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Investigation of the effects of lipopolysaccharide preconditioning on neuropathic pain induced by chronic sciatic nerve constriction injury in rats

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Abstract

Background: Neuropathic pain results from nerve injury and involves pro-inflammatory cytokines that activate pain-sensing neurons, contributing to both the onset and maintenance of pain. This study investigated the effect of lipopolysaccharide (LPS) preconditioning on pain following sciatic nerve constriction in rats.

Methods: Neuropathic pain was induced via the chronic constriction injury (CCI). Rats received 0.1 mg LPS intraperitoneally three days before CCI. They were divided into four groups: control, CCI without LPS, CCI with LPS, and LPS alone. Pain behavior was assessed on days 0, 7, and 14 using acetone and hot plate tests.

Results: LPS alone did not affect pain sensitivity. CCI increased responses to thermal and cold stimuli. However, prior LPS reduced these responses compared to CCI alone.

Conclusion: This study suggests that 0.1 mg LPS can alleviate neuropathic pain by increasing pain threshold after nerve injury.

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Highlights

What is current knowledge?

Current knowledge shows that neuropathic pain involves inflammatory cytokines and activation of microglia, which play key roles in the onset and maintenance of pain after nerve injury. LPS is known to modulate immune responses and has been studied for its effects on neuroinflammation and pain.

What is new here?

This study provides new evidence that preconditioning with a low dose of LPS reduces neuropathic pain behaviors following sciatic nerve injury in rats, suggesting that LPS may have a therapeutic effect on nerve injury-induced pain through modulation of inflammatory responses.

Introduction

Pain can arise from various factors, including trauma, tissue injury, necrosis, inflammation, muscular spasms, and various diseases. Among the types of pain, neuropathic pain is characterized by spontaneous burning pain at the injury site, hyperalgesia (Increased response to a normally painful stimulus), and allodynia (Response to a stimulus that does not normally cause pain) (1,2). Glial cells play a significant role in the pathogenesis of neuropathic pain as they support the central nervous system. These cells produce neuroactive substances such as pro-inflammatory cytokines, nerve growth factors, and enkephalins (3). Furthermore, previous studies have demonstrated the presence of glial cells in the dorsal horn of the spinal cord and their role in pain modulation (4). One of the substances that stimulates microglial cells and the central nervous system's immune system is lipopolysaccharide (LPS). As the main component of the outer membrane of Gram-negative bacteria, LPS is a pattern recognition molecule associated with primary pathogens that triggers the synthesis and release of pro-inflammatory cytokines through Toll-like receptor (TLR) pathways. These pro-inflammatory mediators regulate local and systemic immune responses to pathogens such as LPS and, more importantly in the context of pain, affect the nervous system, behavioral changes, and endocrine stimulation (5). Studies have shown that if LPS is administered in low doses preventively before nerve injury and the onset of inflammatory processes, it can induce a form of preconditioning in the immune system by mildly stimulating the immune response, so that in the event of subsequent severe immune activation, the immune responses do not occur in an exaggerated manner, thereby alleviating related complications (6). The mechanism of preconditioning with LPS is mediated through TLRs. These receptors not only recognize and defend against pathogens but also act as sentinels for damaged tissues, mediating the inflammatory responses of injured tissues. These receptors are expressed in leukocytes and brain cells (Microglia, astrocytes, and neurons). Following brain injury, these receptors are activated and lead to the production of inflammatory mediators such as IL-1β, IL-6, TNF-α, and iNOS. It has been shown that the stimulation of these receptors before ischemia provides neuroprotection (7). In this context, the aim of this study is to investigate the effect of LPS pre-treatment on the acquisition of neuropathic pain following sciatic nerve constriction in the chronic constriction injury (CCI) neuropathic model in male rats.

Methods

Animals and ethical considerations

Adult male Wistar rats, weighing approximately 270 g, were housed at the Pasteur Institute in Amol, Iran, and used in the study. The animals were housed in standard cages at a temperature of 22±3°C under a 12-hour light/dark cycle. During the study, they had unrestricted access to food and water. All experimental procedures were conducted in accordance with institutional guidelines and were approved by the ethical committee [IR.GOUMS.AEC.1403.006]. Efforts were made to minimize animal suffering throughout the study.

Surgical procedures

The neuropathy model was based on the CCI model established by Bennett and Xie (1988) (8). Rats were anesthetized with an intraperitoneal mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg). Once anesthesia was confirmed, the fur on the right thigh was

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shaved, and the surgical area disinfected with alcohol. A 2-cm incision was made on the thigh, followed by a parallel incision along the sciatic nerve through the biceps femoris muscle. The muscle was displaced to expose the sciatic nerve, which was carefully isolated from surrounding tissues.

Induction of nerve injury

Four loose ligatures using chromic gut (Size 0-4) were placed approximately 1 mm apart around the nerve, just proximal to the trifurcation, with knots tightened to partially impair blood flow without complete occlusion. The nerve was then repositioned, and the muscle and skin were sutured separately using silk sutures (Size 0-4). The surgical site was irrigated with sterile saline and disinfected. After recovery from anesthesia, animals were returned to their housing (8,9).

Experimental groups

Each experimental group consisted of five rats.

1. **Control:** No intervention was performed.
2. **CCI**
3. **CCI + 0/1 mg LPS:** A dose of 0.1 mg/kg LPS was injected intraperitoneally. After 72 hours, the rats underwent CCI. Behavioral tests were conducted on days 7 and 14 following sciatic nerve injury.
4. **0/1 mg LPS:** A dose of 0.1 mg/kg LPS was injected intraperitoneally, and behavioral tests began on days 7 and 14 (10-12).

To study pain behavior and evaluate the analgesic effects of LPS on neuropathic pain created by the CCI model, both the acetone test and the hot plate test were used.

Acetone test: Cold allodynia was induced by spraying acetone (Cold induction by evaporation) as proposed by Choi. The rats were placed in cages with a wire mesh floor, and acetone was sprayed onto the soles of the animals' feet five times at three-minute intervals. The number of foot withdrawals was expressed as a percentage and calculated using the following formula. A positive response was considered as a foot withdrawal. The calculations for the acetone test for each animal were conducted using the following equation:

Number of foot withdrawals on day 0 - Number of foot withdrawals on the specified day (12).

Hot plate test: Pain threshold and pain tolerance were measured using a hot plate apparatus. This device consists of a heated plate powered by electricity. In this study, all rats were placed individually on this plate, heated to 55°C, and the time to onset (Zero) was recorded. As soon as the rats began licking their paws or exhibited specific changes in their behavior, their baseline tolerance was noted. When the animals started jumping, their pain tolerance was recorded. To prevent tissue damage, the test for each rat was limited to a maximum of 60 seconds (13).

Statistical analysis

In each experiment, the score of each group was recorded as the mean ± standard error of the mean (Mean ± SEM). To determine the presence of significant differences between the experimental groups, ANOVA and Tukey's test were utilized. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant. GraphPad Prism 9.0.0 software was used for the analysis.

Results

The effect of LPS pretreatment on neuropathic pain in the hot plate test

Figure 1 shows that in the hot plate test, there was a significant decrease in response latency in the CCI and CCI + LPS groups compared with the control group on days 7 and 14 after surgery, indicating increased pain sensitivity. No statistically significant difference was observed between the control group and the group that received 0.1 mg LPS, indicating that LPS at this dose did not alter the pain threshold.

The effect of LPS pretreatment on neuropathic pain in the acetone test

Figure 2 shows the percentage response to acetone. The control group and the group receiving 0.1 mg LPS did not show significant differences on the testing days. However, the CCI and CCI + 0.1 mg LPS groups exhibited a greater response to acetone compared to the control group, indicating a statistically significant difference based on the statistical tests ($P < 0.05$).

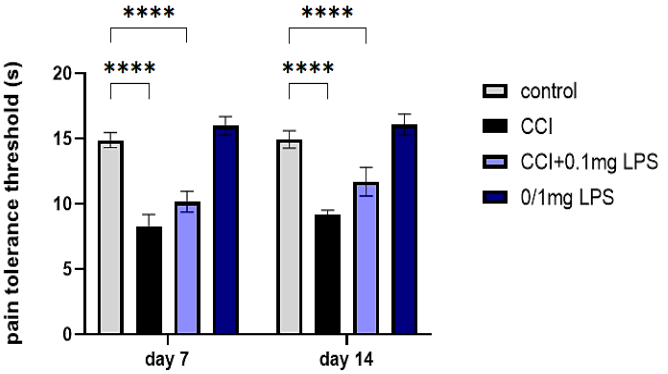

Figure 1. Effect of lipopolysaccharide preconditioning on neuropathic pain in response latency to thermal pain in the hot plate test: Data are expressed as Mean ± SEM for rats in each group. (**** = $p < 0.0001$) (n = 5).

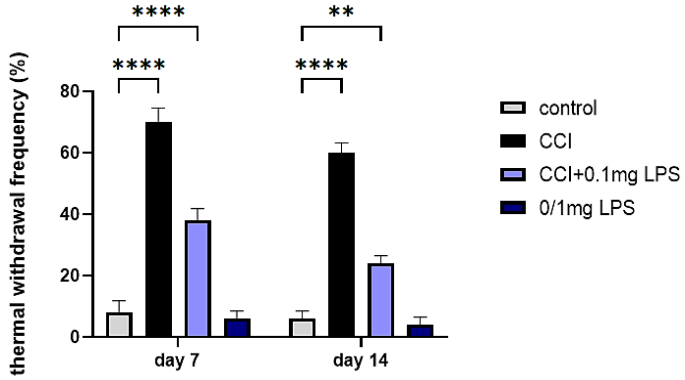

Figure 2. Effect of lipopolysaccharide preconditioning on response percentage to neuropathic pain induced by the acetone test: Data are expressed as Mean ± SEM for rats in each group (**** = $p < 0.0001$, ** = $p < 0.01$) (n = 5).

Discussion

Glial cells, as the supporters of the central nervous system, play a key role in the pathogenesis of neuropathic pain and modulate the function of the nervous system by producing neuroactive substances such as pro-inflammatory cytokines, nerve growth factors, and enkephalins (3). LPS, as a molecular pattern associated with pathogens, increases the synthesis and release of pro-inflammatory cytokines by stimulating TLR receptors and controls local and systemic immune responses (5). Studies have shown that preventive administration of LPS can create a form of preconditioning in the immune system that reduces immune responses upon subsequent stimulation and alleviates related complications (6). In this study, the effects of LPS preconditioning on neuropathic pain resulting from chronic sciatic nerve constriction surgery in rats were investigated. The results showed that the CCI group significantly reduced the pain tolerance threshold and increased the percentage response to acetone compared to the control group. Moreover, treatment with LPS contributed to an increase in the pain tolerance threshold and a decrease in the percentage response to acetone in the rats, indicating the effectiveness of LPS preconditioning in modulating pain responses. In our study, it was observed that preconditioning with LPS can have positive effects on reducing neuropathic pain. Numerous studies have confirmed the neuroprotective and modulating effects of LPS across various models of inflammation and neural injury. Longhi et al. (2011) demonstrated that intracranial injection of 0.1 mg/kg LPS several days prior to brain injury significantly reduces neuronal death, contusion volume, and trauma-induced motor deficits, indicating a prophylactic role in modulating inflammatory responses and preventing severe complications (6). Zhou et al. (2020), in a long-term study, showed that repeated low-dose LPS injections exert neuroprotective effects by decreasing pro-inflammatory cytokine levels and improving cognitive and emotional functions, thereby preventing hyperactivation of the immune system and excessive inflammatory responses during infection and systemic inflammation (14). Additionally, Eslami et al. (2015) reported that pre-injection of LPS at a specific dose, five days before

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inducing brain trauma, could inhibit the trauma-facilitated development of epilepsy, highlighting the prophylactic role of mild immune stimulation in reducing neurological sequelae (15). Furthermore, based on Marsh et al. (2009), low-dose LPS administration not only avoids harmful effects but may also reduce complications related to cerebral ischemia and promote repair processes (7). These findings suggest that low-dose conditioning with LPS can diminish inflammatory responses and neural damage, thus providing long-term neuroprotective effects in models of neurological pathology and reducing outcomes associated with ischemia. These results align with the findings of the present study and support the role of immunomodulatory prophylactic interventions in managing neuroimmune complications and mitigating neurological injury consequences.

Conclusion

Overall, the results of our study demonstrate that preconditioning with LPS has a positive effect on reducing neuropathic pain resulting from chronic sciatic nerve constriction surgery in rats. These findings, based on mechanisms identified in previous research, can be proposed as a new strategy in the management of chronic and neuropathic pain, providing further insights into pain management in clinical settings.

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Ethical statement

This study was approved by the Ethics Committee of Golestan University of Medical Sciences with the approval code (Ethical code: IR.GOUMS.AEC.1403.006).

Conflicts of interest

The authors declared no conflicts of interest.

Author contributions

This article is the result of a research project conducted in collaboration with Ms. Soqra Hesam (Project manager), Fatemeh Piri, Mahshid Moradpour, and Dr. Hamid Sepehri at Golestan University of Medical Sciences.

Data availability statement

Data sharing is not applicable.

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