Influence of zinc supplementation on imipramine effect in a chronic unpredictable stress (CUS) model in rats

Katarzyna Cieślak¹, Bożena Klenk-Majewska¹, Zofia Danilczuk¹, Andrzej Wróbel¹,², Tomasz Łupina³, Grażyna Ossowska¹

¹Department of Pharmacology and Clinical Pharmacology, ²2nd Department of Gynecology, ³Department of Pulmonology, Oncology and Allergology, Medical University of Lublin, Jacekowskiego 8, PL 20-090 Lublin, Poland

Correspondence: Katarzyna Cieślak, e-mail: k.cieślik@am.lublin.pl

Abstract:
Zinc is an endogenous modulator of neuronal activity and may play an important role in the pathogenesis of depression. Recent studies have shown that zinc exhibits antidepressant-like activity in some models of depression in rodents. Our previous studies have shown that the footshock-induced fighting behavior was reduced in the rats subjected to chronic unpredictable stress (CUS). This test is used as the new experimental model of depression. Various antidepressant drugs given repeatedly prevented this kind of behavioral depression.

The aim of the present study was to evaluate the effect of prolonged treatment with zinc hydroaspartate and to examine if zinc supplementation could modulate the imipramine effect in CUS model of behavioral depression in rats. The experiments were carried out on male Wistar rats. Chronic stress (persisting for 16 days) was induced by the modified method described by Katz et al. Zinc hydroaspartate at the dose of 30 mg/kg/day or 15 mg/kg/day and imipramine at the dose of 5 mg/kg/day were administered once daily for 14 days. Imipramine was given (ip) 1 h before every stress session and zinc hydroaspartate (ip) 1 h before the antidepressant. The footshock-induced fighting behavior test was performed 48 h after the last session of the chronic stress. It was demonstrated that in chronically stressed rats the number of fighting attacks was significantly reduced (by about 75%). Zinc hydroaspartate at the dose of 30 mg/kg/day, given alone, prevented the deficit in fighting behavior in chronically stressed rats. Neither imipramine at the dose of 5 mg/kg/day nor zinc hydroaspartate (15 mg/kg/day) administered alone changed the intensity of fighting behavior in chronically stressed rats. However, when imipramine was given at the same dose in the rats pretreated with zinc hydroaspartate (15 mg/kg/day) the deficit of fighting behavior was not observed. The present results indicate that zinc similarly to antidepressants protects the rats against the CUS-induced behavioral depression. Moreover, our findings suggest that zinc supplementation could potentiate the antidepressant effect of imipramine.

Key words:
imipramine, zinc, chronic unpredictable stress (CUS), electric footshock-induced fighting behavior, rats

Introduction
Zinc is present in many regions of mammalian central nervous system (CNS), particularly in the cerebral cortex, pineal gland and the hippocampus [3]. Zinc is an important modulator of inhibitory and excitatory synaptic transmissions [27].

It has been shown that zinc modulates the action of multiple receptors and channels, enhancing AMPA and ATP-sensitive potassium (KATP) channel activity and inhibiting NMDA, GABA_A and voltage-dependent...
channel responses [1, 4, 34]. Zinc may enter the postsynaptic region via calcium permeable AMPA/kainate and NMDA receptor channels or through voltage-dependent calcium channels [11, 30] particularly following excessive zinc release [11, 30].

Early clinical manifestations of human zinc deficiency are growth disturbances in children, immune deficiency, and behavioral disturbances such as taste and smell acuity, impaired cognitive functions or dysphoria [35].

Alterations of brain zinc homeostasis can be also implicated in the mechanism of pathophysiology and therapy of depression [6, 7, 9, 10, 12, 14, 29]. Recently clinical data have revealed lower serum zinc concentration in patients suffering from depression, which may be normalized after effective antidepressant therapy [12, 29].

Among the variety of procedures modeling human depressive-like symptoms in animals, stress procedures belong to the well-validated models [5, 25, 37]. Studies in animals have shown that the chronic stress is able to evoke behavioral changes resembling clinical depression, such as motor activity deficit, reduced food and water consumption and decrease in responsiveness to rewarding stimuli [5, 25, 37].

Our previous findings have demonstrated that CUS (various stressors over 16 days) decreased the footshock-induced fighting behavior in rats. This test is used as the new experimental model of depression in rats. It was shown that antidepressants of different pharmacological profiles, i.e. tricyclic drugs, MAO inhibitors or selective serotonin reuptake inhibitors, given repeatedly, prevented the behavioral depression (the deficit of fighting behavior) induced by CUS [23, 25, 38].

The aim of the present study was to evaluate the effect of zinc hydroaspartate alone and the influence of zinc supplementation on imipramine effect in CUS model of depression in rats.

Materials and Methods

All procedures were conducted according to NIH Animal Care and Use Committee guidelines, and were approved by the Ethics Committee of Medical University of Lublin.

Animals

The study was conducted on male Wistar rats (weighting initially 180–200 g). Animals were housed six per cage under a natural light/dark cycle, temperature + 22°C and humidity 60%. Food and water were provided ad libitum. All experimental procedures were carried out between 8 a.m. and 1 p.m. Rats were experimentally naive and tested once.

Drugs

Zinc hydroaspartate (Farmapol) used in the experiments was from Farmapol, Poland and imipramine was from Polfa, Poland. All drugs were injected intraperitoneally (ip) at the single dose or once daily for 14 days. Imipramine at the dose of 5 mg/kg/day was given 1 h before every stress session and zinc hydroaspartate at the dose of 30 mg/kg/day or 15 mg/kg/day was administered 1 h before the antidepressant administration. The footshock-induced fighting behavior was tested 48 h after the last session of the CUS.

CUS procedure

Chronic unpredictable stress (CUS) procedure was a variant of Katz et al. method [5]. The rats were subjected once daily to the following kinds of unpredictable stressors: 20 s exposure to electric footshock (3 mA, 0.2 s duration every 2 s), 2 h periods of immobilization at 20°C or at 4°C, 5 min exposure to electric bell, 3 min periods of swimming in cold water (12°C) or 5 min periods of illumination (80 ± klx) and 48 periods of food deprivation. Each stressor was repeated 2 times during the 16-day period.

The footshock-induced fighting behavior

The pairs of male rats were placed in the glass cylinder (15 × 23 cm) on a steel grid floor, for 10 min adaptation. Next, fighting was induced by electric footshock (intensity 3 mA, impulse duration 0.2 s, rate 1/2s). The number of fighting attacks were scored during 5 min of painful stimulation. 19–21 days before the fighting behavioral test the pairs of rats were selected for similar intensity of fighting in each group. The footshock-induced fighting behavioral test was performed 48 h after the last session of chronic stress.
Exploratory activity

Exploratory activity (the number of squares traversed and rearings) was observed for 3 min in the “open field”, 15 min before the fighting behavior test.

Statistical analysis

The results of the experiments were expressed as the means ± SEM. The obtained data were evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison test. The criterion for significance was set at p < 0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Squares traversed* (mean ± SEM)</th>
<th>Rearings** (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>24.3 ± 3.3</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>Stress</td>
<td>19.3 ± 2.8</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>Zinc hydroaspartate 15</td>
<td>18.5 ± 3.5</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Stress + Zinc hydroaspartate 15</td>
<td>21.4 ± 2.9</td>
<td>3.8 ± 1.1</td>
</tr>
<tr>
<td>Zinc hydroaspartate 30</td>
<td>20.3 ± 2.7</td>
<td>4.8 ± 0.9</td>
</tr>
<tr>
<td>Stress + Zinc hydroaspartate 30</td>
<td>21.4 ± 3.1</td>
<td>4.4 ± 0.8</td>
</tr>
</tbody>
</table>

Zinc hydroaspartate was administered ip, once daily, for 14 days (in stressed rats – 1 h before every stress session). The last dose of the drug was injected 48 h 45 min before the test. The data are expressed as the means ± SEM of 12 rats per group. *ANOVA, F(5,66) = 0.4420, p = 0.68 NS; **ANOVA, F(5,66) = 0.3759, p = 0.86 NS.
Zinc hydroaspartate given at the single dose of 15 or 30 mg/kg/day (results not shown).

**Effect of prolonged treatment with zinc hydroaspartate on footshock-induced fighting behavior and exploratory activity in CUS model in rats**

Zinc hydroaspartate administered at the doses of 15 or 30 mg/kg/day for 14 days did not change the fighting behavior of normal (unstressed) rats (Fig. 2). On the other hand, in rats exposed to chronic stress, prolonged treatment with zinc hydroaspartate at the dose of 30 mg/kg/day but not 15 mg/kg/day increased the number of fighting attacks when compared with the control stressed rats (Fig. 2). Zinc hydroaspartate (15 or 30 mg/kg/day) given for 14 days changed the exploratory activity (squares transversed and rearings) neither in normal (unstressed) nor in chronically stressed rats (Tab. 1).

**Effect of prolonged co-administration of imipramine and zinc hydroaspartate on footshock-induced fighting behavior and exploratory activity in CUS model in rats**

Imipramine given at the dose of 5 mg/kg/day for 14 days influenced the number of fighting attacks neither

**Results**

- In control (unstressed) pairs of rats, the mean number of fighting attacks was 133/5 min. In rats subjected to CUS procedure, the number of fighting attacks was significantly reduced (by about 75%) when observed 48 h after the last stressor (Fig. 1). Exploratory activity was not changed in these animals (results not shown).

- Zinc hydroaspartate given at the single dose of 15 or 30 mg/kg changed neither the intensity of fighting behavior (Fig. 1) nor exploratory activity in normal (unstressed) and in stressed rats (results not shown).

- **Effect of prolonged treatment with zinc hydroaspartate on footshock-induced fighting behavior and exploratory activity in CUS model in rats**

Tab. 2. Effect of prolonged co-administration of imipramine (5 mg/kg/day) and zinc hydroaspartate (15 mg/kg/day) on exploratory activity in CUS model in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Squares traversed (mean ± SEM)</th>
<th>Rearings (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>19.4 ± 2.8</td>
<td>4.25 ± 0.6</td>
</tr>
<tr>
<td>Stress</td>
<td>23.5 ± 3.2</td>
<td>5.3 ± 0.65</td>
</tr>
<tr>
<td>IMI 5</td>
<td>21.7 ± 2.9</td>
<td>4.6 ± 0.8</td>
</tr>
<tr>
<td>IMI 5 + Zinc hydroaspartate 15</td>
<td>22.3 ± 3.1</td>
<td>3.9 ± 1.1</td>
</tr>
<tr>
<td>Stress + IMI 5</td>
<td>23.4 ± 3.2</td>
<td>4.25 ± 0.69</td>
</tr>
<tr>
<td>Stress + IMI 5 + Zinc</td>
<td>18.8 ± 4.3</td>
<td>3.81 ± 0.97</td>
</tr>
</tbody>
</table>

IMI (5 mg/kg/day) and zinc hydroaspartate (Zn) were administered ip, once daily, for 14 days. IMI was given 1 h before every stress session and Zn 1 h before IMI administration. The data are expressed as the means ± SEM of 12 rats per group. *ANOVA, F(5,66) = 0.3700, p = 0.8 NS; **ANOVA, F(5,66) = 0.4383, p = 0.6 NS
in normal nor in chronically stressed rats. Co-administration of imipramine (5 mg/kg/day) and zinc hydroaspartate (15 mg/kg/day) for 14 days did not influence the fighting behavior of control (unstressed) rats but prevented the decrease in fighting attacks in rats subjected to CUS regimen (Fig. 3).

Imipramine (5 mg/kg/day) administered alone or parallelly with zinc hydroaspartate (15 mg/kg/day) changed the exploratory activity neither in normal (unstressed) nor in chronically stressed rats (Tab. 2).

Discussion

The present study revealed that the rats subjected to CUS procedure exhibited the decrease in the foot-shock-induced fighting behavior by about 75% when observed 48 h after the last session of the chronic stress. The results obtained in the open field test indicated that the motor function of rats was fully efficient after chronic stress, so the deficit in fighting behavior did not depend on locomotor activity reduction. The results are consistent with our previous studies [23, 25, 38].

We have shown also that prolonged (but not acute) treatment with zinc hydroaspartate (30 mg/kg/day) given alone, prevented the deficit of fighting behavior in chronically stressed rats. Neither prolonged treatment with imipramine (5 mg/kg/day) nor zinc hydroaspartate at (15 mg/kg/day) administered alone changed the intensity of fighting behavior in chronically stressed rats. However, after prolonged co-administration of both drugs at the doses ineffective, when given alone, the deficit of fighting behavior was not observed.

The therapeutic effect of conventional antidepressants visible after 2–3 weeks of therapy is commonly known. Likewise, results of our study indicate that also chronic zinc administration prevented the behavioral depression in rats.

The antidepressant-like effect of zinc has been revealed by other authors following acute, subchronic as well as chronic zinc administration in the tests used to screen new antidepressants and in the models of depression [5, 6, 19, 21, 28]. The findings of Nowak et al. [21] have shown the antidepressant-like effect of zinc in olfactory bulbectomy model of depression in rats. Kroczyk et al. [6, 7] have demonstrated that zinc reduces immobility time in forced swim test in rodents and enhances the effect of imipramine in this test. The reduced immobility times in the forced swim test or the tail suspension test in mice after zinc administration and the additive effects of zinc plus imipramine (forced swim test) were reported also by Rosa et al. [28].

Chronic treatment with zinc reversed the reduction in the consumption of 1% sucrose solution caused by chronic mild stress (CMS) a widely accepted model of depression [19].

The results obtained in our study conform to the above-mentioned experimental findings and clearly indicate antidepressant-like effect of zinc salts.

Zinc is a signaling molecule present at high concentrations in the CNS which produces wide variety of neuromodulatory effects. It is an endogenous potent inhibitor of the NMDA receptor complex [2, 18]. Skolnick et al. [31–33] suggested that the inhibition of NMDA receptor complex played a significant role in the antidepressant treatment. Clinically used antidepressant drugs impair the function of the NMDA receptor [31]. On the other hand, functional antagonists of the NMDA receptor exhibit an antidepressant-like effect in animal models of depression [8, 24, 26, 36].

It has been suggested that antidepressant drugs might inhibit function of NMDA receptor by increase in brain-derived neurotrophic factor (BDNF) activity [13]. Likewise, prolonged treatment with zinc increases level of BDNF mRNA level in the rat cerebral cortex [15], so both antidepressants and zinc salts share the same effect.

The modulatory role of zinc in depression was also suggested by some clinical studies. It has been shown that in depressed patients the serum concentration of zinc was reduced but it was normalized after the effective antidepressant treatment [9, 10]. Moreover, the data obtained by Nowak et al. [20] have demonstrated the reduction in the potency of zinc to inhibit NMDA receptor activity in the hippocampus of suicide victims.

Recently, the clinical studies have shown the benefit of zinc supplementation in antidepressant therapy of patients with unipolar depression [17].

Experimental results have revealed the decreased zinc serum concentration in our model of CUS-induced behavioral depression in rats [22]. Moreover, chronic treatment with imipramine or citalopram or chronic electroconvulsive shock raises hippocampal and serum zinc concentration in rats [16].
All these studies, therefore, reveal that zinc can play a critical role in the modulation of pathophysiology and therapy of depression.

The results of the present study indicate that zinc like antidepressant drugs, protects the rats against the CUS-induced behavioral depression. Moreover, our findings confirm suggestions of other authors that zinc supplementation can potentiate the antidepressant effect of imipramine.

References:


Received: