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Original Research Article

Curcumin inhibits expression of brain-derived neurotrophic factor (BDNF) in hippocampus of mice with sleep deprivation

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Abstract

Purpose: *To determine the inhibitory effect of curcumin on the expression of brain-derived neurotrophic factor (BDNF) in hippocampus of mice with sleep deprivation.*
Methods: *Expressions of BDNF and miRNA-206 were evaluated using quantitative real time-polymerase chain reaction (qRT-PCR) in a sleep-deprived mouse model. Interaction of BDNF and miRNA-206 was assessed by dual luciferase and qPCR) assays. The effect of curcumin on BDNF and miRNA-206 was also determined by qRT-PCR.*
Results: *Expressions of BDNF and miRNA-206 were significantly suppressed in hippocampus of mice in sleep deprivation group compared with control mice ($p < 0.05$). Treatment with miRNA-206 significantly suppressed the luciferase activity of wild type (WT) BDNF reporter gene vector ($p < 0.05$) rather than the mutated (mut) vectors. Inhibition of miRNA-206 upregulated RNA and protein levels of BDNF ($p < 0.05$), while curcumin treatment inhibited BDNF expression ($p < 0.01$) and upregulated miRNA-206 level ($p < 0.01$).*
Conclusion: *Curcumin inhibits BDNF expression by upregulating miRNA-206 level in hippocampus tissues of mice in the sleep deprivation model. The relationship between BDNF and miRNA-206 might provide a new direction for the development of new therapeutics for sleep deprivation.*

Keywords: *Curcumin, Brain-derived neurotrophic factor, microRNA 206, Sleep deprivation*

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INTRODUCTION

Over the past decades, the significant biomedical activities of Curcumin have been widely studied in multiple diseases, including cardiovascular diseases and cancers [1-4]. Nevertheless, the role of curcumin in sleep deprivation has not been elucidated yet. Sleep deprivation is a phenomenon that happens when people don't get enough sleep, which has become a common health issue in modern society [5]. It is estimated to affect around one-third of people around the world [6,7]. Lack of sleep directly causes a variety of dysfunctions, such as emotional and cognitive disorders [8]. The hippocampus is an important structure for the maintenance of cognitive memory function [9]. It has been reported that pro-inflammatory and inflammatory

cytokines in the hypothalamus and hippocampus participate in a wide spectrum of pathological disorders such as depressive-like behavior [10]. However, there are relatively few studies on the dynamic changes of cytokines in the hippocampus after sleep deprivation.

Brain-derived neurotrophic factor (BDNF) is a key transducer of antidepressant effects [11,12]. It is reported the up-regulation of BDNF involves sleep deprivation via activating the cerebral cortex and brainstem [11]. Another study revealed that short-term sleep deprivation disrupted the interaction of BDNF and MAPK/pERK signaling pathway [13]. At the same time, sleep deprivation affects both miRNA and BDNF expression and Shiva Shrestha's research has revealed that miRNA-206 can affect the synthesis of BDNF in brain [14]. However, the detailed regulatory order and mechanisms are still unknown. The present work was aimed to determine the differentially expressed genes in sleep deprivation and to investigate the detailed regulatory mechanism between BDNF and miRNA-206 in sleep deprivation of mice.

EXPERIMENTAL

Mouse sleep deprivation model

The C57BJ6 mice (n = 20) obtained from GemPharmatech Co., Ltd., Jiangsu, China were fed for one week to acclimation. The sleep deprivation room was kept in light (9:00 ~ 21:00) and then altered to darkness (21:00 ~ 9:00), and the light was simulated by 40 W lamp. The indoor temperature was kept at 24 ± 1 ℃, and the relative humidity was maintained at 40 - 70 %. Ten mice were used for construction of sleep deprivation group for 3 days via modified multi-platform water environment method (MMPM), and the remaining 10 mice was used as the control group. All experiments were authorized by the Ethic Committee of Jiangnan University (approval no. 2021-3-025) and performed following the updated Guide for the Care and Use of Laboratory Animals of the National Research Council (US) Committee [15].

Sleep-deprived mice and control mice were injected intraperitoneally with 10 % chloral hydrate. After that, the thoracic cavity was cut open and the heart, lung and liver were exposed, then the right atrial appendage was cut and scalp needle was inserted into the left ventricle. The other end of the scalp needle was connected to the left ventricle. Subsequently, pre-cooled PBS (100 mL, 0.1 mol/L) was fast perfused until the liquid becomes clear and colorless and the lung and liver become white, indicating the sufficient perfusion. Next, the perfusion was stopped, and the brain tissues of mice were removed. The hippocampus on both sides were collected to store at -80 °C after quick freezing in liquid nitrogen.

Dual-luciferase reporter gene assay

The 3'-untranslated region (UTR) of mouse BDNF gene was cloned and inserted to a firefly luciferase reporter psi-CHECK2 (Promega, USA) to obtain wild-type luciferase plasmids (wt-BDNF-3′UTR). Similarly, the predicted interaction sequence of miR-206 on BDNF 3' UTR was mutated. After that, the mutated BDNF sequences was inserted into psi-CHECK2 to obtain mutated BDNF luciferase plasmids (mu-BDNF-3' UTR). Then miR-206 mimics and wild-type or mutated luciferase reporter plasmids were co-transfected into HEK293T cells that seeded into a six-well plate. After incubation for 48 h, the luciferase activity was measured by a dual-luciferase reporter assay kit (Promega, USA) in line with manufacturer's description. Renilla reporters were transfected as internal control.

Plasmid construction and transfection

The BDNF gene sequence was obtained from Genebank, and specific primer was designed. pHBLV-CMVIE-Zs Green-Puro vector was digested using EcoR I and BamH I enzyme. The BDNF fragment was inserted into the vectors, *viz*,

m-BDNF-EcoR I-F: gaattc gccacc ATGTTCCACCAGGTCAGAAGA

m-BDNF-BamH I-R: ccgaggatcc TCTTCCCCTTTTAATGGTCAG

Cell transfection

The miRNA-206 mimics, miRNA-206 inhibitors, BDNF siRNA and negative control RNA were synthesized by Genepharma (Shanghai, China). The plasmid and RNAs was transfected into astrocytes using lipo2000 reagent according to the instructions provided by the manufacturer. After 48 h, total RNA of the cell line was extracted, and qPCR was performed to determine the expression of miR-206 and BDNF. The control group used unintentional fragment overexpression vector or overexpression empty.

Quantitative real time-polymerase chain reaction (PCR)

The total RNA was extracted from cells using TRizol reagent (Sigma, USA). The cDNA was synthesized using First strand synthesis kit

(Transgen, China) according to the manufacturer's instruction. Gene expression was measured using the SYBR Green Mixture (Takara, Japan). GAPDH and U6 were used for normalization of mRNA and miRNA, respectively.

Statistical analysis

The experimental processes in this work were performed in triplicates. Results are presented as mean ± standard deviation (SD). Statistical difference was determined using the unpaired Student's *t*-test by SPSS software. $P < 0.05$ was set as statistically significant.

RESULTS

MiRNA-206 and BDNF down-regulation in hippocampus

Sleep deprivation model of mice was established and the levels of miRNA-206 and BDNF were analyzed. It was obvious that sleep deprivation significantly downregulated expressions of miRNA-206 and BDNF in contrast to the control group (Figure 1 A and C). Moreover, the protein level of BDNF was almost half of the control group, which indicated that there existed interaction between miRNA206 and BDNF (Figure 1 A and C).

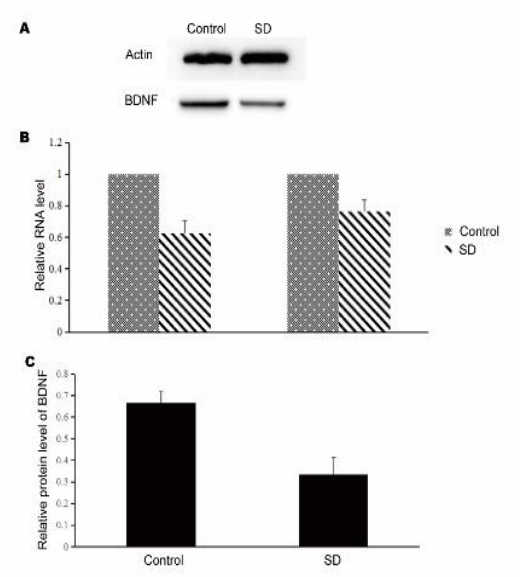

Figure 1: (A) miRNA in hippocampus of mice with or without sleep deprivation. (B and C) mRNA and protein levels of BDNF

Relationship between BDNF miRNA-206 and curcumin

In order to validate the bioinformatics analysis, a dual-luciferase assay was performed in 293T cells. The activity of the reporter plasmid with psi-CHECK2 WT in the presence of miRNA-206 mimics was significantly lower than those of cells transected with another miRNA and psiCHECK2, indicating that BDNF was specifically down-regulated by miRNA 206 (Figure 2). Therefore, it indicated that miRNA-206 can bind to the 3'UTR of BDNF. Besides, overexpression or silence of BDNF did not affect the expression of miRNA-206 (Figure 3), whereas treatment with miRNA-206 mimics can up-regulate the mRNA and protein level of BDNF (Figure 3). Moreover, inhibition of miRNA-206 also significantly down-regulated the mRNA and protein level of BDNF. Significantly, the expression of miRNA-206 was enhanced by curcumin in sleep deprivation mice and the expression of BDNF was reduced by Curcumin, while the inhibition of miRNA-206 reversed the effect of curcumin in the mice (Figure 4).

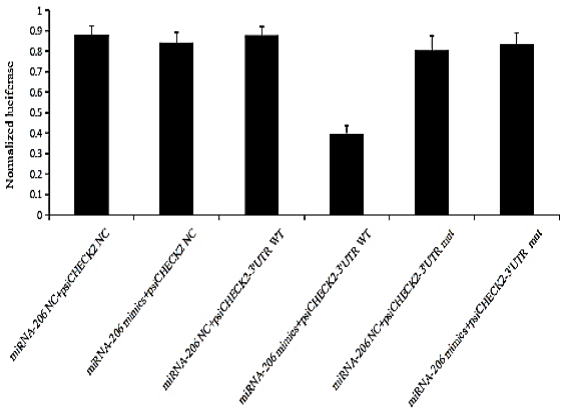

Figure 2: Dual luciferase activity of wild-type and mutant psiCHECK2-3'UTR expression in 293T cells. The psiCHECK2 NC vector was used as a control

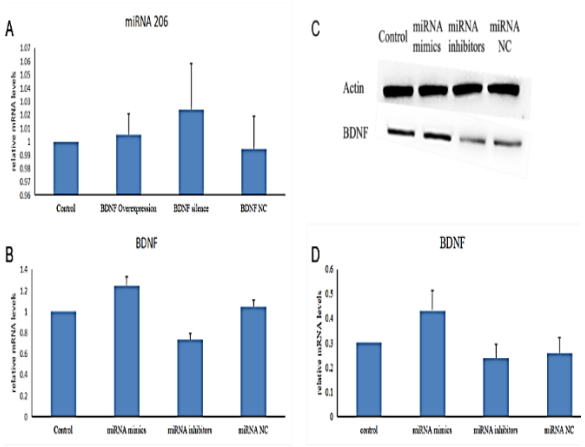

Figure 3: The effect of overexpression/silencing of (A and C) miRNA or (B and D) BDNF on mRNA and protein level of BDNF or miRNA

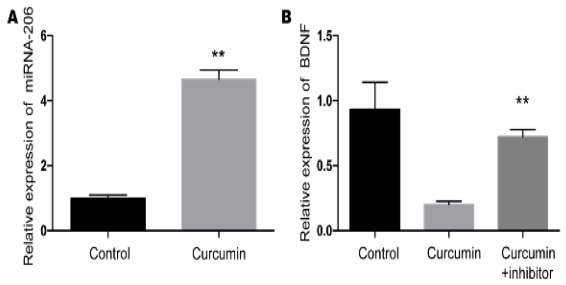

Figure 4: The effect of curcumin on (A) miRNA and (B) BDNF in hippocampus of mice with sleep deprivation

DISCUSSION

Sleep is an imperative process that facilitates the recovery of energy for human beings, whereas pre-competition thoughts or nervousness leads to sleep deprivation [16]. It was reported that sleep deprivation is involved in development of multiple diseases, such as diabetes, obesity, and cardiovascular diseases [17], which has become an important health and public safety issue. Sleep deprivation leads to stress response and is correlated to anxiety, depression and cognitive decline [6]. Studies have proved that BDNF is a central neurotrophic factor that expressed in central nervous system, especially in hippocampus tissues, and there is strong evidence that BDNF plays a critical role in regulating sleep [18]. Increasing number of studies have shown decreased BDNF level in chronic sleep deprivation or stress [19], but acute sleep deprivation or stress was associated with fast increase in serum BDNF levels [20]. Other evidence showed that micro-RNA (miRNA) levels in brain could be altered by sleep deprivation [21]. And the bio-informatics analysis in the study revealed that both BDNF and miRNA-206 were differentially expressed genes in sleep deprivation [22]. Therefore, it was necessary to study the molecular mechanisms underlying miRNA-206 and BDNF-correlated sleep deprivation in mice.

The results from dual luciferase experiment verified that miRNA-206 is capable of binding with the 3'UTR region of BDNF, which was in accordance with previous literature [23]. However, the regulatory order of miRNA-2016 and BDNF is still unknown. Here, the overexpression or silencing of miRNA-206 was performed to determine the alteration of BDNF expression. Results showed that treatment with miRNA-206 decreased BDNF level significantly, whereas inhibition of miRNA-206 elevated the BDNF expression. Similarly, over-expression or inhibition of BDNF was adopted to assess the expression of miRNA-206. However, the over-expression or inhibition of BDNF did not show obvious effects on the level of miRNA-206. Therefore, the data obtained in this study indicated that miRNA-206 regulated the expression of BDNF in the upstream.

CONCLUSION

Curcumin inhibits brain-derived neurotrophic factor (BDNF) expression in sleep deprivation mouse model by modulating miRNA-206 expression. The relationship between BDNF and miRNA-206 might provide a new approach for the treatment of sleep deprivation.

DECLARATIONS

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None provided.

Ethical approval

None provided.

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 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. Sha-sha Ruan and Tao Ma designed the study. Sha-sha Ruan, Pei-cheng He, and Qin-qin Cao performed the experiments. Sha-sha Ruan and Tao Ma wrote the manuscript. All authors read and approved the final version of the manuscript.

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