Research report

Vitamin C attenuates memory loss induced by post-traumatic stress like behavior in a rat model

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ABSTRACT

Oxidative stress is associated with neuronal damage in many brain regions including the hippocampus; an area in the brain responsible of memory processing. Oxidative stress is also linked with many psychiatric conditions including post-traumatic stress disorder (PTSD). PTSD is triggered by traumatic experience and many PTSD patients show signs of memory impairment. Vitamin C is a water-soluble vitamin with antioxidant properties. Herein, we hypothesized that memory impairment observed during PTSD could be a result of oxidative stress in hippocampal tissues and that prophylactic vitamin C administration may reduce oxidative stress in the hippocampus and prevent memory impairment. The above hypothesis was tested in a rat model where PTSD-like behavior was induced through single prolonged stress (SPS). Short and long-term memory was tested using a radial arm water maze (RAWM). We found that SPS induced a significant increase in the oxidized glutathione levels of the hippocampus. This reduction was accompanied with a significant decrease in glutathione peroxidase and catalase enzyme activity, and a significant increase in lipid peroxidation. Intriguingly, vitamin C administration successfully attenuated memory impairment and all of the changes observed in oxidative stress markers. Our findings demonstrate that vitamin C could prevent oxidative stress and memory impairment induced by SPS model of PTSD-like behavior in rat.

1. Introduction

Reactive oxygen species (ROS) are regularly produced by the cellular systems of aerobic organisms [1]. These species are released inadvertently during the process of cellular respiration and are also generated by inflammatory cells to combat invading microorganisms [2]. Despite their involvement in normal physiological functions, ROS can potentially damage cellular systems via oxidation of DNA, proteins and lipids [3]. Indeed, in view of these damaging effects, aerobic organisms have developed multiple defense mechanisms that inactivate ROS and prevent them from destroying cells. The superoxide dismutase (SOD), glutathione and catalase systems are examples of the above defense systems; a cellular state commonly described as “oxidative stress” [4]. Examples of these diseases include diabetes, Alzheimer, atherosclerosis, traumatic brain injury and post-traumatic stress disorder (PTSD) [7–11]. Further evidence on the central role of ROS in mediating the etiology and pathogenesis of the above disorders comes from studies performed on preclinical and clinical models which showed that inactivating ROS or limiting their production is linked with improved treatment outcome and/or prevention of many of the above disorders [12].

In humans, vitamin C (ascorbic acid) is an essential water-soluble nutrient. Vitamin C is a cofactor of enzymes involved in the hydroxylation of proline and lysine residues of collagen; a process mandatory for the folding, maturation and strength of the collagen triple-helix [13]. Furthermore, vitamin C is involved in the synthesis of catecholamines, carnitine, cholesterol, some peptide hormones and amino acids [14]. In addition to its role in the above biosynthetic reactions, vitamin C is considered an antioxidant [15]. The exact mechanism by which vitamin C plays this role is unknown, however, it is thought that vitamin C protects the cellular DNA, proteins and lipids from the oxidative damage of ROS by being oxidized itself. Indeed, the ascorbic acid form of vitamin C can donate up to two electrons to various ROS thereby inactivating them. The antioxidant properties of vitamin C could explain the preventive effects of diets rich in this vitamin (i.e.
fruits and vegetables) on the risk of cardiovascular disease and cancer; conditions strongly linked with oxidative stress [16,17]. Furthermore, vitamin C implemented several beneficial functions such as immunostimulant and anti-inflammatory manners [18,19]. Intriguingly, previous study on mice given LPS and pretreated with vitamin C has shown to prevent pro-inflammatory cytokines involving tumor necrosis factor-alpha and normalizing the signal pathway and malondialdehyde level in the hippocampus of the LPS treated group [20].

Post-traumatic stress disorder (PTSD) is a debilitating psychiatric condition. Development of PTSD is observed in war-veterans; however, the symptoms of PTSD could be precipitated through exposure to any life-threatening event such as sexual assault, home displacement and road traffic accidents [21]. Individuals affected with PTSD develop a number of symptoms including re-experiencing symptoms in which they relive the precipitating trauma and avoidance symptoms in which they modify their behavior in an attempt to avoid any event which may remind them of the trauma, and hyperarousal [22]. Moreover, PTSD is associated with cognitive decline and memory loss that was shown in both human [23] and animal studies [10,24-29]. Memory loss in PTSD patients could be severe enough to affect day to day activities of affected individuals [23].

The exact reason behind the cognitive decline in PTSD patients is under investigation. Several reports indicated that it may be linked with the neurodegeneration of the hippocampus, an area in the brain responsible for processing and storing memory [27,30-32]. Indeed, destruction of the neuronal tissues of the hippocampus is observed in several psychiatric conditions with overlapping symptoms of PTSD such as depression and Alzheimer and is observed on MRI images of the brains of PTSD patients. Preventing or delaying the onset of memory loss in PTSD requires a mechanistic understanding of its pathophysiology at the molecular level. Although still under extensive investigation, several reports indicated that the above memory loss could be related to increased oxidative stress in the hippocampus eventually leading to its neurodegeneration [33,34]. Concomitantly, recent study has observed neurotoxicity in mouse model and deterioration of antioxidant defense system in brain tissue, vitamin C has mitigated these actions by increasing hippocampus neurogenesis and potentiating memory [35]. This hypothesis is supported by several studies which reported a decrease in memory loss in animal models of PTSD-like behavior upon the use of pharmacological reagents that normally scavenge ROS [36,37]. In this report, we investigated the effect of vitamin C administration on the memory loss in an animal model of PTSD-like behavior including the levels of several oxidative markers in the hippocampus.

2. Methods

2.1. Animals and treatment

Male Wistar rats used in this study (150–200 g in weight) were purchased from the animal care facility of Jordan University of Science and Technology (JUST). Rats were kept cages made of plastic (3–4 animals/cage) in a climate-controlled room (24 ± 1°C), they had an ad libitum access to food and water, and 12 h light/dark cycle (light on 7:00 am) with the experimental procedure being performed during the light cycle. Tail labeling was used to distinguish rats of the different treatment group. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of JUST. All lab and animal house personnel who handled rats followed the Institutional Animal Care and Use Committee Guidebook, 2nd edition of 2002.

Four groups (n = 15/group) of animals were randomly allocated: control, vitamin C, single prolonged stress (SPS) group, and vitamin C plus SPS group (SPS + Vitamin C). Vitamin C was administered via oral gavage at a dose of 100 mg/kg/day, six days a week for a total duration of four weeks. Vitamin C was also administered on the day when the behavioral test was performed. The above vitamin C dose was previously shown to preserve cognition in conditions other than PTSD [38-40] and was thus chosen for this study. The SPS and vitamin C-SPS groups were subjected to SPS procedure, an established model of PTSD-like behavior in animals [26,27,29,36,41-45], one week following the initiation of vitamin C administration (Fig. 1). Water alone was administered to animals of the control and SPS groups once daily on the same days the other two groups received vitamin C.

2.2. Single prolonged stress (SPS) procedure

All animals of the SPS or SPS + Vitamin C groups received SPS single prolonged stress (SPS) to introduce PTSD behavior in animals. The SPS was carried out in three stages as described previously [10,26,27,41]. In the first stage, a rodent restrainer (Part # 82, IITC Life Science, Woodland Hills, CA, USA) was used to restrain the animal for two hours. The above procedure guaranteed full immobilization. The second stage, which followed immediately, was a twenty-minute forced swimming performed in a transparent cylinder (height: 50 cm; diameter: 35 cm; water depth: 30 cm; water temperature: 24°C). Right after that, animals were allowed to recuperate in for 15 min in pre-prepared plastic cages. Finally, 1–2 min ether anesthesia until loss of consciousness was applied [10,27,46-48].

2.3. The radial arm water maze (RAWM)

It is used to test spatial learning and memory. Details of the RAWM were previously described [49-51]. The RAWM has 6 stainless steel arms and a central black circular water-filled tube with a hidden
platform located on the target arm, which was always the same arm for each rat during both learning and memory phases. The starting arm was different in each of the learning trials or memory tests. All experiments were carried out in dimly-light room with visual cues that were fixed at the wall of the room during experiment forming a spatial reference for the animal during the learning and memory phase. Water temperature was maintained at 23 ± 1°C. The learning phase was performed prior to the short term and long term memory tests. The learning phase consisted of twelve trials; six trials were performed consecutively followed by a 5 min rest before performing the remaining six trials. Short-term memory testing was performed 30 min after the end of the learning phase, whereas long-term memory testing was performed 5 h and 24 h following the end of the last trial of the learning phase. During the learning phase, each rat was given a one min to swim freely to the target arm but it was guided to the target arm after having one minute without finding the target arm. Each animal was left on the target arm for 15 s to explore their location according to the visual cues. In memory test, each rat was given one minute to locate the hidden platform (with no 15 s chance on the target arm). An error was recorded when the rat entered to an arm other than the target arm.

2.4. Dissection of the Hippocampus

Animals were euthanized and brain was dissected out and put on top of a normal saline soaked filter paper. The filter paper was placed over a crushed ice filled cold glass plate [52]. Both left and right lopes of the hippocampus were separated from the remaining cerebral tissues and directly placed in Eppendorf tube that were pre-labeled for that purpose. The tube was then placed in liquid nitrogen before being stored at -30°C until further analysis.

2.5. Molecular assays

The tissues of the hippocampus were homogenized in phosphate buffer (200 μl). using a plastic pestle, the homogenization buffer was prepared by the reconstitution of one phosphate buffered saline tablet (Sigma Chemical CO., Saint Louis, MO) and two tablets of protease inhibitor (Sigma Chemical CO., Saint Louis, MO) in distilled water (200 ml). Then, mixture of homogenized tissues and buffer were centrifuged at 15000xg for 10 min (4°C) in order to eliminate tissues that failed to dissolve in the buffer and thus remained insoluble. The supernatant from the previous step was recovered and stored for further analysis.
analysis. Total protein concentration in the recovered supernatant was measured using a commercially available kit purchased from Bio-rad (Bio-Rad, USA). For oxidative stress biomarkers, total glutathione, GSSG, GPx, catalase and Thiobarbituric acid reactive substance (TBARs), commercially available kits were utilized as per kit’s manufacturer’s instructions (total glutathione and GSSG: Glutathione assay kit, CS0260, Sigma-Aldrich, MI, USA, GPx: CGP1, Sigma-Aldrich, MI, USA, Catalase: catalase assay kit, Cayman Chem, Ann Arbor, MI, USA, TBARs: Cayman Chem, item № 10009055, Ann arbor, MI. USA). Absorbance for each assay was measured spectrophotometrically using a Epoch Microplate Spectrophotometer at specified wavelength in each of the after-mentioned kits (Bio-tek instruments, Highland Park, Winooski, USA).

2.6. Statistical analysis

The number of errors were compared via two-way ANOVA followed by posttest for multiple comparison. The repeated measures factor was Time and interaction with SPS/no SPS and vitamin C/no vitamin C were independent group dimensions. For the biochemical assays, one-way ANOVA plus Tukey’s posttest was used to achieve comparisons. The statistical software used was GraphPad Prism version 4.0. Significance was set at $P < 0.05$. Values all over the study are reported as mean ± SEM.

3. Results

3.1. The effect of SPS and/or vitamin C on learning and memory

The number of errors in the learning phase was high in the initial trials, however, this number decreased with subsequent trials (Fig. 1). There were, however, no significant differences in the number of errors between different experimental groups whether in the early or late trials. We conclude that neither SPS nor vitamin C significantly affected
learning in our model. However, upon the examination of the effect of SPS and/or vitamin C on short term and long term memory (after 5 or 24 h) a different picture emerged. Here, we found that SPS significantly affected short term (Fig. 2a) and long term memory when tested after 5 h (Fig. 2b) or 24 h (Fig. 2c). Intriguingly, our findings indicated that prophylactic administration of vitamin C attenuated short and long term memory loss induced by SPS although vitamin C by itself had no significant effect on short or long term memory compared to control rats (Fig. 2b and c).

3.2. The effect of SPS and/or vitamin C on the levels of oxidative stress biomarkers in the hippocampus

It was observed in prior studies that memory loss could result from oxidative stress, we therefore measured the levels of several oxidative stress markers in hippocampal tissues recovered from the brains of the rats of each experimental group. These markers included GSH, GSSG and TBARS. Additionally, we measured the activities of two enzymes involved in combating ROS; these included the catalase and glutathione peroxidase (GPx) enzymes. We found that although SPS did not significantly affect the levels of reduced glutathione (GSH) in hippocampal tissues (Fig. 3a), SPS induced a significant increase in oxidized glutathione levels (GSSG) (Fig. 3b) and consequently a significant decrease in GSH/GSSG ratio (Fig. 3c). It was also observed that vitamin C attenuated the effects of SPS on GSSG and GSH/GSSG ratio. Interestingly, vitamin C administration by itself did not significantly change the levels of GSH, GSSG or GSH/GSSG ratio compared to vehicle treated rats. Since changes in the levels of the above biomarkers could be a result of changes in the levels of enzymes expressed in cellular system to combat oxidative stress, we also measured the activity of GPx and catalase enzymes in hippocampus tissues. In this analysis, we found that SPS caused a significant reduction in the levels of GPx (Fig. 4a) as well as in catalase enzyme activity (Fig. 4b). Moreover, analogous to the effects of vitamin C on GSSG and GSH/GSSG ratio, prophylactic

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**Fig. 4.** Effect of vitamin C and/or SPS on activities of GPx and catalase. The GPx and catalase enzymes activities were reduced by the SPS, which was prevented by vitamin C. *Indicates significant difference compared with other groups (P < 0.05). Values are mean ± SEM. N = 15/group.

**Fig. 5.** Effect of vitamin C and/or SPS on levels of TBARS. Significant increase in TBARS levels were induce by the SPS model of PTSD-like behavior. This effect was prevented by administration of vitamin C. *Indicates significant difference compared with other groups (P < 0.05). Values are mean ± SEM. N = 15/group.
treatment with vitamin C prevented the reduction in GPx and catalase activities induced by SPS (Fig. 4a and b). Finally, we found that a SPS resulted in an increase in the levels of TBARs; a marker of lipid peroxidation (Fig. 5). The above increase in TBARs was completely prevented by vitamin C (Fig. 5).

4. Discussion

Current study demonstrated that chronic vitamin C treatment prevented the deleterious effect of oxidative stress biomarkers within the hippocampus during memory impairment in the SPS animal model of PTSD-like behavior. To test our hypothesis, we applied SPS to create cognitive impairment, as well as we tested the spatial and learning memory using the radial arm water maze. Results revealed that SPS induced short- and long-term memory impairments, which were in consistence with previous studies that examined cognition, using the RAWM [10,27,48], and Morris water maze [53,54] in SPS exposed animals. Learning function was not altered due to SPS, which is consistent with previous reports [10,11,27,29].

On the other hand, both short- and long-term memories, which were impaired by SPS model of PTSD-like behavior, were normal with vitamin C treatment. This protective impact of vitamin C on impaired memory shown in present study was in accordance with previous other studies that examined vitamin C protective effects on impairment of cognitive functions in conditions other than PTSD [55,56]. Interestingly, vitamin C deficiency in guinea pigs was shown to evoke spatial memory deterioration that was proposed to be due to the impairment in neurotransmission and synaptic development, which was linked to increase in oxidative stress [57].

The mechanism by which vitamin C alleviated memory was probably by potentiating the antioxidant defense system in the hippocampus [50,56]. Oxidative stress was related to the pathophysiology of PTSD [30]. The hippocampal levels or activities of antioxidant molecules and oxidative capacity enzymes (GSH/GSSG ratio, catalase, and GPx) were shown to be reduced in rats exposed to SPS. Moreover, the levels of GSSG and TBARs were elevated significantly due to SPS. This is in accordance with previously published studies [27,26]. Furthermore, the levels of TBARs and other oxidative stress biomarkers were reduced when vitamin C treatment was instituted on top of memory impairment induced by LPS treatment [20]. These outcomes were put into context the potential effect of oxidative stress in extending PTSD symptoms and related complications. On the contrary, vitamin C normalized GSSG, GPx, catalase, and TBARs levels during SPS. In accordance, vitamin C was shown to possess nephroprotective effects that oppose cisplatin induced nephrotoxicity in rats [58].

In conclusion, oxidative stress imbalance in the hippocampus, which induced memory impairment in the SPS model of PTSD-like behavior might be alleviated by chronic vitamin C treatment.

Declaration of Competing Interest

None declared.

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