This review article highlights the scientifically affirmed connection between infant and prenatal thimerosal exposure and neurological disorders, including tic disorder, which has been shown to be much more prevalent in children with autism. The author also delineates the use of thimerosal in vaccines in developing countries at a greater exposure level than developed countries such as the U.S.
The author of this review article contrasts the medical outcomes of children who receive thimerosal-containing vaccines versus thimerosal-free vaccines. Neurological disorders have been shown to be associated with exposure to thimerosal in vaccines. The author calls for the complete removal of thimerosal in vaccines especially in developing countries where they are used most prevalently.
Thimerosal inhibited cellular production of cobalamin necessary for detoxification and amelioration of oxidative stress. This caused lower methionine synthase activity and an impaired methylation capacity. Autistic subjects in general show cobalamin deficiencies and several impaired methylation.
Results of this case-control study showed that children with ASD had higher urinary levels of mercury and lead as well as porphyrins that are characteristic of mercury toxicity as compared to non-ASD control children. Porphyrins are complex molecules that are processed in the body through a series of chemical reactions. Mercury poisons the enzymes that are needed in the process, causing a build-up in the body of excess levels of specific porphyrins. The porphyrins for mercury toxicity also correlated with autism severity in ASD patients.
The levels of blood mercury and inflammatory-related neuropeptides in the serum are correlated in children with autism spectrum disorder

Gehan Ahmed Mostafa, Geir Bjorklund, Mauricio A. Urbina, Laila Yousef Al-Ayadhi

Abstract Tachykinins (substance P, neurokinin A, and neurokinin B) are pro-inflammatory neuropeptides that may play an important role in some autoimmune neuroinflammatory diseases, including autism spectrum disorder (ASD). Mercury (Hg) is a neurotoxicant, and potentially one of the main environmental triggers for ASD as it induces neuroinflammation with a subsequent release of neuropeptides. This is the first study to explore the potentially causal relationship between levels of serum neurokinin A and blood mercury (BHg) in children with ASD. Levels of serum neurokinin A and BHg were measured in 84 children with ASD, aged between 3 and 10 years, and 84 healthy-matched children. There was a positive linear relationship between the Childhood Autism Rating Scale (CARS) and both serum neurokinin A and BHg. ASD children had significantly higher levels of serum neurokinin A than healthy controls ($P < 0.001$). Increased levels of serum neurokinin A and BHg were respectively found in $54.8\%$ and $42.9\%$ of the two groups. There was significant and positive linear relationship between levels of serum neurokinin A and BHg in children with moderate and severe ASD, but not in healthy control children. It was found that $78.3\%$ of the ASD patients with increased serum levels of neurokinin A had elevated BHg levels ($P < 0.001$). Neuroinflammation, with increased levels of neurokinin A, is seen in some children with ASD, and may be caused by elevated BHg levels. Further research is recommended to determine the pathogenic role of increased levels of serum neurokinin A and BHg in ASD. The therapeutic role of tachykinin receptor antagonists, a potential new class of anti-inflammatory medications, and Hg chelators, should also be studied in ASD.

Keywords Autism - Neurokinin A - Neuroinflammation - Mercury

Introduction

Neurogenic inflammation is a neurally mediated immune inflammation that is orchestrated by a large number of neuro-
Neurodevelopment of Amazonian children exposed to ethylmercury (from Thimerosal in vaccines) and methylmercury (from fish)

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ABSTRACT

Few studies have addressed co-occurring methylmercury (MeHg) from maternal origin and ethylmercury (EtHg) from Thimerosal-containing vaccines (TCVs) during infant’s neurodevelopment. We studied children (n=1130) from the Western Amazon based on combined (low, intermediate, and high) exposure to chronic MeHg from fish consumption and acute TCV-EtHg. Neurodevelopmental outcomes were age of walking and age of talking, and the Bayley Scale of Infant Development (BSID). The Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) were measured at six and 24 months of age. Median hair-Hg (HgH) at birth was 64.4 μg g⁻¹ in mothers, and 194 μg g⁻¹ in newborns; total (pregnancy and infancy) EtHg exposure ranged from 0 to 187.5 μg. The combined (MeHg+EtHg) exposure showed significant differences for MDI but not for PDI; however, there was a significant decrease in both MDI and PDI scores at 24 months. The increase in BSID delays (scores – 80) between six and 24 months was not discernible with regards to EtHg or MeHg exposure. We found a statistically significant increase in neurodevelopmental (BSID) delays related to the combined exposure to Hg (MeHg > EtHg). Neurodevelopmental delays due to low-doses of organic mercury (albeit undiscernible) are not predictable but can be avoided by choosing low-Hg fish and providing Thimerosal-free vaccines.

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1. Introduction

During developmental periods extending from prenatal stages, the brain is more vulnerable to the adverse effects of toxic insults than at more mature stages; however, experience-guided development drives neuro-cognition achievements. Early brain sustenance food contaminated with organic Hg (both MeHg and EtHg) showed impairment derived from neurological examination scores and milestone (age of first walking and first talking) delays (Marsh et al., 1987). Despite strong evidence of potential effects of low-doses of Thimerosal/EtHg (Geier et al., 2015), studies addressing only TCV-EtHg exposures and association with neurodevelopmental delays due to low-doses of organic mercury (albeit undiscernible) are not predictable but can be avoided by choosing low-Hg fish and providing Thimerosal-free vaccines.
Toxicity of organic and inorganic mercury species in differentiated human neurons and human astrocytes

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\section*{ABSTRACT}
Organic mercury (Hg) species exert their toxicity primarily in the central nervous system. The food relevant Hg species methylmercury (MeHg) has been frequently studied regarding its neurotoxic effects in vitro and in vivo. Neurotoxicity of thimerosal, which is used as a preservative in medical preparations, is to date less characterised. Due to dealkylation of organic Hg or oxidation of elemental Hg, inorganic Hg is present in the brain albeit these species are not able to readily cross the blood brain barrier. This study compared for the first time toxic effects of organic MeHg chloride (MeHgCl) and thimerosal as well as inorganic mercury chloride (HgCl\textsubscript{2}) in differentiated human neurons (LUHMES) and human astrocytes (CCF-SITG1). The three Hg species differ in their degree and mechanism of toxicity in those two types of brain cells. Generally, neurons are more susceptible to Hg species induced cytotoxicity as compared to astrocytes. This might be due to the massive cellular mercury uptake in the differentiated neurons. The organic compounds exerted stronger cytotoxic effects as compared to inorganic HgCl\textsubscript{2}. In contrast to HgCl\textsubscript{2} exposure, organic Hg compounds seem to induce the apoptotic cascade in neurons following low-level exposure. No indicators for apoptosis were identified for both inorganic and organic mercury species in astrocytes. Our studies clearly demonstrate species-specific toxic mechanisms. A mixed exposure towards all Hg species in the brain can be assumed. Thus, prospectively coexposure studies as well as cocultures of neurons and astrocytes could provide additional information in the investigation of Hg induced neurotoxicity.

\section*{1. Introduction}

Organic mercury (Hg) compounds are important neurotoxicants capable of damaging the developing and adult nervous system [1]. Due to its accumulation in the aquatic food chain, chronic exposure to methylmercury (MeHg) via seafood intake still poses a risk to human health [2]. Ethylmercury (EtHg) containing thimerosal, used as a preservative in medical preparations including vaccines, is of particular concern since it has been linked to autism [3]. Although organic Hg compounds, especially methylmercury (MeHg), have been extensively studied, the mechanisms of Hg species mediated neurotoxicity remain not completely understood [4]. Inorganic Hg\textsuperscript{2+} does not readily cross the blood brain barrier. Probably therefore effects of inorganic Hg\textsuperscript{2+} species on brain cells are not well characterized [5]. Nevertheless, it should be noted that inorganic Hg is present in the brain due to dealkylation of organic species or an oxidation of elemental Hg, which originates e.g., from the
Toxicological effects of thiomersal and ethylmercury: Inhibition of the thioredoxin system and NADP+-dependent dehydrogenases of the pentose phosphate pathway

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ABSTRACT

Mercury (Hg) is a strong toxicant affecting mainly the central nervous, renal, cardiovascular and immune systems. Thiomersal (TM) is still in use in medical practice as a topical antiseptic and as a preservative in multiple dose vaccines, routinely given to young children in some developing countries, while other forms of mercury such as methylmercury represent an environmental and food hazard. The aim of the present study was to determine the effects of thiomersal (TM) and its breakdown product ethylmercury (EtHg) on the thioredoxin system and NADP+-dependent dehydrogenases of the pentose phosphate pathway. Results show that TM and EtHg inhibited the thioredoxin system enzymes in purified suspensions, being EtHg comparable to methylmercury (MeHg). Also, treatment of neuroblastoma and liver cells with TM or EtHg decreased cell viability (Glo4, 1.5 to 20 μM) and caused a significant (p < 0.05) decrease in the overall activities of thioredoxin (Trx) and thioredoxin reductase (TrxR) in a concentration- and time-dependent manner in cell lysates. Compared to control, the activities of Trx and TrxR in neuroblastoma cells after EtHg incubation were reduced up to 60% and 80% respectively, whereas in hepatoma cells the reduction was almost 100%. In addition, the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were also significantly inhibited by all mercurials, with inhibition intensity of Hg2+ > MeHg ≈ EtHg > TM (p < 0.05). Cell incubation with sodium selenite alleviated the inhibitory effects on TrxR and glucose-6-phosphate dehydrogenase. Thus, the molecular mechanism of toxicity of TM and especially of its metabolite EtHg encompasses the blockade of the electrons from NADPH via the thioredoxin system.

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Introduction

Mercurial compounds have shown a wide range of toxicological effects on human beings, involving especially the central nervous system, causing damage to the brain, but also to the kidneys, the cardiovascular and immune systems (Clarkson et al., 2003; Dórea et al., 2013). Exposure to mercurial compounds such as methylmercury (MeHg) and mercuric mercury (Hg2+) at levels above the toxicity threshold occurs either by regular fish consumption or occupational contact, respectively, and represents a major concern in toxicology (Clarkson et al., 2003; Carvalho et al., 2008a; Nunes et al., 2014). Not less important is mercury exposure in dental practice for both dentists and patients due to the use of dental amalgam fillings that release mercury vapour (Clarkson et al., 2003). Even though the use of mercury compounds such as thiomersal (TM) in medicines and antiseptics is decreasing it is still used as a preservative in some formulas, namely in vaccines (Sykes et al., 2014).

Although mercurial compounds are not new toxicants, there is a significant lack of knowledge about their molecular mechanisms of toxicity especially about TM and its breakdown product.

Thimerosal exposure led to the death of neuroblastoma and liver cells due to inhibition of thioredoxin-based cellular metabolism. This is similar to neuronal damage associated with autistic disorder.
In a comparison of lymphoblast cells from children with autism and matched non-autistic controls, a significantly higher number of “autistic” lymphoblast cell lines showed a reduction in ATP-linked respiration, maximal respiratory capacity and reserve capacity indicative of mitochondrial injury when exposed to thimerosal as compared to control cell lines. This supports the notion that a subset of individuals with autism (approximately 30%) may be vulnerable to mitochondrial dysfunction from thimerosal exposure.
This review article includes a section on numerous papers linking thimerosal exposure via infant vaccines to autism. The publication also includes a critique of studies supported or conducted by the U.S. Centers for Disease Control (CDC) that deny any associations between exposure to thimerosal in vaccines and the subsequent development of autism. The CDC has been criticized by Congress for inherent conflicts of interest related to its vaccine development activities and role in vaccine safety oversight.
In this paper, autistic case children show significantly higher levels of hair mercury as compared to non-autistic control children. In general, autistic children accumulated metals at a much higher level that control children who did not have a diagnosis of autism.
In this case-control study of pervasive development disorder (PDD) in U.S. children, cases were consistently exposed to higher levels of thimerosal via infant vaccines at both 6 months of age and 15 months of age, based specifically on Haemophilus influenzae type b vaccines. Differences between exposures in cases and controls were statistically significant at both ages evaluated.
Transcriptomic Analyses of Neurotoxic Effects in Mouse Brain After Intermittent Neonatal Administration of Thimerosal

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Results of mouse studies showed that thimerosal levels just 20 times of that used in the Chinese infant vaccine schedule is capable of inducing long lasting substantial dysregulation of neural development. The study authors posited that thimerosal could have “causal involvements of autistic-like behavior in mice.”

Thimerosal is a vaccine antimicrobial preservative which has long been suspected an iatrogenic factor possibly contributing to neurodevelopment 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 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 analysis; anterior pituitary; autism.
Blood levels of mercury and lead were found to be much higher in autistic children as compared to normal controls. Upon chelation, the blood levels of these heavy metals decreased and autistic symptoms improved, which strongly supports a causal relationship between metals and the physical manifestations associated with a diagnosis of autism.
This review article shows methodological flaws in six separate CDC studies claiming that thimerosal does not cause autism. In three specific instances (Madsen et al. 2003, Verstraeten et al. 2003 and Price et al. 2010) evidence of malfeasance on the part of CDC scientists is shown. Background data (not reported in print) from these three publications suggest a strong link between thimerosal exposure and autism.
This study involves a comparison of autism reports derived from the Vaccine Adverse Event Reporting System (VAERS), a federally supported surveillance program which compares data obtained from thimerosal-containing versus thimerosal free DTaP formulations. The investigators reported a relative risk of 7.67 (667% increase) for autism when children were exposed to thimerosal via the DTaP vaccine to those who received the thimerosal free formulation.
The protein zinc-metalloprotease-BDNF is upregulated in the presence of organic mercurials, including thimerosal. This protein is implicated in the development of large brains (megencephaly) and cortical hyperconnectivity in the brains of children with autism.
Suppression by Thimerosal of Ex-Vivo CD4⁺ T Cell Response to Influenza Vaccine and Induction of Apoptosis in Primary Memory T Cells

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Abstract
Thimerosal is a preservative used widely in vaccine formulations to prevent bacterial and fungal contamination in multidose vials of vaccine. Thimerosal was included in the multidose non-adjuvanted pandemic 2009 H1N1 vaccine Panenza. In the context of the analysis of the ex-vivo T cell responses directed against influenza vaccine, we discovered the in vitro toxicity of Panenza, due to its content in thimerosal. Because thimerosal may skew the immune response to vaccines, we investigated in detail the ex-vivo effects of thimerosal on the fate and functions of T cells in response to TCR ligation. We report that ex-vivo exposure of quiescent or TCR-activated primary human T cells to thimerosal induced a dose-dependent apoptotic cell death associated with depolarization of mitochondrial membrane, generation of reactive oxygen species, cytochrome c release from the mitochondria and caspase-3 activation. Moreover, exposure to non-toxic concentrations of thimerosal induced cell cycle arrest in G0/G1 phase of TCR-activated T cells, and inhibition of the release of proinflammatory cytokines such as IFN gamma, IL-1 beta, TNF alpha, IL-2, as well as the chemokine MCP1. No shift towards Th2 or Th17 cells was detected. Overall these results underline the proapoptotic effect of thimerosal on primary human lymphocytes at concentrations 100 times less to those contained in the multidose vaccine, and they reveal the inhibitory effect of this preservative on T-cell proliferation and functions at nanomolar concentrations.

Thimerosal at concentration below those in the H1N1 influenza vaccine caused human T-cell death via mitochondrial depolarization. This type of cell death has been previously seen primarily in the peripheral T cells of autistic subjects.
Thimerosal compromises human dendritic cell maturation, IL-12 production, chemokine release, and T-helper polarization

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Keywords: thimerosal, dendritic cells, Th polarization, cytokines, chemokines

Introduction

Adaptive immunity plays a crucial role in natural host defense against pathogens and tumors, and it is central to the long-term protective effect of vaccines. The innate immune system functions to direct the adaptive immune response, both through antigen presentation by dendritic cells and by providing the key signals for the differentiation of naive CD4+ T cells into functionally distinct T helper cell (Th) subtypes.1,2 DCs act as a sentinel population that constantly samples the tissue microenvironment and takes up microbial cells through toll-like receptors (TLRs).3 TLRs can detect multiple pathogen-associated molecular patterns (PAMPs),4 including LPS detected by TLR4, resulting in the activation of NF-κB that drives the production of many proinflammatory cytokines, including IL-1, IL-6, TNF-α, and IL-12.5 TLR-induced IL-12 is the key differentiation factor for Th1 cells.6

Thimerosal is a preservative used in multidose vials of vaccine formulations to prevent bacterial and fungal contamination.9,10 Thimerosal is an ethylmercury-containing pharmaceutical compound that contains 49.6% mercury by weight and metabolizes into ethylmercury (etHg) and thiosalicylate.11 Thimerosal is known as a contact allergen, and caution has been urged regarding significant side effects in therapeutic agents12 and in vaccines9 with specific issues related to infant-CNS.14,15 Thimerosal has been shown to cause a number of toxic changes in vitro, including neuronal mitochondrial cell death,16,17,28 oxidative stress and apoptosis of HeLa S epithelial cells,19 and S phase arrest and apoptosis via inhibition of the PI3K/Akt/survivin pathway on the murine C2C12 myoblast cells.20 Because thimerosal is one of the best-known skin sensitizers, several studies have been performed on human myeloid dendritic cells, which play an essential role in the initiation of allergic contact dermatitis. DC activation and associated immune functions are

Thimerosal at concentrations comparable to those used in the H1N1 influenza vaccine interfered with human T cell function and inhibited human dendritic cell maturation required for a robust immune response. Immune suppression such as that seen in this in vitro study is a characteristic of autistic disorder.
The risk of developmental disorders including PDD was assessed on a per microgram mercury (from thimerosal) basis for exposures during the first six months of life. Both PDD and tic disorder (which is seen much more frequently in children with autism) were significantly correlated to mercury exposure within this time period.

Abstract: A hypothesis testing case-control study evaluated concerns about the toxic effects of organic-mercury (Hg) exposure from thimerosal-containing (49.55% Hg by weight) vaccines on the risk of neurodevelopmental disorders (NDs). Automated medical records were examined to identify cases and controls enrolled from their date-of-birth (1991–2000) in the Vaccine Safety Datalink (VSD) project. ND cases were diagnosed with pervasive developmental disorder (PDD), specific developmental delay, tic disorder or hyperkinetic syndrome of childhood. In addition, putative non-thimerosal-related outcomes of febrile seizure, failure to thrive and cerebral degenerations were examined. The cumulative total dose of Hg exposure from thimerosal-containing hepatitis B vaccine (T-HBV) administered within the first six months of life was calculated. On a per microgram of organic-Hg basis, PDD (odds ratio (OR) = 1.054), specific developmental delay (OR = 1.035), tic disorder (OR = 1.034) and hyperkinetic syndrome of childhood (OR = 1.05) cases were significantly more likely than controls to receive increased organic-Hg exposure. By
A two-phase study evaluating the relationship between Thimerosal-containing vaccine administration and the risk for an autism spectrum disorder diagnosis in the United States

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Abstract

Background: Autism spectrum disorder (ASD) is defined by standardized criteria of qualitative impairments in social interaction, qualitative impairments in communication, and restricted and stereotyped patterns of behavior, interests, and activities. A significant number of children diagnosed with ASD suffer a loss of previously-acquired skills, which is suggestive of neurodegeneration or a type of progressive encephalopathy with an etiological pathogenic basis occurring after birth. To date, the etiology of ASD remains under debate, however, many studies suggest toxicity, especially from mercury (Hg), in individuals diagnosed with an ASD. The present study evaluated concerns about the toxic effects of organic-Hg exposure from Thimerosal (49.55% Hg by weight) in childhood vaccines by conducting a two-phased (hypothesis generating/hypothesis testing) study with documented exposure to varying levels of Thimerosal from vaccinations.

Methods: A hypothesis generating cohort study was undertaken to evaluate the relationship between exposure to organic-Hg from a Thimerosal-containing Diphtheria-Tetanus-acellular-Pertussis (DTaP) vaccine in comparison to a Thimerosal-free DTaP vaccine administered, from 1998 through 2000, for the risk of ASD as reported in the Vaccine Adverse Event Reporting System (VAERS) database (phase I). A hypothesis testing case-control study was undertaken to evaluate the relationship between organic-Hg exposure from Thimerosal-containing hepatitis B vaccines administered at specific intervals in the first six months of life among cases diagnosed with an ASD and controls born between 1991 through 1999 in the Vaccine Safety Datalink (VSD) database (phase II).

Results: In phase I, it was observed that there was a significantly increased risk ratio for the incidence of ASD reported following the Thimerosal-containing DTaP vaccine in comparison to the Thimerosal-free DTaP vaccine. In phase II, it was observed that cases diagnosed with an ASD were significantly more likely than controls to receive increased organic-Hg from Thimerosal-containing hepatitis B vaccine administered within the first, second, and sixth month of life. In phase one of this study, the investigators using Vaccine Adverse Event Reporting System (VAERS) data reported a significantly elevated risk of developing autism after a child received a DTaP vaccine with thimerosal in comparison to children who received a thimerosal-free DTaP vaccine. In the second phase of the study, the investigators using the CDC’s Vaccine Safety Datalink (VSD) program found that children diagnosed with autism were much more likely to have received thimerosal containing hepatitis B vaccine during the first, second and sixth month of life than control children. The VSD database includes hundreds of thousands of children’s electronic health data and is used for vaccine safety monitoring.
Effect of thimerosal on the neurodevelopment of premature rats

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**Background:** This study was undertaken to determine the effect of thimerosal on the neurodevelopment of premature rats.

**Methods:** Thimerosal was injected into premature SD rats at a dose of 32.8, 65.6, 98.4 or 131.2 µg/kg on postnatal day 1. Expression of dopamine D4 receptor (DRD4) and serotonin 2A receptor (5-HT2AR), apoptosis in the prefrontal cortex on post-injection day 49, and learning and memory function were studied and compared with those in a control group injected with saline.

**Results:** Expression of DRD4 and 5-HT2AR and learning function decreased, and apoptosis increased significantly in the 131.2 µg/kg group (P<0.001). Memory function was significantly impaired by 65.6 (P=0.05), 98.4 and 131.2 µg/kg (P<0.001).

**Conclusions:** The negative adverse consequences on neurodevelopment observed in the present study are consistent with previous studies; this study raised serious concerns about adverse neurodevelopmental disorder such as autism in humans following the ongoing worldwide routine administration of thimerosal-containing vaccines to infants.


**Key words:** dopamine D4 receptor; neurodevelopment; serotonin 2A receptor; thimerosal

**Introduction**

Neurological alterations that may result from thimerosal exposure have recently become a hot topic. Thimerosal exposure via vaccination is thought to cause brain disorder. Since there is no appropriate agent to replace, thimerosal is used as a preservative in vaccines. Therefore, it is necessary to determine the appropriate levels of thimerosal for neurodevelopment. Studies have been focused on neurological alterations after exposure to thimerosal in rats, but further study is required to demonstrate the acceptable levels of exposure for neurodevelopment.

Rat model is considered feasible for research in intoxication following metal exposure. Learning and memory are important brain functions. And the prefrontal cortex is a critical region receiving stimulation for the development of learning and memory function, which is mainly executed by neurotransmitters. The variants of dopamine D4 receptor (DRD4) are reported to be associated with memory function of rats, whereas serotonin 2A receptor (5-HT2AR) is correlated with impaired episodic memory performance. It was reported that in the human neuroblastoma cell line, thimerosal induced mitochondria-mediated apoptosis.

In the present study, we investigated whether thimerosal could induce alterations in expression of DRD4 and 5-HT2AR, apoptosis of the prefrontal cortex, and learning and memory functions in the premature rats.

Thimerosal given to premature rat pups resulted in neuronal cell death and delays in development as compared to unexposed premature rats. Doses were comparable to that seen in infants receiving the thimerosal containing birth HepB vaccine. This affirms the neurotoxicological effects of mercury in conjunction with the developmental delays and impaired learning in autism.
This CDC authored publication shows that ASD prevalence rates in Denmark decreased by 30% from 1994 to 2004 after Denmark removed Thimerosal from their vaccines in 1992. This result is directly counter to an earlier study in 2003, the notorious and thoroughly discredited Madsen study that CDC has nevertheless touted as dispositive of the thimerosal/autism link.
This paper found peripheral blood lymphocytes from a subset of autistic children and their unaffected siblings, exhibited greater sensitivity and higher rates of cell death when exposed to thimerosal than those of unaffected, unrelated control children. The exposure levels to thimerosal in this study were the same levels that infants received during vaccinations. These findings are consistent with the only other thimerosal/B-cell study by Rose et al. 2015 that found mitochondria were thimerosal targets and that antioxidant defenses, especially those linked to glutathione, were compromised in ASD cells and were further injured by exposure to thimerosal. This research supports the notion that there is a genetic component to thimerosal hypersensitivity and this hypersensitivity is found in approximately a third of children with ASD.
Studies on H1N1 vaccine-induced monoamines alternations and oxidative stress on brain of adult mice

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ABSTRACT

Over the past decade illness outbreaks have posed a serious threat to human life and well-being. The 2009 outbreak H1N1/A influenza virus also was expected to disproportionately affect healthy young persons under the age of 25 years. A small amount of the preservative thimerosal is routinely added to many vaccine preparations, including H1N1 vaccine. Thimerosal is an organic mercurial containing an ethylmercury moiety attached to the sulfur atom of thiosalicylate. Since the 1930s, thimerosal has been used as an antiseptic and a preservative in a wide variety of products, to investigate the monoamines alternation and oxidative stress induced after H1N1 vaccine injection, adult male Swiss mice were injected with thimerosal, adjuvant, H1N1 antigen and H1N1 vaccine. Results obtained on the present study showed that thimerosal, H1N1 antigen and H1N1 vaccine were caused significant decrease in noradrenaline (NE) and dopamine (DA) contents of hypothalamus, striatum and cerebral cortex. The alternation in NE and DA was associated with significant increase in oxidative markers namely lipid peroxidation and nitric oxide, oxidation induction was extent to cause significant decrease in glutathione level. In conclusion, the present study demonstrated that H1N1 vaccine as a whole and/or its ingredient caused oxidative stress and monoamines alternations in brain of mice. The present observation could be due to the presence of thimerosal.

INTRODUCTION

New communicable disease influenza A (H1N1) affected geographically diverse areas around the world in 2009. Person to person transmission has led to increased numbers of patients. The current H1N1 virus, which was previously referred as Swine Flu is totally a new virus subtype. This new virus subtype is efficiently able to be transmitted from human to human which may cause Pandemic Influenza (Gangurde et al., 2011). Influenza virus infection, one of the most common infectious diseases, is a highly contagious airborne disease that causes an acute febrile illness and pandemic influenza vaccines are of the highest priority in global health security. There are limited immunogenicity and safety data, and no efficacy data would be available when human pandemic influenza vaccines are first administered after a pandemic is declared.

The risks and benefits of pandemic influenza vaccine will need to be studies post marketing (Bouvier and Palese, 2008). Vaccines contain live viruses, killed viruses, purified viral proteins, inactivated bacterial toxins, or bacterial polysaccharides. In addition to these immunogens, vaccines often contain other substances. For example, inactivated influenza virus vaccines are usually adjuvanted with aluminum salts that act as a scaffold for antigen presentation, allowing the vaccine to stimulate the immune system. This study showed that components of the thimerosal-containing H1N1 vaccine caused oxidative stress and lipid peroxidation in the brain tissue of adult male mice. The markers were consistent with previous reports of thimerosal toxicity as well as the high levels of oxidative stress observed in the brains of autistic subjects.
Prenatal exposure to thimerosal led to lasting impairment of brain monoaminergic systems in rats. Impairment was seen in 50 day old adult mice after thimerosal injection on embryonic day 9. This type of dysregulation is also present in the brains of autistic subjects.
Efficacy of DMSA Therapy in a Sample of Arab Children with Autistic Spectrum Disorder

Eleonor BLAACOKE-BUSCH; Omnia R. AMIN; Hani H. DESSOKI; Thanaa RABAH

Abstract

Objective: The aim of this study was to provide evidence that DMSA detoxification treatments cause a reduction in the heavy metal burden in the autistic, and that this reduction lessens neurological symptoms associated with ASD (Autistic Spectrum Disorder).

Method: The participants were 44 children, age 3 to 9 years of age, with Autistic Spectrum Disorder (ASD) according to Diagnostic and Statistical Manual of Mental Disorders 4th Edition, (DSM-IV). The severity of the autistic symptomatology had been measured by the Childhood Autism Rating Scale (SCARS). We collected urine samples before and after the DMSA challenge test, comparing urine metal output. We also compared the results of the DMSA detoxification (urine challenge test) with behavioral effects, typical for ASD.

Results: The DMSA challenge test increased the urine metal output for a number of potentially toxic metals. Statistically significant difference were noted between the baseline urine and DMSA challenge test regarding the level of cadmium, mercury, and lead (P=0.006, P=0.049, and P=0.008 respectively). We also noted that behavioral effects, typical for ASD (autism spectrum disorders) were reduced with this method of detoxification. A comparison between CARS Subscales and Total Score before and after a 6-month chelation program showed greatest improvements for Verbal and nonverbal communication (P <0.001), Taste, Smell and Touch (P 0.001) and Relating to People (P 0.005). Other improvements were noted for Adaptation to Change and Improvement.

Conclusion: DMSA chelation increased the urinary output of toxic and neurotoxic metals. Our data supports evidence that detoxification treatment with oral DMSA has beneficial effect on ASD patients.

DMSA chelation therapy was shown to be effective in detoxifying a number of toxic metals including mercury and resulted in marked improvement in behavioral effects of autistic children. A six month trial of chelation therapy in children with autism resulted in improved verbal and nonverbal communication, smell and touch, improvements in relating to people and adaptation to change.
Administration of Thimerosal to Infant Rats Increases Overflow of Glutamate and Aspartate in the Prefrontal Cortex: Protective Role of Dehydroepiandrosterone Sulfate

Michalina Duszczynk-Budhathoki · Mieszko Oleczak · Małgorzata Lehner · Maria Dorota Majewska

Abstract Thimerosal, a mercury-containing vaccine preservative, is a suspected factor in the etiology of neurodevelopmental disorders. We previously showed that its administration to infant rats causes behavioral, neurochemical, and neuropathological abnormalities similar to those present in autism. Here we examined, using microdialysis, the effect of thimerosal on extracellular levels of neuroactive amino acids in the rat prefrontal cortex (PFC). Thimerosal administration (4 injections, i.m., 240 μg Hg/kg on postnatal days 7, 9, 11, 15) induced lasting changes in amino acid overflow: an increase of glutamate and aspartate accompanied by a decrease of glycine and alanine; measured 10–14 weeks after the injections. Four injections of thimerosal at a dose of 12.5 μg Hg/kg did not alter glutamate and aspartate concentrations at microdialysis time (but based on thimerosal pharmacokinetics, could have been effective soon after its injection). Application of thimerosal to the PFC in perfusion fluid evoked a rapid increase of glutamate overflow. Co-administration of the neurosteroid, dehydroepiandrosterone sulfate (DHEAS; 80 mg/kg; i.p.) prevented the thimerosal effect on glutamate and aspartate; the steroid alone had no influence on these amino acids. Co-application of DHEAS with thimerosal in perfusion fluid also blocked the acute action of thimerosal on glutamate. In contrast, DHEAS alone reduced overflow of glycine and alanine, somewhat potentiating the thimerosal effect on these amino acids. Since excessive accumulation of extracellular glutamate is linked with excitotoxicity, our data imply that neonatal exposure to thimerosal-containing vaccines might induce excitotoxic brain injuries, leading to neurodevelopmental disorders. DHEAS may partially protect against mercurials-induced neurotoxicity.

Keywords Thimerosal · Glutamate · Amino acids · Microdialysis · DHEAS

The study documents that exposure of infant rats to thimerosal on postnatal days 7, 9, 11, 15 modeling the human infant vaccine schedule induced lasting changes in critical brain amino acids which alters the balance between excitatory and inhibitory amino acids in the brain, shifting it toward excessive neuroexcitation and provides a plausible mechanism whereby thimerosal exerts neurotoxic effects in the brain. The authors go on to say that thimerosal—still present in pediatric vaccines in many countries—causes a similar disturbance of excitatory and inhibitory neurotransmitters in the brains of human infants, leading to neurotoxicity, encephalopathies, and in consequence to neurodevelopmental disorders, including autism. Elevated levels of both blood and brain glutamate levels have been documented to occur in children with autism.
Evidence of parallels between mercury intoxication and the brain pathology in autism

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The purpose of this review is to examine the parallels between the effects mercury intoxication on the brain and the brain pathology found in autism spectrum disorder (ASD). This review finds evidence of many parallels between the two, including: (1) microtubule degeneration, specifically large, long-range axon degeneration with subsequent abortive axonal sprouting (short, thin axons); (2) dendritic overgrowth; (3) neuroinflammation; (4) microglial/astrocytic activation; (5) brain immune response activation; (6) elevated glial fibrillary acidic protein; (7) oxidative stress and lipid peroxidation; (8) decreased reduced glutathione levels and elevated oxidized glutathione; (9) mitochondrial dysfunction; (10) disruption in calcium homeostasis and signaling; (11) inhibition of glutamic acid decarboxylase (GAD) activity; (12) disruption of GABAergic and glutamatergic homeostasis; (13) inhibition of IGF-1 and methionine synthase activity; (14) impairment in methylation; (15) vascular endothelial cell dysfunction and pathological changes of the blood vessels; (16) decreased cerebral/cerebellar blood flow; (17) increased amyloid precursor protein; (18) loss of granule and Purkinje neurons in the cerebellum; (19) increased pro-inflammatory cytokine levels in the brain (TNF-α, IFN-γ, IL-1β, IL-8); and (20) aberrant nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB). This review also discusses the ability of mercury to potentiate and work synergistically with other toxins and pathogens in a way that may contribute to the brain pathology in ASD. The evidence suggests that mercury may be either causal or contributory in the brain pathology in ASD, possibly working synergistically with other toxic compounds or pathogens to produce the brain pathology observed in those diagnosed with an ASD.

Key words: autism, autism spectrum disorder (ASD), mercury (Hg), toxicity, brain pathology

INTRODUCTION

Evidence suggests that children with autism spectrum disorder (ASD) have a greater susceptibility to heavy-metal intoxication than typically developing children (Holmes et al. 2003, Kern and Jones 2006, Rose et al. 2008, Nataf et al. 2008, James et al. 2009, Geier et al. 2009a, Majewska et al. 2010, Youn et al. 2011), both of which are critically important for detoxification (Gutman 2002, Kern et al. 2004). Expressions such as “poor detoxifiers” and “poor excretors” have been used in reference to those with ASD (Holmes et al. 2003). In a recent analysis, DeSoto and Hitlan (2010) found that there are 58 research articles which provide empirical evidence relevant to the question of a link between autism and one or more

The study authors discuss 20 specific similarities between mercury intoxication and autism brain pathologies. The evidence suggests that mercury might be causal or contributory in the brain pathology of autism, with a possibility of synergistic interactions with other toxins or pathogens.
Thimerosal significantly damaged the mitochondrial membranes and DNA in human astrocytes and induced oxidative stress, both of which are also documented to occur in autism spectrum disorders. The enzyme caspase-3, which signals cell death, was upregulated 5-fold in the presence of thimerosal and mitochondrial membranes showed significant depolarization and an increase in the levels of mitochondrial DNA nicks and breaks.
Maternal Thimerosal Exposure Results in Aberrant Cerebellar Oxidative Stress, Thyroid Hormone Metabolism, and Motor Behavior in Rat Pups; Sex- and Strain-Dependent Effects

Z. L. Sulkowski · T. Chen · S. Midha · A. M. Zavacki · Elizabeth M. Sajdel-Sulkowska

Abstract Methylmercury (Met-Hg) and ethylmercury (Et-Hg) are powerful toxicants with a range of harmful neurological effects in humans and animals. While Met-Hg is a recognized trigger of oxidative stress and an endocrine disruptor impacting neurodevelopment, the developmental neurotoxicity of Et-Hg, a metabolite of thimerosal (TM), has not been explored. We hypothesized that TM exposure during the perinatal period impairs central nervous system development, and specifically the cerebellum, by the mechanism involving oxidative stress. To test this, spontaneously hypertensive rats (SHR) or Sprague-Dawley (SD) rat dams were exposed to TM (200 μg/kg body weight) during pregnancy (G10–G15) and lactation (P5–P10). Male and female neonates were evaluated for auditory and motor function; cerebella were analyzed for oxidative stress and thyroid metabolism. TM exposure resulted in a delayed startle response in SD neonates and decreased motor learning in SHR male (22.6%), in SD male (29.8%), and in SD female (55.0%) neonates. TM exposure also resulted in a significant increase in cerebellar levels of the oxidative stress marker 3-nitrotyrosine in SHR female (35.1%) and SD male (14.0%) neonates. The activity of cerebellar type 2 deiodinase, responsible for local intra-brain conversion of thyroxine to the active hormone, 3',3,5-triodothyronine (T3), was significantly decreased in TM-exposed SHR male (60.9%) pups. This coincided with an increased (47.0%) expression of a gene negatively regulated by T3, Odf4 suggesting local intracerebellar T3 deficiency. Our data thus demonstrate a negative neurodevelopmental impact of perinatal TM exposure which appears to be both strain- and sex-dependent.

Keywords Ethylmercury · Rat · Cerebellum · Oxidative stress marker 3-nitrotyrosine (3-NT) · Type 2 deiodinase (D2)

Introduction

Environmental toxicants such as heavy metals [1] including mercury Hg [2, 3] have been identified as factors exerting a range of harmful neurological and cognitive effects in humans and experimental animals, and have been implicated in the etiology of a number of neuropsychiatric disorders. The major environmental organic compounds of mercury include methylmercury (Met-Hg) and ethylmercury (Et-Hg). The main exposure to Met-Hg comes from contaminated fish through bioaccumulation of both organic and inorganic of Hg environmental contamination.
In young children with autism spectrum disorder, measured levels of hair mercury correlated significantly with ASD severity, such that more severely affected children had the highest levels of hair mercury. Other hair metals measured including arsenic, antimony, cadmium and lead, among others, were not significantly correlated with ASD severity.

Abstract: Previous studies have found a higher body-burden of toxic metals, particularly mercury (Hg), among subjects diagnosed with an autism spectrum disorder (ASD) in comparison to neurotypical controls. Moreover, Hg body-burden was associated with ASD severity. This cross-sectional study examined the potential correlation between hair toxic metal concentrations and ASD severity in a prospective cohort of participants diagnosed with moderate to severe ASD. The Institutional Review Board at the University of Texas Southwestern Medical Center at Dallas (Dallas, TX) approved the present study. Qualifying study participants (n = 18) were evaluated for ASD severity using the Childhood Autism Rating Scale (CARS) and quantitatively for arsenic, Hg, cadmium, lead, chromium, cobalt, nickel, aluminum, tin, uranium, and manganese using hair toxic element testing by Doctor’s Data (a CLIA-approved laboratory). CARS scoring and hair toxic element testing were blinded to one another. Increasing hair Hg concentrations significantly correlated with increased ASD severity. In contrast, no significant correlations were observed between any other of the hair toxic metals examined and ASD severity. This study helps to provide additional mechanistic support for Hg in the etiology of ASD severity, and is supported by an increasing number of recent critical reviews that provide biological plausibility for the role of Hg exposure in the pathogenesis of ASDs.
Research Article

Mercury Disposition in Suckling Rats: Comparative Assessment Following Parenteral Exposure to Thiomersal and Mercuric Chloride

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Due to the facts that thiomersal-containing vaccine is still in use in many developing countries, and all forms of mercury have recognised neurotoxic, nephrotoxic, and other toxic effects, studies on disposition of ethylmercury and other mercury forms are still justified, especially at young age. Our investigation aimed at comparing mercury distribution and rate of excretion in the early period of life following exposure to either thiomersal (TM) or mercuric chloride (HgCl₂) in suckling rats. Three experimental groups were studied: control, TM, and HgCl₂, with 12 to 18 pups in each. Both forms of mercury were administered subcutaneously in equimolar quantities (0.81 μmol/kg b.w.) three times during the suckling period (on the days of birth 7, 9, and 11) to mimic the vaccination regimen in infants. After the last administration of TM or HgCl₂, total mercury retention and excretion was assessed during following six days. In TM-exposed group mercury retention was higher in the brain, enteral excretion was similar, and urinary excretion was much lower compared to HgCl₂-exposed sucklings. More research is still needed to elucidate all aspects of toxicokinetics and most harmful neurotoxic potential of various forms of mercury, especially in the earliest period of life.

1. Introduction

Mercury is a pervasive environmental contaminant with proven toxic properties in mammals. Major risks recognized due to mercury exposure are dietary methylmercury exposure from fish and seafood, elemental mercury vapour from mercury lies in the fact that the exposure occurs in the most vulnerable period of life, when the brain is developing and growing [8]. Organic forms of mercury are more easily absorbed when ingested and are less readily eliminated from the body than its inorganic forms [1].

By now considerable amount of evidence has been col-

Thimerosal clearance in the body was compared to the clearance of mercuric chloride. Thimerosal showed much higher retention time in the brain, as inorganic mercury. This is consistent with brain studies on autistic subjects that show a significantly greater level of inorganic mercury in the brain.
Embyronic exposure to thimerosal, an organomercury compound, causes abnormal early development of serotonergic neurons.

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Keywords: Thimerosal, Serotonergic neuron, Development, Embryonic exposure

Abstract

Even though neuronal toxicity due to organomercury compounds is well known, thimerosal, an organomercury compound, is widely used in pediatric vaccine preservation. In the present study, we examined whether embryonic exposure to thimerosal affects early development of serotonergic neurons. Thimerosal (1 mg Hg/kg) was intramuscularly administered to pregnant rats on gestational day 9 (susceptible time window for development of fetal serotonergic system), and fetal serotonergic neurons were assessed at embryonic day 15 using anti-serotonin antibodies. A dramatic increase in the number of serotonergic neurons localized to the lateral portion of the caudal raphe was observed in thimerosal group (1.9-fold increase, p<0.01 compared to control). These results indicate that embryonic exposure to thimerosal affects early development of serotonergic neurons.

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Thimerosal, an organomercury compound, is known for its preservative effects on pediatric vaccines [2,12]. Thimerosal biotransforms in vivo to ethylmercury and subsequently to inorganic forms of mercury [19,20], which are toxic to animals [4,7]. Therefore, accumulation of mercury through frequent vaccine administration is a concern [2,26].

The adverse effects of thimerosal have been studied extensively; neonatal administration of thimerosal induces impairment of sensitivity to pain [16] and neurodegeneration of hippocampus [17]. Although fetal organomercury poisoning (fetal Minamata disease) is known to exhibit systemic effects on fetus [5,6], little is known regarding the mechanism of action of thimerosal during the embryonic period.

Serotonergic neurons are one of the earliest neurotransmitter phenotypes to appear during the development of the nervous system [1,8,10]. In the fetal rat, serotonergic neurons were identified at around embryonic day (E) 13 (day of insemination = E1) [1,18]. Thimerosal administration to thalidomide resulted in caudal shift of serotonergic neurons in the dorsal raphe, suggestive of perturbed neuronal migration [13]. The effect of thalidomide was specific for the day of thalidomide administration, demonstrating that embryonic exposure at E9 is specifically crucial in the normal development of serotonergic neurons.

Since the early development of serotonergic neurons is time specific and three-dimensional [1,8,10], precise evaluation of serotonergic neuronal development by conventional immunohistochemical methods is difficult. In the present study, we utilized whole-mount preparation method for embryonic brain [1,9], which facilitates assessment of spatiotemporal data on the development of neurotransmitter system. Using this technique, we investigated whether exposure to thimerosal at E9 affects early development of serotonergic neurons.

Thimerosal administration: Pregnant Wistar rats were purchased by CLEA Japan, Inc. (Tokyo, Japan). Thimerosal (Sigma-Aldrich,
Thimerosal administered to rat neonates showed sex-dependent impairment of brain dopaminergic system, leading to aberrant behaviors similar to autism. Males were more sensitive than females to some neurodisruptive/neurotoxic actions of thimerosal, similar to the higher incidence of autism in males compared to females.
The authors review the route and cellular mechanisms of mercury (Hg) exposure in autism; current research and examples of possible genetic variables that are linked to both Hg sensitivity and autism; the role Hg may play as an environmental toxin fueling the oxidative stress found in autism; the role of mitochondrial dysfunction; and the possible role of Hg in abnormal neuroexcitatory and excitotoxity actions that may play a role in the immune dysregulation found in autism.
The incidence of autism in ancestors of pink disease survivors was significantly higher (1 in 22) than autism incidence in the general population (1 in 160). The results support the hypothesis that Hg sensitivity may be a heritable/genetic risk factor for ASD.
Toxicity biomarkers among US children compared to a similar cohort in France: a blinded study measuring urinary porphyrins

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The purpose of this blinded study was to evaluate potential environmental toxicity in a cohort of neurotypical children (n=28) living in a suburban area of north-central Texas in the United States (US) with a comparable age- and gender-matched cohort of neurotypical children (n=28) living in a suburban area of southeastern France using urinary porphyrin testing: uroporphyrin (uP), heptacarboxyloporphyrin (7cXP), hexacarboxyloporphyrin (6cXP), pentacarboxyloporphyrin (5cXP), protoporphyrin (proP), and coproporphyrin (cP). Results showed significantly elevated 6cXP, proP (an atypical, mercury-specific porphyrin), and cP levels, and increasing trends in 5cXP levels, among neurotypical children in the USA compared to children in France. Data suggest that in US neurotypical children, there is a significantly increased body-burden of mercury (Hg) compared to the body-burden of Hg in the matched neurotypical children in France. The presence of lead contributing to the higher levels of cP also needs to be considered. Further, other factors including genetics can not be completely ruled out.

Keywords: mercury; heavy metal; porphyrins; biomarkers; xenobiotic; lead; toxicity

Introduction

For many years, measuring heavy metal toxicity in children involved a direct measure of the metals in the blood, urine, hair, or fecal matter. A more recent approach is to use urinary porphyrins as a measure of toxic metal body-burden. Previous studies showed that urinary porphyrins (heme precursors formed in the heme synthesis pathway) afford a measure of xenobiotic exposure, particularly mercury (Hg) (Woods 1996; Pingree et al. 2001a; Pingree, Simmonds, and Woods 2001b). Specific patterns of porphyrins suggest the presence of Hg exposure. Mercury toxicity was demonstrated to be associated with

In this study, urinary porphyrins as a consequence of mercury toxicity were compared between children with autism spectrum disorder and “neurotypical” control children. Children with ASD consistently and statistically significantly showed elevated patterns of urinary porphyrins that were consistent with mercury toxicity.
Infant macaques exposed to thimerosal in a dosage mimicking the “birth” hepatitis B vaccine showed delays in reflex development including acquisition of root, snout, and suck reflexes, compared to unexposed animals. This is similar to developmental delays associated with autism.
Influence of pediatric vaccines on amygdala growth and opioid ligand binding in rhesus macaque infants: A pilot study

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This longitudinal, case-control pilot study examined amygdala growth in rhesus macaque infants receiving the complete US childhood vaccine schedule (1994-1999). Longitudinal structural and functional neuroimaging was undertaken to examine central effects of the vaccine regimen on the developing brain. Vaccine-exposed and saline-injected control infants underwent MRI and PET imaging at approximately 4 and 6 months of age, representing two specific timeframes within the vaccination schedule. Volumetric analyses showed that exposed animals did not undergo the maturational changes over time in amygdala volume that was observed in unexposed animals. After controlling for left amygdala volume, the binding of the opioid antagonist [11C]diprenorphine (DPN) in exposed animals remained relatively constant over time, compared with unexposed animals, in which a significant decrease in [11C]DPN binding occurred. These results suggest that maturational changes in amygdala volume and the binding capacity of [11C]DPN in the amygdala were significantly altered in infant macaques receiving the vaccine schedule. The macaque infant is a relevant animal model in which to investigate specific environmental exposures and structural/functional neuroimaging during neurodevelopment.

Key Words: rhesus macaques, Macaca mulatta, non-human primates, animal model, neuroimaging, PET, MRI, amygdala, opioids, ethyl mercury, thimerosal, neurotoxicity

INTRODUCTION

The amygdala, a complexly interconnected limbic system structure located in the temporal lobe of the brain, is thought to play a central role in the expression of emotions (reviewed by Aggleton 1992). In rhesus macaques the amygdala has been associated with the development of social and emotional behavior (reviewed by Prather et al. 2001) but failed to develop an appropriate fear response (Antoniadis et al. 2009), implicating an important role for the amygdala in regulating such responses (reviewed by Amaral and Corbett 2003, Amaral et al. 2008, Machado et al. 2009, Roozendaal et al. 2009). While the human amygdala has been well studied longitudinally, it is not clear if the amygdala lesioned in autism.

In this study, infant macaque monkeys exposed to thimerosal doses comparable to the US vaccine schedule showed arrested amygdala development as compared to unexposed controls where normal amygdala maturation occurred. Changes in amygdala development have been documented in autism.
HEPATITIS B VACCINATION OF MALE NEONATES AND AUTISM DIAGNOSIS, NHIS 1997–2002

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Universal hepatitis B vaccination was recommended for U.S. newborns in 1991; however, safety findings are mixed. The association between hepatitis B vaccination of male neonates and parental report of autism diagnosis was determined. This cross-sectional study used weighted probability samples obtained from National Health Interview Survey 1997–2002 data sets. Vaccination status was determined from the vaccination record. Logistic regression was used to estimate the odds for autism diagnosis associated with neonatal hepatitis B vaccination among boys age 3–17 years, born before 1999, adjusted for race, maternal education, and two-parent household. Boys vaccinated as neonates had threefold greater odds for autism diagnosis compared to boys never vaccinated or vaccinated after the first month of life. Non-Hispanic white boys were 64% less likely to have autism diagnosis relative to nonwhite boys. Findings suggest that U.S. male neonates vaccinated with the hepatitis B vaccine prior to 1999 (from vaccination record) had a threelfold higher risk for parental report of autism diagnosis compared to boys not vaccinated as neonates during that same time period. Nonwhite boys bore a greater risk.

Universal newborn immunization with the hepatitis B vaccination was recommended in 1991 (CDC, 1991). A recent narrative review concluded that hepatitis B vaccines available since 1982 are safe and effective (Demirjian & Levy, 2009); however, safety findings from individual studies are mixed. In Vaccine Safety Datalink studies, Lewis et al. (2001) reported no evidence of a significant association between vaccination at birth and fever or neurological adverse events, Naleway et al. (2009) found an elevated, although reported no association between autism and vaccination with the hepatitis B vaccination during the first month of life. Additionally, Marques et al. (2007) found no association between time of hepatitis B vaccination, i.e., within 24 hrs versus 2–4 days postnatally, and neurodevelopment delays at 6 months of age. In contrast, increased risk for central nervous system inflammatory demyelination in childhood were associated with hepatitis B vaccination (Mikaeloff et al., 2009). Further, hepatitis B vaccination has been associated with acute

The study authors investigated the National Health Inventory Survey (a very large national database) and found that boys receiving the full Hepatitis B series were 3 times as likely to receive an autism diagnosis as compared to those not receiving any HepB vaccine (a statistically significant finding). Non-white boys were found to have a significantly worse outcome after Hep B vaccination.
Blood mercury levels in autism spectrum disorder: Is there a threshold level?

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Mercury (Hg) may significantly impact the pathogenesis of autism spectrum disorders (ASDs). Lab results generated by Vitamin Diagnostics (CLIA-approved), from 2003-2007, were examined among subjects diagnosed with an ASD (n=83) in comparison to neurotypical controls (n=89). Blood Hg levels were determined by analyzing Hg content in red blood cells (RBC) using cold vapor analysis, and consistent Hg measurements were observed between Vitamin Diagnostics and the University of Rochester. Adjusted (age, gender, and date of collection) mean Hg levels were 1.9-fold significantly (P<0.0001) increased among subjects diagnosed with an ASD (21.4 µg/L) in comparison to controls (11.4 µg/L). Further, an adjusted significant (P<0.0005) threshold effect (>15 µg/L) was observed for Hg levels on the risk of a subject being diagnosed with an ASD in comparison to controls (odds ratio=6.4). The weight of scientific evidence supports Hg as a causal factor in subjects diagnosed with an ASD.

Key words: Asperger, autistic, body-burden, neurodevelopmental, PDD

INTRODUCTION

Autism spectrum disorders (ASDs) are neurodevelopmental disorders, presenting in childhood that affect at least 1 in 110 children in the United States (Centers for Disease Control and Prevention 2009). The condition is characterized by severe impairments in socialization, communication, and behavior. Individuals diagnosed with an ASD may display a range of problem behaviors such as hyperactivity, poor attention, impulsivity, aggression, self-injury, and tantrums. Further, these children often display unusual responses to sensory stimuli, such as hypersensitivities to light, sound, color, smell or touch, and have a high threshold for anxiety (Austin et al., 2009). Exposure to Hg can cause immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining/associated with ASDs, and these similarities extend to neuroanatomy, neurotransmitters, and biochemistry (Austin 2008, Geier et al. 2008, 2009a).

DeSoto and Hitlan (2007) postulated that if Hg does play a causal role in facilitating an ASD diagnosis, there would likely be at least some correlation between high Hg measured in the blood and the symptoms of autism, even if an individual’s ability to metabolize mercury mediates the relationship between exposure and neural toxicity. This is because even if exposure is limited, both neurodevelopmental and developmental periods (Austin 2008, Geier et al. 2008, 2009a).

In this study, the authors identify a statistically significant level of mercury in red blood cells of autistic children compared to non-autistic controls. When adjusted for age, gender and date of collection, mercury levels were 90% higher in autistic subjects than controls without autism.
The authors found that low dose injections of thimerosal induced metallothionein in the brains of mice. Based on these findings, in combination with the brain pathology observed in patients diagnosed with autism, the study supports the biological plausibility for how low-dose exposure to mercury from thimerosal-containing vaccines may be associated with the development of autism.
The authors compared autistic children to matched control children and found the autistic children had significantly higher hair mercury levels. This phenomenon was seen particularly in children who received thimerosal-containing vaccines.
Inflammatory mediator release is much more common in the mast cells of autistic children than compared to non-autistic controls. Human cultured mast cells treated with mercuric chloride showed higher levels of inflammatory mediator release as compared to untreated controls. This may provide clues as to how thimerosal exposure could stimulate mastocytosis, which has a higher prevalence in autistic subjects.
Biomarkers of environmental toxicity and susceptibility in autism

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ABSTRACT

Autism spectrum disorders (ASDs) may result from a combination of genetic/biochemical susceptibilities in the form of a reduced ability to excrete mercury and/or increased environmental exposure at key developmental times. Urinary porphyrins and transsulfuration metabolites in participants diagnosed with an ASD were examined. A prospective, blinded study was undertaken to evaluate a cohort of 28 participants with an ASD diagnosis for Childhood Autism Rating Scale (CARS) scores, urinary porphyrins, and transsulfuration metabolites. Testing was conducted using Vitamin Diagnostics, Inc. (CLIA-approved) and Laboratoire Philippe Auguste (ISO-approved). Participants with severe ASDs had significantly increased mercury intoxication-associated urinary porphyrins (pestaxoaxyporphyrin, preproporphyrin, and coproporphyrin) in comparison to participants with mild ASDs, whereas other urinary porphyrins were similar in both groups. Significantly decreased plasma levels of reduced glutathione (GS), cysteine, and sulfate were observed among study participants relative to controls. In contrast, study participants had significantly increased plasma oxidized glutathione (GSSG) relative to controls. Mercury intoxication-associated urinary porphyrins were significantly correlated with increasing CARS scores and GSSG levels, whereas other urinary porphyrins did not show these relationships. The urinary porphyrin and CARS score correlations observed among study participants suggest that mercury intoxication is significantly associated with autistic symptoms. The transsulfuration abnormalities observed among study participants indicate that mercury intoxication was associated with increased oxidative stress and decreased detoxification capacity.

1. Introduction

Autism spectrum disorders (ASDs) are prevalent neurodevelopmental disorders that, based on a recent survey, affect not less than 1 in 150 children born in the US during the early 1990s [1]. ASD diagnoses are characterized by impairments in social relatedness and communication, repetitive behaviors, abnormal movement patterns, and sensory association with an ASD diagnosis include gastrointestinal disease and dysbiosis [3], autoimmune disease [4], and mental retardation [5].

In attempting to understand the underlying pathogenesis in those with an ASD diagnosis, a considerable body of research has been conducted to evaluate potential candidate causal genes. Genetic studies, to date, have not uncovered genes of strong effect. It has recently been postulated that increasing rates and less than 100%
Three types of human cell lines were subjected to increasing concentrations of thimerosal, along with other toxic metal compounds. Thimerosal exhibited the greatest toxicity in each of the cell lines tested and the damage was similar to that observed in autism pathophysiologic studies.
This study focused on correlations between overall body burden of toxic metals, including mercury, and the severity of the autistic disorder. Higher body burden associated strongly with more severe cases of autistic disorder as did low red blood cell levels of glutathione, a molecule that plays a critical role in excreting mercury from the body.
A Prospective Study of Transsulfuration Biomarkers in Autistic Disorders

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Abstract The goal of this study was to evaluate transsulfuration metabolites in participants diagnosed with autism spectrum disorders (ASDs). Transsulfuration metabolites, including: plasma reduced glutathione (GSH), plasma oxidized glutathione (GSSG), plasma cysteine, plasma taurine, plasma sulfate, and plasma free sulfate among participants diagnosed with ASDs (n = 38) in comparison to age-matched neurotypical controls were prospectively evaluated. Testing was conducted using Vitamin Diagnostics, Inc. (CLIA-approved). Participants diagnosed with ASDs had significantly (P < 0.001) increased plasma GSSG relative to controls. The present observations are compatible with increased oxidative stress and a decreased detoxification capacity, particularly of mercury, in patients diagnosed with ASDs. Patients diagnosed with ASDs should be routinely tested to evaluate transsulfuration metabolites, and potential treatment protocols should be evaluated to potentially correct the transsulfuration abnormalities observed.

Keywords Heavy metal • Metabolic endophenotype • Sulfation • Sulfur

Introduction

Autism spectrum disorders (ASDs) are prevalent neurodevelopmental disorders that affect an estimated 1 in 150 children in the US [1]. It has been observed that ASDs are characterized by impairments in social relatedness and communication, repetitive behaviors, abnormal movement patterns, and sensory dysfunction [2]. Further, common co-morbidity conditions often associated with ASDs significantly (P < 0.001) increased plasma GSSG relative to controls. The present observations are compatible with increased oxidative stress and a decreased detoxification capacity, particularly of mercury, in patients diagnosed with ASDs. Patients diagnosed with ASDs should be routinely tested to evaluate transsulfuration metabolites, and potential treatment protocols should be evaluated to potentially correct the transsulfuration abnormalities observed.

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In this study, a drop in autism spectrum disorder prevalence is reported in Denmark after the removal of thimerosal from vaccines in 1992. ASD prevalence drops from 82.0/10000 for children born in 1994-95 to 61.9/10000 for children born in 1998-99. The decreases seen in 1996-97 and 1998-99 are statistically significant.
Thimerosal exposure in infants and neurodevelopmental disorders: An assessment of computerized medical records in the Vaccine Safety Datalink

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Abstract

The study evaluated possible associations between neurodevelopmental disorders (NDs) and exposure to mercury (Hg) from Thimerosal-containing vaccines (TCVs) by examining the automated Vaccine Safety Datalink (VSD). A total of 278,624 subjects were identified in birth cohorts from 1990–1996 that had received their first oral polio vaccination by 3 months of age in the VSD. The birth cohort prevalence rate of medically diagnosed International Classification of Disease, 9th revision (ICD-9) specific NDs and control outcomes were calculated. Exposures to Hg from TCVs were calculated by birth cohort for specific exposure windows from birth-7 months and birth-13 months of age. Poisson regression analysis was used to model the association between the prevalence of outcomes and Hg doses from TCVs. Consistent significantly increased rate ratios were observed for autism, autism spectrum disorders, tics, attention deficit disorder, and emotional disturbances with Hg exposure from TCVs. By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from TCVs. Routine childhood vaccination should be continued to help reduce the morbidity and mortality associated with infectious diseases, but efforts should be undertaken to remove Hg from vaccines. Additional studies should be conducted to further evaluate the relationship between Hg exposure and NDs.

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Keywords: ADD; ADHD autistic disorder; ASD; Ethylmercury; Merthiolate; Thimerosal

1. Introduction

In the last few decades, vaccines—one of the greatest breakthroughs in health sciences—have helped to accomplish striking reductions of infection and disease worldwide [1]. From the 1930s through the early 2000s, many routinely administered childhood vaccines in the United States contained Thimerosal [2]. Thimerosal is an organic mercury-containing compound that is 49.55% mercury (Hg) by weight, and initially metabolized to ethylmercury com-

The study authors found significantly increased risk ratios for autism and autism spectrum disorders as a result of exposure to mercury from thimerosal-containing vaccines using the CDC’s Vaccine Safety Datalink, a robust database of electronic medical records used for monitoring vaccine safety.
Mothers receiving thimerosal containing Rho(D) immune globulin injections while pregnant because they had Rh negative blood type had a significantly higher rate of children who developed autism. Overall, twice as much autism was seen in children exposed to thimerosal prenatally when compared to a control group of children who had no prenatal exposure to thimerosal.
This paper explains how deficits in sulfur metabolism along with toxic heavy metals exposure could lead to the development of autistic disorder. It also points out genetic mutations in sulfur metabolism that hinder heavy metals detoxification occur more frequently in autistic patients.
This paper shows how high levels of glutathione protect against thimerosal toxicity. Previous studies have shown that autism is more common in individuals with lower levels of plasma glutathione and, therefore, diminished ability to detoxify thimerosal.
Children with autism showed significantly higher levels of mercury in their baby teeth (2.1 fold higher or more than double) than non-autistic controls, indicating high exposures to mercury. Baby teeth are a good measure of cumulative exposure to toxic metals during fetal development and early infancy. This study suggests that children with autism had a higher body burden of mercury during gestation and early infancy than children without autism.
A Prospective Study of Mercury Toxicity Biomarkers in Autistic Spectrum Disorders

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Porphyrias are derivatives formed in the heme synthesis pathway and porphyrias afford a measure of xenobiotic exposure. The steps in the heme pathway most vulnerable to heavy metal inhibition are uroporphyrin decarboxylase (UROD) and coproporphyrinogen oxidase (CPOX) reactions. Mercury toxicity was associated with elevations in urinary coproporphyrin (cP), pentacarboxyporphyrin (5cP), and preprotoxoporphyrin (prcP) (also known as keto-isocoproporphyrin) levels. Two cohorts of autistic patients in the United States and France had urine porphyrin levels associated with mercury toxicity. A prospective study of urinary porphyrin testing at LabCorp (United States) and the Laboratoire Philippe Auguste (France) involving 71 autism spectrum disorder (ASD) patients, neurotypical sibling controls, and general population controls was undertaken. ASD patients had significant elevations in urinary levels of cP, 5cP, and prcP relative to controls, and > 50% of ASD patients had urinary cP levels more than 2 standard deviations above the mean values for neurotypical sibling controls. Significant reductions in urinary 5cP and cP levels were observed in ASD patients following chelation. A significant correlation was found between urinary porphyrins measured at LabCorp and those measured at the Laboratoire Philippe Auguste on individual ASD patients. The established developmental neurotoxicity attributed to mercury and biochemical genomic evidence for mercury susceptibility/toxicity in ASDs indicates a causal role for mercury. Urinary porphyrin testing is clinically available, relatively inexpensive, and noninvasive. Porphyrins need to be routinely measured in ASDs to establish if mercury toxicity is a causative factor and to evaluate the effectiveness of chelation therapy.

Porphyrias are derivatives of the heme synthesis pathway that afford a measure of xenobiotic exposure (Brewster, 1988). Heme production primarily occurs in liver, kidneys, and erythroid cells. The synthetic process is summarized in Figure 1 (Nataf et al., 2006). Excess porphyrinogen metabolites are excreted in the urine as oxidized porphyrins, particularly uroporphyrin (uP) and coproporphyrin (cP), the most abundant soluble porphyrin molecules in the kidney cortex (Woods & Miller, 1993). Because these mid-pathway porphyrins are the most water-soluble of all the porphyrins, they are excreted predominantly in urine, whereas the hydrophobic protoporphyrin is predominantly found in the bile and feces.

Excess urinary porphyrin excretion, or porphyrinuria, results from inhibition of key enzymatic steps in conditions including genetic deficiencies in heme production enzymes (Sarkany, 1999), hepatitis, renal disease, and erythroid disease (Gross et al., 2000), as well as by heavy metal inhibition of heme enzyme synthesis (Woods, 1996). Both in experimental animals and in humans exposed to heavy metals, elevated levels of porphyrins are found in the urine (Bowers et al., 1992; Woods, 1996). The steps in the heme pathway most vulnerable to heavy metal inhibition are those in which uroporphyrin decarboxylase (UROD) (Woods & Kardish, 1983) and copro-

Children with autism spectrum disorders showed significantly higher levels of urinary porphyrins associated with mercury toxicity as compared to “neurotypical” control children. Children in the U.S. and France exhibited the same characteristics which were confirmed by U.S. and French clinical laboratories.
Antineuronal antibodies in autistic children: relation to blood mercury

**Background:** It was recently suggested that autism, a severe neurodevelopmental disorder, may involve an autoimmune pathogenesis. Mercury (Hg) is a potential risk factor for autoimmunity in autistic children.

**Objective:** We sought to investigate the expression of antineuronal antibodies, as an index of autoimmunity to brain, in autistic children. The potential relationship between blood mercury and these antibodies was also investigated.

**Methods:** Forty autistic children (20 with mild to moderate and 20 with severe disease) were studied in comparison to 40 healthy children. After complete clinical and neuropsychiatric evaluation, serum antineuronal antibodies and blood Hg levels were estimated.

**Results:** Autistic children had significantly higher seropositivity for antineuronal antibodies (67.5%) than healthy controls (5%). Similarly, the former group had significantly higher blood Hg levels than the latter (p<0.0001). Seropositivity of antineuronal antibodies had a significant positive association with elevated blood Hg, which was found in 70% of autistic children, (p<0.0001). In addition, the two markers were positively associated with some parameters such as the family history of autoimmunity, autistic severity and some important clinical manifestations of autism (mental retardation, behavioral abnormalities and autistic regression) as well as EEG abnormalities.

**Conclusion:** Autism may be, in part, one of the pediatric autoimmune neuropsychiatric disorders. Such autoimmunity may be triggered by environmental Hg exposure. Further studies are warranted to enforce these concepts. If these assumptions could be proved, routine assessment of serum antineuronal antibodies and blood mercury in autistic children would be mandatory. Studies assessing the role of immunotherapy and Hg chelators as new therapeutic modalities for autism are also recommended.

**Keywords:** Antineuronal antibodies; autism; autoimmunity; children; heavy metals; EEG; mercury.

Autistic children showed higher levels of antineuronal antibodies and blood mercury as compared to non-autistic controls. There was a strong, statistically significant correlation between antineuronal antibodies and blood mercury in autistic children.
A prospective study of thimerosal-containing Rho(D)-immune globulin administration as a risk factor for autistic disorders

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(Received 1 May 2006; revised 29 September 2006; accepted 27 November 2006)

Abstract

Background. This study evaluated the relationship between prenatal mercury exposure from thimerosal (49.55% mercury by weight)-containing Rho(D)-immune globulins (TCRs) and autism spectrum disorders (ASDs).

Methods. The Institutional Review Board of the Institute for Chronic Illnesses approved the present study. A total of 53 consecutive non-Jewish Caucasian patients with ASDs (Diagnostic and statistical manual of mental disorders, fourth ed. — DSM IV) born between 1987 and 2001 who presented to the Genetic Centers of America for outpatient genetic/developmental evaluations were prospectively collected from June 1, 2005 through March 31, 2006. Imaging and laboratory testing were conducted on each patient to rule out other causal factors for their ASDs. As race-matched controls, the frequency of Rh negativity was determined from 926 non-Jewish Caucasian pregnant women who had presented for outpatient prenatal genetics care to the Genetic Centers of America between 1980 and 1989.

Results. Children with ASDs (28.30%) were significantly more likely (odds ratio 2.35, 95% confidence interval 1.17–4.52, p < 0.01) to have Rh-negative mothers than controls (14.36%). Each ASD patient’s mother was determined to have been administered a TCR during her pregnancy.

Conclusion. The results provide insights into the potential role prenatal mercury exposure may play in some children with ASDs.

Keywords: Developmental delay, ethylmercury, rhogam, thimerosal, thionmeral

Introduction

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by impairments in social relatedness and communication, repetitive similar to traits defining or associated with autistic disorders, and that these similarities extend to neuroanatomy, neurotransmitters, and biochemistry [4–7]. Rho(D)-immune globulin is an immune globulin

Children with autism were twice as likely as non-autistic controls to be born to mothers who had Rh negative blood type and thus were exposed to thimerosal via Rho(D) immune globulin injections during pregnancy. In 1991 the American College of Ob-Gyn made the universal recommendation that all women with Rh negative blood type (approximately 12% of the population) receive an injection of Rho(D) immune globulin at 28 weeks gestation to prevent Rh incompatibility disease in subsequent pregnancies.
Eight autistic patients were found to be heavily contaminated with mercury. They each excreted significant amounts of mercury following chelation and all showed biochemical evidence of decreased function in their glutathione pathways. None of the subjects had known, significant mercury exposure except from thimerosal-containing vaccines/Rho(D)-immune globulin preparations. In addition, all had alternate causes for their regressive ASDs ruled out.
This study is a correction to a previous study that claimed mercury levels in children’s blood did not correlate with the presence of autism. In this reanalysis, Desoto shows clearly that a statistically significant link appears between blood mercury levels and autistic disorder in children.
This study shows significantly increased risk ratios for autism, speech disorders, mental retardation, infantile spasms, and thinking abnormalities reported to the Vaccine Adverse Event Reporting System (VAERS) following exposure to thimerosal-containing DTP vaccines in comparison to thimerosal-free DTPH vaccines.
Dendritic cells (DCs), a rare cell type widely distributed in the soma, are potent antigen-presenting cells that initiate primary immune responses. DCs rely on intracellular redox state and calcium (Ca²⁺) signals for proper development and function, but the relationship between these two signaling systems is unclear. Thimerosal (THI) is a mercurial used to preserve vaccines and consumer products, and is used experimentally to induce Ca²⁺ release from microsomal stores. We tested adenosine triphosphate (ATP)-mediated Ca²⁺ responses of DCs transiently exposed to nanomolar THI. Transcriptional and immunocytochemical analyses show that murine myeloid immature DCs (iDCs) and mature DCs (mDCs) express inositol 1,4,5-triphosphate receptor (IP₃/R) and ryanodine receptor (RyR) Ca²⁺ channels, known targets of THI. iDCs express the RyR₁ isoform in a punctate distribution that is dense near plasma membranes and within dendritic processes, whereas IP₃/Rs are more generally distributed. RyR₁ positively and negatively regulates purinergic signaling because ryanodine (Ry) blockade a) recruited 80% more ATP responders, b) shortened ATP-mediated Ca²⁺ transients >2-fold, and c) produced a delayed and persistent rise (≥2-fold) in baseline Ca²⁺. THI (100 nM, 5 min) recruited more ATP responders, shortened the ATP-mediated Ca²⁺ transient (≥1.4-fold), and produced a delayed rise (≥3-fold) in the Ca²⁺ baseline, mimicking Ry. THI and Ry, in combination, produced additive effects leading to uncoupling of IP₃/R and RyR₁ signals. THI altered ATP-mediated interleukin-6 secretion, initially enhancing the rate of cytokine secretion but suppressing cytokine secretion overall in DCs. DCs are exquisitely sensitive to THI, with one mechanism involving the uncoupling of positive and negative regulation of Ca²⁺ signals contributed by RyR₁. Key words: calcium, calcium channel, dendritic cell, erythroleukemia, interleukin-6, organic mercury, redox, thimerosal. Environ Health Perspect 114:1083–1091; 2006). doi:10.1289/ehp.8881 available via http://dx.doi.org/ [Online 21 March 2006]

Recent animal and human studies have underscored the strong influence of genetic, epigenetic, and physiological factors in defining susceptibility of the immune system to methylmercury (MeHg) and ethylmercury (EtHg) (Haviranad and Hultman 2005; Lawler et al. 2004; Silbergeld et al. 2005). Immune dysregulation triggered by organic mercury can include suppression, stimulation, loss of tolerance, and generation of autoantibodies. Therefore, the pattern of immunotoxicity induced by organic mercury is likely to depend not only on the chemical form, timing, and dose to which an individual is exposed but also on susceptibility factors that are poorly understood at present. Thus, significant attention is currently focused on identification of a few cases of accidental high-dose poisoning (Cinca et al. 1980; Damjulj 1962; Zhang 1984). Attention has been focused on THI in vaccines, where it is used as a preservative for multiuse formulations. THI was withdrawn from pediatric vaccines starting in 1999 (Centers for Disease Control and Prevention 1999) over concerns that organic mercury is a known neurodevelopmental toxicant. Nevertheless, THI is still used in influenza, diptheria toxoid, diphtheria toxoid and acellular pertussis (DT₃P), and tetanus toxoid vaccines. The hypothesis that THI can cause neurodevelopmental disorders was tested by injecting THI and THI-containing vaccines into inbred strains of young mice (Hornig et al. 2004). Growth, behav
Children with autism were found to have statistically elevated levels of urinary porphyrins that specifically show mercury toxicity due to environmental exposure. This was a large study of 106 children with autism compared to children with Asperger’s and control children. Neither the Asperger’s or control group showed elevations in urinary porphyrin levels.
A meta-analysis epidemiological assessment of neurodevelopmental disorders following vaccines administered from 1994 through 2000 in the United States

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Key words: developmental delay; mercury; merthiolate; thimerosal; thiomersal

Abstract

BACKGROUND: Thimerosal is an ethylmercury-containing compound (49.6% mercury by weight) used as at the preservative level in vaccines (0.005% to 0.01%).

METHODS: Statistical modeling in a meta-analysis epidemiological assessment of the Vaccine Adverse Event Reporting System (VAERS) for neurodevelopment disorders (NDS) reported following Diphtheria-Tetanus-whole-cell-Pertussis (DTP) vaccines in comparison to Diphtheria-Tetanus-whole-cell-Pertussis-Haemophilus Influenzae Type b (DTPH) vaccines (administered: 1994–1997) and following Thimerosal-containing Diphtheria-Tetanus-acellular-Pertussis (DTaP), vaccines in comparison to Thimerosal-free DTaP vaccines (administered: 1997–2000), was undertaken.

RESULTS: Significantly increased adjusted (sex, age, vaccine type, vaccine manufacturer) risks of autism, speech disorders, mental retardation, personality disorders, thinking abnormalities, ataxia, and NDS in general, with minimal systematic error or confounding, were associated with TCV exposure.

CONCLUSION: It is clear from the results of the present epidemiological study and other recently published data associating mercury exposure with childhood NDS, additional ND research should be undertaken in the context of evaluating mercury-associated exposures, especially from Thimerosal-containing vaccines.

Conflict of interests: Dr. Mark Geier has been an expert witness and consultant in vaccine cases before.

Metanalyses completed on the VAERS database show a statistically significant relationship between autism reports and the administration of thimerosal containing DTaP versus thimerosal free DTaP vaccines.
El timerosal y las enfermedades del neurodesarrollo infantil

Luis Maya 1,2, Flora Luna 2

Resumen
Se evalúa la relación causal entre el timerosal (etilmercurio), como preservante en las vacunas pediátricas, y el incremento de casos de enfermedades del neurodesarrollo infantil, como consecuencia de la ampliación de los esquemas de inmunización. Se revisó la información científica, relacionando el timerosal y las evidencias que permitan evaluar una posible asociación causal, con estudios epidemiológicos, ecológicos, biomoleculares y toxicológicos, de bioseguridad, toxicología fetofetal y sobre salud reproductiva. Se encontraron múltiples asociaciones entre la exposición al timerosal y las enfermedades del neurodesarrollo infantil. Tal toxicidad ocurre en los infantes y fetos de gestantes vacunadas por dosis acumulativa de mercurio. Las diversas evidencias implican al timerosal como el agente causante, agravando o disipando la exposición al timerosal del neurodesarrollo infantil. La toxicidad del mercurio obligó al retiro progresivo del timerosal de los medicamentos. Lamentablemente, en las vacunas, ha habido una sustancial demora en la demostración de su impacto negativo. Actualmente, existen vacunas sin timerosal, cuyo uso está ocasionando la disminución de la incidencia de las enfermedades del neurodesarrollo infantil.

Palabras clave: Timerosal; autismo; enfermedades del sistema nervioso; desarrollo infantil; vacunas.

Thimerosal and children’s neurodevelopmental disorders

Abstract
The causal relation of thimerosal (ethylmercury), preservative in pediatric vaccines, and the increase of children’s neurodevelopmental disorders as a result of the increase in immunization schemes is determined. The scientific information on thimerosal and its influence on the child’s neurodevelopmental disorders is reviewed. Evidences found in epidemiological, ecological, biomolecular toxicology, biosecurity, fetal toxicology and reproductive health studies signal the possible causal association of thimerosal exposure and neurodevelopmental disorders of the child. Such neurotoxicity occurs in infants and fetuses of vaccinated pregnant women, due to mercury cumulative doses. The various evidences imply thimerosal as the causal agent, aggravating or

This review article cites epidemiological, ecological, biomolecular, toxicology, fetal toxicology and reproductive health studies that signal the possible causal association of thimerosal exposure and the development of neurodevelopmental disorders in children.
Using VAERS data, the study authors show a downward trend in autism and speech delay reports following the phase out of thimerosal-containing vaccines in the U.S. but prior to the widespread use of thimerosal containing maternal and infant flu vaccines.
The present study demonstrates that micromolar concentrations of thimerosal significantly inhibit (*p<0.01) the transport of glutamate by the Na+-dependent astrocytic glutamate transporters GLAST (EAAC1) and GLT-1 (EAAC2). These data corroborate previous studies by Aschner et al. (1990), Albrecht et al. (1993), and Brooks and Kristt (1989), which showed that exposure to both inorganic and organic mercurials results in a significant decrease in glutamate uptake in primary cultures of rat and mouse cerebral cortical astrocytes. Overall, the study provides direct evidence for the potential of thimerosal to alter glutamate homeostasis in the CNS. Individuals across the spectrum of ASD have regionally specific abnormalities in subcortical glutamatergic neurotransmission that are associated with variation in social development (Horder, et al. 2013).
Large Brains in Autism: The Challenge of Pervasive Abnormality

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The most replicated finding in autism neuroanatomy—a tendency to unusually large brains—has seemed paradoxical in relation to the specificity of the abnormalities in three behavioral domains that define autism. We now know a range of things about this phenomenon, including that brains in autism have a growth spurt shortly after birth and then slow in growth a few short years afterward, that only younger but not older brains are larger in autism than in controls, that white matter contributes disproportionately to this volume increase and in a nonuniform pattern suggesting postnatal pathology, that functional connectivity among regions of autistic brains is diminished, and that neuroinflammation (including microgliosis and astrogliosis) appears to be present in autistic brain tissue from childhood through adulthood. Alongside these pervasive brain tissue and functional abnormalities, there have arisen theories of pervasive or widespread neural information processing or signal coordination abnormalities (such as weak central coherence, impaired complex processing, and underconnectivity), which are argued to underlie the specific observable behavioral features of autism. This convergence of findings and models suggests that a systems- and chronic disease-based reformulation of function and pathophysiology in autism needs to be considered, and it opens the possibility for new treatment targets. NEUROSCIENTIST 11(5):417-440; 2005. DOI: 10.1177/09127005278866

KEY WORDS Autism, Macrocephaly, Connectivity, Neuroinflammation, Complex processing, Brain

Autism is a developmental disorder defined behaviorally by a triad of abnormalities involving language, social interaction, and a marked lack of flexibility that may include repetitive or ritualistic behaviors (American Psychiatric Association, 1994); full criteria must be met by the age of three. The behavioral features of autism appear to be continuously distributed, and autism is part of a spectrum that also includes more mildly affected individuals (Dawson and others 2002).

Given that the atypical behaviors defining autism appear specifically characterizable, there has naturally been the expectation that we will find anatomical correlates for each feature of the behavioral phenotype. Indeed, there are findings in the limbic system and cerebellum (parts of the brain subserving functions that include some impaired in autism) that have been common (Cody and others 2002), yet they are troublingly not consistently encountered. Instead, the most replicated finding in autism, and one that has been found in multiple reliably characterized cohorts and artifact-free samples, has been that the brains are on average unusually large. This finding has had a paradoxical impact. On one hand, the consistency of an anatomical measure was an encouraging sign of convergence upon unraveling the neurobiology of this disorder. On the other hand, large brains did not make sense in terms of neural systems models of autism or brain-behavior correlations. How would such a generalized phenomenon relate to a disorder characterized by three specific classes of atypical behaviors? This conundrum has been sitting in the center of the autism field almost like a zen koan, awaiting a mental frame shift that would allow its obscure significance to become clear.

In the past few years, a series of discoveries about the autistic brain are appearing to converge toward a model that integrates biological, processing, and behavioral levels in autism. These discoveries potentially shed light on large brains regarding both underlying mechanisms and functional consequences. Moreover, these findings point toward a disease model that departs from earlier formulations of autism in having several new levels of potential treatment implications. The recent findings prominently include identification of pervasive volume scaling alterations, widespread reductions in connectivity and perfusion, and neuroinflammation and microgliosis that had previously been unappreciated. Identification of these features of the autistic brain for the most part was driven by investigation of tissue and

The author of this study links large brain size in autism with neuroinflammation. Neuroinflammation is a hallmark of inorganic mercury deposited in the brain tissue.
Comparison of Blood and Brain Mercury Levels in Infant Monkeys Exposed to Methylmercury or Vaccines Containing Thimerosal

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Thimerosal is a preservative that has been used in manufacturing vaccines since the 1930s. Reports have indicated that infants can receive ethylmercury (in the form of thimerosal) at or below the U.S. Environmental Protection Agency guidelines for methylmercury exposure, depending on the exact vaccinations, schedule, and size of the infant. In this study we compared the systemic disposition and brain distribution of total and inorganic mercury in infant monkeys after thimerosal exposure with those exposed to MeHg. Monkeys were exposed to MeHg (via oral gavage) or vaccines containing thimerosal (via intramuscular injection) at birth and 1, 2, and 3 weeks of age. Total blood Hg levels were determined 2, 4, and 7 days after each exposure. Total and inorganic brain Hg levels were assessed 2, 4, 7, or 28 days after the last exposure. The initial and terminal half-life of Hg in blood after thimerosal exposure was 21.5 days, which is significantly shorter than the elimination half-life of Hg after MeHg exposure at 21.5 days. Brain concentrations of total Hg were significantly lower by approximately 3-fold for the thimerosal-exposed monkeys when compared with the MeHg infants, whereas the average brain-to-blood concentration ratio was slightly higher for the thimerosal-exposed monkeys (3.5 ± 0.5 vs. 2.5 ± 0.3). A higher percentage of the total Hg in the brain was in the form of inorganic Hg for the thimerosal-exposed monkeys (34% vs. 7%). The results indicate that MeHg is not a suitable reference for risk assessment from exposure to thimerosal-derived Hg. Knowledge of the toxicokinetics and developmental toxicity of thimerosal is needed to afford a meaningful assessment of the developmental effects of thimerosal-containing vaccines. Key words: brain and blood distribution, elimination half-life, ethylmercury, infant nonhuman primates, methylmercury, thimerosal. Environ Health Perspect 113:1015–1021 (2005). doi:10.1289/ehp.7712 available via http://dx.doi.org/[Online 21 April 2005]

Some cases, exceeds the U.S. EPA guidelines for MeHg exposure during pregnancy (0.1 µg/kg/day). Other estimates (Halsey 1995) have indicated that the schedule could provide repeated doses of ethylmercury from approximately 5 to 20 µg/kg over the first 6 months of life. Studies in preterm infants indicate that blood levels of Hg after just one vaccination (hepatitis B) increase by > 10-fold overestimate Hg in the brain after exposure to ethylmercury and the risk of brain damage is less for ethylmercury than for MeHg. These conclusions are based on only a few studies, none of which included measurements of both blood and brain Hg levels in infant subjects.

We initiated the present study in order to directly compare the blood and brain levels of Hg in infant nonhuman primates exposed orally to MeHg or via intramuscular (im) injections of vaccines containing thimerosal. Nonhuman primates have been used extensively in previous studies of MeHg toxicokinetics and developmental neurotoxicity (Burbacher et al. 1986, 1990b; Gunderson et al. 1986, 1988; Rice and Gilbert 1982, 1990, 1995; Sinon et al. 1989; Vilter et al. 1994, 1995). The routes of administration (oral for MeHg and im injection for thimerosal-containing vaccines) were chosen to mimic the two routes of Hg exposure for humans. The dosages and schedule of administration of Hg were chosen to be comparable with the current immunization schedule for human newborns, taking into consideration the faster growth (~ 4 to 1) of the macaque infant (Gunderson and Sackett 1984). The results of the present study provide important new information regarding the comparative toxicokinetics of these two compounds in newborns and infants.

Infant macaques retained significantly higher levels of inorganic mercury in their brain tissue when exposed to levels of thimerosal resulting from exposure to in infant vaccines versus methylmercury from fish. The half-life of the inorganic mercury resulting from thimerosal exposure was indefinite, as it lasted much longer than the overall testing period. The research literature cites a half-life of 27 years. Inorganic mercury in the brain of adult non-human primates has been associated with an ongoing neuroinflammatory process that is also well documented in brain tissue of individuals with autism.
Thimerosal induces neuronal cell apoptosis by causing cytochrome c and apoptosis-inducing factor release from mitochondria

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Abstract. There is a worldwide increasing concern over the neurological risks of thimerosal (ethylmercury thiosalicylate) which is an organic mercury compound that is commonly used as an antimicrobial preservative. In this study, we show that thimerosal, at nanomolar concentrations, induces neuronal cell death through the mitochondrial pathway. Thimerosal, in a concentration- and time-dependent manner, decreased cell viability as assessed by calcein-ethidium staining and caused apoptosis detected by Hoechst 33258 dye. Thimerosal-induced apoptosis was associated with depolarization of mitochondrial membrane, generation of reactive oxygen species, and release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria to cytosol. Although thimerosal did not affect cellular expression of Bax at the protein level, we observed translocation of Bax from cytosol to mitochondria. Finally, caspase-9 and caspase-3 were activated in the absence of caspase-8 activation. Our data suggest that thimerosal causes apoptosis in neuroblastoma cells by changing the mitochondrial microenvironment.

Introduction

Apoptosis is a physiological form of cell suicide that plays a role in embryogenesis, metamorphosis, cellular homeostasis, and as a defensive mechanism to remove infected, mutated, or damaged cells (1,2). Apoptosis is characterized by loss of cellular contact with the matrix, cytoplasmic contraction, chromatin condensation, plasma membrane blebbing, and DNA fragmentation into large and small oligosomes. Apoptosis takes place through the death receptors (3-6) and/or involvement of the mitochondrial pathway (7,8), with molecular and biochemical steps leading to the activation of common effector or executioner cysteine proteases, the caspases resulting in the cleavage of a number of nuclear and cytoplasmic substrates that culminate in apoptosis. Because of the role of apoptosis in cellular homeostasis, disorders of apoptosis result in either the accumulation of abnormal cells, leading to cancer and autoimmunity, or in the loss of cells, leading to immunodeficiency and neurodegenerative diseases (9).

There is an increasing concern throughout the world about the risks of environmental exposure to mercury, which is ubiquitously found in fish, dental amalgams, and in preservatives (10-17). One of the mercury compounds that has recently come to public attention, because of its wide usage as an antibacterial and antifungal preservative in biomedical products and vaccines, is thimerosal (10-12). Thimerosal (ethylmercury salicylate) contains 49.6% mercury by weight and is metabolized to ethylmercury and thiosalicylate (15). In the body, ethylmercury readily passes through cellular membranes and concentrates in vital tissue and organs, including the central nervous system where it can exert its toxicity over a prolonged period of time (12,16). However, the effects of thimerosal on neuronal cell functions, especially on apoptosis, are poorly understood and largely unexplored.

During the last decade, there has been a better understand-
The study authors report a mechanism for human cell death from accumulation of organic and inorganic mercury in the mitochondria. This was followed by cytochrome c leakage from the mitochondria and caspase 9 activation, which induces mitochondrial depolarization. Mitochondrial dysfunction is also a known co-morbid condition associated with autism.
This study investigated the cellular response to thimerosal toxicity. The authors reported a very profound decrease in intracellular glutathione levels. Earlier research by this same author showed that autistic children had significantly lower glutathione levels as compared to neurotypical control children. Glutathione is the body's natural defense mechanism critical in removing environmental toxicants. Low levels of glutathione in children with autism make them more vulnerable to injury from mercury exposure.
Children with autism have a diminished methylation capacity leading to higher sustained levels of oxidative stress, due to deficiencies primarily in glutathione. Thimerosal containing vaccines produce a very high level of oxidative stress in the body upon administration. Glutathione is essential in processing and effectively removing mercury from the body. Low levels of glutathione put children with autism at increased risk for neurological injury from exposure to mercury.
This study shows that a novel growth factor signalling pathway regulates methionine synthase (MS) activity and thereby modulates methylation reactions. The potent inhibition of this pathway by ethanol, lead, mercury, aluminum and thimerosal suggests that it may be an important target of these neurodevelopmental toxins. Abnormalities in the methionine synthase pathway have previously been implicated in the development of autism.
Neurotoxic effects of postnatal thimerosal are mouse strain dependent

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The developing brain is uniquely susceptible to the neurotoxic hazard posed by mercurials. Host differences in maturation, metabolism, nutrition, sex, and autoimmunity influence outcomes. How population-based variability affects the safety of the ethylmercury-containing vaccine preservative, thimerosal, is unknown. Reported increases in the prevalence of autism, a highly heritable neuropsychiatric condition, are intensifying public focus on environmental exposures such as thimerosal. Immune profiles and family history in autism are frequently consistent with autoimmunity. We hypothesized that autoimmune propensity influences outcomes in mice following thimerosal challenge that mimic routine childhood immunizations. Autoimmune disease-sensitive SJL/J mice showed growth delay; reduced locomotion; exaggerated response to novelty; and densely packed, hyperchromic hippocampal neurons with altered glutamate receptors and transporters. Strains resistant to autoimmunity, C57BL/6J and BALB/cJ, were not susceptible. These findings implicate genetic influences and provide a model for investigating thimerosal-related neurotoxicity.

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Keywords: autistic disorder; thimerosal; neurotoxicity; autoimmunity; inbred mouse strains

As autism spectrum disorders (ASDs) comprise a set of highly heritable conditions with core impairments in social interaction, communication, and imagination. The prevalence of ASDs is reported to be rising worldwide,²⁵ an increase not fully explained by changes in awareness and diagnostic patterns.²³ Environmental susceptibility genes may be determinants of adverse neurodevelopmental outcomes following prenatal or postnatal exposures. One environmental factor may be increased mercury burden through industrial sources, fish, and sodium mercury-related neurodevelopmental damage, we exposed mice of differing MHC (H-2) backgrounds to thimerosal in doses and timing equivalent to the pediatric immunization schedule. Profound behavioral and neuropathologic disturbances were observed after postnatal thimerosal in SJL/J (H-2k) mice, but not in strains without autoimmune sensitivity (BALB/cJ, H-2¹, or C57BL/6J, H-2¹ mice).

Materials and methods

Specific mouse strains showing autoimmune disease sensitivity exhibited autistic behaviors and autistic-like brain pathologies after being exposed to thimerosal. A comparison mouse strain without a genetic predisposition to autoimmunity did not exhibit these abnormal behaviors or neurological features after thimerosal exposure. This study supports the notion of a genetic susceptibility to thimerosal resulting in neurological injury. It has also been hypothesized that a subset of children may be more vulnerable to exposure to thimerosal which is why not all children exposed are harmed.
The study authors investigated Biological Surveillance Summaries of the Centers for Disease Control and Prevention (CDC), the U.S. Department of Education datasets, and the CDC's yearly live birth estimates to look at the prevalence of autism in the 1980s and 90s. This study showed that autism rates correlated with increases in uptake of thimerosal containing vaccines as compared to a baseline level in 1984.
Reduced Levels of Mercury in First Baby Haircuts of Autistic Children

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Reported rates of autism have increased sharply in the United States and the United Kingdom. One possible factor underlying these increases is increased exposure to mercury through thimerosal-containing vaccines, but vaccine exposures need to be evaluated in the context of cumulative exposures during gestation and early infancy. Differential rates of postnatal mercury elimination may explain why similar gestational and infant exposures produce variable neurological effects. First baby haircut samples were obtained from 94 children diagnosed with autism using Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM IV) criteria and 45 age- and gender-matched controls. Information on diet, dental amalgam fillings, vaccine history, Rho D immunoglobulin administration, and autism symptom severity was collected through a maternal survey questionnaire and clinical observation. Hair mercury levels in the autistic group were 0.47 ppm versus 3.63 ppm in controls, a significant difference. The mothers in the autistic group had significantly higher levels of mercury exposure through Rho D immunoglobulin injections and amalgam fillings than control mothers. Within the autistic group, hair mercury levels varied significantly across mildly, moderately, and severely autistic children, with mean group levels of 0.79, 0.46, and 0.21 ppm, respectively. Hair mercury levels among controls were significantly correlated with the number of the mothers' amalgam fillings and their fish consumption as well as exposure to mercury through childhood vaccines, correlations that were absent in the autistic group. Hair excretion patterns among autistic infants were significantly reduced relative to control. These data cast doubt on the efficacy of traditional hair analysis as a measure of total mercury exposure in a subset of the population. In light of the biological plausibility of mercury's role in neurodevelopmental disorders, the present study provides further insight into one possible mechanism.

Autism has been defined by symptoms rather than causes since it was first characterized by Kanner in the 1940s (Eisenberg and Kanner 1956). Since Rutter's (Rutter 1978) further elaboration of diagnostic standards in 1976, the prevailing standards for diagnosis (Diagnostic and Statistical Manual of Mental Disorders, 3rd edition [DSM III] 1980; 3rd edition—revised [DSM-III-R] 1987; 4th edition [DSM IV] 1994) have included impairment in three domains: social relatedness, communication, and behavior. In a small number of cases, either genetic (Wahlstrom et al. 1986; Bolton et al. 2002; Steffenburg et al. 1996) or environmental (Stromland et al. 1994; Williams and Hersh 1997; Aronson, Hagberg, and Gillberg 1997) causes have been established, but the vast majority of cases remain idiopathic.

The need to account for the relative contribution of genetic and environmental causes has taken on increased importance in light of possible sharp increases in the incidence of autism. Early prevalence studies in the United States (Burd, Fisher, and Kerbeshian 1987; Treffert 1970; Ritvo et al. 1989) and the United Kingdom (Lotter 1966; Wing and Gould 1979; Deb and Prasad 1994) reported low rates of autism—generally less than 5 per 10,000—among children born before 1990. Studies of populations born in the 1990s, however, show far higher (Bertrand et al. 2001; Baird et al. 2000) and increasing (Department of Developmental Services 1999; Kaye, del Melero-Montes, and Jick 2001; Taylor et al. 1999) rates of autism and autism spectrum disorders (ASDs), in some cohorts as high as 55 per 10,000 for autism and 80 per 10,000 for ASDs.

This study shows that autistic children are poor excreters of mercury via hair, which is a normal physiological mode of mercury detoxification. Thus, autistic children subjected to mercury exposure would likely experience a longer, sustained toxicological effect. Demographic data from this investigation also found a highly significant findings (p<0.0002) that 43 out of 94 (46%) of the mothers of children with autism had received Rho-D products during the pregnancy compared to only 4 out of 34 (12%) of the control mothers. A majority of these products contained thimerosal.
The study authors found that thimerosal exposure at micromolar levels initiates a cascade of events leading to cell death in human neurons and fibroblasts. Cell death is preceded by DNA breakage, caspase-3 activation and mitochondrial membrane depolarization. The levels of thimerosal exposure in this study were less than four times the levels received that were administered to infants up to 6 months of age receiving the CDC recommended schedule of vaccines in the 1990's.
Overall mercury burden, determined by urinary mercury concentrations following a chelation challenge, was significantly higher in children with autism as compared to matched non-autistic control children. The mercury burden in vaccinated autistic children was 590% greater than in vaccinated non-autistic controls. This difference was statistically significant.
In this case study, an 11-month-old boy regressed into a syndrome with autism-like features after exposure to elemental mercury via a broken thermometer. Symptoms improved after he was treated for acrodynia (a mercury induced illness) with chelation therapy and by two years of age, he was considered to be developmentally normal.
Neurodevelopmental Disorders after Thimerosal-Containing Vaccines: A Brief Communication

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In a study of the VAERS database, incidence of autism reports was studied after the administration of thimerosal-containing versus thimerosal-free DTaP vaccines. The relative risk in this comparison was 6.0 and was highly statistically significant. Mental retardation and speech delays were also significantly correlated to thimerosal exposure via the DTaP vaccine.

We were initially highly skeptical that differences in the concentrations of thimerosal in vaccines would have any effect on the incidence rate of neurodevelopmental disorders after childhood immunization. This study presents the first epidemiologic evidence, based upon tens of millions of doses of vaccine administered in the United States, that associates increasing thimerosal from vaccines with neurodevelopmental disorders. Specifically, an analysis of the Vaccine Adverse Events Reporting System (VAERS) database showed statistical increases in the incidence rate of autism (relative risk [RR] = 6.0), mental retardation (RR = 6.1), and speech disorders (RR = 2.2) after thimerosal-containing diphtheria, tetanus, and acellular pertussis (DTaP) vaccines in comparison with thimerosal-free DTaP vaccines. The male/female ratio indicated that autism (17) and speech disorders (2.3) were reported more in males than females after thimerosal-containing DTaP vaccines, whereas mental retardation (1.2) was more evenly reported among male and female vaccine recipients. Controls were employed to determine if biases were present in the data, but none were found.

It was determined that overall adverse reactions were reported in similar-aged populations after thimerosal-containing DTaP (2.4 ± 3.2 years old) and thimerosal-free DTaP (2.1 ± 2.8 years old) vaccinations. Acute control adverse reactions such as deaths (RR = 1.0), vasculitis (RR = 1.2), seizures (RR = 1.6), ED visits (RR = 1.4), total adverse reactions (RR = 1.4), and gastroenteritis (RR = 1.1) were reported similarly after thimerosal-containing and thimerosal-free DTaP vaccines. An association between neurodevelopmental disorders and thimerosal-containing DTaP vaccines was found, but additional studies should be conducted to confirm and extend this study. Exp Biol Med 228:660-664, 2003

Key words: autism; neurodevelopmental disorders; thimerosal; VAERS

In recent years, thimerosal, an organic mercury compound that is metabolized to ethylmercury and thiosalicylate and has been present since the 1930s as a preservative in some vaccines and pharmaceutical products to prevent bacterial and fungal contamination, has come under scrutiny. It was determined by the U.S. Food and Drug Administration (FDA) in 1999 under the recommended childhood immunization schedule that infants might be exposed to cumulative doses of ethylmercury that exceed some federal safety guidelines established for exposure to methylmercury, another form of organic mercury (1).

The hypothesis that exposure to thimerosal-containing vaccines could be associated with neurodevelopmental disorders is not established and rests on indirect and incomplete information, primarily from analogies with methylmercury and levels of maximum mercury exposure from vaccines given in children. The hypothesis is biologically possible, but the possible relationship between thimerosal from vaccines and neurodevelopmental disorders of autism, attention deficit/hyperactivity disorder (ADHD), and speech or language delay remains seriously suspect. As of the present, there are no peer-reviewed epidemiological studies in the scientific literature examining the potential association between thimerosal-containing vaccines and neurodevelopmental disorders. Here, we show the first epidemiologic evidence, based upon tens of millions of doses of vaccine administered in the United States, that associates increasing thimerosal from vaccines with neurodevelopmental disorders.

Materials and Methods

In this study, the incidence of neurodevelopmental disorders in a comparative examination between thimerosal-containing and thimerosal-free DTaP vaccine use was studied.
The study found “little” difference between methylmercury (from eating fish and environmental exposures) and ethylmercury (breakdown product of thimerosal) toxicity to cells counter to CDC sponsored studies that declared that ethylmercury was the “safe mercury” in contrast to methyl mercury.
This study shows that thimerosal causes cell death in T lymphocytes (immune cells) via a mitochondrial depolarization mechanism. Mitochondrial depolarization is a hallmark of mitochondrial dysfunction which affects up to 30% of children with autism.
This paper reports that traits of autism spectrum disorders are known to arise from mercury exposure, onset of autistic symptoms are temporally associated with administration of mercury containing vaccines, the increasing prevalence of autism follows the introduction of of mercury containing vaccines in infants, and elevated levels of mercury have been documented in autistic children.
The researchers report that several dozen signs and symptoms of mercury poisoning were identical to the symptoms used to define and diagnose autism. A comprehensive analysis is included on the comorbidities of autism and their corresponding analogs due to mercury exposure.
This study investigated prenatal, perinatal and postnatal factors that might contribute to autism. One of the significant findings was a higher than expected incidence of Rh negative blood type in the mothers of the children diagnosed with autism. A vast majority of Rho(D) immune globulins administered to women with Rh negative blood type during pregnancy contained thimerosal.
Verstraeten, Thomas M., MD, NIP, Division of Epidemiology and Surveillance, Vaccine Safety and Development Branch, Mailstop E-61, 770-639-8327.
EIS Class Year of Entry: 1999
No previous EIS Conference presentations
Mackel Award consideration: No
Number of abstracts submitted: 2, priority this abstract: 1
Strong preference for poster presentation: No

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*Increased risk of developmental neurologic impairment after high exposure to thimerosal-containing vaccine in first month of life.*

**Background:** Concern has risen on the presence of the ethylmercury containing preservative thimerosal in vaccines. We assessed the risk for neurologic and renal impairment associated with past exposure to thimerosal-containing vaccine using automated data from the Vaccine Safety Datalink (VSD). VSD is a large linked database from four health maintenance organizations in Washington, Oregon and California, containing immunization, medical visit and demographic data on over 400,000 infants born between ’91 and ’97.

**Methods:** We categorized the cumulative ethylmercury exposure from thimerosal containing vaccines after one month of life and assessed the subsequent risk of degenerative and developmental neurologic disorders and renal disorders before the age of six. We applied proportional hazard models adjusting for HMO, year of birth, and gender, excluding premature babies.

**Results:** We identified 286 children with degenerative and 3702 with developmental neurologic disorders, and 310 with renal disorders. The relative risk (RR) of developing a neurologic development disorder was 1.8 (95% confidence intervals [CI] = 1.1-2.8) when comparing the highest exposure group at 1 month of age (cumulative dose > 25 ug) to the unexposed group. Within this group we also found an elevated risk for the following disorders: autism (RR 7.6, 95% CI = 1.8-31.5), nonorganic sleep disorders (RR 5.0, 95% CI = 1.6-15.9), and speech disorders (RR 2.1, 95% CI =1.1-4.0). For the neurologic degenerative and renal disorders group we found no significantly increased risk or a decreased risk.

**Conclusion:** This analysis suggests that high exposure to ethylmercury from thimerosal-containing vaccines in the first month of life increases the risk of subsequent development of neurologic development impairment, but not of neurologic degenerative or renal impairment. Further confirmatory studies are needed.

This original version of the Verstraeten et al. paper shows a 7.6-fold risk of an autism diagnosis for children exposed to thimerosal in the first month of life compared to unexposed controls. Prior to the final publication of the paper statistical methods were altered which resulted in a decrease in the statistical power of the study to detect associations. The author of the paper labeled the study results as being neutral and called for more research. This study is often touted by the CDC as being evidence that thimerosal exposure is not associated with autism.