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Zinc and imipramine reverse the depression-like behavior in mice induced by chronic restraint stress

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ABSTRACT

Depression is a common psychopathological disorders. Studies of depression have indicated that zinc play a role in the depression pathophysiology and treatment. In present study, we examined the effects of zinc and imipramine supplement alone or combination of zinc and imipramine in mice induced by chronic restraint stress (CRS). Moreover, the possible roles of zinc receptor (G protein-coupled receptor 39, GPR39)-related pathway was investigated. Decreased weight and increased corticosterone (CORT) were observed after 3 weeks CRS exposure. It was shown that CRS induced lower serum zinc, higher hippocampal zinc, increased immobility time in tail suspension test and decreased movement distance in spontaneous activity test, which could be normalized by zinc (30 mg/kg) and imipramine (20 mg/kg) supplement alone and combination of zinc (15 mg/kg) and imipramine (5 mg/kg) for 3 weeks after CRS exposure. Moreover, the changes in mRNA expressions of GPR39, cAMP-response element binding protein (CREB), brain-derived neurotropic factor (BDNF) and n-methytl-D-aspartate receptors (NMDAR) could be reversed by the same treatment mentioned above. These results suggested that zinc dyshomeostasis in serum and hippocampus and depression-like behavior in CRS exposure animals observed in present study could be normalized by zinc and imipramine. The combination of zinc and imipramine in low dose has synergetic effects. The possible mechanism might be correlated to GPR39 receptor-related pathway.

1. Introduction

Depression is a mental disorder with high morbidity and mortality; more than 350 million people of all ages worldwide suffer from depression. Depression is a major risk factor for self-inflicted injury, at its worst, can lead to suicide. It has been estimated that suicide is responsible for 1 million deaths every year (Belzung et al., 2015; Bschor et al., 2014; Kuo et al., 2015). Even though there are treatments for depression, pharmacotherapy is usually costly. Medications have the potential for adverse side effects and a significant proportion of people fail to achieve a reduction in their depressive symptoms (Bschor et al., 2014; Mauskopf et al., 2009). Hence, there is a need to investigate alternative treatments and prevention strategies.

Stress has attracted much attention because its significant negative effects can increase the risk of various diseases, including diabetes, cardiovascular neurodegenerative diseases, and aging (Hemmerle et al., 2014; Naji et al., 2015; Srivastava and Kumar, 2015). For many years, studies of depression have indicated that stress abnormalities are likely involved in the disease pathogenesis. Activation of the hypothalamic–pituitary–adrenal (HPA) axis because of various stress results in increased glucocorticoids secretion. However, repeated HPA axis activation can produce damaging physiological effects and exert a profound impact on brain function (McEwen, 2000). For example, repeated stress and HPA axis hyperactivation have been associated with the development of depression. Up to 50% depression patients showed a 24-h hypersecretion of cortisol, which could be normalized with antidepressant (Bosch et al., 2012). Nowadays, more and more studies support the fact that repeated corticosterone (CORT) injections can induce depression-like behavior (Iijima et al., 2010; Takeda et al., 2012; Ulloa et al., 2010).

Interestingly, zinc-deficient diet also can induce high level of serum CORT concentration in rats (Takeda et al., 2012). Zinc, as an essential trace element, is very important to organisms. Both clinical studies and animal experiments have demonstrated that zinc deficiency is connected to clinical depression and the depression-like behavior of animals (Młyniec et al., 2012; Młyniec and Nowak, 2012). There are strong evidences that depression is

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accompanied by lower serum zinc. The zinc dyshomeostasis could result in clinical depression, changes in behavior and mental functions (Młyniec et al., 2012; Młyniec and Nowak, 2012; Młyniec, 2015) The hippocampus has the most abundant zinc in the brain. Our previous studies showed that the depression-like behavior in psychological stress-treated rats might be correlated with decreased zinc contents and increased free zinc in hippocampus (Dou et al. 2014).

The antidepressant activity of zinc was demonstrated in both clinical and preclinical studies, even there were some inconsistent results. However, the roles of zinc in depression are still being debated. Therefore, in present study, we examined the effects of zinc supplement alone or co-supplement of zinc and imipramine in mice induced by chronic restraint stress (CRS). Moreover, the possible role of zinc receptor (G protein-coupled receptor 39, GPR39)-related pathway was investigated.

2. Materials and methods

2.1. Animals

Male ICR mice from Shanghai-BK Co., Ltd., Shanghai, China, eight weeks old, weighed 20 ± 2 g, one in a cage at a temperature of 25 ± 1 °C, a humidity of 55 ± 5% in a natural light/dark cycle, and free access to food and water. All the objects treated according to the international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals, and the experiments carried out under the supervision of the Committee of Experimental Animal Administration of the Second Military Medical University. All animals were fed with a commercially AIN-93G diet (SLACOM, Shanghai, China) and the content of zinc in the diet was 30 mg/kg. After an adaptive phase of 3 d, the mice were randomly divided into control and CRS groups. In CRS experiments, mice were restrained in polypropylene cylinder (3 cm inner diameter, with air vents at the nasal end of the cylinder and length adjusted for each animal) 3 h/d (8:00–11:00 am) for three weeks.

2.2. Drug treatment

Following 3 weeks of CRS exposure, the CRS animals were further divided into subgroups. Different doses imipramine (5, 20 mg/kg; Sigma, Shanghai, China) and $ZnSO_4$ (15, 30 mg/kg; Sigma, Shanghai, China) were administered intraperitoneally everyday after CRS for 3 weeks (Fig. 1). Imipramine was administered 1 h after $ZnSO_4$ supplement.

2.3. Tissue preparation

24 h after the last dose of zinc and imipramine treatment in every stage, the mice were deeply anesthetized by intraperitoneal injection of 7% chloral hydrate as soon as possible. Blood samples were collected from the heart followed by centrifuging at 3000*g* for 15 min, and the supernatants were obtained and stored at −80 °C for further determination. Then the mice were perfused through the left cardiac ventricle with ice-cold phosphate-buffered saline (pH 7.4). Then the hippocampus was quickly removed and snaps frozen in liquid nitrogen, then kept in a −80 °C freezer till use.

2.4. CORT measurement in serum

CORT in serum was analyzed using commercially available enzyme-linked immunosorbent assay kits suitable for rat (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions.

2.5. Tail suspension test (TST)

The mice used in our study were securely fastened with medical adhesive tape by the tip of the tail to a flat surface and suspended for 6 min approximately 30 cm below the surface. The total time of immobility was measured during the last 5 min of the testing session. Immobility was defined when the animals hung passively without limb movement and was scored manually.

2.6. Spontaneous activity test

The spontaneous activity is used to measure the behavior and locomotor activity of animals. The arena (50 cm × 50 cm × 70 cm) made of black plastic box, in which the subject had never been placed in before, illuminated with an infrared camera. Test of each animal in the arena lasting for 3 min was recorded by the camera and the image was transmitted to the computer. The Ethovision XT 4.0 (Noldus, Wageningen, Netherlands) software had been used to analysis the videos. The arena had been cleaned between tests.

2.7. Zinc assay

Each sample was weighed and wet-acid digested with a concentrated nitric/perchloric acid mixture (4:1 ratio) for 24 h. Zinc contents in the hippocampus were measured using a zinc flame atomic absorption spectrophotometer (AAS, Z-8100, Hitachi, Tokyo, Japan). The zinc concentration in 1 mL serum was also measured in the same manner. The zinc contents were expressed as micrograms of zinc per gram of wet tissue or nanograms of zinc per milliliter serum.

2.8. Real-time PCR

The Trizol reagent (Invitrogen, Carlsbad, USA) has been used to

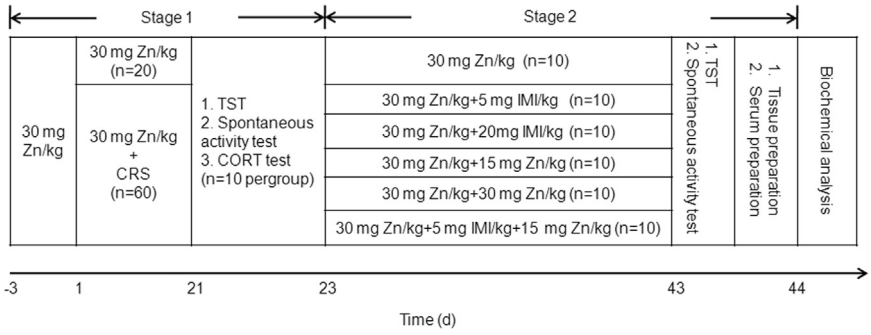

Fig. 1. Experimental paradigm.