The role of zinc and copper in autism spectrum disorders

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Autism spectrum disorders (ASDs) are a group of developmental disabilities that can cause significant social, communication and behavioral challenges. Several studies have suggested a disturbance in the copper (Cu) and zinc (Zn) metabolism in ASDs. Zinc deficiency, excess Cu levels, and low Zn/Cu ratio are common in children diagnosed with an ASD. The literature also suggests that mercury accumulation may occur as a cause or consequence of metallothionein (MT) dysfunction in children diagnosed with an ASD, which may be one of the causes of Zn deficiency. MTs are proteins with important functions in metal metabolism and protection. Zinc and Cu bind to and participate in the control of the synthesis of MT proteins. Studies indicate that the GABAergic system may be involved in ASDs, and that Zn and Cu may play a role in this system.

Key words: Asperger syndrome, autism, pervasive developmental disorder, neurodevelopmental

INTRODUCTION

Autism spectrum disorders (ASDs) are lifelong developmental disabilities. This group of disorders includes autistic disorder, Asperger syndrome, and pervasive developmental delay not otherwise specified. These disorders are presented from birth or very early during development and affect essential human behaviors such as social interaction, the ability to communicate ideas and feelings, imagination, and the establishment of relationships with others. Infantile autism and Asperger syndrome are two closely related forms of developmental disorders (Bjørklund 1998). ASDs are a group of systemic disorders with multiple etiology, including both genetic and environmental/lifestyle factors. This article focuses on some of the changes in trace element metabolism commonly found in ASD patients.

Bioelements play important roles in the central nervous system. The lack or excess of essential minerals and trace elements are known to cause a variety of health problems, and could contribute to the etiology of ASDs (Lakshmi Priya and Geetha 2011, Blaurock-Busch et al. 2012). Similarly, mercury (Hg) and other toxins may play a role in the pathogenesis of ASDs (Bernard et al. 2001, Geier et al. 2009, 2010, 2012, Blanchard et al. 2011, Blaurock-Busch et al. 2012, Kern et al. 2012). Children are, due to their behavior, more exposed to environmental toxins than adults, but often also have higher intestinal absorption rates and lower detoxification ability (Grandjean and Landrigan 2006). Many studies show that autistic children have a higher body-burden of toxic metals compared to neurotypical controls (Desoto and Hitlan 2010, Geier et al. 2010, 2012, Lakshmi Priya and Geetha 2011, Obrenovich et al. 2011, Elsheshtawy et al. 2011, Adams et al. 2013, Al-Farsi et al. 2013). These studies indicate that children with autism have a decreased ability to excrete toxic metals, leading to a higher body burden. Moreover, studies show that Hg body-burden is associated with increased ASD severity (Lakshmi Priya and Geetha 2011, Elsheshtawy et al. 2011, Geier et al. 2012, Adams et al. 2013).

Reduced activity of transketolase has been reported in some patients with ASDs (Lonsdale et al. 2011, Obrenovich et al. 2011). The level of transketolase is used as a diagnostic test of thiamine deficiency. Of 91 examined children with ASD, 19 (21%) suffered from lack of transketolase and thiamine, defined by enhancement of the activity of transketolase from blood samples by more than 18% after supplementation with thiamine (Obrenovich et al. 2011). It is an unresolved

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question if a saturation of transketolase in the blood also means that the transketolase is saturated in the central nervous system. It should not be taken for granted that the transport rate for thiamin across the blood-brain barrier always will be sufficient to compensate for enhanced local degradation of the vitamin in the brain because of locally enhanced oxidative stress (which is common in several different brain diseases), even when there is no thiamin deficiency in the blood cells.

According to Filipek and coworkers (1999), autistic infants appear in some cases to develop normally until age 1 to 3 years. Then sudden changes occur that indicate the presence of an ASD (Filipek et al. 1999). Possible causes for this are toxic metal exposure in combination with an inadequate nutritional status (Blaurock-Busch et al. 2012), at the same time as nutritional requirements might be different than in normal children as a consequence of genetic or epigenetic disturbances (Schanen 2006, Yasuda et al. 2011). Other possible causes might be changes in the intestinal flora because of weaning and/or introduction of other foods than breast milk, or the progressive, cumulative effect as a function of time of disturbances of brain development and/or learning processes that have started only after the birth of the child.

Given the importance of zinc (Zn) and copper (Cu) metabolism for healthy neurological functioning and detoxification of heavy metals, including Hg, it is thought that these two trace elements may contribute in the pathogenesis of ASDs (Faber et al. 2009). Working with a Romanian team, we investigated the levels of Zn and Cu in whole blood, as well as the Cu/Zn ratio in a group of 28 children with autism (Crăciun et al. 2009). The patient group was compared with healthy age and sex matched control subjects. The concentrations of Cu and Zn were measured in whole blood with inductively coupled plasma-mass spectrometry. We found that the Zn level was decreased ($P<0.001$), and the Cu/Zn ratio increased ($P<0.001$) in autistic children, compared with a healthy control group.

Zinc

Zinc is an essential trace element, an important antioxidant (Russo and deVito 2011) in spite of not being a free radical scavenger, and a metalloenzyme required for the catalytic activity of at least 300 enzymes

(Prasad 2012). It plays a role in immune system functioning (Prasad 1995), protein synthesis (Prasad 1995), wound healing (Heyneman 1996), DNA synthesis (IOM/FNB 2001), and cell division (Prasad 1995). Zinc is required for proper sense of taste and smell (Prasad et al. 1997). Zinc is part of a very large number of transcription factors, far more than the number of Zn-containing enzymes that are known (Oteiza and Mackenzie 2005, Prasad 2012).

Zinc supports normal growth and development during pregnancy, childhood, and adolescence (Simmer and Thompson 1985, Fabris and Mocchegiani 1995). Prolonged Zn deficiency may therefore cause growth impairment (Sandstead et al. 1967, Prasad 2012). A child who is born with decreased Zn stores will remain at risk for Zn deficiency throughout childhood (Faber et al. 2009).

Zinc is naturally present in some foods, is added to others, and is available as a dietary supplement (Lifschitz 2012). The recommended intake for Zn is 11 mg/day for men and 8 mg/day for women (Trumbo et al. 2001). Lower Zn intake is recommended for infants (2–3 mg/day) and children (5–9 mg/day) because of their lower average body weights (Trumbo et al. 2001).

Zinc deficiency occurs not only as a result of nutritional factors, but also in various disease states, including malabsorption syndromes, alcoholism and cirrhosis of the liver, acrodermatitis enteropathica, Crohn's disease, and immune dysregulation (Prasad 1983, Faber et al. 2009, Russo and deVito 2011). Changes in the intestinal flora and function are common in autistic patients (de Theije et al. 2011, Finegold et al. 2012, MacFabe 2012, Midtvedt 2012); it is therefore conceivable that malabsorption due to pathological changes in the intestinal mucosa may play an important role as one of the causes of Zn deficiency in autism. Low intracellular Zn has been associated with DNA damage, which might be due to a combination of oxidative stress, impairment of antioxidant defences, and impairment of DNA repair (Russo and deVito 2011). An important clinical point to note is that, because Zn is primarily an intracellular nutrient, serum Zn levels can be normal in states of mild deficiency (Bales et al. 1994, Salgueiro et al. 2001).

The most common cause of Zn deficiency is dietary factors that reduce the availability of Zn, but inherited metabolic disturbances and intestinal diseases can also result in reduced Zn. Both these types of Zn deficiency

can produce similar symptoms, such as dermatitis, diarrhea, alopecia, and loss of appetite (Hambidge et al. 1986, Russo and deVito 2011). These are symptoms of severe Zn deficiency. More moderate Zn deficiency, which leads to impaired immune function and increased mortality due to infections, as well as brain damage in the fetus when it affects pregnant women, are more common (Hambidge et al. 1986, Fischer Walker and Black 2004). Individuals with Zn deficiency often have suppressed immune function and frequent infections (Shankar and Prasad 1998, Lakshmi Priya and Geetha 2011) with the degree of immunosuppression depending on the severity of Zn deficiency.

The overall frequency for Zn deficiency worldwide is thought to be higher than 20% (Wuehler et al. 2005). In developing countries it may affect more than 2 billion people (Plum et al. 2010). It has further been estimated that only 42.5% of U.S. elderly ($\geq$71 years) have adequate Zn intake (Briefel et al. 2000). While many elderly have low intakes of Zn, it is also possible that the Zn requirement increases with age because of the age-related accumulation of mutations in mitochondrial DNA, leading to enhancement of mitochondrial production of reactive oxygen species (ROS), which in turn enhances the synthesis of Zn-binding apometallothionein in various cell types and organs (Moxnes and Christophersen 2008).

Since only exposure to high doses of Zn has toxic effects, acute Zn intoxication is rare (Plum et al. 2010). Copper and Zn are metabolic antagonists (Underwood 1977). Copper absorption is depressed when Zn is given in high excess of Cu, or when Zn therapy is given for a long time without Cu supplementation. Many of the toxic effects of Zn are in fact due to Cu deficiency (Plum et al. 2010). On the other hand, a low level of Zn exacerbates Cu toxicity (Blaurock-Busch et al. 2012).

Copper

Copper is an essential trace element for living cells, and plays an important role in redox reactions. It is easily converted from Cu^+ to Cu^{++} and back to Cu^+. Copper and Zn interact at the intestinal mucosal level, affecting Cu and Zn absorption (Underwood 1977). Wapnir and Balkman (1991) investigated these concepts using a duodenal-jejunal single-pass perfusion process in rats. They found that Cu absorption decreased with increasing Zn presence. Copper is mainly transported in the bloodstream by ceruloplasmin, a Cu-binding antioxidant protein. Ceruloplasmin is synthesized in several tissues, including the brain (Russo and deVito 2011).

Copper is an important cofactor in many metalloenzymes including Cu/Zn-superoxide dismutase, cytochrome c oxidase, and lysyl oxidase (Underwood 1977, Davis and Mertz 1987). Copper deficiency may cause impairment of oxidative phosphorylation, cellular antioxidant defense, and collagen and elastin biosynthesis (Underwood 1977, Davis and Mertz 1987). Copper deficiency causes depression of tissue levels of cytochrome c oxidase and Cu/Zn-dependent superoxide dismutase (Davis and Mertz 1987), but may also affect the levels of selenium-dependent glutathione peroxidase (Medeiros et al. 2009). Ceruloplasmin, which is the most abundant Cu-binding protein in blood plasma (Underwood 1977, Davis and Mertz 1987) is one of the acute phase proteins that are synthesized more abundantly in the liver during infectious diseases (Wyatt and Wilson 2013). Serum Cu concentrations increase therefore in several diseases because of cytokine-induced enhancement of the synthesis of ceruloplasmin in the liver. Severe Cu intoxication is much rarer in humans than in sheep since humans, but not sheep, normally have good capacity to excrete surplus Cu through the bile (Davis and Mertz 1987). Adult humans contain less than 100 mg of stored Cu (Faber et al. 2009).

Common symptoms of Cu deficiency include hypocupremia, impaired iron mobilization, anemia, leukopenia, neutropenia, decreased superoxide dismutase, ceruloplasmin as well as cytochrome c oxidase, but also increased plasma cholesterol and LDL/HDL cholesterol ratio and abnormal cardiac function (Davis and Mertz 1987, Plum et al. 2010). Copper levels can be low due to malnutrition, malabsorption, and Menke's kinky hair syndrome (Davis and Mertz 1987). Elevated Cu levels are associated with excessive dietary intake, infections, inflammation, Wilson's disease, trauma, systemic lupus erythematosus, as well as autism (Russo and deVito 2011). Copper is a cofactor in proteins connected with neurological diseases including amyotrophic lateral sclerosis, Alzheimer's disease, and Creutzfeldt–Jacob disease (Faber et al. 2009).

It is important to control Cu homeostasis very closely, because Cu becomes toxic in higher than normal concentrations. Abnormally elevated Cu concentrations can cause oxidative damage to lipids, nucleic acids and proteins (Faber et al. 2009). In Cu-poisoned sheep, Cu is released from necrotic areas in the liver,

causing hemolytic anemia (Davis and Mertz 1987). Tissues that contain abnormally large amounts of redox-active transition metals, including Cu, have increased free radical concentrations (Ozdemir et al. 2009). Copper excretion is regulated by the liver by changing the Cu concentration in the bile (Davis and Mertz 1987, Faber et al. 2009). As already mentioned, Cu and Zn are antagonists. Excess Cu disturbs Zn balance and interferes with adrenal hormone production, thus weakening the immune system, which is observed in autistic individuals (Lakshmi Priya and Geetha 2011).

Copper and molybdenum (Mo) are also antagonists in ruminant diets (Davis and Mertz 1987), and Mo deficiency in the diet may be a risk factor for Cu toxicity. The antagonistic interaction between Mo and Cu has been explained as a consequence of thiomolybdate formation in the rumen, with thiomolybdate being next absorbed and functioning as an inhibitor of Cu-dependent enzymes (Gould and Kendall 2011). It is conceivable, however, that thiomolybdate might also react with Cu in the gastrointestinal tract to form a very heavily soluble compound that would lead to malabsorption of both elements. The same can not happen in humans, since we are not ruminants. However, it is conceivable that pathological disturbances in the intestinal mucosa, as might happen in many autists, could lead to reduction of the O_2 flux from blood to the intestinal lumen, favouring anaerobic bacteria there and enhancing the microbial production of H_2S, which might in turn lead to formation of thiomolybdate. A study by Blaurock-Busch and coworkers (Blaurock-Busch et al. 2012) has been thought to suggest that low levels of Mo and Zn directly affect the Cu and lead status and ASD symptomatology. An alternative interpretation of the same data could be that Mo malabsorption caused by abnormal H_2S production in the intestine might be a problem in ASD patients, with the severity of this problem being enhanced in patients with more severe symptoms. In animal nutrition it is well known and documented that excess Cu storage in the liver of sheep can be prevented by adding a few milligrams of Mo to their feed (Dick 1953, Davis and Mertz 1987).

Zinc, copper and the human mind

Individuals who suffer from severe Zn deficiency can develop neuropsychological changes such as emotional instability, irritability and depression (Halsted et al. 1972, Prasad 1991, Vallee and Falchuk 1993, Hambidge 2000, Russo and deVito 2011). Zinc deficiency interferes with cognitive performance (Black 1998). Zinc is also involved in glutamatergic transmission with short-term and long-term effects that may go in opposite directions, e.g., it blocks NMDA receptors (Sensi et al. 2011). The activation of the glutamatergic neurotransmitter system depends on Zn uptake, and synaptic neurotransmission needs Zn as an intracellular signal factor due to its involvement with numerous proteins (Takeda et al. 2006a). There are also some observations from patients with fragile X syndrome suggesting that interactions between Zn and glutamate may exist (Siller and Broadie 2012).

It appears that Zn functions as a cotransmitter with glutamate because Zn is previously stored in secretory granules which empty out into the synapse simultaneously with glutamate (Qian and Noebels 2005). This does not necessarily mean that glutamate and Zn act in the same direction relative to the postsynaptic neuron, although they enter the synapse simultaneously. It is not inherently implausible that there might be simultaneous release of a neurotransmitter functioning as an activator and another neurotransmitter functioning as an inhibitor to prevent tissue damage caused by overstimulation of the postsynaptic neuron – and excitotoxicity is a well known phenomenon that is very scary.

When Zn this way is discharged into the synapse, this not only need to be because it affects the electrophysiological processes immediately afterwards. It is also conceivable that Zn after being absorbed by the postsynaptic neuron can act as an important trophic factor, i.e. as a nutrient that can later be used as a cofactor for enzymes with anabolic function, when there is an increased protein synthesis in a synapse area as part of permanent learning processes.

Copper toxicity has a powerful effect on the mind. Depending on the severity of the toxicity and the susceptibility of the person, Cu at toxic levels can affect the mind moderately or very severely (Lakshmi Priya and Geetha 2011). Potential neurotoxic effects of Cu in excess quantity include depression, irritability, fear, nervousness, learning and behavioral disorders (Madsen and Gitlin 2007), and perhaps also Alzheimer disease (Brewer 2012).

Copper is a cofactor required for the activity of dopamine-β-hydroxylase (DBH) (Deinum et al. 2004, Rahman et al. 2009). This neurotransmitter synthesiz-

ing enzyme converts dopamine to norepinephrine. Increased norepinephrine levels have been found in autistic individuals (Lake et al. 1977). This might perhaps partly be explained by excess Cu. Increased levels of Cu and ceruloplasmin have been found to be associated with inhibition of the enzyme hydroxytryptophan decarboxylase, which decreases the production of the neurotransmitter serotonin (Lakshmi Priya and Geetha 2011). A state of hypercupremia appears to be associated with depression and perceptual disturbances in schizophrenias (Lakshmi Priya and Geetha 2011).

According to Pagano and Castello (2012), Down syndrome is one of the best documented cases of a human disorder etiologically related to the redox imbalance that has long been attributed to overexpression of Cu/Zn-superoxide dismutase (SOD-1), encoded by trisomic chromosome 21. Too much Cu/Zn-dependent superoxide dismutase paradoxically causes increased oxidative stress, may be due to reactions with hydrogen peroxide (H_2O_2) (Midorikawa and Kawanishi 2001) and/or peroxynitrite. The involvement of oxidative stress has been reported both in genes located elsewhere than at chromosome 21 and in transcriptional regulation of genes located at other chromosomes (Pagano and Castello 2012). Copper is bound to amyloid β-peptide (Abeta) in senile plaque of Alzheimer's disease (Syme et al. 2004). Copper is also linked with the neurotoxicity of Abeta and free radical damage (Syme et al. 2004).

Abnormal metallothionein function

Metallothioneins (MTs) are small, low-molecular weight, intracellular proteins containing 61–68 amino acids with an unusually high concentration of cysteine. MTs are the most common intracellular proteins which bind to metals (Andrews 2000). These proteins have an extraordinary metal-binding capability [due to their many sulfhydryl (–SH) groups], and are essential to heavy metal detoxification (Aschner 1996). MTs guard the brain and gastrointestinal tract against heavy metal overload. Metals that form less soluble sulphides [e.g., cadmium (Cd) compared to Zn] are bound to metallothionein more strongly than those forming more soluble sulphides, and they are commonly also more potent inducers of apometallothionein synthesis. Copper might be a partial exception to this rule, when comparing the strength of Cu and Zn as apometallothionein

inducers, due to stronger complex binding of Cu to amino acids, peptides and other proteins. Zinc is most abundant among the MT-inducing metals (Park et al. 2001), but Cu is also very important as an MT-inducing element. Toxic metals such as Cd and Hg can, in spite of lower abundance, lead to significant disturbance of Zn and Cu metabolism due to their potency as MT inducers (Underwood 1977).

Mammalian MTs are part of a gene cluster on human chromosome 16q13, which contains four isoforms (MT1, MT2, MT3, and MT4) and 17 subtypes of MT genes. MT1 and MT2 isoforms are ubiquitously expressed in most tissues, including the brain, whereas MT3 is only expressed in the brain, and MT4 is expressed in certain stratified squamous epithelia (Aschner et al. 2006). MT1 and MT2 are induced following Hg exposure, presumably to protect essential cellular functions and enhance survival. They can function as antioxidants, and are up-regulated in response to other divalent metals (Aschner et al. 2006). Mercury compounds may replace Zn in the metal-binding sites of metallothioneins, which may be regarded as a mechanism of detoxification because it hinders the binding of Hg to the active sites of enzymes (Faber et al. 2009). But it also counteracts the loss of Hg by excretion processes, similar to that which also happens with other toxic metals, such as Cd, when they bind to MT. Observations on the abundance of MTs within the central nervous system (CNS) and the identification of a brain-specific isoform, MT3, suggest that it might have important neurophysiological and neuromodulatory functions (Aschner et al. 2006). Increased concentrations of vesicular Zn induce MT3 production in the hippocampus, amygdala and pyriform cortex (Faber et al. 2009).

It might be speculated that one important reason for synthesis of apometallothionein in the brain could be for temporary storage of Zn that has been transported from one nerve cell to another during synaptic transmission until the Zn later can be used as for anabolic purposes (i.e., as a cofactor for enzymes) during microanatomical remodeling being part of the long-term learning process. Pathological overstimulation of metallothionein synthesis in the brain, e.g., because of too much Cu accumulation, might then interfere with the subsequent mobilization of Zn that has been temporarily stored, thus hindering Zn-dependent anabolic processes that are needed for normal learning.

MTs are able to modulate Hg neurotoxicity, a neurotoxin that also has been proposed to play an etiologic role in ASDs (Filipek et al. 1999, Bernard et al. 2001, Palmer et al. 2006, 2009, Russo 2008, Geier et al. 2009, 2010, Blanchard et al. 2011, Kern et al. 2012). Stress on the metallothionein system triggered by heavy metal exposure may cause faster depletion of Zn reserves (Faber et al. 2009). It has been proposed that allergic autoimmune reactions occurring after exposure to heavy metals, may contribute to some symptoms associated with autism. This means that abnormalities in MT concentration and/or structure, as well as presence of anti-MT antibodies, may be associated with autism (Russo 2008).

Copper is involved in the regulation of synthesis of MT proteins. These proteins have a high attraction for Cu, and in times of high Cu loads, MT levels rise (Arredondo and Núñez 2005, Faber et al. 2009). MTs trap Cu within intestinal cells and prevent its systemic absorption (Russo and deVito 2011). The same also happens with Zn, which is one of the reasons why excess Cu hinders the intestinal absorption of Zn and *vice versa*.

Zinc is important for the metal-responsive transcription factor 1 (MTF-1), which enhances the transcription of MT genes in response to heavy metal load. MTF-1 up- and downregulates many genes and enzymes responsible for the elimination of heavy metals (Wang et al. 2004, Wimmer et al. 2005).

Redox regulation of zinc and copper metabolism

The expression of metallothionein is not only metal-regulated, but also redox-regulated (Andrews 2000), so that increased oxidative stress increases the expression of metallothionein, making both Zn and Cu more strongly bound to metallothionein, and less available to be used as cofactors in enzymes or other proteins. Increased expression of metallothionein in enterocytes will probably lead to impaired absorption of Zn and Cu in the intestine, and increased expression of metallothionein in the liver will lead to poorer mobilization from there to other organs. This could probably explain the age changes in Zn metabolism − because the aging of mitochondrial DNA through accumulation of mutations leads to increased ROS production in mitochondria, and this can then lead to increased expression of metallothionein, causing the Zn concentration in blood plasma to go down (Moxnes and Christophersen 2008).

But the same can also be expected to happen if ROS production in mitochondria is elevated for other reasons, such as a congenital metabolic disorder. This may be relevant to ASDs, and it is not unreasonable to suggest that it could be one of the main causes of Zn deficiency in autism. However, when Zn deficiency has occurred, whatever the cause, it will of course worsen the patient's general condition, including that it may be detrimental to brain function (Lakshmi Priya and Geetha 2011).

Enhanced apometallothionein induction due to oxidative stress in organs such as the liver and the kidney must be expected to lead to enhanced retention of toxic metals such as Cd and Hg in these organs. It is possible that this could be the most important cause of enhanced levels of Hg or other toxic metals observed in ASD patients, rather than abnormally high exposure compared with the rest of the population. But the toxic metals will, once they have accumulated, have important deleterious effects, e.g., by interfering with Zn metabolism. It is thus possible that autistic patients may have less tolerance to toxic metal exposure, compared to the rest of the population.

Similarly, it is also possible that enhanced apometallothionein induction due to oxidative stress in the liver might interfere with the capacity for homeostatic regulation of the excretion of Cu through the bile, leading to a tendency for abnormal accumulation of this element in the liver. It is not impossible that enhanced MT induction due to oxidative stress might have opposite net effects on Cu and Zn metabolism, leading to enhanced severity of Zn deficiency, at the same time as it might also lead to enhanced Cu accumulation and therefore Cu toxicity.

Zinc, copper and severity of autism

The frequency of Zn deficiency and Cu intoxication is high in children diagnosed with an ASD (Faber et al. 2009). In a study by Lakshmi Priya and Geetha (2011) a significant variation was found for Zn in both hair and nails of low functioning autism group children when compared to a control group and other study groups. Subjects of the study were 45 autistic children with different grades of severity and 50 healthy children (age and sex matched). The children with different grades of autism showed highly significant differences ($P<0.001$) in the level of Cu in their hair and nail samples when compared to healthy controls. The level

of Cu in the autistic children could be correlated with the severity of their symptoms. The children in the low functioning autism group showed higher levels of Cu in both hair and nail samples when compared to middle and high functioning autism. This result could suggest that the higher the level of Cu toxicity, the more severe is the autism in children (Lakshmi Priya and Geetha 2011).

It might be speculated that Zn deficiency in autism arises mainly as a secondary consequence of other metabolic disturbances leading to enhanced oxidative stress, e.g., due to enhanced mitochondrial ROS production, while Cu excess might occur both as a consequence of enhanced oxidative stress and abnormally enhanced cytokine levels (enhancing the synthesis of ceruloplasmin in the liver), perhaps due to intestinal dysfunction and changes in the intestinal microflora. However, one should not exclude the possibility of other inherited biochemical lesions directly affecting Cu metabolism.

Zinc to copper ratio

Zinc maintains a balance with Cu in the blood, where changes in these two trace elements tend to be inversely related. This can in large measure be explained as a consequence of cytokine regulation of the metabolism of the two elements, with the same cytokines causing enhancement of the cellular uptake of Zn and enhancement of the production of ceruloplasmin in the liver. A low plasma Zn concentration is nearly always associated with a high serum Cu concentration. According to published studies, the normal Zn to Cu ratio in children and adults is close to 1:1 (Van Weyenbergh et al. 2004, Faber et al. 2009). It has been proposed that the plasma Zn/serum Cu ratio may be used as a rapid method of determining the functional state of the metallothionein system (Faber et al. 2009).

Faber and coworkers (2009) performed a retrospective review of plasma Zn, serum Cu and Zn/Cu on data from 230 children [179 male, 51 female, mean age 6.3, standard deviation (SD) of 3.67] with autistic disorder, pervasive developmental delay not otherwise specified, and Asperger syndrome. The entire cohort's mean Zn level was 77.2 μg dl^{-1}, the mean Cu level 131.5 μg dl^{-1}, and the mean Zn/Cu 0.608, which was below the 0.7 cut-off of the lowest 2.5% of healthy children (Faber et al. 2009).

Lower Zn/Cu ratios may reflect total body Zn deficiency or accumulation of Zn-antagonistic toxic metals. It has been proposed that Hg toxicity may be a major cause of MT dysfunction in children diagnosed with an ASD, which may be reflected in the Zn/Cu ratio (Aschner et al. 2006, Faber et al. 2009). It is not impossible that the toxic metals Hg and Cd, similar to that proposed above for oxidative stress due to genetic disturbances, might have opposite net effects on Zn and Cu metabolism because enhanced MT induction in the liver might affect Cu excretion via the bile more than it affects the mobilization of this element from the liver to the blood, while for Zn it is the rate of mobilization to the blood which is more strongly affected.

GABA

γ-Aminobutyric acid (GABA) is responsible for synaptic inhibition in the brain. Alterations in levels of GABA and GABA receptors indicate that the GABAergic system may be involved in autism (Ma et al. 2005, Ashley-Koch et al. 2006, Collins et al. 2006). Autistic individuals frequently have lower levels of Zn and significantly higher levels of Cu when compared to normal controls (Russo and deVito 2011). It has been suggested that low Zn and high Cu levels may modulate GABA synthesis or membrane transport, ultimately causing a lowering of transmitter concentration in the synaptic cleft. A high Cu level may also be associated with high norepinephrine levels found in autistic children, and low GABA and high epinephrine levels may, in turn, manifest as excitability and hyperactivity associated autistic symptoms (Russo and deVito 2011).

GABA is synthesized by the enzyme glutamate decarboxylase (GAD) using pyridoxal phosphate (which is the active form of vitamin B$_6$) as a cofactor (Bown and Shelp 1997, Schousboe and Waagepetersen 2007). GAD plays a very important role in maintaining excitatory-inhibitory balance of the central nervous system (Li et al. 2008). It was known already during the late 1960s that laboratory animals that were exposed to hyperbaric oxygen had decreased levels of GAD in the brain (Haugaard 1968). Newer research has shown that there are (at least) two different isozymes of GAD (Davis et al. 2001, Li et al. 2008, Wei and Wu 2008). The recombinant forms of these two isozymes, GAD65 and GAD67, are potently and reversibly inhibited by molecular oxygen (K$_i$=0.46 and

0.29 mM, respectively) (Davis et al. 2001). GAD65 contains 15 cysteyl groups and GAD67 13 cysteyl groups (Battaglioli et al. 2005), which may presumably explain the sensitivity of these enzymes both to oxidative stress, toxic heavy metals and other toxic agents attacking the thiol groups. It is a plausible speculation that *S*-glutathionylation, similarly as for creatine kinase (Reddy et al. 2000), might be an important mechanism of oxidative inhibition of these enzymes. It is likely that GAD primarily is inhibited because of reactions between the enzyme and various ROS, including hydroxyl radicals (Khan et al. 2009), rather than with molecular oxygen itself. Copper may play a role in oxidative attack on GAD of ROS through Fenton-like reactions (Trigwell et al. 2001). GAD67 is apparently much more vulnerable to oxidative stress than GAD65 (Li et al. 2008).

Research suggests strongly that Zn and Cu might play a role in the GABAergic system, and Zn also in the glutamatergic system. Approximately 10% of total Zn in the brain exists in synaptic vesicles of glutamatergic neurons (Takeda et al. 2004). Zinc has been found to be associated with GABA and glutamate regulation, particularly through anxiolytic activity, modulating GABAergic inhibition and seizure susceptibility (Ben-Ari and Cherubini 1991, Xie and Smart 1993, Takeda et al. 2003). Zinc deficiency has also been found to be associated with GABAergic impairment (Takeda et al. 2006b). Copper is a potent inhibitor of GABA-evoked responses, particularly in Purkinje cells (Russo and deVito 2011). Zinc and Cu might interact with each other and with GABA$_A$ receptor complex and participate in modulation of synaptic transmission (Kim and Macdonald 2003).

CONCLUSIONS

Children with ASDs appear to be at risk for Zn deficiency, Cu toxicity, have often low Zn/Cu ratio, and often disturbed metallothionein (MT) system functioning. Zinc, copper, various toxic heavy metals and oxidative stress up-regulate the gene expression of the metallothioneins. MTs are important proteins implicated in heavy metal, including Hg, detoxification and in the elimination of free radicals throughout the body. MT induction by oxidative stress, excessive Cu levels, Hg or other toxic metals may lead to Zn deficiency by interfering with the intestinal absorption of this element and most likely also with its mobilization from the liver to other parts of the body. For Cu, however, it is possible that the effects of MT induction by oxidative stress or toxic heavy metals on the rate of biliary excretion may be more important than the effect on the rates of intestinal absorption and mobilization from the liver to the blood. It is therefore possible that enhanced MT induction by oxidative stress might have opposite net effects on functional Zn and Cu status, with Zn deficiency being combined with a tendency for Cu excess. In the brain, it is possible that MT over-induction caused by excess Cu may interfere with normal temporary storage in synaptic regions followed by mobilization of Zn for use as a cofactor of enzymes needed for long-term learning processes (because Cu might interfere with the second release phase of the storage-release process). This suggests that providing Zn to autistic children may be an important component of a treatment protocol, especially in children with Zn deficiency. It is important to monitor and follow the values for both Cu and Zn together during Zn therapy, because these two trace elements are both antagonists in function, and essential for living cells. Studies indicate that the GABAergic system may be involved in ASDs, and that Zn and Cu may play a role in this system.

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