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Tempol prevents post-traumatic stress disorder induced memory impairment

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ABSTRACT

Post-traumatic stress disorder (PTSD) is a mental health disorder that can develop after a terrifying or life threatening event. Multiple symptoms are noticed in patients with PTSD including cognitive impairment, which was shown to be is associated with oxidative stress. Tempol is a highly efficient membrane-permeable antioxidant. In this study, we investigated the possible protective effect of tempol on PTSD-induced memory impairment. To test this hypothesis, we used single prolonged stress (SPS) model (2 h restrain, 20 min forced swimming, 15 min rest, and 1–2 min diethyl ether exposure) as a model of PTSD. Rats were randomly assigned into four groups: control (provided distilled water), tempol (provided tempol; 80 mg/kg/day by oral gavage for 4 weeks), SPS (exposed to prolonged stress and administered distilled water) and tempol/SPS (exposed to prolonged stress and administered tempol for 4 weeks). We used radial arm water maze to test spatial learning and memory functions and enzyme-linked immunosorbant assay (ELISA) to measure levels of oxidative stress biomarkers in the hippocampus. Results showed that SPS model of PTSD impaired both short and long-term memories ($P < 0.05$), and chronic tempol administration prevented such effect. Tempol also prevented decreases in hippocampal catalase, and SOD activities, GSH/GSSG ratio and increases TBARS levels, which were all impaired by SPS model of PTSD ($P < 0.05$). In conclusion, we suggest a protective effect of tempol administration against SPS model of PTSD–induced short– and long- term memory impairment, and we believe that this protective effect of tempol is accomplished, at least partly, through prevention of alternation in oxidative stress in the hippocampus.

1. Introduction

Post-traumatic stress disorder (PTSD) is a disabling trauma and stressor anxiety psychiatric disorder that is developed after experiencing or witnessing traumatic events such as natural disasters, war disasters, violent attacks or sexual assault [24,48]. Cognitive symptoms of PTSD include intrusive memories, avoidance of thoughts, feelings, or reminders of the trauma, and inability to recall parts of the trauma [31]. Deterioration of cognitive function was also seen in PTSD patients [33]. Several reports have shown verbal declarative memory deficits during PTSD, among adult patients with PTSD who have been through combat [45], childhood abuse [10], rape [26], and political violence [27]. On the other hand, studies on PTSD animal models reported structural changes in hippocampus in relation to PTSD [21,35–37,47]. Additionally, PTSD animal models have shown hippocampus memory deficits that was evaluated by different tasks, such as eight-arm radial maze [35], Morris water maze [44,55,56], and radial arm water maze [15,42].

The exact mechanism by which PTSD-induced memory impairment is not clearly understood. Using PTSD animal models, several studies linked the clinical manifestations of PTSD to the increased oxidative stress and the suppressed antioxidant mechanisms in the brain [18,20,60,63]. Thus, PTSD potentiates oxidative stress that accelerates cellular aging [39]. Using predator animal model of PTSD, a progressive increase in oxidative stress was shown in a time-dependent pattern during the development of PTSD [60]. It was also reported that PTSD patients possess lower antioxidant system activity (GPx, SOD) and higher lipid peroxidation level compared to healthy control subjects [8,9], and therefore, oxidative stress is a possible casual factor for the clinical symptoms of PTSD. Among PTSD patients, a statistically reliable correlations has been reported between the incidence and symptomatic severity of PTSD and oxidative stress biomarkers [54]. PTSD animal models also showed increased reactive oxygen species in hippocampus tissues [60].

Tempol (4-hydroxy-2, 2, 6, 6-tetramethylpiperidine-*N*-oxyl) is a potent antioxidant that belongs to the nitroxide compounds family

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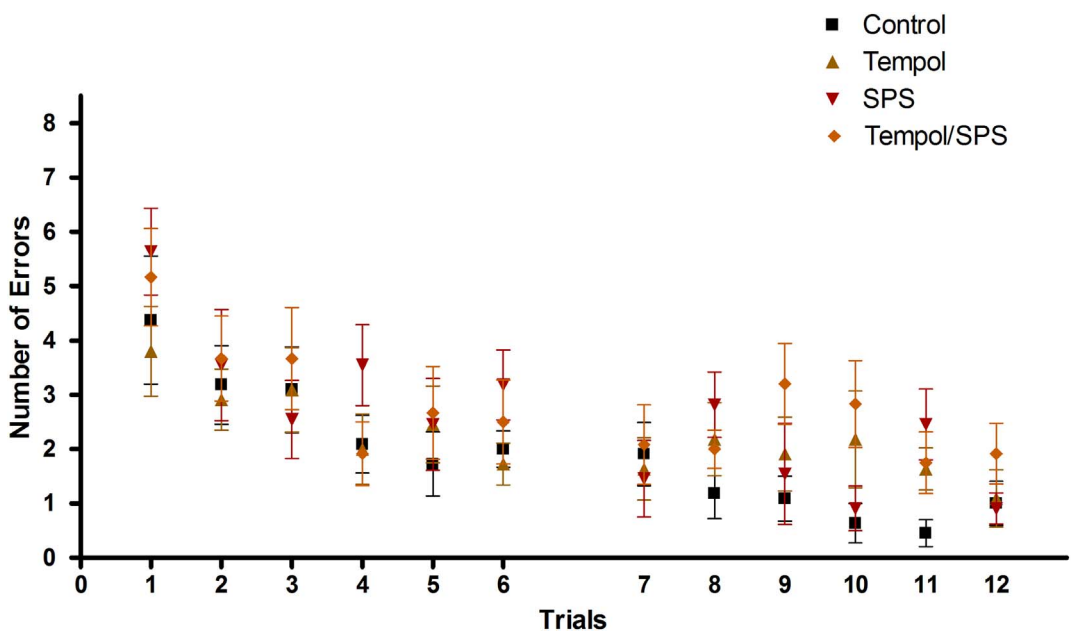

Fig. 1. Tempol and/or SPS model of PTSD did not affect learning performance. Number of errors made by each animal decreased with continued learning trials (1 through 12) without significant difference among experimental groups. Each point is the mean ± SEM of 15 rats.

[51,57]. Tempol works as a superoxide dismutase (SOD)-mimetic [46]. The greatest efficacy of tempol is shown in preventing the damaging effects of the hydroxyl radical (·OH) on cells compared to its efficacy on metabolizing cellular (O_2^-) and (H_2O_2) [57,58]. Additionally, tempol prevents the generation of hydroxyl radical (˙OH) from (H_2O_2) by transition metals in the fenton reaction [40]. The antioxidant effects of tempol were demonstrated in many studies using cell cultures [61], and animal models [11,22,64]. Furthermore, tempol improved memory and cognitive function in animal models of different diseases [2,19,25]. In this study, the effects of tempol on PTSD-induced oxidative stress and memory impairment were evaluated.

2. Materials and methods

2.1. Animals and treatment

Male Wistar rats weighing 150–175 g were obtained from animal house at Jordan University of Science and Technology (JUST). Animals were housed in plastic cages (five animals per cage) under hygienic conditions in a climate controlled room (24 ± 1 ˚C) with free access to rat chow and water. Rats were identified by tail labeling and housed in 12 h light/dark cycle (light on 7:00 am). All of the experimental work applied at the light phase. The study was approved by the Institutional Animal Care and Use Committee of the Jordan University Science and Technology.

Animals were randomly distributed into four groups (15 rat/group): control, tempol, SPS, and tempol/SPS. The tempol and tempol/SPS groups were administered tempol (Sigma-Aldrich, St. Louis, MI, USA) at dose of 80 mg/kg via oral gavage for 6 days per week for a total of 5 weeks, starting 1 weeks before the application of SPS. The tempol dose was used in previous studies to prevent cognitive impairment [2]. Tempol was dissolved in distilled water (80 mg/ml), and administered to animals as per their body weight (Alzoubi et al. [2]).The SPS and tempol/SPS groups were subjected to SPS model of PTSD on day 8 of tempol treatment. The control and SPS groups were administered distilled water 0.2 ml via oral gavage once daily at the same days tempol was administrated to tempol and tempol/SPS.

2.2. Induction of single prolonged stress (SPS) model

All animals were exposed to a one-time combined stress paradigm. Simply each animal was placed in double-layered plastic Ziploc bag. The animal was then wrapped by the bag, and fixed in immobile positon for 2 h by duct tape placed around the raped bag. A small whole was made in the bag right at the animal's nose to ensure continued breathing. This was followed by a forced swimming for 20 min in transparent cylindrical container 50 cm in height; 35 cm in diameter; 35 cm water (24 ± 1 ˚C) depth. After 20 min of forced swimming, the animal was placed in a cage for 15 min followed by ether anesthesia for 1–2 min until loss of consciousness [42,50]. Animals were monitored after loss of consciousness via observing respiratory pattern, and mucus membranes color.

2.3. The radial arm water maze (RAWM)

The RAWM procedure was carried out 4 weeks after SPS. The RAWM was used to evaluate spatial learning and memory among all four groups. This paradigm along with detailed procedure was previously described in details [3–7,38]. Briefly, the RAWM is circular water top with six-arms radiating from a central area. Learning phase consisted of twelve consecutive trials; the first six trials followed by 5 min rest then another six trials. Short-term memory testing was done 30 min, whereas long-term memory testing was done at 5 h and 24 h, after the end of the last trial of the learning phase. The above-described sequence was done only one time in one day. In each trial, the animal was allowed 1 min to swim freely in the maze to find a hidden escape platform at the far end of one of the six radiating arms (the goal arm). The animal was started in each trial at the far end of one of the six radiating arms (except the goal arm). Once the animal was on the escape platform, the rat was allowed 15 s to observe visual cues hanged on the walls of the experimental room before the next trial. At the learning trials, if a rat was unable to find the platform within the 1 min period allowed, it was guided toward the platform for a 15 s stay. No guidance was offered at the memory tests as all animals were able to find the hidden platform within the allowed 1 min. During the 1 min period, each time the animal entered an arm other than the goal arm, an error was counted. Entry occurred when the whole body of the rat (not including the tail) was inside the arm. The RAWM training was carried