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Role of Zinc in the Pathogenesis of Attention-Deficit Hyperactivity Disorder

Implications for Research and Treatment

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Abstract

The dopamine transporter is regulated by zinc (Zn^{2+}), which directly interacts with the transporter protein as a potent non-competitive blocker of substrate translocation (dopamine transport inward and outward). The fact that dysfunction of the dopamine transporter is involved in the pathogenesis of attention-deficit hyperactivity disorder (ADHD) is interesting in the context of studies that suggest the involvement of zinc deficiency in patients with ADHD. In this article, we present a hypothesis exploring the causative mechanism of zinc deficiency in ADHD and why zinc might be beneficial as a supplementary medication and/or adjunct to psychostimulants (methylphenidate, amfetamine) in zinc-deficient ADHD patients. The hypothesis is based on published *in vitro* observations that the human dopamine transporter contains a high-affinity zinc binding site (His-193, His-375, Glu-396) on its extracellular face that modulates transporter function, and *in vivo* studies suggesting that response to stimulants is reduced in zinc-deficient ADHD patients. It seems likely that zinc supplementation in zinc-deficient ADHD patients improves the binding status of insufficiently occupied zinc binding sites on the dopamine transporter. We propose to test our hypothesis by recruiting zinc-deficient ADHD patients who will undergo positron emission tomography with the ^{11}C-raclopride displacement method to investigate whether zinc increases extracellular dopamine levels.

1. Introduction

The dopamine transporter is a presynaptic plasma membrane protein specifically expressed by dopaminergic neurons. It is essential for the maintenance of normal dopamine homeostasis in the synaptic cleft and mediates the action of psychostimulants.[1] The dopamine transporter is regulated by zinc (Zn^{2+}), which directly interacts with the transporter protein as a potent non-competitive blocker of substrate translocation (dopamine transport inward and outward).[2,3] The fact that dysfunction of the dopamine transporter is involved in the pathogenesis of attention-deficit hyperactivity disorder (ADHD) is interesting in the context of studies suggesting the involvement of zinc deficiency in patients with ADHD.[4]

ADHD is a very common and heterogeneous childhood-onset psychiatric condition, affecting about 4% of school-aged children worldwide and

about 2% of adults.[5] The key features include persistent and chronic symptoms of inattention, hyperactivity and impulsive behaviour. There is a significant social and economic cost associated with ADHD,[6,7] which makes it highly clinically relevant to find adequate treatment. Untreated severe ADHD has a poor prognosis in terms of long-term outcome, including conduct and educational problems, and a strong association with adult antisocial personality disorder, substance misuse and mood disorders.[8] Furthermore, it is still unclear why about 15% of ADHD patients do not seem to respond to stimulant medication,[9,10] rendering the treatment of a significant minority of patients difficult and often ineffective.

Both animal data and human findings suggest the involvement of zinc deficiency in ADHD.[11,12] There are preliminary data suggesting that many children with ADHD have lower than average zinc levels (e.g. measured in blood or hair, urine or nails).[4] Two placebo-controlled, Middle-Eastern studies found that zinc as a supplementary medication and/or adjunct to the psychostimulant methylphenidate might be beneficial in the treatment of ADHD.[13,14] Further evidence suggests that an optimal clinical response to psychostimulant (amfetamine) therapy may depend on adequate zinc levels.[15]

The neurobiological mechanism of zinc in ADHD remains unclear. Zinc is an essential cofactor for over 100 enzymes (metallo-enzymes and metal-enzyme complexes) in the brain, required for the metabolism of carbohydrates, fatty acids, proteins and nucleic acids (relevant for maintaining brain structure and function).[16] Next to iron, zinc is the most abundant trace mineral in the body. Dietary changes and nutritional supplements have been used to treat ADHD with partial success.[17-20] Some of these results suggest a link between zinc deficiencies and low levels of essential fatty acids.[21] Zinc is also necessary for the production and modulation of the pineal hormone melatonin, which helps regulate dopamine function,[22,23] widely believed to be a key factor in ADHD and its treatment. In fact, Sandyk[22] hypothesized in 1990 that para-sympathomimetic stimulants, such as amfetamines, work in ADHD partly through their effects on melatonin. Diurnal synthesis and release of melatonin influences the dosing time-dependent actions of cocaine and amfetamines.[24] Dosing time-dependent actions relate to the differential action a drug can have at various times of the day. It is proposed that the dosing time-dependent characteristics of psychostimulant action and the contribution of the melatonergic system may have clinical implications for the treatment of ADHD.[24-26]

Methylphenidate, the most commonly used and first-line drug in the treatment of ADHD, exerts its effects by inhibiting the dopamine transporter.[27] In fact, its therapeutic effect is probably related to an increase in extracellular levels of dopamine, especially in brain regions with high levels of dopamine transporter such as the striatum.[28] An abundance of research shows that abnormalities in frontostriatal and cortico-cerebellar circuits with imbalances in both dopaminergic and noradrenergic (catecholaminergic) systems are implicated in the aetiology and maintenance of ADHD (see review by Biederman and Faraone[29]).

The action of methylphenidate on the dopamine transporter, and neuroimaging findings demonstrating that ADHD patients have increased striatal dopamine transporter densities and that dopamine transporter density is decreased after treatment with methylphenidate,[30,31] indicate that the dopamine transporter plays a major role in ADHD. Furthermore, genetic studies[32-34] have suggested that the dopamine transporter gene (DAT1; SLC6A3 locus) may be a susceptibility gene for ADHD.

2. Zinc and the Dopamine Transporter

The human dopamine transporter is a presynaptic plasma membrane protein with high prevalence in the striatum. It tightly regulates the extracellular level of dopamine by transporting released dopamine back into the presynaptic neuronal terminals (inward transport) [figure 1]. The dopamine transporter-mediated reuptake system limits not only the intensity but also the duration of action of dopamine at pre- and postsynaptic receptors. It is also the molecular target

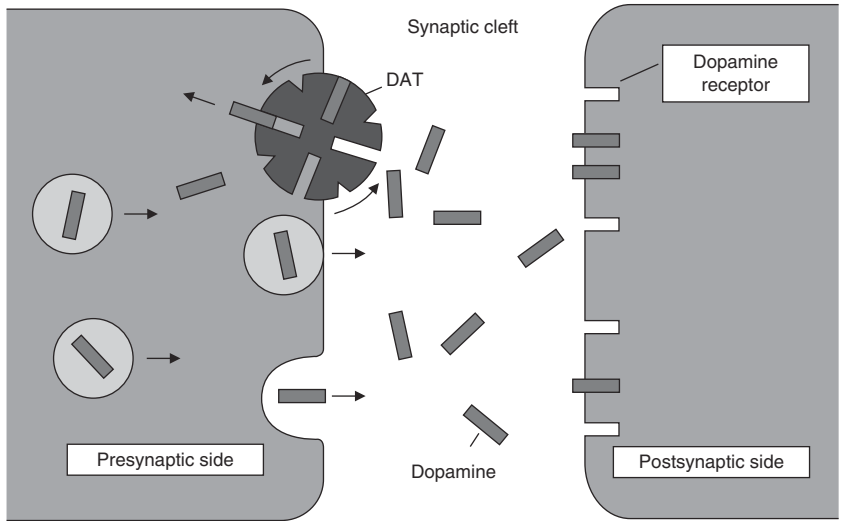

Fig. 1. Schematic depiction of the human dopamine transporter (DAT) – dopamine inward transport.

for therapeutic agents used in the treatment of mental disorders, such as ADHD (methylphenidate) and depression (bupropion).[1] Therapeutic doses of the psychostimulant methylphenidate effectively attenuate ADHD symptoms. The methylphenidate-induced surge in extracellular dopamine levels resulting from dopamine transporter inhibition in the striatum has been confirmed in living human brain by positron emission tomography (PET).[35] Oral methylphenidate achieves peak concentrations in the brain 60–90 minutes after administration, blocks more than 50% of dopamine transporter proteins and, thus, significantly enhances extracellular dopamine levels in the basal ganglia.[35] Moreover, the dopamine transporter is the principal target for the psychostimulatory addictive drugs, cocaine and amfetamine.[1,36,37] Just like methylphenidate, amfetamine raises extracellular dopamine levels by inhibiting the dopamine transporter. However, amfetamine modulates dopamine levels with greater complexity than methylphenidate. Cocaine is also believed to work by blocking the dopamine transporter and thereby increasing the availability of free dopamine within the brain, especially in the striatum.[38]

A growing number of cations and anions have been shown to interact with the dopamine transporter.[39] *In vitro* neurochemistry studies show that chiefly zinc, applied at physiological levels (10–30 μM), inhibits dopamine reuptake and potentiates the binding affinity of cocaine and cocaine analogues at the dopamine transporter.[2,40,41] Some investigations have reported novel insights into the dopamine translocation mechanism by systematically studying the endogenous zinc binding sites on the human dopamine transporter.[2,3,42,43] These studies revealed that the human dopamine transporter contains an endogenous high-affinity zinc binding site on its extracellular face. The high-affinity zinc binding site in the human dopamine transporter (wild-type hDAT) was mapped to three coordinating residues situated on the external face of the transporter: His-193 in the large, second extracellular loop between transmembrane segments (TM) 3 and 4, His-375 at the external end of TM 7 and Glu-396 at the external end of TM 8. The dopamine transporter, with the closely related noradrenaline (norepinephrine) and serotonin transporters, forms a subfamily within the large family of Na^+/Cl^--dependent transporters.

The binding of zinc on the dopamine transporter leads to potent inhibition of dopamine re-uptake through inhibition of the inward translocation process by enhancement of carrier-mediated dopamine

efflux (facilitated outward/reverse transport).[2,3,43] Zinc specifically acts as a potent non-competitive blocker of substrate translocation (dopamine transport inward and outward), whereas dopamine can bind with unchanged affinity to the zinc-occupied transporter.[2] This leads to the conjecture that zinc imposes a conformational constraint on the dopamine transporter, which impedes movements critical for the inward and outward transport of dopamine.

3. Zinc and Attention-Deficit Hyperactivity Disorder

There are data to suggest that zinc deficiency may be involved in ADHD. In the US, Arnold et al.[44] found that in middle-class American children with ADHD, zinc deficiency was positively correlated with more severe symptoms of ADHD. They concluded that: ''These findings add to accumulating evidence for a possible role of zinc in ADHD, even for middle-class Americans.'' A number of European and Israeli studies have also shown evidence of zinc deficiency in children diagnosed with ADHD.[45-49] Kozielec et al.[45] and Starobrat-Hermelin[46] in Poland reported serum zinc levels in ADHD children to be significantly deficient compared to controls. Interestingly, hair zinc level was lower in ADHD children with co-morbid oppositional-defiant or conduct disorder than in children with ADHD alone or with anxiety.[46] Ward et al.[47] in the UK found significantly lower zinc levels in urine, hair, serum and nails in 20 hyperactive boys compared to 20 age-matched controls. In another larger study, Ward[48] found lower zinc and iron levels in a sample of 486 hyperactive children compared with 172 healthy controls. Toren et al.[49] in Israel reported significantly lower serum zinc levels and more variance in 39 boys and 4 girls with ADHD aged between 6 and 17 years compared to age-matched healthy controls.

In view of the data reviewed in section 2, we hypothesize that in zinc-deficient ADHD patients the available zinc binding sites on the extracellular face of the dopamine transporter are insufficiently occupied, resulting in elevated dopamine transporter activity (reduced inhibition). The neurochemistry data obtained *in vitro* using physiological levels of zinc provide clear evidence that the effects of zinc on the human dopamine transporter are consistent with its role as a modulator of the human dopamine transporter *in vivo*.[2] It is highly likely that free zinc is present in the synaptic cleft at levels required to modulate dopamine transporter function. Physiologically, the extracellular level of zinc may reach about 10–20 μM.[50]

We suggest that zinc deficiency in zinc-deficient ADHD patients leads to decreased striatal extracellular dopamine levels through the disinhibition (acceleration) of the dopamine inward translocation process by inhibition (deceleration) of the carrier-mediated dopamine efflux. On the other hand, we suggest that zinc supplementation in zinc-deficient ADHD patients improves the binding status of insufficiently occupied (unsaturated) zinc binding sites on the dopamine transporter, resulting in reduced dopamine transporter activity, and thus a higher availability of dopamine in the synaptic cleft. The *in vitro* experiments by Norregaard and colleagues[2] demonstrated that fully saturated dopamine transporter zinc binding sites (about 100 μM of zinc) cause 60–70% inhibition of dopamine reuptake. It could equally be hypothesized that zinc supplementation in zinc-deficient ADHD patients inhibits the dopamine inward transport by facilitating the outward transport, resulting in an increased extracellular dopamine level.

In this context, it is clinically important to note that one study has suggested that children who are unresponsive to psychostimulants are more likely to be zinc deficient than children who respond favourably to these medications.[15] To explore the relationship between zinc nutrition and essential fatty acid supplements and their effect on the efficacy of psychostimulants in the treatment of ADHD, Arnold and colleagues[15] reanalysed data from an 18-subject, double-blind, crossover, placebo-controlled treatment comparison of d-amfetamine with Efamol® (evening primrose oil, rich in γ-linolenic acid). Subjects were categorized as zinc adequate (n = 5), borderline zinc deficient (n = 5) and zinc deficient (n = 8). They analysed hair, red cell and urine for

zinc levels. For each category, they calculated mean differences between placebo and active treatment groups based on teachers' ratings as an outcome measure (Conners' 10-item Hyperactivity Index). The placebo-controlled d-amfetamine response appeared linear to nutritional zinc levels; in other words, higher baseline zinc predicted a better placebo-controlled response to d-amfetamine. This study suggests that zinc nutrition may be important for the treatment of ADHD with psychostimulants, as sufficient zinc levels may aid stimulant response.

Essentially, both zinc and methylphenidate enhance the extracellular dopamine level by dopamine transporter inhibition (antagonism), an action responsible for the therapeutic properties of methylphenidate in patients with ADHD.[51] Moreover, it is known that zinc *in vitro* potentiates the binding affinity of cocaine and cocaine analogues on the dopamine transporter.[2,40,41] It is also most likely that the application of zinc as an adjunct to psychostimulants in zinc-deficient ADHD patients improves the binding of these drugs to the dopamine transporter. It is also conceivable that the improved psychostimulant binding is caused by conformational changes on the dopamine transporter protein through the action of zinc on its dopamine transporter binding sites. We assume that this effect is particularly pronounced in patients with very low zinc levels, i.e. plasma/serum zinc levels <60 μg/dL (normal about 65–120 μg/dL).

In this way, zinc could potentially be used as a therapeutic tool in the treatment of zinc-deficient ADHD patients. Improving zinc nutritional status might even have a beneficial therapeutic effect on zinc-deficient ADHD patients independent of psychostimulant treatment, might improve the response to psychostimulants or, at least, might lower the psychostimulant dose needed to successfully treat ADHD. We believe that the unexplained beneficial effects of zinc in zinc-deficient ADHD patients might be mediated by dopamine transporter inhibition, resulting in increased extracellular dopamine level in the striatum.

Neuro-imaging studies such as PET should also be undertaken to investigate the *in vivo* action of zinc in patients with ADHD. The difficulty in conducting such studies is that PET is not available ubiquitously, often only being available in university hospitals. It also needs to be emphasized that at this time supplementation with zinc is not integrated in any ADHD treatment algorithm. At present there are only two known controlled studies investigating the supplementation with zinc in ADHD.[13,14] Akhondzadeh and colleagues[14] examined the effect of zinc supplementation in Iranian children with ADHD treated with methylphenidate in a randomized double-blind trial. Forty-four children, aged between 5 and 11 years, were treated with methylphenidate 1 mg/kg/day in two divided doses. They were randomly assigned to additional zinc sulphate 55 mg/day (n = 22) or placebo (n = 22) for 6 weeks. On the DuPaul ADHD rating scale (both parent and teacher ratings), those assigned to methylphenidate plus supplemental zinc improved significantly more than those assigned to methylphenidate plus placebo. Bilici et al.[13] reported the only published, double-blind, placebo-controlled trial of zinc supplementation as sole treatment for ADHD. 400 Turkish ADHD patients (328 boys, 72 girls, mean age 9.61 ± 1.7 years) were randomly assigned in a ratio of 1 : 1 to 12 weeks of double-blind treatment with either zinc sulphate 150 mg/day (n = 202) or placebo (n = 198). After 12 weeks of treatment, ADHD scales (the Attention Deficit Hyperactivity Scale, the Conners' Teachers Questionnaire and the DuPaul Parent Ratings of ADHD scale) showed that the supplemented group (n = 95 completers) improved significantly more compared to the placebo group (n = 98 completers). The significant difference was a result of an effect on the hyperactivity, impulsivity and impaired socialisation subscales, with no effect on the attention-deficit subscale. The largest effect was seen in those who were of older age, had higher body mass indices and had lower zinc and free fatty acids levels. The study had some limitations. The dropout rate after randomization was relatively high (207 of 400), and no intent-to-treat analysis was performed. Those randomized to zinc supplementation received zinc sulphate 150 mg/day for 12 weeks, which is a dosage higher than the normally recommended upper limit. Interestingly, both positive clinical trials of zinc

supplementation came from an area with suspected endemic zinc deficiency (Turkey[13] and Iran[14]).

4. Implications for Research

To measure the hypothesized zinc-induced changes in extracellular dopamine level we propose that zinc-deficient ADHD patients undergo a PET scan with ^{11}C-raclopride before (baseline) and following 2 months of zinc supplementation (a fixed oral dose of zinc sulphate 90 mg daily with an approximately 24.5 mg zinc element). ^{11}C-raclopride represents a dopamine D_2/D_3 receptor radioligand whose binding is sensitive to competition by endogenous dopamine in brain regions of high D_2/D_3 receptor density such as the striatum (^{11}C-raclopride displacement method).[52-54] A significant difference in ^{11}C-raclopride binding measured as D_2/D_3 receptor availability (B_{max}/K_d; number of receptors/affinity of receptors) between PET scans at baseline and post-treatment with zinc would lend support to the suggestion that zinc increases extracellular dopamine levels (baseline B_{max}/K_d – zinc B_{max}/K_d = >0, tested with paired t-tests of significance revealed as a reduction in ^{11}C-raclopride binding after zinc supplementation). To test the related hypothesis that zinc potentiates the binding affinity of cocaine and cocaine analogues on the dopamine transporter, we propose using PET with ^{11}C-cocaine and/or ^{11}C-d-threo-methlphenidate in different scans before (baseline) and after zinc supplementation in zinc-deficient ADHD patients.[53] A significantly higher binding value (B_{max}/K_d) of ^{11}C-cocaine and/or ^{11}C-d-threo-methylphenidate in the post-zinc supplementation scans versus baseline scans would lend support to the suggestion that zinc potentiates the binding of cocaine and cocaine analogues (methylphenidate) on the dopamine transporter of zinc-deficient ADHD patients.

In summary, the evidence appears strong enough to warrant further controlled study in well diagnosed samples.[4] Therefore, more double-blind, placebo-controlled studies should be carried out to clarify the potential value of zinc supplementation in ADHD. In this way, we could also arouse researchers' interest in imaging studies with zinc in patients with ADHD as described above.

5. Implications for Treatment

Zinc as additional therapy in zinc-deficient ADHD patients has the potential to enhance outcome in those who are currently only partially responding to psychostimulants. It may also have a role to play in those who currently do not respond to stimulants at all. This would not only improve the prognosis of childhood ADHD, but at the same time help avoid significant adulthood complications of ADHD.[55] Zinc is relatively cheap (e.g. Solvazinc® 45 mg zinc, 30 tablets cost £4.32 or €5.18 [2010 costing in the UK]). It is well tolerated and readily available.[14]

6. Conclusion

Results such as the unexpected benefits of zinc in zinc-deficient ADHD patients, detailed here, led to advances in our knowledge of ADHD. The action of zinc in ADHD should be looked at objectively *in vivo* similarly to research conducted on methylphenidate. Our research suggestions yield an opportunity to expand thinking in ADHD treatment research. The confirmation of zinc as an *in vivo* dopamine transporter inhibitor in ADHD interacting with psychostimulants would, if correct, be of important public interest in regard to hundreds of thousands of ADHD patients who take psychostimulants every day.

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