The effects of captopril on lipopolysaccharide-induced sickness behaviors in rats

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Article Info	Abstract
	Neuro-immune mediators play an important role in the development of sickness behaviors. In the present study, the effect of captopril on sickness behaviors caused by lipopolysaccharide (LPS) was studied in the rats. The animals were randomized into the following groups: control, sham, 10 mg kg^{-1} captopril - LPS (Capto 10-LPS), 50 mg kg^{-1} captopril - LPS (Capto 50-LPS), and 100 mg kg^{-1} captopril - LPS (Capto 100-LPS). Behavioral tests including open-field (OF), elevated plus maze (EPM) and forced swimming (FS) test were performed, and the serum level of interleukin-6 (IL-6) was assessed. In OF, the number of crossings in the central zone in Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups was higher than that of the sham group. In EPM, the open arm entry numbers in the sham group were lower compared to the control group. Furthermore, pretreatment by captopril increased the entries to the open arms. In FS test, the immobility time of the sham group was longer than that of the control group. In Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups, immobility was shorter compared to the sham group. In addition, the IL-6 level was higher in the sham group compared to the control group, and treatment with 50 and 100 mg kg^{-1} of captopril restored the IL-6 level in comparison with the sham group. Results confirmed that pretreatment with captopril ameliorated LPS-caused sickness behaviors and attenuated IL-6 as an inflammatory marker in the rats.

Introduction

The renin-angiotensin system (RAS) is well known as a circulating hormonal system in mammals. A locally independent RAS has also been reported in the central nervous system (CNS).[1] Angiotensin II (Ag II) as the principal effector of RAS is synthesized in the brain[2] and can regulate the release of multiple neurotransmitters such as gamma-aminobutyric acid (GABA), norepinephrine, 5-hydroxytryptamine (5HT), and acetylcholine in the CNS.[3] It has also been proposed that Ag II may exert certain effects on cognitive functions and memory which can be attenuated by angiotensin-converting enzyme (ACE) inhibitors.[4-6] In addition, a relationship between RAS components such as Ag II and inflammation has been suggested.[7,8] Recent studies revealed that Ag II and its AT1 receptors are involved in the neuro-inflammation caused by lipo-polysaccharide (LPS).[9,10]

Scientific evidences also demonstrated that some uncontrolled generations of inflammatory cytokines contribute to several behaviors in rodents which are generally named sickness behaviors.[11] Sickness behaviors occurring following putrefaction, and tissue damage are characterized by disquiet, lack of interest in social interactions, narcosis, decreased locomotor activity, decreased grooming behaviors, a decline in reproductive fulfillment, drowsiness, loss of appetite, loss of weight, decentralization, and anguish.[12,13] Researchers suggest that some sickness behaviors are similar to depression behaviors.[14] Depression is manifested by a combination of

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the states of sadness, loneliness, irritability, despair, confusion, and reduction of locomotor activity.[15] Depression is also proposed to be as an immune-inflammatory disorder, and neuro-immune stimulators play a crucial role in its pathogenesis.[16] In addition, the anti-neuroinflammatory properties of antidepressant drugs and their decreasing effects on cytokine levels may confirm the important role of neuro-inflammation in sickness behaviors and depression.[17]

The LPS, a potent bacterial endotoxin, induces an inflammation status via triggering the over-generation of inflammatory cytokines.[18] The results of other studies revealed that the systemic injection of LPS induces a sickness behavior in rodents.[19] It has also been reported that LPS induces a depression-like behavior when injected intracerebroventricularly or peripherally.[15] Since LPS-induced inflammation triggers sickness behaviors, and regarding the effects of RAS components on both inflammation and brain disorders such as Alzheimer's disease (AD), anxiety, and depression, the present study was conducted to further define the role of captopril in sickness behaviors induced by LPS.

Materials and Methods

Animals and drugs. This study was conducted on healthy male Wistar rats weighing 240.00 ± 10.00 g. The animals were provided by the Laboratory Animal Center of Mashhad University of Medical Sciences, Iran. They were kept under standard conditions (22.00 ± 2.00 °C and 12 hr light/dark cycle). The rats had free access to sufficient amounts of food and water. Animal studies were performed in accordance with the approved procedures and supervised by the Committee on Animal Research of Mashhad University of Medical Sciences (ir.mums.fm.rec. 1394.39m:16.03.1394). The animals were randomly divided into the following groups (n=10): 1) control, 2) sham, 3) 10 mg kg^{-1} captopril -LPS (Capto 10-LPS), 4) 50 mg kg^{-1} captopril -LPS (Capto 50-LPS), and 5) 100 mg kg^{-1} captopril -LPS (Capto 100-LPS). The animals in the sham, Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups received LPS (1.00 mg kg^{-1}; intraperitoneally) 120 min before behavioral experiments.[20] Captopril (10, 50, or 100 mg kg^{-1}) was administered to Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups three days before starting the behavioral experiments. Injection of captopril was made 30 min before LPS injetion.[20] The sham group was treated by saline instead of captopril. Rats of the control group were administered 1.00 mL kg^{-1} of saline instead of both captopril and LPS. The LPS derived from *Escherichia coli* 055:B5 was provided by Sigma-Aldrich Chemical Co., (Darmstadt, Germany), and captopril was kindly provided by Daroupakhsh Company, Tehran, Iran.

Open-field (OF) test. After the animals were got used to the OF apparatus (Noavarane Sanaie Amoozeshi,

Mashhad, Iran), they were freed in the center of the apparatus. The movement was recorded using a digital camera for 5 min. The number of crossings in the central zone and the total number of crossings were recorded.

Elevated plus maze (EPM) test. A standard EPM apparatus (Noavarane Sanaie Amoozeshi) with four arms (two open arms and two closed arms) 50 cm in length and 10 cm in width was utilized. The height of the arms was approximately 100 cm. The apparatus was equipped with a digital camera. The test was performed by placing the animals in front of the closed arm. The recorded parameters were the time elapsed and the number of entries into the open arms.

Forced swimming (FS) test. The FS test was executed for all animals. In summary, each rat was forced to swim in a glass cylindrical tank (60 cm in height and 38 cm in width). Then, the tank was filled with water (24.00 ± 1.00 °C) to 40 cm. The duration of immobility and active times was calculated for 5 min by an observer blind to the treatment.

Measurement of Interleukin 6 (IL-6). After completing the behavioral tests, the rats were deeply anesthetized by urethane (Acros Organics, Morris Plains, USA), and their blood was taken from the heart to assess the serum level of IL-6 by an ELISA kit (eBioscience, San Diego, USA). The assessment was performed based on the guideline provided in the kit. Finally, absorbance was read using a microplate reader (Biotek, Winooski, USA). Serum IL-6 concentration was determined by comparing the absorbance of the samples with an established curve made by different standard concentrations.

Statistical analysis. The data were presented as mean ± SEM. Data were analyzed using SPSS (version 19.0; SPSS Inc., Chicago, USA) and using one-way ANOVA followed by Tukey post hoc test, with $p < 0.05$ set as the significance level.

Results

OF test. The number of crossings in the central zone was significantly higher in the animals of Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups than the sham group ($p < 0.001$; Fig. 1A). However, no significant difference was observed between sham and control groups. Additionally, the number of central crossings of Capto 50-LPS ($p < 0.01$) and Capto 100-LPS ($p < 0.001$) groups was higher than that of the Capto 10-LPS group. The results of OF also indicated that the total number of crossings was lower in the sham group with respect to the control group ($p < 0.01$; Fig. 1B). The total number of crossings in Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups was significantly more than that of the sham group ($p < 0.001$; Fig. 1B). In addition, the total number of crossings was significantly higher in Capto 50-LPS and Capto 100-LPS groups than the Capto 10-LPS group ($p < 0.01$).
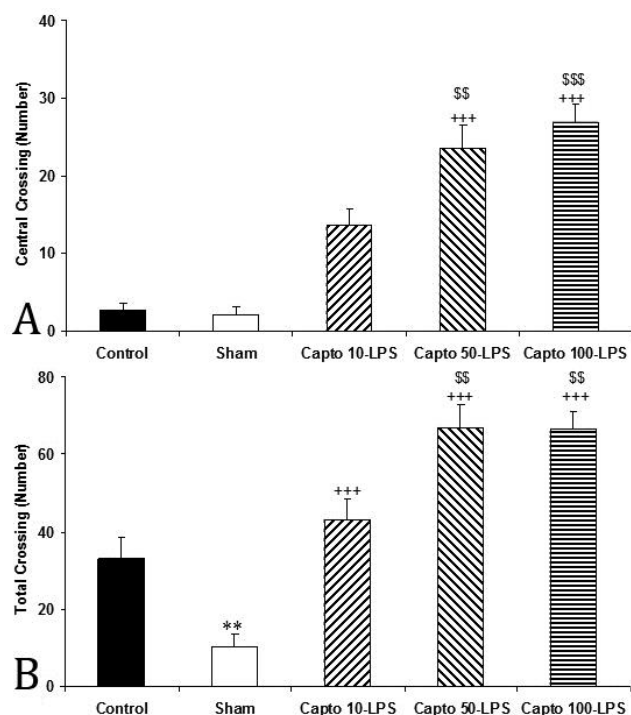
Fig. 1. Comparison of the numbers of central (A) and total (B) crossing in the OF test among the four groups. Data are expressed as mean ± SEM (n = 10 in each group). ** $p < 0.01$ shows the difference between sham and control groups. +++ $p < 0.001$ shows the difference between Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups compared to the sham group. $$ $p < 0.01$ $$$ $p < 0.001$ shows the difference between Capto 50-LPS and Capto 100-LPS groups compared to the Capto 10-LPS group.

EPM test. The open arm entries in the sham group were low compared to the control group ($p < 0.01$). In Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups, the entries to the open arm were more than those of the sham group ($p < 0.001$; Fig. 2A).

In this study, no significant difference was found among the three captopril-treated groups. Findings also demonstrated that the time spent in the open arm in the sham group was lower than that of the control group ($p < 0.001$). The rats of Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups spent more time in the open arm than the LPS group ($p < 0.01$ and $p < 0.001$; Fig. 2B). The data also indicated that the rats of the Capto 50-LPS group spent a shorter time in the open arm than the Capto 10-LPS group ($p < 0.01$). Additionally, the time spent in the Capto 100-LPS group in the open arm was longer than that of both Capto 10-LPS and Capto 50-LPS groups ($p < 0.001$).

FS test. Based on the results of the FS test, the immobility time in the sham group was more than that of the control group ($p < 0.001$). The immobility time of the animals in Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups was lower than that of the sham group ($p < 0.001$; Fig. 3A).

The results did not show any significant difference among the Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups in terms of immobility time. In addition, results of FS test revealed that the active time in the sham group was less than that of the control group ($p < 0.001$; Fig. 3B). The active time for the rats of Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups was higher with respect to the sham group ($p < 0.001$; Fig. 3B). According to the findings of this study, no significant difference was observed between Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups in terms of active time.
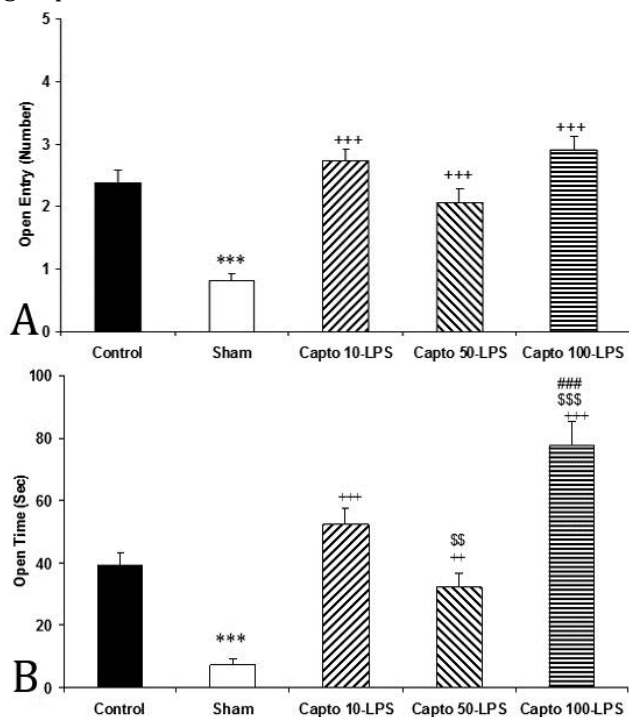

Fig. 2. Comparison of the number of entries to (A) and the time spent in (B) the open arm of the EPM test across the four groups. Data are expressed as mean ± SEM (n = 10 in each group). *** $p < 0.001$ indicate the difference between sham and control groups. ++ $p < 0.01$ +++ $p < 0.001$ indicate the difference between Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups compared to the sham group. $$ $p < 0.01$ $$$ $p < 0.001$ indicate the difference between Capto 50-LPS and Capto 100-LPS groups compared to the Capto 10-LPS group. ### $p < 0.001$ indicates the difference between Capto 100-LPS and Capto 50- LPS groups.

IL-6 level. Injection of LPS increased the level of IL-6 in the serum of the LPS group compared to the control group ($p < 0.001$). The data also revealed that treatment with two higher doses of captopril decreased serum concentration in Capto 50-LPS and Capto 100-LPS groups with respect to the LPS group ($p < 0.01$ to $p < 0.001$, receptively). However, data did not indicate any significant difference between Capto 10-LPS and LPS groups (Fig. 4). Additionally, no significant difference was observed between Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups.
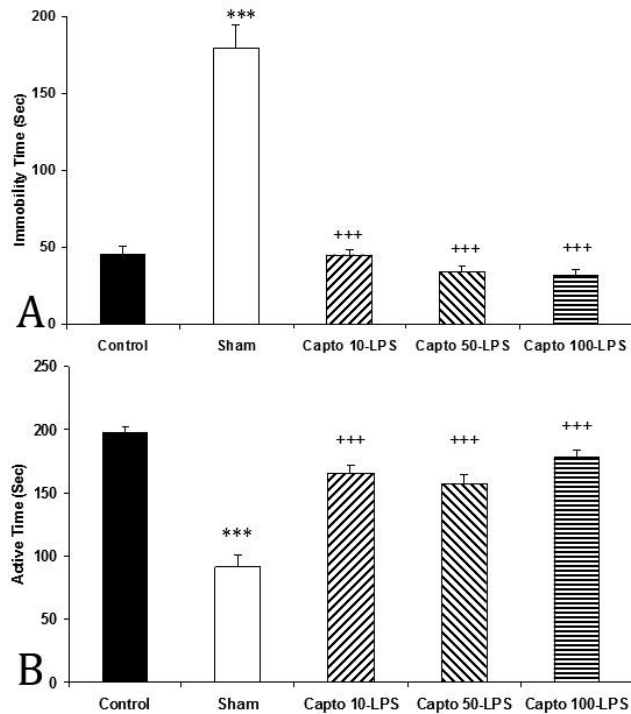
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Fig. 3. Comparison of immobility (A) and active (B) time in the FS test across the four groups. Data are expressed as mean ± SEM (n=10 in each group). *** $p < 0.001$ shows the difference between control and sham groups. +++ $p < 0.001$ shows the difference between Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups compared to the control group.
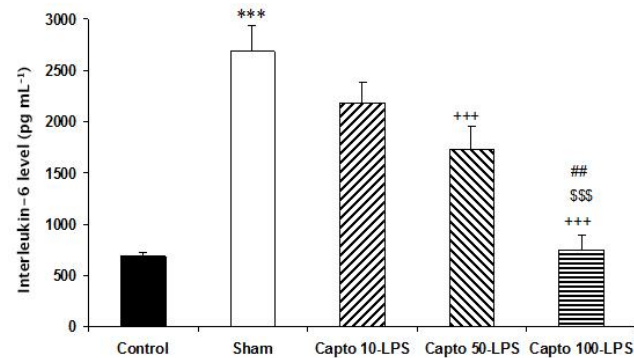

Fig. 4. Comparison of serum interleukin-6 levels among the four groups. Data are expressed as mean±SEM (n = 10 in each group). *** $p < 0.001$ indicates the difference between control and sham groups. +++ $p < 0.001$ indicates the difference between Capto 10-LPS, Capto 50-LPS, and Capto 100-LPS groups compared to the control group. $$$ $p < 0.001$ indicates the difference between Capto 50-LPS and Capto 100-LPS groups compared to the Capto 10-LPS group. ## $p < 0.01$ indicates the difference between Capto 100-LPS and Capto 50-LPS groups.

Discussion

As a structural component of most Gram-negative bacteria, LPS has the potential to induce the generation of various pro-inflammatory mediators in the brain tissue and serum, leading to inflammation.[21] Based on the results of this study, LPS injection resulted in the induction of inflammatory responses presented by an enhanced level of IL-6 in the serum of the rats. Previously, studies reported that LPS induced a sickness behavior accompanied by a decrease in appetite, body weight, libido and sexual behavior, inhibition of exploratory and social activities, induction of fatigue, cognitive impairment, and depression- and anxiety-like behaviors.[22,23] It has also been reported that sickness behaviors are observable 2 hr after LPS injection.[11] In the present study, the injection of LPS resulted in depressive symptoms and sickness behaviors in rats, presented by an increase in immobility times in the sham group compared to the control rats when the animals were tested 2 hr later in the FS test. LPS also decreased the activity of the rats compared to the control group. The LPS has been proposed to increase the metabolism of many neurotransmitters such as norepinephrine and serotonin in the brain which are essential for the regulation of emotions, psychomotor functions, and reward.[24,25] Interestingly, it has been reported that these changes may occur shortly after LPS injection.[26,27] Supporting these ideas, offspring rats exposed to LPS have been reported to show depression-related behaviors accompanied by a low level of serotonin in the hippocampus.[28] The effect of LPS on depression and sickness behaviors seen in the present study might also be explained by altered concentrations of these neurotransmitters in the brain which, of course, requires further investigation. Based on previous studies, LPS promoted the production of inflammatory cytokines in the serum and multiple regions of the brain, including hippocampus formations, hypothalamus, and some diencephalic areas, thus playing a role in depression.[12,17] It has also been indicated that an enhanced level of inflammatory cytokines following the administration of LPS was accompanied by depression-like behaviors in rats.[29]

One of the signs of sickness behaviors caused by LPS in rodents is anxiety.[30] EPM is a well-known tool frequently employed for the evaluation of anxiety-like behaviors in which the time spent in open arms was compared among groups.[31,32] Using the EPM test, LPS was observed to induce anxiety-like behavior in rats.[33] In the present study, the number of entries into open arms and the time spent in open arms were decreased in LPS-treated rats compared to those treated with saline. These results may provide another evidence for the emergence of sickness behaviors due to the administration of LPS. In the present study, LPS injection was followed by a reduced number of total crossings in the OF test which may be considered as another evidence for sickness behavior.[31] Nevertheless, the central crossing was affected by LPS.

A large body of evidence suggests that the administration of ACE inhibitors such as captopril exerts

antidepressant-like effects.[34] Researchers also indicated that the reduction of RAS activity in the brain has positive effects on the cognitive and neurotransmitter release, including acetylcholine and serotonin.[35] In our study, the injection of captopril before LPS improved sickness behaviors presented by a decrease in immobility, however, an increase in active time in the FS test. The number of crossings in the central area of the OF test is considered as an indicator for depression.[36,37] In this study, captopril increased the number of crossings in the central arena of the OF test which may confirm its improving effects on depression-like behaviors. RAS has also been suggested to play a role in the etiology and remedy of depression.[38,39] The mechanism(s) responsible for the effects of RAS components has not been fully understood. Research suggests that there is an interaction between brain RAS and the release of neurotransmitters such as dopamine,[40,41] GABA, norepinephrine, serotonin, and acetylcholine.[42,43] Multiple neurotransmitter systems such as 5HT, dopamine, and norepinephrine have been suggested to be involved in the etiology of sickness behaviors.[44] The beneficial effect of captopril on LPS-induced sickness behavior observed in the present study might be explained by both anti-inflammatory effects and the interaction with brain neurotransmitters.

Using EPM test, it was revealed that pre-treatment with captopril increased the time spent in and the entries into the open arms compared to the sham group, a manifestation for the beneficial effects of anxiety-like behaviors induced by LPS. All three doses of captopril prolonged the time spent in the open arms of the EPM tests, confirming the anti-anxiety effects of captopril in the LPS model in rats. Supporting our data, it has been indicated that captopril and losartan decreased the anguish state in animals in EPM.[45] The number of crossings in the central area of OF was higher in the groups pretreated by all doses of captopril than the LPS group, confirming its improving effects on sickness behaviors induced by LPS. These results along with the prolonging effects of captopril on total crossing in OF may be considered as an effect on motor activity and another explanation for the beneficial effects of captopril on sickness behaviors induced by LPS.

Furthermore, the beneficial effects of ACE inhibitors are suggested to be due to their anti-oxidative and anti-inflammatory properties.[39] Besides blood pressure and body fluid regulation, Ag II as an important effector peptide in RAS has been shown to play an important role in inflammatory responses.[46] Other studies have suggested the involvement of Ag II in the progression of inflammatory processes induced by LPS in rats.[47] It has been reported that brain Ag II and AT1 receptors are responsible for IL-1β production and LPS-induced fever.[9] Meanwhile, the injection of LPS leads to the activation of transcription factors influencing the expression of inflammatory cytokines such as nuclear factor-kappa B (NF-κB) in macrophages inhibited by losartan.[48] On the other hand, NF-κB plays an essential role in Ag II-induced damages and hypertension.[49] Administration of Ag II also causes the up-regulation of NF-κB and pro-inflammatory cytokines and finally induces oxidative stress.[50] In the present study, the two higher doses of captopril decreased IL-6 concentration in the serum. Therefore, it may be assumed that the inhibition of RAS by captopril along with the reduction of immune responses and oxidative stress are responsible for the results of the present study.

Additionally, an interaction between brain RAS and endogenous opioid systems has been suggested which may be involved in the mood-improving effects of captopril observed in this study.[1] The effects of Ag II and captopril on the rewarding properties of morphine in rats may be evidence for the interaction of RAS with the opioid system.[3] Therefore, it seems that the endogenous opioid system may have contributed to the results of this research. Still, more studies are needed to clarify the exact possible mechanism(s).

In summary, similar to previous studies, LPS induced a sickness behavior presented by depression- and anxiety-like behaviors accompanied by a low level of activity. In addition, the results of this study indicated that captopril could restore LPS-induced sickness behaviors.

Acknowledgments

Conflict of interest

We announce that there is no conflict of interest.

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