonical pathways involving neuronal development, neuronal synaptic function, and the dysregulation of endocrine system. Intriguingly, the elevation of anterior pituitary secreting hormones occurred exclusively in male but not in female thimerosal-treated mice, demonstrating for the first time the gender bias of thimerosal-mercury toxicity with regard to endocrine system. Our results indicate that higher dose of neonatal thimerosal-mercury (20× higher than that used in human) is capable of inducing long-lasting substantial dysregulation of neurodevelopment, synaptic function, and endocrine system, which could be the causal involvements of autistic-like behavior in mice.

Key words: thimerosal; transcriptomic analyses; anterior pituitary; hormone; neurotoxicity; autistic disorder.

Thimerosal (sodium ethylmercury thiosalicylate, 49.6% mercury (Hg) by weight) has been used as an antimicrobial preservative in many vaccines and medicinal preparations since 1930s (Pless and Risher, 2000). It rapidly metabolizes to ethylmercury and subsequently to inorganic mercury forms which accumulate in different organs/tissues including the brain for months or years (Qvarnstrom *et al.*, 2003). The neurotoxicity of ethylmercury has been well known (Zhang, 1984). Because the blood-brain barrier of newborns is not well-developed, and the developing brain is uniquely vulnerable to neurotoxic hazard exposure, thimerosal-mercurials are suspected pathogenic factors in the etiology of several neurodevelopmental disorders, including autism (Bernard *et al.*, 2001; Geier and Geier, 2003, 2005, 2006b; Hewitson *et al.*, 2010; Majewska *et al.*, 2010; Young *et al.*, 2008). However, the association between thimerosal exposure via childhood vaccinations and neurodevelopmental disorders such as autism remains an open question (Blaxill *et al.*, 2004; Kern *et al.*, 2012; Nelson and Bauman, 2003). Several independent epidemiological investigations support a hypothesis linking this disorder with postnatal exposure to mercurials (Gallagher and Goodman, 2010; Geier and Geier, 2003, 2004, 2006a,b; Mutter *et al.*, 2005; Young *et al.*, 2008), whereas the others do not support such a relationship (Heron and Golding, 2004; Hviid *et al.*, 2003; Immunization Safety Review Committee, 2004; Madsen *et al.*, 2003; Stehr-Green *et al.*, 2003; Thompson *et al.*, 2007; Verstraeten *et al.*, 2003). Nevertheless, due to concern of increased mercury exposure and elevated body burdens in children (Ball *et al.*, 2001), thimerosal has been removed from mandatory childhood vaccines in the United States (American Academy of Pediatrics and United States Public Health Service, 1999).

Thimerosal-preserved vaccines are still widely used outside of the United States especially in developing countries such as Brazil and China, where the advantages of multiuse vials of thimerosal-preserved vaccines take precedence over perceived mercury hazards (Dorea, 2007; WHO IRIS, 2002). According to 2001 United States vaccination schedule, each 1-year-old U.S. child could have been exposed to a total of 237.5 µg Hg from vaccines distributed at 2, 4, 6, and 12 months