Medical Hypotheses (2004) x, xxx–xxx

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medical hypotheses

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The potential importance of steroids in the treatment of autistic spectrum disorders and other disorders involving mercury toxicity

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Received 9 November 2004; accepted 10 November 2004

Summary Autism is a neurodevelopmental disorder that according to the Centers for Disease Control and Prevention (CDC) affects 1 in 150 children in the United States. Autism is characterized by impairments in social relatedness and communication, repetitive behaviors, abnormal movements, and sensory dysfunction. Recently emerging evidence suggests that mercury, especially from childhood vaccines, appears to be a factor in the development of the autistic disorders, and that autistic children have higher than normal body-burdens of mercury. In considering mercury toxicity, it has previously been shown that testosterone significantly potentates mercury toxicity, whereas estrogen is protective. Examination of autistic children has shown that the severity of autistic disorders correlates with the amount of testosterone present in the amniotic fluid, and an examination of a case-series of autistic children has shown that some have plasma testosterone levels that were significantly elevated in comparison neurotypical control children. A review of some of the current biomedical therapies for autistics, such as glutathione and cysteine, chelation, secretin, and growth hormone, suggests that they may in fact lower testosterone levels. We put forward the medical hypothesis that autistic disorders, in fact, represents a form of testosterone mercury toxicity, and based upon this observation, one can design novel treatments for autistics directed towards higher testosterone levels in autistic children. We suggest a series of experiments that need to be conducted in order to evaluate the exact mechanisms for mercury–testosterone toxicity, and various types of clinical manipulations that may be employed to control testosterone levels. It is hoped by devising therapies that address the steroid hormone pathways, in addition to the current treatments that successful lower heavy metal body-burdens of mercury, will work synergistically to improve clinical outcomes. In light of the fact that there are a number of other diseases that may have a chronic mercury toxicity component, such as Alzheimer's disease, heart disease, obesity, ALS, asthma, and other various forms of autoimmune disorders, it is imperative that further research should be conducted to understand mercury–testosterone toxicity.

Introduction

Autism is a neurodevelopmental disorder characterized by impairments in social relatedness and

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0306-9877/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.mehy.2004.11.018

communication, repetitive behaviors, abnormal movements, and sensory dysfunction. According to the most recent estimates published by the Centers for Disease Control and Prevention (CDC), it has been reported that approximately 1 in 150 children in the United States suffers from an autistic disorder [1,2]. Recent studies have reported that 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 [3–5].

Thimerosal, a preservative added to many vaccines, has become a major source of mercury among children in the United States who, within their first two years of life, may have received a quantity of mercury that exceeded Federal Safety Guidelines [6,7]. According to the CDC recommended immunization schedule in the United States during the 1990s, infants may have been exposed to 12.5 μg of ethylmercury at birth, 62.5 μg of ethylmercury at two months, 50 μg of ethylmercury at four months, 62.5 μg of ethylmercury at six months, and 50 μg of ethylmercury at 18 months, for a total of 237.5 μg of ethylmercury during the first 18 months of life, if all thimerosal-containing vaccines were administered [6,7].

Redwood et al. have estimated hair mercury concentrations expected to result from the recommended CDC childhood immunization schedule during the 1990s utilizing a one compartment pharmacokinetic model. The authors determined that modeled hair mercury concentrations in infants exposed to vicinal thimerosal were in excess of the Environmental Protection Agency (EPA)'s safety guidelines of 1 part-per-million (ppm) for up to the first 365 days, with several peak concentrations within this period. We have evaluated doses of mercury from thimerosal-containing childhood vaccines administered in accordance with the recommended CDC childhood immunization schedule during the 1990s in comparison the EPA and the Food and Drug Administration (FDA) safety guidelines for the oral ingestion of methylmercury, a similar compound to the ethylmercury [6]. We have reported that children received instantaneous doses of mercury from thimerosal-containing childhood vaccines that were many-fold in excess of the Federal Safety Guidelines [8,9].

Epidemiological studies conducted in the United States have examined the relationship between thimerosal-containing childhood vaccines and neurodevelopmental disorders. It has been shown that children receiving thimerosal-containing childhood vaccines were two- to sixfold statistically significantly more likely to develop neurodevelopmental disorders, depending upon the specific conditions or symptoms examined, in comparison to children receiving thimerosal-free childhood vaccines [8–12].

Several recent studies have clinically evaluated the body-burden of heavy metals present in children with autistic disorders in comparison to normal children. Bradstreet et al. have evaluated urinary heavy metals following three days of oral chelation with meso-2,3-dimercaptosuccinic acid (DMSA) in children with autistic disorders in comparison to a control population [13]. It was determined that autistic children had statistically significantly approximately sixfold higher urinary mercury concentrations than matched normal controls, whereas other heavy metals were present in similar urinary concentrations following three days of oral chelation with DMSA. In addition, in this study, urinary mercury concentrations were compared following three days of oral chelation with DMSA in matched vaccinated and unvaccinated normal children. It was observed that there were similar concentrations of urinary mercury in both groups following DMSA treatment. Holmes et al. have evaluated mercury excretion rates in autistic and normal children by evaluating mercury levels in first baby haircuts [14]. It was observed that the mercury levels in the first baby haircuts of children were inversely related to the severity of the autistic disorders of the children (i.e. the more severely affected the children were – the less mercury levels were present in their first baby haircut). It has been hypothesized that these results are consistent with autistic children having biochemical differences than normal children, possible as a result of genetic polymorphisms, resulting in children with autistic disorders have an increased body-burden of mercury in comparison to normal children [13].

Boris et al. have recently conducted genomic studies of children with autistic disorders in comparison to normal control populations [15]. The authors have examined genes in pathways that are responsible for the synthesis of key biochemical molecules that are of functional relevance in the excretion and/or oxidative stress protection of mercury from the body. Specifically, the authors demonstrated that there was approximately a twofold statistically significant increase in the homozygous methylenetetrahydrofolate reductase (MTHFR) 677TT gene among children with autistic disorders in comparison to controls. This of particular relevance because MTHFR 677TT is one of the key genes in the biochemical pathway involved with the synthesis of glutathione, a key molecule in the body's natural defenses against mercury,

and those with the MTHFR 677 TT gene have been found to have an enzyme with only 32% of the activity of normal [16].

The understanding of the cause of the autism epidemic has allowed for the design of treatment modalities that address the mercury toxic component of these disorders. These therapies include methods to remove the mercury by such techniques as the use of chelating agents and by corrections in various biochemical pathways that lead to sulfhydryl-containing compounds that the body uses to rid itself of the mercury [17,18]. Clearly much more clinical research in this area is needed. However, even at this early stage in the development of these types of therapies, it is clear that many if not most children only have a partial response or do not respond at all to such therapies. What is needed is another modality of clinical treatment to complement the work on eliminating mercury from these mercury toxic individuals.

We believe that the data from the autism epidemic itself suggests another method of attacking the problem in affected individuals. This potential therapeutic mode stems from the observation that autism affects males four to five times as often as females [1,2]. In fact, closer observation indicates that the more severely affected the group of autistics studied the higher the male to female ratio. In very severe autistics males may outnumber females by 15 to 1 or even more [14].

Mercury toxicity and testosterone

Furthermore, Clarkson et al. have developed a mouse model to evaluate the neurotoxic effects of alkyl mercury exposure on different sexes [19]. The authors reported that two-day-old mice were administered alkyl mercury at 4 mg of mercury/kg/bodyweight (low dose), 8 mg of mercury/kg/bodyweight (high dose), or no mercury. Animals were sacrificed 24 h later, and matched sections of brain were prepared. The total number of mitotic figures in the external granule layer of the cerebellar cortex were recorded and classified as early (prophase and metaphase) or late anaphase and telophase). Mercury concentrations in the brain for both males and females were 2.7 μg of mercury/g at the high dose exposure and 1.8 μg of mercury at the low dose exposure. The authors determined that at the high dose, male and female mice had similarly reduced percentages of late mitotic figures compared with controls. At the lower dose, female mice were significantly much less affected in their percentages of late mitotic figures compared with male mice. The authors concluded males are considerably more sensitive to the neurotoxic effects of mercury, and that in some human fetal/infant population exposures to low dose alkyl mercury, it has been observed that males were more sensitive than females to psychomotor retardation [20]. Muraoka and Itoh have investigated sex differences in the effects of mercury exposure on other organ systems [21]. The authors reported that when doses of 0.3—2 mg/kg of mercuric chloride were intravenously administered to rats of the JCL-SD strain, acute renal tubular necrosis was produced in the straight portion of the proximal tubules with a pronounced sex difference, the male being more susceptible. Necrosis was inhibited by castration of male rats and promoted by testosterone pretreatment.

Testosterone levels in autistic children

In considering testosterone levels among autistic children, Manning et al. have investigated prenatal testosterone levels in children with autistic spectrum disorders [22,23]. The authors examined 72 children with autism, including 23 children with Asperger syndrome (i.e. these children have less serve autistic disorders), 34 siblings, 88 fathers, 88 mothers, and sex and age-matched controls. The authors demonstrated that the more severely affected the children were the higher the levels of prenatal testosterone. In addition, Tordjman et al. have reported on a case-series of 12 prepubertal autistic children (6—10 years old) in their inpatient child psychiatry department, four of whom the researchers observed to have precocious secondary sexual characteristics (growth of pubic hair, increase of testis volume) that suggest high androgenic activity in autistic disorders [24]. In order to investigate the hyperandrogeny and autism association, the authors measured plasma testosterone and adrenal androgen in nine drug-free inpatients with DSM-IV autism and 62 normal subjects of same age, sex, weight (within 2 kg), and stage of puberty. Results showed that three of the nine autistic subjects had an abnormally high plasma testosterone concentration (over two standard deviations above the mean for the comparison subjects), with values above that of the highest in the comparison subjects. Among the autistic subjects, plasma testosterone concentration values (ng/ml) were 0.64 for a prepubertal 10-year-old boy, 8.8 for the pubertal 17-year-old boy, and 0.5 for the pubertal 13-year-old girl, whereas the appropriate comparison group means were

0.06 ± 0.03 (range = 0.01—0.15), 5.51 ± 1.27 (range = 0.27—7.50), and 0.12 ± 0.09 (range = 0.01—0.25), respectively. These three children all showed aggression against others. High plasma testosterone concentrations were present in all autistic subjects who exhibited aggression against others. The 10-year-old boy exhibited pubic hair growth. The 13-year old girl had a level of adrenal androgen (4.40 ng/ml) that was 500% higher than the mean level of the comparison subjects (mean = 0.88 ± 0.39, range = 0.36—1.70).

Medical hypothesis — tesosterone and mercury interaction

We believe that the potentiating effect of testosterone and the protective effect of estrogen need to be studied in far more detail because many of the seemingly unrelated clinical treatments that have had some reported success in the treatment of autism can actually be seen to have one thing in common, i.e. they all in one way or another lower testosterone [17,18]. In Table 1 is a summary of some of the treatments of autistics reported to have some beneficial affects. The kinetics of these effects need to be determined. Additionally, the precursors to testosterone and estrogen in the steroidgenic pathway as shown in Table 2 need to be tested for their effects on the toxicity of mercury compounds on neuron survival in tissue culture.

It is interesting to note that one of the enzymes in the pathway to synthesize testosterone, hydroxysteroid transferase (HST), which converts DHEA to DHEA-S, is known to have glutathione as a cofactor, and HST is known to be inhibited by mercury compounds (Fig. 1) [25]. Published studies have shown that glutathione levels tend to be lower in autistics and mercury levels are much higher [13,14,26]. Thus, in these individuals the HST may well be inhibited. Normally, most of the DHEA that is produced in the testosterone synthesis pathway is stored as DHEA-S, reducing the amount that goes on to be made into androstrenediol and then into testosterone, itself. In autistics, if the HST is blocked by low glutathione and high mercury, then the pathway would be shifted to produce more testosterone and subsequently more testosterone breakdown products. It is quite possible that the success that has been observed by various manipulations of the glutathione pathway in autistics may actually work by removing the block in the conversion of DHEA to DHEA-S which would result in a significant shift in the steroid pathway. Chelation may also work in the same way. It may also prove to be

Table 1 A summary of some treatments reported for autistic disorders and how they potentially effect testosterone levels

Clinical abnormality	Causes	Testosterone effect	Treatments	Testosterone effect
Mercury toxicity	Thimerosal, mercury (inability to eliminate mercury)	↑	Chelation [DMSA], biochemical treatments	↓
Biochemical abnormalities in glutathione, and other sulfhydryl pathways	Pre-existing genetic polymorphisms, low glutathione pathway — enzymes, substrates, and DNA polymorphisms	↑	Various biochemical manipulations of the pathway	↓
Autism spectrum disorders	Thimerosal, mercury (inability to eliminate mercury)	↑	Secretin therapy down regulates hypothalamus–pituitary–adrenal axis	↓
Autism spectrum disorders	Thimerosal, mercury (inability to eliminate mercury)	↑	Growth hormone down regulates hypothalamus–pituitary–adrenal axis	↓
Aggressive behavior	Thimerosal, mercury (inability to eliminate mercury)	↑	All of the above	↓

Table 2 The steroidogenic pathway

Cholesterol			DHEA-S	
↓	**Progestogens**		↑↓	**Androgens**
Pregnenolone	→ 17-hydroxy-pregnenolone	→	DHEA	⇄ Androstenediol
↓	↓		↓	↓
Progesterone	→ 17 hydroxy-progesterone	→	Androstenedione	⇄ Testosterone
↓	↓		↓	↓
11 deoxy-corticosterone	11-deoxycortisol		Estrone	⇄ Estradiol
↓	↓		↘	↙
Corticosterone	Cortisol		**Estrogens**	Estriol
↓	**Corticoids**			

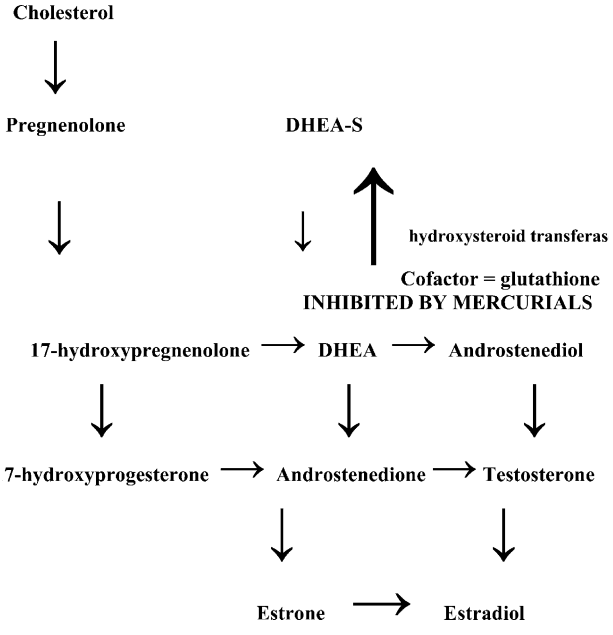

Figure 1 Mercury and glutathione in the testosterone pathway.

important to test the other enzymes in the steroid synthesis and metabolic pathways to determine if they are inhibited by mercurials or if they have sulphydryl containing cofactors.

Interestingly, the unexpected neurological improvements observed following the administration of secretin or growth hormone to some autistic children may also work by influencing the steroidogenic pathway. This is because secretin, glucogon, gastric inhibitory polypeptide, and parathyroid hormone along with vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase activating polypeptide belong to a family of polypeptides called the VIP-secretin-glucogon family, which also includes growth hormone releasing hormone and extendins. All members of this polypeptide family possess a remarkable amino-acid sequence homology, and bind to G-protein-coupled receptors, whose signaling mechanism primarily involves AC/protein kinase A and phospholipase C/protein kinase C cascades and these among others have been shown to inhibit the regulation of the hypothalamus–pituitary–adrenal axis, regulation as shown in Fig. 2 [27]. Thus secretin and growth hormone may inhibit gonadotropin-releasing hormone,

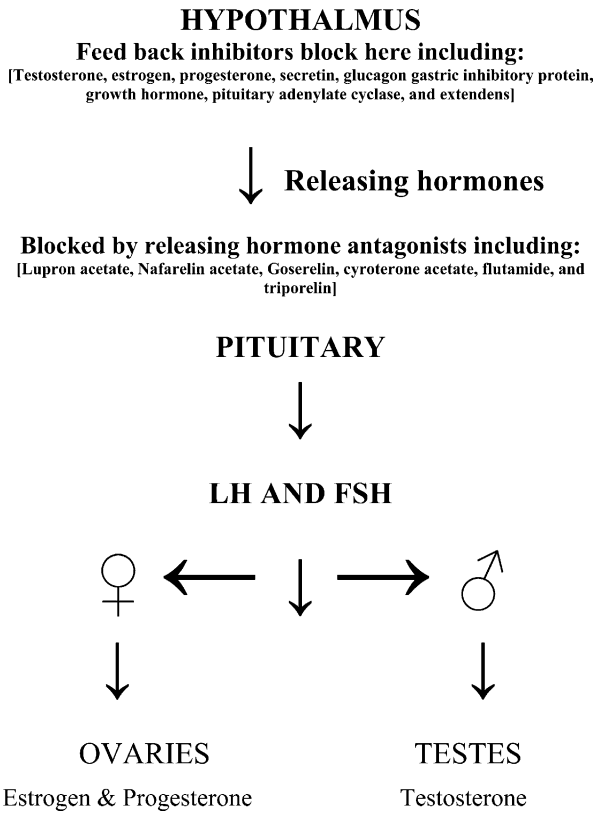

Figure 2 Regulation of the hypothalamus–pituitary axis.

(GnRH). Along these lines triptorelin, cyproterone, and flutamide, Lupron acetate, Nafarelin acetate, Goserelin all of which are gonadotropin-releasing hormone agonists, may be of use in manipulating the steroidogenic pathway.

Megson has reported on using Urocholine, an α muscarinic receptor agonist, in autistic children [28]. Urocholine stimulates the post-synaptic cell membranes via receptors for acetylcholine (ACh), a neurotransmitter in the parasympathetic system. It has previously been shown that ACh acts through different cholinergic receptor subtypes to exert inhibitory effects on GnRH release [29]. Megson has reported that her experience with this treatment in autistic children resulted in dramatic, immediate improvements in language, vision, attention, and social interaction in some children [28].

Realmuto and Ruble have described a case report of an autistic child that highlights the difficulties of managing severe behavioral disorders in autistics [30]. The authors reported that after failure of behavioral and educational programs, leuprolide, an injectable anti-androgen, resulted in suppression of the autistics' severe behavioral disorders and retention in his community placement. Follow-up by the authors for almost three years showed no abnormal physical effects. The dosage administered to the child was tapered over that period to a low but effective dose.

In further considering the hypothalamus–pituitary–adrenal axis, it has been shown that the anterior pituitary, hypothalamus, preoptic area and brain cortex of castrated male rats have been found to possess specific androgen binding proteins [31]. The physico-chemical characteristics of these binding proteins appear to be very similar. Thus, they were excluded by Sephadex G-100 gel and had a sedimentation coefficient of 6-7S by sucrose gradient centrifugation. The protein nature of the androgen binding components was supported by the fact that protease, but not DNase and RNase eliminated the binding of androgens. In addition, the authors reported elimination of the binding by 1 mM *p*-chloro-meruriphenylsulfonate (PCMPS) indicated that free sulfhydryl groups were necessary for androgen binding.

The breakdown products of testosterone and estrogen also need to be tested to determine their effects on the neurotoxicity of mercury and mercury compounds. The break down pathway for testosterone is shown (Fig. 3). Testosterone breakdown products are well known to play a major toxic role in male pattern baldness and in the development of benign prostatic hypertrophy. The FDA approved drug Finasteride which blocks

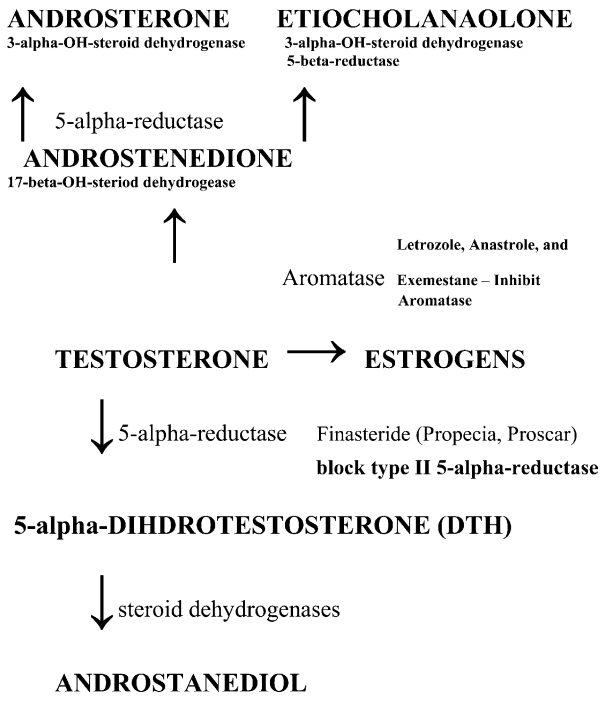

Figure 3 Testosterone metabolism.

the break down of testosterone into 5-α-dihydrotestosterone (DTH) has been shown to be highly effective in preventing and treating these conditions. If these testosterone metabolic compounds are shown to potentate mercury neurotoxcity it is possible the same drugs might be effective in the treatment of neurodevelopmental disorders.

In further considering the effects of thimerosal, it has been shown by Waly et al. that methylation events play a critical role in the ability of growth factors to promote normal development [32]. Neurodevelopmental toxins, such as ethanol and heavy metals, interrupt growth factor signaling, raising the possibility that they might exert adverse effects on methylation. The researchers 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 exert their activity, 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. The authors reported that their findings outline a novel growth factor signaling pathway that regulates MS activity and thereby modulates methylation reactions, including DNA methylation. The authors concluded that the potent inhibition of this pathway by ethanol, lead, mercury, aluminum and thimerosal suggests that it may be an important target of neurodevelopmental toxins.

In actual treatment of patients with male pattern baldness, it has recently been shown that Finasteride up-regulated production of IGF-1 [33]. Specifically, biopsy specimens were collected from 9 male patients from both the balding area and nonbalding occipital area before and after four months of Finasteride therapy. Dermal papilla (DP) were microdissected and total RNA was extracted from an equal number of DP from each biopsy specimen. The expression of various cytokines, including insulin-like growth factor (IGF)-1, was determined by reverse transcription polymerase chain reaction. The signals were detected by autoradiography. All nine patients were given Finasteride for one year and evaluated for efficacy at month 12. It was shown that IGF-1 was up-regulated by Finasteride treatment in four of nine patients. Among the patients with increased IGF-1 expression, three of them showed moderate clinical improvement after 12 months of treatment and another patient remained unchanged. In contrast, three patients with decreased IGF-1 expression in the balding scalp showed clinical worsening after 12 months. The other two patients without noticeable change in IGF-1 expression showed either slight improvement or no change in their hair condition. The authors concluded that in their small uncontrolled study of nine patients with androgenetic alopecia (AGA), an increased expression of IGF-1 messenger RNA levels in the DP was associated with patient response to Finasteride. Therefore, treatment with Finasteride may be a means to re-stimulate production of IGF-1 that has been down regulated by exposure to thimerosal in children that have developed autistic disorders, and also serve to lower levels of DHT in autistic children.

Biochemical manipulations that favor the conversion of testosterone to estrogen also might well be shown in the tissue culture system to protect neurons from damage by mercurials. FDA approved anti-androgens such as Bicalutamide, Nolvadex, Nilandron, and Flutamide might also protect neurons from damage by mercurials. Even the introduction or manipulation of the related corticosteroid pathways might be found to alter the neurotoxicity of mercury compounds. Biochemical strategies which are found to ameliorate the neurotoxic effects of thimerosal and other mercury compounds in tissue culture could then be tried in the recently developed Hornig et al. thimerosal-induced mouse model of autism [34], prior to trials in humans.

Conclusion

Experience from many areas of medicine has shown that, in diseases that can be attacked in multiple ways, the effects of such therapies are not only additive but are often strongly synergistic. Given the magnitude of the current epidemic of neurodevelopmental disorders, it seems imperative that the avenue of potential treatment by manipulation of the steroid hormone pathways be explored immediately. Knowledge on how to

treat chronic mercury toxicity might also be useful in other diseases that also may have a mercury toxic component such as Alzheimer's disease, diabetes, heart disease, obesity, ALS, asthma, and various other forms of autoimmune disorders all of which are very common in our mercury toxic population.

Potential conflict of interest

Dr. Mark Geier has been an expert witness and consultant in vaccine cases before the no-fault National Vaccine Injury Compensation Program (NVICP) and in civil litigation. David Geier has been a consultant in vaccine cases before the no-fault NVICP and in civil litigation.

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