

Original Article

Effects of duloxetine on microRNA expression profile in frontal lobe and hippocampus in a mouse model of depression

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Abstract: Depression is a major mood disorder affecting people worldwide. The posttranscriptional gene regulation mediated by microRNAs (miRNAs) which may have critical roles in the pathogenesis of depression. However, to date, little is known about the effects of the antidepressant drug duloxetine on miRNA expression profile in chronic unpredictable mild stress (CUMS)-induced depression model in mice. Healthy adult male Kunming mice were randomly divided into three groups: control group, model group and duloxetine group. Sucrose preference test and open field test were used to represent the behavioral change. MiRNAs levels in frontal lobe and hippocampus of mice were analyzed using miRNA microarrays assay. We observed that long-term treatment with duloxetine significantly ameliorated the CUMS procedure-induced sucrose preference decreases and mice treated with duloxetine demonstrated a reversal of the number of crossings, and rearings reduced by CUMS. A significant upregulation of miR-132 and miR-18a in hippocampus in the duloxetine treatment group compared with model group, whereas the levels of miR-134 and miR-124a were significantly downregulated. Furthermore, miR-18a showed significant upregulation in frontal lobe in the duloxetine treatment group relative to model group. Our data showed that miRNA expression profile in frontal lobe and hippocampus was affected by duloxetine in mice model of depression. The effect was especially pronounced in the hippocampus, suggesting that hippocampus might be the action site of duloxetine, which presumably worked by regulating the expression of miRNA levels.

Keywords: CUMS Duloxetine, miRNA, depression, frontal lobe, hippocampus

Introduction

Depression is a major mood disorder characterized by chronic low mood, disturbance of sleep and appetite, as well as feelings of inferiority, despair, and suicide [1]. Recently, studies on the mechanisms of depression are mainly concentrated on the genetic, neural and biochemical factors, neuroendocrine function, electroencephalogram (EEG) dynamics, neuroimaging and psychosocial problems. Despite much effort, the molecular underpinnings of depression has not been fully understood, which thus remains to be elucidated.

MicroRNAs (miRNAs) are a class of small non-coding RNAs of 20-25 nucleotides (nt) that function principally by disrupting the expression of target messenger RNAs [2]. MiRNAs

highly conserved in evolution and expressed in almost all tissues under tight spatial and temporal regulation. MiRNAs play a key role in regulation of gene expression at the post-transcriptional level. It has been estimated that hundreds of miRNAs in the human genome may regulate approximately 30% of human genes [3, 4]. Increasing evidences have shown that miRNAs are closely related to the pathogenesis of a variety of diseases, including diabetes, cardiovascular disease, cancer, and autoimmune diseases [5-8]. Moreover, multiple researches expound that certain brain-specific individual miRNAs, such as miR134 [9], miR132 [9, 10] and miR124 [11], could play a critical role in the translational regulation at synaptic plasticity, the perturbation of some intracellular mechanisms as well as impaired assembly, localization, and translational regulation of specific

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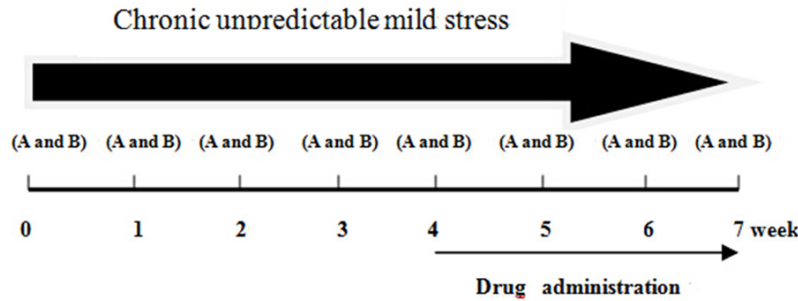


Figure 1. CUMS procedure. A: Body weight; B: Sucrose consumption test.

RNA binding proteins may affect learning and memory [12]. Given the powerful effects of miRNA on the central nervous system in literature [13, 14], there is a compelling rationale to examine the potential contribution of miRNA to depressive etiology.

Region-specific expression patterns of some miRNAs coincide with regional expression of their predicted target mRNAs [15]. MiR-8 family, miR-182/miR-96/miR-183 cluster, and miR-212/miR-312 cluster overexpressed in frontal cortex and miR-34 family overexpressed in hippocampus [16]. It is likely proved that some regulatory networks involving mRNAs have region-specific expression patterns.

More and more antidepressant drugs are devised for depression and become the most common treatment for depressive episodes. But the mechanism of different drugs is still unknown. MiR-1202 regulates expression of the gene encoding metabotropic glutamate receptor-4 (GRM4) and predicts antidepressant response at baseline [17]. Another study found that oleanolic acid led to the BDNF-related phosphorylation and activation of extracellular signal-regulated kinases (ERK) and cyclic adenosine monophosphate response element binding protein (CREB), which was associated with the upregulation of miR-132 and hippocampal neuronal proliferation [18]. All these data suggest that different antidepressant medications may be associated with specific miRNAs in pathophysiology of depression.

Duloxetine, a selective serotonin and norepinephrine reuptake inhibitor (SNRI), has been used to treat major depressive disorder in clinical application [19-21]. In addition to its antidepressant effect, duloxetine also has strong analgesic effects, especially to persistent chronic pain [22]. Therefore, the efficacy of duloxetine is better than other antidepressants

since a large percentage of the depression often accompanies chronic pain symptoms. However, the effects of duloxetine on miRNAs expression profile have not been explored.

The chronic unpredictable mild stress (CUMS) model in mice has played a crucial role in the understanding of depression [23]. In this study, the differentially expressed miRNAs induced by Duloxetine in the frontal lobe and hippocampus in CUMS model of mice were investigated. We performed miRNA microarray assay to detect differentially expressed miRNAs.

Materials and methods

Reagents and instruments

The following experimental reagents and instruments were used in this study: duloxetine hydrochloride enteric capsule (also known as Cymbalta) was obtained from Eli Lilly and Company with approval document number H10983237. Trizol reagent (Merck, Germany); Ambion's miRNA isolation kit (Ambion, USA); Biotin RNA Labeling Kit (Sigma, USA); Hybridization Control kit (Affymetrix, USA); Spectrophotometer (Shimadzu, Japan); High level oscillator (Shanghai Pure Industrial Co., Ltd, China); UV transmission detector (Shanghai Jun Rui Biotechnology Co., China); Affymetrix's miRNA V1 chip (Capital Bio Co, China); Luxscan 10 K/A (Capital Bio Co, China); Genepix 4000B (Molecular Devices, USA).

Experimental animals

Healthy adult Kunming male mice were purchased from the Comparative Medical Center of Yangzhou University (License number: SCXR (Su) 2012-0004). After 1 week of habituation, 30 animals were randomly divided into 3 groups: control group, model group, and duloxetine group. All experiments were carried out in accordance with the Zhejiang University of Health's Guide for the Care and Use of Laboratory Animals and the animal use protocol was approved by the Institutional Animal Care and Use Committee.

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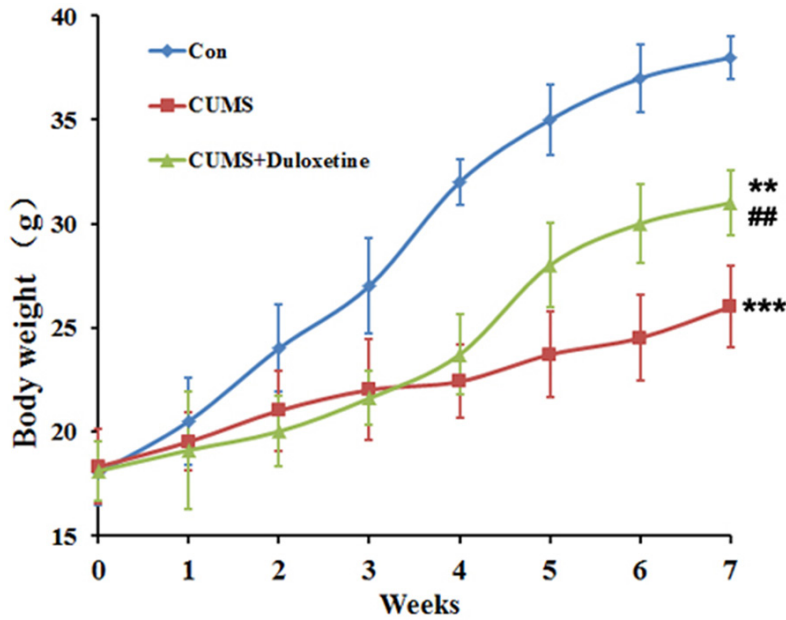


Figure 2. Effects of duloxetine on body weight in mice. Values are means \pm SEM (n = 10). **P < 0.01, ***P < 0.001 vs. control group; ##P < 0.01 vs. CUMS model group.

Chronic unpredictable mild stress (CUMS) procedure

The CUMS procedure was performed as described previously with minor modifications [23]. Briefly, the mice were exposed to a set of chronic unpredictable mild stressors as follows: (1) 1 h of resistant stress; (2) 12 h of cage tilting (45°C); (3) 24 h of food deprivation; (4) 24 h of water deprivation; (5) Inversion of light and dark cycle; (6) 12 h of damp sawdust; (7) 12 h of crowds; (8) swimming in 40-45°C hot water for 4 min; (9) swimming in 4°C cold water for 4 min, (10) 20 min of high horizontal vibration (160 times/min). One stressor was applied per day with a completely random order, Mice received one of these stresses per day, and the same stressor was not applied in 2 consecutive days. Mice in the control group were not subject to the CUMS procedure. The design of experiments is provided in **Figure 1**.

Duloxetine administration

The duloxetine group was treated with stress plus duloxetine at the dose of 9 g/kg via gastric intubation each day, while the control and model groups were administered the same amount of distilled water. Mice were subjected to various mild stressors for 4 weeks and then

administered duloxetine for 3 weeks. The animals were then sacrificed and samples were processed for analysis.

MiRNA microarray assay

Total RNA was extracted from the frontal lobe and hippocampus in mice using Trizol reagent according to the manufacturer's instructions; (2) RNA integrity was examined by formaldehyde denaturalization agarose gel electrophoresis; (3) miRNAs were isolated from 50-100 μ g of total RNA using Ambion's miRNA isolation kit following the manufacturer's instructions. (4) miRNAs samples were labeled with Cy3; (5) miRNAs microarray hybridization was performed according to the manufacturer's protocol; (6) Microarray scanning; (7) After microarray scanning, differential miRNAs were selected using Significance Analysis of Microarrays (SAM, version 2.1) and analyzed by Genepix Pro 6.0. We reported only miRNAs with significant differential expression (fold change > or < 2, P < 0.05) compared with the indicated group.

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Sucrose preference test (SPT)

Mice were deprived of water and food for 24 h, then the mice were given the choice to drink from two bottles for 12 h: one was filled with a sucrose solution (1% w/v), and the other was filled with water. The positions of the bottles in the cage were switched after 6 h. The consumption of water and the sucrose solution and the total intake of liquids were estimated by weighing the bottles. The preference for sucrose was measured as the percentage of consumed sucrose solution relative to the total amount of liquid intake.

Open field test (OFT)

To assess the MTN treatment effects on spontaneous locomotor activities, mice were subjected to the open-field paradigm as described.

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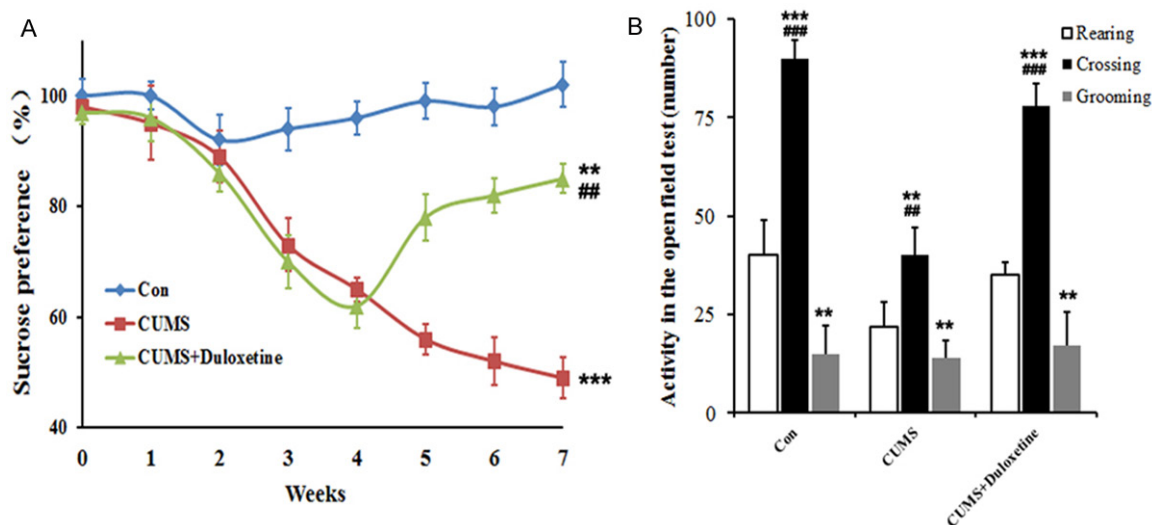


Figure 3. Behavioral changes of CUMS mice and treatments. A. The sucrose preference test was performed every week during the CUMS procedure. B. The open field test (the number of rearings, squares crossed and groomings) was evaluated after the UCMS procedure. $n = 10$, values represent the means \pm SEM. ** $P < 0.01$, *** $P < 0.001$ vs. control group; ## $P < 0.01$, ### $P < 0.001$, vs. CUMS model group.

The apparatus (40 × 60 × 50 cm) was divided into 12 equal squares and the number of crossings was recorded. The test began by placing the mouse in the center of the device so that it was acclimatized with its environment for 2 min. The number of crossings (the number of squares crossed), rearings (standing on its hind legs) and groomings (licking, cleaning and scratching its face with the forepaws) was recorded for 4 min. The apparatus was then cleaned with a 10% alcohol solution and dried after occupancy by each mouse.

Statistical analysis

Data were presented as mean \pm SEM. Statistical analysis was performed using SPSS 20.0 software. Differences between any two groups were compared by one-way analysis of variance (ANOVA). $P < 0.05$ was considered to be statistically significant.

Results

Duloxetine increased the body weight of CUMS mice

As shown in **Figure 2**, body weight was measured at the beginning of the experiment and at every 7 days thereafter. At the beginning of the experiment (Day 0), the body weight among each group tends to accordance. During the stress sessions, the control group mice gained

more weight than the model group mice in the whole experiment. Moreover, after 1 week of duloxetine treatment, the body weight of the duloxetine-treated group mice were rapidly increased than the model group mice.

Duloxetine improves depression-related behaviors in the CUMS mice

As shown in **Figure 3A** at the beginning of the test, there were no significant differences among the three groups. After 3 weeks of the experiment, the CUMS procedure significantly decreased the sucrose preference of mice compared with control mice. Long-term treatment with Duloxetine significantly ameliorated the CUMS procedure-induced sucrose preference decreases at 4 week. Spontaneous locomotor activities were measured in the open field test. As shown in **Figure 3B**, mice exposed to stressors showed a significant decrease in the number of crossings and rearings, but not the number of groomings. We observed that mice treated with duloxetine demonstrated a reversal of the number of crossings, and rearings induced by CUMS.

Detection of differentially expressed miRNAs in the frontal lobe of mice with CUMS-induced depression

As shown in **Table 1**, miR-18a level in the model group was significantly lower compared with

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Table 1. miRNA microarray analysis of the differentially expressed miRNAs in the frontal lobe. Up or down in parenthesis denotes up-regulated miRNA and down-regulated miRNA, respectively

miRNA	Fold change (Model group/ Control group)	Fold change (Duloxetine group/ Control group)	Fold change (Duloxetine group/ Model group)
miR-134	-	-	-
miR-132	1.006	0.689	-1.154
miR-124a	-1.237	1.042	0.985
miR-18a	-4.206 (down)	-3.987 (down)	2.125 (up)
miR-183	1.782	-1.436	0.893

Table 2. MiRNA microarray analysis of the differentially expressed miRNAs in the hippocampus. Up or down in parenthesis denotes up-regulated miRNA and down-regulated miRNA, respectively

miRNA	Fold change (Model group/ Control group)	Fold change (Duloxetine group/ Control group)	Fold change (Duloxetine group/ Model group)
miR-134	5.358 (up)	1.012	-5.481 (down)
miR-132	-4.481 (down)	-2.215 (down)	3.416 (up)
miR-124a	3.973 (up)	3.084 (up)	-2.975 (down)
miR-18a	-4.057 (down)	-3.682 (down)	2.435 (up)
miR-183	1.681	0.857	-1.437

the control group. After administrated with duloxetine, miR-18a level was significantly upregulated than that in the model group. However, miR-134 was not found in the frontal lobe in any of the three groups of mice. In addition, there were no significant differences in the expression level of miR-132, miR-124a and miR-183 among the three groups.

Identification of differentially expressed miRNAs in the hippocampus in mice with CUMS-induced depression

As shown in **Table 2**, the expression of miR-132 and miR-18a in the hippocampus in the model group were significantly lower compared with the control group, whereas the level of miR-134 and miR-124a were significantly higher in the model group than that in the control group. After administrated with duloxetine, the expression of miR-132 and miR-18a was significantly upregulated in the hippocampus relative to model group, while the level of miR-134 and miR-124a was significantly downregulated compared with model group. In addition, No significant difference in miR-183 level was identified among the three groups.

Discussion

Depression has become one of the major concerns of the society. After numerous basic and clinical researches, the drug therapy has been proven to be one of the most important treatment methods [24]. As a selective serotonin and norepinephrine reuptake inhibitor (SNRI), duloxetine has been widely used to treat persistent chronic pain [14] and strong antidepressant [11]. However, the effects of duloxetine on regulatory networks involved in mRNAs expression profile have not been explored in depression.

In this work, we selected the sucrose preference test to represent the behavioral change. Sucrose intake was decreased when mice suffered from CUMS, while duloxetine treatment reversed the reduction in sucrose consumption in the model group. In addition, to assess the effects of duloxetine on locomotor activity and exploratory

behaviors, we recorded the number of crossings, rearings and groomings. Mice in the CUMS group exhibited a decreased number of crossing and rearing times compared with the control group in the OFT. Long-term administration of duloxetine could also reverse these alterations. Taken together, these data provide that duloxetine improves depression-related behaviors in the CUMS mice.

The studies on the mechanisms of depression are mainly concentrated on circadian rhythm disorders, neuroprogression, activation of immune-inflammatory pathways, Oxidative and nitrosative stress [25-28]. Recent study revealed aberrant expression of miRNAs in the brains of patients with neuropsychiatric diseases, especially in the frontal lobe and hippocampus [29, 30]. However, there are few reports about the different expression of specific miRNA between in frontal lobe and hippocampus. Our study revealed that miR-134 is only expressed in the hippocampus and miR-132 is mainly differential expressed in the hippocampus with no obvious change in the frontal lobe. It is reported that chronic stress promotes cog-

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nitive impairment and dendritic spine loss, miR-134 reduces LIMK1 levels which resulting in spine shrinkage while miR-132 activates RAC1 which promoting spine formation in hippocampal neurons, however, in the apical dendrites of CA1 pyramidal neurons, there is no effect on the levels of miR-132 or miR-134 a reduction in spine number [31]. We therefore speculated that the major regulatory networks involving miR-132 and miR-134 have region-specific expression patterns in depression.

There are currently multiple evidence demonstrating that different antidepressant medications may act restoring specific miRNAs regulated networks in several neuropsychiatric diseases [17, 18]. Duloxetine, is an effective of antidepressants in clinic, little is known about the effects of duloxetine on miRNAs expression profile in depression. In this study, we investigated the effects of duloxetine on miRNAs expression profile in the frontal lobe and hippocampus in CUMS-induced mice model of depression. We observed that miR-134 and miR-124a level were significantly increased in hippocampus in the model group, and the elevated expressions were dropped after administration with duloxetine. Furthermore, miR-132 and miR18a level were significantly downregulated in hippocampus in the model group compared with the duloxetine groups. MiR-18a level was significantly downregulated in frontal lobe in the model group compared with the duloxetine groups. These data suggested that the function of duloxetine might be mediated by the modulation of miRNAs expression. However, the functions of these differently expressed miRNAs remain to be verified.

An understanding of miRNAs expression profiles associated with pharmacological treatments is important as these may open up new pathways for targeting depression. In summary, we confirmed that miR-132 and miR-134 have region-specific expression patterns, and duloxetine affected the expression profile of miRNAs in the frontal lobe, and especially in the hippocampus in mice model of depression. Our results suggested that the hippocampus was probably the action site of duloxetine, which presumably worked by regulating the expression levels of relevant miRNAs. Our findings provide a new basis for potential drug targets in

depression, and thus contribute to the diagnosis and treatment of depression at the miRNAs level. However, further in-depth studies will be needed to extend our current findings. For instance, it is necessary for us to expand specific function of these differently expressed miRNAs or under different doses of duloxetine in mice depression model. Such researches may facilitate the understanding of the characteristics of duloxetine including the mechanisms, action site and dosage, which will be extremely useful for medication guidance, especially for patients with different sex or a family history of depression.

Our data showed that miRNAs expression profile in frontal lobe and hippocampus was affected by duloxetine in mice model of depression. The effect was especially pronounced in the hippocampus, suggesting that hippocampus might be the action site of duloxetine, which presumably worked by regulating the expression of miRNAs levels.

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Disclosure of conflict of interest

None.

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