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Research report

Pentoxifylline prevents post-traumatic stress disorder induced memory impairment

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ABSTRACT

Posttraumatic stress disorder (PTSD) is a disabling prevalent and difficult-to-treat psychiatric disorder, which can develop after the exposure to severe traumatic events such as those occurring during wars and natural disasters. Pentoxifylline (PTX) is a potent antioxidant, which has an important role in prevention of cognitive dysfunctions. In the present study, the effect of PTX on memory impairment induced by PTSD was investigated using the rat animal model. PTSD-like behavior was induced in animals using a single-prolonged stress (SPS) rat model of PTSD (2 h restrain, 20 min forced swimming, 15 min rest, 1–2 min diethyl ether exposure). PTX was administered intraperitoneally at a dose of 100 mg/kg/day. Spatial learning and memory were assessed using the radial arm water maze (RAWM). Changes in oxidative stress biomarkers, brain derived neuroptrophic factor (BDNF), and epigenetics (histones) in the hippocampus following treatments were measured using enzymatic assays. The result revealed that SPS impaired both short- and long- term memory ($P < 0.05$). Use of PTX prevented memory impairment induced by SPS. Furthermore, PTX normalized SPS induced changes in the hippocampus GSH/GSSG ratio, activity of catalase, and glutathione peroxidase (GPx), BDNF, and certain histones levels. In conclusion, the SPS model of PTSD-like behavior induced memory impairment, whereas PTX prevented this impairment possibly through normalizing antioxidant mechanisms, BDNF and epigenetic changes in the hippocampus.

1. Introduction

Post-traumatic stress disorder (PTSD) is a disabling, prevalent and difficult-to-treat psychiatric disorder that can develop upon the exposure to severe traumatic events such as those occurring during war and natural disasters (Perlick et al., 2017; Yatham et al., 2017). PTSD is marked by clear biological changes and psychological symptoms (LoSavio et al., 2017). It is complicated by conjunction with related disorders such as depression, substance abuse, memory impairments, and other physical and mental disabilities (LoSavio et al., 2017; Shalev et al., 2017). It is also associated with impairment of ability to function in social and family life, causing occupational instability, marital problems and other social problems (Rees et al., 2013).

It is believed that these major disabilities are related to oxidative stress, which is generated through the psychological or physical traumatic events (Miller and Sadeh, 2014; Schiavone et al., 2013). Several data exhibit strong correlations between the oxidative stress and PTSD complications, especially its harmful implication on cognitive properties (Diehl et al., 2012; Wilson et al., 2013). Evidence showed that oxidative brain damage as a consequence of trauma-induced mitochondrial oxidative stress, influenced hippocampal neural, structural and functional plasticity (Alzoubi et al., 2018b; Duchen, 2004; Raha et al., 2000; Ros-Simo et al., 2013; Sun et al., 2016). PTSD has also been shown to modulate levels of BDNF in the brain via a number of pathways, including oxidative stress and epigenetic mechanisms (Kim et al., 2017; Shafia et al., 2017).

Among the variety of PTSD animal models, the SPS model reproduces most of the neural symptoms presented in PTSD patients (Liberzon et al., 1997). This model was developed by Liberzon et al. (Liberzon et al., 1997). The SPS model consists of three different types of stresses: 2 h restraint, which is psychological, 20 min forced swim, which is physical and ether anesthesia, which is endocrinological. The combined institution of these stresses accomplishes severity of symptoms that resembles most of the symptoms of PTSD (PTSD-like behavior), and elevates corticosterone (Yamamoto et al., 2009; Yehuda and Antelman, 1993). Studies have shown that animals exposed to SPS suffer from increased anxiety, impairment of social and object recognition memory (Eagle et al., 2013) and present with changes in

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glucocorticoid and mineralocorticoid receptors involved in the HPA system (Knox et al., 2012; Zhang et al., 2012).

Pentoxifylline (PTX) has a potent antioxidant activity, where it acts as a competitive nonselective phosphodiesterase inhibitor, which increases cyclic adenosine monophosphate and reduces inflammation (An et al., 2015; Karatay et al., 2017). PTX had been approved for the prevention of intermittent claudication in chronic occlusive arterial disease (Ward and Clissold, 1987). It improves red blood cell deformability, and reduces blood viscosity. Additionally, it exhibits beneficial effects on cognitive functions during status epilepticus (Tariq et al., 2008), brain ischemia (Movassaghi et al., 2012), glutamate lesions (Cunha et al., 2000), aging (Hu et al., 2007), and sleep deprivation (Alzoubi et al., 2013b). Other studies showed that PTX diminished both streptozotocin and malathion induced oxidative stress (Davila-Esqueda and Martinez-Morales, 2004; Ranjbar et al., 2009). Overall, these data suggest that variety of pharmacological benefits of PTX are related to its antioxidant and anti-inflammatory effects. In this study, we investigated the possible protective effect of PTX on learning ability and memory functions in a rat model of PTSD-like behavior. In addition, the effect of PTX on oxidative stress, BDNF and histones biomarkers that have been shown to be modulated by this model were also investigated. The results of this investigation can shed light on a possible use for PTX in the management of PTSD.

2. Materials and methods

Male Wister rats weighing 150–200 g were obtained from the animal facility at Jordan University of Science and Technology (JUST) and were used in this study. Animals were housed in plastic cages (six animals per cage) under hygienic conditions in acclimate-controlled room (24 ± 1 °C) at libitum access to rat chow and water. Rats were identified by tail labelling and they were housed in 12 h light/dark cycle (light on 7:00 am). All of the experimental work was applied at the light cycle. The protocol of the study was by Institutional Animal Care and Use Committee of Jordan University of Science and Technology.

2.1. Animal groups and treatments

Animals were randomly assigned into four groups (n = 12–15/group): control, sustained prolonged stress (SPS) as a model of PTSD-like behavior, pentoxifylline (PTX), pentoxifylline plus SPS (PTX-SPS). Animals were acclimatized for 1 week. Pentoxifylline (Sigma Chemical CO., Saint Louis, MO) was administered by intraperitoneal injection to animals in the PTX and the PTX-SPS groups in a dose of 100 mg/kg six days per week for four weeks and during the behavioral testing day (Alzoubi et al., 2013b; Mayyas et al., 2015; Nouri et al., 2016; Vashghani Farahani et al., 2017). The SPS and PTX-SPS groups were subjected to SPS as a model of PTSD one week after the start of PTX administration. The control and SPS groups were administered distilled water (vehicle in the same volume) via the intraperitoneal route once daily at the same days PTX was administrated to PTX and PTX-SPS groups. The control group was not exposed to SPS procedure.

2.2. Induction of Single Prolonged Stress Model (SPS)

The SPS, which is a well-established model for PTSD-like behavior (Alzoubi et al., 2017a; Alzoubi et al., 2018b; Li et al., 2010; Patki et al., 2014; Yamamoto et al., 2009), was applied to animals of the SPS and PTX-SPS groups. Each animal was placed in double layered a plastic Ziploc bag and covered by duct tape in order to ensure complete immobilization for two hours. Followed by a forced swimming for twenty minutes in a transparent cylindrical container (50 cm in height; 35 cm in diameter; 35 water depths). After that, each animal was placed in a cage for 15 min, followed by ether anesthesia for 1–2 min until loss of consciousness

2.3. Radial Arm Water Maze (RAWM)

The RAWM was used to test spatial learning and memory (Alzoubi et al., 2013c; Alzoubi et al., 2018a; Alzoubi et al., 2017b; Alzoubi et al., 2017c; Rababa'h et al., 2017). It consists of six arms radiating out to an open central area to form six swimming paths with escape platform located at the end of a goal arm that is kept constant for each particular rat during all trials/tests with different starting arm at each trial/test. All four groups were tested using the RAWM for spatial learning ability and memory performance after completing one month treatment period (Alhaider et al., 2010; Alzoubi et al., 2009; Diamond et al., 1999; Gerges et al., 2004; Park et al., 2001). All experiments were carried out in a dimly lit room with visual cues fixed on the walls of the room during the experiment. Water temperature was maintained at 23 ± 1 °C. Each animal had to find the submerged platform (2 cm beneath water level) located at the end of the one swimming arm (goal arm) in one minute. There are two phases, the learning phase and the testing phase. The learning phase consists of two sessions, each session consists of six trials one minute/trial, and 5 min rest between the two sessions. During each trial, the animal was allowed to freely swim to find the submerged platform. However, it was guided to the platform after spending one minute of swimming without finding hidden platform. Once on the platform, the animal was left there for 15 s to observe visual cues on the walls before the next trial was started. In memory tests, the animal was neither guided to the platform nor given 15 s on the platform. Each rat had to undergo 12 learning trial. The short-term memory test was done 30 min, whereas the long-term memory tests were done 5 h and 24 h, after the last learning trial. In memory tests, each rat was given one minute to locate the hidden platform. An error was recorded when the rat entered to any arm other than the goal arm.

2.4. Brain dissection

The brain was dissected immediately after killing the animals. It was then, placed over a filter paper impeded with normal saline over a cold glass dish filled with a crushed ice. The hippocampus was isolated and placed immediately in a previously labeled Eppendorf tube and transferred to a container filled with liquid nitrogen. Eppendorf tubes were placed at −30 °C until analysis.

2.5. Molecular assays

The obtained hippocampus tissues were homogenized using 200 µl of homogenization buffer prepared by reconstitution of one tablet of phosphate buffered saline (Sigma Chemical CO., Saint Louis, MO) and two protease inhibitor tablets (Sigma Chemical CO., Saint Louis, MO) in 200 ml of distilled water using plastic pestle (Mhaidat et al., 2015). The homogenized tissues were centrifuged 15000xg for 10 min at 4 °C in order to remove insoluble materials. The supernatant was obtained and stored for further analysis. Total protein concentration in the obtained supernatant was estimated using an available commercial kit (Bio-Rad, Hercules, CA, USA).

To quantify total glutathione, tissues homogenates were deproteinized with 5% of 5-sulfosalicylic acid (SSA) Solution, centrifuged at 10,000 xg for 10 min, at 4 °C to remove the precipitated protein, and then assayed photometrically for glutathione according to the kit's instructions (Glutathione assay kit, Sigma-Aldrich, MI, USA). GSSG measurement, 10 µl of 1 M 2-vinylpyridine (Glutathione assay kit, Sigma-Aldrich, MI, USA) was added per 1 ml of supernatant of the sample, then the procedure was carried as described above for total glutathione. GSH was then calculated by subtracting GSSG value from total glutathione. Glutathione peroxidase (GPx) activity was determined using cellular activity assay kit (CGP1, Sigma Aldrich, MI, USA). Catalase activity was measured using commercially available kits according to manufacturer instructions (Cayman Chem, Ann Arbor, MI, USA). The H3 and H4 histones levels were measured using