Original Article

The Effects of Pentoxifylline on Serum Levels of Interleukin 10 and Interferon Gamma and Memory Function in Lipopolysaccharide-induced Inflammation in Rats

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Abstract

Background: Studies have shown that pentoxifylline (PTX) in addition to protective effects on blood vessels probably has positive influence against the brain inflammation. Therefore, the aim of this study was to evaluate the effects of PTX on serum levels of interleukin 10 (IL-10) and interferon gamma (IFN-γ) and passive avoidance learning in lipopolysaccharide (LPS)-induced inflammation in rats. **Materials and Methods:** Inflammation was induced by intraperitoneal (i.p.) injection of LPS (0.5 and 5 mg/kg) in male Wistar rats. After a week, PTX (25 mg/kg; i.p.) was injected for 14 days. Passive avoidance learning test was used for evaluation of learning and memory. Serum levels of cytokines were measured by enzyme-linked immunosorbent assay. **Results:** The behavioral results did not show any significant effect of LPS and PTX on learning and memory. Both doses of LPS (0.5 and 5 mg/kg) decreased IL-10 significantly ($P < 0.05$). PTX prevented this reduction just in the LPS 0.5 mg/kg + PTX 25 mg/kg group. Serum level of IFN-γ was increased only in the LPS 0.5 mg/kg + PTX 25 mg/kg group comparing to the LPS 0.5 mg/kg group ($P < 0.05$). **Conclusions:** The results showed that LPS-induced inflammation decreased the serum levels of IL-10. PTX could prevent these decreases only in mild inflammation. Both PTX and LPS-induced inflammation had no significant effects on learning and memory; therefore, their effects on CNS require further study.

Keywords: *Inflammation, interferon gamma, interleukin 10, learning and memory, lipopolysaccharide, pentoxifylline*

Introduction

Pentoxifylline (PTX) is a xanthine analogs that are known as an inhibitor of phosphodiesterase (which is released in inflammation).[1] PTX has been demonstrated that increases the filterability of red blood cells (RBCs)[2] and improves oxygen delivery to tissues; furthermore, it decreases the adherence of RBCs to endothelial cells, blood viscosity, platelet aggregation, fibrinogen levels, and acts as a vasodilator.[3,4] In addition, protective effects of PTX against ischemia-induced brain trauma and loss of oxygen (hypoxic ischemia) have also been reported.[4-6] Studies have shown that PTX has modulatory effects on inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin 10 (IL-10), and interferon gamma (IFN-γ). It has been demonstrated in some of neuroinflammatory diseases that there is an impairment in brain circulation that damages neuronal survivals, such as Alzheimer's; PTX has produced protective effects.[7,8] It has been demonstrated that PTX improved memory impairment that caused by cerebral ischemia.[5]

Studies have shown that damage of peripheral tissues and systemic infection could increase inflammatory cytokines in the brain and by activation of microglia impairs brain functions.[9] It has been demonstrated that systemic lipopolysaccharide (LPS) administration caused a significant but transient increase in pro- and anti-inflammatory cytokines in the periphery that is coincided with neuroinflammation and elevated cytokine levels in the brain.[10] Intraperitoneal (i.p.) injection of LPS can lead to a systemic inflammation and increase of inflammatory cytokines in the peripheral circulation. Both LPS and inflammatory factors can damage blood–brain barrier and facilitate the entry of the cytokines to the central nervous system (CNS).[9,10] Therefore,

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systemic inflammation may damage CNS. In this study, we aimed to evaluate the effects of PTX on learning, memory, and serum levels of IL-10 and IFN-γ in LPS-induced inflammation in rats.

Materials and Methods

Subjects

The subjects were male Wistar rats (200–300 g) that were housed four per cage and maintained on a 12 h light–dark cycle in an air conditioned constant temperature (23°C ± 1°C) room, with food and water made available *ad libitum*. The Ethic Committee for animal experiments at Institute for Cognitive Science Studies approved the study and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023) revised 1996. Experimental groups were the control, LPS - 0.5 mg/kg, LPS - 5 mg/kg, LPS - 0.5 mg/kg + PTX 25 mg/kg, LPS - 5 mg/kg + PTX 25 mg/kg (n = 10 in each experimental group).

LPS was dissolved in saline and was injected intraperitoneally (a single injection of 0.5 or 5 mg/kg; Sigma, St. Louis, USA).[11,12] One week after the injection,[13] the rats in the treated groups started to receive daily injection of PTX 25 mg/kg at 2:00 PM for 14 days (i.p., dissolved in saline; Sigma, St. Louis, USA).[14,15] Animals in the control and LPS groups received same volume of placebo.

Learning and memory performance were evaluated using passive avoidance learning test in the rats. The apparatus consists of two separate chambers connected through a guillotine door. One chamber was illuminated, while the other was dark. The floor of both the chambers consists of steel grids, used to deliver electric shocks. In the acquisition trail, 4 h after the last PTX injection, each rat was placed in the illuminated chamber while its back was to the guillotine door. After 60 s of habituation, the guillotine door separating the illuminated and dark chambers was opened and the initial latency to enter the dark chamber was recorded. The guillotine door was closed immediately after the rat enters the dark chamber and an electric foot shock (75 V, 0.5 mA, 50 Hz) was delivered to the floor grids for 2 s. Then, the rat was removed from the dark chamber and returned to its home cage. Twenty-four hours later, retention latency time to enter the dark chamber was taken in the same way as in the acquisition trail, but the foot shock was not delivered, and the latency time was recorded up to a maximum of 600 s.[16]

At the end of behavioral study, the rats were anesthetized by i.p. injection of chloral hydrate and after decapitation by guillotine, the trunk blood was collected. After blood clotting, the samples were centrifuged (20 min, 6000 rpm), and serums were collected and were kept at −80°C. Serum levels of IL-10 and IFN-γ were determined by enzyme-linked immunosorbent assay (ELISA) using IL-10 rat ELISA kit and IFN-γ rat ELISA kit (Abcam; ab100765 and ab46107, respectively) according to the manufacturer's instructions. Each sample was double checked and the average was reported.

Data were analyzed using the SPSS Version 21 for Windows (IBM Corporation). Behavioral results were analyzed statistically using Kruskal–Wallis test (nonparametric ANOVA) and Dunn's multiple comparisons for posttest. The serum levels of IL-10 and IFN-γ were analyzed using one-way ANOVA and Tukey for posttest. The significant level was set at $P < 0.05$. Results are expressed as mean ± standard error of mean.

Results

As seen in Figure 1, in LPS groups, both doses of LPS (0.5 and 5 mg/kg) decreased IL-10 significantly comparing to the control group (470 ± 13.41, 450.71 ± 42.45, and 556.37 ± 41.06 pg/ml, respectively; $P < 0.05$). PTX increased IL-10 in the LPS 0.5 mg/kg + PTX 25 mg/kg group (519.14 ± 36.46 pg/ml) as there were no significant differences comparing to the control group. In the LPS 5 mg/kg + PTX 25 mg/kg group, PTX could not affect the decreased level of IL-10 and it was different (435 ± 35.08 pg/ml; $P < 0.05$) with respect to the control group.

As seen in Figure 2, serum levels of IFN-γ were not different in the LPS and LPS-treated groups with respect to the control group. However, PTX increased the levels of IFN-γ significantly in LPS 0.5 mg/kg + PTX 25 mg/kg group comparing to LPS 0.5 mg/kg group (73.19 ± 16.54 and 32.45 ± 10.1 pg/ml, respectively; $P < 0.05$).

In the acquisition trail, all rats entered to the dark chamber. With respect to control group, the low dose of LPS significantly decreased the mean of initial latency ($P < 0.05$), but high dose of LPS had no significant effects. The initial latency was significantly increased in the LPS 5 mg/kg + PTX 25 mg/kg group ($P < 0.05$) [Figure 3]. The evaluation of the retention latency showed no significant differences between the groups [Figure 4].

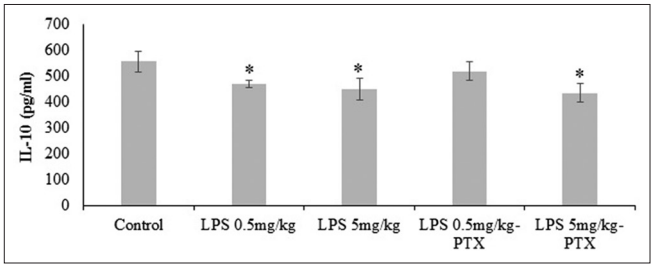

Figure 1: Effect of pentoxiphyline on serum levels of interleukin 10 after interpritoneal injection of lipopolysaccharide in rats. Values are shown as mean ± standard error mean. *$P < 0.05$ with respect to the control group (n = 10)

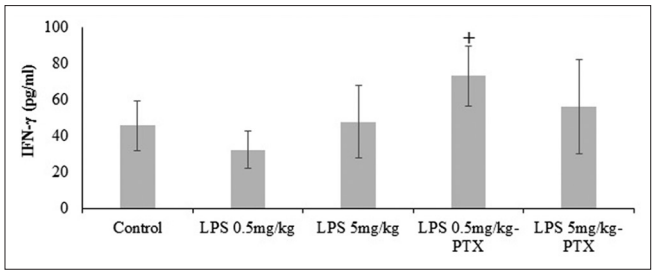

Figure 2: Effect of pentoxiphyline on serum levels of interferon-γ after interpritoneal injection of lipopolysaccharide in rats. Values are shown as mean ± standard error mean, $^{+}P < 0.05$ with respect to the control group ($n = 10$)

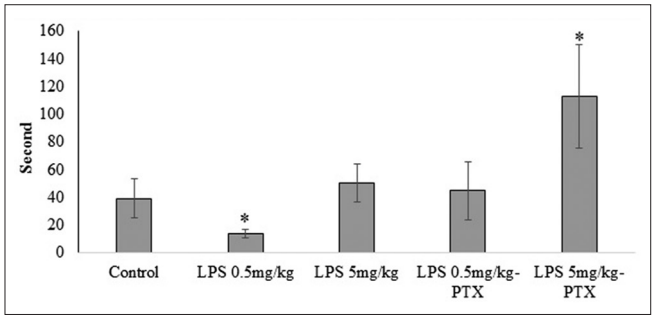

Figure 3: Effect of pentoxiphyline on initial latency in passive avoidance test after interpritoneal injection of lipopolysaccharide in rats. Data are expressed as mean ± standard error mean. *$P < 0.05$ with respect to the control group ($n = 10$)

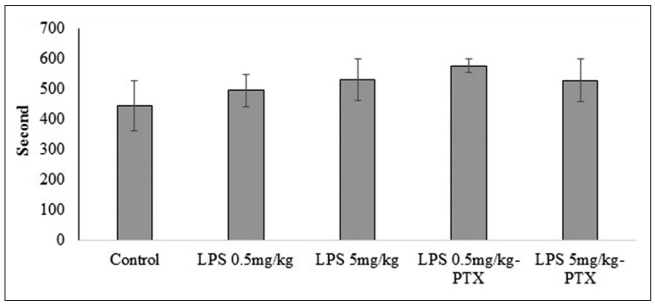

Figure 4: Effect of pentoxiphyline on retention in passive avoidance test, 24 h after the shock, after interpritoneal injection of lipopolysaccharide in rats. Data are expressed as mean ± standard error mean ($n = 10$)

Discussion

The results showed that both different doses of LPS after 3 weeks decreased serum levels of IL-10 but had no significant effect on serum levels of IFN-γ.

Following inflammation, one of the responses of the immune system is enhancement of inflammatory cytokines. However, with elimination of the cause of inflammation, serum levels of cytokines and immune response return to normal conditions. IL-10 is an anti-inflammatory cytokine that modulates immune responses and can suppress production of pro-inflammatory cytokines such as TNFα and IFN-γ.[17] IFN-γ is a pro-inflammatory cytokine and has an important role in immune responses. IFN-γ, by affecting the immune cells, induces production of different cytokines that their abnormal expression causes autoimmune disorders.[18]

LPS strongly stimulates immune system, macrophage activity, and product of inflammatory cytokines. LPS is the major part of the outer membrane of Gram-negative bacteria that stimulates the production of inflammatory cytokines and leads to excessive production of free radicals and oxidative stress.[19-21] Although production of cytokines is a defensive mechanism against bacterial infection, their overexpression may have complications.[22]

One of anti-inflammatory cytokines in the immune responses is IL-10 that performs inflammatory actions. This cytokine can increase acutely following LPS administration.[10] However, the results of this study show that IL-10 was significantly reduced after 3 weeks of systemic injection of LPS; hence, its reduction over the long-term can consider as one of the driving factors in secondary complications of this model of inflammation although its levels may be increased in the short-term.[10] IL-10 suppresses production of pro-inflammatory cytokines such as IFN-γ.[17] Therefore, following the reduction of IL-10, IFN-γ can be increased. However, as seen in Figure 2, LPS did not change the serum levels of IFN-γ after 3 weeks in the present study.

It has been demonstrated that LPS can alter the profile of released cytokines by activated T-cells toward T helper (Th) 2 cytokines rather than TH1 cytokines.[23] Therefore, IFN-γ, the main effectors cytokine of Th1,[24] can be expected to decrease following the injection of LPS. However, the levels of IL-10, one of the effector cytokines of Th2 helper cells,[25] were also decreased following the LPS injection. Studies have shown that LPS inhibited TH2 response in lung inflammation.[26,27] Hence, LPS has different effects on the immune cells over the time and in the different tissues of the body.

Exposure to LPS has been demonstrated affected CNS and its function.[28] Studies have shown that exposure to LPS through affecting expression of inflammatory cytokines causes expression of amyloid beta, neuroinflammation, neuronal death, and memory dysfunction.[29,30] Our behavioral study showed that before electrical shock in the initial latency, the rats in the LPS 0.5 mg/kg group had little tendency for staying in the light room due to the probable inflammation and resulting restlessness. In the LPS 5 mg/kg group, although a severe weakness was observed, no significant differences were observed comparing to the control group. However, in the PTX-treated groups, the times that have spent in the light room were increased, probably due to the effects of this drug in reduction of inflammation and stress in the rats. LPS had no significant effects on learning and memory in this study; however, some studies showed memory impairments during acute application[31] or 7 weeks after it.[32] This difference may be due to the time because we evaluated memory 3 weeks

after the LPS injection and it has been demonstrated that LPS effects can be time dependent. However, we have seen that the rats in LPS groups showed a high reluctance and decreased motor activity and most of them slept in a corner of the light room of shuttle box and spent time in this way, especially in retention trail, which reflects the lethargy caused by inflammation.

As a secondary observation, our study demonstrates that PTX could prevent suppressive effects of LPS on Th1 and Th2 cells, and reduction of IL-10 and IFN-γ that was induced by low dose of LPS; however, PTX had no protective effects in the severe inflammation that was induced by high-dose LPS.

In behavioral study, LPS had no significant effects on passive avoidance learning; however, we think this response is caused by inflammation-induced weakness and malaise rather than the lack of effects of inflammation on learning and memory; this suggestion is confirmed by previous studies that showed sickness and depressive-like behavior following systemic LPS administration.[10] But, we have seen that PTX improved these lethargy and weakness, and the staying in the light room presented the better performance in the treated groups rather than the lethargy.

Conclusion

In the present results, we have shown that following LPS-induced inflammation, the serum levels of IL-10 were decreased. PTX could prevent these decreases only in mild inflammation that induced by low dose of LPS. Both PTX and LPS-induced inflammation had no significant effects on learning and memory. These behavioral responses are probably due to inflammation-induced weakness; therefore, their effects on CNS require further study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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