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Long-Term Treatment with Buprenorphine Increased TLR1 Receptor Expression in Methamphetamine Rats' Brainstems

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ABSTRACT

Background & Objective: Toll-like receptors (TLRs) are proteins that play key roles in inflammation. METH and buprenorphine (BUP) both modulate pain, but the exact mechanism underlying their antinociceptive effects is unknown. As a result, the expression of TLR1 and TLR2 genes was examined in METH rats that had been treated with or without BUP.

Materials & Methods: A total of 77 rats were classified into 11 subtypes (n = 7): control (saline), BUP 6 or 10 mg/kg, METH (10 mg/kg), METH+BUP 6 or 10 mg/kg, or withdrawal groups. The treatments were intraperitoneally administered for 5 or 14 days. RT-PCR evaluated genes expressed in the brain stem area.

Results: The results showed that TLR1 gene expression in the METH group (10 mg/kg; 5 days) considerably improved compared with the control group. Furthermore, BUP injection (10 mg/kg) acutely decreased TLR2 gene expression compared with the METH group. In the METH + BUP (10 mg/kg; 14 days) group, TLR1 expression was higher than in the METH group. The coadministration of METH+BUP (10 mg/kg) acutely decreased TLR2 gene expression compared with METH.

Conclusion: There are limited changes in these genes, and their role in METH consumption and inflammation is unclear. Due to the presence of these two genes in the inflammatory pain and addiction signaling pathways, they may have more clear roles in other parts of the nervous system.

Keywords: Buprenorphine, Methamphetamine, Brainstem, Toll-like receptors, Inflammation

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1. Introduction

For acute or chronic opioid dependence, the FAD approves the BUP. This combination minimizes opioid withdrawal symptoms. Patients are kept off opioids through this treatment plan, reducing morbidity and mortality (1).

BUP's maximum effect is less than that of other opioid agonists, such as heroin and methadone (2). Because it binds strongly to Mu receptors, it can compete with other opioids to stop them from working. This separates morphine, methadone, and other opioids from their receptors (3, 4). This is why morphine causes withdrawal symptoms in people who are still using it. Other Mu receptor agonists cannot dissociate BUP from the receptor, so they cannot have an opioid agonist effect on receptors already occupied by BUP (5).

There are various receptors on body cells that detect pathogens and induce an immune response against them. One of these receptors that reacts to the coating of a particular group of bacteria (poly-lipo-saccharides present in Gram-negative bacteria) is called TLR. This receptor also responds to viruses and body proteins. One of the actions of this receptor is to transmit inflammation inside the cell. This inflammation affects leukocytes and other cells (6).

To date, 28 vertebrate TLRs have been identified. TLR1–13 is found in mammals. Humans have only ten TLRs (TLR1–10), whereas mice have all TLRs, and TLR10 is non-functional (6, 7). TLRs are mostly in groups. A group of receptors, such as TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11, are expressed on the outer surface of cells and

distinct bacterial membrane components (8). The TLR2 receptor attaches to the peptidoglycan of gram-positive bacteria and sends signals into the cell. These signals set off a chain of events that activate the cytokine gene-inducing factor and make the gene's products. This interaction results in defense cell activation and pathogen destruction (9). TLR2 forms a heterodimer to act on TLR1 or TLR6. This pathway activates the IRF3, IRF1, MAPK, and IRF7 cascades to stimulate the production of cytokines, including type 1 interferons and pro-inflammatory cytokines (9, 10). According to the mentioned evidence and the lack of information about the role of TLR1 and TLR2 receptors during METH addiction in the presence/absence of BUP, these two receptors were investigated in this study.

2. Materials and Methods

2.1 Groups

The Pasteur Institute of Iran provided 77 male rats (250 ± 50 g) for the experiments. A 12 h dark/12 h dark cycle was applied (lights on at 7 am) to the animals in a temperature-controlled room. All rats were acclimated to the changing environment and handled for at least one week before their research. The research committees of Tabriz and Hamadan University have approved a protocol for using animals (IR.Umsha.REC.1394.200).

2.2 The rats were randomized into 11 groups (n = 7), including:

1. Control group
2. METH group: The animals received METH at 10 mg/kg (11) for 5 or 14 consecutive days (12).
3-6. BUP (6 or 10 mg/kg; 5 or 14 days) groups: During 5 or 14 days, the animals received BUP at a dose of 6 or 10 mg/kg.
7-10. METH+BUP (6 or 10 mg/kg; 5 or 14 days) groups: A combination of METH (10 mg/kg) and BUP (6 or 10 mg/kg) was administered to the animals for 5 or 14 days.
11. For the withdrawal group, METH was given for five days. There was an assessment after a 72-hour drug-free period (12).

2.3 Procedure for inducing addiction

10 mg/kg METH was injected intraperitoneally (11) for 5 or 14 days (13).

2.4 BUP prescription

Injections of BUP (6 mg/kg or 10 mg/kg) were given intraperitoneally for 5 or 14 days alone or with METH (12).

2.5 The RT-PCR assessment

First, mRNA from brain stems was extracted with Trizol buffer in chloroform and isopropanol (14). Samples were centrifuged at 4°C for 15 min and at 13,000 rpm. After centrifugation, remove the clear-phase sample (RNA). In the next step, cold isopropanol was added to the microtube, mixed, and frozen for one hour. Using the same centrifuge conditions, pour into a microtube, discard the supernatant, add 75% ethanol to the precipitate, and centrifuge for 10 minutes at 8000 rpm (13). As soon as the clear liquid had been centrifuged, the microtube was turned upside down to dry, and DEPC-treated water was poured into it. The microtube was placed in a pan for 10 minutes at 65 0C. A nanodrop spectrophotometer was used to check purity and concentration. The BIONEER reverse transcription kit was used in the final step. A triple real-time PCR procedure using gene-specific primers mediated by cDNA probes was performed to measure TLR1 and TLR2 mRNA expression levels (Table 1). Next, relative expressions were recorded. A SYBR Green (Takara Bio Inc.) was used in this step to perform RT-PCR. In addition, real-time quantitative PCR utilizing the comparative CT method was conducted, and a mathematical formula was applied to calculate the relative expression of the target RNA compared to the reference value (15).

2.6 Analyses of statistics

A mean and standard error of the mean (SEM) were used for all data. Differences between groups were measured using SPSS version 16.0 using one-way analysis of variance with the Tukey posthoc multiple comparisons test. Statistically significant results were evaluated using a threshold of $P<0.05$.

3. Results

3.1 Comparison of TLR1 gene expression changes between groups on days 5 or 14

A one-way ANOVA test demonstrated that TLR1 gene expression levels differed between groups. A significant increase was observed in the METH group compared to the control group ($P < 0.05$; Fig. 1).
Co-administration of BUP (10 mg/kg; 14 days) + METH improved this parameter compared to the METH group ($P<0.05$). The drug withdrawal syndrome group also revealed a significant decrease in this parameter compared to the METH group ($P< 0.05$; Fig. 1). In addition, no significant difference was shown between the other groups.
The level of TLR2 in the METH + BUP (6 mg/kg; for 14 days) increased ($P<0.05$) and the BUP (6 mg/kg; for 5 days) decreased ($P<0.05$) compared with the control group (Fig. 2). The level of this factor was decreased in the METH+BUP (10 mg/kg; 5 days) or BUP (6 or 10 mg/kg; 5 days) group compared to the METH group ($P<0.05$; Fig. 2). In addition, no statistical differences were observed between the other groups.

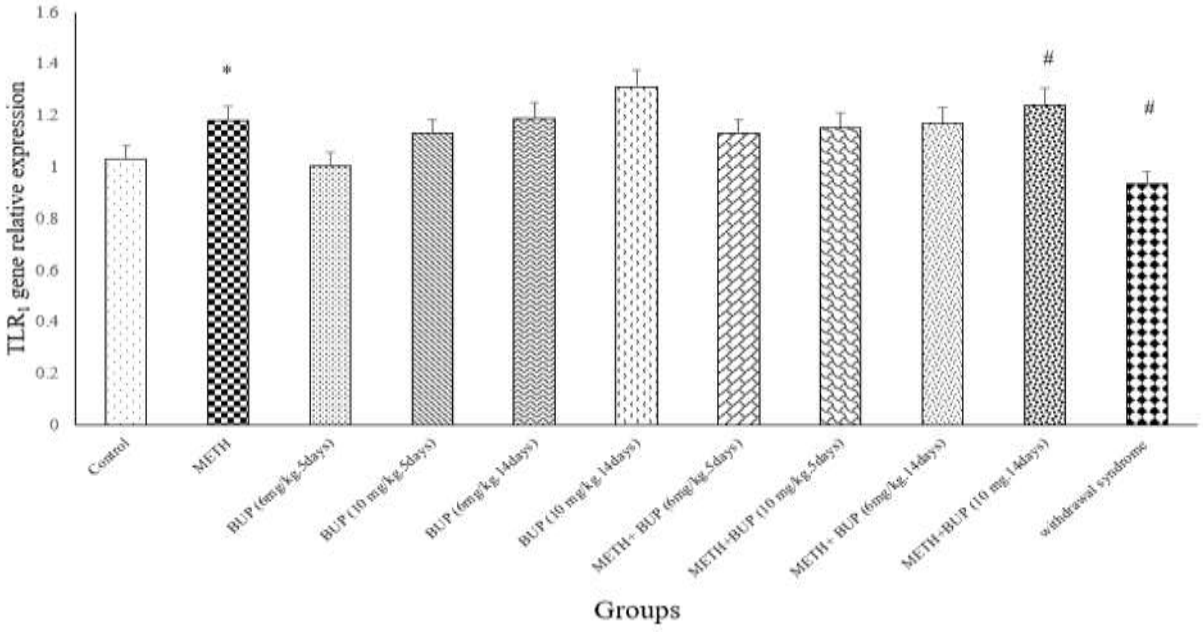

Figure 1. The amount of TLR1 gene expression changes between groups. *P < 0.05; control vs. other groups; # vs. METH group.

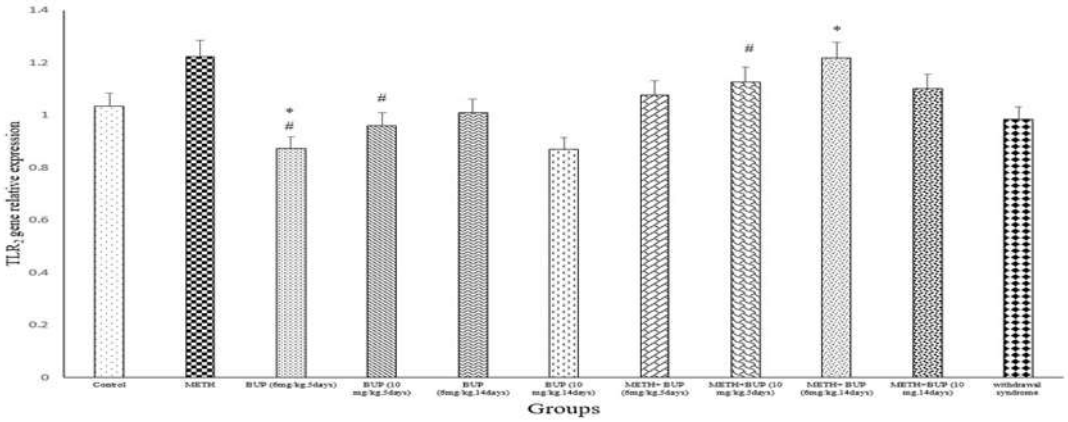

Figure 2. The amount of TLR2 gene expression changes between groups. *P < 0.05; control vs. other groups; # vs. METH group.

4. Discussion

In this study, we investigated alterations in the gene expression of TLR1 and TLR2 in the brain stem in response to inflammatory pain in male rats exposed to METH, with or without BUP treatment. Our findings revealed several noteworthy observations:

1) METH administration at 10 mg/kg for 5 days remarkably improved the expression of the TLR1 gene in the brain stem compared to the control group in a 5-day experimental period.

2) It's interesting that giving both METH (10 mg/kg) and BUP (10 mg/kg) for 14 days together increased the expression of TLR1 even more than giving METH alone.

3) Acute injection of 6 or 10 mg/kg BUP resulted in a notable reduction in TLR2 gene expression when compared to the METH-treated group.4) In a different set of experiments, giving METH and BUP at the same time for 5 days at a dose of 10 mg/kg increased the expression of the TLR2 gene more than giving METH alone. Toll-like receptors (TLRs) are key mediators of inflammatory pathways (16) and may be a suitable target for treating inflammation caused by METH (17). Previous research by Acioglu et al. (2022) has indicated that endogenous TLR agonists may serve as pivotal signaling pathways between microglia, other glial cells, and neurons (18). Our results showed that the TLR1 gene expression in the brain stem of the METH group (10 mg/kg; 5 days) was notably improved compared with the control group. Our study aligns with existing literature demonstrating the influence of various stressors (19), alcohol (20), and other substances of abuse (21) on TLRs, thereby

impacting innate immunity signaling molecules within the brain (22).

Furthermore, our research concurs with previous studies highlighting the interplay between TLRs and HMGB1 proteins, which function as endogenous TLR agonists. For example, expression of HMGB1 and several TLRs within the after-death human orbitofrontal cortex is related to lifetime alcohol intake (23).

Preclinical models have shown that ethanol increases HMGB1 and TLR expression in the brain, which persists during prolonged abstinence (24). Current evidence suggests that ethanol induces multiple TLRs and endogenous TLR agonists in the brain. Although currently poorly understood, research suggests that increased activation and signaling of this system contribute to progressive behavioral control associated with increased ventral striatal responses to reward-seeking behavior. It has been suggested that loss, increased impulsivity and anxiety, and negative emotions and desires are promoted, increasing the risk of developing alcohol dependence (23, 24).

Notably, the administration of BUP, especially for five days at a dose of 10 mg/kg, resulted in a decrease in TLR2 expression, offering a potential avenue for modulating TLR-related inflammatory responses in rats treated with methamphetamine or without it.

Existing evidence supports the involvement of TLR2 in inflammatory pain and neuroinflammatory mechanisms within pain signaling pathways (25, 26). Mice lacking functional TLR2 have been observed to exhibit a lack of pain responses (27), while chronic ethanol exposure has been shown to elevate TLR2 expression in the mouse brain (28). In contrast, acute ethanol exposure inhibits the LPS-induced responses, independent of TLR4 or TLR2 expression (29).

In line with our findings, studies by Wang (2021) have linked increased TLR4 expression to methamphetamine addiction, ultimately triggering pro-inflammatory responses. Methamphetamine binds to MD-2, one of the essential TLR4 receptors, and activates NF-κB through LPS, which also transcriptions pro-inflammatory factors and cytokines (30).

Also, in the research of Zhu (2018), while examining the changes in TLR3 expression, it was found that during cocaine addiction, the expression of this gene increased significantly. By activating initial pathways such as NFkB-p65 (Ser536) phosphate, and finally, pro-inflammatory cytokines are produced (31).

So far, no direct study has been conducted regarding the changes in TLR2 gene expression during METH addiction and BUP treatment in the brain stem or other body tissues; therefore, this study was performed.

5. Conclusion

Injection of METH slightly enhanced TLR1 expression and did not change TLR2 expression in the brain stem. Feedback mechanisms may return these genes' expression to control levels. Although, due to the small amount of these changes, a significant role for these genes in connection with METH addiction and inflammatory pain is not conceivable, considering the presence of these two genes in the signaling pathways of inflammatory pain and addiction, it is most likely that in other parts of the nervous system, they will have more prominent roles.

6. Declaration

6.1 Acknowledgments

This manuscript is derived from a Master of Science dissertation, and we thank the Vice Chancellor's Office at Tabriz University for funding this research.

6.2 Authors' Contribution

HH & DSh: Conceived and designed the experiments; Analyzed and interpreted the data NA: Performed the experiments; Analyzed and interpreted the data MSh & RS: wrote the paper.

6.3 Conflict of Interest

There are no competing interests.

6.4 Funding

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