

Original Research Article

Venlafaxine inhibits neuronal apoptosis in a depression rat model via ERK1/ERK2 pathway

Yanbin Hou¹, Zhongze Lou¹, Yunxin Ji¹, Liemin Ruan¹, He Gao^{2*}

¹Department of Psychosomatics, Ningbo First Hospital, ²Department of Psychiatry, Ningbo Kangning Hospital, Ningbo, China

*For correspondence: **Email:** 812478044@qq.com; **Tel:** +86-015958272157

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Abstract

Purpose: To investigate the effects and mechanism of action of venlafaxine on neuronal apoptosis of depressed rats.

Methods: Rats were randomly divided into normal control (NC) group, depressed rats (depression) group or venlafaxine-treatment group. Changes in body weight and sucrose preference ratio were recorded and behaviors in open field test (OFT) were observed in each group. Pathological changes in and the apoptosis rate of the cerebral neurons, and the activity of extracellular signal-regulated kinase 1 (ERK1)/ERK2 pathway were observed under a microscope.

Results: At weeks 2 and 4, the body weight and water consumption of rats in depression group dropped below those of rats in NC group. On the other hand, at week 2, the body weight and water consumption of rats in venlafaxine-treatment group were significantly higher than those of rats in depression group ($p < 0.05$). Besides, depression group had randomly arranged neuron cells and a thinner cell layer, while venlafaxine-treatment group had a relatively regular hippocampal neural cell arrangement and a thicker cell layer. Moreover, cell apoptosis rate was higher in depression group than in that NC group, and lower in venlafaxine-treatment group than that in depression group ($p < 0.05$). Finally, the protein expressions of phosphorylated (p)-ERK1 and p-ERK2 were significantly higher in depression group than those in NC group ($p < 0.05$), and distinctly lower in venlafaxine-treatment group than those in depression group ($p < 0.05$).

Conclusion: By suppressing the activity of ERK1/ERK2 pathway, venlafaxine relieves the symptoms of depression and repairs neuronal injuries in rats, thereby suppressing neuronal apoptosis. Thus, these findings provide a novel approach for the development of new antidepressants.

Keywords: Venlafaxine, ERK1/ERK2, Depression, Neuronal apoptosis, Hippocampus, Cerebral neurons

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INTRODUCTION

Although depression is listed as one of the major causes of disabilities worldwide [1,2], its mechanism and the exact drugs for its treatment remain unclear. The hippocampus is an

important region of the brain that regulates emotions and cognition [3]. It has been reported that neurochemical changes mainly occur in the hippocampus of patients with depression [4]. According to pathological studies, neuronal damage in the hippocampus leads to depression,

and antidepressants can reverse this damage. Hippocampal atrophy has been observed in patients with depression [5,6], and changes in the hippocampus may be triggered by neuronal injuries [7,8]. Venlafaxine is widely used to treat patients with depression, and possesses unique chemical characteristics. Compared with fluoxetine, it can inhibit the resorption of 5-hydroxytryptamine (5-HT) and noradrenaline synaptosome, and increase the level of cerebral 5-HT, thus facilitating signal transduction [9]. However, clinically, the antidepressant effect develops slowly within a few weeks of continuous medication [10], which suggests that the efficacy of antidepressants shall not be explained only by its effect on monoaminergic systems. According to recent studies, with the increase of monoamines, changes in gene expression are very important for the efficacy of antidepressant treatment.

Mitogen-activated protein kinases (MAPKs) play an important role in the process of nuclear signal transduction. They are widespread in the central nervous system, participating in the regulation of multiple biological processes such as cell proliferation, differentiation, apoptosis and synaptic plasticity [11,12]. Among them, the best-studied MAPKs are extracellular signal-regulated kinase 1 (ERK1) and ERK2. In recent years, the effect of the ERK pathway on the molecular mechanism of depression has been enhanced. Mounting evidence has manifested that ERK pathway may be involved in the neuronal modulation in depression.

Currently, venlafaxine is a widely used antidepressant, with obvious efficacy in improving the depressive symptoms. Despite its short half-life, a better understanding of its mechanism of action will greatly help with the research and development of novel antidepressants with similar structures.

EXPERIMENTAL

Materials

Sprague-Dawley (SD) rats were provided by Shanghai Laboratory Animal Center (Shanghai, China), while venlafaxine was purchased from Anhui Jingke Biology Co., Ltd. (Anqing, China). Antibodies against phosphorylated (p)-ERK1, p-ERK2, ERK1 and ERK2 were sourced from Abcam (Cambridge, MA, USA). Bicinchoninic acid (BCA) protein assay kits were provided by Beyotime (Shanghai, China), and apoptosis assay kits were supplied by Solarbio (Beijing, China).

Animal grouping

The rats were equally divided into normal control (NC) group, depression model (depression group) and venlafaxine-treatment group.

Treatments

No stimuli were administered on rats in NC group, while rats in depression group randomly received one of the following stimulus within 28 consecutive days, with no stimulus used continuously: 1) swimming in cold water (4 °C) for 5 min, 2) swimming in hot water (48 °C) for 5 min, 3) starving for 48 h, 4) fasting for 24 h, 5) day-night reversal for 24 h, 6) tail flick and shaking for 2 min, or 7) electric shock on the sole at 1 mA for 10 s with an interval of 1 min. After successfully establishing a depressed rat model, the rats in Venlafaxine-treatment group were intraperitoneally injected with venlafaxine (1 mg/100 g, 2 mg/mL), while those in depression group were injected with normal saline of the same volume. The behaviors of rats were observed in each group. This study was approved by the Animal Ethics Committee of Ningbo First Hospital Animal Center (approval no. NZLSC201903-0132), and all procedures were conducted in accordance with Animal Research: Reporting *In vivo* Experiments, Guidelines 2.0 [13].

Hematoxylin-eosin (HE) staining of brain tissues

After anesthesia, the brain tissues of rats were dissected, embedded in paraffin, and cut into 4 μm-thick sections. After treatment with gradient xylene and ethanol, HE staining was conducted. The severity of pathological changes in brain tissues was observed, and evaluated with a 0-3-point rating system, in which 0 = no change, 1 = slight change, 2 = moderate change and 3 = severe change.

Sucrose preference test

As previously stated, sucrose preference test was performed [14-16]. Reduction of sucrose intake can be used to simulate the core symptoms of anhedonia in patients with depression. After deprivation of water and food for 12 h (21:00 - 09:00), the rats were allowed to freely take 1 % sucrose solution or water in two separate bottles. After 30 min, the positions of the two bottles were swapped, and the rats were kept in separate cages. One hour later, the volumes of sucrose solution and water consumed were recorded. The sucrose preference ratio (SPR) was calculated according

to the formula: $SPR = \text{consumption of sucrose} / [\text{consumption of sucrose (mL)} + \text{consumption of water (mL)}]$.

Open field test (OFT)

The rats were placed in a central compartment in an open area (100 cm × 100 cm × 60 cm) of a quiet, soft-lighted room. The bottom of the central compartment was equally divided into 25 compartments. The time of stay in the central compartment, the frequencies of standing up and tidying up the hair and the number of compartments passed through were observed.

Western blotting

Cerebral tissues were physically ground, added with protein extraction reagent, made into a homogenate, and then centrifuged to extract proteins. Next, the proteins were separated by electrophoresis, transferred onto a membrane, and incubated with specific antibodies at 4°C overnight. After the membrane was washed, images were developed.

Determination of neuronal apoptosis

Brain tissues were physically ground into a single cell suspension, added with pre-cooled medium, and centrifuged at 1,000 rpm for 10 min. After the supernatant was discarded, the resulting cells were centrifuged again as above and washed twice to obtain a cerebral cell suspension. 1 mL of binding buffer was added to 1 mL of cell suspension. Then 200 µL of suspension was added with 10 µL of fluorescein isothiocyanate (FITC) and 10 µL of Propidium Iodide (PI) separately, incubated in the dark for 10 min, and then added with 400 µL of binding buffer before detection.

Statistical analysis

Statistical Product and Service Solutions (SPSS) 17.0 Software (SPSS Inc., Chicago, IL, USA) was utilized for significance analysis of data and plotting. *t*-test was used for comparison between two groups. $P < 0.05$ and $p < 0.01$ were considered statistically significant as appropriate.

RESULTS

Effect of venlafaxine treatment on body weight and sucrose water consumption of depressed rats

Anorexia is one of the clinical manifestations of depression. Changes in the body weight of the rats were recorded for four consecutive weeks.

Compared with those in NC group, the water consumption and body weight of rats in depression group began to decrease from the 2nd week, while the intake of food started to increase in Venlafaxine-treatment group ($p < 0.05$) (Figure 1 A). Then the SPRs were compared. It was found that from the 2nd week, depression group had a lower SPR than NC group, while venlafaxine-treatment group had a higher SPR than depression group ($p < 0.05$) (Figure 1 B).

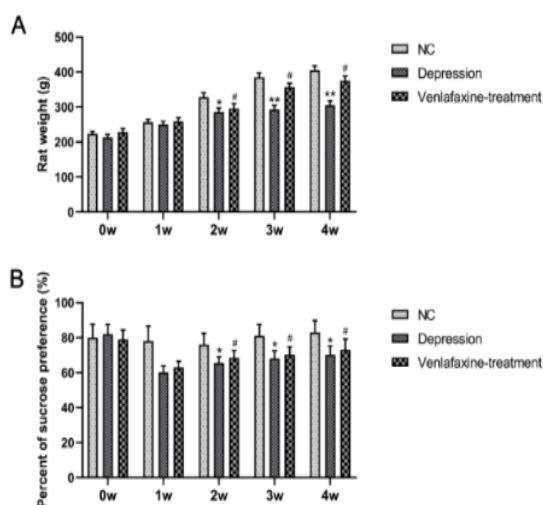


Figure 1: Changes in body weight and sucrose water consumption of depressed rats before and after Venlafaxine treatment. (A) Effect of venlafaxine on body weight of rats Compared with that of rats in NC group, the body weight of rats began to decline in depression group, but started to rise in venlafaxine-treatment group from the 2nd week ($p < 0.05$). (B) Changes in SPR of each group of rats From the 2nd week, the rats in depression group had a lower SPR than those in NC group, while the SPR of rats in venlafaxine treatment group was higher than that in depression group ($p < 0.05$). * $P < 0.05$ vs. NC group, and # $p < 0.05$ vs. depression group

Behavior of rats in OFT

The severity of depression and anxiety in rats was reflected by the observed changes in behaviors of rats in each group. Compared with those in NC group, the time of stay in the central compartment was extended markedly, and the frequencies of standing up, tidying up the hair and passing through compartment in depression group distinctly reduced, while in venlafaxine treatment group, the time of stay in the central compartment was shortened, and the frequencies of standing up, tidying up the hair, and passing through compartment increased. The above results indicate that venlafaxine can relieve the symptoms of depression (Figure 2).

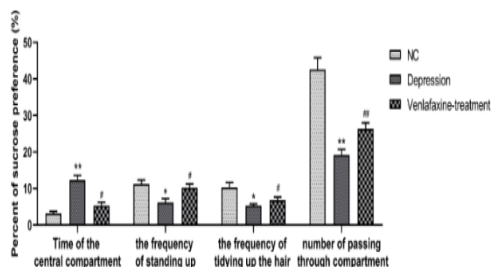


Figure 2: Changes in behavior of the three groups of rats during OFT. Compared with those in NC group, the time of stay in the central compartment was extended and the frequencies of standing up, tidying up the hair, and passing through compartment in depression group decreased, while in venlafaxine treatment group, the time of stay in the central compartment was shortened and the frequencies of standing up, tidying up the hair, and passing through compartment increased ($p < 0.05$) ($^*p < 0.05$ and $^*p < 0.01$ vs. NC group, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. depression group)

Pathological changes in hippocampal pyramidal cells of rats

To evaluate the changes in cerebral neuronal injuries, the hippocampus was chosen for HE staining. Under a microscope, it was found that the rats in NC group had a thicker hippocampal pyramidal cellular layer, with regular cellular arrangement, while those in depression group had a thin hippocampal pyramidal cellular layer, with fewer and irregularly arranged cells, severe pyramidal cell apoptosis and damage to the hippocampus. In contrast, the rats in venlafaxine-treatment group had a thicker layer of hippocampal pyramidal cells and narrowed intercellular space, implying that venlafaxine repaired the damage to the hippocampus to some extent (Figure 3).

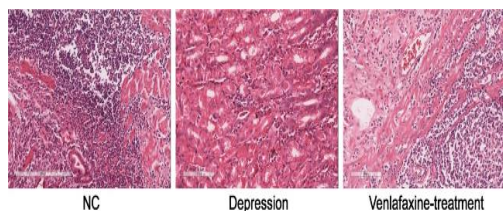


Figure 3: Pathological changes in hippocampal pyramidal cells under a microscope (magnification: 400 ×). NC group had a thicker layer of pyramidal cells and a regular cell arrangement. The rats in depression group had a thinner layer of pyramidal cells, with irregular cell arrangement, incomplete structure and increased cell apoptosis. Compared with those before treatment, the rats in venlafaxine-treatment group had a more regular cell arrangement, complete cell morphology and a declined cell apoptosis rate

Changes in neuronal apoptosis

The results of flow cytometry indicated that venlafaxine remarkably suppressed the apoptosis of hippocampal cells. Compared with that of rats in NC group, the cell apoptosis rate in rats in Depression group was significantly elevated ($p < 0.05$), while after venlafaxine treatment, it declined significantly ($p < 0.05$), suggesting that venlafaxine reduces neuronal injuries (Figure 4).

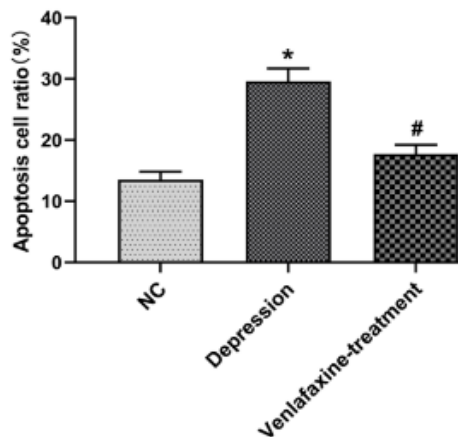


Figure 4: Changes in neuronal apoptosis in each group of rats. The cell apoptosis rate of rats in depression group was remarkably higher than that in venlafaxine-treatment group and NC group. ($^*p < 0.05$ vs. NC group, and $^{\#}p < 0.05$ vs. depression group)

Venlafaxine inhibited the activity of ERK1/ERK2 pathway in brain tissues

The role of ERK pathway in the molecular mechanism of depression is increasing, and mounting evidence has shown that ERK pathway may be involved in the modulation of neurons in depression. To investigate the mechanism of action of venlafaxine, the changes in the activity of ERK1/ERK2 pathway were verified. It was revealed that compared with those in NC group, the protein expressions of p-ERK1 and p-ERK2 rose remarkably in rats in depression group, while there were no differences in the protein expressions of ERK1 and ERK2 between the two groups of rats. In addition, the protein expressions of p-ERK1 and p-ERK2 of rats in venlafaxine-treatment group were lower than those in Depression group. Therefore, venlafaxine alleviates the symptoms of depression by regulating the activity of ERK1/ERK2 pathway (Figure 5).

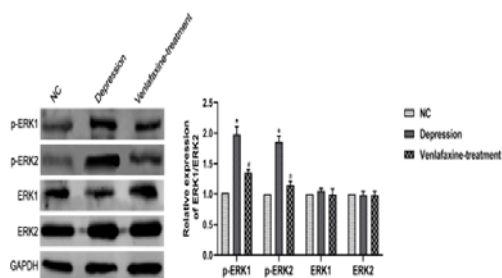


Figure 5: Changes in the activity of the ERK1/ERK2 pathway in the cerebral tissue of each group of rats. Compared with those in NC group, the expressions of p-ERK1/ERK1 and p-ERK2/ERK2 were elevated in rats in depression group ($p < 0.05$). The expressions of p-ERK1/ERK1 and p-ERK2/ERK2 were higher in venlafaxine-treatment group than those in Depression group ($p < 0.05$). (* $p < 0.05$ vs. NC group, and # $p < 0.05$ vs. depression group)

DISCUSSION

Depression is the most important causative factor of non-fatal health loss globally and one of the most common severe emotional disorders [3,17]. Venlafaxine is a dual reuptake inhibitor of 5-hydroxytryptamine and noradrenaline, which improves the activity of some neurotransmitters. Besides, it is associated with neuronal regeneration and involved in neuronal apoptosis.

In the present study, after stimulation, the rats exhibited such behavioral changes as loss of interest, a reduction in tolerance to activities, changes in sleep patterns and body weight loss. In most research, SPR is used as an effective parameter to evaluate the anhedonia in depression models. In this study, the results indicated that after administration of chronic mild stress, the SPR declined distinctly from the 2nd week to the 4th week, which was consistent with the results of the previous study of stress-induced depression model. After taking venlafaxine, the rats in Depression group had an increased SPR, implying that venlafaxine can alleviate the symptoms of depression.

The OFT may reflect the degrees of depression and anxiety of depressed patients. It can also be used to observe the exploratory abilities, emotional state and tolerance to activities of patients. Specifically, the distance of horizontal movement can reflect the endurance for movement and mobility, and the distances of vertical movement and central movement can reflect the exploratory ability and degree of anxiety in a new environment. In the OFT, the rats in depression group stayed longer in the central compartment, and the frequencies of standing up, tidying up the hair, and passing

through compartment decreased. After venlafaxine treatment, the time of stay in the central compartment was shortened markedly, and the frequencies of standing up, tidying up the hair, and passing through compartment increased. It has been found in most OFTs that chronic mild stress can reduce the mobility and exploratory ability of rats. However, it was also revealed in some other OFTs that the mobility and exploratory ability of rats increase under chronic mild stress, while there are no obvious changes in the overall mobility. Such conflicting results suggest that the behavioral changes of rats in the OFTs are not always the same. It is consistent with clinical findings that some patients with depression have decreased spontaneous activities, while the others have increased spontaneous activity.

Several studies have reported that there is hippocampal atrophy in depressed patients as well as in depression model animals. Antidepressants can ameliorate the symptoms by increasing neural regeneration and restoring the volume of damaged hippocampus. Therefore, most researchers think that the occurrence of depression may be associated with the abnormal regeneration of hippocampal neurons, indicating that the neural regeneration of hippocampus occurs in all life cycles. In this study, after venlafaxine treatment, it was observed under a microscope that hippocampal cells had morphological changes, the cell layer became thicker, and the cell arrangement was more regular than that before treatment. Moreover, the results of flow cytometry revealed that the cell apoptosis rate was significantly lower than that before treatment.

The role of ERK pathway in the molecular mechanism of depression is increasingly important, and there is mounting evidence that ERK pathway may be involved in the modulation of neurons in depression [18]. It was found in the present study that the protein expressions of p-ERK1 and p-ERK2 were significantly higher in rats in depression group than those in NC group, and their protein expressions decreased after venlafaxine treatment, in consistency with the hypothesis that ERK pathway is hyperstimulated in depression [19]. In addition, depression-like behaviors induced by stress is associated with the increase in hippocampal p-ERK1. Long-term desipramine therapy can prevent the depression-like behaviors and the elevation in p-ERK1, and intrahippocampal injection of U0126, an inhibitor of MEK, can have antidepressant effects [20,21], suggesting that blocking the signaling pathway is one of the mechanisms by which the drugs exert antidepressant effects.

CONCLUSION

The findings of this study demonstrate that by suppressing the activity of ERK1/ERK2 pathway, venlafaxine relieves the symptoms of depression and repairs neuronal injuries in rats, thereby suppressing neuronal apoptosis. Thus, these findings provide a novel strategy for the development of new antidepressants.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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