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Protective role of alpha-lipoic acid in impairments of social and stereotyped behaviors induced by early postnatal administration of thimerosal in male rat

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ABSTRACT

Aim

Thimerosal, a mercury-containing preservative has been widely used in a number of biological and drug products, including many vaccines, and has been studied as a possible etiological factor for some neurodevelopmental disabilities. Here, the protective effects of Alpha Lipoic Acid (ALA), an organosulfur compound derived from Octanoic Acid, on Thimerosal-induced behavioral abnormalities in rat were examined.

Methods: 108 male Wistar rats were divided into three cohorts and treated as follows: 1) Thimerosal at different doses (30, 300, or 3000 μg Hg/kg) in four i.m. injections on 7, 9, 11, 15 postnatal days. 2) ALA (at doses of 5, 10 and 20 mg/kg), following the same order; 3) single dose of Thimerosal (3000 μg Hg/kg) plus ALA at different doses (5, 10 or 20 mg/kg), by the previously described method. A saline treated control group and a ALA vehicle control (0.1% NaOH) were also included. At 5 and 8 weeks after birth, rats were evaluated with behavioral tests, to assess locomotor activity, social interactions and stereotyped behaviors, respectively.

Results: The data showed that Thimerosal at all doses (30, 300 and 3000 μg Hg/kg) significantly impacted locomotor activity. Thimerosal at doses of 300 and 3000 but not 30 μg Hg/kg impaired social and stereotyped behaviors. In contrast, ALA (at doses of 5, 10 and 20 mg/kg) did not alter behaviors by itself, at doses of 20 mg/kg, it reduced social interaction deficits induced by the highest dose of Thimerosal (3000 μg Hg/kg). Moreover, ALA, at all doses prevented the adverse effects of Thimerosal on stereotyped behaviors.

Conclusions: the results of this preclinical study, consistent with previous studies on mice and rats, reveals that neonatal dose-dependent exposure to Thimerosal mimicking the childhood vaccine schedule can induce abnormal social interactions and stereotyped behaviors similar to those observed in neurodevelopmental disorders such as autism, and, for the first time, revealed that these abnormalities may be ameliorated by ALA. This indicates that ALA may protect against mercurial-induced abnormal behaviors.

1. Introduction

Thimerosal (THIM, sodium ethyl-mercurithiosalicylate; 49.55% mercury by weight), found in vaccines and some other pharmaceutical products, is a significant source of mercury exposure for many infants (Geier et al., 2015a). THIM, rapidly disassociates in saline solutions into ethyl-mercury chloride and ethyl-mercury hydroxide, and is further metabolized in the body into inorganic mercury compounds, which may be stored in the brain and other vital structures for many years following exposure (Barregard et al., 2011; Dorea et al., 2012; Geier et al., 2015b).

Mercury is a neurotoxicant that has destructive effects on brain structures, depletes glutathione (GSH) and other antioxidants, reduces antioxidant defenses, damages the mitochondria and makes extensive changes in three-dimensional structures of proteins, which sometimes results in autoimmunity (Kidd, 2002).

Indeed, due to the high sensitivity of the nervous system to heavy metals neurotoxicity, a wide range of reactions occur in the brain after exposure to mercury.

The subject of THIM as a possible cause of autism spectrum disorder

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(ASD) and neurodevelopmental disorders (NDDs) has been a debatable issue since 1999 (Adamson et al., 2011; Gallagher and Goodman, 2008; Young et al., 2008).

Autism or autism spectrum disorder (ASD) is a complex neurodevelopmental disorder, which is characterized by impairments in social interactions and verbal and nonverbal communication, and stereotyped, repetitive behaviors and interests (Association). Recently, some studies have shown that increasing heavy metal exposure/body burden significantly correlates with increased symptoms severity in persons diagnosed with ASD (Adams et al., 2009; Elsheshtawy et al., 2011; Geier and Geier, 2007; Geier et al., 2009; Nataf et al., 2008; Priya and Geetha, 2011). Although numerous studies have been conducted on the relationship between ASD and heavy metals such as cadmium, lead and arsenic (Priya and Geetha, 2011), a number of studies have focused on the role that mercury may play in the etiology of this prevalent developmental disability (Kern et al., 2012).

According to many studies, mammalians are more sensitive to the neurotoxic effects of THIM (Ball et al., 2001; Barile et al., 2011; Dórea et al., 2011). In a study by Hornig et al. (2004), several neurological deficits associated with ASD diagnosis were observed in an autoimmune disease-sensitive mouse strain treated during the neonatal period with THIM doses comparable to those used in infant vaccines (Hornig et al., 2004). Similarly, Li et al. (2014) observed that neonatal THIM administration was capable of inducing long-lasting substantial dysregulation of neurodevelopment, synaptic function, and endocrine system, which could be causally related to autistic-like behaviors in mice (Li et al., 2014). Hewitson et al. reported that infant monkeys injected with a single neonatal dose of THIM-containing hepatitis B vaccine comparable to that received by infants revealed delay in the attainment of some survival, motor, and sensorimotor reflexes. They recommended that this model provides a suitable way for assessing adverse neurodevelopmental results from neonatal THIM-containing hepatitis B vaccine exposure, mainly in infants of lower gestational age or birth weight. (Hewitson et al., 2010).

Olczak et al. showed that early postnatal THIM administration comparable to those used in infant vaccines caused lasting neurobehavioral impairments and neuro chemical variations in the brain, based on dose and sex. They concluded that if similar changes occur in THIM/mercurial-exposed children, they could contribute to neurodevelopmental disorders like ASD (Olczak et al., 2011). However, there are many pieces of evidence that do not support a relationship between THIM and childhood neurodevelopment disorders (Andrews et al., 2004; Madsen et al., 2003; Price et al., 2010; Schultz, 2010; Thompson et al., 2007). Pichichero et al. in a study on infants aged 6 months and younger, concluded that THIM in vaccines causes very little risk to full-term infants, but these vaccines should not be applied at birth to very low birth weight premature infants (Pichichero et al., 2002). Also, Gadad et al. showed that administration of THIM-containing vaccines to infant rhesus macaques did not result in autism-like behavior or neuropathology. These data indicate that exposure to low-dose of THIM via vaccination did not significantly impact behavior (Gadad et al., 2015). Similar data were obtained in Curtis et al. study (Curtis et al., 2015). So far, this problem requires further exploration.

According to these results, utilizing a substance that can affect the action mechanism of mercury in the brain may be effective in reducing THIM-induced behavioral abnormalities. Alpha Lipoic acid (ALA; 8, 6-dithio-octanoic acid) is a natural material that in small volumes, is synthesized by some plants and animals and even human. This strong antioxidant is generally utilized for the treatment of numerous neurological disorders such as diabetic polyneuropathy (Grbovic et al., 2016; Kim and Choi, 2010; Packer et al., 1995), multiple sclerosis (Durastanti et al., 2016; Hume, 2016; Wu et al., 2016) and stroke (Wu et al., 2016). ALA is known to impact several cellular processes including direct radical scavenging, metal chelation, regeneration of endogenous antioxidants and modulation of transcription factor activities. Studies showed that ALA improves endothelial function and blood flow, and accelerates the synthesis of glutathione (GSH), which plays an essential role in regulating the expression of several antioxidant and anti-inflammatory genes (Biewenga et al., 1997; Gorąca et al., 2011; Guo et al., 2008; Moini et al., 2002). In addition, ALA can reduce the production of ROS and nitric oxide (NO) (Kleinkauf-Rocha et al., 2013). These findings suggest that low doses of ALA can have considerable therapeutic potential in neurological diseases which are related to oxidative stress.

In order to evaluate the possible role of THIM in neurodevelopmental impairments, its neurotoxic effects in a series of behavioral studies in rat model system were examined. Further, the potential protective effect of ALA treatment on the neurotoxicity of THIM administration was tested. The hypothesis tested in this study was that THIM administration mimicking the childhood vaccine schedule would in a dose-dependent fashion induce autistic-like behaviors in a rat model system. Further, it was hypothesized that ALA treatment in a dose-dependent fashion would help to significantly ameliorate THIM induced abnormal behaviors in a rat model system.

2. Materials and methods

2.1. Animals

A total of 108 male Wistar rats were obtained from pregnant rats purchased from Pasteur Institute of Iran (the Production and Research Complex, Tehran, Iran.) Then, they were kept in cages with dimensions of$20 \times 20 \times 40$ in a room under standard conditions ($22 \pm 1\,^{\circ}C$, relative humidity of 60%, 12 h–12 h light-dark cycle (lights on at 7: 00 A.M). These animals were not provided with environmental enrichment. Standard laboratory chow (Javaneh Khorasan Co.Toos Industrial City, Mashhad, Iran) and Tap water were presented ad libitum. All experiments were conducted according to the Ethics Committee of Shahid Beheshti University of Medical Sciences (code no: IR. SBMU. MSP.REC.1395.167). The trial groups for different examinations comprised of collective rats from various litters. Tests were performed in the process of light cycle.

2.2. Drugs

Thimerosal and Alpha Lipoic Acid, respectively, were purchased from Merck (The Merck group, Germany) and Acros (Acros organic, Thermo Fisher Scientific, United States) companies. THIM, at different doses dissolved in Saline, was injected into experimental groups on postnatal days 7, 9, 11 and 15 in three doses (30, 300, and 3000 μg Hg/kg) (per kg) in a volume of 50 μl, i.m. into the glutei maximi. This schedule was firstly presented by Hornig et al. to imitate the childhood vaccine schedule (Carneiro et al., 2014; Hornig et al., 2004). By considering the controversial effect of low dosage THIM on animals (Carneiro et al., 2014; Hornig et al., 2004) and lesser toxic sensitivity of rat compared to human, the doses of THIM were chosen at least 2 fold higher than human infant vaccine dose to mimic the THIM exposure from childhood vaccine schedule used in some societies.

ALA's vehicle was 0.1% NaOH. As regards ALA, at low doses are used as a treatment in neurological disorders (Shay et al., 2009), and in order to find effective and subthreshold dosage, ALA was administered at 5, 10 and 20 mg/kg per injection.

2.3. Experimental design

The newborn animals were randomly distributed into three main cohorts and each cohort had four or five subgroups (eight rats each) and treated as follows:

2.3.1. Cohort 1: THIM

There were four sub-groups in THIM cohort, including: