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Lycium barbarum polysaccharide improves traumatic cognition via reversing imbalance of apoptosis/regeneration in hippocampal neurons after stress

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ABSTRACT

Aims: Previous studies in our laboratory have demonstrated the increased neuronal apoptosis in the hippocampus and abnormal hippocampal morphology after severe stress, which directly correlates to the pathogenesis of post-traumatic stress disorder (PTSD). This study aims to investigate the effects of *Lycium barbarum* polysaccharide (LBP) on intrusive memory of posttraumatic stress in rats, and to analyze the mechanism of regeneration/apoptosis balance in the hippocampal neurons.
Main methods: The experimental rats received 20 inescapable electric foot shocks in an enclosed box for six times in three days. The rats were treated by intragastric administration of LBP (20 mg/kg/day) for 3 days before stress in the stress plus prophylactic group, and for 28 days after stress in the stress plus therapeutic group. The emotion, intrusive memory-related behavior (freezing, open field, pain latency, spatial cognition), hippocampus cell morphology, and relation of neurogenesis and apoptosis in dental gyrus of the hippocampus were observed. The hippocampus volume was evaluated by stereology. Meanwhile, the neurogenesis and apoptosis were analyzed with 5-bromo-2′-deoxyuridine and terminal deoxylnucleotidyl transferase mediated-dUTP nick end labeling (TUNEL) method.
Key findings: The treatment of LBP in pre-stress and post-stress had obvious beneficial effect on the behaviors and neurogenesis. The stressed rats showed improvement of intrusive memory related cognition defect, alleviation of the apoptosis in the hippocampus and recovery for the neurogenesis, which was related to the hippocampus volume after LBP treatment.
Significance: LBP treatment might effectively improve the traumatic cognition defect induced by severe stress and be useful for the intrusive memory-related cognition recovery.

Introduction

Intrusive memory is a severe symptom affecting people in traumatic stress. It also constitutes the key clinical manifestation in posttraumatic stress disorder (PTSD). Growing evidence has witnessed that the hippocampus acts as the advanced center and most sensitive brain region in stress responses [28]. It is involved in not only the courses of emotion, learning and memory, but also in the neurogenesis in the mammalian brain. Hippocampal CA and dental gyrus (DG) regions are mainly composed of pyramidal cells and granular cells respectively. Granular cells in DG region possess the neurotization potential ([5,9]). Since stress is the chief regulatory factors in the memory formation in the hippocampus [8], the morbid forgetfulness, some detail reinforcement, flash back and solidifying in intrusive memory may be related to hippocampal neurogenesis.

Apoptosis is a cascade process of programmed cell death regulated by endogenous genes, enzymes and intracellular signaling. Recently, the relation between non-traumatic stress and neuronal apoptosis has attracted much attention. Also, clinical imageology data from single photon emission tomography, positron emission tomography and magnetic resonance imaging revealed that the functional pathological

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changes and structure abnormality, such as regional blood flow changes, cortex thickness decrease or atrophy and fissure increase [19,27,36]. Animal studies imitating the PTSD symptom also showed the neuronal apoptosis [3,25]. However, whether apoptosis and neurogenesis in the hippocampus could be influenced reciprocally remains to be elucidated. The potential hippocampal neurogenesis/apoptosis interrelationship is of great value for the clarification of intrusive memory in PTSD.

Concerning the high incidence and sophisticated outcome in post-traumatic stress, plant essence isolated from traditional Chinese plants has been paid much attention due to its efficiency and biosafety. *Lycium barbarum* belongs to nightshade as a valuable Chinese plant both for drug and food. Till now, Ningxia *L. barbarum* is the only species recorded in Chinese pharmacopeia. *L. barbarum* polysaccharide (LBP) is the main active constituent. Previous studies showed that LBP have the bioactivity to protect liver function, anti-radiation, anti-fatigue, anti-tumor and anti-oxidation [32,40,44]. It had been used for improving the blunted neurogenesis for manganism mice, stroke ischemia and free radical clearance in brain injury [4,37,39], suggesting that LBP may play a prominent role in anti-stress in trauma.

Thus, we hypothesized that regeneration/apoptosis imbalance in the hippocampal neurons is involved in the intrusive memory of PTSD, which could be reversed by LBP treatment. To test this hypothesis, we use a PTSD-like rat model induced by short time-electric foot shocks to evaluate the protective effects of LBP on intrusive memory and other key symptoms in PTSD, and investigate its possible mechanism in the hippocampus (Fig. 1). These data may provide new insights into the effect of LBP on PTSD, which could contribute to the rehabilitation of PTSD patients.

Materials and methods

Animals and grouping

A total of 126 male Sprague–Dawley rats weighing 180–218 g (age 4–6 months) were purchased from Third Military Medical University (Chongqing, China) and given at least seven days of acclimation. The rats lived in acrylic boxes 44 × 33 × 20 cm (four per box) at constant room temperature (23 ± 2 °C) and humidity (60%) with a 12 h/12 h light–dark cycle. The rats were randomly assigned to control, stress, stress plus prophylactic (Pre-LBP + stress) and stress plus therapeutic (Post-LBP + stress) groups. Animals (control: n = 10, stress, stress plus prophylaxis and stress plus therapeutic groups: n = 8 per group) were used for weight and behavioral tests such as freezing, pain latency, elevated plus maze (EPM), open field, and water maze tests. Previous rats except for those in water maze tests were randomly assigned for morphological, apoptosis, and 5-bromo-2′-deoxyuridine (n = 6 per group for morphological and terminal deoxylnucleotidyl transferase mediated-dUTP nick end labeling (TUNEL) analysis, n = 6 per group for neurogeneration analysis, n = 6 per group for volume evaluation).

LBP treatment

The lyophilized powder of LBP from Ningxia Rubygoji Ltd is freshly dissolved in saline. Animals were treated with LBP by intragastric administration. The rats in the prophylactic group were treated with LBP solution at 20 mg/kg/day for 3 days before stress, while those in the therapeutic group were treated with LBP at 20 mg/kg/day for 28 days after stress. The rats in the control and stressed groups were treated with equal volume of saline. The treat time is fixed at the 8:00 a.m.

Procedure for stress

All experiments were performed between 08:00 a.m. and 04:00 p.m. After habituation, each rat was placed individually in an enclosed opaque box (17.0 × 8.0 × 40 cm) above a stainless steel grid floor. The stress box was connected to a scrambler controller that delivers shocks to the metallic floor. On days 1, 2, and 3, rats in the stress group were given stimulus twice a day, with an interval of >4 h. For traumatic stress, rats were confined to an enclosed box (stress box) for 30 min

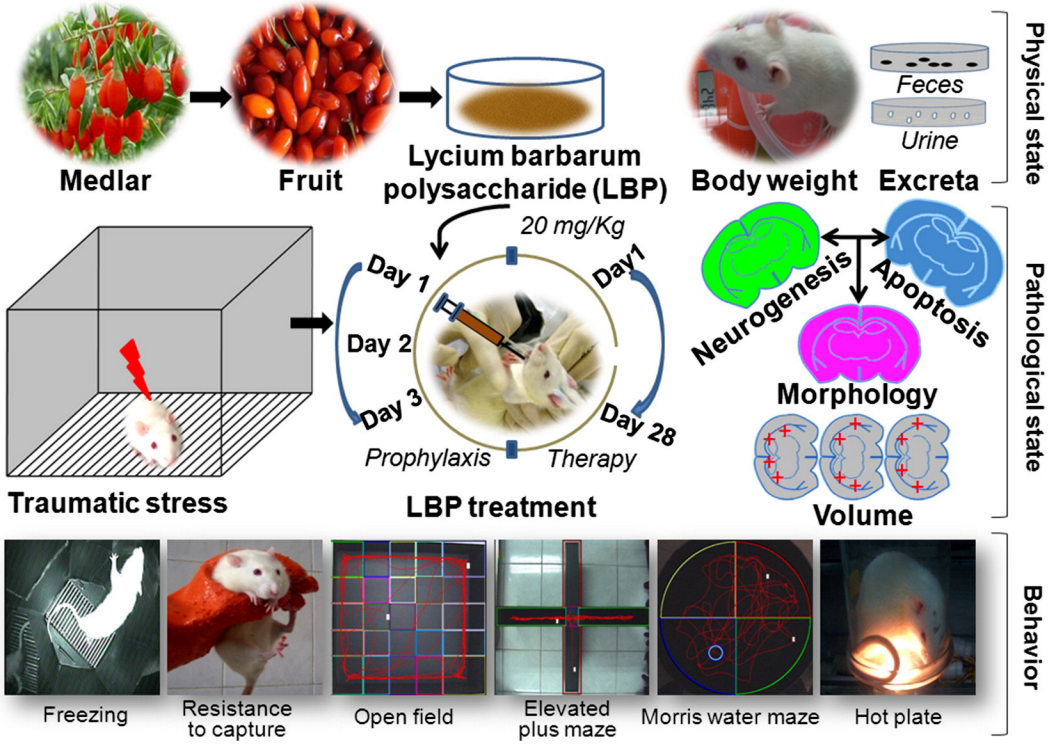

Fig. 1. Experimental design. Male rats in the stress group began daily two times of inescapable electric shock in enclosed box in 3 consecutive days. The rats in the stress plus prophylactic group were treated with 20 mg/kg/day LBP for 3 days before stress, and the rats in the stress plus therapeutic group were given 20 mg/kg/day LBP for 28 days after stress. In the first and fourth weeks after stress, the body weight changes, values of resistance to the capture, and behavior in contextual fear conditioning, open field, elevated plus maze, hot plate and Morris water maze were tested. Meanwhile, tissues of the hippocampus were collected for detection of apoptosis and regeneration of dental gyrus, morphology and volume evaluation.

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