

Original Research Article

Edaravone mitigates cognitive impairment and hippocampal injury in juvenile rats with obstructive sleep apnea hypopnea syndrome via regulation of cAMP/PKA-CREB pathway

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Abstract

Purpose: To investigate the influence of edaravone on cognitive impairment and hippocampal injury in juvenile rats with obstructive sleep apnea hypopnea syndrome (OSAHS), and the mechanism involved.

Methods: Fifty-four young Wistar rats were randomly selected into control, intermittent hypoxia and edaravone groups. The contents of the antioxidants CAT, Mn-SOD, Cu/Zn SOD and oxidative stress products malondialdehyde (MDA) in hippocampus were assayed and compared. The expressions of brain-derived neurotrophic factor (BDNF), Bcl-2, CREB, p-CREB and PKAc were determined.

Results: The times taken to cross the target quadrant and the platform; levels of CAT and Mn-SOD, as well as protein levels of BDNF, Bcl-2, p-CREB and PKAc were markedly lower in intermittent hypoxia group than in controls; and MDA contents, 8-OHdG and protein hydroxyl were markedly higher in intermittent hypoxic rats group than in controls. Time taken to cross the platform and quadrant; activities of CAT and Mn-SOD, and protein concentrations of BDNF, Bcl-2, p-CREB and PKAc were markedly higher in the edaravone-treated rats than in intermittent hypoxia rats.

Conclusion: Edaravone significantly mitigated cognitive damage and hippocampal lesions in OSAHS rats via a mechanism related to alleviation of oxidative stress and up-regulation of the expressions of p-CREB and its downstream proteins BDNF and Bcl-2. This finding provides a theoretical basis for research and development of new drugs against OSAHS.

Keywords: Obstructive sleep apnea hypopnea syndrome, Edaravone, cAMP, PKA, CREB

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INTRODUCTION

In children, OSAHS is characterized by incomplete or total blockage of the upper respiratory tract during sleep. It causes damage

to many organs, and leads to memory deterioration, mental retardation, cognitive and behavioral disorders, elevated blood pressure, metabolic disorders and changes in the heart structure [1,2]. A survey has shown that the

prevalence of OSAHS in China is about 3.4 %. Proadenoidal tonsil hypertrophy in children is the main cause of OSAHS. Therefore, clinical treatment of the disease involves tonsillectomy and adenoidectomy which effectively improve ventilation [3,4]. However, factors that induce OSAHS in children are multiple levels of obesity and neuromuscular lesions, leading to the higher incidence of persistent OSAHS which require tonsillectomy and adenoidectomy [5]. Therefore, it is important to understand the pathogenesis of OSAHS so as to evolve new treatment methods with improved clinical efficacy.

Edaravone is a free radical-scavenging and antioxidant drug with small molecular weight. At present, it is mainly used in clinical treatment of acute cerebral infarction and other diseases, with ideal clinical efficacy and high degree of safety [6]. Clinical studies have confirmed that edaravone relieves impairment in neurobehavior and cognitive function caused by ischemia and hypoxia [7]. However, not much has been reported on its effect on OSAHS-induced cognitive impairment. Clinical reports confirm that the OSAHS-induced organ damage is associated with oxidative stress response, while cAMP/protein kinase A (PKA) reaction element binding (CREB) route is associated with cognitive function [8, 9]. However, there are limited studies on the involvement of this pathway in OSAHS. This research was aimed at studying the effect of edaravone on OSAHS-induced cognitive impairment and hippocampal injury in juvenile rats, and the role of hippocampal cAMP/PKA-CREB route in the process.

EXPERIMENTAL

Animals

Fifty-four juvenile Wistar rats aged 3 - 4 weeks were purchased from Zhaoyan New Drug Research Center Co. Ltd [production license no. SCXK (Su) 2020-0015]. All rats were kept in the same environment at a temperature at 25 °C and humidity of 40 %, with free access to feed and water. This study received approval from the Animal Ethics Committee of West China Second Hospital and was conducted in line with "Principles of Laboratory Animal Care" [10].

Main reagents and instruments

Edaravone was purchased from Beijing Eta Biotechnology Co. Ltd. Paraformaldehyde (4%) was obtained from Lanzhou Weiboxin Biotechnology Co. Ltd. Hematoxylin dyeing solution was bought from Chuzhou Snorda Biological Technology Co. Ltd. BCA protein

quantitative kit was product of Shenyang Wanjie Biotechnology Co. Ltd; BDNF and Bcl-2 were purchased from Shanghai Fanwei Biotechnology Co. Ltd, while CREB antibody was obtained from Shanghai Fanwei Biotechnology Co. Ltd. The p-CREB antibody was purchased from Jiangxi Jiang Blue Pure Biological Reagent Co. Ltd, while PKAC antibody was bought from Shanghai Caiyou Industrial Co. Ltd.

Oxygen meter was purchased from Lubo Weiye Environmental Protection Technology Co. Ltd. Low-temperature high speed centrifuge was product of Shanghai Hengfei Biotechnology Co. Ltd. Precision balance was purchased from Nanjing Baden Medical Co. Ltd. Morris Water Maze and its imaging system were bought from Shanghai Sieger Biotechnology Co. Ltd. Optical microscope was purchased from Wuhan Purity Biotechnology Co. Ltd. Automatic embedding machine was product of Hubei Botai Electronic Technology Co. Ltd.

Treatments

A total of 18 out of the 54 juvenile Wistar rats were randomly selected as the control group. The rats were placed in a hypoxia chamber from 9:00 a.m. to 5:00 p.m. daily, with intermittent air treatment, but without any drug intervention. The remaining 36 rats were placed in the hypoxia chamber from 9:00 a.m. to 5:00 p.m. every day, for establishment of intermittent hypoxia. The intermittent hypoxia model rats were randomly divided into edaravone group and intermittent hypoxia group, with 18 rats in each group. Rats in the intermittent hypoxia group did not receive any drug intervention. The edaravone group was given edaravone at a dose of 5 mg/kg via intraperitoneal injection before daily exposure to intermittent hypoxia.

Evaluation of biochemical parameters

After 5 days of treatment, positioning navigation and space exploration tests were used to determine the time taken to cross the target quadrant, the number of times the rats crossed the platform, as well as their learning and memory abilities were evaluated.

H & E staining

The rats were sacrificed under intratracheal instillation anesthesia using the abdominal aorta depletion method. Whole brains were excised and fixed in 10 % neutral formalin for 48 h. Then, the bilateral hippocampal tissues were removed and separated. Pathological changes in neurons

in CA1 and CA3 hippocampal areas were analyzed using H & E dyes.

Evaluation of hippocampal tissues

The contents of catalase (CAT), malondialdehyde (MDA) and protein hydroxyl group in hippocampal tissues of rats were determined using CAT decomposition reaction, thiobarbituric acid method, and Levine's method, respectively. The activities of Mn-SOD and Cu/Zn-SOD, as well as levels of 8-OHDG in hippocampal tissues of rats were determined using ELISA. The hippocampal tissues of rats in each group were subjected to western blot assay for determination of protein expression levels of brain-derived neurotrophic factor (BDNF), Bcl-2, CREB, p-CREB and PKAC. The kits used were purchased from Jianchen Biotech (Nanjing, China).

Statistical analysis

Measurement data for activities CAT, Mn-SOD and Cu/Zn-SOD which conformed to normal distribution are presented as mean \pm SD. Comparison amongst groups was done using one-way ANOVA. Two-group comparison was done with SNK-Q test. All statistical analyses were done with SPSS version 22.0. Values of $p < 0.05$ indicated significant differences.

RESULTS

Learning and memory abilities

The times taken to cross the target quadrant and the platform in the intermittent hypoxia group were markedly lower than those in controls ($p < 0.05$). In contrast, the time taken to cross the target quadrant and the platform were markedly higher in edaravone-exposed rats than in intermittent hypoxic rats. These results are presented in Table 1.

Pathological changes in hippocampal tissues

In the control group, neurons in hippocampal CA1 and CA3 zones were arranged neatly, the cytoplasm and nucleus were visible, and there were no obvious pathological changes. In the

intermittent hypoxia group, the spaces around the neurons in hippocampal CA1 and CA3 regions were increased, and the morphological integrity of the cells was damaged; the nuclei were degenerated, and the nucleoli were unclear. In contrast, the morphology and structure of hippocampal CA1 and CA3 neurons in edaravone group were significantly improved, when compared with the intermittent hypoxia group. These results are shown in Figure 1.

Table 1: Learning and memory abilities amongst the groups (mean \pm SD, n = 18)

Group	Time taken to cross the target quadrant (s)	Time taken to cross the platform (s)
Control	43.21 \pm 5.22	5.96 \pm 1.46
Intermittent hypoxia	30.22 \pm 7.48*	3.41 \pm 1.23*
Edaravone	39.90 \pm 8.07#	5.26 \pm 1.60#
F	16.590	15.11

* $P < 0.05$, vs control; # $p < 0.05$, vs intermittent hypoxic rats

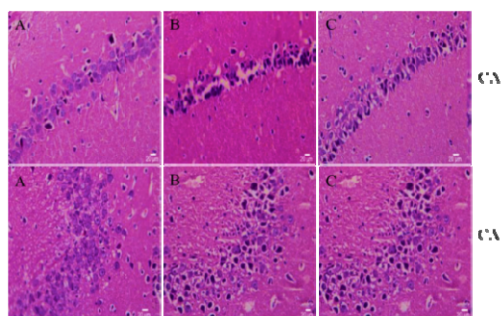


Figure 1: Pathological and morphological changes in the hippocampus (H&E staining, $\times 200$). A: control; B: intermittent hypoxia; C: edaravone-treated

Levels of antioxidant enzymes in hippocampal tissues

The contents of CAT and Mn-SOD in intermittent hypoxic rats were markedly reduced, relative to control. However, CAT and Mn-SOD contents in edaravone-exposed rats were markedly higher than the corresponding levels in intermittent hypoxic rats. There was no significant difference in Cu/Zn-SOD content amongst the groups ($p > 0.05$). These results are shown in Table 2.

Table 2: Comparison of CAT, Mn-SOD and Cu/Zn-SOD contents in hippocampus (mean \pm SD, n = 18)

Group	CAT (U/mg)	Mn-SOD (pg/mL)	Cu/Zn-SOD (pg/mL)
Control	24.10 \pm 3.11	150.93 \pm 11.69	1441.72 \pm 223.43
Intermittent hypoxia	14.90 \pm 2.40*	119.32 \pm 11.05*	1322.50 \pm 233.02
Edaravone	21.15 \pm 4.28#	140.61 \pm 9.31#	1259.78 \pm 229.22
F	35.310	40.620	2.940

* $P < 0.05$, vs control; # $p < 0.05$, vs intermittent hypoxic rats

Hippocampal levels of oxidative stress products

The levels of MDA, 8-OHDG and protein hydroxyl in hypoxic rats were markedly higher than the control values. However, MDA, 8-OHDG and protein hydroxyl contents in edaravone group were markedly lower than the corresponding levels in intermittent hypoxia groups ($p < 0.05$; Table 3).

Hippocampal tissue protein levels of PKAC, CREB, p-CREB, BDNF and Bcl-2

There were significantly downregulated protein levels of BDNF, Bcl-2, p-CREB and PKAC in intermittent hypoxic rats, relative to control rats. However, the expressions of BDNF, Bcl-2, p-CREB and PKAC in the edaravone group were markedly up-regulated, relative to the corresponding levels in intermittent hypoxic rats. There was no appreciable difference in CREB protein level between the control and intermittent hypoxia groups. These results are shown in Table 4.

DISCUSSION

It has been reported that edaravone significantly inhibited tissue edema induced by ischemia or embolism in animals [11, 12]. In addition, edaravone significantly mitigated hippocampal oxidative stress-induced damage caused by sleep deprivation, through a mechanism linked to the normalization of oxidative balance [13]. However, there are limited studies on the effect of edaravone on OSAHS at present. Therefore, it is of great significance to investigate the therapeutic effect of edaravone on cognitive dysfunction in children with OSAHS.

The results from this research revealed that the times taken to cross the target quadrant and the platform were markedly shorter in the hypoxia group than the corresponding times in control group. However, the times taken to cross the target quadrant and the platform in the edaravone group were significantly longer than those in intermittent hypoxia group. These results suggest that the decline in memory of young mice as a result of intermittent hypoxia was alleviated using edaravone intervention. The morphology and structure of neurons in hippocampal CA1 and CA3, which were seriously damaged in intermittent hypoxia group, were significantly improved by edaravone. These results suggest that intermittent hypoxia caused severe damage to hippocampal nerve cells in young rats, but this damage was significantly alleviated with edaravone.

Oxidative stress occurs in OSAHS patients due to chronic intermittent hypoxia and reoxygenation, and it is usually manifested as an raised superoxide content and reduced antioxidant enzyme content. These lead to a decrease in total antioxidant capacity in the body, resulting in damage to tissues and organs [14,15]. In this study, SOD and antioxidant enzyme-related indices were used to evaluate the degree of oxidative stress in rats in each group. The results showed that the contents of CAT and Mn-SOD were markedly reduced in the intermittent hypoxic rats, relative to the control group, while MDA, 8-OHDG and protein hydroxyl groups were significantly higher.

However, CAT and Mn-SOD contents were markedly higher in edaravone group than in intermittent hypoxia group. This indicates that intermittent hypoxia provoked oxidation-linked stress response in the animals, but edaravone neutralized the ROS and oxidative imbalance,

Table 3: Comparison of levels of MDA, 8-OHDG and protein hydroxyl groups in hippocampus (mean \pm SD)

Group	MDA (nmol/mg)	8-OHDG (ng/mL)	Protein hydroxyl (nmol/mg)
Control	1.75 \pm 0.33	0.49 \pm 0.07	3.62 \pm 0.64
Intermittent hypoxia	5.26 \pm 0.49*	0.64 \pm 0.09*	5.73 \pm 0.95*
Edaravone	2.04 \pm 0.42#	0.52 \pm 0.06#	3.98 \pm 0.81#
F	390.090	20.490	40.720

* $P < 0.05$, vs control; # $p < 0.05$, vs intermittent hypoxia group

Table 4: Comparison of protein expression levels of BDNF, Bcl-2, CREB, p-CREB and PKAC in hippocampal tissues (mean \pm SD)

Group	BDNF	Bcl-2	p-CREB	CREB	PKAc
Control	0.78 \pm 0.02	0.36 \pm 0.02	0.75 \pm 0.03	0.86 \pm 0.05	0.52 \pm 0.03
Intermittent hypoxia	0.63 \pm 0.01*	0.22 \pm 0.01*	0.38 \pm 0.02*	0.87 \pm 0.01	0.28 \pm 0.02*
Edaravone	0.79 \pm 0.03#	0.33 \pm 0.03#	0.71 \pm 0.04#	0.85 \pm 0.01#	0.48 \pm 0.04#
F	309.860	209.570	767.790	0.670	307.860
P-value	<0.001	<0.001	<0.001	0.518	<0.001

* $P < 0.05$, vs control; # $p < 0.05$, vs intermittent hypoxia group

thereby mitigating hippocampal injury. It has been reported that hypoxia induced decreases in p-CREB expression, leading to a significant decrease in learning and memory ability of animals in water maze and avoidance tests [16]. However, treatment with N-methyl-D-aspartic acid receptor antagonist significantly increased the expression level of p-CREB [16].

Activation of cAMP/PKA pathway led to increased mitochondrial activity, thereby reducing the release of ROS, indicating that edaravone not only eliminated ROS through single electron transfer, but also increased the ROS clearance intensity through activation of this pathway [17-20]. It is known that BDNF is a downstream molecule of phosphorylated CREB gene transcription, and its levels are highest in CNS, hippocampus and cortex. Moreover, BDNF regulates the protrusion plasticity of hippocampal neurons and mediates long-term enhancement of related links in the hippocampus. Dead weight leads to changes in learning and memory ability. Another downstream molecule of CREB-mediated gene transduction is Bcl-2 which is an anti-apoptotic factor. It has been reported that overexpression of Bcl-2 significantly reduced the release of oxygen-free radicals and generation of lipid peroxides through suppression of the release of superoxide anions.

The results of this study showed that the protein expression levels of BDNF, Bcl-2, p-CREB and PKAC in the intermittent hypoxia group were markedly smaller than control values, but they were markedly up-regulated in edaravone group, relative to intermittent hypoxia group. These results suggest that the cAMP/PKA-CREB pathway may be involved in the development of cognitive dysfunction caused by intermittent hypoxia, and edaravone may play a mitigating role in cognitive impairment and hippocampal injury in young mice by regulating this pathway.

CONCLUSION

Edaravone significantly alleviates cognitive dysfunction and hippocampal injury in OSAHS rats via mechanisms involving mitigation of oxidative stress response and up-regulation of p-CREB and its downstream molecules, BDNF and Bcl-2. This finding provides a theoretical basis for research and development of new drugs against OSAHS.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors 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 them.

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