Nutrition and Metabolic Insights

ORIGINAL RESEARCH

Increased Copper in Individuals with Autism Normalizes Post Zinc Therapy More Efficiently in Individuals with Concurrent GI Disease

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Abstract

Aim: To assess plasma zinc and copper concentration in individuals with autism.

Subjects and methods: Plasma from 79 autistic individuals, and 18 age and gender similar neurotypical controls, were tested for plasma zinc and copper using inductively-coupled plasma-mass spectrometry.

Results: Autistic individuals had significantly elevated plasma levels of copper and Cu/Zn and lower, but not significantly lower, plasma Zn compared to neurotypical controls.

Zn levels increased significantly in autistic individuals with and without GI disease after zinc therapy. Cu decreased significantly after zinc therapy in the GI disease group but not in the autistic group without GI disease.

Autistic children significantly improved with respect to hyperactivity and stimming after zinc therapy in autistic children with GI disease. Autistic children without GI disease did not improve in these symptoms after the same therapy.

Discussion: These results suggest an association between zinc and copper plasma levels and autism, and they suggest that zinc therapy may be most effective at lowering copper levels in autistic children with GI disease.

Keywords: autism, zinc, copper

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Introduction

Autism is a complex, behaviorally defined neurodevelopmental disorder characterized by social deficits, language impairments, and repetitive behaviors. There has been a dramatic increase in the diagnosis of autism over the past decade.[1,2]

The etiology of this complex disease is highly heritable, but likely involves environmental factors.[3] Twin studies demonstrate concordance rates of 82%–92% in monozygotic twins and 1%–10% concordance rate in dizygotic twins.[1] Sibling recurrence risk (6%–8%) is 35 times the population prevalence.[1,4]

Genetic analysis suggests that as many as 15 genes might be involved in autism spectrum disorders (ASD), including variants on chromosomes 2q, 7q, 15q, and 17q.[5–8]

Children with ASD frequently have accompanying gastrointestinal, immunological, or nonspecific neurological symptoms.[9–15]

Zinc has a unique and extensive role in biological processes. Since the discovery of this element as an essential nutrient for living organisms,[16–18] many diverse biochemical roles for it have been identified. These include roles in enzyme function,[19] nucleic acid metabolism,[20,21] cell signaling[22] and apoptosis.[23] Zinc is essential for physiological processes including growth and development,[24] lipid metabolism,[25] brain and immune function.[24,26]

Dietary factors that reduce the availability of zinc are the most common cause of zinc deficiency. However, inherited defects can also result in reduced zinc. Both nutritional and inherited zinc deficiency produce similar symptoms, such as dermatitis, diarrhea, alopecia and loss of appetite.[27] With more prolonged deficiency causing growth impairment and neuropsychological changes such as emotional instability, irritability and depression.[28–31]

Deficiency of zinc in man has now been recognized to occur not only as a result of nutritional factors, but also in various disease states, including malabsorption syndromes, acrodermatitis enteropathica, Crohn's disease, alcoholism and cirrhosis of the liver.[59,60]

Low intracellular zinc has been found to be associated with DNA damage, oxidative stress, antioxidant defenses, and DNA repair,[32,33] and zinc may serve as an important anti-oxidant.[34]

Copper (Cu), a trace metal, is also an essential element for living cells. It plays an important role in redox reactions because of its easy conversion from Cu+ to Cu++. Copper is transported mainly by ceruloplasmin, a copper-binding antioxidant protein that is synthesized in several tissues, including brain.[35,36]

Copper levels are low in Menke's kinky hair syndrome,[37] malnutrition[38] and Malabsorption.[39] Elevated copper levels are associated with infections,[40] inflammation,[41] trauma,[42] Wilson's disease,[43] excessive dietary intake[44] systemic lupus erythematosus,[45] as well as autism.[46]

Because of the potential association between Zn and Cu levels and autism, we tested patients with autism for plasma concentration of these elements and then compared those levels with severity of disease symptoms in autistic children with concurrent GI disease and those without GI disease.

Materials and Methods

Subjects

Experimental and control

Plasma from consecutive individuals with diagnosed autism (n = 73; 36 male; mean age 38 years) and controls (n = 16; 7 male; mean age 42 years) was obtained from patients presenting at the Health Research Institute/Pfeiffer Treatment Center. These individuals meet the DSM-IV criteria and many were diagnosed using The Autism Diagnostic Interview-Revised—ADI-R before presenting for treatment at the Pfeiffer Treatment Center, Warrenville, Il.[a]

Twenty-five of the autistic patients in this study had documented GI disease (7 had chronic constipation; 3 had GERD; 7 had gluten intolerance; 1 with IBS, 1 with Colitis and 1 with Celiac disease; 5 had generalized GI disease).

Patient consent was obtained from all patients involved in this study and this study was approved by the IRB of the Health Research Institute/Pfeiffer Treatment Center.

Severity of disease

An autism questionnaire was used to evaluate symptoms. The questionnaire (Pfeiffer Questionnaire) asked parents or caregivers to assess the severity of the following symptoms: Awareness, Expressive Language, Receptive Language, (Conversational)

[a]The Pfeiffer Treatment Center is a comprehensive treatment and research center, specializing in the care of with neurological disorders, including Depression.

Pragmatic Language, Focus, Attention, Hyperactivity, Impulsivity, Perseveration, Fine Motor Skills, Gross Motor Skills, Hypotonia (low muscle tone), Tip Toeing, Rocking/Pacing, Stimming, Obsessions/Fixations, Eye Contact, Sound Sensitivity, Light Sensitivity, Tactile Sensitivity, Pica/eats dirt, metal, Tics and Seizures. The symptoms were rated on a scale of 0–5 (5 being the highest severity) for each of these behaviors.

Zinc and anti-oxidant therapy

Individuals in this study who presented to the Pfeiffer Treatment Center with autism were tested for zinc, copper and anti-oxidant levels. Based on deficiencies, they were then prescribed the appropriate dose of anti-oxidants. Pre-therapy patients represent those who were tested when they first presented and were not previously taking any zinc or anti-oxidants. Post-Therapy patients received anti-oxidant therapy (Vitamin C, E, B-6 as well as Magnesium, and Manganese if warranted), and zinc supplementation (as zinc picolinate), daily, for a minimum of 8 weeks.

Serum/plasma

All experimental and control plasmas were treated in an identical fashion—refrigerated (4C) immediately after collection and cell/serum separation, then used within 4 hours for inductively-coupled plasma-mass spectrometry.

Statistics

Inferential statistics were derived from *t*-test with 95% confidence intervals.

Results

Plasma from 79 autistic individuals (diagnosed by the Autism Diagnostic Interview-Revised—ADI-R), and 18 age and gender similar neurotypical controls, were tested for plasma zinc and copper using inductively-coupled plasma-mass spectrometry.

Autistic individuals had significantly elevated plasma levels of copper ($P = 0.0133$) and Cu/Zn (Copper Zinc ratio) ($P = 0.05065$) and lower, but not significantly lower Zn ($P = 0.3541$) compared to neurotypical controls (Table 1).

There was no difference in Cu and Zn levels based on type of GI disease (ANOVA $P = 0.74$ Cu levels; $P = 0.84$ Zn levels). There was not enough data to adequately assess any differences in symptom severity between these groups.

Table 1. Significant differences in zinc and copper concentrations (mg/dL) and Cu/Zn between individuals with autism and neurotypical controls.

	Controls Cu	**Autistic Cu**
Mean	90.42	111.50
SD	19.55	27.73
	$P = 0.0133$	
	Controls Zn	**Autistic Zn**
Mean	84.42	78.36
SD	24.18	20.32
	$P = 0.3541$	
	Controls Cu/Zn	**Autism Cu/Zn**
Mean	1.18	1.46
SD	0.50	0.44
	$P = 0.05065$	

Zn levels increased in autistic individuals with GI Disease (N = 25) ($P = 0.0003$) and without GI disease (N = 54) ($P = 0.0001$) (Table 3) after zinc therapy. Cu decreased significantly after zinc therapy in the GI disease group ($P = 0.02425$) but not in the autistic group without GI disease ($P = 0.0839$) (Table 2).

Autistic children significantly improved with respect to hyperactivity ($P = 0.00491$) and stimming ($P = 0.05594$) after zinc therapy in autistic children with GI disease. Autistic children without GI disease did not improve in hyperactivity ($P = 0.1937$) or stimming ($P = 0.97406$) after the same therapy (Table 4).

Table 2. Plasma copper decreases significantly in autistic children with GI disease.

	Autistic Cu pre therapy	**Autistic Cu post therapy**
Mean	111.50	98.78
SD	27.73	24.86
	$P = 0.00972$	
	GI Cu pre therapy	**GI Cu post therapy**
Mean	112.74	95.80
SD	23.82	17.86
	$P = 0.02425$	
	Non GI Cu pre therapy	**Non GI Cu post therapy**
Mean	110.94	100.03
SD	29.53	27.38
	$P = 0.0839$	

Table 3. Plasma zinc increases significantly in autistic children with and without GI disease.

	Autistic Zn pre therapy	Autistic post therapy
Mean	78.36	102.59
SD	20.32	28.14
	$P = 0.0001$	
	GI Zn pre therapy	**GI Zn post therapy**
Mean	74.30	112.27
SD	26.66	31.86
	$P = 0.0003$	
	Non GI Zn pre therapy	**Non GI post therapy**
Mean	80.20	98.56
SD	16.71	25.85
	$P = 0.0001$	

No other symptoms improved significantly post therapy.

Discussion

There is much support for the role of GABA in the etiology of autism. Alterations in levels of GABA and GABA receptors in autistic patients indicate that the GABAergic system, which is responsible for synaptic inhibition in the adult brain, may be involved in autism.[47–49]

Zinc has been found to be associated with GABA and glutamate regulation, particularly through anxiolytic activity, modulating GABAergic inhibition and seizure susceptibility.[50–52] Zinc deficiency has also been found to be associated with GABAergic impairment.[53]

Copper, on the other hand, has been found to be a potent inhibitor of GABA-evoked responses, particularly in Purkinje cells. Copper toxicity, notably in Wilson's disease, could result, to some extent, from chronic GABAA receptor blockade.[54] Data strongly suggest that Cu and Zn might interact with each other with $GABA_A$ receptor complex and participate in modulation of synaptic transmission.[55]

Dopamine-β-hydroxylase (DBH) is a neurotransmitter, synthesizing enzyme which catalyzes the formation of norepinephrine from dopamine. Copper is a co-factor required for this enzyme's activity.[57,58] Increased norepinephrine levels have been found in autistic individuals,[56] which, at least in part, could be explained by excess copper.

Our study shows that autistic individuals have lower levels of zinc and significantly higher levels of copper when compared to neurotypical controls. We suggest that the low zinc and high copper may modulate GABA, ultimately causing a lowering of transmitter concentration. High copper may also be associated with high norepinephrine found in autistic children, and low GABA and high epinephrine may, in turn, manifest as excitability and hyperactivity associated autistic symptoms. To evaluate this relationship, future studies will assess more patients with autism and evaluate GABA and norepinephrine levels, as they are associated with Cu and Zn levels.

Our data also showed that, post zinc therapy, zinc levels normalized in both autistic children with GI disease and those without GI disease. However, copper only decreased significantly (normalized) in the GI group and this decrease correlated with symptom (hyperactivity and stimming) improvement. This suggests that copper normalization after zinc supplementation is most effective in autistic children with GI Disease. This may be associated with concurrent improvement of GI and immune functionality or related to a dysfunctional carrier, such as metallothionein, in these patients.

Table 4. Hyperactivity and stimming improve significantly in autistic children with GI disease post zinc therapy.

Hyperactivity		
	GI pre therapy	**GI post therapy**
Mean	4.13	1.57
SD	1.03	1.13
	$P = 0.00491$	
	Non GI pre therapy	**Non GI post therapy**
Mean	3.25	2.24
SD	1.72	1.58
	$P = 0.1937$	
Stimming		
	GI pre therapy	**GI post therapy**
Mean	3.63	1.25
SD	0.95	2.05
	$P = 0.05594$	
	Non GI pre therapy	**Non GI post therapy**
Mean	2.00	2.03
SD	2.02	1.74
	$P = 0.97406$	

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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