## Original Research Article

# Captopril reverses chronic unpredictable mild stress-induced depression-like behavior in rats via bradykinin-B2r signaling pathway

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## Abstract

*Purpose:* To investigate the effect of captopril on chronic unpredictable mild stress (CUMS)-induced depression-like behavior in mice, and the involvement of the bradykinin-B2r signaling pathway in the process.
*Methods:* Sixty healthy male C57BL/6J mice were assigned to control, model and high-, medium- and low-dose captopril groups and given the drug at doses of 9, 18 and 36 mg/kg, respectively. Open field and elevated cross maze tests were carried out, and escape latency in Morris water maze test was also test. The expressions of bradykinin B2R signal pathway proteins were assayed.
*Results:* Open arm residence time and open arm entry times were significantly higher in captopril-exposed mice than in model mice, while 5-day escape latency values were significantly less in captopril-treated mice than in model group (p < 0.05). Protein expressions of B2R, bpnf and Cdc42 in captopril groups were significantly higher than those in model group (p < 0.05).
*Conclusion:* Captopril mitigates CUMS-mediated depression-like disease in mice by regulating bradykinin B2R signal pathway. Therefore, captopril may play an antidepressant role by activating the expressions of B2R, bpnf and Cdc42.

*Keywords:* Captopril, Bradykinin-B2r, Chronic unpredictable mild stress, Depression

## INTRODUCTION

Depression is an affective mental disorder with low mood as the main symptom, in addition to mood and cognitive disorders of varying degrees [1]. A survey has revealed that the incidence of depression is increasing year by year, with serious physical and psychological consequences on the affected persons [2]. People with depression often exhibit persistent low mood and suicidal tendencies, and depression is a major inducer of suicide [3]. Depression is an emotional disorder caused by multiple factors. In clinical practice, physical diseases such as pain, malignant tumor and cardiovascular disease may be accompanied by depression [4]. Studies have found that depression-like symptoms are most common in hypertensive patients [5].

Captopril is an angiotensin-converting enzyme inhibitor (ACEI). It inhibits the transformation of Angiotensin 1 (Ang 1) and reduces the level of Ang 11, thereby producing a hypotensive effect [6]. Studies outside China have shown that when patients receive single antihypertensive drug therapy, the use of ACEI drugs reduced the effect on patients with affective disorders, while commonly used β -blockers or calcium channel blockers increase the risk in patients with the disease [7]. Studies have shown that ACEI degrades bradykinin (BK) by inhibiting the cleavage of Ang 1, resulting in the accumulation of bradykinin receptor 2 (B2R) signaling pathway [8]. However, there are limited studies on CUMS-induced depression-like behavior in mice. Therefore, the purpose of the present research was to study the impact of captopril on CUMS-induced depression-like behavior in mice, and the role of the bradykinin-B2R signaling pathway in the process.

# EXPERIMENTAL

## Animals

Sixty healthy male C57BL/6J mice of mean age $5.33 \pm 1.22$ weeks and mean body weight of $56.78 \pm 3.45$ g were used. The mice were reared in cleaned cages at ambient temperature of 22 - 25 ℃ and ambient humidity of 55 - 60 %, and kept in an environment with 12-h day/12-h night cycle using an automatic lighting system; the animals were ventilated regularly. There were 3 - 5 mice/cage, and the animals were fed adaptively for 7 days and given unrestricted supply of feed and water. Approval for this study was received from the Animal Ethical Authority of Tianjin Anding Psychiatric Hospital (approval no. 20220122), and the study was performed in line with the guidelines of "Principles of Laboratory Animal Care" [9].

## Instruments and reagents

The instruments used, and sources were: Electronic balance (Shandong Boyu Medical Instrument Co. Ltd), cryogenic slicer (Jinhua Huiyou Instrument Equipment Co. Ltd.), low-speed, high-temperature centrifuge (Shanghai Beckman Coulter International Trade Co. Ltd), small animal brain stereolocator (Shenzhen Rayward Life Technology Co. Ltd), automatic biological tissue slicer (Germany LLS ROWIAK Company), and pipette gun (Mettler Toledo International Co. Ltd).

The main experimental reagents were PBS solution (Thermo Fisher Technologies Co. Ltd.), and Mouse Bradykinin ELISA kit (Shanghai Enzyme-linked Biotechnology Co. Ltd.).

## Establishment of mouse model of CUMS and animal grouping

A mouse model of chronic unpredictable mild stress (CUMS) was established, which is a universally accepted animal model of depression. After 7 days of adaptive feeding, the mice were transferred to a single cage and given repeated mild stress (reversal of day and night cycle, bondage, high temperature, cold, electric shock to the plantar, fasting and water deprivation, swimming in ice-cold water, tail clipping, and frequency flash source stimulation) continuously for 35 days. The mice were subjected to two types of mild stress each day. During the treatment, the mild stress was randomly ranked to prevent the mice from anticipating what was coming next. Twelve of the C57 mice were assigned to control (n = 12) and CUMS groups (n = 48). The mice were given the 9 stresses indicated above every 7 days. Mice in the control group were not subjected to any stress treatment, but were subjected to behavioral examination. The CUMS mice comprised model, high-dose, medium-dose and low-dose captopril groups, each with 12 animals. Twenty-four hours (24 h) after establishment of the CUMS model, the mice were treated with drugs. Mice in control group and model group were given intraperitoneal injection of normal saline once a day, while those in 3 captopril treatments received intraperitoneal injections of captopril at doses of 9, 18 or 36 mg/kg.

## Sugar water preference test

Mice adapted to the process were trained to drink sugar water. Two water pipes (one containing sucrose solution and the other containing tap water) were placed in each cage, and the water pipes were changed every 24 h. *Ad libitum* provision for drinking water was made for 48 h, after which water was withdrawn. After 24 h of water deprivation, the two drinking pipes (sucrose solution and tap water) were placed again, and the mice were weighed only 2 h after drinking water (the mice were allowed to eat freely during the experiment). The degree of sugar water preference (SWP) was calculated using Eq 1, after weighing the two drinking pipes.

$$SWP\ (\%) = \{QS/(QT+QS)100\} \ \dots \dots \dots \ (1)$$

Where QS is the quantity of sucrose solution consumed and QT is the quantity of tap water consumed.

### Forced swimming test

The temperature of PVC transparent water tank was set at 19 - 23 ℃, and the duration of immobility of mouse in each group was observed and recorded in a 7-min forced swimming experiment after pre-swimming for 15 min.

### Morris water maze test

#### *Positioning navigation experiment*

Each mouse was placed head-down in water in 4 quadrants, and the escape latency was recorded, that is, the time taken by the mouse to find the scaffold within 120 sec. If the mouse could not locate the scaffold within 120 sec, the experimenter gave it traction and allowed it on the platform for 10 sec, and the escape incubation period was recorded as 2 min. The mice were kept warm twice a day for five days.

#### *Space exploration experiment*

On the sixth day of the experiment, the jumping platform was removed, and the mice were put into water in four quadrants. The time taken by each mouse to cross the original jumping platform position within 2 min was recorded.

### Elevated cross maze test

The elevated cross maze device was set, and the animals were positioned centrally on the device such that they faced the open wing. The population of animals going into the open wing and shut wings, and duration of stay in the open, were observed and recorded within 5 min.

### Open-field test

A box of dimensions 60 cm × 60 cm × 50 cm was used. The bottom of the box was divided into 9 small squares. The durations of time used by mouse for straddling, vertical standing and modification were counted and recorded in the box for 8 min.

### Bradykinase-linked analysis

Each mouse was given pentobarbital sodium injection *i.p.* (40 mg/kg) to ensure that the animals could breathe normally but were unconscious. Fully-anesthetized mice were decapitated and their brains were quickly exfoliated. After weighing the brain tissue, PBS solution was added to it and placed on ice. This was followed by centrifugation for 20 min to obtain a supernatant solution. The bradykinase

assay kit was rewarmed, and tissue lysis solution was added and mixed, followed by incubation, dilution and washing. Finally, light avoidance detection was conducted.

### Statistical analysis

The SPSS 20.0 statistical software was used. Measured data are expressed in the form of mean ± standard deviation (SD), and paired comparison was done using *t*-test. Statistical significance was set at $p < 0.05$.

## RESULTS

### Sugar water preference and immobile swimming time

Table 1 shows that the percentage preference for sugar water was less in model mice than in control mice. However, the percentage preference values for sugar water in the 3 captopril groups were significantly higher than that in model group ($p < 0.05$).

There was higher immobility time in the model group than in control group, but immobility times in the 3 captopril groups of mice were significantly less than that of control mice ($p < 0.05$).

### Open field text results

The data on Table 2 show that the times used for straddling, vertical standing and modification in model mice were significantly less than those in control mice. However, the times used for straddling, vertical standing and modification in the 3 captopril groups were significantly longer than the corresponding times in model mice.

### Results of elevated cross maze test

The open wing withholding time ratio and open wing entry time ratio in model mice were significantly higher than control mice values. Moreover, ratio of open wing stay time and open wing entry time were significantly higher in captopril-treated mice than in model mice (Table 3).

### Escape latency

Table 4 shows that the 5-day escape latency was significantly higher in model mice than in control mice. However, 5-day escape latency values in all captopril-exposed mice were significantly less than model mice value.

**Table 1**: Sugar water preference and immobility time during swimming in the 3 groups (n = 12)

| Group | Sugar water preference (%) | Swimming immobility time (s) |
|---|---|---|
| Control | 78.36±2.31 | 46.89±3.46 |
| Model | 41.35±2.45* | 81.33±5.61* |
| High-dose captopril | 72.58±2.11# | 55.78±4.33# |
| Medium-dose captopril | 63.78±1.68# | 62.35±4.08# |
| Low-dose captopril | 55.34±1.35# | 72.36±5.33# |

*#$P < 0.05$: *vs control; #vs model

**Table 2**: Open field test results amongst the 3 groups of mice (mean ± SD, n = 12)

| Group | Across the cell number | Number of vertical standing | Number of retouching |
|---|---|---|---|
| Control | 78.33±3.62 | 31.69±2.69 | 21.35±1.99 |
| Model | 41.68±3.15* | 9.36±1.39* | 8.48±1.08* |
| High-dose captopril | 70.39±3.98# | 24.33±2.11# | 18.37±1.57# |
| Medium-dose captopril | 65.77±3.26# | 18.34±2.01# | 13.48±1.22# |
| Low-dose captopril | 58.66±3.07# | 12.34±1.78# | 10.64±1.54# |

*#$P < 0.05$: *vs control; #vs model

**Table 3**: Results of elevated cross maze test for each group of mice (mean ± SD, n = 12)

| Group | Open arm retention time ratio (%) | Open arm entry ratio (%) |
|---|---|---|
| Control | 16.85±6.36 | 42.31±4.05 |
| Model | 3.58±1.38* | 18.24±2.64* |
| High-dose captopril | 12.64±5.33# | 35.41±3.85# |
| Medium-dose captopril | 8.62±4.31# | 28.15±3.22# |
| Low-dose captopril | 6.14±3.08# | 21.35±3.04# |

*#$P < 0.05$: *vs control; #vs model

**Table 4:** Escape latency amongst the 3 groups of mice (mean ± SD, n = 12)

| Group | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| Control | 51.25±3.65 | 41.85±4.45 | 35.48±3.58 | 29.78±3.05 | 24.33±2.46 |
| Model | 75.61±5.31* | 67.12±5.42* | 58.64±4.67* | 49.31±3.15* | 40.33±3.12* |
| High-dose captopril | 58.64±4.21# | 49.31±3.64# | 40.33±3.07# | 32.11±2.38# | 26.38±2.34# |
| Medium-dose captopril | 63.48±5.11# | 55.36±3.47# | 46.11±3.16# | 36.18±3.86# | 29.36±2.64# |
| Low-dose captopril | 69.15±5.17# | 61.08±4.61# | 51.33±3.46# | 41.34±3.08# | 33.56±2.99# |

*#$P < 0.05$: *vs control; #vs model

**Table 5**: Expressions of bradykinin-B2R-related signaling pathway proteins (mean ± SD, n = 12)

| Group | B2R | BPNF | CDC42 |
|---|---|---|---|
| Control | 431.25±53.45 | 91.35±5.94 | 89.36±5.31 |
| Model | 211.62±37.45* | 55.39±3.67* | 20.34±3.45* |
| High-dose | 391.75±42.01# | 86.64±5.78# | 75.61±3.02# |
| Medium-dose captopril | 364.15±35.75# | 77.54±4.36# | 61.33±3.62# |
| Low-dose captopril | 332.17±31.05# | 65.74±4.12# | 49.35±3.04# |

*#$P < 0.05$: *vs control; #vs model

**Bradykinin-B2R-related signaling pathway protein expressions**

The expression levels of B2R, BPNF and CDC42 in model group were significantly less than the corresponding control values ($p < 0.05$). However, the expression levels of these signal pathway proteins in all captopril-treated mice were significantly higher than those in model mice ($p < 0.05$; Table 5).

## DISCUSSION

Diverse sources of pressure of daily life have led to accentuation of incidence of depression [10]. Patients with severe depression not only have psychological diseases, but also suffer from neurological impairment and changes in physical function [11]. The main treatment strategy used for depression is drug therapy. The drugs used comprise tricyclic antidepressants and monoamine oxidase inhibitors. However, these

drugs have slow onset, and they are effective only in some patients [12]. Several studies have shown that some antihypertensive drugs increase the incidence of depressive symptoms in patients, while others mitigate these symptoms.

The water maze experiment established by Morris, a British psychologist, is effectively used for evaluating the spatial learning ability of animals. Indeed, the Morris water maze experiment has become the most commonly used method for studying learning and memory [13]. In the positioning navigation experiment, mice are trained to locate the hidden platform through visual spatial clues, and then escape. This test was applied in this study.

Spatial memory ability of mice was evaluated using the space exploration experiment [14]. The 5-day escape latency values of the three captopril groups were significantly lower than that of the model group. This suggests that captopril improved spatial learning and memory ability of CUMS mice, thereby exerting an anti-depression effect. Neurochemical changes caused by chronic stress are involved in the occurrence and development of depression, as a result of changes in levels of neuroendocrine, neurotransmitter and neuroplasticity [15].

The CUMS mouse model exhibits symptoms which closely resemble those of depression in humans with chronic multiple stressors. In this study, relative to model mice, the degrees of preference for sugar water in the 3 captopril doses were significantly higher. Moreover, immobility time during swimming was significantly lower in the three captopril mice groups than in control mice. The number of straddles and vertical standings, and the number of modifications in the captopril groups were significantly higher than those in the model group. In addition, the open wing withholding time ratio and open wing entry time ratio in model mice were significantly higher than in the control mice. Moreover, ratio of open wing stay time and open wing entry time was significantly higher in captopril-treated mice than in model mice. These results suggest that captopril reversed CUMS-induced depression-like behavior in mice, indicating that captopril has antidepressant property.

The effect of bradykinin is mediated by B1R and B2R receptors in patients. It is known that B2R is expressed mainly in peripheral blood vessels and central nervous system, usually under pathological conditions [16]. Studies have shown that B2R mediates most physiological functions of bradykinin and plays a neuroprotective role in the central system [17]. A number of studies have demonstrated that bradykinin activation increases the expression of CDC42 in the small G protein Rho GTPase family, and that CDC42 plays a regulatory role in cell differentiation and cytoskeleton remodeling [18]. Moreover, CDC42 plays a regulatory role in the dendritic actin cytoskeleton by regulating the structure and function of dendrites, thereby influencing learning and memory ability [19].

It has been reported that activation of CDC42-MTOR pathway led to increased expression of BPNF in mTOR pathway, which promoted synaptic regeneration and reversed dendritic spine lesions, thereby ultimately playing an antidepressant role [20]. In the present study, it was found that the expressions of B2R, BPNF and CDC42 in all captopril-treated mice were significantly higher than those in model mice, indicating that captopril stimulated the expression of B2R, activated the CDC42-MTOR pathway, accelerated synaptic genesis, and alleviated depression-like behaviors in the animals.

## CONCLUSION

Captopril mitigate CUMS-mediated depression-like attitude in mice by regulating bradykinin-B2R signaling pathway, and it exerts an antidepressant effect by up-regulating the expressions of B2R, BPNF and CDC42. This provides a possible direction for further research on other antidepressants.

## DECLARATIONS

### *Availability of data and materials*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### *Conflict of Interest*

No conflict of interest associated with this work.

## Contribution of Authors

We declare that this work was performed 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. Shaofeng Zhang designed the study, supervised the data collection, and analyzed the data. Shaofeng Zhang and Lin He interpreted the data and prepared the draft manuscript for publication. Shaofeng Zhang and Lin He reviewed the draft manuscript and approved it for submission.

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