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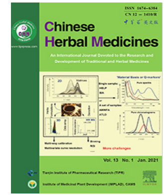

Original Article

Berberine exerts antidepressant-like effects via regulating miR-34a-synaptotagmin1/Bcl-2 axis

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ABSTRACT

Objective: Berberine, a cationic alkaloid first isolated in 1917, has been approved by the China Drug Administration for decades. Accumulating evidence demonstrated its antidepressant-like activities *in vivo*. Our previous study has shown that chronic stress leads to the upregulation of miR-34a in the hippocampus of mice. This study aims to evaluate the underlying miR-34a mediated mechanism of berberine in chronic stress-induced depression in mice.
Methods: In the present study, mice were administered with berberine during chronic stress. Levels of miR-34a, dendritic density, mitochondrial morphology, and neurogenesis were assessed in the hippocampus. Subsequently, miR-34a agomir was used as a pharmacological intervention for the investigation of berberine.
Results: The results showed that berberine reversed the decrease in sucrose preference and the increase in latency to feed without altering total food consumption. Furthermore, chronic stress-induced overexpression of miR-34a decreased synaptotagmin-1 and Bcl-2 levels, thereby impairing spinal morphology, mitochondria and neurogenesis. Berberine inhibited miR-34a expression, in turn restored synaptotagmin-1 and Bcl-2 levels, and thus improved spinal morphology, mitochondria and neurogenesis in the hippocampus. However, the improvements induced by berberine were totally blocked by the pretreatment of miR-34a agomir, which caused the elevation of miR-34a levels in the hippocampus.
Conclusion: This finding demonstrated that miR-34a downregulation was involved in the antidepressant-like effects of berberine in mice exposed to chronic stress.
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1. Introduction

Depression is a common mental disorder in which a patient feels depressed, loses interest or enjoyment, produces guilt, or lacks self-esteem, sleep and appetite disorders, physical exhaustion, and difficulty concentrating. The etiology and pathogenesis of depression are still unclear until now. The current view is that the disease results from multiple factors such as genetics, biochemistry, and psychosocial culture.

MicroRNA (miRNA) is a class of endogenous non-coding small RNAs of about 22 nucleotides in length that often regulate gene expression at the post-transcriptional level. miRNA can regulate about 30% of intracellular genes (Filipowicz, Bhattacharyya, & Sonenberg, 2008), not only participate in the normal physiological processes of cells, but also closely mediate the pathogenesis of diseases such as diabetes, autoimmune diseases and mental diseases (Hung, Wu, Tsai, Huang, & Kang, 2019). Therefore, miRNAs are regarded as a critical factor in regulating gene expression and are receiving more and more attention. Studies have shown that a large number of miRNAs are specifically expressed or enriched in the brain and that the neurological disorders of the brain in both depressive patients and depressive-like animals are accompanied by many miRNA expression abnormalities (Camkurt et al., 2019; Yi et al., 2014, 2018). In this way, identifying changes in miRNA profiles in depression and revealing the corresponding target genes/proteins through miRNAs will further provide new insight into the pathophysiology of depression.

Recently, there was evidence showing that total abdominal exposure can impair cognitive function by increasing miR-34a levels (Cui et al., 2017), while miR-34a gene silencing could improve spatial cognition and protect hippocampal neurogenesis (Zhang, Li, & Zhang, 2018). Another study also showed that miR-34a inhibition could improve anesthesia-induced learning and

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memory dysfunction by inhibiting apoptosis (Li, Li, Zhuang, & Li, 2018a). More importantly, through the use of miRNA array and qPCR validation, we found that miR-34a-5p was elevated in the hippocampus of mice exposed to chronic stress (Liu et al., 2015). These findings suggested that the lack of normal neuronal proliferation and survival may be related to the overexpression of miR-34a in depression.

Berberine is a plant-derived alkaloid with various pharmacological properties, including neuroprotection, antioxidants, anti-apoptotic and anti-inflammatory effects. Accumulating studies have indicated the antidepressant-like effects of berberine (Fan et al., 2017; Liu et al., 2017). In addition, it has been shown that berberine can penetrate blood–brain barrier and enter the brain regions such as the hippocampus (Wang et al., 2005), which exhibited its therapeutic potential for mental diseases. However, whether miR-34a mediated gene silencing involving in the antidepressant-like effects of berberine remains unclear. The present study evaluated whether berberine could improve spine and mitochondria morphology, as well as neurogenesis via decreasing miR-34a to regulate the expression of synaptotagmin-1 and Bcl-2 in the hippocampus of depressive-like mice.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (8 weeks old, bodyweight 20–22 g, Shanghai Slac Animal Center, China) were housed in four per plastic cage (320 × 180 × 160 mm). It was maintained in a standard experimental animal environment [ambient temperature: (22 ± 2)°C; relative humidity: (55 ± 5)%; light/dark cycle: 12 h/12 h]. It was kept that mice can have access to food and drinking water freely except the special illustration. All the mice were freed for one week before the experimental treatment to adapt to the new environment. All experimental protocols were approved by the Ethics Committee of Biomedical College of Huaqiao University and performed in accordance with the National Regulations for the Administration of Affairs Concerning Experimental Animals in China (Approved by the State Council on 31 October 1988 and promulgated by Decree No. 2 of the State Science and Technology Commission on 14 November 1988).

2.2. Reagents

Berberine (14050) and the anti-β-actin antibody (A3854) connected with HRP were purchased from Sigma (St. Louis, USA). Doublecortin (DCX, ab18723), Bcl-2 (ab692) and synaptotagmin-1 (ab13259) antibodies were purchased from Abcam (Cambridge, USA). All miRNA related reagents were purchased from Ribobio Co., Ltd. (Guangzhou, China). The sense strand of miR-34a agomir is UGGCAGUGUCUUAGCUGGUUGU. On the other hand, the sequence of cel-miR-239b-5p (UUUGUACUACACAAAAGUACUG) was used for sense strand of mouse agomir control, as this sequence does not target any genes in mice.

2.3. Chronic stress procedure

The method of establishing the chronic stress model was mainly described in the relevant literature (Li et al., 2013a) and combined with the laboratory conditions. The sucrose adaptation was carried out for three times before modeling. The stress groups received a variety of different mild stressors every day, such as water deprivation, empty bottle exposure, soiled cage, light/dark succession every 2 h, space reduction, 45° cage tilt, overnight illumination and predator sounds and ensuring that each stressor does not occur continuously, forming an unpredictable status (Table 1). After four weeks of modeling, the mice were evaluated for depression by sucrose preference and then were treated with the corresponding drugs. In addition, the control groups did not receive any stressors and were housed separately.

2.4. Drug administration

Mice were randomly divided into four groups after four weeks of chronic stress (n = 12): the control-vehicle group, the control-berberine group, the stress-vehicle group, and the stress-berberine group. Berberine (100 mg/kg) was dissolved in the saline, and thus saline was used as a vehicle. Both berberine and vehicle were orally administrated once a day in the last four weeks of chronic stress. The dose of berberine was selected according to our previous study (Liu et al., 2017). For the pharmacological intervention experiment, mice were randomly divided into five groups after four weeks of chronic stress (n = 8): the control-vehicle group, the chronic stress-vehicle group, the chronic stress-berberine group, the chronic stress-miR-34a agonist group, and the chronic stress-miR-34a agonist-berberine group. Berberine was orally administrated once a day. Our preliminary experiment showed that single miR-34a agomir intracerebroventricular treatment increased miR-34a levels after one week (Fig. 1), miR-34a agomir and its control (2 nmol) were dissolved in artificial cerebrospinal fluid (4 μL at an injecting rate of 0.5 μL/min) and intracerebroventricularly infused (0.6 mm caudal to bregma, 1.5 mm from the midline and 1.5 mm below the dural surface) once a week during the last four weeks of chronic stress.

2.5. Sucrose preference test

The sucrose preference test was carried out after the sucrose training test. Briefly, the mice were separated, housed, and trained to adapt the sucrose solution in their home-cage (two bottles of sucrose solution access during first day; one bottle of sucrose solution and one bottle of water access during the second day). After water deprivation but not food for 12 h, two identical bottles containing sucrose solution and water were placed in each cage at the same time. The consumption of sucrose solution and water within the 24 h was measured. The sucrose preference, which is used to reflect anhedonia, was calculated by sucrose consumption/total liquid consumption × 100%.

2.6. Novelty-suppressed feeding test

The mice were deprived of food but not water for 24 h before the test. A single pellet of the same size at every test session was placed in the plastic box (50 × 50 × 20 cm). Mouse was put in the corner of the box and allowed to get the pellet within 8 min. The time was recorded from the release to feeding. The criterion for feeding is that the animal starts chewing food instead of sniffing or fiddling with food. The antidepressant-like activity of the drug was determined by taking the latency to feed as a parameter. If the mouse did not take the food during 8 min, the latency to feed was recorded as 8 min. After the test, the mouse was put back into its home-cage to measure food consumption for 15 min. This food consumption was used as a control value for latency to feed.

2.7. qPCR

The brain tissue samples were dissected and stored at −80 °C. A Sangon's qPCR miRNA kit was used for detection. Briefly, miRNA was reverse-transcribed to cDNA with the RT primer. And then, cDNA was subjected to 40 cycles of amplification with the corresponding PCR primers, in which SYBR Green I was used as

Table 1
Schematic presentation of chronic stress procedures.

Day	Stressor	Day	Stressor	Day	Stressor	Day	Stressor
1	Empty bottle exposure	15	Empty bottle exposure	29	Predator sounds	43	Empty bottle exposure
2	Soiled cage	16	Predator sounds	30	45° cage tilt	44	Space reduction
3	Space reduction	17	Empty bottle exposure	31	Soiled cage	45	Water deprivation
4	Predator sounds	18	Light/dark succession	32	Empty bottle exposure	46	Empty bottle exposure
5	Empty bottle exposure	19	Space reduction	33	45° cage tilt	47	Light/dark succession
6	Light/dark succession	20	Soiled cage	34	Overnight illumination	48	45° cage tilt
7	Space reduction	21	Water deprivation	35	Predator sounds	49	Overnight illumination
8	Water deprivation	22	Light/dark succession	36	Light/dark succession	50	Predator sounds
9	Overnight illumination	23	Predator sounds	37	Empty bottle exposure	51	Light/dark succession
10	Water deprivation	24	Overnight illumination	38	Soiled cage	52	45° cage tilt
11	45° cage tilt	25	Space reduction	39	Light/dark succession	53	Water deprivation
12	Empty bottle exposure	26	45° cage tilt	40	Overnight illumination	54	Space reduction
13	Predator sounds	27	Predator sounds	41	Light/dark succession	55	Soiled cage
14	Space reduction	28	Water deprivation	42	Predator sounds	56	45° cage tilt

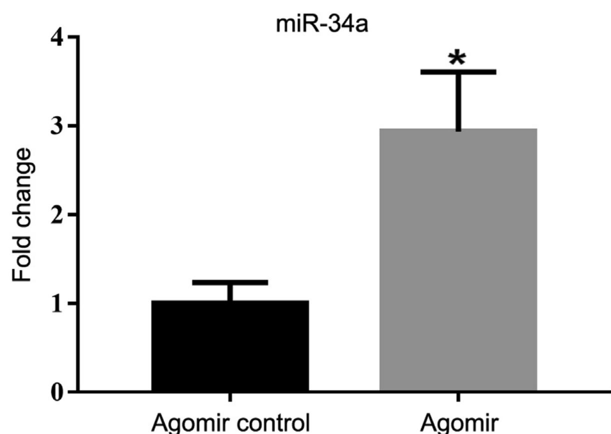

Fig. 1. Effects of single miR-34a agomir intracerebroventricular treatment on miR-34a levels in hippocampus of normal mice after 1 week (mean ± SEM, $n = 4$). $^{*}P < 0.05$ *vs* agomir control group.

the signal fluorescence and ROX as the control fluorescence. Three sample replicates per group were included in qPCR miRNA validation experiment. U6 (F: 5′-CTCGCTTCGGCAGCACA-3′; R: 5′-AACGCTTCACGAATTTGCGT-3′) was used as an internal reference to normalize the relative expression of miR-34a (F: 5′-TGGCAGTGTCTTAGCTGGTTGT-3′; R: 5′-GCGAGCACAGAATTAATACGAC-3′). The method of $2^{-\Delta\Delta Ct}$ was used for calculation.

2.8. Western blot

The brain tissue samples were dissected and stored at −80 °C. RIPA buffer was added to the tissues. Then the brain tissues were homogenized by a sonicator until no apparent tissue appeared in the lysate. After the homogenization, the lysate was centrifuged at 12,000 × g for 15 min at 4 °C, and the supernatants were taken into a new tube. The protein concentration of the supernatants was assayed by a standard BCA kit. Subsequently, denatured supernatants containing 20–50 μg protein were loaded into the gel and separated. After that, the gel was transferred to the PVDF membrane. The membrane was blocked in 5% BSA/TBST buffer at room temperature for 1 h and followed by incubation in 5% BSA/TBST buffer with the primary antibodies (anti-synaptotagmin-1: 1:500, anti-Bcl-2: 1:1000) at 4 °C overnight. After removing the primary antibody solution and PBS rinsing, the membranes were subsequently incubated with a secondary antibody labeled with HRP. Finally, the membrane was placed on the Chemiluminescence Imager for detecting and imaging. The expression of β-actin (1:5000) was used as an internal reference for synaptotagmin-1 and Bcl-2.

2.9. Brain extraction and immunofluorescence

Mice were anesthetized with chloral hydrate (0.35 g/kg), fixed in a supine position on a foam board, and the heart was completely exposed. The perfusion needle was inserted into the aorta along the ventricle. The perfusion needle was held with a hemostatic clip to secure it. Then the right atrial appendage was cut and quickly injected with PBS. Subsequently, the mouse was perfused with heparinized 0.9% saline, followed by ice-cold 4% paraformaldehyde. At the end of the perfusion, the brain was decapitated and placed in a 4% paraformaldehyde solution for post-fixation at 4 °C overnight. The brain tissue was then dehydrated sequentially with 10%, 20%, and 30% sucrose solution at 4 °C until the specimen was bottomed entirely. Coronal sections were taken using a Leica CM1900 cryostat. The thickness of the brain slices was 15 μm, and then the slices were stored in PBS at 4 °C. Subsequently, the slices were rinsed with 0.01 mol/L PBS and blocked with a blocking solution for 1 h at room temperature. The sections were then incubated with primary antibody at 4 °C shaker at night (DCX: 1:500). On the next day, the primary antibody was discarded, the slices were rinsed with PBS, the secondary antibody was then incubated. After the incubation, slices were rinsed with PBS and then stained with DAPI (1:5000). Then the DAPI solution was discarded. Finally, photos of the slices were taken by a Leica TCS SP8 confocal microscope.

2.10. Golgi staining

The entire brain was immersed in the Golgi-Cox solution for 14 d. Then the Golgi-Cox solution was replaced by a 30% sucrose solution in the following three days. The coronal slices of the hippocampus were performed (slices 50 μm thick) using a Leica CM1900 cryostat. The tissue sections were placed on a 1% gelatin-pretreated glass slide for Golgi staining. Three intact neuron pyramidal cells were selected on three slices at A/P levels (Bregma −2.06 mm, −2.46 mm, −2.80 mm approximately) according to the brain diagram of mouse (Franklin & Paxinos, 2008). The dendritic spines were observed under a 10 × 100-fold oil microscope. The dendritic spine density in the CA3 subregion of the hippocampus was expressed as the number of spines per 10 μm of dendritic length.

2.11. Mitochondria observation by transmission electron microscope

Mice were immediately anesthetized with chloral hydrate (0.35 g/kg) and then were sacrificed by intracardial perfusion with PBS followed by ice-cold 4% Polyoxymethylene mixture. The hip-

pocampus was carefully dissected from each hemisphere and incised about 1 mm^3, then quickly fixed in 2.5% glutaraldehyde for 4 h. The hippocampal tissue was washed twice for 15 min with 0.1 mol/L phosphate buffer, fixed with 1% osmic acid for 1 h, flushed with 0.1 mol/L phosphate buffer, then acetone dehydration for three times, following epoxy resin 618 soaked 2 h after embedding, oven polymerization. Thin sections (50 nm) were cut on a Reichert Ultracut E microtome and stained with uranyl acetate and lead citrate for observation under a transmission electron microscope.

2.12. Data and statistical analysis

All data were expressed as mean ± SEM. Kolmogorov-Smirnov test was used for verification of normal distribution before the ANOVA. A two-way or one-way ANOVA followed by a Tukey post-hoc test was performed. A value of $P < 0.05$ was considered statistically significant for analysis.

3. Results

3.1. Berberine reversed depressive-like behaviors in mice induced by chronic stress

To confirm the antidepressant-like effects of berberine in mice (Fig. 2A), sucrose preference test was first used to assess animals' anhedonia (Fig. 2B). Chronic stress exposure caused a significant reduction of sucrose preference ($P < 0.01$), confirming the depressive-like symptoms in mice. In parallel to our previous study, berberine administration (100 mg/kg) reversed the reduction of sucrose preference in mice exposed to chronic stress ($P < 0.01$). Subsequently, novelty-suppressed feeding test was performed to further demonstrate the effects of berberine (Fig. 2C). Chronic stress caused a significant elevation of latency to feed in the test ($P < 0.01$), while berberine completely reversed the increase ($P < 0.01$). Finally, there was no significant difference among the total food consumption (Fig. 2D), suggesting that the change of latency to feed was not caused by appetite of the animals.

3.2. Berberine decreased miR-34a levels in hippocampus of chronic stress-mice

Using microarray array and qPCR analysis, our previous study identified that miR-34a levels were increased in the hippocampus of depressive-like mice. In accordance with our previous study, the present study also found that chronic stress-induced an elevation of miR-34a levels ($P < 0.01$) (Fig. 3). Administration with berberine for four weeks significantly decreased the levels of miR-34a in the hippocampus ($P < 0.01$).

3.3. Berberine alleviated reduction of synaptotagmin-1 and impaired spinal morphology in hippocampus

As shown in Fig. 4A, chronic stress significantly decreased synaptotagmin-1 levels in the hippocampus ($P < 0.01$). Berberine treatment significantly increased synaptotagmin-1 levels ($P < 0.01$). Considering that synaptotagmin-1 is involved in the synaptogenesis in the brain, spinal morphology was detected subsequently. As shown in Fig. 4B, chronic stress displayed significantly less density of dendritic spine ($P < 0.05$), as compared with the control-vehicle group. However, this deficiency was attenuated by the administration of berberine ($P < 0.05$).

3.4. Berberine restored inhibition of Bcl-2, mitochondria morphology, and neurogenesis in hippocampus

Besides cancer, depression has been linked to dysregulation of Bcl-2. Fig. 5A showed the effect of berberine on the Bcl-2 levels in the hippocampus of chronic stress-induced mice. Chronic stress reduced the anti-apoptotic Bcl-2 levels ($P < 0.01$), which was reversed by berberine ($P < 0.01$). Next, we detected the mitochondria morphology as the Bcl-2 focuses much of their efforts at the mitochondria level. As shown in Fig. 5B, chronic stress-induced

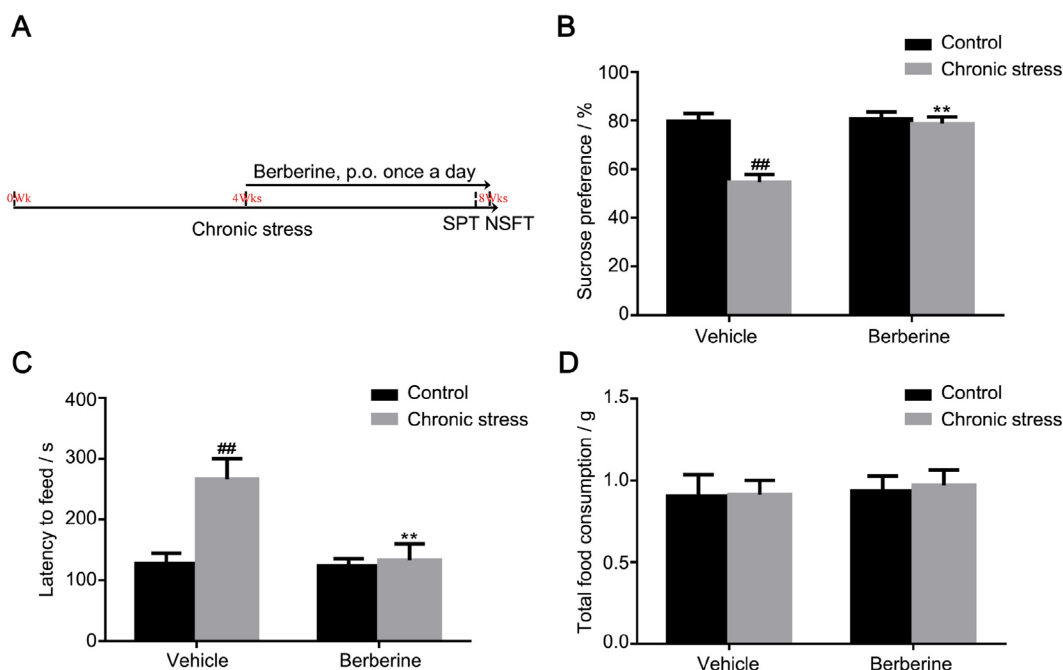

Fig. 2. Effects of berberine on depressive-like behaviors in mice induced by chronic stress (mean ± SEM, $n = 12$). (A) Timeline of experiment. (B) Sucrose preference. (C) Latency to feed. (D) Total food consumption. $^{\#\#}P < 0.01$ *vs* control-vehicle group. $^{**}P < 0.01$ *vs* chronic stress-vehicle group.

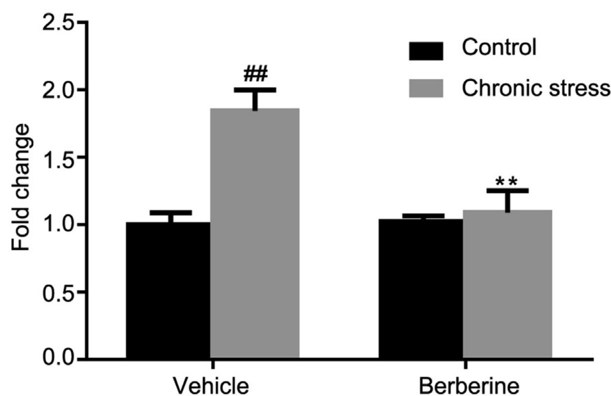

Fig. 3. Berberine inhibited miR-34a expression in hippocampus of mice induced by chronic stress (mean ± SEM, n = 6). ##P < 0.01 *vs* control-vehicle group. **P < 0.01 *vs* chronic stress-vehicle group.

damage in mitochondria was markedly reversed by berberine. On the contrary, neurogenesis in the hippocampus is considered as a compensatory adaptive response to excessive apoptosis. In this way, neurogenesis was also assessed in the present study. As shown in Fig. 5C, chronic stress significantly decreased DCX positive cells in the dentate gyrus of the hippocampus (P < 0.01). When berberine was administrated, the number of DCX positive cells was restored compared to control-vehicle animals, indicating that berberine can improve neurogenesis in the dentate gyrus (P < 0.01).

3.5. miR-34a agomir blocks effects of berberine in behavioral tests

To evaluate whether miR-34a regulation was required for the antidepressant-like effects of berberine, miR-34a overexpression induced by miR-34a agomir was pretreated prior to berberine administration (Fig. 6A). As shown in Fig. 6B – D, chronic stress-induced a decrease of sucrose preference (P < 0.01) and an increase of latency to feed (P < 0.01), while berberine reversed the abnormalities (sucrose preference: P < 0.01; latency to feed: P < 0.05). However, miR-34a pretreatment partly blocked the antidepressant-like effects of berberine (sucrose preference: P < 0.05; latency to feed: P = 0.077). In addition, the effects of miR-34a agomir were verified as the increased expression of miR-34a (Fig. 6E).

4. Discussion

Our results demonstrated a novel antidepressant-like mechanism of berberine in the present study, which inhibits miR-34a to restore synaptotagmin-1 and Bcl-2 expression, resulting in the improvement of spinal morphology, mitochondria, and neurogenesis in the hippocampus.

Chronic stress, a depressive-like animal model, is widely used to mimic the process of depression in human beings (Willner, 2017). The results from the present study showed an anhedonia symptom in mice induced by chronic stress; However, the anhedonia attenuated by berberine treatment as the sucrose preference was increased. Consistently, latency to feed in the novelty-suppressed feeding test was increased by chronic stress but reversed by berberine. The behavioral results above suggested the role of berberine in depression treatment.

miR-34a, as a critical and highly conserved miRNA associated with brain development and cognitive regulation, is involved in the pathophysiology of mental disease. For example, overexpression of miR-34a caused severe cognitive impairment, associated with Aβ accumulation and Tau hyperphosphorylation in the brain, which is the typical neuropathology of Alzheimer's disease (Sarkar et al., 2019). Moreover, another study also found an upregulation of miR-34a in the hippocampus of the triple transgenic mice of Alzheimer's disease (Zhang et al., 2016). Similar with the results in Alzheimer's disease, miR-34a has been previously found to be

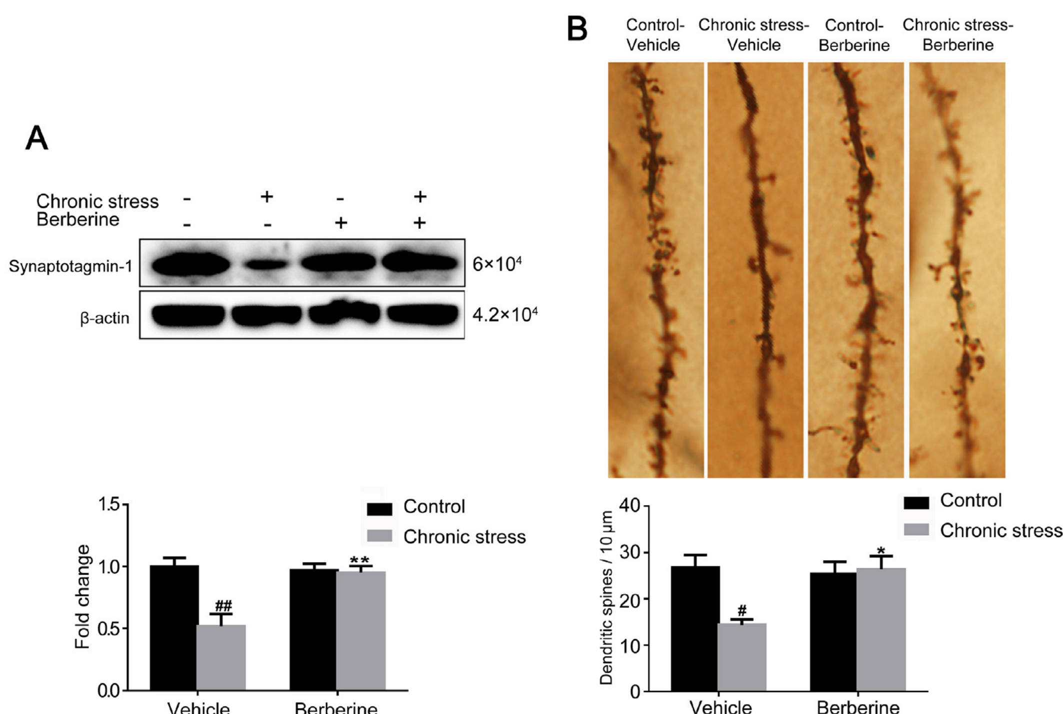

Fig. 4. Berberine promoted synaptotagmin-1 expression and improved spinal morphology in hippocampus (mean ± SEM, n = 3–4). (A) Representative photomicrograph and summary data of Western blot. (B) Representative photomicrograph and summary data of Golgi staining. #P < 0.05 *vs* control-vehicle group. *P < 0.05 *vs* chronic stress-vehicle group.

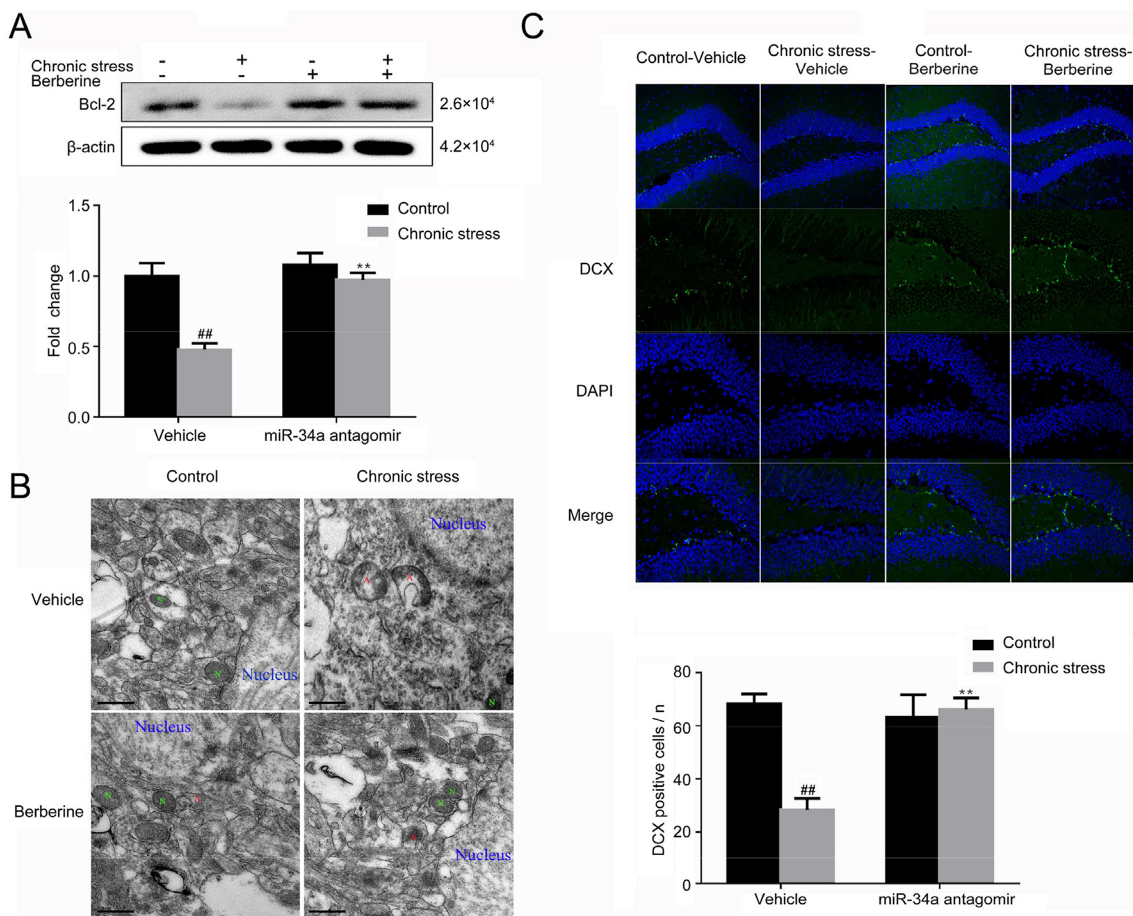

Fig. 5. Berberine induced Bcl-2 expression and enhanced mitochondria morphology and neurogenesis in hippocampus (mean ± SEM, n = 3–4). (A) Representative photomicrograph and summary data of Western blot. (B) Representative photomicrograph of mitochondria. N: normal mitochondria; A: abnormal mitochondria. (C) Representative photomicrograph and summary data of DCX positive cells. $^{\#\#}P < 0.01$ *vs* control-vehicle group. $^{**}P < 0.01$ *vs* chronic stress-vehicle group.

increased in depression-like animals (Liu et al., 2015). More importantly, a clinical investigation indicated that both cerebrospinal fluid and serum miR-34a levels in depressive patients were significantly higher than those in control subjects, which suggested miR-34a as a therapeutic target for further investigation (Wan et al., 2015). Consistent with these observations, we also found that miR-34a expression was abnormally elevated after CUMS, adding the evidence that excessive expression of miR-34a was correlated with the pathophysiology of depression. On the contrary, berberine could reverse the elevation. The data above supported the evidence that the reduction of hippocampal miR-34a expression was involved in the antidepressant-like effects of berberine.

According to Targetscan database, synaptotagmin-1 and Bcl-2 are two potential functional targets of miR-34a as several binding sequences for miR-34a in the 3′-UTRs of the synaptotagmin-1 and Bcl-2 mRNAs. A luciferase reporter assay verified the binding sites between miR-34a and synaptotagmin-1/Bcl-2 *in vitro* in our previous study (Yi et al., 2020). In this respect, the effects of berberine on the synaptotagmin and Bcl-2 were investigated.

Synaptotagmin is a family of important proteins playing a role in synaptic transmission. It belongs to the presynaptic vesicle protein and is involved in neurotransmitters. Synaptotagmin exerts physiological functions via controlling neurotransmitter releases and thus regulates the learning and memory of the brain (Sudhof, 1995). Synaptotagmin-1, the most abundant of the synaptotagmin family, which is mainly distributed in the cerebral cortex and hippocampus, plays a crucial role in synaptic vesicle fusion during neurotransmitter release (Sollner, Bennett, Whiteheart, Scheller, & Rothman, 1993). It has been shown that the synchronous release of neurotransmitters was inhibited in synaptotagmin-1 knockout mouse hippocampal neurons (Nishiki & Augustine, 2004). There was evidence showing that learning and memory were positively correlated with the expression of synaptotagmin-1 in the hippocampus (Zhang et al., 2014). On the other hand, it has been verified that synaptotagmin-1 is the target of miR-34a *in vitro* (Agostini et al., 2011). miR-34a modulates neuronal development neural stem cell differentiation by targeting synaptic proteins such as synaptotagmin-1 (Agostini et al., 2011; Wan et al., 2015). In the present study, we found that chronic stress decreased the levels of synaptotagmin-1 in the hippocampus, which was in parallel to a previous study showing that levels of synaptotagmin-1 were decreased in the hippocampus of mice exposed to chronic stress (Han et al., 2018). On the contrary, administration with berberine significantly increased the levels of synaptotagmin-1. Actually, the effects of the SSRI antidepressant fluoxetine on behaviors were associated with its function on promoting neurotransmitter trafficking and release via enhancing hippocampal synaptotagmin-1 expression (Popova, Castren, & Taira, 2017). Similarly, resveratrol treatment could produce the antidepressant-like effects and upregulate the expression of both BDNF and synaptotagmin-1 in the hippocampus of rats (Ge et al., 2015; Shen, Qu, Xu, Sun, & Zhang, 2019). These results suggested that berberine might produce the antidepressant-like via miR-34a/synaptotagmin-1 axis. Furthermore, as spinal morphology is regulated by miR-34a/synaptotagmin-1 axis (Agostini et al., 2011), spinal morphology was investigated by Golgi staining. We

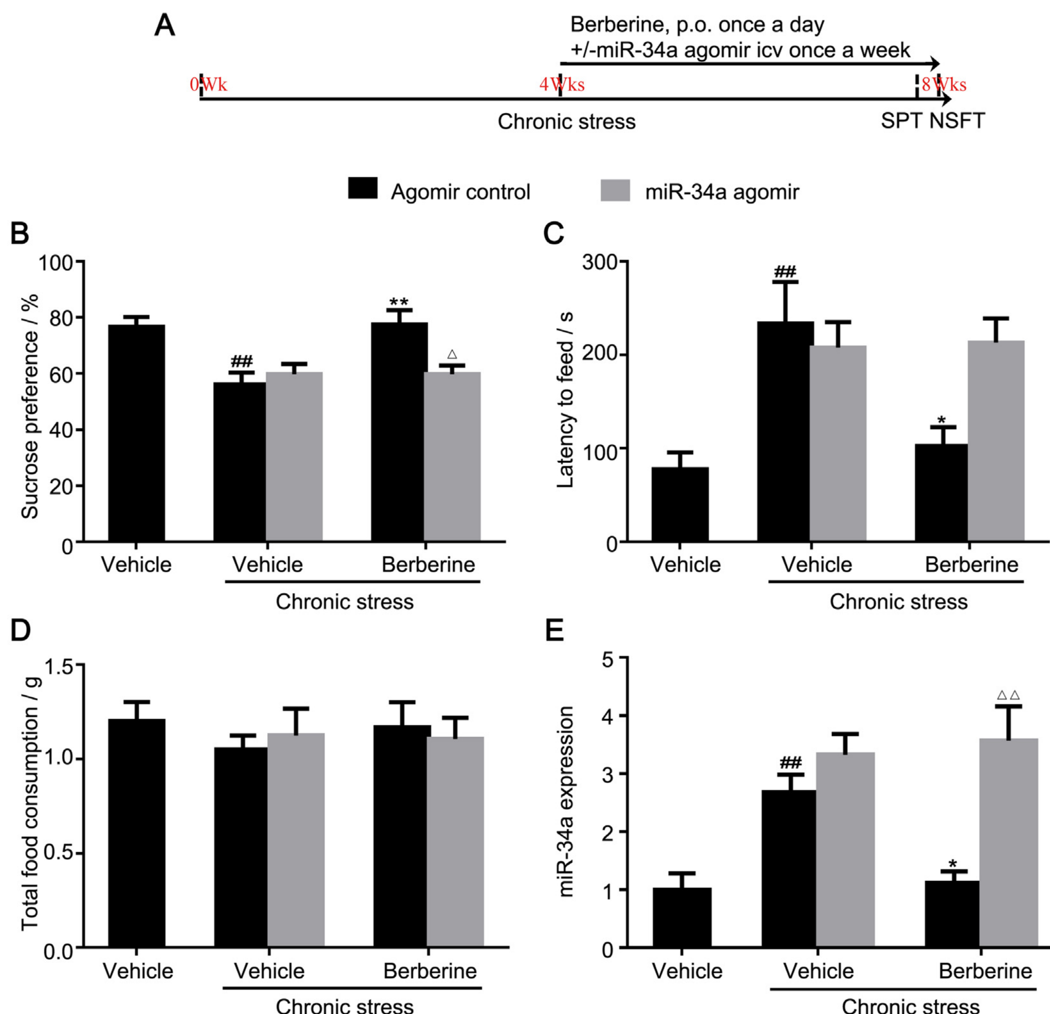

Fig. 6. miR-34a agomir prevented effects of berberine in chronic stress mice (mean ± SEM, n = 8). (A) Timeline of experiment. (B) Sucrose preference. (C) Latency to feed. (D) Total food consumption. (E) miR-34a expression. $^{\#\#}P$ < 0.01 *vs* control-vehicle group. $^{*}P$ < 0.05 and $^{**}P$ < 0.01 *vs* chronic stress-vehicle group. $^{\triangle}P$ < 0.05 and $^{\triangle\triangle}P$ < 0.01 *vs* chronic stress-berberine group.

found that chronic stress caused the loss of the synaptic spine density, while berberine attenuated this reduction. The results demonstrated that the change of dendritic spine density was positively correlated with the change of synaptotagmin-1 but negatively correlated with the change of miR-34a.

Besides synaptotagmin-1, Bcl-2 was demonstrated to be another target of miR-34a as well (Li et al., 2013b), as both the bioinformatics and luciferase reporter assays found that miR-34a targeted the 3′-UTR of the Bcl-2 mRNA and represses its translation (Yang et al., 2014). Bcl-2 is an anti-apoptotic protein involved in the regulation of cellular apoptosis processes in mammals. Its overexpression is correlated with neuroprotective activity and maintains neurogenesis (Xu et al., 2006). According to several post-mortem studies, bipolar disorder showed an increase of apoptosis genes (Benes, Matzilevich, Burke, & Walsh, 2006) and a decrease of anti-apoptotic genes (Jarskog, Gilmore, Selinger, & Lieberman, 2000; Kim, Rapoport, & Rao, 2010). In agreement with the findings in clinical investigation, experimental studies also showed that different depression-like animals exhibited a reduction of Bcl-2 in the hippocampus (Li et al., 2018b; Wang et al., 2016). In the present study, we found that chronic stress decreased the levels of Bcl-2 in the hippocampus, while berberine restored it. The results were partly consistent with the previous reports that mRNA expression of hippocampal Bcl-2 was decreased in depression-like animals exposed to chronic stress (Shen et al., 2019) and berberine increased the protein levels of hippocampal Bcl-2 in mice with Parkinson's disease (Kim et al., 2014). In accordance with the overexpression of miR-34a impaired neurogenesis in mouse cortical neural progenitor cells (Fineberg, Datta, Stein, & Davidson, 2012), the neurogenesis inhibited by chronic stress was attenuated by administration with berberine. Meanwhile, as miR-34a/Bcl-2 axis is involved in the function of mitochondria (Bukeirat et al., 2016; Liao et al., 2016), the mitochondria morphology was evaluated. We can clearly find that berberine restored the impaired mitochondria in the hippocampus. Similarly, a previous study also indicated that berberine prevented Aβ25-35-induced neuronal apoptosis via an improved mitochondria-related function, such as increasing Bcl-2 levels (Liang et al., 2017).

Next, to further confirm the results, the experiment using miR-34a agomir was performed as well. The results demonstrated that berberine increased sucrose preference and decreased latency to feed, consistent with the experimental data above. More importantly, the effects of berberine could be abrogated by overexpression of miR-34a by pretreatment with miR-34a agomir. Therefore, it can be speculated that berberine could produce antidepressant-like effects through miR-34a-synaptotagmin/Bcl2 axis.

5. Conclusion

The current study indicated that berberine exerted the antidepressant-like effects via upregulating miR-34a expression and restored miR-34a- synaptotagmin/ Bcl2 levels. These findings further provide new evidence that berberine may be served as a potential antidepressant from the natural product.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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