Behavioral Assessment of Mice Treated with Zinc and Paroxetine

HASAN S. AL AMRY, M.D.; SSC-Psych
The Department of Medicine, College of Medicine, King Khalid University, KSA

Abstract

Background: Less than half of older patients with major depression disorder (MDD) achieve remission with antidepressant medications and rates of remission are even poorer for those with comorbid condition. The addition of another medication to the antidepressant regimen of patients not responding adequately to their treatment has become a common intervention.

Aim of Study: To examine the behavioral changes of acute and subacute intraperitoneal administration of zinc in the forced swimming test (FST) in mice; to investigate if addition of zinc to selective serotonin reuptake inhibitor “paroxetine” would enhance the antidepressant effect of paroxetine; and to study the effects of combined administration of zinc and paroxetine on spontaneous locomotor activity in mice.

Material and Methods: It has been hypothesized that addition of zinc with its multiple pharmacological effects to the antidepressants may modulate and enhance their efficacy. We investigated the behavioral changes of acute and subacute interaction of zinc with paroxetine in the FST in mice. Mice were injected with either paroxetine (20mg/kg); zinc sulfate (40mg/kg) or paroxetine in combination with zinc for one day and one week (once daily).

Results: Significant antidepressant effect of paroxetine alone or zinc alone has been shown in a decrease of immobility and increase of swimming behavior. Also, results showed a significant decrease in the immobility time and increase in the swimming behavior time of the animals treated with zinc in combination with paroxetine as compared with animals treated with either paroxetine or zinc alone. There was no significant difference in the animals’ behavior between acute and sub-acute treatment with zinc or even upon its addition to paroxetine. None of the treatment regimens have shown any significant changes in the animals’ motor activities.

Conclusion: The present study supports the notion that administration of zinc may offer additional antidepressant activity. This combination may have a significant clinical application in psychiatric patients particularly in geriatric patients or other population where zinc level has shown dramatic decrease.

Key Words: Zinc treatment – Paroxetine – Forced swim test – Mice – Animal experiment.

Introduction

MAJOR depression disorder (MDD) is responsible for high morbidity in the overall population. It is an overwhelming illness that affects 17% of the population at some point in life, resulting in major social and economic consequences [1]. It is a chronic disorder, frequently characterized by relapses and recurrences. Suicide is estimated to be the cause of death in up to 15% of individuals with depression, and many other deleterious health-related effects have been recognized. Despite the devastating impact of depression, the available treatments are either not effective in many patients or have many adverse effects [2,3].

Paroxetine, a selective serotonin reuptake inhibitor (SSRI) antidepressant, is used to treat major depression, obsessive-compulsive disorder, panic disorder, social anxiety, and generalized anxiety disorder in adult outpatients [4]. In adults, the efficacy of paroxetine for depression is comparable to that of older tricyclic antidepressants, with fewer side effects and lower toxicity. Differences with newer antidepressants are subtler and mostly confined to side effects. Paroxetine shares the common side effects and contraindications of other SSRIs, with high rates of nausea, somnolence and sexual side effects [5].

Partial response and non-response to antidepressant medications are common problems. Between 10% and 30% of depressed patients taking an antidepressant are partially or totally resistant to the treatment [6,7]. Recurrence of depression while still taking medication (i.e., breakthrough) can also occur [8].

With advancing age, there is a higher incidence of chronic major depressive disorders and many bear a nutritional deficiency component, either as a cause or as a result of that condition. The trace elements, such as zinc and copper, are constituent
components of many metalloproteins and metalloenzymes and a deficiency of one of these elements could lead to an alteration of cellular function particularly at the neuronal level [9].

Zinc is necessary for many enzymatic activities, DNA replication, transcription and protein synthesis. It is present in the whole body, but the brain (especially the hippocampus and cerebral cortex) is the organ with the highest zinc concentrations [10-12]. In the brain, all neurons bearing synaptic zinc are glutamatergic. However, not all glutamatergic neurons contain this metal [13,14]. Alterations in brain zinc homeostasis are associated with behavioral disturbances, such as anorexia, dysphoria, impaired learning and cognitive function and with some neurological disorders (e.g., epilepsy, Alzheimer’s disease) due to its potent inhibitor effect of the N-methyl-D-aspartate (NMDA) receptor complex [15,16].

Zinc is implicated in the mechanism of pathophysiology and therapy of depression [17,18]. Also, chronic zinc administration increases brain-derived neurotrophic factor (BDNF) gene expression in the rat brain [18,19]. That feature correlates with the fact that chronic treatment with classical antidepressants increases BDNF [20] and induces neurogenesis [21] in rats. Moreover, zinc has been shown to modulate intracellular signaling cascades, such as mitogen-activated protein kinases, protein kinase C, protein tyrosine phosphatases, Ca $^{2+}/$ calmodulin activated protein kinase II, participating in cell proliferation, differentiation, and death [22].

Clinical observations demonstrated a reduced blood zinc level in depressed patients [23]. This reduced level has been normalized only after successful antidepressant therapy [24]. Chronic antidepressant treatment elevates (i.e., redistributes) zinc concentration in rat hippocampus [25]. Chronic treatment with imipramine increases the potency of zinc to inhibit the NMDA receptor activity in the mouse cerebral cortex [26]. These alterations may lead to a reduction in the activity of the NMDA receptor complex. Zinc is active in the forced swimming test, commonly used for evaluation of antidepressant activity [27]. Moreover, zinc exhibits antidepressant-like effects in animal models of depression, such as chronic mild stress and olfactory bulbectomy models of depression [28].

The aim of the present study was to examine the behavioral changes of acute and subacute intraperitoneal administration of zinc in the forced swimming test (FST) in mice; and to investigate if addition of zinc to selective serotonin reuptake inhibitor “paroxetine” would enhance the antidepressant effect of paroxetine. We also aimed to study the effects of combined administration of zinc and paroxetine on spontaneous locomotor activity in mice.

Material and Methods

All procedures were conducted according to the general Animal Care and Use Committee guidelines, and were approved by the Ethics Committee of the Pharmacology Department, King Khalid University. The experiments were carried out on male Albino Swiss mice (25-30gm). The animals were kept under a natural day-night cycle with free access to food and water. Each experimental group consisted of 12 animals. Zinc sulfate and paroxetine hydrochloride were administered by ip injection following the initial pre-swim test, three times approximately at 23.5, 7 and 1 hour prior to the 5 minutes FST in the acute study and in the subacute study, the animals received the treatment once daily for one week. Control animals received a vehicle (saline).

Forced swimming test (FST):

The studies were carried out according to the method of Porsolt et al. [29] Mice were dropped individually into glass cylinders (height 25cm, diameter 10cm) filled with water to a height of 10cm, (maintained at 23-25 $^\circ$C), and animals were exposed to a pretest for 15 minutes, 24 hours prior to the 5-minute swim test. The pretest facilitates the development of immobility during the test session and increases the sensitivity for detecting antidepressant behavioral effects. Animals were removed from the water, dried by the experimenter and placed into plastic cages with a heating pad.

Behavioral scoring was performed according to Detke et al. [30] and during the 5-minute test session, three different behaviors were rated:

- Immobility: An animal was judged to be immobile if it remained floating passively in the water.
- Swimming: An animal was judged to be swimming if it was making active swimming motions, more than necessary to solely maintain their head above water.
- Climbing: An animal was judged to be climbing when they were making active movements in and out of the water with their forepaws, usually directed against the walls.

Locomotor activity:

Locomotor activity was measured using photoresistor actometers (circular cages, 25cm in diameter, two light sources, and two photoresistors).
The animals were placed individually in an actometer for 10 minutes. Activity was measured at 5-minute intervals to characterize dynamics of changes. The number of light beams crossed by an animal was recorded as the locomotor activity.

**Data analysis:**
The obtained data were evaluated by the one way analysis of variance (ANOVA) test, followed by Dunnett’s multiple comparisons test. *p*-values <0.05 were considered as statistically significant.

**Results**
Zinc sulfate at doses of 10, 20, 30 and 40mg/kg (respectively) was tested in the FST in mice and in the locomotor activity test. Zinc significantly reduced the immobility time in the FST at doses of 30 and 40mg/kg and had no effect at doses of 10 or 20mg/kg (Table 1). Therefore, the dose of 40mg/kg was chosen for use in the studies of interaction of antidepressants with zinc.

In the acute study, zinc sulfate at a dose of 40mg/kg was tested in the FST. Zinc significantly reduced the immobility time and increased the swimming behavior in the FST. Also paroxetine administered alone at the dose of 20mg/kg reduced the immobility time and increased the swimming. Combined administration of paroxetine (20mg/kg) and zinc (40mg/kg) in the acute study, induced statistically significant reduction of the immobility time in mice and increased the swimming durations as compared with either paroxetine- or zinc-treated animals, as shown in Fig. (1). On the other hand, combined administration of zinc with paroxetine had no effect on the locomotor activity in mice (as shown in Fig. 2).

In the sub-chronic study, zinc (40mg/kg) significantly reduced the immobility time and increased the swimming activity in the FST (Fig. 2). Also paroxetine administered alone at the dose of 20mg/kg reduced the immobility time and increased the swimming behavior durations (Fig. 3).

Combined administration of paroxetine (20mg/kg) and zinc (40mg/kg) in the sub-chronic study, induced statistically significant reduction of the immobility time in mice and increased the swimming durations as compared with either paroxetine or zinc treated animals, as shown in Fig. (2). On the other hand, combined administration of zinc with paroxetine had no effect on the locomotor activity in mice, Fig. (2).

The effects due to either zinc administration or paroxetine or combined administration of paroxetine and zinc on total duration of immobility of acute and sub-chronic studies in mice are shown in Table (2). The results showed no significant difference between acute and sub-chronic administration of all treatments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Immobility time (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>37.12±5.91</td>
<td></td>
</tr>
<tr>
<td>Zinc sulfate</td>
<td>10</td>
<td>34.70±7.32</td>
</tr>
<tr>
<td>Zinc sulfate</td>
<td>20</td>
<td>35.18±5.64</td>
</tr>
<tr>
<td>Zinc sulfate</td>
<td>30</td>
<td>29.42±4.16*</td>
</tr>
<tr>
<td>Zinc sulfate</td>
<td>40</td>
<td>27.40±4.20*</td>
</tr>
</tbody>
</table>

*p*<0.05, compared with saline-treated animals.

Table (2): Effects of acute or sub-chronic zinc sulfate (40mg/kg) administration alone and/or in combination with paroxetine (20mg/kg) on the duration of immobility in mice.

<table>
<thead>
<tr>
<th>Immobility time</th>
<th>Zinc sulfate (40mg/kg)</th>
<th>Paroxetine (20mg/kg)</th>
<th>Zinc sulfate + Paroxetine (40mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute (24 hours)</td>
<td>29.25±4.80</td>
<td>21.87±4.15</td>
<td>17.20±7.52</td>
</tr>
<tr>
<td>Subchronic (7 days)</td>
<td>28.50±4.40</td>
<td>23.50±4.65</td>
<td>19.00±4.13</td>
</tr>
</tbody>
</table>

*p*<0.05

Fig. (1): Effect of Acute zinc sulfate (40 mg/kg) administration alone and/or in combination with paroxetine (20mg/kg) for 24 hours. Mice received their treatments three times following the initial 15min pre-test swim, at 23.5, 7 and one hour before the 5 minutes forced-swim test. Data are presented as mean ± S.D. of total number of intervals spent in each specific behavior (N= 12 per group), sampled every 5 sec, during a 5 min FST period. There were significant differences (*p*<0.05) within each behavior, as compared with (a) saline treatment, (b) 40mg/kg zinc, (c) 20 mg/kg Paroxetine and (d) Paroxetine (20mg/kg) + zinc (40mg/kg).
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**Fig. (2):** Effect of different treatments on locomotor activity in the open-field test. Mice received saline (n=11), Paroxetine (20mg/kg; n=10) and/or zinc sulfate (40mg/kg) by IP injection prior to the 10 min open-field test. Data are presented as mean ± S.D. number of crossings in the open-field test in response to saline or drug treatments. No significant difference was found between saline and zinc, paroxetine and zinc + paroxetine treated animals. Treated mice had no significant activity scores compared with saline-treated animals.

**Fig. (3):** Effect of subchronic zinc sulfate (40mg/kg) administration alone and/or in combination with Paroxetine (20mg/kg) for one week, once daily in the forced-swim test. Mice received the last dose 24 hours before the 5 minutes forced-swim test. Results are presented as mean ± SD of total number of intervals spent in each specific behavior (n=12 per group), sampled every 5 sec, during a 5min FST period. There were significant differences (p<0.05) within each behavior, as compared with (a) saline treatment, (b) 40mg/kg zinc, (c) 20mg/kg Paroxetine and (d) Paroxetine (20mg/kg) + zinc (40mg/kg).

**Discussion**

Several classes of antidepressants are available, which act as selective biogenicamine reuptake inhibitors [31]. Unfortunately, commonly used antidepressant therapy is effective in only 60-70% of patients and produces a variety of unwanted side effects [32]. Thus, the search for new more effective therapeutic strategies represents one of the priorities for treatment of special populations as well as resistant patients.

In the present study, we demonstrated the behavioral effects of zinc sulfate either alone or in combination with paroxetine after acute and sub-chronic administration in mice. Our results indicate that zinc administered once at the dose of 40mg/kg reduced the immobility time of mice in the FST and increased the swimming activity. Concurrently, when zinc is given jointly with paroxetine (20mg/kg), a potent reduction in the immobility time has been observed with concomitant increase in the swimming activity without change in the locomotor activity. Since these treatments (paroxetine plus zinc) do not affect locomotor activity, the results indicate potentiation of antidepressant-like activity by such combined treatments. These results confirmed that zinc produced an antidepressant-like effect and demonstrated a zinc induced enhancement of the antidepressant effect of paroxetine in the Porsolt’s test without stimulation of locomotor activity.

Similarly, previous results of Trullas and Skolnick [30] and Maj et al. [12] demonstrated that the blockade of the NMDA receptor produced antidepressant-like action and enhanced the effect of antidepressant drugs [26].

It was shown that chronic zinc administration increases the BDNF gene expression, which is the effect shared by most of clinically effective antidepressants. However, it is possible that other zinc doses and treatment schedules might also cause the hippocampal BDNF alterations. Since zinc profoundly affects glutamate transmission, mainly by antagonizing the NMDA receptors [33], this glutamate-dependent mechanism is likely to be responsible for zinc-induced changes in BDNF gene expression.

Also, an increase in the swimming but not climbing parameter of the mice, FST observed following zinc administration indicates the serotonergic pathway participation. Therefore, these results suggest that the antidepressant-like effect of zinc seems to be mediated through an interaction with the serotonergic rather than the noradrenergic
system. This may be a specific phenomenon for the FST, since the study of Cunha et al. [34] showed the synergistic effects of zinc with no less "serotonergic" but also with "noradrenergic" and "dopaminergic" profile antidepressants in the mouse tail suspension test. Zinc may indirectly release 5-hydroxy tryptamine (5HT), which in turn, activates 5HT receptors as part of the mechanism of antidepressant activity.

The present results show that zinc has an antidepressant effect that can be observed following its acute as well as sub-chronic administration. This antidepressant action did not increase after repeated administration. It could be assumed that its effects have a serotonergic component that also appears from the first administration. The effect of zinc on swimming remains the same after 7 days of treatment. This may suggest that predominance of zinc effects as NMDA antagonist over the other time consuming effects like BDNF induction that require chronic administration.

It could be concluded from this study that zinc possesses antidepressant activity. Further studies are needed to assess the role of chronic zinc administration in augmenting the activity of antidepressants as well as its underlying mechanism.

Acknowledgement:

It is my privilege to express my sincerest appreciation to Dr Hesham El Refae, Associate Professor of Pharmacology for his valuable inputs, ample guidance, encouragement, whole-hearted cooperation and constructive criticism throughout this study. I would also like to thank Dr. Ossama A. Mostafa, Associate Professor of Family and Community Medicine, for his highly valuable and expert support.

References