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Research paper

Z-guggulsterone negatively controls microglia-mediated neuroinflammation via blocking IκB-α–NF-κB signals

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HIGHLIGHTS

- Z-guggulsterone exhibits anti-inflammatory effects in microglia.
- Z-guggulsterone inhibits neuroinflammation-induced behavioral abnormalities.
- Z-guggulsterone attenuates LPS-induced IκB-α degradation in microglia.
- Z-guggulsterone prevents LPS-induced NF-κB nuclear translocation in microglia.
- Z-guggulsterone does not influence LPS-induced NF-κB phosphorylation in microglia.

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ABSTRACT

Induction of pro-inflammatory factors is one of the characteristics of microglial activation and can be regulated by numerous active agents extracted from plants. Suppression of pro-inflammatory factors is beneficial to alleviate neuroinflammation. Z-guggulsterone, a compound extracted from the gum resin of the tree commiphora mukul, exhibits numerous anti-inflammatory effects. However, the role and mechanism of Z-guggulsterone in pro-inflammatory responses in microglia remains unclear. This study addressed this issue in *in vitro* murine microglia and *in vivo* neuroinflammation models. Results showed that Z-guggulsterone reduced inducible nitric oxide (iNOS) protein expression as well as nitric oxide (NO), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) production in LPS-stimulated BV-2 cells. Z-guggulsterone also reduced the mRNA level of iNOS, TNF-α, and IL-6. Mechanistic studies revealed that Z-guggulsterone attenuated the LPS-induced degradation of inhibitor κ B-α (IκB-α) as well as the LPS-induced nuclear translocation of nuclear factor-κB (NF-κB). Z-guggulsterone, however, failed to reduce the LPS-induced increase in NF-κB phosphorylation level. These major findings were ascertained in primary microglia where the LPS-induced increases in iNOS expression, NO content, and IκB-α degradation were diminished by Z-guggulsterone treatment. In a mouse model of neuroinflammation, Z-guggulsterone exhibited significant anti-inflammatory effects, which were exemplified by the attenuation of microglial activation and neuroinflammation-induced behavioral abnormalities in Z-guggulsterone-treated mice. Taken together, these studies demonstrate that Z-guggulsterone attenuates the LPS-mediated induction of pro-inflammatory factors in microglia via inhibition of IκB-α–NF-κB signals, providing evidence to uncover the potential role of Z-guggulsterone in neuroinflammation-associated disorder therapies.

Abbreviations: CNS, central nervous system; NO, nitric oxide; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; eNOS, endothelial NOS; nNOS, neuronal NOS; iNOS, inducible NOS; LPS, lipopolysaccharide; IKK, inhibitor of κB kinase; IκB-α, inhibitor of κB α; NF-κB, nuclear factor κB; GAPDH, glyceral-dehyde-3-phosphate dehydrogenase; DMSO, dimethylsulfoxide; TST, tail suspension test; FST, forced swimming test.

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1. Introduction

Microglia, the resident macrophage in the central nervous system (CNS), usually serve as a stimuli sensor under pathophysiological conditions [1,2], and this sensing leads to activation of microglia, which then produce trophic factors that are important for neuronal recovery and bacterial killing [3,4]. However, overactivation of microglia may trigger neurotoxicity through overproduction of cytokines such as nitric oxide (NO), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [5–7]. NO is synthesized by a family of NO synthase (NOS) consisting of three isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) [8,9]. The eNOS/nNOS-derived NO mediates numerous physiological processes in the cardiovascular and neuronal system [10,11]. Both NO over-produced from nNOS or iNOS promotes the development of neuroinflammation-associated disorders such as Parkinson's disease [12], major depression [13] and Alzheimer's disease [14]. Similarly, TNF-α and IL-6 have been shown to exert numerous protective effects [15–17], and their overaccumulation alternatively enhances cell toxicity [18,19] and mediates behavioral abnormalities [20,21]. Therefore, induction of microglia-derived inflammatory factors should be tightly controlled in order to keep the homeostasis of host defense.

Lipopolysaccharide (LPS), also known as endotoxin, is a typical inducer of pro-inflammatory factors in immune cells [22]. In most settings, it stimulates gene transcription of pro-inflammatory factors through the classical inhibitor of κB kinase (IKK) inhibitor of κB α (IκB-α)-nuclear factor κB (NF-κB) signals. LPS binds with the Toll-like receptors leading to IκB-α degradation through the ubiquitin-proteasome system [23]. The removal of IκB-α liberates transcriptional factor NF-κB [24]. The active NF-κB is then free for translocation to the nucleus, where it initiates gene transcription [23,25]. Interfering with the IκB-α–NF-κB signal should be beneficial to cope with neuroinflammation-associated disorders.

Guggulsterone is an active compound extracted from the gum resin of the tree commiphora mukul that has been used for thousands of years to treat arthritis, obesity, lipid metabolism disorders and hypothyroidism. Since these disorders are tightly associated with inflammation, researchers focus a lot on the effects of guggulsterone on pro-inflammatory responses. In fact, published studies have reported numerous anti-inflammatory effects of guggulsterone such as amelioration of T cell-induced colitis [26], prevention of endotoxin-induced uveitis in rats, suppression of LPS-induced inflammation in human middle ear epithelial cells [28] and ethanol-induced gastric mucosal lesions in mice [29], and blockage of IL-1β-mediated inflammatory responses in fibroblast-like synoviocytes [30]. However, the effects of guggulsterone in microglia have not yet been described. Here, we investigated the role and possible mechanism of Z-Guggulsterone in LPS-stimulated inflammatory responses in murine microglia *in vitro* and *in vivo*. Results showed that Z-guggulsterone suppressed the microglial activation, pro-inflammatory factors production, and neuroinflammation-induced behavioral abnormalities through attenuation of IκB-α–NF-κB signals.

2. Materials and methods

2.1. Materials

Z-guggulsterone was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). LPS was the product of Sigma (Saint Louis, MO, USA). Antibodies against iNOS (#13120), IκB-α (#9247), NF-κB p65 (#3034), *p*-NF-κB p65 (Ser536) (#3033), Histone H2A (#12349) and glyceral-dehyde-3-phosphate dehydrogenase (GAPDH) (#12349) were purchased from Cell Signaling Technology (Beverly, MA, USA). The antibody against Iba-1 (#ab49999) was purchased from Abcam (Cambridge, MA, USA). Other related agents were purchased from commercial suppliers. Z-guggulsterone was dissolved in dimethylsulfoxide (DMSO), and then stored at −20 °C. The final concentration of DMSO was <0.05%.

2.2. Cell preparation

BV-2 cells were grown in DMEM/F12 with 10% fetal bovine serum (FBS, Gibco). Mouse primary cultured brain cells were prepared as described previously with some modifications [18]. Briefly, newborn (day 0–1) C57BL/6 mice were decapitated, cortex was then removed and digested with 0.125% trypsin for 15 min at 37 °C. Followed by trituration and centrifugation at 118*g* for 5 min, cells were re-suspended and plated on poly-L-lysine (0.1 mg/mL)-coated culture flasks. The single cell suspension was cultured in DMEM/F12 supplement with 10% heat-inactivated FBS and 1% penicillin-streptomycin (100 U/mL). For isolation of primary microglia, the medium was changed to fresh medium after 24 h and replaced every 3 days. After 12 days, mixed cells were shaken gently 2 h at 37 °C, and then the supernatants were collected and plated on the new poly-L-lysine (0.1 mg/mL)-coated culture flasks. The Iba-1 antibody was used to identify the primary microglia (purity > 99%). All cells were maintained in a 37 °C incubator containing 95% air and 5% CO_2. After treatment, cell supernatants from BV-2 cells or primary microglia were collected and frozen at −80 °C for NO or cytokine detection.

2.3. Cell viability assay

Cell viability was measured using MTT Cell Proliferation and Cytotoxicity Assay Kit (Bi Yuntian Biological Technology Institution, Shanghai, China). 5 mg/mL of methylthiazolyldiphenyl tetrazolium bromide was dissolved in prepared MTT-dissolved solutions and kept at −20 °C. After washing with PBS, the cells were added 20 μL of MTT solutions and kept at 37 °C for 4 h. The blue crystals were dissolved in formazan-dissolved solutions, and the absorbance was read at 570 nm.

2.4. Animals and experimental protocol

6–8 weeks old male C57BL/6 mice were randomly divided into six groups (n = 10 per group). Mice pre-received an intraperitoneal injection of vehicle or Z-guggulsterone (10 or 30 mg/kg) for 3 consecutive days. On the third day, after injection of vehicle or Z-guggulsterone (10 or 30 mg/kg) for 2 h, mice were intraperitoneally injected with saline or LPS (0.83 mg/kg) for another 5 consecutive days. During that time, mice were still accepted Z-guggulsterone (10 or 30 mg/kg) treatment. The LPS dosage was selected because it has been reported to induce pro-inflammatory responses in the brain and leads to behavioral abnormalities in adult mice [31,32]. After behavioral experiments, some mice were sacrificed and were prepared to perform the perfusion-fixation experiment. The use of mice was approved by the University Animal Ethics Committee of Nantong University (Permit Number: 2110836).

2.5. Tail suspention test (TST)

The TST was performed in C57BL/6J mice according to the method described by Steru et al. [33]. Briefly, 2 h after the last drug injection, mice (n = 10 per group) were suspended 50 cm above the floor for 6 min by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded during the last 4-min by an investigator blind to the study. Mice were considered immobile only when they hung passively and were completely