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Original article

# Evidence for the involvement of heme oxygenase-1 in the antidepressant-like effect of zinc

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ABSTRACT

*Background:* Considering that heme oxygenase-1 (HO-1) and the brain-derived neurotrophic factor (BDNF)-mediated pathway are involved in the pathophysiology of depression and that zinc has been shown to exert beneficial effects in the management of depression, this study investigated the influence of these targets on the antidepressant-like effect of zinc.
*Methods:* Mice were treated with sub-effective or effective doses of zinc chloride ($ZnCl_2$, 10 mg/kg, *po*), and 45 min later, they received intracerebroventricular (*icv*) injections of sub-effective doses of either zinc protoporphyrin IX (ZnPP, 10 μg/mouse, HO-1 inhibitor), cobalt protoporphyrin IX (CoPP, 0.01 μg/mouse, HO-1 inducer) or K-252a (1 μg/mouse, TrkB receptor antagonist). Immobility time and locomotor activity were evaluated through the tail suspension test (TST) and open-field test (OFT), respectively. HO-1 immunocontents were evaluated in the prefrontal cortex and hippocampus 60 min after $ZnCl_2$ (10 mg/kg, *po*) treatment.
*Results:* The antidepressant-like effect of $ZnCl_2$ was prevented by the treatment with ZnPP and K-252a. Furthermore, sub-effective doses of CoPP and $ZnCl_2$ produced a synergistic antidepressant-like effect in the TST. None of the treatments altered locomotor activity. $ZnCl_2$ administration increased HO-1 immunocontents only in the prefrontal cortex.
*Conclusions:* The results indicate that the antidepressant-like effect of $ZnCl_2$ in the TST may depend on the induction of HO-1, and activation of TrkB receptor.


## Introduction

Depression is an incapacitating psychiatric disorder related to decreases in monoamines [1] and neurotrophic factors, particularly the brain-derived neurotrophic factor (BDNF) [2], as well as increased inflammation and oxidative stress [3], and alterations in neuronal intracellular signaling pathways [4].

The erythroid 2-derived-like 2 factor (Nrf2)/heme oxygenase-1 (HO-1)-mediated pathway has been suggested to be implicated in the pathophysiology of depression [5]. The Nrf2 transcription factor induces the expression of cytoprotective and detoxification genes [6]. Heme oxygenase is one of the proteins translated as a consequence of the activation of this factor [7]. There are the two catalytically active forms of this enzyme, an inducible form (HO-1) and a constitutive form (HO-2) [8]. HO-1 is the rate-limiting enzyme involved in the degradation of heme to generate carbon monoxide, iron, and biliverdin-IX alpha [7]. Some signaling pathways can lead to the activation of Nrf2, including extracellular-regulated protein kinase [9], phosphoinositide 3-kinase [10], protein kinase B [11], and protein kinase C [12]. Noteworthy, these signaling pathways are related to the increased production of BDNF [13].

BDNF is a neurotrophin well-known to be involved in the pathophysiology of depression [14]. Chronic treatments with antidepressants lead to increased hippocampal BDNF levels [15]. However, the actions of neurotrophins depend on transmembrane-receptor signaling, particularly the tropomyosin-related receptor kinase B (TrkB) receptors [16].

*Abbreviations:* BDNF, brain-derived neurotrophic factor; CoPP, cobalt protoporphyrin IX; HO-1, heme oxygenase-1; icv, intracerebroventricular; Nrf2, erythroid 2-derived-like 2 factor; OFT, open-field test; po, per os; SDS, sodium dodecyl sulfate; SEM, standard error of the mean; TRIS2, amino-2-hydroxymethylpropane-13-diol; TrkB, tropomyosin-related receptor kinase B; TST, tail suspension test; $ZnCl_2$, zinc chloride; ZnPP, zinc protoporphyrin IX.

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Taking into account that antidepressant treatment has some limitations [17], nutraceuticals, including zinc, have been proposed as strategies to improve the treatment of depression [18]. Several preclinical [19,20] and clinical [21,22] studies have underscored the role of zinc in depression. Although much still remains to be uncovered on this issue, the reduction of oxidative stress [23,24], and the activation of signaling pathways related to increased BDNF levels appears to be crucial for the antidepressant-like effect of zinc [25,26]. In addition, zinc may cause Nrf2-dependent stimulation of glutathione synthesis in endothelial cells [27]. Therefore, this study was aimed at investigating whether HO-1 and BDNF receptors are involved in the antidepressant-like effect of zinc. For this purpose, we investigated the influence of administering HO-1 inhibitor zinc protoporphyrin IX, HO-1 inducer cobalt protoporphyrin IX, and TrkB receptor antagonist K-252a on the effect elicited by zinc in the tail suspension test (TST). This experimental protocol is efficient to allow these drugs to reach brain structures rapidly [28], following zinc administration by oral route.

## Material and methods

### *Animals*

Female Swiss mice (30–40 g, 55–60 days old) were maintained at 20–22 °C with free access to water and food, under a 12:12 h light:dark cycle (lights on at 7.00 a.m.). All behavioral tests were carried out between 9.00 a.m. and 04.00 p.m. Animals were acclimatized to the experimental room for at least 12 h before testing. The animals were used according to the NIH Guide for the Care and Use of Laboratory Animals. All the experiments were approved by the Ethics Committee of the Institution and all efforts were made to minimize animal suffering.

### *Drugs and treatment*

Zinc chloride ($ZnCl_2$), cobalt protoporphyrin IX (CoPP), zinc protoporphyrin IX (ZnPP), and K-252a obtained from Sigma Chemical Co. (St. Louis, USA) were freshly prepared before administration. $ZnCl_2$ was dissolved in distilled water and was given orally by gavage in a volume of 10 ml/kg body weight. ZnPP and CoPP were dissolved in a final concentration of 0.1% dimethyl sulfoxide, and K-252a in a final concentration of 30% of this solvent. They were administered by intracerebroventricular (*icv*) route, in a volume of 5 μl per mouse, as described previously [29]. Control groups of mice treated with correspondent vehicle were also assessed simultaneously.

### *Pharmacological treatment*

To investigate the involvement of HO-1 in the antidepressant-like effect of $ZnCl_2$ in the TST, mice received an active dose of $ZnCl_2$ (10 mg/kg) or vehicle and after 45 min they were treated with the HO-1 inhibitor ZnPP (10 μg/mouse) or vehicle. In another set of experiments, mice were treated with a sub-effective dose of $ZnCl_2$ (1 mg/kg) or vehicle and 45 min after, received a sub-effective dose of the HO-1 inducer CoPP (0.01 μg/mouse) or vehicle. Animals were tested in the TST 15 min after *icv* administrations.

To test the hypothesis that the antidepressant-like effect of zinc in the TST is dependent on the activation of TrkB receptors, mice were pretreated with an effective dose of $ZnCl_2$ (10 mg/kg) or vehicle and 45 min later, they were administered with the TrkB receptor antagonist K-252a (1 μg/mouse) or vehicle. A further 15 min were allowed to elapse before the animals were tested in the TST.

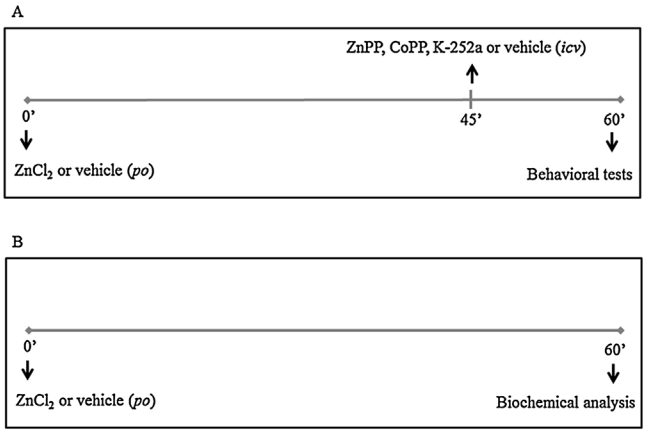


**Fig. 1.** Diagram of the experimental protocol. For the behavioral tests (panel A), Swiss female mice received active or sub-effective doses of $ZnCl_2$ (10 or 1 mg/kg, *po*) or vehicle and after 45 min they were treated with the HO-1 inhibitor ZnPP (10 μg/mouse, *icv*), HO-1 inducer CoPP (0.01 μg/mouse), TrkB receptor antagonist K-252a (1 μg/mouse, *icv*) or vehicle. After 15 min, the animals were tested in the TST and open field test (OFT). For the biochemical analysis (panel B), independent groups of mice received $ZnCl_2$ (10 mg/kg, *po*) or vehicle. After 60 min, prefrontal cortices and hippocampi were used for Western blotting analyses.

The experimental protocols used for behavioral tests are given in Fig. 1A.

The doses of $ZnCl_2$ used were chosen based on previous data that established sub-effective and effective doses in the TST [19,29]. The doses of ZnPP, CoPP and K-252a were chosen based on previous results that show their effectiveness as pharmacological tools [30–32].

### *Behavioral tests*

#### *TST*

Total immobility time induced by tail suspension was registered according to the method described by Steru et al. [33] in a 6-min period. Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Mice were considered immobile only when they hung passively and were completely motionless, and decreased immobility time was considered as antidepressant-like effect [29].

#### *Open-field test (OFT)*

After the TST, the same mice were subjected to OFT in a wooden box (40 × 60 × 50 cm high) with the floor of the arena divided into 12 equal squares [30]. The number of squares crossed with all four paws (crossings) was manually counted in a 6 min session.

### *Western blotting*

Independent groups of mice were used for biochemical analysis. Animals received (*po*) vehicle or $ZnCl_2$ (10 mg/kg). After 60 min, the animals were decapitated for biochemical analysis, as shown in Fig. 1B.

After decapitation, prefrontal cortices and hippocampi were quickly dissected (4 °C), placed in liquid nitrogen and stored at −80 °C until use. Samples were homogenized as previously described. [34]. Protein content was estimated using bovine serum albumin as a standard [35].

Samples (60 μg protein/track) were electrophoresed in 10% SDS-PAGE minigels and transferred to nitrocellulose membranes using a semi-dry blotting apparatus (1.2 mA/$cm^2$; 1.5 h) [34]. To verify transfer efficiency, gels were stained with Coomassie blue