

Roles of BDNF/TrkB Signaling Pathway in Anti-Depressant Effect of Vortioxetine

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Abstract: Objective To investigate the effects of vortioxetine on BDNF/TrkB signal pathway. Methods Kunming mice were randomized into control group and chronic unpredictable mild stress (CUMS) group. After establishment of depressive models verified by sucrose preference test, the mice in CUMS group were divided into model group and vortioxetine group. The antidepressant effect of vortioxetine was analyzed by Y-maze test, forced swim test and open field test. The expressions of brain-derived neurotrophic factor (BDNF) and TrkB were evaluated with Western blotting. Results Vortioxetine significantly shortened the immobility time of the depressive mice in forced swim test without affecting the locomotor activity of the mice in open fields, suggesting the antidepressant effect of against depression in mice. Vortioxetine significantly improved the depression-like behavior. Vortioxetine significantly increased the expression of BDNF in the hippocampus of the mice ($p < 0.01$). Conclusion Vortioxetine improves the behaviors of mice with depression possibly by affecting the BDNF/TrkB signal pathway.

Keywords: Vortioxetine, TrkB, Brain-Derived Neurotrophic Factor, Depression

1. Introduction

Depression has now developed into a global health problem that seriously affects the physical and mental health of people in all countries and in all social classes.[1] It is predicted that by 2020 it will rise to the second most devastating disease, which greatly aggravates the economic burden of the society.[2, 3] Currently clinically used in the treatment of depression are selective serotonin reuptake inhibitors (SSRIs), selective serotonin norepinephrine reuptake inhibitors (SNRIs) and so on. [4, 5] Studies have found that classic 5-HT drugs such as fluoxetine in addition to increase the role of 5-HT in the synaptic gap play an antidepressant effect can also repair the damaged BDNF/TrkB signaling pathway to promote neuronal growth and development to improve depression symptom.[6] BDNF plays an important role in the process of neuronal growth, survival and

differentiation.[7] Abnormal BDNF can cause depression-anxiety-like symptoms.[8] Vortioxetine is a new multi-modal anti-depressant drug used to treat depression in 2013 in Europe and the United States. At present, the antidepressant effect of vortioxetine is closely related to the 5-HT receptor and 5-HT transporter. Vortioxetine can exert antidepressant effect by regulating 5-HT system, but its downstream events are still unclear. It is unclear which signal pathways exert antidepressant effect. Studies have shown that depression in patients with plasma BDNF levels decreased; taking vortioxetine depression in patients with plasma BDNF levels were significantly higher.[9] The purpose of this study was to investigate whether vortioxetine may have an antidepressant effect on the BDNF/TrkB signaling pathway in a classic CUMS-induced depression animal model. This research will elucidate the mechanism of action of



vortioxetine from the perspective of molecular biology.

2. Animals

Adult male Kunming (Swiss) mice (18-22g, 4 weeks old) were obtained from the Laboratory Animal Centre, Qingdao Food and Drug Inspection Institute (Qingdao, China). During the experiment, the animals were kept in the SPF animal house of Qingdao University for a sufficient amount of standard feed and clean drinking water. Before the experiment, the animals were allowed to adapt to one week's time. During the adaptation period, the circadian rhythm was maintained. The room temperature was 23-25°C and the humidity was (55 ± 10) %.

3. Groups and Drug administration

According to the results of sucrose preference test, male Kunming mice were divided into 5 groups: normal group, CUMS model group, CUMS + vortioxetine group (5 mg/kg), CUMS + K252α group, CUMS + vortioxetine + K252α group. Mice in normal group and CUMS model group were given intragastric administration of distilled water, intraperitoneal injection of 0.9% saline for 30 min before intragastric administration, and intraperitoneal injection of 0.9% saline intraperitoneally for 30 min before intragastric administration. CUMS + K252α group, CUMS + vortioxetine + K252α group were given intragastric administration of distilled water and low dose of drugs, intraperitoneal injection of K252α (25 μg/kg) 30min before intragastric administration.

4. Experimental protocol

The CUMS model of mice was established by chronic unpredictable mild stimulation. The main stress factors were: fasting forbidden (12 h), restraint, empty bottle, day and night reversed, inclined cage, empty cage plus water, wet cage, foreign body stimulation. Mice are given 1 or 2 stimuli per day. Settings to avoid having an adaptive stress program in animals should be consistent with unpredictable behavior. The stimulus lasts for 4 weeks.

5. Behavioral evaluation

5.1 Open field experiments

The experiments were carried out in an open box of 100 cm × 100 cm × 42 cm. The open box was divided into four equal parts and the inner wall of the box was black. The mice were placed in the middle of the box using the video analysis software smart3.0 video analysis of mice in the middle and the edge of the area time, horizontal motion and vertical motion score, each mouse test time was 5min. After the experiment, all the boxes need to be wiped with 75% absolute ethanol. During the experiment, the dark ambient light is weak yellow incandescent light.

5.2 Sucrose preference test

The mice were all housed in a single cage during the sweet-water preference test. Before the sucrose preference test (SPT), the sugar-water adaptation was required. Each mouse was given 2 bottles of 0.1% sucrose for 24 h. The adaptation process changed the positions of the bottles every 12 h. After adapted to 24 h, mice were deprived of water and food 12 h, and then each cage of mice given pre-weighed 1 bottle of 0.1% sucrose water and 1 bottle of water for sugar preference test, this experiment main to test CUMS model loss of symptoms of pleasure. After weighing the test calculation drinking bottle sucrose consumption, water consumption, the preference index calculation sucrose: sucrose preference percentage (%) = sucrose consumption / total liquid consumption.

5.3 Forced swimming test

According to the method established by Prosolt [10] et al to conducted forced swimming test (FST). The mice were placed in forced swimming equipment (30 cm in height, 11 cm in diameter and 15 cm in depth) in a transparent plastic bucket at a water temperature of 22-25°C. Using smart3.0 image real-time monitoring and analysis system to automatically record and record the behavior of mice swimming in water. Before the end of adaptation time in the first 2 minutes, the system automatically records the immobility time of the mice at 4 minutes after the analysis. Mice floating in the water, limbs struggling or floating on the water as a

immobility criterion.

6. Western blotting

After the experiment, the mice were quickly decapitated, and the required hippocampus tissue were rapidly stripped from the brain on ice and immediately placed in liquid nitrogen tank. Subsequently, the hippocampus and prefrontal cortex were treated with protease inhibitor and phosphatase inhibitor tissue lysate homogenate, $12000 \times g$, $4^{\circ}C$, 10min after the supernatant. Protein content was determined using the BCA reagent protein concentration assay and 6x protein loading buffer was added at a 5: 1 ratio of protein sample solution to protein loading buffer (6x SDS-PAGE loading buffer). $95^{\circ}C$ boiled denatured 10min and then reserve. Treated protein samples were loaded on $50\mu g$ per well for polyacrylamide gel electrophoresis, and then transferred to PVDF membrane, blocked with 5% BSA for 2h and then transferred to $4^{\circ}C$ refrigerator to incubate primary antibody overnight. β -actin (1: 5000, proteintech), BDNF (1: 1000, abcam). Subsequently, the primary antibody-incubated PVDF membrane was washed with TBST, washed once every 10 minutes. After three washes, the secondary antibody was incubated for 2 h at room temperature (1: 10,000, Proteintech). After rinsing three times, ECL luminescence was added drop wise and developed in a dark room.

7. Data analysis

The experimental data were expressed as mean \pm SEM. Multiple samples need to be tested for homogeneity of homogeneity between groups. Tukey's post-hoc method is used if the variance is the same, and Dunnett's T 3 method is used if the samples are not homogeneous. $P < 0.05$ is considered as significant.

8. Results

8.1 Behavior

The results shown in Figure1, CUMS group compared with the normal control group, the degree of preference for sugar was significantly reduced ($p < 0.01$), indicating that the experiment model of depression in animal models of success. Compared with the CUMS

group, the preference of sugar and water in all the doses of vortioxetine increased, and the preference of sugar in the CUMS + vortioxetine group increased significantly ($p < 0.01$). Compared with CUMS group and CUMS + vortioxetine + K252a group, SPT in CUMS + vortioxetine group was significantly increased. There was no significant difference in SPT between CUMS + vortioxetine + K252a group, CUMS + K252a group and CUMS group.

The results shown in Figure 2, compared with the normal control group CUMS model mice forced swimming test immobility time was significantly longer, with statistical significance ($p < 0.001$). The CUMS + vortioxetine 5 mg/kg significantly shortened the immobility time in forced swimming test ($p < 0.05$), indicating that vortioxetine has antidepressant effect. Compared with CUMS group and CUMS + vortioxetine + K252a group, the immobility time in CUMS + vortioxetine group was significantly decreased. There was no significant difference immobility time between CUMS + vortioxetine + K252a group, CUMS + K252a group and CUMS group.

8.2 western blot

As shown in Figure 3, the BDNF protein levels in mice in the CUMS model group were significantly lower compared to the control group. Compared with CUMS group and CUMS + vortioxetine + K252a group, the expression of BDNF in CUMS + vortioxetine group was significantly increased. There was no significant difference in the expression of BDNF protein between CUMS + vortioxetine + K252a group, CUMS + K252a group and CUMS group.

9. Discussion

Vortioxetine is a novel antidepressant developed by Lundbeck and Takeda Pharmaceuticals for the treatment of major depression. Current studies on the pharmacological mechanism of vortioxetine have focused on the 5-HT receptor regulatory system and the serotonin transporter reuptake inhibitors. The mechanism of action of drugs and the body is very

complex, so the drug's use of its powerful pharmacological effects is not a simple mechanism of action. The time to market for vortioxetine is short and the number of people taking it is limited. Its potential mechanism of action is worth further investigation. At present, many studies on the pathogenesis of depression have shifted from traditional nor epinephrine and serotonin transport systems to neural signal pathways. The trial used a series of chronic unpredictable stimuli to establish a classic CUMS-induced depression mouse model.[11] The CUMS model is currently recognized as a chronic stress model similar to the occurrence of depression in humans. Drinking sucrose water is the indexes for mice that can make mice feel excited. The sugar-water preference experiment is mainly to test the experience of anhedonia.[12] The mice were born with exploration behavior. When the mice were depressed after being stimulated with CUMS, the mice lost interest in new things. The Y maze was mainly used to evaluate the exploratory behavior of mice. Forced swimming test is a behavioral despair test for evaluating mice's desire to survive.[13] The results of this experiment show that the CUMS + K252 α group, the CUMS + K252 α group and the CUMS group have a significantly lower preference for sugar water in the sugar water test, the immobility time in the FST increases significantly, and the residence time in the M-arm in the Y-maze. The CUMS + vortioxetine group was significantly increased, the immobility time in the FST experiment was significantly shortened, and the residence time in the M-arm was significantly longer in the Y-maze test. Vortioxetine can significantly improve the behavioral performance of mice, but the behavioral index of mice given CUMS + vortioxetine group and CUMS + vortioxetine + K252 α group at the same time did not improve. At the same time western blot showed that vortioxetine can significantly increase the BDNF protein expression level, while the BDNF level given to both CUMS + vortioxetine group and CUMS + K252 α group was still very low and there was no significant change. This indicates that the BDNF/TrkB

signaling pathway is the pharmacological mechanism of the antidepressant effect of vortioxetine. Some papers showed that BDNF/TrkB signal pathway play an important role in depression. [14] Studies have shown that BDNF levels in the brain of depressed patients are decreasing, and studies have also shown that BDNF levels in serum of patients receiving vortioxetine are significantly increased. BDNF/TrkB signaling pathway plays an important role in the development of depression.

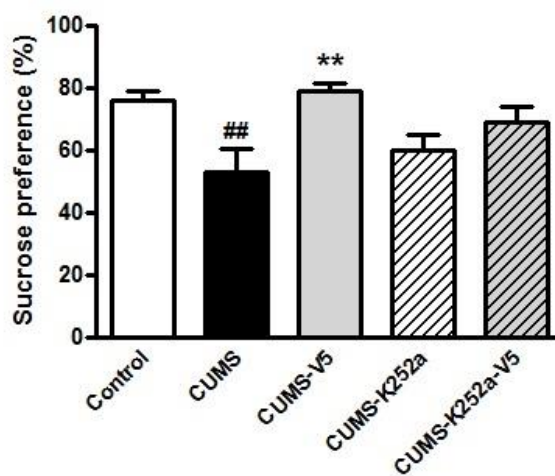


Figure 1. Effect of pretreatment with K252 α (25 μ g/kg) on vortioxetine (CUMS-V5, 5mg/kg)-induced sucrose preference in mice (n=7). ## $p < 0.01$ versus the control group, ** $p < 0.01$ versus the CUMS group.

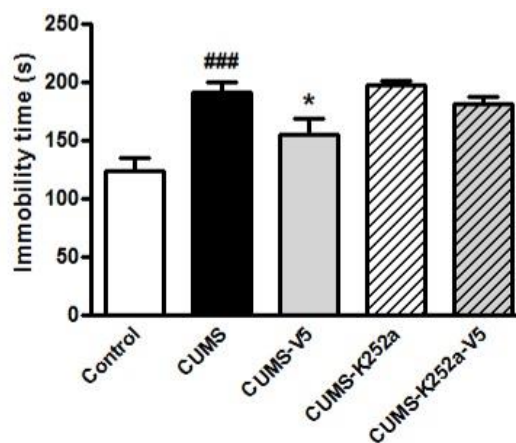


Figure 2. Effect of pretreatment with K252 α (25 μ g/kg) on vortioxetine (CUMS-V5, 5mg/kg)-induced immobility time in mice (n=7). ## $p < 0.01$ versus the control group, ** $p < 0.01$ versus the CUMS group.

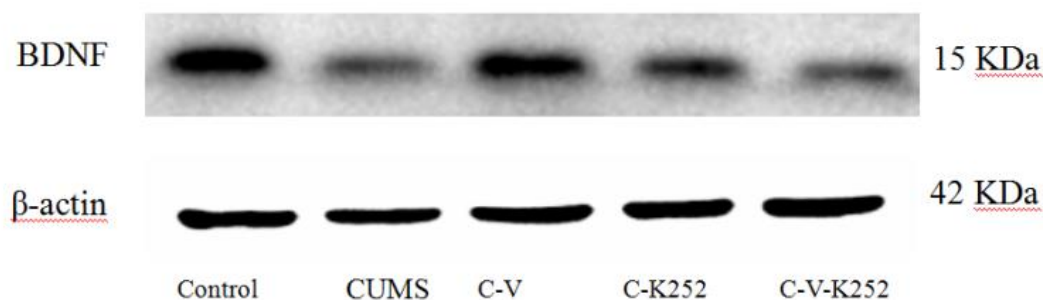


Figure 3. Treatment with vortioxetine can reverse BDNF protein expression induced by CUMS. Control represents protein expression in control mice; CUMS represent protein expression in CUMS mice; “ C-V ” represent protein expression in CUMS + vortioxetine (5 mg/kg) group mice. “ C-K252 ” represent protein expression in CUMS + K252 α group mice. “ C-V-K252 ” represents protein expression in CUMS + vortioxetine (5 mg/kg) + K252 α group mice.

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