Zinc Supplementation: Neuroprotective or Neurotoxic?

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While zinc is essential for normal brain function and repair, recent work has implicated this trace element in the neuronal damage and death that follow traumatic brain injury, stroke, and seizure. Therefore, the development of new zinc-based therapeutic strategies will need to consider the emerging roles of zinc in the central nervous system.

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Traumatic and Ischemic Brain Injury

Every year, millions of people suffer from neuronal injury related to stroke or traumatic brain injury (TBI). In the United States alone, it has been estimated that 5.3 million people are currently living with disabilities resulting from TBI caused by automobile accidents, violence, workplace accidents, and falls. The cost of TBI in the United States is estimated to be $48.3 billion annually. An additional 500,000 people per year sustain brain injury due to a reduction in oxygen supply after a stroke. Unfortunately, even this number may be too low because cardiac arrest can also contribute to brain oxygen deprivation, raising the number of yearly ischemic brain injuries to over 750,000.

While the causes of ischemic brain injury and TBI are different, we now know that both of these injuries result in the release of cellular zinc, triggering neuronal death. Thus, an understanding of zinc, not only as an essential nutrient but also as a neurotoxic element, requires that we examine the role of dietary zinc and zinc supplementation in neuronal injury, particularly after stroke or TBI.

Zinc in Neuronal Injury and Death

While most of the zinc in the central nervous system (CNS) is bound to proteins, approximately 10% is “free zinc.” It is now accepted that free zinc, which is not associated with proteins or amino acid ligands, can be released from vesicular pools, where it is co-localized with the neurotransmitter glutamate. Under normal conditions, vesicular zinc acts to modulate post-synaptic glutamate receptors. However, there is a large and growing body of evidence showing that after CNS injury, large quantities of free zinc can be released, not just from pre-synaptic vesicles, but also from metalloproteins and from mitochondrial zinc pools, resulting in neuronal damage and death. Two recent informative reviews on the source and role of free zinc in the CNS are from Sensi and Jeng and Frederickson et al.

A significant amount of elegant in vitro work has established the neurotoxicity of zinc. For example, when primary cultures of rat and mouse cortical neurons were treated with zinc at concentrations as low as 30 to 35 μM for 24 h, there was clear evidence of neuronal death with characteristics of both apoptosis and necrosis. This is consistent with a report showing that the accumulation of high concentrations of free zinc leads to the loss of NAD+ and the inhibition of glycolysis.

Free zinc accumulation leading directly to neuronal death has been reported in vivo after ischemia, TBI, and seizure (Table 1). While the release of vesicular zinc has clearly been implicated in neuronal damage, studies have shown an increase in metallothionein-III (MT-III) after TBI, suggesting that other pools of free zinc such as this may also mobilized after injury. TBI from either mechanical cortical trauma or fluid percussion injury resulted in neuronal death in the dentate gyrus, hilus, and CA1 regions of the hippocampus that could be largely prevented by treatment with the zinc chelator CaEDTA (Table 1). This is also true for ischemic injury. When Long Evans rats were subjected to 10 minutes of transient forebrain ischemia, 24 to 72 hours later there was a significant increase in neuronal zinc accumulation in the hippocampus that was followed by post-synaptic...
neuronal death. Three intracerebroventricular treatment with CaEDTA 30 minutes prior to the ischemia prevented both the post-synaptic zinc accumulation and the subsequent neuronal degeneration. Three others have shown that intracerebroventricular administration of CaEDTA prior to transient focal ischemia for 30 minutes reduced the infarct volume measured after 3 days compared with the administration of either the vehicle or ZnEDTA. While these reports certainly suggest that zinc chelation may protect neurons after injury, the data are complicated by the fact that 14 days after reperfusion, chelation appeared to have no effect on infarct size. This was true even when the chelator was infused continuously for the entire 14-day period.

Zinc Supplementation after TBI

Following TBI, patients, particularly those who are immobilized, are at risk for the development of moderate to severe zinc deficiency. Urinary zinc excretion is increased and serum zinc is significantly depressed in patients with TBI. An early report showed that urinary zinc excretion was proportional to the severity of the injury such that in the most severely injured patients, mean urinary zinc was 14 times normal values. Furthermore, patients with severe head injuries also develop hypoalbuminemia, most likely due to increased interleukin-1-mediated transendothelial movement of albumin, and evidence of inflammatory processes such as an increase in acute-phase proteins such as ceruloplasmin and C-reactive protein and elevated white blood cell counts. The impact of these factors on zinc metabolism and availability is potentially significant. Albumin is the major serum transport protein for zinc, so hypoalbuminemia would potentially impair zinc transport and availability. Furthermore, the induction of cytokines such as interleukin-1 may further tax zinc availability that is already significantly compromised by increased urinary excretion.

In support of this hypothesis, we used a rat model of TBI to show that the zinc deficiency that develops after TBI can have deleterious consequences. Moderate zinc deficiency significantly increased cell death, as measured by TUNEL labeling, at the site of cortical injury. In this model, there was evidence of both necrotic and apoptotic cell death. More severe zinc deficiency, which resulted in anorexia, increased the number of apoptotic cells at the site of injury. Interestingly, the cell death was not just an acute problem associated with injury, but in zinc-deficient animals persisted for at least 4 weeks after the initial trauma.

While these data make it clear that systemic zinc deficiency after CNS injury should be avoided, our understanding of the role of free zinc in neuronal death raises the question of whether clinicians should be treating brain-injured patients with supplemental zinc. We have attempted to answer this question using a rat model of TBI that consisted of a cortical stab wound. We were particularly interested in this model because we wanted to test the effect of zinc supplementation in a model in which we knew that the blood-brain barrier, which regulates brain zinc uptake, had been disrupted. We showed that 4 weeks of dietary zinc supplementation did not significantly increase TUNEL labeling in any region of the CNS after TBI. However, caution should be used when interpreting these results, because the zinc supplementation in this study was supplied as dietary zinc (180 ppm). Thus, we would expect that zinc uptake and distribution would be under the control of the intestine and liver, where absorption and storage of zinc primarily occur. In patients with severe TBI, zinc supplementation is necessarily administered parenterally, where it may cross the injured blood-brain barrier in an unregulated fashion.

<table>
<thead>
<tr>
<th>Insult</th>
<th>Model</th>
<th>Zn Accumulation and Neurodegeneration</th>
<th>Effect of Chelation (CaEDTA)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia</td>
<td>Rat, transient forebrain ischemia, 10 min</td>
<td>Hippocampal, 24–72 h after insult</td>
<td>Prevented Zn influx and neuronal death</td>
<td>Koh et al., 1996</td>
</tr>
<tr>
<td>Ischemia</td>
<td>Rat, middle cerebral artery occlusion, 30 min</td>
<td>Hippocampal, 3 d after reperfusion</td>
<td>Protective at d 3 but not at d 14</td>
<td>Lee et al., 2002</td>
</tr>
<tr>
<td>Trauma</td>
<td>Rat, cortical trauma</td>
<td>Hippocampal, 1–24 h</td>
<td>Neuronal death reduced 50%–75%</td>
<td>Suh et al., 2000</td>
</tr>
<tr>
<td>Trauma</td>
<td>Rat, fluid percussion injury</td>
<td>2 h, 24 h, 7 d</td>
<td>Neuronal death reduced up to 85%</td>
<td>Hellmich et al., 2004</td>
</tr>
<tr>
<td>Seizure</td>
<td>Mice, kainate-induced seizure</td>
<td>Hippocampal (CA1, CA3), 24 h</td>
<td>Neuroprotective</td>
<td>Lee et al., 2009</td>
</tr>
<tr>
<td>Seizure</td>
<td>Mice, pilocarpine-induced seizure</td>
<td>Hippocampal (CA1, CA3), 24 h</td>
<td>Neuroprotective</td>
<td>Suh et al., 2001</td>
</tr>
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This issue was partially addressed in a study designed to examine the effects of zinc supplementation on neurological outcomes of brain-injured adults. Sixty-eight patients with TBI were randomly assigned to either an adequate zinc (2.5 mg/d) or supplemental zinc (12 mg/d) treatment group within 72 hours of injury. For the initial 15 days after injury, these zinc levels were administered intravenously in a double-blind fashion as zinc sulfate along with total parenteral nutrition. After the initial period of total parenteral nutrition, 22 mg of zinc (as zinc gluconate) or placebo was then supplied orally for the remainder of the study. After approximately 3 weeks of zinc treatment, there were increases in serum prealbumin and retinal-binding protein in the supplemented group, indicating improved protein synthesis.

This study suggests that supplemental zinc may be useful in maintaining visceral protein in patients with brain trauma. Most interestingly, by 2 weeks after injury, patients in the zinc-supplemented group had improved recovery scores on the Glasgow Coma Scale compared with patients who were provided adequate zinc \( (p = 0.005) \). Improvements persisted throughout the study, and were seen at 21 days \( (p = 0.02) \) and 28 days \( (p = 0.09) \) post injury. The differences were seen despite the fact that serum zinc concentrations and cerebrospinal fluid zinc levels were not measurably changed by supplementation. These data suggest that, rather than being dangerous, zinc supplementation after TBI, at least at these levels, may be beneficial to both visceral protein synthesis and neurological outcomes.

**Zinc Supplementation after Ischemia**

While most work in animal models now show that zinc chelation with CaEDTA is neuroprotective after ischemia, three early studies showed that pretreatment with zinc-protoporphyrin complexes reduced the size of the infarct and the edema associated with transient cerebral ischemia (2 hours of middle cerebral artery occlusion). Zinc-protoporphyrin was not, however, an effective treatment in models of permanent occlusion. These results led others to test the effect of zinc without protoporphyrin in transient ischemia. Intraperitoneal injections of zinc chloride (10.9 mg/kg) prior to 2 hours of ischemia significantly reduced the infarct size 24 hours later. While zinc was not effective in reducing the cerebral edema associated with ischemia, combined with the apparent efficacy of zinc supplementation after TBI, these data opened the door to the hypothesis that zinc could be used as a successful clinical treatment after ischemic damage to the CNS.

Despite these encouraging results, a recent report suggested that caution is warranted before we begin the testing of zinc supplementation during recovery from stroke. The effects of zinc chloride (10.9 mg/kg) administered intraperitoneally 30 min prior to embolization of the middle cerebral artery were tested in adult male rats. Because GABA has been hypothesized to be neuroprotective, the GABA antagonist bicuculline or a combination of zinc and bicuculline was also tested. One hour and 24 hours after ischemia, neurological deficits, as determined by the Bederson’s scoring system, were not different between the groups. However, by 48 hours post ischemia, zinc pretreatment resulted in a significant worsening of behavioral outcomes. These neurological deficits were consistent with a significant increase in infarct volume that was found at this time point in zinc-treated rats. Interestingly, bicuculline alone did not increase infarct volume or brain swelling, and did not impair behavioral outcomes compared with vehicle-treated controls. However, at 48 hours, the combination of zinc and bicuculline treatment appeared to be more damaging (as measured by all three indices) than either treatment alone.

Therefore, we have one report of improved outcomes with supplemental zinc and another where zinc impaired recovery. Both studies used the same zinc dose and form, 10.9 mg ZnCl\(_2\)/kg body weight, which is approximately 5 mg Zn\(^{2+}\)/kg. However, the methods used to induce transient ischemia and reperfusion were different. In the initial work, transient arterial occlusion using a filament threaded into the middle cerebral artery permitted a rapid end to ischemia and immediate reperfusion. In contrast, the more recent work used an embolization procedure that mimics the gradual reperfusion often observed clinically. Furthermore, this method is known to produce smaller secondary regions of ischemia following clot dissolution.

It should also be noted that most of the studies examining the efficacy of zinc have used zinc pretreatment. While this may help us to understand the role of zinc mechanistically, it does little to help develop strategies to treat stroke after it occurs. In the one study in which zinc was administered 2 or 4 hours after transient ischemia, zinc treatment did not reduce edema, cerebral blood flow, or infarct size 22 hours after reperfusion. It is not known whether this treatment would actually increase infarct size at 48 hours, as was suggested by the data of Shabanzadeh et al.

**Conclusion**

In conclusion, it is now clear that the development of new treatment strategies for the treatment of CNS insults needs to take into account the role of zinc in neuronal function, damage, repair, apoptosis, and necrosis. In addition to testing the use of zinc supplementation, there is an abundance of data suggesting that central zinc chelation may also be a future strategy. Regardless of the
approach, systemic zinc supplementation or central zinc chelation, future work that seeks to explore the possible efficacy of zinc modulation in stroke, cerebral ischemia, TBI, and seizure disorders should employ clinically relevant models to permit not only the development of drug therapies, but also the clarification of dietary recommendations after injury.

References
