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The effects of captopril on lipopolysaccharide induced learning and memory impairments and the brain cytokine levels and oxidative damage in rats

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ABSTRACT

Aim: Renin-angiotensin system has a role in inflammation and also involves in learning and memory. In the present study, the effects of captopril on lipopolysaccharide (LPS) induced learning and memory impairments, hippocampal cytokine levels and brain tissues oxidative damage was investigated.
Materials and methods: The rats were divided and treated : [1] saline (Control), [2] LPS (1 mg/kg), [3-5] 10, 50 or 100 mg/kg captopril 30 min before LPS. The treatment was started since six days before the behavioral experiments and continued during the behavioral tests (LPS injection two h before each behavioral experiment).
Results: Administration of LPS prolonged the escape latency and traveled path to find the platform in Morris water maze (MWM) test ($P < 0.01$–$P < 0.001$) while, shortened the latency to enter the dark compartment in passive avoidance (PA) test ($P < 0.001$). Pretreatment by all doses of captopril improved performances of the rats in MWM ($P < 0.05$–$P < 0.001$) and also prolonged the latency to enter the dark in PA test ($P < 0.001$). LPS also increased IL-6, TNF-α, malondialdehyde (MDA) and nitric oxide(NO) metabolites in the hippocampal tissues ($P < 0.05$–$P < 0.001$) which were prevented by captopril ($P < 0.05$–$P < 0.001$). The thiol, superoxide dismutase(SOD) and catalase(CAT) in the hippocampus of LPS group were lower than the control ($P < 0.001$) while, they were enhanced when the aniamls were pretraeted by captopril ($P < 0.01$–$P < 0.001$).
Conclusion: The results of present study showed that captopril improved the LPS-induced learning and memory impairments in rats which were accompanied with attenuating hippocampal cytokine levels and improving the brain tissues oxidative damage criteria.

1. Introduction

The renin-angiotensin system (RAS) is one of the neuropeptide systems in the brain. The substrate of this system, angiotensinogen, is suggested to be synthetized in several regions of the brain and is cleaved by the enzyme renin to form the decapeptide angiotensin (Ang I) [1]. Ang I is then converted to an octapeptide Ang II by angiotensin converting enzyme (ACE) [2] which is extensively located within the central nervous system (CNS) areas [3]. Ang II as the main effector of RAS binds to specific receptors to perform multiple actions in the brain [4]. The brain RAS has also been shown to have a role in Alzheimer's disease(AD) and the other diseases associated with memory impairments including stroke, depression and emotional stress [1,5] which are suggested to be preventable by angiotensin converting enzymes (ACE) inhibitors including captopril [6].

Also, RAS has a role in inflammation responses. In recent studies, Ang II and AT_1 receptors have been suggested to implicate in inflammation responses and lipopolysaccharide (LPS) -mediated microglial activation [7]. It has also been reported that Ang II in a dose dependent manner enhances interlukin 6 (IL-6) release from smooth muscle cells [8]. ACE inhibitors have also been reported to have anti-inflammatory effects in both in vitro and in vivo conditions through reducing inflammatory cytokines such as tumor necrosis factor α (TNFα) and interlukin1 (IL-1) [9]. It has also been reported that treatment with

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captopril decreases inflammation responses, improves glomerulonephritis in mice and reduces symptoms in patients with rheumatoid arthritis [10].

Lipopolysaccharide (LPS), as a potent bacterial endotoxin [11], has frequently used to induce a neuroinflammation model in rodents. LPS triggers immune responses and promotes the generation of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (12). When administered systemically or centrally, LPS was reported to be able to affect on cognitive, learning and memory [12–16]. These effects are attributed to a cascade of cytokines which are generated and released from immune cells such as macrophages. The primarily released cytokines due to inflammation induced by LPS are interleukin- 1β (IL-1β) and TNF_{α} [16,17]. It has been well-documented that activation of immune cells, including macrophages and neutrophils by LPS, is associated with memory and synaptic plasticity impairment [18]. An increased level of IL-1β followed by LPS administration is reported to lead to cognitive dysfunction and spatial learning deficits in Morris water maze (MWM) [19]. In another study, researchers showed that administration of LPS leads to spatial memory impairment and cognition, reduction of antioxidant defense, and an increased level of inflammatory cytokines such as TNF-α and IL-1β in the hippocampus [13–15,19]. In addition, ACE inhibitors are suggested to affect the generation of proinflammatory and anti-inflammatory cytokines induced by LPS [20,21].

We, therefore, decided to test the effects of captopril on lipopolysaccharide induced learning and memory impairments, hippocampal cytokine levels and brain tissues oxidative damage in rats.

2. Materials and methods

2.1. Animals and drugs

Male Wistar rats (8 weeks old and weighing 200–250 g) were obtained from animal house of Mashhad University of Medical Sciences, Mashhad, Iran. The animals were housed in the animal house with standard temperature (22 ± 2 °C) and 12 h light/dark cycle. The rats were freely allowed to food and water. Animal examinations were carried out by procedures approved by the Committee on Animal Research of Mashhad University of Medical Sciences. The animals were allocated to five groups: [1]Control [2] LPS, [3] LPS-captopril 10(LPS-Capto 10), [4] LPS-captopril 50 (LPS-Capto 50) and [5] LPS- captopril 100(LPS-Capto 100) ($n = 10$ in each group). These animals were treated by drugs or vehicle for 6 days and used for behavioral tests. The drugs were dissolved in saline and injected IP (LPS 1 mg/kg in groups 2–5, captopril 10, 50 and 100 mg/kg in groups 3,4 and five respectively). LPS was also administered two h before the behavioral experiments and captopril were injected 30 min before LPS. In the LPS group, the animals were treated with saline (2 ml/kg) instead of captopril. The animals of the control group received 2 ml/kg of saline instead of LPS. All drugs were prepared freshly. LPS (E.coli 055: B5), and captopril were purchased from Sigma (Sigma Aldrich Chemical Co.). The chemical agents which were used for biochemical assessments were purchased from Merck Company.

2.2. Morris Water Maze (MWM) test

MWM apparatus was consisted of a cylindrical tank 136 cm in diameter, 60 cm in high and 30 cm in deep with boundaries of the four quadrants. The tank was filled with water (23–25 °C) until a circular platform 10 cm in diameter and 28 cm in high was submerged 2 cm beneath the surface of the water in the center of the Northwest quadrant. Fixed visual cues at several locations around the maze and on walls of the room determined the navigation path. Before each experiment, for familiarizing with the apparatuses, the animals were placed in filled maze with water without a platform for 30 s. In the hidden platform acquisition test, the animals were released randomly in the tank at one of four positions (north, east, south, and west) and allowed to swim freely to find the hidden platform within 60 s. The position of the animals was detected by a camera that was hung above the center of the pool. The camera signals were transformed to a computerized tracking system that monitored and stored the location of the animals [22]. The time spent, traveled distance to reach the platform and the swimming speed was recorded. If the rat found the platform within 60 s, it was allowed to remain on the platform for 20 s before the next trial otherwise, it was guided to the platform by the experimenter and permitted to stay on it for 20 s. The experiments were repeated with four trials in each day for five consecutive days. The mean of the time spent and traveled distance were measured to evaluate the spatial learning ability. Twenty-four hours after acquisition test, the platform was removed, and a probe test was performed. The time spent and the traveled path in the target quadrant (Q1) was compared between groups [23].

2.3. Passive avoidance (PA) test

PA apparatus was made of a light and a dark chamber separated by a guillotine door. During habituation trial, the animals were placed into the light compartment and permitted to move freely between two chambers for 5 min. In acquisition trial, after entering the animal into the dark chamber, the guillotine door was closed, and an electrical shock (2 mA, 2 s) was delivered to the animal's feet. At 3, 24, 48 and 72 h later, the animals were again placed into the light compartment and the latencies to enter the dark room as well as the time spent by the animals in the dark compartment were recorded and defined as retention trial [24].

2.4. Biochemical assessment

After the last session of the behavioral test the animals were deeply anesthetized and were then killed, and their hippocampal and cortical tissues were removed, weighed, and submitted to determine biochemical assessment. Total thiol (SH), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were measured in both the hippocampal and cortical tissues. NO metabolites (NO_2 or NO_3), TNF-α and IL-6 concentrations were determined in hippocampal tissues.

2.4.1. Determination of hippocampal TNFα and IL-6 contents

Hippocampal tissues TNFα and IL-6 contents determination were performed with specific rat ELISA kits (ebioscience Co., San Diego, CA, USA) according to the manufacturer instructions. The absorbance was measured using a microplate reader (Biotek, USA) and concentration of TNFα and IL-6 was calculated by comparison curve established in the same measurement.

2.4.2. Determination of MDA, total thiol, and NO metabolites

The concentration of MDA, as an index of lipid peroxidation, and total thiol groups content were determined in the hippocampal and cortical tissues according to a protocol that we described previously [24]. Briefly, after reacting MDA with thiobarbituric acid (TBA) and producing a red complex, the absorbance was read at 535 nm. DTNB (2, 2′-dinitro-5, 5′-dithiodibenzoic acid) as a reagent reacts with the SH group and produces a yellow complex that has a peak absorbance at 412 nm. Hippocampal tissues NO metabolites (NO_2/NO_3) were measured according to the Griess reagent methods. In brief, after adding 100 μl supernatant to the Griess reagent, contents were transferred to a 96-well flat-bottomed micro-plate and absorbance was read at 520 nm using a micro-plate reader, and the final values were calculated from standard calibration plots [25–27].

2.4.3. The enzymatic assays

SOD activity in both cortical and hippocampal tissues was determined by the method of Madesh and Balasubramanian [28]. In a colorimetric assay, the SOD activity was measured at 570 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the rate