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The role of trace elements, thiamin(e) in autism and autistic spectrum disorder

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1. ABSTRACT

There has been much research into autism or autistic spectrum disorder (ASD) and room for considerable conjecture regarding the etiology of these disorders remain. ASD is marked by a complex interaction between environmental factors and genetic predisposition, including epistasis. This manuscript argues that changes in oxidative metabolism, thiamine homeostasis, heavy metal deposition and cellular immunity have a role in the etiopathogenesis of autism and ASD. Recent evidence for abnormal thiol metabolism, marked by significant alteration in the deposition of several trace heavy metal species is provided here. We hypothesize that altered thiol metabolism from heavy metal toxicity, one of the key mechanisms for oxidative stress production, may be responsible for the biochemical alterations in transketolase, dysautonomia and abnormal thiamine homeostasis. It is unknown why these particular metals accumulate; but we suspect that children with ASD and forms of autism may have particular trouble excreting thiol-toxic heavy metal species. We maintain divalent cation accumulation is evidence of altered clearance, which leads to oxidative stress, offering intriguing component or possible mechanism for oxidative stress-mediated neurodegeneration in ASD patients.

2. INTRODUCTION

Autism and Autistic Spectrum Disorder (ASD) are serious problems and present particular challenges for any discipline of medicine. The presenting symptoms are quite diverse, which often creates difficulty when assigning a specific diagnostic category for patients. Rather than taking the prevailing view, where genetics play the largest role in the etiology of the disease, we propose a continuum model to the etiology of this broad spectrum of disorders. However, a better diagnostic acumen notwithstanding, the fact remains that prevalence of Autism and ASD is growing at an exponential rate in the United States. The prevalence today is 1–2 individuals per 1,000 births for autism and over 10 per 1,000 for ASD, which was ~40% increase over previous years (1). This number continues to rise at an alarming rate. Exponential increases of this sort suggest a strong environmental component to

the etiology of the disease, as mutation rates are μ=1E6 or one in a million, arguing against genetics alone as the predominant cause of the disease. Unfortunately, the exact etiology of autism and ASD remains obscure, although occurrence among families demonstrates that heredity plays a role. In that regard, it is unknown whether the heritable traits are Mendelian, Epigenetic or Mitochondrial in basis (2, 3). Thus the diverse nature of the autistic spectrum and other related disease entities might be considered a general representation of disordered brain metabolism in simplest terms and as inflammation generally (1). ASD patients do in fact present a varied constellation of the behaviors and alterations in biochemistry associated with the disease. We propose that genetic and epistatic interactions are mediated through metabolism, offering a new avenue for research and treatment for these growing diseases (4, 5, 16). In this review we outline a unifying hypothesis for the etiology of Autism and ASD, which takes into account the numerous apparently divergent and controversial findings with this diverse spectrum of diseases and offer an argument for the restoration of thiol loss and reducing equivalents through various forms of thiamine administration.

3. GENETIC MUTATIONS ASSOCIATED WITH ASD AND AUTISM

We have shown that metal ion homeostasis, particularly the deposition of several divalent cations is altered in ASD (16). In support of this hypothesis, mechanisms involving alterations in metal ion transport have been implicated in a complex autosomal dominant disorder characterized by ASD, which is known as Timothy syndrome (6). There are numerous other potential confounders with thiamine and various channel activities in neurons, which should be studied further (7-9). TTP, at a concentration of 1 microM, activates chloride channels of large unitary conductance (the so-called maxi-Cl- channels). In particular, the de novo missense mutation in a calcium channel gene is localized to select affected tissues and is characterized by multi-organ dysfunction, cognitive abnormalities and autism (10). Studies by this group have implicated mutations in the divalent calcium channels CACNA1C (L-type Cav1.2) and CACNA1H (T-type Cav3.2) genes with development of ASD (6, 10). Also implicated are CACNA1C, an L-type calcium channel for the Cav1.2 protein and CACNA1H, the T-type Cav3.2 channel. Both proteins can localize to neurons, and display selective permeability to calcium ions and perhaps other divalent cations, allowing the flow of calcium down their electrochemical gradients. Splawski and colleagues found that mutations in these channels cause abnormal calcium signaling and could explain severe deficits of language and social development. These studies look at depolarization, thereby demonstrating that such mutation in ASD reduces available Ca2 current and neuronal excitability. Moreover, 20 mutations on CACNA1H were reported to be associated with childhood absence epilepsy (CAE) (11-15). These mutations cause increased calcium channel activity and associated increased neuronal excitability. CAE is a complex polygenic disorder, and often manifests as ASD. Missense mutations in CACNA1H increase susceptibility to CAE, mainly because T-type calcium channels are abundant in the thalamus where many seizures are believed to originate. Mutations in other regions of the CACNA1H gene have been identified in childhood absence epilepsy, linked in one third of ASD patients.

However, for the CACNA1H mutation only 6 of 461 ASD subjects were found to have a channel less voltage sensitive and thus this particular mutation was not responsible for phenotype alone as these same mutations were also absent in 480 ethnically matched individuals unaffected by ASD. Several different polymorphisms were identified in CACNA1H including A-C, A-W, G-C, with some allele frequency for ASD as high as 6.1% vs. 5.7% for controls. Moreover, when 3 of 6 families were tested, an affected child did not carry a CACNA1H mutation. At the same time one sibling and several parents were not affected but did carry the mutations. The impression one can take away from these studies is that while a mutation may modify a phenotype, it is not by itself causative. The highest expression of the mutation was in the hippocampus, amygdala, and putamen, also known to be associated with ASD.

Although a mechanism for altered heavy metal homeostasis cannot be implicitly implied from the small subset of affected patients in our study, it certainly demonstrates the diversity underlying the etiology of ASD. We feel that a similar mechanism may involve the loss of regulation in the flux of most divalent cations in general. Our data suggest that this may indeed be the case. Divalent cation transport defects could arise from thiamine deficiency (16). Conversely, polymorphisms in channels or transport proteins also may explain some of altered

redox-active metal deposition evident in our ASD patient population and the oxidative stress in the pathogenesis of the disease. Indeed, there appears to be a defect in cellular divalent cation flux, which may offer another epigenetic mechanism for the etiology of autism and ASD. The channel involved was identified as CACNA1C (L-type Ca(V)1.2 cardiac calcium channel and is important for excitation and contraction of the heart. The role for this transporter in other tissues is unclear. However, irregularities in Ca(2+) signaling and flux is thought to be important in autism as well and may have a role in thiamine transport. Interestingly, several authors (17, 18) have shown, in animal studies, that thiamine administration causes the excretion of the divalent cation lead. We suggest that thiamine administration deserves more study in this disease process and we propose our findings offer a unifying mechanism for the associated damage known to occur in the vulnerable neurons of the ASD patient. At the same time, thiamine administration offers one potential treatment strategy for this devastating and growing disease with little else seemingly on the horizon.

4. SEVERAL ASD RELATIONSHIPS EXPLORED

4.1. The ASD relationship with divalent cation transport

In regard to divalent cation transport disregulation, we explored the hair and urine in ASD patients and age-matched controls for dysregulation and distribution of select heavy metal species of 26 children with a diagnosis of ASD compared with those of 39 healthy children (16). The data show manganese and mercury was significantly lower ($p<0.001$) in the hair of ASD patients as compared to controls, while arsenic was significantly elevated ($P<0.0001$) in the same group and nearly absent in hair of control patients, leading to questions as to the source of this environmental toxin. There were few statistically significant differences in most of the other elements examined. However, concentrations of select metals that can have a valency of +2, including mercury ($p<0.001$), manganese ($p<0.05$), copper ($p<0.05$) and iron ($p<0.07$), were different between the groups. These elements are particularly redox-active or thiol-toxic but were lower in the hair of the ASD group of children. We also took the ratios of sodium/potassium, and magnesium/calcium, which were not significantly different for the divalent species, but were significant for sodium/potassium ($p<0.05$) and lower in controls as compared to the ASD group. Taken together, one could argue the transport deficit lies not in a channel as much as in an antiporter or symporter. However, this remains to be established.

4.2. The ASD relationship with thiamine and its transport

One of the earliest vitamins to be discovered and synthesized was thiamine, which was thought to be a “vital amine”, but was found later not to be an amine at all. Nevertheless, thiamine is a vital substance and its transport and the role of heavy metal toxicity can be associated with neurologic sequalae, as thiamine deficiency has shown. Its biochemical properties have been implicated in many diseases, including autism and autistic spectrum disorder (ASD) (16). Thiamine deficiency results in neurological disorders such as beriberi, Wernicke-Korsakoff syndrome and may be indicated in other neurological disorders. Perhaps the key to emphasize is the effect of beriberi on the autonomic nervous system (19-21). There is loss of forebrain cholinergic neurons and alterations in stimulated acetylcholine (ACh) levels in the hippocampus and cortex in animal models of diencephalic amnesia associated with thiamine deficiency. The study used the pyrithiamine-induced thiamine deficiency rat model to assess the functional relationships between thalamic pathology, behavioral impairment, ACh efflux and cholinergic innervation of the hippocampus and cortex (22, 23). The conclusion was that it supported thiamine tetrahydrofurfuryl disulfide (TTFD) as having cholinergic action (24, 37). There is evidence for a central cholinergic effect of high dose thiamine (37). Acetylcholine deficiency accrues from TD affecting the inflammatory reflex / inflammation controlled by the cholinergic system (25).

In assessing the role of thiamine deficiency in the modern world, early Japanese investigators found that the arterial oxygen concentration in beriberi patients was relatively low while venous oxygen concentration was relatively high (26). This is altered early in the disease and produces changes in vasomotor function. The diastolic blood pressure falls and may even reach zero. Thiamine can correct some of these deficits (27). Although the mechanism is unknown, it has been suggested that such phenomena might be related to instability and imbalance of autonomic nervous system signaling. At any rate, it certainly introduces the fact that beriberi symptomatology is extremely complex and not a matter of simply giving a few milligrams of thiamine. In fact, it took huge doses of the vitamin given over a period of months to abolish the symptoms. We believe that these facts continue to be relevant today and can

provide help when confronted with symptoms and signs that can be confusing in a differential diagnosis.

5. TRANSKETOLASE AND THE REDOX STATE

Along with others, we have published findings in a private practice setting, showing a large proportion of autistic children have, at any given time, an abnormal erythrocyte transketolase, which reveals a deficiency of thiamin(e) pyrophosphate (7). Decreased transketolase activity contributes to impaired hippocampal neurogenesis induced by thiamine deficiency (28). An uncontrolled pilot study was also published in which 8 of 10 children showed clinical improvement from the administration of TTFD, an open ring derivative of thiamine (29). A key paper was recently published reporting evidence of neuroinflammation, including activated microglia and astrocytes at post mortem and irregular proinflammatory cytokine profiles in the brain and cerebrospinal fluid of children with ASD, where neuroinflammation is considered part of the pathobiology in this disease. Further neurologic effects, such as loss of Purkinje cells in ASD, is accompanied by gliosis and increased glial fibrillary acid protein (GFAP), suggesting glia and astrocytes are also affected. To further support our hypothesis that redox active metals or mercury toxicity play a role in the disease, recent research suggests that glutathione depletion can play a role in microglia-mediated neurotoxicity.

Toxicity of glyoxals plays a role in oxidative stress, metabolic detoxification and thiamine deficiency (30). One way the cellular redox state can be assessed biochemically is by measuring transsulfuration metabolites in plasma. Important sulfur-rich enzymes, some of which are major antioxidant enzymes, and small thiol compounds can be assessed in plasma and include reduced glutathione, oxidized glutathione, cysteine, taurine, sulfate, and free sulfate metabolites. When these metabolites were evaluated in ASD-diagnosed individuals and compared to neurotypical age-matched controls, the individuals diagnosed with ASD had significantly ($p < 0.001$) decreased plasma-reduced glutathione, cysteine, taurine, sulfate, and free sulfate relative to controls (46). It is the pentose phosphate pathway that provides a major source of reducing equivalents important for the reduction of glutathione. Studies by Waring and associates reported sulfur depletion in the plasma of ASD children (47) as well as abnormal sulfur concentrations in urine, which suggests that heavy metal toxicity may play a role in ASD as well as autism and offers an additional mechanism for the oxidative stress formation in these diseases. Further, James and associates (48) published findings, which implicate oxidative stress and impaired methylation capacity as contributing factors in ASD as did Ogier et al. (61) Taken together, these findings strongly indicate a state of increased oxidative stress in ASD and a decreased detoxification capacity, particularly for mercury.

High GSSG/GSH ratios (oxidized glutathione) can be used as an indicator of oxidative stress. Studies have shown that children with ASD have inadequate GSH production (46, 47). What we have not known until now is the etiology of this decrease. In addition to oxidative stress, if GSH cannot be readily regenerated, low GSH availability may stimulate microglia activation, leading to a cascade of events that potentiates itself and feeds forward a reaction with numerous down-stream consequences. Low selenium levels may be found in locally grown foods in some areas of the United States, particularly around the Great Lakes region. Heavy metals, coupled with the low selenium levels, can contribute to the oxidative stress mechanisms involved in ASD and other diseases. Many studies suggest that there are pharmaceutical and nutraceutical treatments that can reduce microglial activation and/or their associated inflammatory cytokines. Such studies to examine the use of these potential therapies in ASD have not been done.

6. THIMAMINE TRANSPORT AND HOMEOSTASIS

It is well known that free thiamine is transported across plasma membranes by high affinity transporters. Thus, work began in the 1930's and continued into the 1950's to establish a variety of lipophilic thiamine derivatives. These compounds pass through plasma membrane by diffusion, bypassing the transport system required for free thiamine. Once incorporated into the cells, the lipophilic derivatives are converted to thiamine through enzymatic or non-enzymatic processes. We found that patients treated with thiamine tetrahydrofurfuryl disulfide (TTFD) administration gave a mostly positive clinical response and increase in transketolase activity. There were no reports of side effects or adverse interaction noted from the administration of TTFD. It is important to note that it may not be possible to correct the abnormal

TPPE acceleration without the administration of magnesium (5).

Thiamine transport was studied in vitro by Bettendorff and colleagues, who found uptake occurred through a high affinity mechanism (Km = 35 nM) and was saturable and at high extracellular concentrations. Thiamine uptake occurs by a low affinity mechanism (Km = 0.8 mM) as well (31). With low external concentrations, thiamine uptake could be blocked by low concentrations of Na+ channel activators, suggesting that these channels are not the mechanism of inhibition. However, the low affinity transport could be blocked by divalent cations and in both cases uptake was independent of external sodium, sensitive to metabolic inhibitors and mutations in Ca(V)3.2 that significantly reduced channel activity (31). The same group demonstrated that a secondary active transport mechanism for thiamine was thiamine phosphorylation rather than a sodium gradient. Our findings implicate a significant relationship between the Na+/K+ ratio and ASD, which was nearly 3-fold higher as compared to controls (16). Functional expression studies demonstrated the mutation led to a near complete loss of voltage-dependent channel inactivation and may induce intracellular Ca(2+) overload and cytotoxicity. However, when the mutations were identified in only a few ASD patients with demonstrably Timothy Syndrome, we were led to ask whether other divalent cation transporters could play a role in these transport issues. Further, a little known use of (Transketolase) TKA is its use in depicting abnormal B12 homeostasis and differentiating folate deficiency vs. B12 deficiency (32). These authors reported that an increase in TKA occurred with B12 deficient pernicious anemia and differentiated it from that caused by folate deficiency. To our knowledge, this has never been followed up and further study might provide clues to the abnormal chemistry of ASD. It is therefore appropriate to point out that only a very few of our population study of 91 patients had a TKA below the normal range and several more were thiamine deficient although again a smaller subset. There were 7 patients with TKA values above the “normal” range, suggesting that abnormal B12 homeostasis might play a part in some ASD cases as well (16).

Abnormal thiamine homeostasis, which appears to be corrected easily with TTFD therapy, may be an important part of the etiology in a subset of children with ASD. To determine thiamine deficiency, we utilize transketolase as a diagnostic tool. There are currently at least three aspects to the use of transketolase as a diagnostic tool and we exploit the Thiamine Pyrophosphate Effect (TPPE). A TPP Effect is well accepted as revealing deficiency of TPP (33). The cutoff of 18% acceleration is used. If the enzyme is saturated with its cofactor, we should expect no acceleration when TPP is added to the reaction. It might be more practical to think of the TPPE, as it increases, as representing a gradual movement from thiamine adequacy to thiamine deficiency. The TPPE range in our laboratory was derived from asymptomatic subjects who were considered to be healthy. It may well be that a 15% acceleration in one person is more significant clinically than, for example, a 21% acceleration in another. It is well known that the worst example of deficiency is when the TKA is below the normal range and the TPPE is accelerated above the acceptable range of 18%. Low activity of TKA without abnormal TPPE might suggest apoenzyme defects and a screen of the genome for genetic polymorphisms may reveal a mechanism for inborn metabolic dysfunction of this enzyme. Apart from using transketolase for testing, it must be pointed out that this enzyme occurs twice in the hexose monophosphate shunt and is a vital link between glucose and purine metabolism. Its dysfunction could have profound effects on symptomology.

7. FORMS OF THIAMINE AS PUTATIVE TREATMENT MODALITIES

Thiamine-dependent processes and treatment strategies in neurodegeneration have been published (34). Thiamine deficiency-induced partial necrosis and mitochondrial uncoupling in neuroblastoma cells and was rapidly reversed by addition of thiamine (35). In a number of diseases, beneficial effects of the administration of free, unphosphorylated thiamine have been reported with high-dose thiamine therapy along with Benfotiamine (S-benzoylthiamine O-monophosphate), an amphiphilic S-acyl thiamine derivative known to improve diabetic nephropathy and diabetic dyslipidaemia in high doses (36). Benfotiamine, has a different pharmacological profile than the other lipid-soluble thiamine disulfide derivatives in experimental diabetes in the rat (36, 37). Thornalley et al., showed that Benfotiamine appeared to prevent diabetic nephropathy (35). Other reports suggested that thiamine might protect against free-radical mediated neurotoxicity through mitochondria (69). Shin and colleagues find that thiamine may have a cytoprotective effect on cultured neonatal rat

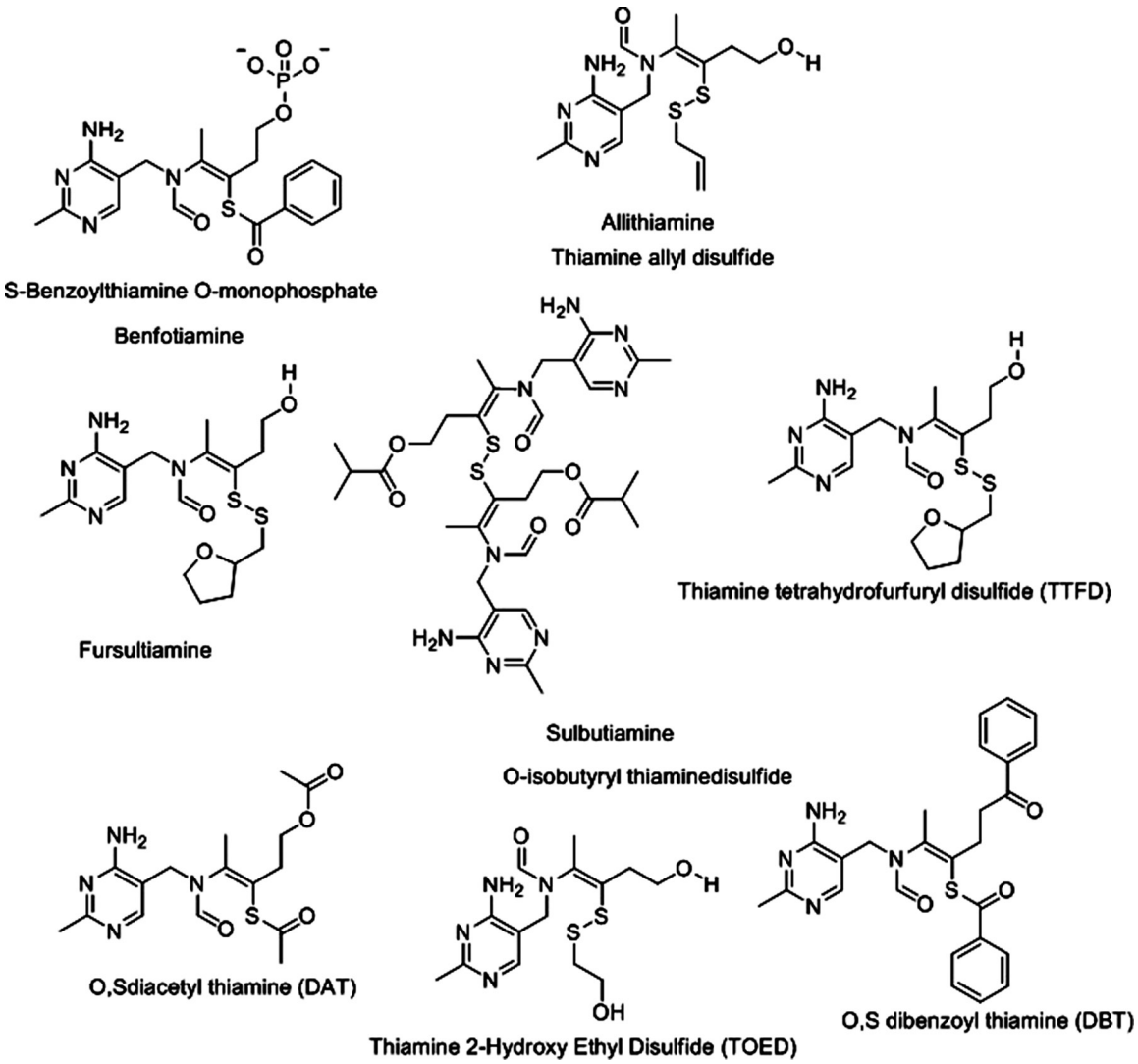

Figure 1. Activity of transketolase in the hexose mono phosphate (HMP) shunt in relation to glycolysis. Reproduced with permission (reference 16).

cardiomyocytes under hypoxic insult (70), arguing that any imbalances or frank deficiency may lead to additional stress. For example, Shangaria and colleagues showed that the effects of partial thiamine deficiency and oxidative stress (i.e., glyoxal and methylglyoxal) on the levels of alpha-oxo-aldehyde plasma protein adducts in Fischer 344 rat occurred regardless of the form of treatment (29). Nevertheless, it is imperative to establish whether thiamine or any of its derivatives are suitable therapeutic modalities. Brownlee and colleagues found that Benfotiamine prevented the progression of diabetic complications, through blocking the three major pathways of hyperglycemic damage and prevented experimental diabetic retinopathy (68), as did Thornalley and colleagues (35), most likely through enhanced transketolase activity (see Figure 1). Many of the forms of thiamine and derivatives are illustrated below in Figure 2.

One of the first lipophilic thiamine derivatives was an allyl disulfide (Figure 2), which was isolated from garlic (Allium sativum) in the1950s and thus was referred to as “allithiamine”. Several analogs of this molecule were synthesized with the expectation of improved bioavailability

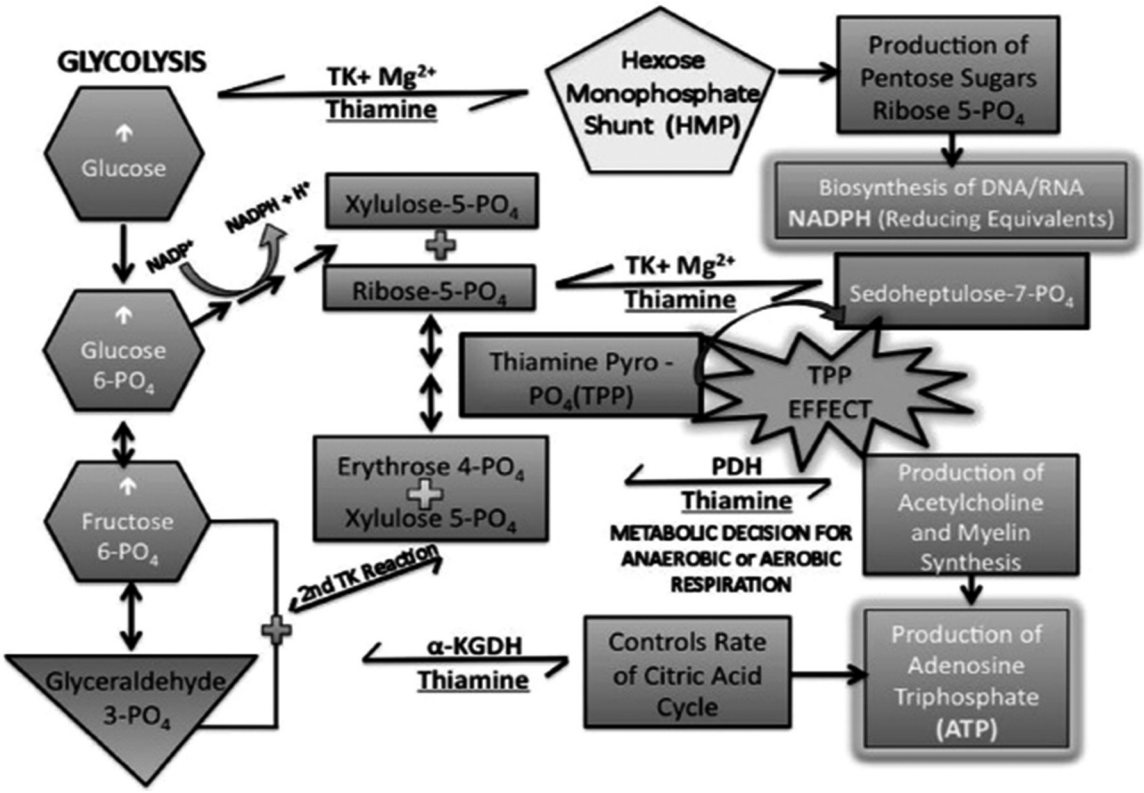

Figure 2. The forms of thiamine, disulfide-containing vitamin B1 and derivatives.

and absorption than thiamine hydrochloride or mononitrate. Currently, two lipophilic disulfide derivatives are used as therapeutic agents, thiamine tetrahydrofurfuryl disulfide (fursultiamine) or TTFD and O-isobutyrylthiamine disulfide (sulbutiamine). Sulbutiamine, has been used as psychotropic drug for the treatment of functional asthenias and related symptoms. Long-term treatment at a therapeutic dose of 52 mg/kg, i.p. was found to increase thiamine, ThMP, ThDP and ThTP levels in the rat brain as well as in peripheral tissues (36). It was shown that administration of sulbutiamine improves long-term memory formation in mice possibly by cholinergic mediation (38).

As the brain is particularly sensitive to thiamine deficiency, it is crucial to determine whether intracellular thiamine and thiamine phosphate levels are increased in the brain after oral Benfotiamine administration. Bettendorf and colleagues explored thiamine derivative distribution, which were increased significantly in the liver but not in the brain, compared to controls (36). Volvert et al., discovered that Benfotiamine has different mechanisms of action and a different pharmacological profile than the lipid-soluble thiamine disulfide derivatives (37). In addition, they incubated neuroblastoma cells with 10 μM Benfotiamine and found it did not lead to increased intracellular thiamine levels. Moreover, when neuroblastoma cells were thiamine-depleted, intracellular thiamine levels increased more rapidly after addition of thiamine than after addition of Benfotiamine to the culture medium. It is thought that the symptoms of thiamine deficiency arise from decreased activity of thiamine diphosphate (ThDP)-dependent enzymes such as transketolase and pyruvate and oxoglutarate dehydrogenases, with subsequent impairment of carbohydrate metabolism in the brain. It is not known to what extent, if any, the decrease in other thiamine derivatives such as thiamine triphosphate (ThTP) or adenosine thiamine triphosphate (AThTP) are involved in the appearance of these symptoms. Thus, better methods for assessing the phosphorylated forms in vivo are needed. These protective effects may be due to increased tissue ThDP levels after

thiamine treatment, but effects mediated by other phosphorylated thiamine derivatives such as ThTP and AThTP have not been ruled out. Structures of the S-acyl derivative benfotiamine and the disulfide compounds allithiamine, fursultiamine and sulbutiamine. Benfotiamine contains a thioester bond, while allithiamine, fursultiamine and sulbutiamine contain a disulfide bond. Allithiamine contains the allyl group, as illustrated in Figure 2.

The S-Acyl thiamine derivatives are: O,S-diacetyl Thiamine (DAT) and O,S-dibenoyl Thiamine (DBT), S-benzoylThiamine Monophosphate (BTMP). Allithiamine or Thiamine allyl disulfide ((TAD) is a lipid-soluble form of vitamin B1 found in garlic bulbs. Other disulfides are Thiamine 2-Hydroxy Ethyl Disulfide (TOED) was identified in 1954, Thiamine Propyl Disulfide (TPD). O,S-Diacetyl Thiamine (DAT) were identified in 1953 and O,S-Dibenoyl Thiamine (DBT) in 1957 as was S-Benzoylthimaine O-Monophosphate (BTMP) in 1960. Synthetic Lipid-soluble Thiamine Derivatives S-Benzolythiamine O-monophosphate (Befotiamine) was discovered in 1935. Sulbutiamine (O-Isobutry Thiamine Disulfide); Fursultiamine (Thiamine Tetrahydrofurfuryl Disulfide) (TTFD) was discovered in 1957.

8. EFFECTS OF OXIDATIVE STRESS

8.1. Oxidative stress mediated neurotoxicity

Long implicated in the generation of reactive oxygen species, especially in vulnerable neurons, is the role of the mitochondria (67). The complex biochemistry involved in ASD and autism indeed may have roots in altered energy metabolism through dysfunction originating in the mitochondria. It has been established that brain endothelial nitric oxide synthase expression decreases in thiamine deficiency: it's relationship to selective vulnerability and the mitochondria can produce much of the cellular reactive oxygen and nitrogen species that alter the cellular redox state and damage cells (39). We showed the same in other neurodegenerative disorders (40, 66).

Mitochondrial dysfunction has been implicated in many diseases, including neurodegenerative diseases, diabetes and aging and some neurons are particularly vulnerable to oxidative stress (41, 66). We suggest this same neurodegenerative process may apply to autism and ASD as well. Similar sources of oxidative stress are suggested by ischemic and reperfusion events in early development. Since hypoxia during birth is a risk factor for subsequent development of autism (42), the oxidative stress due to ischemic reperfusion events or hypoperfusion *in utero* may also play a role in this disease (43). In that regard, severe hypoxia-ischemia is implicated in Wernicke's encephalopathy, as Vortmeyer and colleagues demonstrated. Hypoxia-ischemia and thiamine deficiency, together with thiamine deficiency showed extensive lesions in the cortex, the thalamus and other regions, which was indistinguishable from Wernicke's encephalopathy (42). This demonstrated that the morphological changes in the mammillary bodies due to thiamine deficiency and those due to hypoxia-ischemia might be identical (42).

Defenses against oxidative stress largely employ key endogenous antioxidant enzymes, many of which require redox-sensitive thiol groups in their active sites. Impaired oxidative metabolism and energy failure due to loss of reducing equivalents eventually leads to selective region-specific neuronal loss and diminished thiamine-dependent enzymes. The key enzyme involved here is transketolase (EC 2.2.1.1) (TK), which is a thiamine diphosphate-dependent enzyme that couples the non-oxidative branch of the pentose phosphate pathway (PPP) with the glycolytic pathway. The sugar phosphates generated by PPP are used for intermediary biosynthesis, nucleic acid synthesis and NADPH for reductive biosynthesis (43). Since thiamine is critical for transketolase function, abnormal thiamine homeostasis diminishes thiamine-dependent enzyme level, disrupts mitochondrial function, and contributes to diminished energy metabolism and the production of NADPH. In addition to being critical for energy metabolism, thiamine deficiency can lead to oxidative stress and neurodegeneration. Gibson and Zhang found that interactions involving oxidative stress, together with abnormal thiamine homeostasis, promoted neurodegeneration in a number of chronic neurological diseases (44).

One way the cellular redox state can be assessed biochemically is by measuring transsulfuration metabolites in plasma. Important sulfur-rich enzymes, some of which are major antioxidant enzymes, and small thiol compounds can be assessed in plasma and include: reduced glutathione (GSH), oxidized glutathione (GSSG), cysteine, taurine, sulfate, and free sulfate metabolites. When these metabolites were evaluated in ASD-diagnosed individuals and compared to neurotypical age-matched controls, the individuals diagnosed with ASD (according to DSM-IV-TR

criteria) (45) had significantly ($P < 0.001$) decreased plasma reduced glutathione, cysteine, taurine, sulfate, and free sulfate relative to controls (46). The pentose phosphate pathway provides a major source of reducing equivalents important for the reduction of glutathione. Studies by Waring and associates reported sulfur depletion in the plasma of ASD children (47) as well as abnormal sulfur concentrations in urine.

This suggests that heavy metal toxicity may play a role in ASD as well as autism and offers an additional mechanism for the oxidative stress formation in these diseases. Further, James and associates (48) published findings, which implicate oxidative stress and impaired methylation capacity as contributing factors in ASD. Taken together, these findings strongly indicate a state of increased oxidative stress in ASD and a decreased detoxification capacity, particularly for mercury.

8.2. Oxidative stress and impaired oxidative metabolism

In a review of oxidative stress in relation to metabolism, one finds dysautonomia, frequently in the medical literature, where it is hypothesized that dysfunctional oxidative metabolism was the connecting link between the functional changes in the autonomic nervous system and the associated organicity. Here, these findings reveal pathology, which strongly suggests that oxidative stress is a common denominator affecting the central control mechanisms that govern the limbic system/ autonomic/ endocrine axis, and other systems globally in ASD (16, 49). An interruption of cognitive processes, such as hard wiring of the brain during early development, would result in the chronological immaturity that is displayed frequently in ASD children and with thiamine deficiency (50-52).

It has been suggested that thiamine deficiency-induced neurodegenerative processes could be reversible (53). This group of investigators looked at a mouse model of TD, where brain regions most sensitive to TD revealed that a 29% neuronal loss occurred after 8 or 9 days of thiamine deficiency, which increased to near 90% by day 10 and 11 (54). These authors found the number of microglia increased by 16% to 400% over the same respective time period. When the authors administered thiamine on day 8, they found neuronal death was blocked and the neuronal effects observed with TD on day 9 could be partially reversed. This time period represents a point of no return, as the thiamine administration was ineffective for cell death at later time points. The finding above was associated with microglia activation that was coupled with concomitant production of heme-oxygenase (HO-1), which is a stress-induced protein that is known to enhance mitochondrial transport carriers and cytochrome C oxidase (54). This enzyme also is implicated in protecting cells from oxidative stress. Moreover, thiamine deficiency has been found to induce oxidative stress (55).

Oxidative stress indeed may be one mechanism responsible for mediating the neurodegeneration observed in ASD patients, which involves the loss of key antioxidant defenses (56, 57, 58), largely through loss of antioxidant capacity mediated by thiol-rich enzymes and enzymes with redox-active thiol groups. It is hypothesized that oxidative stress, reported to be an important part of etiology in ASD, is responsible for the changes in control mechanisms mediated through the highly oxygen sensitive limbic system of the brain. It is suggested that this results in a chaotic state of adaptive mechanisms via the limbic/ autonomic/endocrine axis. One culprit also implicated in generating oxidative stress is through redox-active and heavy metal catalyzed reactions (59). Further, oxidative stress also underscores the importance of thiamine in providing reducing equivalents through the hexose monophosphate shunt that is also responsible for regenerating key thiol-containing enzymes such as glutathione peroxidase.

Surveys of heavy metal deposition in hair, particularly mercury, were significantly lower in the ASD patient as compared to control patients. These findings strongly suggest that an alteration in natural trace elements is implicated in the etiology of the disease. Further, many groups have reported variations in heavy metal load with ASD and autism. However, this is not a settled issue (60) as the results have been mixed. Results of hair analysis in our subjects demonstrated lower concentrations of copper, mercury and iron, but higher concentrations of arsenic in ASD. One group, Fido and Al-Saad, reported that 40 boys with autism had significantly higher concentrations of lead, mercury and uranium in hair when compared with healthy children (60). Likewise, we reported a heavy metal imbalance in the urine of autistic children (16) as did other investigators (29). The source of the heavy metals involved remains unknown, but local age-matched children would be expected to have similar exposure, similar diets and similar environments,

i.e. playground equipment or other exposure. Nevertheless, heavy metal toxicity research remains of interest, especially since heavy metal burden continues to be implicated in ASD and our data, as well as that of others, support such a notion. It has been emphasized that thiol (SH)-reactive metal intoxication (62) is a potential stressor in etiology of ASD, and Quig (62) reviewed its effects on cysteine metabolism. Impaired methylation capacity has already been noted in ASD (63, 64). Many complementary alternative practitioners now use various forms of chelation therapy aimed at the removal of SH-reactive metals. Since we and other groups (60, 61, 62) found sulfhydryl-reactive metals to be lower in hair or urine in ASD (60, 62), we hypothesize that the excretion mechanisms for mercury and other divalent cations into the hair follicle or elsewhere may be impaired (16).

We previously reported a significant relationship between the presence of aluminum and increased sodium/potassium ratios in the hair of a variety of symptomatic patients seen in our clinic. Another group found endoplasmic stress and a role for calcium induced by thiamine deficiency, which suggests a role for thiamine in membrane portential and the ER (63). Gorgoglione and coworkers demonstrated the protective effect of magnesium and potassium on external mitochondrial membrane permeability (64). Their work underlines the importance of magnesium and thiols in preserving cellular energy homeostasis. Whang and Whang (65) reviewed the mechanism by which magnesium modulates intracellular potassium through select ATP-dependent membrane pumps. In that regard, there was a statistically significant increase in the sodium/ potassium ratio in ASD patients, while the calcium/magnesium ratio remained unchanged. Taken together with the deposition in heavy metals, this suggests that that these children may have particular trouble excreting divalent cations, particularly thiol-toxic heavy metal species and is a reflection of a dysfunctional membrane pump.

9. DISCUSSION

ASD is recognized by a continuum of symptoms, presenting as a diverse spectrum of related disorders (ASD). This accounts for the difficulty inherent in the attempt to isolate the specific identifying characteristics of the disease. Thus researchers have been thwarted in attempts to identify a single comprehensive definition. This ambiguity in definition further complicates attempts to isolate possible mechanisms of etiology. Rather than continue attempts to isolate discrete definitions, a better approach is to view the disease as a continuum. Research focusing on specific areas of the Autism spectrum would foster research on isolated aspects as opposed to attempt to develop an all-inclusive hypothesis of causality. Nevertheless, we offer a unified hypothesis that takes into account the diverse findings and poses a mechanism for neurodegeneration that is not exclusive of any current prevailing view of etiology. We now have shown evidence that heavy metal toxicity, apparently through increased divalent cation accumulation in ASD patients, may play a significant role in the loss of thiamine and lead to oxidative stress, the effects of which seemed to be ameliorated or improved by thiamin administration. We suggest the mechanisms are multifactorial and thiamine could correct for the loss of thiols through increasing reducing equivalents, improve brain energy and metabolism, as well as affect mitochondrial respiration.

As early as 1998, it was hypothesized that autism was a mitochondrial disorder (2), which has gained support in more recent years and implicates metabolism in the disease. We have also established a relationship between mitochondrial abnormalities and neurodegeneration through stained hypoperfusion promoting oxidative stress of brain tissues, which could stimulate secondary damage via the overexpression of inducible and neuronal specific nitric oxide synthase (iNOS and nNOS, respectively) and endothelin-1 (ET-1) in brain cells (39, 66, 67) and increased brain endothelial nitric oxide synthase expression in thiamine deficiency has a relationship to selective vulnerability. When we explored Thiamine and TTFD for ASD treatment in a pilot study, the addition of thiamine tetrahydrofurfuryl disulfide (TTFD) to the existing treatment regimen provided evidence of clinical improvement in 8 of the 10 children studied (29). The rationale for adding this compound was further supported due to its antioxidant and neuroprotective properties. Both TTFD and vitamin B1 activate the transketolase enzyme, and thiamine is important in glucose metabolism as it drives the flux of metabolites through the hexose mono-phosphate shunt. Further, since sulfur depletion had been observed in autistic children, TTFD was expected to act as a sulfate donor during its metabolism. It is suggested that TTFD acts as a metal-chelating agent as well (69). Thus, the addition of thiamine, particularly in its disulfide form, may be an important regimen for treating a subset

ASD population and address the putative causal factors through several potential mechanisms of action at the same time. For example, a role for heavy metals and metal-mediated toxicity has been suggested to be important in the pathology of autism and ASD, which also was a rationale behind TTFD administration as a good candidate treatment approach.

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