Children's
Health Defense

Peer-Reviewed, Published Research Showing Adverse Effects of Mercury

The literature showing the toxicity of mercury goes well beyond its associations with autism. This document includes the abstracts for 89 studies that show the harmful effects of mercury, from both thimerosal and environmental sources, on brain cells, immune cells and other body systems. These include cellular, animal and human studies. There can be no justification for any intentional use of mercury given the extent of this literature.

The following pages are abstracts from the peer reviewed 89 studies.

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Research Article

Assessment of Hair Aluminum, Lead, and Mercury in a Sample of Autistic Egyptian Children: Environmental Risk Factors of Heavy Metals in Autism

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Background and Aims. The etiological factors involved in the etiology of autism remain elusive and controversial, but both genetic and environmental factors have been implicated. The aim of this study was to assess the levels and possible environmental risk factors and sources of exposure to mercury, lead, and aluminum in children with autism spectrum disorder (ASD) as compared to their matched controls. *Methods.* One hundred ASD children were studied in comparison to 100 controls. All participants were subjected to clinical evaluation and measurement of mercury, lead, and aluminum through hair analysis which reflects past exposure. *Results.* The mean Levels of mercury, lead, and aluminum in hair of the autistic patients were significantly higher than controls. Mercury, lead, and aluminum levels were positively correlated with maternal fish consumptions, living nearby gasoline stations, and the usage of aluminum pans, respectively. *Conclusion.* Levels of mercury, lead, and aluminum in the hair of autistic children are higher than controls. Environmental exposure to these toxic heavy metals, at key times in development, may play a causal role in autism.

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.

A Case-Control Study of Mercury Burden in Children with Autistic Spectrum Disorders

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ABSTRACT

Large autism epidemics have recently been reported in the United States and the United Kingdom. Emerging epidemiologic evidence and biologic plausibility suggest an association between autistic spectrum disorders and mercury exposure.

This study compares mercury excretion after a three-day treatment with an oral chelating agent, meso-2,3-dimercaptosuccinic acid (DMSA), in children with autistic spectrum disorders and a matched control population. Overall, urinary mercury concentrations were significantly higher in 221 children with autistic spectrum disorders than in 18 normal controls (Relative Increase (RI)=3.15; $P < 0.0002$). Additionally, vaccinated cases showed a significantly higher urinary mercury concentration than did vaccinated controls (RI=5.94; $P < 0.005$). Similar urinary mercury concentrations were observed among matched vaccinated and unvaccinated controls, and no association was found between urinary cadmium or lead concentrations and autistic spectrum disorders.

The observed urinary concentrations of mercury could plausibly have resulted from thimerosal in childhood vaccines, although other environmental sources and thimerosal in Rh_o(D) immune globulin administered to mothers may be contributory.

Regardless of the mechanism by which children with autistic spectrum disorders have high urinary mercury concentrations, the DMSA treatment described in this study might be useful to diagnose their present burden of mercury.

KEY WORDS: autism, autistic spectrum disorders, chelation, DMSA, mercury, thimerosal

Background

Recent studies have analyzed the prevalence of autism from the mid-1980s through 2002 in the United States and the United Kingdom.[1-5] The prevalence of autism is estimated to have risen from one in about 2,500 children in the mid-1980s to as common as

link between exposure to mercury from thimerosal contained in childhood vaccines and neurodevelopment disorders.[7-9]

The purpose of this study was to evaluate the concentration of mercury in the urine following a three-day treatment with an oral chelating agent, meso-2,3-dimercaptosuccinic acid (DMSA), in children with autistic spectrum disorders in comparison to a control population. Forman et al.[10] have reported on the use of oral treatment with DMSA in children exposed to metallic mercury. The authors found that oral chelation with DMSA produced a significant mercury diuresis in these children. They observed no adverse side effects of treatment. The authors concluded that DMSA appears to be an effective and safe chelating agent for treatment of pediatric overexposure to metallic mercury. In addition, extensive literature supports its safety in the chelation of lead from exposed children.

Methods

This study is a retrospective analysis of 221 consecutive children with previously established autism spectrum disorders referred and admitted to the International Child Development Resource Center (ICDRC). Each child had been diagnosed with autism (DSM-IV 299.00) or pervasive developmental disorder (DSM-IV 299.80) by outside physicians. A control population of 18 children was also identified without autism spectrum disorders in themselves or among their siblings or their first-degree family members. These healthy children presented to the ICDRC for elective determination of their levels of environmental mercury exposure at the request of their families, and are included here for case comparison. The Arizona State University Institutional Review Board approved our retrospective examination of cases and controls in this study.

All children were examined to exclude those who had dental amalgams. Among the 221 cases, all had received their full scheduled childhood immunizations appropriate for their respective ages. Among the 18 controls, 10 children had received their full childhood immunization schedules, and 8 children had received no childhood immunizations because of religious objections.

Informed consent was obtained from both cases and controls for DMSA chelation treatment. Controls and cases were both challenged with a three-day oral treatment of DMSA (10 mg/kg per

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.

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ORIGINAL ARTICLE

The levels of blood mercury and inflammatory-related neuropeptides in the serum are correlated in children with autism spectrum disorder

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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

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Introduction

Neurogenic inflammation is a neurally mediated immune inflammation that is orchestrated by a large number of neuro-

Blood mercury levels and tachykinins (neuropeptides that cause inflammation) were correlated in children with ASD and statistically significantly higher than neurotypical control children. It has been shown that mercury exposure can elicit tachykinin formation which has been implicated in neuroinflammatory disorders including autism.

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Article

Hair Toxic Metal Concentrations and Autism Spectrum Disorder Severity in Young Children

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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.

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Research Article

The Severity of Autism Is Associated with Toxic Metal Body Burden and Red Blood Cell Glutathione Levels

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This study investigated the relationship of children's autism symptoms with their toxic metal body burden and red blood cell (RBC) glutathione levels. In children ages 3–8 years, the severity of autism was assessed using four tools: ADOS, PDD-BI, ATEC, and SAS. Toxic metal body burden was assessed by measuring urinary excretion of toxic metals, both before and after oral dimercaptosuccinic acid (DMSA). Multiple positive correlations were found between the severity of autism and the urinary excretion of toxic metals. Variations in the severity of autism measurements could be explained, in part, by regression analyses of urinary excretion of toxic metals before and after DMSA and the level of RBC glutathione (adjusted R^2 of 0.22–0.45, $P < .005$ in all cases). This study demonstrates a significant positive association between the severity of autism and the relative body burden of toxic metals.

1. Background

Autism is a severe developmental disorder which involves social withdrawal, communication deficits, and stereotypic/repetitive behaviour. The pathophysiological etiologies which precipitate autism symptoms remain elusive and con- of autism. A small study by Adams et al. [4] found that children with autism had a 2-time higher level of mercury in their baby teeth than typical children. A study by Bradstreet et al. [5] investigated the body burden of toxic metals by giving dimercaptosuccinic acid (DMSA), an oral chelation medication approved by the FDA for treating infantile lead

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.

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Mercury, Lead, and Zinc in Baby Teeth of Children with Autism Versus Controls

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This study determined the level of mercury, lead, and zinc in baby teeth of children with autism spectrum disorder (n = 15, age 6.1 ± 2.2 yr) and typically developing children (n = 11, age = 7 ± 1.7 yr). Children with autism had significantly (2.1-fold) higher levels of mercury but similar levels of lead and similar levels of zinc. Children with autism also had significantly higher usage of oral antibiotics during their first 12 mo of life, and possibly higher usage of oral antibiotics during their first 36 mo of life. Baby teeth are a good measure of cumulative exposure to toxic metals during fetal development and early infancy, so this study suggests that children with autism had a higher body burden of mercury during fetal/infant development. Antibiotic use is known to almost completely inhibit excretion of mercury in rats due to alteration of gut flora. Thus, higher use of oral antiobiotics in the children with autism may have reduced their ability to excrete mercury, and hence may partially explain the higher level in baby teeth. Higher usage of oral antibiotics in infancy may also partially explain the high incidence of chronic gastrointestinal problems in individuals with autism.

Autism is a severe developmental disorder that involves social withdrawal, communication deficits, and stereotypic/

including especially language/communication problems and social withdrawal. Therefore, they suggested that autism was primarily due to infantile exposure to mercury. Their hypothesis is plausible because mercury exposure at hazardous levels is common in the United States and other countries; the Food and Drug Administration (FDA) estimates that 1 in 6 women in the United States have mercury levels that increase the risk of neurological damage to their children. (Mahaffey et al., 2004) The major sources of mercury exposure for infants are (1) maternal seafood consumption, (2) maternal mercury amalgam dental fillings, and (3) thimerosal (an ethylmercury compound) in childhood vaccines and in anti-RhoD immune globulins given to Rh-negative mothers during pregnancy. Thimerosal was largely but not totally removed from childhood vaccines by 2004.

Mercury toxicity might occur either due to high exposure, or due to a decreased ability to excrete mercury, with the latter case seeming to be the primary issue in autism. The primary mechanism for excreting mercury involves its binding to glutathione and then being excreted in the bile (Ballatori & Clarkson, 1985). Infants are poor excretors because they produce less glutathione (Ballatori & Clarkson, 1984) and because they are

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.

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ORIGINAL PAPER

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 ($P < 0.001$) decreased plasma reduced GSH, plasma cysteine, plasma taurine, plasma sulfate, and plasma free sulfate relative to controls. By contrast, 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

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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

This study focuses on biomarkers associated with mercury detoxification. Autistic patients showed metabolic profiles consistent with increased oxidative stress (including increased levels of oxidized glutathione) and decreased capacity to detoxify mercury as compared to matched controls. Such findings are consistent with prior or ongoing mercury exposure and a reduced capacity to effectively excrete mercury from the body effectively.

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ORIGINAL ARTICLE

Altered urinary porphyrins and mercury exposure as biomarkers for autism severity in Egyptian children with autism spectrum disorder

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Abstract Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that affects social, communication, and behavioral development. Recent evidence supported but also questioned the hypothetical role of compounds containing mercury (Hg) as contributors to the development of ASD. Specific alterations in the urinary excretion of porphyrin-containing ring catabolites have been associated with exposure to Hg in ASD patients. In the present study, the level of urinary porphyrins, as biomarkers of Hg toxicity in children with ASD, was evaluated, and its correlation with severity of the autistic behavior further explored. A total of 100 children was enrolled in the present study. They were classified into three groups: children with ASD (40), healthy controls (40), and healthy siblings of the ASD children (20). Children with ASD were diagnosed using DSM-IV-TR, ADI-R, and CARS tests. Urinary porphyrins were evaluated within the three groups using high-performance liquid chromatography (HPLC), after plasma evaluation of mercury (Hg) and lead (Pb) in the same groups. Results showed that children with ASD had significantly higher levels of Hg, Pb, and the porphyrins pentacarboxyporphyrin, coproporphyrin, precoproporphyrin, uroporphyrins, and hexacarboxyporphyrin compared to healthy controls and healthy siblings of the ASD children. However, there was no significant statistical difference in the level of heptacarboxyporphyrin among the three groups, while a significant positive correlation between the levels of coproporphyrin and precoproporphyrin and autism severity was observed. Mothers of ASD children showed a higher percentage of dental amalgam restorations compared to the mothers of healthy controls suggesting that high Hg levels in children with ASD may relate to the increased exposure to Hg from maternal dental amalgam during pregnancy and lactation. The results showed that the ASD children in the present study had increased blood Hg and Pb levels compared with healthy control children indicating that disordered porphyrin metabolism might interfere with the pathology associated with the autistic neurologic phenotype. The present study indicates that coproporphyrin and precoproporhyrin may be utilized as possible biomarkers for heavy metal exposure and autism severity in children with ASD.

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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 buildup in the body of excess levels of specific porphyrins. The porphyrins for mercury toxicity also correlated with autism severity in ASD patients.

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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), heptacarboxyporphyrin (7cxP), hexacarboxyporphyrin (6cxP), pentacarboxyporphyrin (5cxP), precoproporphyrin (prcP), and coproporphyrin (cP). Results showed significantly elevated 6cxP, prcP (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.

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Porphyrinuria in childhood autistic disorder: Implications for environmental toxicity

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Abstract

To address a possible environmental contribution to autism, we carried out a retrospective study on urinary porphyrin levels, a biomarker of environmental toxicity, in 269 children with neurodevelopmental and related disorders referred to a Paris clinic (2002–2004), including 106 with autistic disorder. Urinary porphyrin levels determined by high-performance liquid chromatography were compared between diagnostic groups including internal and external control groups. Coproporphyrin levels were elevated in children with autistic disorder relative to control groups. Elevation was maintained on normalization for age or to a control heme pathway metabolite (uroporphyrin) in the same samples. The elevation was significant ($P < 0.001$). Porphyrin levels were unchanged in Asperger's disorder, distinguishing it from autistic disorder. The atypical molecule precoproporphyrin, a specific indicator of heavy metal toxicity, was also elevated in autistic disorder ($P < 0.001$) but not significantly in Asperger's. A subgroup with autistic disorder was treated with oral dimercaptosuccinic acid (DMSA) with a view to heavy metal removal. Following DMSA there was a significant ($P = 0.002$) drop in urinary porphyrin excretion. These data implicate environmental toxicity in childhood autistic disorder.

Keywords: Autism; Asperger; Porphyrin; Mercury; Pervasive Developmental Disorder

Introduction

Autism is a disorder of reciprocal social interaction, behavioral repertoire, and language and communication. Because the phenotype ranges from manifest disability to specific performance elevation, the term Autistic Spectrum Disorder (ASD) (Wing, Barbaresi et al., 2005), as reviewed (Blaxill, 2004), is suggestive of an environmental contribution. Changes in awareness and diagnostic criteria may explain some of the rise (Croen et al., 2002; Rutter, 2005), but a true increase in prevalence has not been excluded (Rutter, 2005). Elevated ASD rates in urban versus rural areas (Deb and Prasad, 1994; Palmer et al., 2006; Williams et al.,

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.

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Original article

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.

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INTRODUCTION

Autism is a severe neurodevelopmental disorder characterized by impaired communication, social interaction and imagination that is often accompanied by repetitive and stereotyped behavior[1]. It develops before the 36 month of age and persists into adulthood causing life long disability[2]. The prevalence of autism has surged in

pediatric vaccine preservative thimerosal; (2) methyl mercury, is most commonly the result of consumption of contaminated food, particularly fish; (3) inorganic Hg, through the use of topical Hg-based skin creams and in infant teething powders; (4) metalic Hg in dental amalgams, which release Hg vapors[6]. In 2006, Palmer and associates[7] reported that for each 1.000 lb of environmentally released Hg, there was a 61% increase in the rate of

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.

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A Prospective Study of Mercury Toxicity Biomarkers in Autistic Spectrum Disorders

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Porphyrins are derivatives formed in the heme synthesis pathway and porphyrins 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 (5cxP), and precoproporphyrin (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, 5cxP, 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 5cxP 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.

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Porphyrins 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.

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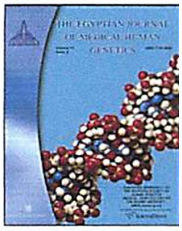

ORIGINAL ARTICLE

Hair mercury measurement in Egyptian autistic children

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KEYWORDS
Autism;
Mercury;
Hair analysis;
Toxic metals

Abstract *Background:* A review of medical literature has shown that exposure to mercury, whether organic or inorganic, can give rise to the symptoms and traits defining or commonly found in autism spectrum disorders (ASD). Mercury can cause impairments in social interaction, communication difficulties, and repetitive and stereotyped patterns of behavior, which comprise the three DSM-IV diagnostic criteria of autism. The aim of this work was to measure the concentration of total mercury trace elements in the hair of some Egyptian autistic children and to correlate these levels with severity of the disease.

Methods: Thirty- two patients diagnosed by DSM-IV-TR criteria (diagnostic and statistical manual of mental disorders, 4th edition criteria, text revised) were subjected to hair mercury measurement using Atomic Absorption Spectrometry (AAS) and were compared to hair mercury measurement of fifteen, age and sex matched healthy children.

Results: Results revealed a highly significant increase in the mean hair mercury level in autistic patients than the control group (0.79 ± 0.51 vs 0.12 ± 0.086 ppm) respectively, ($P < 0.001$). There

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.

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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

developmental periods (Austin 2008, Geier et al. 2008, 2009e). 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, 2009e).

DeSoto and Hitlan (2007) postulated that if Hg does play any 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

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.

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A Case Series of Children with Apparent Mercury Toxic Encephalopathies Manifesting with Clinical Symptoms of Regressive Autistic Disorders

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Impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movement patterns characterize autism spectrum disorders (ASDs). It is clear that while genetic factors are important to the pathogenesis of ASDs, mercury exposure can induce immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with ASDs. The Institutional Review Board of the Institute for Chronic Illnesses (Office for Human Research Protections, U.S. Department of Health and Human Services, IRB number IRB00005375) approved the present study. A case series of nine patients who presented to the Genetic Centers of America for a genetic/developmental evaluation are discussed. Eight of nine patients (one patient was found to have an ASD due to Rett's syndrome) (a) had regressive ASDs; (b) had elevated levels of androgens; (c) excreted significant amounts of mercury post chelation challenge; (d) had biochemical evidence of decreased function in their glutathione pathways; (e) had no known significant mercury exposure except from Thimerosal-containing vaccines/Rho(D)-immune globulin preparations; and (f) had alternate causes for their regressive ASDs ruled out. There was a significant dose-response relationship between the severity of the regressive ASDs observed and the total mercury dose children received from Thimerosal-containing vaccines/Rho (D)-immune globulin preparations. Based upon differential diagnoses, 8 of 9 patients examined were exposed to significant mercury from Thimerosal-containing biologic/vaccine preparations during their fetal/infant developmental periods, and subsequently, between 12 mercury intoxication should be considered in the differential diagnosis as contributing to some regressive ASDs.

Autism is a neurodevelopmental syndrome characterized by impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movement patterns (California Department of Developmental Services, 2003). While genetic factors are recognized as being important in the pathogenesis of autistic disorders, the role for environmental factors has received considerable attention. Researchers have previously reported that exposure to mercury can produce immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with autistic disorders, and these similarities extend to neuroanatomy, neurotransmitters, and biochemistry (Bernard et al., 2001, 2002; Blaxill et al., 2004; Redwood et al., 2001). Furthermore, recent research observing children's communicative, social, affective and repetitive behaviors and toy play coded from videotapes of the toddlers' first and second birthday parties revealed there are children with regressive autistic disorders that manifest between the ages of 12 and 24 mo (Werner & Dawson, 2005), a temporal period concurrent with exposure of

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.

Special Article

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Blood Levels of Mercury Are Related to Diagnosis of Autism: A Reanalysis of an Important Data Set

M. Catherine DeSoto, PhD, and Robert T. Hitlan, PhD

The question of what is leading to the apparent increase in autism is of great importance. Like the link between aspirin and heart attack, even a small effect can have major health implications. If there is any link between autism and mercury, it is absolutely crucial that the first reports of the question are not falsely stating that no link occurs. We have reanalyzed the data set originally reported by Ip et al. in 2004 and have found that the original p value was in error and that a significant relation does exist between the blood levels of mercury and diagnosis of an autism spectrum disorder. Moreover, the hair sample analysis results offer some support for the idea that persons with autism may be less efficient and more variable at eliminating mercury from the blood.

Keywords: autism; mercury; environmental health; neurotoxin; neurodevelopment; blood

There is a marked increase in the diagnosis of autism. The question of what is (and is not) related to this increase is crucial to millions of persons affected by the disorder. This article reanalyzes an original data set regarding the relation between blood levels of mercury and diagnosis of an autism spectrum disorder (ASD) by Ip et al. based on our finding of discrepancies in the original article.[1]

A review of what is known about the neurotoxic effects of mercury is beyond the scope of this paper,[2] but the observable symptoms of acute mercury poisoning have been reported to match up with many of the problems observed in autism.[3] Furthermore, mercury poisoning has sometimes been presumptively diagnosed as autism of unknown etiology until the mercury poisoning has been uncovered.[4] Because there has been a several-fold increase in environmental mercury exposure, the hypothesis that the rise in autism could be related to an environmental increase in mercury levels is a reasonable one to pursue. Autism may result from a combination of genetic susceptibility (perhaps in the form of reduced ability to remove mercury or other neurotoxins from the system) and environmental exposure at key times in development.[5-7] This would mean a generalized [illegible] exposed to relatively high mercury would not be affected if, for example, their bodies were very efficient eliminators of such toxins. Only if an exposed infant or fetus also had a genetic susceptibility that makes one less able to remove mercury (or other heavy metals) would normal levels of mercury exposure lead to problems. Alternatively, it could be that genes that help detoxify get switched on and start to express themselves a little later than normal in those genetically predisposed to autism; or perhaps, autism results from some combination of these theories.

Nevertheless, if mercury does play any causal role in facilitating a diagnosis of autism, there would likely be at least some relation between high mercury measured in the blood and symptoms of autism even if ability to metabolize mediates the relationship between exposure and neural toxicity. This is because even if exposure is identical, those who remove mercury less effectively should still have higher levels in the blood. Interestingly, results of hair samples could be expected to be somewhat mixed. The level of mercury in hair may be better understood as an indication of how much mercury has been removed by the body as opposed to the level in the body.[6] If people are approximately equal in their [illegible]

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.

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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) dentritic 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 (NF-κB). 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. 2010, Kern et al. 2011, Fernandez-Morales 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.

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Research Article

Increased Susceptibility to Ethylmercury-Induced Mitochondrial Dysfunction in a Subset of Autism Lymphoblastoid Cell Lines

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The association of autism spectrum disorders with oxidative stress, redox imbalance, and mitochondrial dysfunction has become increasingly recognized. In this study, extracellular flux analysis was used to compare mitochondrial respiration in lymphoblastoid cell lines (LCLs) from individuals with autism and unaffected controls exposed to ethylmercury, an environmental toxin known to deplete glutathione and induce oxidative stress and mitochondrial dysfunction. We also tested whether pretreating the autism LCLs with N-acetyl cysteine (NAC) to increase glutathione concentrations conferred protection from ethylmercury. Examination of 16 autism/control LCL pairs revealed that a subgroup (31%) of autism LCLs exhibited a greater reduction in ATP-linked respiration, maximal respiratory capacity, and reserve capacity when exposed to ethylmercury, compared to control LCLs. These respiratory parameters were significantly elevated at baseline in the ethylmercury-sensitive autism subgroup as compared to control LCLs. NAC pretreatment of the sensitive subgroup reduced (normalized) baseline respiratory parameters and blunted the exaggerated ethylmercury-induced reserve capacity depletion. These findings suggest that the epidemiological link between environmental mercury exposure and an increased risk of developing autism may be mediated through mitochondrial dysfunction and support the notion that a subset of individuals with autism may be vulnerable to environmental influences with detrimental effects on development through mitochondrial dysfunction.

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.

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Research Article

B-Lymphocytes from a Population of Children with Autism Spectrum Disorder and Their Unaffected Siblings Exhibit Hypersensitivity to Thimerosal

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The role of thimerosal containing vaccines in the development of autism spectrum disorder (ASD) has been an area of intense debate, as has the presence of mercury dental amalgams and fish ingestion by pregnant mothers. We studied the effects of thimerosal on cell proliferation and mitochondrial function from B-lymphocytes taken from individuals with autism, their nonautistic twins, and their nontwin siblings. Eleven families were examined and compared to matched controls. B-cells were grown with increasing levels of thimerosal, and various assays (LDH, XTT, DCFH, etc.) were performed to examine the effects on cellular proliferation and mitochondrial function. A subpopulation of eight individuals (4 ASD, 2 twins, and 2 siblings) from four of the families showed thimerosal hypersensitivity, whereas none of the control individuals displayed this response. The thimerosal concentration required to inhibit cell proliferation in these individuals was only 40% of controls. Cells hypersensitive to thimerosal also had higher levels of oxidative stress markers, protein carbonyls, and oxidant generation. This suggests certain individuals with a mild mitochondrial defect may be highly susceptible to mitochondrial specific toxins like the vaccine preservative thimerosal.

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.

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http://www.molecularbrain.com/content/7/1/64

Molecular Brain

REVIEW Open Access

Abnormalities in the zinc-metalloprotease-BDNF axis may contribute to megalencephaly and cortical hyperconnectivity in young autism spectrum disorder patients

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Abstract

Whereas aberrant brain connectivity is likely the core pathology of autism-spectrum disorder (ASD), studies do not agree as to whether hypo- or hyper-connectivity is the main underlying problem. Recent functional imaging studies have shown that, in most young ASD patients, cerebral cortical regions appear hyperconnected, and cortical thickness/brain size is increased. Collectively, these findings indicate that developing ASD brains may exist in an altered neurotrophic milieu. Consistently, some ASD patients, as well as some animal models of ASD, show increased levels of brain-derived neurotrophic factor (BDNF). However, how BDNF is upregulated in ASD is unknown. To address this question, we propose the novel hypothesis that a putative zinc-metalloprotease-BDNF (ZMB) axis in the forebrain plays a pivotal role in the development of hyperconnectivity and megalencephaly in ASD.

We have previously demonstrated that extracellular zinc at micromolar concentrations can rapidly increase BDNF levels and phosphorylate the receptor tyrosine kinase TrkB via the activation of metalloproteases. The role of metalloproteases in ASD is still uncertain, but in fragile X syndrome, a monogenic disease with an autistic phenotype, the levels of MMP are increased. Early exposure to lipopolysaccharides (LPS) and other MMP activators such as organic mercurials also have been implicated in ASD pathogenesis. The resultant increases in BDNF levels at synapses, especially those involved in the zinc-containing, associative glutamatergic system may produce abnormal brain circuit development. Various genetic mutations that lead to ASD are also known to affect BDNF signaling: some down-regulate, and others up-regulate it. We hypothesize that, although both up- and down-regulation of BDNF may induce autism symptoms, only BDNF up-regulation is associated with the hyperconnectivity and large brain size observed in most young idiopathic ASD patients.

To test this hypothesis, we propose to examine the ZMB axis in animal models of ASD. Synaptic zinc can be examined by fluorescence zinc staining. MMP activation can be measured by in situ zymography and Western blot analysis. Finally, regional levels of BDNF can be measured. Validating this hypothesis may shed light on the central pathogenic mechanism of ASD and aid in the identification of useful biomarkers and the development of preventive/therapeutic strategies.

Keywords: Autism spectrum disorder (ASD), Zinc, Metalloprotease, Brain-derived neurotrophic factor (BDNF)

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 (megalencephaly) and cortical hyperconnectivity in the brains of children with autism.

MÆDICA - a Journal of Clinical Medicine

ORIGINAL PAPERS

Efficacy of DMSA Therapy in a Sample of Arab Children with Autistic Spectrum Disorder

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ABSTRACT

Objective: *the aim of this study was to provide evidence that DMSA detoxification treatments cause a reduction of 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 4t Edition, (DMS-IV). The severity of the autistics symptomatologiy 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(=the 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 non-verbal communication, smell and touch, improvements in relating to people and adaptation to change.

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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 (pentacarboxyporphyrin, precoproporphyrin, 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 (GSH), 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 associated 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%

The authors assessed mercury toxicity in a cohort of 28 children with autism. The cohort showed significantly higher levels of urinary porphyrins associated with mercury toxicity as well as decreased plasma levels of reduced glutathione, cysteine and sulfate, indicating active mercury toxicity in autistic children and an inability to detoxify heavy metals.

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YOUR DIAGNOSIS

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An 11-month-old boy with psychomotor regression and auto-aggressive behaviour

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Clinical information

An 11-month-old Swiss boy was brought to his paediatrician. He had been in good health and had developed normally until then, but his parents mentioned that over the previous 2 weeks, the child no longer laughed or played, was becoming more and more restless, and slept only 1 to 2 h a night. He was no longer able to crawl and to stand up, and he had lost weight. Clinical examination revealed swollen hands and feet with skin desquamation, axial hypotonia and brisk reflexes. The child sweated profusely, refused to crawl or stand, showed stereotypic movements of the hands (kneading) and repeatedly bit objects or his own hands.

Upon admission to the regional hospital, blood cell count, electrolytes, blood gas analysis, thyroid hormones and cerebrospinal fluid were normal. Liver and kidney parameters were normal, but plasma LDH and CK were increased to 724 U/l (normal < 296 U/l) and to 270 U/l (normal < 234 U/l), respectively. EEG, chest X-ray film, abdominal sonography, cranial CT scan and a muscle biopsy were all normal. Differential diagnosis at that point included a viral infection, neuroborreliosis, and coeliac disease, but laboratory investigations remained inconclusive.

Two further weeks later, the symptoms persisted and the child was referred to a secondary centre. Clinical findings were unchanged; routine laboratory studies were again normal. EEG, brain MRI, and investigations for a metabolic disorder (ammonia, uric acid, amino acids, organic acids, acylcarnitines and mucopolysaccharides) were normal. The diagnosis remained obscure. The child was referred for further evaluation of severe psychomotor regression with autistic features of unknown aetiology.

The child was admitted to our hospital at age 14 months. Length and weight, that had been at P50 at age 11 months, had declined to P3–10. The child appeared exhausted and irritable and sweated profusely. The hands and feet were swollen, erythematous and tender (Fig. 1). A fine maculo-papular exanthema was present on the trunk. There was muscular hypotrophy with peripheral oedema and axial hypotonia. Physical findings included tachycardia (160–180/min) and arterial hypertension (140/80 mmHg). The boy refused to stand, but would sit in a crouched position, had a sad and apathetic look, took little interest in his surroundings and would occasionally bite his hands and feet. A review of a videotape obtained during the first hospitalisation several weeks earlier showed that all features had been even more marked at that time.

Abdominal sonography showed no signs of a tumour or renal disease; chest radiography and MIBG-scintigraphy showed no signs of a tumour. Echocardiography showed no signs of cardiomyopathy. An ophthalmological examination did not reveal corneal or lenticular abnormalities. An afebrile seizure occurred during hospitalisation: EEG and cerebral spinal fluid were again normal. Arterial hypertension and tachycardia had a good response to beta-blockers. Thyroid hormones were normal, but urinary excretion of epinephrine (epinephrine/creatinine: 86 nmol/mmol; normal < 25 nmol/mmol), norepinephrine (norepinephrine/ creatinine: 263 nmol/mmol; normal < 55 nmol/mmol), dopamine (dopamine/creatinine 1192 nmol/mmol; normal < 590 nmol/mmol) and HMA and VMA (HMA/creatinine 6.9 µmol/mmol; normal < 4.7 µmol/mmol) were elevated.

Because of developmental regression, muscular hypotonia, anorexia, and auto-aggressive behaviour with

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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.

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: 10.1177/0091270005278866

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

This work was supported by the Cure Autism Now Foundation. Lisa McCoy contributed in research assistance.

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.

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.

Prenatal, Perinatal, and Neonatal Factors in Autism, Pervasive Developmental Disorder-Not Otherwise Specified, and the General Population

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ABSTRACT. *Objectives.* To examine various pre-, peri-, and neonatal factors in autistic participants and in pervasive developmental disorder-not otherwise specified (PDD-NOS) participants and to compare the incidence of each factor to that of the normal population.

Methods. Seventy-four participants (66 males, 8 females) were diagnosed with autism at 2.5 through 4 years of age using the most accurate and up-to-date methods, including the *Diagnostic and Statistical Manual of Mental Disorders* and the Autism Diagnostic Interview-Revised. At age 5, all participants were reevaluated using the *Diagnostic and Statistical Manual of Mental Disorders*, the Autism Diagnostic Interview-Revised, the Childhood Autism Rating Scale, and the Autism Diagnostic Observation Schedule-Revised, resulting in 61 autistic and 13 PDD-NOS participants. Twenty-eight pre-, peri-, and neonatal factors were examined in these 2 groups using both medical records and parental interviews. Incidences were compared with those of the US population as reported in the *Report of Final Natality Statistics, 1995.* This grand scale population group was used to closely approximate comparison to a normal, unbiased population. Results were analyzed using the binomial probability test, with a P value of $<.05$, constituting a significant difference in incidence. A Bonferroni correction was applied to the data to adjust for the number of factors investigated.

Results. Although most of the factors showed comparable incidences between the index and control groups, several factors showed statistically significant differences. Following the Bonferroni correction, the autism group was found to have a significantly higher incidence of uterine bleeding, a lower incidence of maternal vaginal infection, and less maternal use of contraceptives during conception when compared with the general population. Similarly, the PDD-NOS group showed a higher incidence of hyperbilirubinemia when compared with the general population.

Conclusions. The results of this study support previous findings suggesting a consistent association of unfavorable events in pregnancy, delivery, and the neonatal phase and the pervasive developmental disorders. However, interpretation of the meaningfulness of these results is difficult, as the specific complications that carried the highest risk of autism and PDD-NOS represented various forms of pathologic processes with no presently apparent unifying feature. Additional studies are needed to corroborate and strengthen these associations, as well as to determine the possibility of an underlying unifying pathological process.

This study's analysis of obstetric and neonatal complications in combination with the use of participants diagnosed at an early age provides some interesting concepts to consider. Perhaps future research will confirm certain pre-, peri-, and neonatal associations that could be used to generate a high-risk historical profile with which to use in conjunction with currently employed diagnostic tools. This may, in turn, help to determine the reliability of a diagnosis of autism in younger children, leading to earlier intervention and assistance for an improved outcome in long-term functionality and quality of life. *Pediatrics* 2001;107(4). URL: http://www.pediatrics.org/cgi/content/full/107/4/e63; ***autism, pervasive developmental disorder, pregnancy, delivery, risk factors, neonatal.***

ABBREVIATIONS. PDD, pervasive developmental disorder; DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders, IV*; ADI-R, Autism Diagnostic Interview-Revised; PDD-NOS, pervasive developmental disorder-not otherwise specified.

Autism is a behavioral syndrome with a reported prevalence of ~10 per 10 000 and an approximate 4:1 ratio of affected males to females.[1] It is classified under the category of pervasive developmental disorder (PDD) and results from a neurologic disorder affecting various functions of the brain. Autism is characterized by impairments in reciprocal social interaction, impairments in verbal and nonverbal communication, lack of imaginative play, and a pattern of repetitive, stereotypical behaviors and interests. Like most other behavioral syndromes, it seems to be a causally heterogeneous disorder. In the literature there is varying support for a wide spectrum of hypotheses regarding the cause of autism: from genetic studies showing a high concordance rate in monozygotic twins[2] to the relationship between environmental events and the development

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.

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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 ($n=1139$) from the Western Amazon based on combined (low, intermediate, and high) exposure to chronic MeHg from fish consumption and acute TCV- EtHg. Neurodevelopment 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 (HHg) at birth was 6.4 $\mu g\, g^{-1}$ in mothers, and 1.94 $\mu g\, g^{-1}$ 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). Neurodevelopment 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.

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 sus-

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 neurodevelop-

Amazonian children exposed to high and low levels of both ethyl and methylmercury were assessed using the Mental Developmental Index and Psychomotor Developmental Index. Statistically significant differences were seen in the high exposure group at 24 months in the mental developmental index only. Combined exposures led to developmental delays including the age of talking and the age of walking.

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ANCESTRY OF PINK DISEASE (INFANTILE ACRODYNIA) IDENTIFIED AS A RISK FACTOR FOR AUTISM SPECTRUM DISORDERS

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Pink disease (infantile acrodynia) was especially prevalent in the first half of the 20th century. Primarily attributed to exposure to mercury (Hg) commonly found in teething powders, the condition was developed by approximately 1 in 500 exposed children. The differential risk factor was identified as an idiosyncratic sensitivity to Hg. Autism spectrum disorders (ASD) have also been postulated to be produced by Hg. Analogous to the pink disease experience, Hg exposure is widespread yet only a fraction of exposed children develop an ASD, suggesting sensitivity to Hg may also be present in children with an ASD. The objective of this study was to test the hypothesis that individuals with a known hypersensitivity to Hg (pink disease survivors) may be more likely to have descendants with an ASD. Five hundred and twenty-two participants who had previously been diagnosed with pink disease completed a survey on the health outcomes of their descendants. The prevalence rates of ASD and a variety of other clinical conditions diagnosed in childhood (attention deficit hyperactivity disorder, epilepsy, Fragile X syndrome, and Down syndrome) were compared to well-established general population prevalence rates. The results showed the prevalence rate of ASD among the grandchildren of pink disease survivors (1 in 25) to be significantly higher than the comparable general population prevalence rate (1 in 160). The results support the hypothesis that Hg sensitivity may be a heritable/genetic risk factor for ASD.

Pink disease, or infantile acrodynia as it was also known (primarily in Europe and America), was an especially prevalent condition in Australia, North America, and Central Europe in the first half of the 20th century (Rocaz 1933). The first description of pink disease in the literature dates back to 1903 by Selter, a German physician, although cases in Australia predate this time by at least two decades (Selter 1903; Wood and Wood 1935). Pink disease remained in relative obscurity in the greater medical community until 1914, when it was again described, this time by Swift, an Australian-born physician, at an Australasian medical congress in New Zealand (Swift 1914).

Case studies provided a comprehensive clinical picture of pink disease long before its etiology was established. The most commonly reported symptoms included: irritability, neurosis, photophobia (light sensitivity), hyperhidrosis (excessive sweating), hypotonia (low muscle tone), ataxia (lack of coordination), digestive problems (including loss of weight, loss of appetite, vomiting, and constipation), anemia, excessive salivation, respiratory problems, lethargy, extreme misery, slurring/loss

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.

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TOXICOLOGY OF NEURODEVELOPMENTAL DISORDERS

The role of mercury in the pathogenesis of autism

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder of unknown etiology in most cases. Studies of monozygotic twins report an average 60% concordance rate, indicating a role for both genetic and environmental factors in disease expression.[1] Recent reviews in environmental health have suggested that early exposure to hazardous substances may underlie some cases of neurodevelopmental disorders, including ADHD, learning disabilities, and speech/language difficulties.[2] In 1999, thimerosal used as a vaccine preservative was identified as a widespread source of organic mercury exposure in infants.[3] Mercury (Hg), a heavy metal, is considered highly neurotoxic.[4] The amount of mercury in vaccines, while small, exceeded USEPA safety guidelines on a cumulative basis.[3] Certain individuals may exhibit severe adverse reactions to low doses of Hg which are otherwise largely benign to the majority of those exposed.[5] Some individuals with idiopathic autism spectrum disorder may represent such a sensitive population. As summarized in this paper, disease characteristics suggest this possibility: (a) ASD traits are known to arise from mercury exposure; (b) onset of ASD symptoms is temporally associated with administration of immunizations; (c) the reported increase in the prevalence of autism in the 1990s closely follows the introduction of two mercury-containing vaccines; and (d) elevated mercury has been detected in biological samples of autistic patients. Since ASD may now affect as many as one in 150 US children,[6] and since thimerosal is still used in many products worldwide, confirmation of thimerosal as an environmental agent in autism pathogenesis has important societal and patient implications.

Thimerosal is comprised of 49.6% ethylmercury (EtHg) by weight. Until early 2001, it was a component of most Hepatitis B, *Haemophilus influenzae* type B (HiB), and Diphtheria/Tetanus/Pertussis (DTP or DTaP) the EtHg from the recommended vaccines is predicted to raise hair mercury levels above USEPA guidelines of 1 ppm for up to one year and, in some infants, to elevate Hg levels to 10 ppm, which is the lowest threshold for adverse outcomes in children exposed prenatally to MeHg.[4,7] That thimerosal-containing vaccines can significantly raise blood Hg levels in infants has been demonstrated *in vivo*.[8] Endpoints for adverse effects at low doses of MeHg have been in domains characteristic of ASD and include lowered performance on tests of attention, memory, language, and fine motor skills.[9–11] A CDC analysis of computerized HMO medical records found statistically significant associations between increased exposure to thimerosal from infant immunizations and attention deficit disorder, speech/language delay, and tics.[12] Traits characteristic of these disorders are common features of ASD.[10,11]

A review of medical literature has shown that exposure to mercury, whether organic or inorganic, can give rise to the symptoms and traits defining or commonly found in ASD individuals.[13] Mercury can cause impairments in social interaction, communication difficulties, and repetitive and stereotyped patterns of behavior, which comprise the three DSM-IV autism diagnostic criteria. Additionally, mercury can induce features prominent in ASD such as sensory abnormalities, emotional/psychological changes, movement disorder, impairments in abstract or complex thinking, severe sleep disturbances, and self injurious behavior. Males are more affected than females in both conditions. Physiological abnormalities more common in ASD populations and known to be caused by mercury exposure include gastrointestinal problems, autonomic nervous system disturbance, unusual EEG activity, immune system alterations, irregularities in neurotransmitter systems, and non-specific brain lesions.

The discovery and increase in the reported prevalence of autism parallels the introduction and spread of thimerosal-containing vaccines. Autism was first described in 1943 among children born in the 1930s.[14]

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.

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Autism: a novel form of mercury poisoning

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Summary Autism is a syndrome characterized by impairments in social relatedness and communication, repetitive behaviors, abnormal movements, and sensory dysfunction. Recent epidemiological studies suggest that autism may affect 1 in 150 US children. Exposure to mercury can cause immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with autism, and the similarities extend to neuroanatomy, neurotransmitters, and biochemistry. Thimerosal, a preservative added to many vaccines, has become a major source of mercury in children who, within their first two years, may have received a quantity of mercury that exceeds safety guidelines. A review of medical literature and US government data suggests that: (i) many cases of idiopathic autism are induced by early mercury exposure from thimerosal; (ii) this type of autism represents an unrecognized mercurial syndrome; and (iii) genetic and non-genetic factors establish a predisposition whereby thimerosal's adverse effects occur only in some children. © 2001 Harcourt Publishers Ltd

INTRODUCTION

Austic spectrum disorder (ASD) is a neurodevelopmental syndrome with onset prior to age 36 months. Diagnostic criteria consist of impairments in sociality and communication plus repetitive and stereotypic behaviors (1). Traits strongly associated with autism include movement disorders and sensory dysfunctions (2). Although autism may be apparent soon after birth, most autistic children experience at least several months, even a year or more of normal development – followed by regression, defined as loss of function or failure to progress (2–4).

The neurotoxicity of mercury (Hg) has long been recognized (5). Primary data derive from victims of contaminated fish (Japan – Minamata disease) or grain (Iraq, Guatemala, Russia); from acrodynia (Pink disease) induced by Hg in teething powders; and from individual mechanisms of Hg toxicity. More recently, the Food and Drug Administration (FDA) and the American Academy of Pediatrics (AAP) have determined that the typical amount of Hg injected into infants and toddlers via childhood immunizations has exceeded government safety guidelines on an individual (6) and cumulative vaccine basis (7). The mercury in vaccines derives from thimerosal (TMS), a preservative which is 49.6% ethylmercury (eHg) (7).

Past cases of HgP have presented with much inter-individual variation, depending on the dose, type of mercury, method of administration, duration of exposure, and individual sensitivity. Thus, while commonalities exist across the various instances of HgP, each set of variables has given rise to a different disease manifestation (8–11). It is hypothesized that the regressive form of autism represents another form of mercury poisoning,

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 comordities of autism and their corresponding analogs due to mercury exposure.

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TOXICOLOGY OF NEURODEVELOPMENTAL DISORDERS

The neuropathogenesis of mercury toxicity

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Elemental and inorganic mercury (Hg) are found in scientific instruments, electrical equipment, dental amalgams, felt making, disinfectants, production of caustic soda, and disk batteries. Environmental Hg contamination has increased dramatically since the beginning of the Industrial Revolution. The largest contributor to atmospheric contamination is coal burning. Mining, smelting, and refining processes also contribute locally to Hg contamination. Through natural processes and direct commercial discharge, most Hg settles in the marine environment. In the waterways, methylation of inorganic mercury to methylmercury (MeHg) leads to its accumulation in the food chain. Anthropogenic sources resulting in the acidification of freshwater streams and lakes, and the impoundment of water for large hydroelectric schemes have led to increasingly higher MeHg concentrations in fish. A considerable degree of bio-concentration takes place as MeHg moves up the aquatic food chain. Most marine fish contain <0.5 ppm MeHg, but sharks, sailfish, marlin, and other billfish frequently have levels of over 1 ppm. When local waters are polluted with MeHg, levels may be much higher.

The clinical manifestations of organic mercury poisoning depend on the type of Hg compound involved. Ethylmercury (EthylHg; the additive in thimerosal) mimics the neurotoxicity of MeHg, whereas phenylmercury mimics the toxicity of inorganic Hg salts. MeHg, at high concentrations, causes a toxic encephalopathy with severe congenital form resulting from prenatal exposure. The correlation between clinical symptoms and whole blood Hg depends both on the Hg species and/or the duration of exposure. Whole-blood mercury levels are the best measure of recent inorganic Hg and elemental Hg vapor absorption. Normal blood levels of Hg do not exceed 1–3 $\mu g\ dl^{-1}$. Hair analysis indicates past exposure, and the Hg blood to hair ratio is ~2:250.

Comparative studies on the neurotoxicity of MeHg and EthylHg are limited. Three or 10 days after the last of five treatments with either 8.0 or 9.6 mg EthylHg kg^{-1}, rats had higher total or organic Hg concentrations in blood, and lower concentrations of Hg in brain than little difference in the neurotoxicity of MeHg and EthylHg.[1]

Given that contaminated fish represent a common source for human MeHg exposure, considerable attention in the scientific and health policy fora is focused on the question of whether MeHg intake from a diet high in fish is associated with aberrant CNS function. The human database on the neurodevelopmental effects of MeHg is extensive, and has been recently summarized by the National Research Council.[2] These studies provide little evidence that the ages at which children achieve major language and motor milestones are affected appreciably by low-dose prenatal MeHg exposure.[2] An association of low-dose MeHg exposure on early childhood development was reported in only two of the four studies using the Denver Developmental Screening Test. The Faroes study reports associations between low-dose prenatal MeHg exposure and children's performance on standardized neurobehavioral tests, particularly in the domains of attention, fine-motor function, confrontational naming, visual-spatial abilities, and verbal memory, but it is the only one to report such effects of the three major prospective long-term studies. In contrast, the Seychelles study failed to reveal such associations. The smaller New Zealand study also observed associations, as did a large pilot study conducted in the Seychelles.[2]

In 1957, Margoshes and Vallee reported the isolation of a protein from horse kidney, which showed a high affinity for cadmium.[3] This protein was subsequently biochemically characterized, and, due to its high content of metals and cysteine residues, it was named metallothionein (MT). Putative physiological functions, such as transport and storage of essential heavy metals (zinc, copper) and detoxification of non-essential ones (mercury) were proposed. The ability of heavy metals to induce MT synthesis was described by Piscator,[4] demonstrating increased MT levels in the livers of rabbits exposed to cadmium. This form of regulation has been recognized in all species and cells that synthesize MTs. MT synthesis is inducible in several tissues by a variety of heavy metal ions, such as Cd, Pb, Zn, Co and Hg.[5] Although the number of studies on the ability of MTs to protect against neurotoxicity is rather limited, recent reports corroborate the cytoprotective effects of these proteins. For example, in an ischemic experi-

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.

Molecular Psychiatry (2004), 1–13

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IMMEDIATE COMMUNICATION

Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and thimerosal

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Methylation events play a critical role in the ability of growth factors to promote normal development. Neurodevelopmental toxins, such as ethanol and heavy metals, interrupt growth factor signaling, raising the possibility that they might exert adverse effects on methylation. We found that insulin-like growth factor-1 (IGF-1)- and dopamine-stimulated methionine synthase (MS) activity and folate-dependent methylation of phospholipids in SH-SY5Y human neuroblastoma cells, via a PI3-kinase- and MAP-kinase-dependent mechanism. The stimulation of this pathway increased DNA methylation, while its inhibition increased methylation-sensitive gene expression. Ethanol potently interfered with IGF-1 activation of MS and blocked its effect on DNA methylation, whereas it did not inhibit the effects of dopamine. Metal ions potently affected IGF-1 and dopamine-stimulated MS activity, as well as folate-dependent phospholipid methylation: Cu^{2+} promoted enzyme activity and methylation, while Cu^{+}, Pb^{2+}, Hg^{2+} and Al^{3+} were inhibitory. The ethylmercury-containing preservative thimerosal inhibited both IGF-1- and dopamine-stimulated methylation with an IC_{50} of 1 nM and eliminated MS activity. Our findings outline a novel growth factor signaling pathway that regulates MS activity and thereby modulates methylation reactions, including DNA methylation. The potent inhibition of this pathway by ethanol, lead, mercury, aluminum and thimerosal suggests that it may be an important target of neurodevelopmental toxins.
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Keywords: autism; attention deficit hyperactivity disorder; P13-kinase; D4 dopamine receptor; DNA methylation; phospholipid methylation; lead; mercury

Introduction

Developmental disorders include a spectrum of neurological conditions characterized by deficits in attention, cognition and learning, frequently accompanied by abnormal behaviors. Severe deficits may be recognized at birth, but a failure to achieve standard milestones during initial years of life remains the primary basis of diagnosis in most cases. While the underlying cause(s) remains obscure for many volved. The development disorders can also be caused by exposure to toxins (eg ethanol, in fetal alcohol syndrome; heavy metals, in lead poisoning),[5,6] although the precise mechanisms underlying their toxicity are not known. The recent increase in the incidence of autism has led to the speculation that environmental exposures including vaccine additives (ie aluminum and the ethylmercury-containing preservative thimerosal) might contribute to the triggering of this developmental

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.

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Biochemical and molecular basis of thimerosal-induced apoptosis in T cells: a major role of mitochondrial pathway

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The major source of thimerosal (ethyl mercury thiosalicylate) exposure is childhood vaccines. It is believed that the children are exposed to significant accumulative dosage of thimerosal during the first 2 years of life via immunization. Because of health-related concerns for exposure to mercury, we examined the effects of thimerosal on the biochemical and molecular steps of mitochondrial pathway of apoptosis in Jurkat T cells. Thimerosal and not thiosalcylic acid (non-mercury component of thimerosal), in a concentration-dependent manner, induced apoptosis in T cells as determined by TUNEL and propidium iodide assays, suggesting a role of mercury in T cell apoptosis. Apoptosis was associated with depolarization of mitochondrial membrane, release of cytochrome c and apoptosis inducing factor (AIF) from the mitochondria, and activation of caspase-9 and caspase-3, but not of caspase-8. In addition, thimerosal in a concentration-dependent manner inhibited the expression of XIAP, cIAP-1 but did not influence cIAP-2 expression. Furthermore, thimerosal enhanced intracellular reactive oxygen species and reduced intracellular glutathione (GSH). Finally, exogenous glutathione protected T cells from thimerosal-induced apoptosis by upregulation of XIAP and cIAP1 and by inhibiting activation of both caspase-9 and caspase-3. These data suggest that thimerosal induces apoptosis in T cells via mitochondrial pathway by inducing oxidative stress and depletion of GSH.
Genes and Immunity (2002) 3, 270–278. doi:10.1038/sj.gene.6363854

Keywords: *mitochondria; glutathione; caspases; cytochrome c; apoptosis-inducing factor; oxidative stress*

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. In the immune system, apoptosis plays an important role in the selection of T cell repertoire, deletion of self-reactive lymphocytes, natural killer (NK)- and T cell-mediated cytotoxicity, and termination of response at the end of an immune response.[1,2] There are two major pathways of apoptosis: the death receptor pathway and the mitochondrial pathway.[3–11] There is recent evidence to suggest that these two pathways may be linked in certain cell types.[1,10,11] In both pathways, a series of molecular and biochemical steps leads to the activation of common effector or executioner cysteine proteases, the caspases resulting in the cleavage of a number of nuclear and cytoplasmic substrates, including those responsible for the maintenance of nuclear integrity, cell cycle progression, and DNA repair.

sodium ethylmercuri-thiosalicylate) is a water-soluble derivative of thiosalicylic acid with antimicrobial activity. Thimerosal is used as an antimicrobial agent and a preservative in cleaning solutions for eye lenses, cosmetics, and vaccines. It is estimated that an infant could be exposed to as high as 187.5 μg of ethylmercury from the routine immunization schedule.[12] Because of health-related concern from exposure to Hg from thimerosal-containing vaccines, the American Academy of Family Physicians, the American Academy of Pediatrics, the Advisory Committee on Immunization Practices (ACIP), and the United States Public Health Service have published their recommendations to remove and greatly reduce thimerosal from vaccines a soon as possible.[12] Hg has been shown to induce a number of immunological and neurotoxic changes, including increased production of Th2 cytokines, increased levels of IgE, decreased activity of T cells and NK cell, suppression of IgG, production of autoantibodies to a variety of self antigens (eg, neural antigens), and apoptosis in microglia and astro-

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.

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JOURNAL OF NEUROINFLAMMATION

RESEARCH Open Access

Mercury induces inflammatory mediator release from human mast cells

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Abstract

Background: Mercury is known to be neurotoxic, but its effects on the immune system are less well known. Mast cells are involved in allergic reactions, but also in innate and acquired immunity, as well as in inflammation. Many patients with Autism Spectrum Disorders (ASD) have "allergic" symptoms; moreover, the prevalence of ASD in patients with mastocytosis, characterized by numerous hyperactive mast cells in most tissues, is 10-fold higher than the general population suggesting mast cell involvement. We, therefore, investigated the effect of mercuric chloride ($HgCl_2$) on human mast cell activation.

Methods: Human leukemic cultured LAD2 mast cells and normal human umbilical cord blood-derived cultured mast cells (hCBMCs) were stimulated by HgCl2 (0.1-10 μM) for either 10 min for beta-hexosaminidase release or 24 hr for measuring vascular endothelial growth factor (VEGF) and IL-6 release by ELISA.

Results: $HgCl_2$ induced a 2-fold increase in β-hexosaminidase release, and also significant VEGF release at 0.1 and 1 μM (311 ± 32 pg/10^6 cells and 443 ± 143 pg/10^6 cells, respectively) from LAD2 mast cells compared to control cells (227 ± 17 pg/10^6 cells, n = 5, $p < 0.05$). Addition of $HgCl_2$ (0.1 μM) to the proinflammatory neuropeptide substance P (SP, 0.1 μM) had synergestic action in inducing VEGF from LAD2 mast cells. $HgCl_2$ also stimulated significant VEGF release (360 ± 100 pg/10^6 cells at 1 μM, n = 5, $p < 0.05$) from hCBMCs compared to control cells (182 ± 57 pg/10^6 cells), and IL-6 release (466 ± 57 pg/10^6 cells at 0.1 μM) compared to untreated cells (13 ± 25 pg/10^6 cells, n = 5, $p < 0.05$). Addition of $HgCl_2$ (0.1 μM) to SP (5 μM) further increased IL-6 release.

Conclusions: $HgCl_2$ stimulates VEGF and IL-6 release from human mast cells. This phenomenon could disrupt the blood-brain-barrier and permit brain inflammation. As a result, the findings of the present study provide a biological mechanism for how low levels of mercury may contribute to ASD pathogenesis.

Background

Heavy metals such as mercury result in neurological injury that may lead to developmental defects, peripheral neuropathies, and enhanced neurodegenerative behavioral dysfunction similar to those associated with Autism Spectrum Disorders (ASD) [2]. The possible role of mercury used as preservative in vaccines [2] has been debated extensively, but most epidemiological studies do

Inflammatory mediator release is much more common in the mast cells of autistic chidren 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.

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Thimerosal Induces DNA Breaks, Caspase-3 Activation, Membrane Damage, and Cell Death in Cultured Human Neurons and Fibroblasts

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Thimerosal is an organic mercurial compound used as a preservative in biomedical preparations. Little is known about the reactions of human neuronal and skin cells to its micro- and nanomolar concentrations, which can occur after using thimerosal-containing products. A useful combination of fluorescent techniques for the assessment of thimerosal toxicity is introduced. Short-term thimerosal toxicity was investigated in cultured human cerebral cortical neurons and in normal human fibroblasts. Cells were incubated with 125-nM to 250-μM concentrations of thimerosal for 45 min to 24 h. A 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) dye exclusion test was used to identify nonviable cells and terminal transferase-based nick-end labeling (TUNEL) to label DNA damage. Detection of active caspase-3 was performed in live cell cultures using a cell-permeable fluorescent caspase inhibitor. The morphology of fluorescently labeled nuclei was analyzed. After 6 h of incubation, the thimerosal toxicity was observed at 2 μM based on the manual detection of the fluorescent attached cells and at a 1-μM level with the more sensitive GENios Plus Multi-Detection Microplate Reader with Enhanced Fluorescence. The lower limit did not change after 24 h of incubation. Cortical neurons demonstrated higher sensitivity to thimerosal compared to fibroblasts. The first sign of toxicity was an increase in membrane permeability to DAPI after 2 h of incubation with 250 μM thimerosal. A 6-h incubation resulted in failure to exclude DAPI, generation of DNA breaks, caspase-3 activation, and development of morphological signs of apoptosis. We demonstrate that thimerosal in micromolar concentrations rapidly induce membrane and DNA damage and initiate caspase-3–dependent apoptosis in human neurons and fibroblasts. We conclude that a proposed combination of fluorescent techniques can be useful in analyzing the toxicity of thimerosal.

Key Words: thimerosal; active caspase-3; apoptosis; toxicity; neurons; fibroblasts; DNA breaks; membrane damage; DAPI.

ranging from 0.003 to 0.01% (30–100 μg/ml) (Ball *et al.*, 2001). Thimerosal contains 49.6 % mercury by weight and releases ethylmercury as a metabolite. In the body, ethylmercury can be converted to inorganic mercury, which then preferentially accumulates in the kidneys and brain (Blair *et al.*, 1975). Inorganic mercury is known to induce membrane and DNA damage (Ferrat *et al.*, 2002; Ben-Ozer *et al.*, 2000), and in cell culture conditions it was shown to be mutagenic and generate DNA breaks in concentrations below 500 nM (Schurz *et al.*, 2000). Ethylmercury can significantly increase the concentration of inorganic mercury in many organs (Magos *et al.*, 1985). After *in vivo* administration, ethylmercury passes through cellular membranes and concentrates in cells in vital organs, including the brain, where it releases inorganic mercury, raising its concentrations higher than equimolar doses of its close and highly toxic relative methylmercury (Magos *et al.*, 1985).

However, little is known about acute reactions of various types of human cells following short-time exposure to thimerosal in micro- and nanomolar concentrations.

In this paper we used a convenient and easily reproducible combination of fluorescent techniques analyzing various markers of DNA and membrane damage, and investigated the toxicity of micromolar and nanomolar concentrations of thimerosal (125 nM–250 μM) occurring in the first 24 h of exposure in cultures of human cortical neuronal cells and in human fibroblasts.

We found that thimerosal in micromolar concentrations rapidly decreased cellular viability. Within several h after thimerosal administration, cells lost their capability to exclude the fluorescent dye 4′,6-diamidino-2-phenylindole dihydrochlo-

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.

The American Journal of Clinical Nutrition

Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism[1,2]

S Jill James, Paul Cutler, Stepan Melnyk, Stefanie Jernigan, Laurette Janak, David W Gaylor, and James A Neubrander

ABSTRACT

Background: Autism is a complex neurodevelopmental disorder that usually presents in early childhood and that is thought to be influenced by genetic and environmental factors. Although abnormal metabolism of methionine and homocysteine has been associated with other neurologic diseases, these pathways have not been evaluated in persons with autism.

Objective: The purpose of this study was to evaluate plasma concentrations of metabolites in the methionine transmethylation and transsulfuration pathways in children diagnosed with autism.

Design: Plasma concentrations of methionine, *S*-adenosylmethionine (SAM), *S*-adenosylhomocysteine (SAH), adenosine, homocysteine, cystathionine, cysteine, and oxidized and reduced glutathione were measured in 20 children with autism and in 33 control children. On the basis of the abnormal metabolic profile, a targeted nutritional intervention trial with folinic acid, betaine, and methylcobalamin was initiated in a subset of the autistic children.

Results: Relative to the control children, the children with autism had significantly lower baseline plasma concentrations of methionine, SAM, homocysteine, cystathionine, cysteine, and total glutathione and significantly higher concentrations of SAH, adenosine, and oxidized glutathione. This metabolic profile is consistent with impaired capacity for methylation (significantly lower ratio of SAM to SAH) and increased oxidative stress (significantly lower redox ratio of reduced glutathione to oxidized glutathione) in children with autism. The intervention trial was effective in normalizing the metabolic imbalance in the autistic children.

Conclusions: An increased vulnerability to oxidative stress and a decreased capacity for methylation may contribute to the development and clinical manifestation of autism. *Am J Clin Nutr* 2004; 80:1611–7.

KEY WORDS Autistic disorder, biomarkers, oxidative stress, methylation, methionine, *S*-adenosylmethionine, *S*-adenosylhomocysteine, adenosine, cysteine, glutathione

males than females, occurring at a ratio of 4:1. A significant role for genetics in the etiology of the autistic disorder is supported by a high concordance of autism between monozygotic twins and increased risks among siblings of affected children and of autistic symptoms associated with several heritable genetic diseases [*see*: Online Mendelian Inheritance in Man (OMIM) #209850 (autism; 5)]. Autism has been reported to be a comorbid condition associated with Rett syndrome (5), fragile X (6), phenylketonurea (7), adenylosuccinate lyase deficiency (8), dihydropyrimidine dehydrogenase deficiency (9), and 5′-nucleotidase hyperactivity (10); however, these genetic diseases account for <10% of cases of autism. Nonetheless, the association of autism with genetic deficits in specific enzymes suggests the possibility that the genetic component of primary autism could be expressed as a chronic metabolic imbalance that impairs normal neurodevelopment and immunologic function. The possibility that autism has a metabolic phenotype is less widely accepted but has been supported by several small studies (9, 11–14).

The current study was prompted by the serendipitous observation in a previous study that the metabolic profiles of dizygotic twins—one with Down syndrome and one with autism—were virtually identical with respect to methionine cycle and transsulfuration metabolites (15). Down syndrome, or trisomy 21, is a complex genetic and metabolic disease due to the presence of 3 copies of chromosome 21 and associated with an increased frequency of autism (16). In our previous study, children with Down syndrome had lower concentrations of metabolites in the methionine cycle and significantly lower glutathione concentrations than did control children (15).

The methionine cycle involves the regeneration of methionine via the vitamin B-12–dependent transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine in the methionine synthase reaction. Methionine may then be activated by methionine adenosyltransferase to form *S*-adenosylmethionine (SAM), the primary methyl donor for most cellular methytransferase reactions including the methylation of DNA, RNA, proteins, phospholipids,

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.

ELSEVIER

NeuroToxicology 26 (2005) 1–8

NeuroToxicology

Thimerosal Neurotoxicity is Associated with Glutathione Depletion: Protection with Glutathione Precursors

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Abstract

Thimerosol is an antiseptic containing 49.5% ethyl mercury that has been used for years as a preservative in many infant vaccines and in flu vaccines. Environmental methyl mercury has been shown to be highly neurotoxic, especially to the developing brain. Because mercury has a high affinity for thiol (sulfhydryl (–SH)) groups, the thiol-containing antioxidant, glutathione (GSH), provides the major intracellular defense against mercury-induced neurotoxicity. Cultured neuroblastoma cells were found to have lower levels of GSH and increased sensitivity to thimerosol toxicity compared to glioblastoma cells that have higher basal levels of intracellular GSH. Thimerosal-induced cytotoxicity was associated with depletion of intracellular GSH in both cell lines. Pretreatment with 100 μM glutathione ethyl ester or N-acetylcysteine (NAC), but not methionine, resulted in a significant increase in intracellular GSH in both cell types. Further, pretreatment of the cells with glutathione ethyl ester or NAC prevented cytotoxicity with exposure to 15 μM Thimerosal. Although Thimerosal has been recently removed from most children's vaccines, it is still present in flu vaccines given to pregnant women, the elderly, and to children in developing countries. The potential protective effect of GSH or NAC against mercury toxicity warrants further research as possible adjunct therapy to individuals still receiving Thimerosal-containing vaccinations.

Keywords: Thimerosal; Neurotoxicity; Glutathione; *N*-acetylcysteine

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 bodies 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.

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NeuroToxicology 26 (2005) 407–416

NeuroToxicology

Mitochondrial Mediated Thimerosal-Induced Apoptosis in a Human Neuroblastoma Cell Line (SK-N-SH)

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Abstract

Environmental exposure to mercurials continues to be a public health issue due to their deleterious effects on immune, renal and neurological function. Recently the safety of thimerosal, an ethyl mercury-containing preservative used in vaccines, has been questioned due to exposure of infants during immunization. Mercurials have been reported to cause apoptosis in cultured neurons; however, the signaling pathways resulting in cell death have not been well characterized. Therefore, the objective of this study was to identify the mode of cell death in an in vitro model of thimerosal-induced neurotoxicity, and more specifically, to elucidate signaling pathways which might serve as pharmacological targets. Within 2 h of thimerosal exposure (5 μM) to the human neuroblastoma cell line, SK-N-SH, morphological changes, including membrane alterations and cell shrinkage, were observed. Cell viability, assessed by measurement of lactate dehydrogenase (LDH) activity in the medium, as well as the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, showed a time- and concentration-dependent decrease in cell survival upon thimerosal exposure. In cells treated for 24 h with thimerosal, fluorescence microscopy indicated cells undergoing both apoptosis and oncosis/necrosis. To identify the apoptotic pathway associated with thimerosal-mediated cell death, we first evaluated the mitochondrial cascade, as both inorganic and organic mercurials have been reported to accumulate in the organelle. Cytochrome c was shown to leak from the mitochondria, followed by caspase 9 cleavage within 8 h of treatment. In addition, poly(ADP-ribose) polymerase (PARP) was cleaved to form a 85 kDa fragment following maximal caspase 3 activation at 24 h. Taken together these findings suggest deleterious effects on the cytoarchitecture by thimerosal and initiation of mitochondrial-mediated apoptosis.

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.

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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 under-

Thimerosal at levels comparable to infant exposure from vaccines caused neuronal cell death through changing the mitochondrial microenvironment. Thimerosal induced cell death was associated with mitochondrial depolarization and a significant level of reactive oxidative stress in the cells. Both mitochondrial dysfunction and increased levels of oxidative stress have been documented to occur in individuals with autism.

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In Vitro Uptake of Glutamate in GLAST- and GLT-1-Transfected Mutant CHO-K1 Cells Is Inhibited by the Ethylmercury-Containing Preservative Thimerosal

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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).

Uncoupling of ATP-Mediated Calcium Signaling and Dysregulated Interleukin-6 Secretion in Dendritic Cells by Nanomolar Thimerosal

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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 (Ca^{2+}) 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 Ca^{2+} release from microsomal stores. We tested adenosine triphosphate (ATP)-mediated Ca^{2+} 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-trisphosphate receptor (IP_3R) and ryanodine receptor (RyR) Ca^{2+} channels, known targets of THI. IDCs express the RyR1 isoform in a punctate distribution that is densest near plasma membranes and within dendritic processes, whereas IP_3Rs 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 Ca^{2+} transients > 2-fold, and *c*) produced a delayed and persistent rise (≥ 2-fold) in baseline Ca^{2+}. THI (100 nM, 5 min) recruited more ATP responders, shortened the ATP-mediated Ca^{2+} transient (≥ 1.4-fold), and produced a delayed rise (≥ 3-fold) in the Ca^{2+} baseline, mimicking Ry. THI and Ry, in combination, produced additive effects leading to uncoupling of IP_3R 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 Ca^{2+} signals contributed by RyR1. *Key words:* calcium, calcium channel, dendritic cell, ethyl mercury, immunotoxicity, 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 physiologic factors in defining susceptibility of the immune system to methylmercury (MeHg) and ethylmercury (EtHg) (Havarinasab 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 identi-

consist of a few cases of accidental high-dose poisoning (Cinca et al. 1980; Damluji 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, diphtheria toxoid, diphtheria toxoid and acellular pertussis (DTaP), 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-

to naive and resting T cells (Banchereau and Steinman 1998). This hypothesis stems from the fact that ambient oxygen (O_2) tension or thiol concentration directly influences DC secretion of interferon-γ (IFN-γ) and interleukin-12 (IL-12) (Murata et al. 2002), enhances expression of FcεR1, the high affinity receptor for IgE (Novak et al. 2002), and regulates surface class II major histocompatibility complex (MHC) expression (Goth et al. 2006) *in vitro*. In this regard, Ca^{2+} contributes essential signals for DC function and maturation. Differentiation (Bagley et al. 2004), pro-inflammatory cytokine secretion (Gardella et al. 2000), apoptotic cell phagocytosis (Poggi et al. 1998), and migrational responsiveness to purine nucleotides or chemokines (Partida-Sanchez et al. 2004; Scandella et al. 2004) are Ca^{2+}-dependent processes. DCs rely on changes in intracellular redox state and Ca^{2+} signals for proper development and function, but the relationship between these signaling systems in DCs is unclear.

THI contains an oxidized mercury atom (Hg^{2+}) whose redox properties can enhance the activity of the inositol 1,4,5-trisphosphate receptor (IP_3R) and ryanodine receptor

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Dendritic cells treated with low levels of thimerosal, comparable to that seen via vaccine exposure, showed dysregulation in immune cytokine production. Dendritic cells play a role the determination of "self" and "non-self" cells and therefore in autoimmunity.

Thiol-Modulated Mechanisms of the Cytotoxicity of Thimerosal and Inhibition of DNA Topoisomerase IIα

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Thimerosal is an organic mercury compound that is widely used as a preservative in vaccines and other solution formulations. The use of thimerosal has caused concern about its ability to cause neurological abnormalities due to mercury accumulation during a normal schedule of childhood vaccinations. While the chemistry and the biological effects of methylmercury have been well-studied, those of thimerosal have not. Thimerosal reacted rapidly with cysteine, GSH, human serum albumin, and single-stranded DNA to form ethylmercury adducts that were detectable by mass spectrometry. These results indicated that thimerosal would be quickly metabolized in vivo because of its reactions with protein and nonprotein thiols. Thimerosal also potently inhibited the decatenation activity of DNA topoisomerase IIα, likely through reaction with critical free cysteine thiol groups. Thimerosal, however, did not act as a topoisomerase II poison and the lack of cross-resistance with a K562 cell line with a decreased level of topoisomerase IIα (K/VP.5 cells) suggested that inhibition of topoisomerase IIα was not a significant mechanism for the inhibition of cell growth. Depletion of intracellular GSH with buthionine sulfoximine treatment greatly increased the K562 cell growth inhibitory effects of thimerosal, which showed that intracellular glutathione had a major role in protecting cells from thimerosal. Pretreatment of thimerosal with glutathione did not, however, change its K562 cell growth inhibitory effects, a result consistent with the rapid exchange of the ethylmercury adduct among various thiol-containing cellular reactants. Thimerosal-induced single and double strand breaks in K562 cells were consistent with a rapid induction of apoptosis. In conclusion, these studies have elucidated some of the chemistry and biological activities of the interaction of thimerosal with topoisomerase IIα and protein and nonprotein thiols and with DNA.

Introduction

Thimerosal (Figure 1) is an organic mercury compound with bactericidal and fungicidal properties that is widely used as a preservative in multiuse vials of vaccines, ophthalmic, otic, nasal, and topical products (*1*–*3*). There has been a public perception that thimerosal use in vaccines is unsafe after suggestions that it caused a predisposition to autism in children (*1*, *4*). However, recent epidemiological studies have not supported this hypothesis (*4*). On the basis of the risk assessment assumption that the dose–effect and dose–response relationships of ethylmercury, the presumed metabolite of thimerosal, and methylmercury were the same, thimerosal was removed from most pediatric vaccines in the United States in 2001 (*1*, *3*). Prior to 2001, by 18 months of age, a child in the United States undergoing a routine schedule of immunizations would have received a cumulative dose of 200 μg of mercury (*3*). The fact that the cumulative exposure to mercury from thimerosal in less is known about the effects of thimerosal or its presumed metabolite, ethylmercury (*1*, *3*, *5*, *6*). The initial distribution of ethylmercury in neonatal mice is similar to that of methylmercury, but they differ sharply in their tissue deposition and their metabolism to Hg^{2+} (*4*). This suggests that the data on methylmercury may not be suitable for risk assessment for thimerosal (*1*, *5*). Methylmercury reacts rapidly with and has a very high affinity for protein and nonprotein thiols (*1*, *7*8), and ethylmercury is likely similar in this regard.

Thus, to elucidate some of the basic chemistry and biochemistry of thimerosal, the reactions of thimerosal with nonprotein and protein thiols and the cellular effects of thimerosal have been studied. While the reaction of thimerosal with thiols has been assumed to be an exchange reaction to yield an ethylmercury-thiol adduct (Figure 1), this does not seem to have been shown. In this study, we showed by MS that thimerosal undergoes an exchange reaction with cysteine, GSH, and human

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.

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Mitochondrial dysfunction, impaired oxidative-reduction activity, degeneration, and death in human neuronal and fetal cells induced by low-level exposure to thimerosal and other metal compounds

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(*Final version received 4 June 2008*)

Thimerosal (ethylmercurithiosalicylic acid), an ethylmercury (EtHg)-releasing compound (49.55% mercury (Hg)), was used in a range of medical products for more than 70 years. Of particular recent concern, routine administering of Thimerosal-containing biologics/childhood vaccines have become significant sources of Hg exposure for some fetuses/infants. This study was undertaken to investigate cellular damage among *in vitro* human neuronal (SH-SY-5Y neuroblastoma and 1321N1 astrocytoma) and fetal (nontransformed) model systems using cell vitality assays and microscope-based digital image capture techniques to assess potential damage induced by Thimerosal and other metal compounds (aluminum (Al) sulfate, lead (Pb)(II) acetate, methylmercury (MeHg) hydroxide, and mercury (Hg)(II) chloride) where the cation was reported to exert adverse effects on developing cells. Thimerosal-associated cellular damage was also evaluated for similarity to pathophysiological findings observed in patients diagnosed with autistic disorders (ADs). Thimerosal-induced cellular damage as evidenced by concentration- and time-dependent mitochondrial damage, reduced oxidative–reduction activity, cellular degeneration, and cell death in the *in vitro* human neuronal and fetal model systems studied. Thimerosal at low nanomolar (nM) concentrations induced significant cellular toxicity in human neuronal and fetal cells. Thimerosal-induced cytoxicity is similar to that observed in AD pathophysiologic studies. Thimerosal was found to be significantly more toxic than the other metal compounds examined. Future studies need to be conducted to evaluate additional mechanisms underlying Thimerosal-induced cellular damage and assess potential co-exposures to other compounds that may increase or decrease Thimerosal-mediated toxicity.

Keywords: autism; glial; lead; mercury; mercuric; neurodevelopmental

Introduction

Thimerosal (ethylmercurithiosalicylic acid) is an ethylmercury (EtHg)-releasing compound that has been used in a range of medical products for more than 70 years (Geier et al. 2007). Thimerosal contains 49.55% mercury (Hg) and, in aqueous solutions, is

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.

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Research Article

Thimerosal-Derived Ethylmercury Is a Mitochondrial Toxin in Human Astrocytes: Possible Role of Fenton Chemistry in the Oxidation and Breakage of mtDNA

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Thimerosal generates ethylmercury in aqueous solution and is widely used as preservative. We have investigated the toxicology of Thimerosal in normal human astrocytes, paying particular attention to mitochondrial function and the generation of specific oxidants. We find that ethylmercury not only inhibits mitochondrial respiration leading to a drop in the steady state membrane potential, but also concurrent with these phenomena increases the formation of superoxide, hydrogen peroxide, and Fenton/Haber-Weiss generated hydroxyl radical. These oxidants increase the levels of cellular aldehyde/ketones. Additionally, we find a five-fold increase in the levels of oxidant damaged mitochondrial DNA bases and increases in the levels of mtDNA nicks and blunt-ended breaks. Highly damaged mitochondria are characterized by having very low membrane potentials, increased superoxide/hydrogen peroxide production, and extensively damaged mtDNA and proteins. These mitochondria appear to have undergone a permeability transition, an observation supported by the five-fold increase in Caspase-3 activity observed after Thimerosal treatment.

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.

RESEARCH PAPER

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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

Thimerosal is a preservative used in multidose vials of vaccine formulations to prevent bacterial and fungal contamination. We recently reported that nanomolar concentrations of thimerosal induce cell cycle arrest of human T cells activated via the TCR and inhibition of proinflammatory cytokine production, thus interfering with T-cell functions. Given the essential role of dendritic cells (DCs) in T-cell polarization and vaccine immunity, we studied the influence of non-toxic concentrations of thimerosal on DC maturation and functions. Ex-vivo exposure of human monocyte-derived DCs to nanomolar concentrations of thimerosal prevented LPS-induced DC maturation, as evidenced by the inhibition of morphological changes and a decreased expression of the maturation markers CD86 and HLA-DR. In addition thimerosal dampened their proinflammatory response, in particular the production of the Th1 polarizing cytokine IL-12, as well as TNF-α and IL-6. DC-dependent T helper polarization was altered, leading to a decreased production of IFN-γ IP10 and GM-CSF and increased levels of IL-8, IL-9, and MIP-1α. Although multi-dose vials of vaccines containing thimerosal remain important for vaccine delivery, our results alert about the ex-vivo immunomodulatory effects of thimerosal on DCs, a key player for the induction of an adaptive response

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 agents[12] and in vaccines[13] 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,[16,17,18] 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.

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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 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.

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Competing Interests: CrossJect provided the academic research/private research partnership to fund a CIFRE fellowship used in this study. There are no patents, products in development or marketed products to declare. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

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Introduction

Thimerosal is a preservative used widely in vaccine formulations to prevent bacterial and fungal contamination in multidose vials of vaccine [1] [2]. Thimerosal, named also thiomersal or merthiolate in clinical studies, is an ethylmercury-containing pharmaceutical compound that contains 49.6% mercury by weight and metabolizes into ethylymercury (etHg) and thiosalicylate [3]. Thimerosal has served as a preservative in vaccines since 1930, but in the late

line following exposure to μM concentrations of thimerosal [10]. The deleterious effects of thimerosal were also reported on HeLa S epithelial cells, inducing an oxidative stress and cell death that were completely suppressed by pretreating the cells with N-acetyl-l-cysteine (NAC), a radical scavenger [11]. Thimerosal could also cause S phase arrest followed by mitochondrial apoptosis in murine myoblast cells that occurred via inhibition of the PI3K/Akt/survivin signaling pathway [12]. Surprisingly, little is known

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.

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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 (GI_{50}: 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 Hg^{2+} > 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 blockage of the electrons from NADPH *via* the thioredoxin system.

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 (Hg^{2+}) 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

Abbreviations: 6PGDH, 6-phosphogluconate dehydrogenase; EtHg, ethylmercury; G6PDH, glucose-6-phosphate dehydrogenase; Hg^{2+}, mercuric mercury; MeHg, methylmercury; Se^{2-}, selenide; SeO_3^{2-}/Se (IV), selenite; Se, selenium; TCV, thiomersal-

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.

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Toxicology

Toxicity of organic and inorganic mercury species in differentiated human neurons and human astrocytes

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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 thiomersal, 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 thiomersal as well as inorganic mercury chloride ($HgCl_2$) in differentiated human neurons (LUHMES) and human astrocytes (CCF-STTG1). 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 $HgCl_2$. In contrast to $HgCl_2$ 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.

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 thiomersal, 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^{2+} does not readily cross the blood brain barrier. Probably therefore effects of inorganic Hg^{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

Thimerosal and methyl mercury caused cell death in differentiated human neurons and astrocytes. Differentiated neurons showed a massive uptake of ethylmercury (degradation product of thimerosal). This affirms the type of neural damage seen in patients with autism.

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Research Article

Alternatively Spliced Methionine Synthase in SH-SY5Y Neuroblastoma Cells: Cobalamin and GSH Dependence and Inhibitory Effects of Neurotoxic Metals and Thimerosal

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The folate and cobalamin (Cbl-) dependent enzyme methionine synthase (MS) is highly sensitive to oxidation and its activity affects all methylation reactions. Recent studies have revealed alternative splicing of MS mRNA in human brain and patient-derived fibroblasts. Here we show that MS mRNA in SH-SY5Y human neuroblastoma cells is alternatively spliced, resulting in three primary protein species, thus providing a useful model to examine cofactor dependence of these variant enzymes. MS activity was dependent upon methylcobalamin (MeCbl) or the combination of hydroxocobalamin (OHCbl) and S-adenosylmethionine (SAM). OHCbl-based activity was eliminated by depletion of the antioxidant glutathione (GSH) but could be rescued by provision of either glutathionylcobalamin (GSCbl) or MeCbl. Pretreatment of cells with lead, arsenic, aluminum, mercury, or the ethylmercury-containing preservative thimerosal lowered GSH levels and inhibited MS activity in association with decreased uptake of cysteine, which is rate-limiting for GSH synthesis. Thimerosal treatment decreased cellular levels of GSCbl and MeCbl. These findings indicate that the alternatively spliced form of MS expressed in SH-SY5Y human neuronal cells is sensitive to inhibition by thimerosal and neurotoxic metals, and lower GSH levels contribute to their inhibitory action.

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.

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Low-dose Thimerosal in pediatric vaccines: Adverse effects in perspective

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Neurodevelopment

ABSTRACT

Vaccines are prophylactics used as the first line of intervention to prevent, control and eradicate infectious diseases. Young children (before the age of six months) are the demographic group most exposed to recommended/mandatory vaccines preserved with Thimerosal and its metabolite ethylmercury (EtHg). Particularly in the less-developed countries, newborns, neonates, and young children are exposed to EtHg because it is still in several of their pediatric vaccines and mothers are often immunized with Thimerosal-containing vaccines (TCVs) during pregnancy. While the immunogenic component of the product has undergone more rigorous testing, Thimerosal, known to have neurotoxic effects even at low doses, has not been scrutinized for the limit of tolerance alone or in combination with adjuvant-Al during immaturity or developmental periods (pregnant women, newborns, infants, and young children). Scientific evidence has shown the potential hazards of Thimerosal in experiments that modeled vaccine-EtHg concentrations. Observational population studies have revealed uncertainties related to neurological effects. However, consistently, they showed a link of EtHg with risk of certain neurodevelopment disorders, such as tic disorder, while clearly revealing the benefits of removing Thimerosal from children's vaccines (associated with immunological reactions) in developed countries. So far, only rich countries have benefited from withdrawing the risk of exposing young children to EtHg. Regarding Thimerosal administered to the very young, we have sufficient studies that characterize a state of uncertainty: the collective evidence strongly suggests that Thimerosal exposure is associated with adverse neurodevelopmental outcomes. It is claimed that the continued use of Thimerosal in the less-developed countries is due to the cost to change to another preservative, such as 2-phenoxyethanol. However, the estimated cost increase per child in the first year of life is lower than estimated lifetime cost of caring for a child with a neurodevelopmental disorder, such tic disorder. The evidence indicates that Thimerosal-free vaccine options should be made available in developing countries.

1. Introduction

Vaccines are prophylactics used as the first line of intervention to prevent, control, and eradicate infectious diseases. Young children (before the age of 6 months) are the demographic group most exposed to recommended/mandatory vaccines that are preserved with Thimerosal and its metabolite ethylmercury (EtHg). Furthermore, in less-developed countries, this vulnerable demographic range (newborns, neonates, young children) is additionally exposed to EtHg when mothers are immunized with Thimerosal-containing vaccines (TCVs) during pregnancy (Dórea, 2011a). Indeed, in certain circumstances, six TC… in he… ults sta… al., et … sal, res… nts …The

when bottled in multi-dose vials, a preservative may be justified. In order to be manufactured, vaccines have to be formulated to resist contamination in the production line and during handling and application from multi-dose vials. As a result, some vaccines contain both preservative-Thimerosal and adjuvant-Al.

During vaccine production, no modern toxicity studies are required to detect specific aspects of low-dose EtHg (alone or in combination with Aluminum) in susceptible individuals; rather, non-specific toxicity tests such as body weight changes are frequently used (Sharma et al., 2012). Albeit at low doses, toxic ingredients (such as Thimerosal and adjuvant-Al) are intrinsically part of the vaccine's development and

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.

htt… Rec… Ava… 001…

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 above 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 2.1 and 8.6 days, respectively, which are 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]

Public perception of the safety and efficacy of childhood vaccines has a direct impact on immunization rates (Biroscak et al. 2003; Thomas et al. 2004). The current debate linking the use of thimerosal in vaccines to autism and other developmental disorders [Institute of Medicine (IOM) 2001, 2004] has led many families to question whether the potential risks associated with early childhood immunization, in some cases, exceeds the U.S. EPA guidelines for MeHg exposure during pregnancy (0.1 μg/kg/day). Other estimates (Halsey 1999) 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 *c*) because ethylmercury decomposes faster than MeHg, 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; Stinson et al. 1989; Vahter 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.

ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research paper Acta Neurobiol Exp 2010, 70: 147–164

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 [^{11}C]diprenorphine (DPN) in exposed animals remained relatively constant over time, compared with unexposed animals, in which a significant decrease in [^{11}C]DPN binding occurred. These results suggest that maturational changes in amygdala volume and the binding capacity of [^{11}C]DPN in the amygdala was 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 amygdala lesions were still capable of interpreting and generating social behaviors (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 longitudi-

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.

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DELAYED ACQUISITION OF NEONATAL REFLEXES IN NEWBORN PRIMATES RECEIVING A THIMEROSAL-CONTAINING HEPATITIS B VACCINE: INFLUENCE OF GESTATIONAL AGE AND BIRTH WEIGHT

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This study examined whether acquisition of neonatal reflexes in newborn rhesus macaques was influenced by receipt of a single neonatal dose of hepatitis B vaccine containing the preservative thimerosal (Th). Hepatitis B vaccine containing a weight-adjusted Th dose was administered to male macaques within 24 h of birth ($n = 13$). Unexposed animals received saline placebo ($n = 4$) or no injection ($n = 3$). Infants were tested daily for acquisition of nine survival, motor, and sensorimotor reflexes. In exposed animals there was a significant delay in the acquisition of *root*, *snout*, and *suck* reflexes, compared with unexposed animals. No neonatal responses were significantly delayed in unexposed animals. Gestational age (GA) and birth weight (BW) were not significantly correlated. Cox regression models were used to evaluate main effects and interactions of exposure with BW and GA as independent predictors and time-invariant covariates. Significant main effects remained for exposure on *root* and *suck* when controlling for GA and BW, such that exposed animals were relatively delayed in time-to-criterion. Interaction models indicated there were various interactions between exposure, GA, and BW and that inclusion of the relevant interaction terms significantly improved model fit. This, in turn, indicated that lower BW and/or lower GA exacerbated the adverse effects following vaccine exposure. This primate model provides a possible means of assessing adverse neurodevelopmental outcomes from neonatal Th-containing hepatitis B vaccine exposure, particularly in infants of lower GA or BW. The mechanisms underlying these effects and the requirements for Th requires further study.

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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.

Molecular Psychiatry (2004) 9, 833–845

www.nature.com/mp

IMMEDIATE COMMUNICATION

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 challenges 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.
Molecular Psychiatry (2004) **9**, 833–845. doi:10.1038/sj.mp.4001529
Published online 8 June 2004

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

Autism spectrum disorders (ASDs) comprise a set of highly heritable conditions[1] with core impairments in social interaction, communication, and imagination. The prevalence of ASDs is reported to be rising worldwide,[2–4] an increase not fully explained by changes in awareness and diagnostic patterns.[2,3,5,6] Environmental susceptibility genes may be determinants of adverse neurodevelopmental outcomes following pre- or postnatal exposures. One environmental factor may be increased mercury burden through industrial sources, fish, and sodium

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.

mercury-related neurodevelopmental damage, we exposed mice of differing MHC (H-2) backgrounds[20] 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 ($H\text{-}2^s$) mice, but not in strains without autoimmune sensitivity (BALB/cJ, $H\text{-}2^d$, or C57BL/6J, $H\text{-}2^b$ mice).

Materials and methods

Cell Biol Toxicol
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Induction of metallothionein in mouse cerebellum and cerebrum with low-dose thimerosal injection

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Abstract Thimerosal, an ethyl mercury compound, is used worldwide as a vaccine preservative. We previously observed that the mercury concentration in mouse brains did not increase with the clinical dose of thimerosal injection, but the concentration increased in the brain after the injection of thimerosal with lipopolysaccharide, even if a low dose of thimerosal was administered. Thimerosal may penetrate the brain, but is undetectable when a clinical dose of thimerosal is injected; therefore, the induction of metallothionein (MT) messenger RNA (mRNA) and protein was observed in the cerebellum and cerebrum of mice after thimerosal injection, as MT is an inducible protein. MT-1 mRNA was expressed at 6 and 9 h in both the cerebrum and cerebellum, but MT-1 mRNA expression in the cerebellum was three times higher than that in the cerebrum after the injection of 12 μg/kg thimerosal. MT-2 mRNA was not expressed until 24 h in both organs. MT-3 mRNA was expressed in the cerebellum from 6 to 15 h after the injection, but not in the cerebrum until 24 h. MT-1 and MT-3 mRNAs were expressed in the cerebellum in a dose-dependent manner. Furthermore, MT-1 protein was detected from 6 to 72 h in the cerebellum after 12 μg/kg of thimerosal was injected and peaked at 10 h. MT-2 was detected in the cerebellum only at 10 h. In the cerebrum, little MT-1 protein was detected at 10 and 24 h, and there were no peaks of MT-2 protein in the cerebrum. In conclusion, MT-1 and MT-3 mRNAs but not MT-2 mRNA are easily expressed in the cerebellum rather than in the cerebrum by the injection of low-dose thimerosal. It is thought that the cerebellum is a sensitive organ against thimerosal. As a result of the present findings, in combination with the brain pathology observed in patients diagnosed with autism, the present study helps to support the possible biological plausibility for how low-dose exposure to mercury from thimerosal-containing vaccines may be associated with autism.

The authors found that low dose injections of thimerosal induced metallothione 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.

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Research report

Persistent behavioral impairments and alterations of brain dopamine system after early postnatal administration of thimerosal in rats

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ABSTRACT

The neurotoxic organomercurial thimerosal (THIM), used for decades as vaccine preservative, is a suspected factor in the pathogenesis of some neurodevelopmental disorders. Previously we showed that neonatal administration of THIM at doses equivalent to those used in infant vaccines or higher, causes lasting alterations in the brain opioid system in rats. Here we investigated neonatal treatment with THIM (at doses 12, 240, 1440 and 3000 μg Hg/kg) on behaviors, which are characteristically altered in autism, such as locomotor activity, anxiety, social interactions, spatial learning, and on the brain dopaminergic system in Wistar rats of both sexes. Adult male and female rats, which were exposed to the entire range of THIM doses during the early postnatal life, manifested impairments of locomotor activity and increased anxiety/neophobia in the open field test. In animals of both sexes treated with the highest THIM dose, the frequency of prosocial interactions was reduced, while the frequency of asocial/antisocial interactions was increased in males, but decreased in females. Neonatal THIM treatment did not significantly affect spatial learning and memory. THIM-exposed rats also manifested reduced haloperidol-induced catalepsy, accompanied by a marked decline in the density of striatal D_2 receptors, measured by immunohistochemical staining, suggesting alterations to the brain dopaminergic system. Males were more sensitive than females to some neurodisruptive/neurotoxic actions of THIM. These data document that early postnatal THIM administration causes lasting neurobehavioral impairments and neurochemical alterations in the brain, dependent on dose and sex. If similar changes occur in THIM/mercurial-exposed children, they could contribute do neurodevelopmental disorders.

1. Introduction

organs [1,2]. With increasing numbers of vaccines injected to pro-

Thimerosal administered to rat neonates showed sex-dependent impairment of brain dopaminergic system, leading to aberant behaviors similar to autism. Males were more sensitive than females to some neurodisruptive/neurotoxic actions of thimerosal, similar to the higher incide of autism in males compared to females.

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Plenary article

Embryonic exposure to thimerosal, an organomercury compound, causes abnormal early development of serotonergic neurons

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ARTICLE INFO

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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.

Thimerosal, an organomercury compound, is known for its preservative effects on pediatric vaccines [2,12]. Thimerosal biotransforms *in vivo* to ethylmercury and subsequently into 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]. rats 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 given to rat embryos (on day 9 of gestation) led to abnormal distribution of serotonergic neurons. Serotonin levels have been shown to be dysregulated in autistic subjects, particularly males.

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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 ($HgCl_2$) in suckling rats. Three experimental groups were studied: control, TM, and $HgCl_2$, with 12 to18 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 $HgCl_2$, 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 $HgCl_2$-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.

Cerebellum
DOI 10.1007/s12311-011-0319-5

ORIGINAL PAPER

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 intrabrain conversion of thyroxine to the active hormone, 3′,3,5-triiodothyronine (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-nitrotrosine (3-NT) · Type 2 deiodinase (D2)

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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

Rat pups exposed to thimerosal levels in utero (similar to the maternal flu shot) exhibited aberrant brain oxidative stress (in the cerebellum) as well as autistic like behaviors. These negative neurodevelopmental impacts were both sex and rat strain dependant. Recent studies have implicated cerebellar pathology in the etiology of ASD.

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ORIGINAL PAPER

Administration of Thimerosal to Infant Rats Increases Overflow of Glutamate and Aspartate in the Prefrontal Cortex: Protective Role of Dehydroepiandrosterone Sulfate

Michalina Duszczyk-Budhathoki · Mieszko Olczak · Malgorzata Lehner · Maria Dorota Majewska

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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. Coadministration 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. Coapplication 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 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.

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Original article

Prenatal exposure to organomercury, thimerosal, persistently impairs the serotonergic and dopaminergic systems in the rat brain: Implications for association with developmental disorders

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Abstract

Thimerosal, an organomercury compound, has been widely used as a preservative. Therefore, concerns have been raised about its neurotoxicity. We recently demonstrated perturbation of early serotonergic development by prenatal exposure to thimerosal (Ida-Eto et al. (2011) [11]). Here, we investigated whether prenatal thimerosal exposure causes persistent impairment after birth. Analysis on postnatal day 50 showed significant increase in hippocampal serotonin following thimerosal administration on embryonic day 9. Furthermore, not only serotonin, striatal dopamine was significantly increased. These results indicate that embryonic exposure to thimerosal produces lasting impairment of brain monoaminergic system, and thus every effort should be made to avoid the use of thimerosal.

Keywords: Thimerosal; Serotonin; Dopamine; Embryonic exposure; Developmental disorders; Rat

1. Introduction

Thimerosal, an organomercury compound, has been widely used as a preservative [1]. Thimerosal is metabolized first to ethylmercury and further to inorganic mercury, both of which accumulate in the brain and other organs and have neurotoxic activity [2,3]. Accordingly, use of thimerosal such as vaccines is of great concern, particularly on infants and fetuses [4,5], and therefore, efforts have been made to reduce thimerosal from vaccines [6].

The adverse effects of thimerosal after neonatal

hippocampal neurodegeneration [8], and changes in the dopamine system with subsequent behavioral disorders [9]. In addition, thimerosal was shown to affect neurite extension of neuroblastoma cells *in vitro*, therefore, it is evident that thimerosal leads to neurological abnormalities [10]. However, little is known regarding the prenatal effects of thimerosal. We recently reported that exposure of pregnant rats at gestational day 9 (E9) to thimerosal increased the number of serotonergic neurons in the lateral portion of the caudal raphe in E15 rat hindbrain and thus prenatal thimerosal exposure

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.

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Studies on H1N1 vaccine-induced monoamines alternations and oxidative stress on brain of adult mice

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H1N1 vaccine; Thiomerosal; Monoamines; Oxidative stress; Histopathology.

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 thiomerosal, adjuvant, H1N1 antigen and H1N1 vaccine. Results obtain on the present study showed that thiomerosal, H1N1 antigen and H1N1 vaccine were caused significant decrease in norepinephrine (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 thiomerosal.

INTRODUCTION

New communicable disease influenza A (H1N1) affected geographically diverse areas around the world in 2009. Person to person transmission has led to increase the 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

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.

Effect of thimerosal on the neurodevelopment of premature rats

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Original article

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.**

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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.[1] 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[2,3] 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,[4] which is mainly executed by neurotransmitters. The variants of dopamine D4 receptor (DRD4) are reported to be associated with memory function of rats,[5] whereas serotonin 2A receptor (5-HT2AR) is correlated with impaired episodic memory performance.[6] It was reported that in the human neuroblastoma cell line, thimerosal induced mitochondria-mediated apoptosis.[7]

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.

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

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

Key words: thimerosal; transcriptomic analyses; anterior pitu-

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

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."

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Invited critical review

Thimerosal: Clinical, epidemiologic and biochemical studies☆

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ABSTRACT

Introduction: Thimerosal (or Thiomersal) is a trade name for an organomercurial compound (sodium ethylmercury (Hg) thiosalicylate) that is 49.55% Hg by weight, which rapidly decomposes in aqueous saline solutions into ethyl-Hg hydroxide and ethyl-Hg chloride. Developed in 1927, it has been and is still being used as a preservative in some cosmetics, topical pharmaceuticals, and biological drug products, including vaccines. Concerns have been voiced about its use because it is toxic to human cells. Although it is banned in several countries, it continues to be added to some vaccines in the United States and many vaccines in the developing world.

Discussion: This critical review focuses on the clinical, epidemiological, and biochemical studies of adverse effects from Thimerosal in developing humans. This review will include research that examines fetal, infant, and childhood death; birth defects; neurodevelopmental testing deficits in children; and neurodevelopmental disorders (attention deficit/hyperactivity disorder, autism spectrum disorder, tic disorder, and specific developmental delays). The review will also look at the research that examined the outcomes of acute accidental ethyl-Hg poisoning in humans. The studies that examine the underlying biochemical insights into the neuronal cellular damage will also be explored.

Conclusion: The culmination of the research that examines the effects of Thimerosal in humans indicates that it is a poison at minute levels with a plethora of deleterious consequences, even at the levels currently administered in vaccines.

Contents

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.

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Temas de Debate

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, toxicológicos fetales y sobre salud reproductiva. Se encontró múltiples asociaciones entre la exposición a timerosal y las enfermedades del neurodesarrollo infantil. Tal neurotoxicidad ocurre en los infantes y fetos de gestantes vacunadas por dosis acumulativa de mercurio. Las diversas evidencias implican al timerosal como el agente causante, agravante o disparador de las enfermedades 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 exposition 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

Currently vaccines without thimerosal are causing less incidence of children's neurodevelopmental disorders.

Key words: Thimerosal; autism; nervous system diseases; child development; vaccines.

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.

INTRODUCCIÓN

En el año 2004, la *American Academy of Pediatrics* (AAP) y el *Department of Health and Human Services* de los Estados Unidos de Norteamérica (EE.UU.) lanzaron una alerta epidémica, impresionados por el número cada vez más alarmante de casos de autismo y otros desórdenes difusos del neurodesarrollo infantil, señalando que para entonces 1 de cada 6 niños norteamericanos tenía una trastorno del desarrollo o de la conducta y que 1 de cada 166 niños tenía

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Review

How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis

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Abstract

Recently higher rates of autism diagnosis suggest involvement of environmental factors in causing this developmental disorder, in concert with genetic risk factors. Autistic children exhibit evidence of oxidative stress and impaired methylation, which may reflect effects of toxic exposure on sulfur metabolism. We review the metabolic relationship between oxidative stress and methylation, with particular emphasis on adaptive responses that limit activity of cobalamin and folate-dependent methionine synthase. Methionine synthase activity is required for dopamine-stimulated phospholipid methylation, a unique membrane-delimited signaling process mediated by the D4 dopamine receptor that promotes neuronal synchronization and attention, and synchrony is impaired in autism. Genetic polymorphisms adversely affecting sulfur metabolism, methylation, detoxification, dopamine signaling and the formation of neuronal networks occur more frequently in autistic subjects. On the basis of these observations, a "redox/methylation hypothesis of autism" is described, in which oxidative stress, initiated by environment factors in genetically vulnerable individuals, leads to impaired methylation and neurological deficits secondary to reductions in the capacity for synchronizing neural networks.

Keywords: Arsenic; Attention; Attention-deficit hyperactivity disorder (ADHD); D4 dopamine receptor; Folic acid; Heavy metal; Lead; Mercury; Oxidative stress; Neuronal synchronization; Pesticide; Phospholipid methylation; Thimerosal; Vitamin B_{12}; Xenobiotic

Contents

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.

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The plausibility of a role for mercury in the etiology of autism: a cellular perspective

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Autism is defined by a behavioral set of stereotypic and repetitious behavioral patterns in combination with social and communication deficits. There is emerging evidence supporting the hypothesis that autism may result from a combination of genetic susceptibility and exposure to environmental toxins at critical moments in development. Mercury (Hg) is recognized as a ubiquitous environmental neurotoxin and there is mounting evidence linking it to neurodevelopmental disorders, including autism. Of course, the evidence is not derived from experimental trials with humans but rather from methods focusing on biomarkers of Hg damage, measurements of Hg exposure, epidemiological data, and animal studies. For ethical reasons, controlled Hg exposure in humans will never be conducted. Therefore, to properly evaluate the Hg-autism etiological hypothesis, it is essential to first establish the biological plausibility of the hypothesis. This review examines the plausibility of Hg as the primary etiological agent driving the cellular mechanisms by which Hg-induced neurotoxicity may result in the physiological attributes of autism. Key areas of focus include: (1) route and cellular mechanisms of Hg exposure in autism; (2) current research and examples of possible genetic variables that are linked to both Hg sensitivity and autism; (3) the role Hg may play as an environmental toxin fueling the oxidative stress found in autism; (4) role of mitochondrial dysfunction; and (5) possible role of Hg in abnormal neuroexcitory and excitotoxity that may play a role in the immune dysregulation found in autism. Future research directions that would assist in addressing the gaps in our knowledge are proposed.

Keywords: autism; mercury; cellular; oxidative stress; mitochondrial; immune dysfunction

Introduction

Rather than critically examining the extensive literature relating to the possible role of Hg

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 neuroexcitory and excitotoxity actions that may play a role in the immune dysregulation found in autism.

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REVIEW PAPER

Abating Mercury Exposure in Young Children Should Include Thimerosal-Free Vaccines

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Abstract Pediatric immunization is essential to prevent, control and eradicate children`s infectious diseases. Newborns and infants in less developed countries have a concentrated schedule of Thimerosal-containing vaccines (TCVs); pregnant mothers are also immunized with TCVs. Metabolic changes during early development are demonstrably an important risk factor for ethylmercury (EtHg) effects on neurodevelopment, while exposure to Thimerosal sensitizes susceptible individuals to life-long contact dermatitis. Concerns regarding toxicity of Hg have moved rich nations to withdraw it from medicines and, in particular, Thimerosal from pediatric vaccines; it has been more than 20 years since rich countries started using Thimerosal-free vaccines. TCVs and Thimerosal-free vaccines show dissimilar profiles of adverse effects. Thimerosal-free vaccines have shown a decrease in contact dermatitis, while TCVs showed a significant association with increased risk of tic disorders; in some circumstances, EtHg in combination with other neurotoxic substances negatively impacted neurobehavioral tests. In studies that explored vaccines and risk of tics, Thimerosal was a necessary factor. However, when the binary exposure to organic Hg forms (TCV–EtHg and fish-MeHg) was considered, effects on neurobehavioral tests were inconsistent. Conclusions: (a) The indiscriminate use of pediatric-TCVs in less developed countries carries an unjustifiable and excessive EtHg exposure with an unnecessary risk of neurotoxicity to the developing brain; (b) measurable benefits (of Thimerosal-free) and measurable risks of tic disorders have been associated with the (Thimerosal-containing) type of vaccine; (c) Thimerosal-free vaccines are clinically and toxicologically justifiable and they should be available to children in less developed countries.

Keywords Thimerosal-free vaccines · Ethylmercury · Infants · Contact dermatitis · Tic disorders

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.

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Original Investigation

Recurrence of Autism Spectrum Disorders in Full- and Half-Siblings and Trends Over Time
A Population-Based Cohort Study

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Supplemental content at jamapediatrics.com

IMPORTANCE To date, this is the first population-based study to examine the recurrence risk for autism spectrum disorders (ASDs), including time trends, and the first study to consider the ASDs recurrence risk for full- and half-siblings.

OBJECTIVES To estimate the relative recurrence risk for ASDs in a Danish population, including recurrence in full- and half-siblings, and to examine time trends in ASDs relative to the recurrence risk.

DESIGN, SETTING, AND PARTICIPANTS Population-based cohort study in Denmark. All children (about 1.5 million) born in Denmark between January 1, 1980, and December 31, 2004, were identified and followed up to December 31, 2010. We identified a maternal sibling subcohort derived from mothers with at least 2 children and a paternal sibling subcohort derived from fathers with at least 2 children.

EXPOSURES Children having an older sibling with ASDs are compared with children not having an older sibling with ASDs.

MAIN OUTCOMES AND MEASURES The adjusted hazard ratio for ASDs among children having an older sibling with ASDs compared with children not having an older sibling with ASDs.

RESULTS The overall relative recurrence risk for ASDs was 6.9 (95% CI, 6.1-7.8), and it did not change significantly over time; similar risks were observed in maternal and paternal full-siblings. The relative recurrence risks were 2.4 (95% CI, 1.4-4.1) for maternal half-siblings and 1.5 (95% CI, 0.7-3.4) for paternal half-siblings.

CONCLUSIONS AND RELEVANCE Our population-based recurrence risk estimate is lower than the recently reported estimates from clinical samples. Our results demonstrate no time trend in the ASDs recurrence risk as seen in the ASDs prevalence. The difference in the recurrence risk between full- and half-siblings supports the role of genetics in ASDs, while the significant recurrence risk in maternal half-siblings may support the role of factors associated with pregnancy and the maternal intrauterine environment in ASDs.

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.

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Article

A Dose-Response Relationship between Organic Mercury Exposure from Thimerosal-Containing Vaccines and Neurodevelopmental Disorders

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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 signficantly 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

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Review Article

Methodological Issues and Evidence of Malfeasance in Research Purporting to Show Thimerosal in Vaccines Is Safe

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There are over 165 studies that have focused on Thimerosal, an organic-mercury (Hg) based compound, used as a preservative in many childhood vaccines, and found it to be harmful. Of these, 16 were conducted to specifically examine the effects of Thimerosal on human infants or children with reported outcomes of death; acrodynia; poisoning; allergic reaction; malformations; autoimmune reaction; Well's syndrome; developmental delay; and neurodevelopmental disorders, including tics, speech delay, language delay, attention deficit disorder, and autism. In contrast, the United States Centers for Disease Control and Prevention states that Thimerosal is safe and there is "no relationship between [T]himerosal[-]containing vaccines and autism rates in children." This is puzzling because, in a study conducted directly by CDC epidemiologists, a 7.6-fold increased risk of autism from exposure to Thimerosal during infancy was found. The CDC's current stance that Thimerosal is safe and that there is no relationship between Thimerosal and autism is based on six specific published epidemiological studies coauthored and sponsored by the CDC. The purpose of this review is to examine these six publications and analyze possible reasons why their published outcomes are so different from the results of investigations by multiple independent research groups over the past 75+ years.

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.

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Autism: A form of lead and mercury toxicity

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ABSTRACT

Aim: Autism is a developmental disability characterized by severe deficits in social interaction and communication. The definite cause of autism is still unknown. The aim of this study is to find out the relation between exposure to Lead and/or mercury as heavy metals and autistic symptoms, dealing with the heavy metals with chelating agents can improve the autistic symptoms.

Method: Blood and hair samples were obtained from 45 children from Upper Egypt with autism between the ages of 2 and 10 years and 45 children served as controls in the same age range, after taken an informed consent and fill a questionnaire to assess the risk factors. The samples were analyzed blindly for lead and mercury by using atomic absorption and ICP-MS. Data from the two groups were compared, then follow up of the autistic children after treatment with chelating agents were done.

Results: The results obtained showed significant difference among the two groups, there was high level of mercury and lead among those kids with autism. Significant decline in the blood level of lead and mercury with the use of DMSA as a chelating agent. In addition, there was decline in the autistic symptoms with the decrease in the lead and mercury level in blood.

Conclusion: Lead and mercury considered as one of the main causes of autism. Environmental exposure as well as defect in heavy metal metabolism is responsible for the high level of heavy metals. Detoxification by chelating agents had great role in improvement of those kids.

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.

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Translational Neurodegeneration

RESEARCH Open Access

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.

Conclusions: Routine childhood vaccination is an important public health tool to reduce the morbidity and mortality associated with infectious diseases, but the present study provides new epidemiological evidence supporting an association between increasing organic-Hg exposure from Thimerosal-containing childhood vaccines and the subsequent risk of an ASD diagnosis.

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.

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Thomas M. Verstraeten, R. Davies, D. Gu, F DeStefano

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.

Neurodevelopmental Disorders after Thimerosal-Containing Vaccines: A Brief Communication

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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 diphtheria, tetanus, and acellular pertussis

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.

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A comparative evaluation of the effects of MMR immunization and mercury doses from thimerosal--containing childhood vaccines on the population prevalence of autism

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Potential conflict of interest: Dr. Mark Geier has been an expert witness and a consultant in cases involving adverse reactions to vaccines before the U.S. Vaccine Compensation Act and in civil litigation. David Geier has been a consultant in cases involving adverse reactions to vaccines before the U.S. Vaccine Compensation Act and in civil litigation.

Summary

Background: The purpose of the study was to evaluate the effects of MMR immunization and mercury from thimerosal-containing childhood vaccines on the prevalence of autism.

Material/Methods: Evaluations of the 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 were undertaken.

Results: It was determined that there was a close correlation between mercury doses from thimerosal--containing childhood vaccines and the prevalence of autism from the late 1980s through the mid-1990s. In contrast, there was a potential correlation between the number of primary pediatric measles-containing vaccines administered and the prevalence of autism during the 1980s. In addition, it was found that there were statistically significant odds ratios for the development of autism following increasing doses of mercury from thimerosal-containing vaccines (birth cohorts: 1985 and 1990–1995) in comparison to a baseline measurement (birth cohort: 1984). The contribution of thimerosal from childhood vaccines (>50% effect) was greater than MMR vaccine on the prevalence of autism observed in this study.

Conclusions: The results of this study agree with a number of previously published studies. These studies have shown that there is biological plausibility and epidemiological evidence showing a direct relationship between increasing doses of mercury from thimerosal-containing vaccines and neurodevelopmental disorders, and measles-containing vaccines and serious neurological disorders. It is recommended that thimerosal be removed from all vaccines, and additional research be undertaken to produce a MMR vaccine with an improved safety profile.

key words: autism • ethylmercury • MMR • neurodevelopmental disorders • thimerosal

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.

Early Downward Trends in Neurodevelopmental Disorders Following Removal of Thimerosal-Containing Vaccines

David A. Geier, B.A.
Mark R. Geier, M.D., Ph.D.

ABSTRACT

Contemporaneously with the epidemic rise in neurodevelopmental disorders (NDs), first observed in the United States during the 1990s, the childhood immunization schedule was expanded by the U.S. Centers for Disease Control and Prevention (CDC) to include several additional thimerosal-containing vaccines (TCVs). On July 7, 1999, a joint recommendation was made by the American Academy of Pediatrics (AAP) and the U.S. Public Health Service (PHS) to remove thimerosal from vaccines. A two-phase study was undertaken to evaluate trends in diagnosis of new NDs entered into the Vaccine Adverse Event Reporting System (VAERS) and the California Department of Developmental Services (CDDS) databases on a reporting quarter basis, from 1994 through 2005. Significant increasing trends in newly diagnosed NDs were observed in both databases 1994 through mid-2002. Significant decreasing trends in newly diagnosed NDs were observed in both databases from mid-2002 through 2005. The results indicate that the trends in newly diagnosed NDs correspond directly to the expansion and subsequent contraction of the cumulative mercury dose to which children were exposed from TCVs through the U.S. immunization schedule.

Background

In 2004, the Department of Health and Human Services and the American Academy of Pediatrics (AAP) issued an Autism A.L.A.R.M., stating that 1 in 166 children currently have an autistic disorder, and 1 in 6 children have a developmental and/or behavioral disorder. Autism, once rare, is now more prevalent than childhood cancer, diabetes, and Down syndrome.[1] Epidemic trends in neurodevelopmental disorders (NDs) were first observed in the United States during the 1990s,[1-3] and cannot be explained by immigration, changed diagnostic criteria, or improved identification.[1,4-8]

Autism is an ND characterized by impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movements.[1] While genetic factors are important in the pathogenesis of autistic disorders, a role for environmental factors has received considerable attention.

Thimerosal is an ethylmercury-containing compound (49.6% mercury by weight) that was historically added to many vaccines at the preservative level (0.005% to 0.01%). The U.S. Centers for Disease Control and Prevention (CDC), from the late 1980s through the 1990s, expanded the number of doses of TCVs to be administered to U.S. infants. To five doses of diptheria-tetanus-whole-cell-pertussis (DTP) vaccine were added three doses of hepatitis B (Hep b) vaccine and four of *Haemophilus influenzae* type b (Hib) vaccine. Additionally, the CDC began recommending three doses of influenza vaccine for certain infant populations. An infant who received all of these vaccines on schedule could have received as much as 200 micrograms (μg) of mercury during the first 6 months of life.[1-4]

In response to theoretical concerns about the cumulative doses of mercury from TCVs, the AAP and the U.S. Public Health Service (PHS) issued a joint statement on July 7, 1999, calling for the removal of thimerosal from all vaccines.[1,4] It has been estimated that the last thimerosal-containing Hep b, diphtheria-tetanus-acellular-pertussis (DTaP) and Hib vaccines were manufactured in 2000-2001 and expired at the end of 2002 (or early 2003).[1,4] Table 1 summarizes significant historical dates in the use of pediatric TCVs in the United States.

Considering all significant environmental exposures to mercury, such as through breast milk, TCVs represent almost 50% of the total mercury dose some infants received.[1,5] The 187.5 μg of mercury through TCVs plus the average of 164 μg from breast milk during the first 6 months exceeded the methylmercury safety guidelines established by the U.S. Environmental Protection Agency (EPA), Health Canada, the World Health Organization (WHO), the Agency for Toxic Substances Disease Registry (ATSDR), and the U.S. Food and Drug Administration (FDA).[1,5] With no additional exposure from any source, these doses also exceeded the methylmercury guidelines for the first year of life set by all of these agencies except the FDA.[1,5]

Despite its removal from many childhood vaccines, thimerosal is still routinely added to some formulations of influenza vaccine administered to U.S. infants, as well as to several other vaccines (e.g. tetanus-diphtheria and monovalent tetanus) administered to older children and adults. In 2004, the Institute of Medicine (IOM) of the U.S. National Academy of Sciences (NAS) retreated from the stated 1999 goal of the AAP and the PHS to remove thimerosal from U.S. vaccines as soon as possible.[1,6] Furthermore, many nations still add thimerosal to many of their pediatric vaccines, and WHO and

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.

A meta-analysis epidemiological assessment of neurodevelopmental disorders following vaccines administered from 1994 through 2000 in the United States

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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 versue thimerosal free DTaP vaccines.

REVIEW ARTICLE

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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 (pentacarboxyporphyrin, precoproporphyrin, 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 (GSH), 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.

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.

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Abnormal Brain Connectivity Spectrum Disorders Following Thimerosal Administration: A Prospective Longitudinal Case–Control Assessment of Medical Records in the Vaccine Safety Datalink

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Abstract

Background: Autism spectrum disorder (ASD), tic disorder (TD), and hyperkinetic syndrome of childhood (attention deficit disorder [ADD]/attention deficit hyperactivity disorder [ADHD]) are disorders recently defined as abnormal connectivity spectrum disorders (ACSDs) because they show a similar pattern of abnormal brain connectivity. This study examines whether these disorders are associated with exposure to thimerosal, a mercury (Hg)-based preservative.

Methods: A hypothesis testing case-control study evaluated the Vaccine Safety Datalink for the potential dose-dependent odds ratios (ORs) for diagnoses of ASD, TD, and ADD/ADHD compared to controls, following exposure to Hg from thimerosal-containing *Haemophilus influenzae* type b vaccines administrated within the first 15 months of life. Febrile seizures, cerebral degeneration, and unspecified disorders of metabolism, which are not biologically plausibly linked to thimerosal, were examined as control outcomes.

Results: On a per 25 μg Hg basis, cases diagnosed with ASD (OR = 1.493), TD (OR = 1.428), or ADD/ADHD (OR = 1.503) were significantly ($P < .001$) more likely than controls to have received increased Hg exposure. Similar relationships were observed when separated by gender. Cases diagnosed with control outcomes were no more likely than controls to have received increased Hg exposure.

Conclusion: The results suggest that Hg exposure from thimerosal is significantly associated with the ACSDs of ASD, TD, and ADD/ADHD.

Keywords

Asperger, autism, ethylmercury, PDD-NOS, thimerosal, Tourette, ADD/ADHD, Mercury

Introduction

Autism spectrum disorder (ASD), tic disorder (TD), and hyperkinetic syndrome of childhood (also known as atten-

It has also been hypothesized that the etiological basis of ACSDs is plausibly related to neuronal insult (eg,

In this study, the CDC's Vaccine Safety Datalink was employed to show that ASD patients received higher levels of thimerosal in their infant vaccines. The odds ratio was determined as 1.493 and the result was statistically significant. This trend was also observed for patients diagnosed with tic disorder and hyperkinetic disorder in children.

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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 threefold 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.c

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A Case-Control Study Evaluating the Relationship Between Thimerosal-Containing *Haemophilus influenzae* Type b Vaccine Administration and the Risk for a Pervasive Developmental Disorder Diagnosis in the United States

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Abstract Thimerosal is an organic mercury (Hg)-containing compound (49.55 % Hg by weight) historically added to many multi-dose vials of vaccine as a preservative. A hypothesis testing case-control study evaluated automated medical records in the Vaccine Safety Datalink (VSD) for organic Hg exposure from Thimerosal in *Haemophilus influenzae* type b (Hib)-containing vaccines administered at specific times within the first 15 months of life among subjects diagnosed with pervasive developmental disorder (PDD) (n=534) in comparison to controls. The generally accepted biologically non-plausible linkage between Thimerosal exposure and subsequent diagnosis of febrile seizure (n=5886) was examined as a control outcome. Cases diagnosed with PDD received significantly more organic Hg within the first 6 months of life (odds ratio (OR)=1.97, p<0.001) and first 15 months of life (OR=3.94, p<0.0001) than controls, whereas cases diagnosed with febrile seizure were no more likely than controls to have received increased organic Hg. On a per microgram of organic Hg basis, cases diagnosed with a PDD in comparison to controls were at significantly greater odds (OR=1.0197, p<0.0001) of receiving increasing organic Hg exposure within the first 15 months of life, whereas cases diagnosed febrile seizure were no more likely than controls (OR=0.999, p>0.20) to have received increasing organic Hg exposure within the first 15 months of life. Routine childhood vaccination is an important public health tool to reduce the morbidity and mortality associated with infectious diseases, but the present study provides new epidemiological evidence of a significant relationship between increasing organic Hg exposure from Thimerosal-containing vaccines and the subsequent risk of PDD diagnosis in males and females.

Keywords Autism · Ethylmercury · Merthiolate · Thimerosal · Thiomersal · Vaccine

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Introduction

Thimerosal is an organic mercury (Hg)-containing compound (49.55 % Hg by weight) historically added to many multi-dose vials of vaccine as a preservative since the 1930s [1]. Thimerosal is initially metabolized into ethyl-Hg compounds and thiosalicylate and rapidly binds onto thiol groups found on many proteins in human blood [2]. It is then actively transported across the blood brain barrier, including by the L-type neutral amino acid carrier transport (LAT) system, into human neuronal cells [3, 4], where it significantly accumulates and persists for many months following exposure and alters numbers of neurons in the dentate gyrus of the hippocampus and thalamus [5, 6].

In 2008, an ecological birth cohort assessment of Thimer-

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.

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A Two-Phase Case-Control Study of Autism Risk Among Children Born From the Late 1990s Through the Early 2000s in the United States

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: This study evaluated the hypothesis that the 1999 recommendation by the American Academy of Pediatrics (AAP) and US Public Health Service (PHS) to reduce exposure to mercury (Hg) from Thimerosal in US vaccines would be associated with a reduction in the long-term risk of being diagnosed with autism.

Material/Methods: A two-phase assessment utilizing a case (n=73) -control (n=11,783) study in the Vaccine Adverse Event Reporting System (VAERS) database (for hypothesis generating) and a more rigorous, independent matched case (n=40) -control (n=40) study (hypothesis testing) was undertaken.

Results: Analysis of the VAERS database using logistic regression revealed that the odds ratio (OR) for being an autism case in the VAERS database significantly decreased with a more recent year of vaccination in comparison to controls (OR=0.65) from 1998 to 2003. Sex-separated analyses revealed similar significant effects for males (OR=0.62) and females (OR=0.71). Analyses of the matched case-control data revealed, using the t-test statistic, that the mean date of birth among cases diagnosed with an autism spectrum disorder (ASD) (2000.5±1.2) was significantly more in the past than in controls (2001.1±1.3). Logistic regression also revealed that the OR for being diagnosed with ASD significantly decreased with a more recent date of birth in comparison to controls (OR=0.67) from 1998–2003.

Conclusions: This study reveals that the risk of autism during from the late1990s to early 2000s in the US significantly decreased with reductions in Hg exposure from Thimerosal-containing childhood vaccines, but future studies should examine this phenomenon in other US populations. Vaccine programs have significantly reduced the morbidity and mortality associated with infectious disease, but Thimerosal should be removed from all vaccines.

MeSH Keywords: **Autistic Disorder • Child Development Disorders, Pervasive • Ethylmercury Compounds • Thimerosal**

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/900257

In this study, the VAERS database was examined for children born between 1998 and 2003. This period of time corresponds to a decrease in overall thimerosal exposure to infants in the U.S. due to the phase out of its use in the hepatitis B, Hib and DTaP/DTP vaccines. Over this time period, there was a statistically significant decline in autism reporting and the average year of birth among cases was earlier than that for non-autistic controls.

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Research Article

The risk of neurodevelopmental disorders following a Thimerosal-preserved DTaP formulation in comparison to its Thimerosal-reduced formulation in the vaccine adverse event reporting system (VAERS)

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Abstract: Mercury (Hg) exposure in human infants and fetuses has long been known to be significantly associated with neurodevelopmental disorders (NDs). Thimerosal (49.55% Hg by weight) is an ethyl-Hg containing compound added to many childhood vaccines as a preservative. A hypothesis testing case-control study was undertaken in the Vaccine Adverse Event Reporting System (VAERS) database (updated through September 2013) by examining 5,591 adverse event reports entered following Thimerosal-preserved Diphtheria-Tetanus-acellular-Pertussis (DTaP) (Tripedia™, Sanofi) administered from 1997-1999 (exposed) and following Thimerosal-reduced DTaP (Tripedia™, Sanofi) administered from 2004-2006 (unexposed). Cases were defined as individuals with adverse event reports with the outcomes of autism, speech disorder, mental retardation, or ND (at least of one these aforementioned specific outcomes being mentioned in the adverse event report). Controls were defined as individuals with adverse event reports without any mention of the specific case outcomes examined. Cases reported with the outcomes of autism (odds ratio = 7.67, $p < 0.0001$), speech disorders (odds ratio = 3.49, $p < 0.02$), mental retardation (odds ratio = 8.73, $p < 0.0005$), or ND (odds ratio = 4.82, $p < 0.0001$) were significantly more likely than controls to have received Thimerosal-preserved DTaP vaccine (exposed) in comparison to Thimerosal-reduced DTaP vaccine (unexposed). Though routine childhood vaccination is considered an important public health tool to reduce the morbidity and mortality associated with certain infectious diseases, this study supports a significant relationship between increased organic-Hg exposure from Thimerosal-preserved childhood vaccines and the child's subsequent risk of a ND diagnosis.

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.

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AN EVALUATION OF THE EFFECTS OF THIMEROSAL ON NEURODEVELOPMENTAL DISORDERS REPORTED FOLLOWING DTP AND Hib VACCINES IN COMPARISON TO DTPH VACCINE IN THE UNITED STATES

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Thimerosal is an ethylmercury (49.55% mercury by weight) preservative historically added to some vaccines. Toxicokinetic studies showed children in the United States received doses of mercury from Thimerosal-containing vaccines (TCVs) in excess of safety guidelines. In the United States during the 1990s, diphtheria–tetanus–pertussis (DTP) and Haemophilus influenzae *type b (Hib) vaccines (maximally, 50 μg mercury per joint administration) and diphtheria–tetanus–pertussis–*Haemophilus influenzae *type b (DTPH) vaccines (25 μg mercury per administration) were given to children in the same childhood vaccination schedule at 2, 4, 6, and 15–18 mo, so that children receiving DTP and Hib vaccines may have maximally received an additional 100 μg more mercury exposure from TCVs than children administered DTPH vaccines. A case-control epidemiological study of neurodevelopmental disorders (NDs) reported to the Vaccine Adverse Event Reporting System (VAERS) (online public access version; updated 31 August 2004) following administration of DTP vaccines in comparison DTPH vaccines manufactured by Lederle Laboratories (Pearl River, NY) from 1994 through 1998 was undertaken. Significantly increased odds ratios for autism, speech disorders, mental retardation, infantile spasms, and thinking abnormalities reported to VAERS were found following DTP vaccines in comparison to DTPH vaccines with minimal bias or systematic error. Additional ND research should be undertaken in the context of evaluating mercury-associated exposures, especially since in 2005 the Institute of Medicine issued a report calling into question handling of vaccine safety data by the National Immunization Program of the Centers for Disease Control and Prevention.*

Thimerosal, an ethylmercurial preservative (49.55% mercury by weight) historically added to many vaccines, may have represented a significant source of mercury exposure in susceptible children (Ball et al., 2001). Thimerosal is still routinely added to required vaccines administered to U.S. infants (e.g., for influenza), and the Institute of Medicine (2004) of the U.S. National Academy

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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.

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healthcare

A prospective study of thimerosal-containing Rho(D)-immune globulin administration as a risk factor for autistic disorders

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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, thiomersal*

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.

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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.

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

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-

Abbreviations: ADD, Attention Deficit Disorder; ADHD, Attention

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.

ARTICLE

Autism Prevalence Trends Over Time in Denmark

Changes in Prevalence and Age at Diagnosis

Erik T. Parner, PhD; Diana E. Schendel, PhD; Poul Thorsen, PhD

Objective: To examine the effect of changing age at diagnosis on the diagnosed prevalence of autism among different birth cohorts.

Design: Population-based cohort study.

Setting: Children were identified in the Danish Medical Birth Registry and psychiatric outcomes were obtained via linkage with the Danish National Psychiatric Register.

Participants: All children born in Denmark from January 1, 1994, through December 31, 1999 (N=407 458).

Main Outcome Measures: The age-specific prevalence, hazard ratio, and relative risk by age.

Results: Statistically significant shifts in age at diagnosis were observed for autism spectrum disorder; children diagnosed before age 9 years in the cohorts born between January 1, 1994, and December 31, 1995, between January 1, 1996, and December 31, 1997, and between January 1, 1998, and December 31, 1999, were on average diagnosed at ages 5.9 (95% confidence interval [CI], 5.8-6.0), 5.8 (95% CI, 5.7-5.9), and 5.3 (95% CI, 5.2-5.4) years, respectively. The relative risk comparing the 1996-1997 birth cohort with the 1994-1995 birth cohort at age 3 years was 1.20 (95% CI, 0.86-1.67), which decreased to 1.10 (95% CI, 1.00-1.20) at age 11 years. Similarly, the relative risk comparing the 1998-1999 birth cohort with the 1994-1995 birth cohort at age 3 years was 1.69 (95% CI, 1.24-2.31), which decreased to 1.23 (95% CI, 1.11-1.37) at age 11 years. Similar results were observed for childhood autism.

Conclusions: Shifts in age at diagnosis inflated the observed prevalence of autism in young children in the more recent cohorts compared with the oldest cohort. This study supports the argument that the apparent increase in autism in recent years is at least in part attributable to decreases in the age at diagnosis over time.

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A RECENT REVIEW OF THE EPIdemiology of autism documented the large number of studies that have shown an increase in the age-specific prevalence of reported autism cases in the last 2 decades.[1] Once considered rare, the current prevalence estimates for all autism spectrum disorders of about 6 cases per 1000 children indicate that autism spectrum disorder may be the sec- is increasing and that the increase may be due to adverse exposures such as childhood vaccines.[7]

The increase in the prevalence of reported cases may be caused by many factors such as heightened public awareness, changes in referral pattern, changes in diagnostic criteria, case identification, or reporting methods. In the few studies that have investigated the effect of these potential confounders the main focus has

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.