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Research report

The neuroprotective effect of vitamin E on chronic sleep deprivation-induced memory impairment: The role of oxidative stress

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ABSTRACT

Sleep deprivation induces oxidative stress and impairs learning and memory processes. Vitamin E, on the other hand, is a strong antioxidant that has neuroprotective effect on the brain. In this study, we examined the potential protective effect of chronic administration of vitamin E on chronic sleep deprivation-induced cognitive impairment. In addition, possible molecular targets for vitamin E effects on chronic sleep deprivation-induced cognitive impairment were determined. Sleep deprivation was induced in rats using modified multiple platform model. Vitamin E (100 mg/kg) was administered to animals by oral gavage. Behavioral study was conducted to test the spatial learning and memory using the radial arm water maze (RAWM). In addition, the hippocampus was dissected out and antioxidant markers including glutathione (GSH), oxidized glutathione (GSSG) and GSH/GSSG, glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD) were assessed. The results of this project revealed that chronic sleep deprivation impaired both (short- and long-term) memories ($P < 0.05$), while vitamin E treatment prevented such effect. Additionally, vitamin E normalized chronic sleep deprivation-induced reduction in the hippocampus GSH/GSSG ratio, and activity of catalase, SOD, and GPx. In conclusion, sleep deprivation induces memory impairment, and treatment with vitamin E prevented this impairment probably through its antioxidant action in the hippocampus.

1. Introduction

Humans spend one-third of their life sleeping, which reflects the fact that sleep seems to be vitally important for human being. Sleep consists mainly of two stages non-rapid eye movement, and rapid eye movement (REM) [1]. A central cognitive function of sleep is to consolidate newly acquired memories for long-term storage [2]. Many studies proved the link of learning and memory consolidation to REM sleep through the observation that the duration of REM sleep increases after learning tasks [3,4].

Sleep deprivation has been shown to induce memory deficit in animal studies [5–10]. The mechanism of such impairment is still unknown but in general, sleep deprivation increases oxidative stress in the hippocampus and many regions in the brain [11–14] that is usually detoxified by sleep [15].

Vitamin E is an essential nutrient in humans and well known antioxidant substance. It reduces free radicals and reactive oxygen species activity. Like other antioxidants, vitamin E slows or prevents memory impairments that accompany several conditions such as mental stress [16], diabetes [17], cerebral ischemic injury [18], Alzheimer's disease [19,20], stroke [21] and aging [22]. On the other hand, studies showed a shared link between endogenous plasma concentration of vitamin E and cognition status [23,24].

In this study, we investigated the hypothesis that chronic vitamin E supplementation prevents chronic sleep deprivation-induced impairment of hippocampal learning and memory via its anti-oxidative properties. Both behavioral approach using the radial arm water maze (RAWM) to test learning and memory functions, and molecular enzymatic assays approach were used to test this hypothesis.

2. Methods

2.1. Animals and treatments

Young adult male Wistar rats weighing 150–250 g were used in this study. The animals were housed in metal cages (six rats per cage) under hygienic conditions and maintained at 24 °C and 12 h light/dark cycle (light on at 8 am) with free access to food and water. All experimental procedures were performed during the light cycle. Before starting the experiment, animals were allowed to stay in the same cage for two weeks to establish a social hierarchy within the group. All procedures

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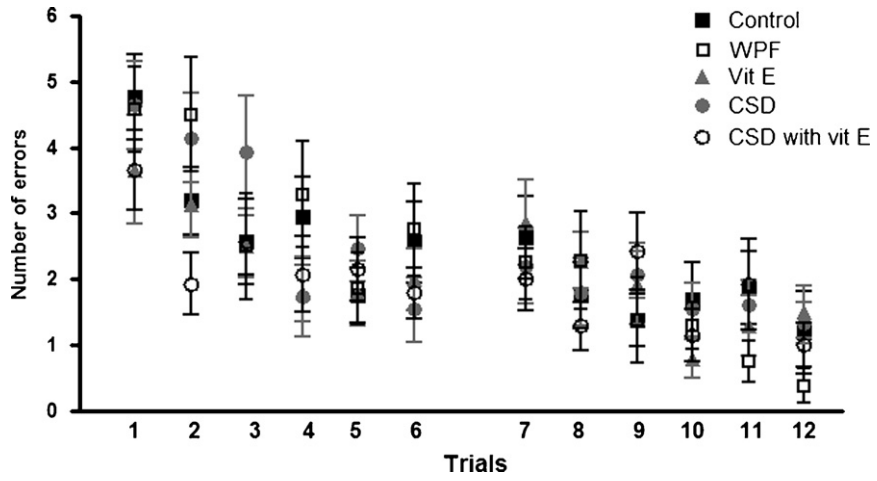

Fig. 1. Animal learning performance in the radial arm water maze. Comparison of control (control), wide platform (WPF), vitamin E (Vit E) 100 mg/kg, chronic sleep deprivation (CSD) and chronic sleep deprivation with vitamin E (CSD with Vit E). *Indicates significant difference from other groups, ($P < 0.05$; $n = 12$–14/group).

were approved by Animal Care and Use Committee (ACUC) at Jordan University of Science and Technology.

Animals were randomly assigned into five groups, each containing 12–14 rats. Control, vitamin E (Vit E), chronic sleep deprivation (CSD), chronic sleep deprivation with vitamin E, and wide platform form (WPF). The Vit E and the CSD with Vit E groups were treated with vitamin E (α-tocopherol, Sigma, St. Louis, MO) at a dose of 100 mg/kg once daily via oral gavage for 6 weeks. Similar dose, route of administration, and duration were previously used by other groups, and were shown to be protective for cognitive functions [17,25,26]. The control, CSD and WPF groups were administered vehicle once daily for 6 weeks by oral gavage. All the treatments were administered between 8:00 and 9:00 am. Right after dosing, both the CSD and CSD with Vit E groups were subjected to REM-SD; 8 h/day for 6 weeks.

2.2. Induction of sleep deprivation

Chronic sleep deprivation was induced using columns-in-water (modified multiple platform) model as described [5–8,27]. Briefly, animals were placed on platforms (20 platforms; 20 cm high and 5 cm diameter, 7 cm apart edge-to-edge) surrounded by water ($24 \pm 1\,^{\circ}C$) in an aquarium where water and food were accessible to animals. The water level in the aquarium was about 4 cm below the edge of the platform. This method has been reported to interfere with total sleep, but it mainly eliminates REM sleep [28]. Loss of muscle tone during REM sleep caused animals to fall into the water and waken. Furthermore, to test the possible effects of stresses of the tank environment, wide platforms (diameter: 12 cm) were used to allow the WPF rats to sleep without falling in the water.

2.3. Behavioral test: radial arm water maze

All animal groups were tested for spatial learning and memory performance on the radial arm water maze [29–33]. This test was carried out only once after six weeks of sleep deprivation and/or vitamin E administration. The radial arm water maze contains six swim paths (stainless steel arms) extending out of black central area tube. With a hidden platform located at the end of one arm (the goal arm). The experiments were done in dimly lit room with two different pictures on the walls which serve as cues for the rats. The animals must find the hidden platform on the goal arm. The goal arm was not changed for a particular rat in a single day. At the morning of the testing day, rats were allowed six consequent trials separated by 5 min rest, then another six consequent trials (acquisition phase), followed by 30 min short-term memory and 5 h and 24 h long-term memory tests. Every trial starts in different arm except the goal arm for a particular rat. In each trial, the rat was allowed to swim freely in the maze for 1 min or until the rat finds the hidden platform. Once the rat is on the platform, the rat was allowed 15 s to observe visual cues before the next trial. When the rat was unable to find the hidden platform the allowed 1 min period, the experimenter guided it toward the platform where it was allowed to remain for 15 s to observe visual cues. Each time the rat enter an arm other than goal arm, an error is counted. Correct entry occurred when the whole body of the rat (not including the tail) is inside the arm.

2.4. Hippocampus dissection

Animals were killed after 6 weeks of vitamin E and/or CSD. Dissection was carried out as described in Refs. [34,35]. Briefly, the brains were removed immediately from the skull, and placed on a filter paper containing 0.2 M ice-cold sucrose solution, over a glass plate filled with crushed ice. Dissected hippocampus parts were placed in test tubes then, immediately, transferred into liquid nitrogen and stored frozen until time of tissue processing.

2.5. Calorimetric immunoassays

To determine activities or levels of oxidative stress enzymes, hippocampus tissues were homogenized manually using small pestle in lysis buffer (137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% Nonyl phenyl polyethylene glycol ether with 20 molecules ethylene oxide, 10% glycerol, 0.5 mM sodium vanadate, 1 mM polymethane sulfonyl floride), and protease inhibitor cocktail (Sigma–Aldrich Corp., MI, USA). Homogenates were centrifuged to remove insoluble material ($14{,}000 \times g$ for 5 min, $4\,^{\circ}C$). Thereafter, homogenates were divided into several aliquots; each of them was used for only a single assay. All the work was carried out over crushed ice. Total protein concentration was estimated using commercially available kit (BioRAD, Hercules, CA, USA). To quantify total GSH, tissues homogenates were deproteinized with 5% of 5-sulfosalicylic acid (SSA), centrifuged ($10{,}000 \times g$ for 10 min, $4\,^{\circ}C$) to remove the precipitated protein, and then assayed photometrically for glutathione according to manufacturer's instructions (Glutathione Assay Kit, Sensitivity: 1 nmol/mL, Sigma–Aldrich Corp., MI, USA). Colors were read at 405 nm using an automated plate reader (ELx800, Bio-teak instruments, plate reader, Highland park, Winooski, USA). For GSSG measurement, 10 μL of 1 M 2-vinylpyridine (Glutathione Assay Kit, Sigma–Aldrich Corp., MI, USA) was added per 1 mL of supernatant of the sample, then the procedure was carried as described above for GSH. For all the assays and to ensure minimum variability between experimental runs, each single plate contained samples from every experimental groups. GSH was calculated by subtracting total glutathione species value from GSSG value.

Activity of GPx was determined using Glutathione Peroxidase Cellular Activity Assay Kit according to manufacturer's instruction (CGP1, Sensitivity: 0.005 units/mL, Sigma–Aldrich, MI, USA). In brief, 10 μL of tissue homogenate was added to a 990 μL reaction mixture containing 0.25 mM NADPH, 2.1 mM reduced glutathione, 0.5 units/mL glutathione reductase, and 30 mM tert-butyl hydroperoxide. The change in absorbance of the reaction product was kinetically quantified every 10 s for 1 min at 340 nm using spectrophotometry (UV–VIS spectrophotometer, UV-1800, Shimadzu, Japan).

Catalase activity was measured using commercially available kits according to manufacturer's instructions (Catalase: Sensitivity: 2.5 units/mL, Cell Biolabs, San Diego, USA). In brief, 20 μL of each tissue homogenate was added to 50 μL hydrogen peroxide working solution, which contains 12 mM H_2O_2 in 100 mM potassium phosphate buffer, pH7.0. Then, the reaction was incubated for 1 min, and stopped by adding 50 μL of the catalase quencher provided by the kit. Finally, 200 μL of the catalase chromagen (4-amino-3-hydrazine-5-mercapto-1,2,4-triazole) was added to the mixture. After 40 min of vigorous shaking of the mixture, ELISA plates were read at 540 nm.

Activity of SOD was measured using commercially available kits according to manufacturer's instructions (SOD kit; Sensitivity: 0.001 units/mL: Sigma–Aldrich Corp., MI, USA). The assay used the Dojindo's tetrazolium salt, WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium). In brief, tissue homogenate (20 μL) was mixed with 200 μL of WST-1 solution. Then, 20 μL of SOD enzyme working solution (Sigma–Aldrich Corp., MI, USA) was added to the mixture, and incubated for 20 min at $37\,^{\circ}C$. Thereafter, plates were read at 450 nm using an automated reader (ELx800, Bio-tek instruments, plate reader, Highland Park, Winooski, USA).