ORIGINAL ARTICLE

Oxidative Stress is not Involved in Motion Sickness in Mice

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Keywords

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SUMMARY

Aims: Some indirect evidences indicate a possible correlation between oxidative stress and motion sickness. The aim of this research was to investigate whether oxidative stress contributing to motion sickness in mice or not. **Methods:** We examined the mRNA levels of peroxiredoxin 6 (PRDX6), catalase, and enzyme superoxide dismutase 1 (SOD1); reactive oxygen species (ROS); and total antioxidant capacity and SOD activity in different brain regions after rotary stimulation. Mice motion sickness index was recorded after rotation when pretreated with paraquat, vitamin C, or vitamin E. **Results:** The ROS level and antioxidant capacity were both increased in cerebellum plus brainstem (CB) after rotation, a critical region determines motion sickness. However, manipulation of oxidants or antioxidants using pharmacological method *in vivo* had no influence on motion sickness index in mice. **Conclusion:** Oxidative stress is not involved in the development of motion sickness in mice.

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The first two authors contributed equally to
this work.

Introduction

Motion sickness (e.g., sea sickness, air sickness, car sickness, and space sickness) is a common disease and can occur in any person with a functional vestibular system [1,2]. Many theories have tried to explain the pathogenesis of motion sickness, such as "neural mismatch theory," "neurotransmitters theory," and "neurotoxins theory" [3–5]. However, the exact mechanism of motion sickness is still unclear.

Oxidative stress is a term used to describe the situation that the organism fails to neutralize the production of oxidants, which can be defined as an increase in oxidants levels and/or a decrease in antioxidant capacity. Oxidative stress can result in damages to cell membranes, lipids, nucleic acids, proteins, and constituents of the extracellular matrix. In humans, oxidative stress has been observed in a number of acute and chronic diseases of the central nervous system, such as epilepsy, migraine, and Parkinson's disease [6–9]. So far, little is known about whether oxidative stress occurs in motion sickness or not.

It has been demonstrated that migraine may be associated with increased oxidative stress [10,11]. Alp et al. [7] reported total oxidant/antioxidant was increased in patients with migraine without aura. Antioxidant enzyme superoxide dismutase (SOD) activities were lower, whereas peroxide levels were higher in migraine sufferers [12,13]. These findings indicate possible exposure to oxidative stress in migraine patients. It is known that motion sickness is commendably linked with migraine. Motion sickness and migraine exhibit common symptoms such as nausea, dizziness, and headache. Moreover, migraine patients have an augmented vulnerability to motion sickness [14]. In addition, high level of oxidative stress is one of the contributing causes of vertigo [15]. Hence, it is possible that oxidative stress may occur in the progress of motion sickness.

The present work was therefore designed to investigate gene expression of antioxidant enzymes including peroxiredoxin 6 (PRDX6), catalase, and SOD1; the reactive oxygen species (ROS) content; total antioxidant capacity and SOD activity in different brain regions of mice after rotary stimulation; and

the effect of oxidant paraquat and antioxidant vitamin E or vitamin C on motion sickness degree induced by biaxial rotation in mice.

Materials and Methods

Materials

1,1'-Dimethyl-4,4'-bipyridinium dichloride (paraquat), vitamin E, vitamin C, and 2',7'-dichlorofluorescein-diacetate (DCFH-DA) were purchased from Sigma-Aldrich (Shanghai, China). Total superoxide dismutase assay kit was purchased from Jiancheng Bioengineering Institute (Nanjing, China). Total antioxidant capacity assay kit was purchased from Beyotime (Haimen, China). Trizol, oligo d(T)₁₈ primers, dNTP mixture, ribonuclease inhibitor, reverse transcriptase M-MLV, premix Ex Taq, and SYBR premix Ex Taq were all purchased from TaKaRa Biotechnology (Dalian, China).

Animals

C57BL/6J male mice weighing 18–22 g were purchased from SLRC Laboratory Animal Ltd (Shanghai, China). Mice were housed at a controlled temperature of $22 \pm 2^\circ\text{C}$, relative humidity of 50–60%, lighting (8:00–20:00), and allowed free access to standard dry diet and tap water *ad libitum*. All animals received humane care, and experimental procedures were in compliance with institutional animal care guidelines.

Motion Stimulus Execution and MS Symptoms Measurement

Rotational stimulation was performed according to the procedure described as before. Briefly, each mouse was enclosed in an individual centrifuge cage (diameter = 20 cm, height = 25 cm). The swing arm on which the animal cage was suspended was mounted 60 cm from the axis of a turntable driven by a servo-controlled torque motor. The device started to rotate in the clockwise direction at a constant angular acceleration of $40^\circ/\text{s}^2$. When the angular velocity reached $240^\circ/\text{s}$, it began to slow down at a constant angular deceleration of $40^\circ/\text{s}^2$. Without pause, the device rotated again in the counterclockwise direction in the same manner. Mice were stimulated by rotating for 40 min and then put on the ground for 5 min. Motion sickness symptoms were observed and recorded. The score of motion sickness symptoms was obtained according to the previously depicted evaluation criteria [16–18].

Quantitative Real-Time RT-PCR

Total RNA was extracted from tissue homogenates of mouse different brain regions with Trizol. After reverse transcription, complementary DNA was used as templates for PCR. Quantitative real-time RT-PCR was carried out on 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) by using the following primers (forward and reverse, respectively) as shown in Table 1. The housekeeping gene, GAPDH, was used to normalize all tested genes, and quantification of mRNA level was performed using the

Table 1 Primer sequences (forward and reverse, respectively) of antioxidants

Genes	Primer sequences
<i>PRDX6</i>	f 5' GGGCAGGAACCTTGATGAGATT 3' r 5' TTCGGAGAGGGTGGGAACCTA 3'
<i>Catalase</i>	f 5' TTTGACAGAGAGCGGATTC 3' r 5' ACACCTTTGCCTTGAGTAT 3'
<i>SOD1</i>	f 5' AGGAGAGCATTCCATCATTG 3' r 5' CCCAGCATTTCCAGTCTTT 3'
<i>GAPDH</i>	f 5' AAATGGTGAAGGTCGGTGTG 3' r 5' AGGTCAATGAAGGGTCGTT 3'

PRDX6, peroxiredoxin 6; *SOD1*, superoxide dismutase 1.

Ct method. The value of each control sample was set at 1 and used to calculate the fold change of target genes.

Tissues ROS Assay

Dichlorofluorescein-diacetate was used as chromogenic agent. DCFH-DA can freely pass through the cell membranes into the cells, which can be hydrolyzed to 2',7'-dichlorofluorescein (DCFH). ROS oxidize DCFH to DCF with green fluorescence. ROS activity was defined as the amount of fluorescence intensity carried out using infiniteM200 Multi-Mode Microplate Reader (TECAN, Grödig, Austria). H_2O_2 was used to establish standard curve. ROS amount was equivalent to H_2O_2 concentration.

Total Antioxidant Capacity Assay

The determination of antioxidant capacity assay was performed by using commercial kit. 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS)) was used as chromogenic agent. Oxidants oxidize ABTS to ABTS^+ with green color. The assay for total antioxidant capacity was based on its ability to inhibit the generation of ABTS^+ . Antioxidant capacity was defined as the amount that reduced the absorbance at 405 nm. Trolox was used to establish standard curve. Total antioxidant capacity was equivalent to Trolox concentration named Trolox equivalent antioxidant capacity (TEAC).

Total SOD Activity Assay

The determination of SOD activity was performed by using commercial total SOD kit with nitroblue tetrazolium, which could be reduced by O_2^- to formazan. The later has an absorbance at 560 nm. The assay for total SOD activity was based on its ability to clear the superoxide anion free radical of O_2^- , which is generated by the xanthine-xanthine oxidase system. SOD activity was defined as the amount that reduced the absorbance at 560 nm.

Pharmacological Interference of Oxidants and Antioxidants in Mice

Experiment I

0.2 mL of paraquat (30 mg/L or 300 mg/L, intragastric) was given to each mouse 1 h before rotary stimulus. Control group was

given same amounts of 0.9% NaCl saline. Motion sickness index was recorded after a 40-min biaxial rotation.

Experiment II

Vitamin C (200 mg/kg/day, intragastric) was given to each mouse for 2 weeks. Control group was given same amounts of 0.9% NaCl saline. Motion sickness index was recorded after a 40-min biaxial rotation.

Experiment III

Vitamin E (100 mg/kg/day, intragastric) was given to each mouse for 2 weeks. Control group was given same amounts of corn oil. Motion sickness index was recorded after a 40-min biaxial rotation [19].

Results

Antioxidant Enzyme mRNA Levels in Motion Sickness

After 40-min rotary stimulation, mice were killed by cervical dislocation and the brains were gained. Then, the brain was divided into three pieces: cerebellum plus brainstem (CB), cerebral cortex, and others (Figure 1). We examined *PRDX6*, catalase, and *SOD1* mRNA levels in different brain regions between control and rotation group. It was found that *PRDX6* was increased in CB dramatically after rotation (Figure 2A). *Catalase* was increased in CB and decreased in cerebral cortex significantly after rotation, respectively (Figure 2B). *SOD1* mRNA level was changed in all three regions after rotation, with an increase in CB and decreases in cerebral cortex and others (Figure 2C). The increases of *PRDX6*, *catalase*, and *SOD1* indicated a lifting of antioxidant activity in CB after rotary stimulation.

ROS Content and Total Antioxidant Capacity in Motion Sickness

The increased antioxidants mRNA levels in CB after rotation suggested oxidative stress possibly participated in motion sickness. We next examined ROS level in the three brain regions after motion stimulus. It was showed a significant increase of ROS content in CB after 40-min rotation (Figure 3A), which indicated oxidant levels were increased in CB after rotary stimulation. However, total antioxidant capacity was also increased in CB, which might defense the oxidants damage (Figure 3B).

Total SOD Activity in Motion Sickness

We have known the mRNA level of *SOD1* enzymes was increased in CB after rotation. We also detected the practical SOD activity in brain and serum in motion sickness. SOD activity was increased in CB (Figure 4A). Meanwhile, SOD activities in cerebral cortex and other brain region were decreased after stimulation (Figure 4A). As for serum, no difference was found between control and rotation group (Figure 4B).

Effect of Paraquat, Vitamin C, and Vitamin E in Motion Sickness

Considering the possible role of oxidative stress in motion sickness, we next observed the effects of antioxidant drugs (vitamin C and vitamin E) and oxidation drug (paraquat) in the development of motion sickness. Disappointingly, paraquat in 30 mg/L and 300 mg/L both failed to show any effect in increasing motion sickness index (Figure 5A). Meanwhile, vitamin C and vitamin E continuous administration of 2 weeks also could not decrease motion sickness index in mice (Figure 5B,C).

Figure 1 Mouse brain was divided into three pieces: cerebellum plus brainstem (green color), cerebral cortex (red color), and others (blue color).

Figure 2 Gene expressions of PRDX6 (A), catalase (B), and superoxide dismutase 1 (SOD1) (C) in different brain regions including cerebellum and brainstem (CB), cerebral cortex, and others after rotation stimulation. $n = 6$ mice in each group. * $P < 0.05$ compared with control group.

Figure 3 Reactive oxygen species (ROS) levels (A) and Trolox equivalent antioxidant capacity (TEAC) (B) in different brain regions including cerebellum and brainstem (CB), cerebral cortex, and others after rotation stimulation. $n = 6$ mice in each group. * $P < 0.05$ compared with control group.

Figure 4 (A) Total superoxide dismutase (SOD) activity in different brain regions including cerebellum and brainstem (CB), cerebral cortex, and others after rotation stimulation. (B) Total SOD activity in blood serum after rotation stimulation. $n = 6$ mice in each group. * $P < 0.05$ compared with control group.

Discussion

Here, we proved that the oxidation level and the antioxidant ability were both increased in CB after rotary stimulation in mice. However, the balance of oxidant/antioxidant was not tended to oxidant. These results indicated mice against excessive ROS by antioxidant compensatory when motion sickness occurred.

The vestibular system provides the leading contribution on movement and sense of balance or spatial orientation [20]. Signals

from the vestibular system also project to the cerebellum, a structure that also plays an important role in motor control and motion sickness. Vestibular nuclei and cerebellum are situated at the floor and the ceiling of the 4th ventricle, respectively. In addition, nuclei involved in autonomic regulation (e.g., nucleus of the tractus solitarius, dorsal motor nucleus of the vagus, reticular formation, and area postrema) are situated immediately below the 4th ventricle (Figure 1). Vestibular nuclei project to these nuclei to form vestibular-autonomic reflex [21]. All these nuclei and brain

Figure 5 Effects of paraquat (A), vitamin C (B), and vitamin E (C) on motion sickness index. $n = 6$ mice in each group.

regions are connected closely in anatomical position and regulate neural activity during motion sickness [22]. ROS levels in these regions were elevated after rotary stimulation. However, the antioxidative capacity was also increased unexpectedly. SOD is part of the first line of antioxidative defense through its elimination of O_2^- by converting it into H_2O_2 and O_2 [23]. Unlike that in migraine suffers [24], SOD activity after rotation was increased but not decreased. It is more like an antioxidant compensatory rather than a real oxidative stress developed in motion sickness. Interestingly, a report about increases of peroxidation levels and SOD activity in chloroplasts of pea during clino-rotation was coincided with the results in mice [25,26]. We also observed decreased SOD1 mRNA level and SOD activity in cerebral cortex and other brain regions, which indicated possible role of oxidative stress in motion sickness. However, vitamin C and vitamin E failed to ease motion sickness symptoms that confirmed a negative role of oxidative stress in motion sickness.

The increased ROS might be explained by enhanced glutamate release in vestibular nuclei and cerebellum. Glutamate induces ROS level increase in neurons [27]. Primary vestibular afferents that project to the vestibular nuclei and cerebellum use glutamate as their excitatory neurotransmitter [28]. Cai et al. also demonstrated excitatory glutamatergic vestibular nucleus–solitary

nucleus and vestibular nucleus–parabrachial nucleus transmission pathways involved in vestibulo-autonomic reflexes during rotary stimulation [29]. Hence, we guess the glutamate released during motion sickness in vestibular nuclei and cerebellum is adequate to increase ROS level, but not enough to decrease antioxidative enzymes' activities.

Taken together, our results proved oxidative stress was not involved in motion sickness, although an increase in ROS level was observed in CB. The oxidants induced by rotation could be offset by increased antioxidants. Moreover, strategy against oxidative stress is useless to treat motion sickness in mice.

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Conflict of Interest

The authors declare no conflict of interest.

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