Effect of zinc in ischemic brain injury in an embolic model of stroke in rats

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Abstract

Zinc is prevalent in the mammalian central nervous system and its role in ischemic brain injury is still controversial. In the present study, the effect of zinc in ischemic brain injury was examined in an embolic model of stroke in rats. Furthermore, the effect of zinc in combination with bicuculline, a GABAa antagonist, was also examined in the ischemic injury. Treatment with zinc or zinc plus bicuculline increased infarct volume significantly and also worsened neurological deficits. Moreover, treatment with zinc plus bicuculline also enhanced ischemic brain edema. These results thus support the hypothesis that administration of zinc i.p. worsens the outcome of ischemic brain injury in the embolic model of stroke in rats.

Keywords: Stroke; Zinc; GABA; Ischemic brain injury

Zinc is prevalent in the mammalian central nervous system (CNS). The physiological significance of neuronal zinc release in the CNS is not clear [5] and its role in ischemic brain injury is controversial [11]. In focal ischemic brain injury, zinc either increased or reduced ischemic neuronal death [12,19]. These apparently controversial results prompted us to re-examine the functional role of zinc in ischemic brain injury. Previous studies, including our own, showed that during ischemic brain injury an increase in extracellular glutamate causes cell death and an increase in extracellular GABA is protective [16]. Although it has been shown that zinc modifies central signaling via a glutamate/GABA system, no study has examined what zinc does when the balance of the glutamate/GABA system is changed. In the present study, we examined the effects of zinc in ischemic brain injury using a clinically relevant model of stroke in rats. We also examined the effects of zinc in combination with bicuculline, a GABA antagonist, in the ischemic brain injury.

Male Sprague–Dawley rats weighing 250–300 g were used. Focal cerebral ischemia was induced by embolizing a preformed clot into the MCA, as described previously [17]. Infarct volume and brain edema were measured 48 h later after MCA occlusion, as detailed previously [14]. Neurological deficits were determined using a modified Bederson’s scoring system at 1, 24 and 48 h after MCA occlusion [2]: 0, no observable deficit; 1, forelimb flexion; 2, forelimb flexion plus decreased resistance to lateral push; 3, unidirectional circling; 4, unidirectional circling plus decreased level of consciousness. There were four groups in this study and the animals were randomly assigned into each group (n = 12). The animals of each group received saline, bicuculline 5.4 µmol/kg [3], zinc chloride 80 µmol/kg [19] or zinc chloride 80 µmol/kg plus bicuculline 5.4 µmol/kg. Saline or drug was administered (i.p.) at 30 min before MCA occlusion. Brain infarct volume and swelling were analyzed with one-way ANOVA followed by Tukey test. Neurological scores were analyzed with the Kruskal–Wallis test and Wilcoxon Signed Ranks Test and expressed with an interquartile range. P < 0.05 was considered statistically significant.

Changes of infarct volume at 48 h after MCA occlusion in different groups are shown in Fig. 1. In the control group, infarct volume was 19.34 ± 9.80% (mean ± SD). Compared to the control group, treatment with zinc increased infarct volume significantly (P < 0.01). Infarct volume in the zinc plus bicuculline group was also significantly larger than that in the control group (P < 0.01). However, infarct volume in the bicuculline group did not differ from the control group significantly.

Brain swelling in the control group was 1.13 ± 0.07% at
bicuculline. When compared with the rats that received treatment with saline, zinc or zinc plus bicuculline, brain edema was measured at 48 h after MCA occlusion. Compared with the control group, treatment with zinc or zinc plus bicuculline increased brain edema significantly.

48 h after ischemic injury (Fig. 2). Compared to the control group, treatment with zinc plus bicuculline increased the brain swelling significantly (P < 0.001). Brain swelling in the zinc plus bicuculline group also increased significantly when compared with the zinc group (P < 0.01) or the bicuculline group (P < 0.01). Additionally, there was also a strong positive correlation between infarct volumes and swelling at 48 h after MCA occlusion (r = 0.78).

At 1 and 24 h after MCA occlusion, all animals showed significant motor deficits with a median score of 3 in all treatment groups (Table 1). At 48 h after MCA occlusion the median score of neurological deficits was 2 in the control group. Treatment with zinc significantly worsened the neurological deficits (P < 0.05). The neurological deficits in the rats that received treatment with zinc plus bicuculline were also more severe than in controls (P < 0.05).

The present results showed that treatment with zinc alone or zinc plus bicuculline increased infarct volume significantly, measured at 48 h after MCA occlusion. Treatment with zinc or zinc plus bicuculline also worsened impairment due to neurological deficits. In addition, treatment with zinc plus bicuculline also increased brain edema significantly. The present results thus are in agreement with observations that zinc plays a significant role in neuronal death after transient global ischemic brain injury [11,15]. In these observations, ischemic injury resulted in an elevation of zinc concentration and also caused cell death in selected populations of neurons. Blocking the zinc elevation prevented cell death. Our data, however, contradict the finding from a study in which focal ischemic brain injury was induced by inserting a suture into the MCA [19]. The reasons for these differences are not clear. One possible reason is that pathological processes of our embolic model are different from the suture model. For example, in the suture model reperfusion started immediately after suture withdrawal. In the embolic model, reperfusion started gradually after MCA occlusion and secondary occlusions were also observed after dissolution of the clots used for MCA occlusion [18]. In the present study, infarct volumes were measured at 48 h after MCA occlusion. In the suture model study, infarct volumes were measured at 24 h after reperfusion [19]; however, this is an unlikely cause for the differences.

Since we did not measure the zinc concentration, we were unable to definitely determine if ischemic brain injury worsened due to increasing zinc concentrations in the brain after the administration of zinc i.p. It is possible that zinc acts on other systems, such as the cardiovascular system, which in turn causes detrimental effects on the brain. The worsening outcome induced by the administration of zinc, however, might also be via several other mechanisms. (1) Extracellular zinc stimulates zinc-containing neurons to release their stores of the ion and this produces a toxic environment for adjacent neurons [4,13]. (2) Rising intracellular free zinc can induce production of cellular reactive oxygen species and loss of mitochondrial membrane potential. These changes in turn cause restriction of production of cellular energy and contribute to ischemic cell death [1,6]. (3) An increase of extracellular or intracellular zinc in neural cells can inhibit bicarbonate exchangers and cause a decrease of intracellular pH. The low pH deranges the function of neurons and enhances ischemic cell death [7].

The present results are at different time points after MCA occlusion and are expressed as median values; interquartile ranges, the 25–75th percentile, are shown in parentheses (n = 12). * denotes significantly different from the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>1 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>3 (3–3)</td>
<td>3 (2–3)</td>
<td>2 (2–2)</td>
</tr>
<tr>
<td>Zinc</td>
<td>3 (3–3.8)</td>
<td>3 (2.2–3)</td>
<td>2.5 (2–3)*</td>
</tr>
<tr>
<td>Bicuculline</td>
<td>3 (2.3–3.8)</td>
<td>3 (2–3)</td>
<td>2 (1.3–3)</td>
</tr>
<tr>
<td>Zinc + bicuculline</td>
<td>3 (3–3)</td>
<td>3 (2.3–3)</td>
<td>3 (2–3)*</td>
</tr>
</tbody>
</table>

The neurological deficit scores are at different time points after MCA occlusion and are expressed as median values; interquartile ranges, the 25–75th percentile, are shown in parentheses (n = 12). * denotes significantly different from the control group.
induced edema by increasing acidosis in neural cells [9,10]. Furthermore, bicuculline also has an important role in inducing elevation of calcium and amplifying the release of arachidonic acid in ischemia [8,9], which may also contribute to the zinc-induced ischemic edema. Nevertheless, to ascertain how zinc interacts with the GABA system, further studies need to be conducted such as investigating the interactions between GABA agonists or antagonists in combination with zinc.

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References