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Lidocaine attenuates lipopolysaccharide-induced inflammatory responses in microglia

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ABSTRACT

Background: Lidocaine has been used as a local anesthetic with anti-inflammatory properties, but its effects on neuroinflammation have not been well defined. In the present study, we investigated the prophylactic effects of lidocaine on lipopolysaccharide (LPS)-activated microglia and explored the underlying mechanisms.

Materials and methods: Microglial cells were incubated with or without 1 μg/mL LPS in the presence or absence of lidocaine, a p38 mitogen–activated protein kinase (p38 MAPK) inhibitor (SB203580), a nuclear factor-kappa B (NF-κB) inhibitor (pyrrolidine dithiocarbamate), or small interfering RNA. The protein and expression levels of inflammatory mediators, such as monocyte chemotactic protein 1, nitric oxide, prostaglandin E_2, interleukin 1β, and tumor necrosis factor α were measured using enzyme-linked immunosorbent assays and real-time polymerase chain reaction. The effect of lidocaine on NF-κB and p38 MAPK activation was evaluated using enzyme-linked immunosorbent assays, Western blot analysis, and electrophoretic mobility shift assay.

Results: Lidocaine (≥2 μg/mL) significantly inhibited the release and expression of nitric oxide, monocyte chemotactic protein 1, prostaglandin E_2, interleukin 1β, and tumor necrosis factor α in LPS-activated microglia. Treatment with lidocaine also significantly inhibited the phosphorylation of p38 MAPK and the nuclear translocation of NF-κB p50/p65, increased the protein levels of inhibitor kappa B-α. Furthermore, our study shows that the LPS-induced release of inflammatory mediators was suppressed by SB203580, pyrrolidine dithiocarbamate, and small interfering RNA.

Conclusions: Prophylactic treatment with lidocaine inhibits LPS-induced release of inflammatory mediators from microglia, and these effects may be mediated by blockade of p38 MAPK and NF-κB signaling pathways.

1. Introduction

Microglial cells are activated during neuropathologic conditions (infection, inflammation, or injury) to restore central nervous system homeostasis [1] and participate in host defense and inflammation in the brain [2]. Once activated, microglia can promote neuronal injury through the release of inflammatory mediators, including nitric oxide (NO), monocyte chemotactic protein 1 (MCP-1), prostaglandin E_2 (PGE_2), interleukin 1β (IL-1β), and tumor necrosis factor α (TNF-α) [3–5]. Studies have demonstrated that inhibiting the release of inflammatory mediators from microglia can attenuate the

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severity of Alzheimer disease, Parkinson disease, atherosclerosis, and multiple sclerosis [6,7]. Thus, anti-inflammatory treatment via inhibition of microglial activation offers a potentially effective therapeutic approach to mitigate the progression of neuroinflammatory diseases.

In vitro studies have shown that lidocaine, a common local anesthetic drug, has significant anti-inflammatory properties on various cell types, including monocytes, macrophages, and neutrophils [8–10]. Lidocaine has also been shown to modulate inflammatory cascades and provide protection from ischemia–reperfusion injury [8,11,12] and septic peritonitis [13] in *in vivo* studies. Jeong *et al.* [14] reported that lidocaine significantly inhibited some of the inflammatory responses in microglia stimulated by lipopolysaccharide (LPS), although the detailed underlying mechanism has not yet been completely resolved.

Recently, it was reported that the protective effect of lidocaine was associated with the inhibition of p38 mitogen–activated protein kinase (p38 MAPK) phosphorylation and the downstream activation of nuclear factor-kappa B (NF-κB) signaling [15–18]. Therefore, the anti-inflammatory effects of lidocaine treatment may be due to the inhibition of these signaling pathways. In this study, we aimed to elucidate the mechanisms underlying these neuroprotective and anti-inflammatory actions of lidocaine by prophylactic treatment of LPS-activated primary microglial cultures.

2. Materials and methods

2.1. Reagents

Lidocaine, SB203580 (p38 MAPK inhibitor), pyrrolidine dithiocarbamate (PDTC; NF-κB inhibitor), and LPS (*Escherichia coli* 0111:B4) were purchased from Sigma–Aldrich (St. Louis, MO). Small interfering RNA (siRNA) for NF-κB p65, IκB-α, or p38 MAPK were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Cell culture and treatment

Sprague–Dawley (SD) rats were obtained from the Experimental Animal Center of Central South University (Changsha, Hunan, China). Experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Bioethics Committee of Central South University. Primary microglia were isolated from SD rats as previously described [19] and cultured in Dulbecco Modified Eagle Medium (Gibco, Grand Island, NE) supplemented with 10% fetal calf serum (Gibco), 100 U/mL penicillin, and 100 mg/mL streptomycin (Gibco) and maintained in a 5% CO_2 incubator at 37°C. After overnight incubation, the cells were washed three times and then transferred to six-well polystyrene culture plates at 1×10^5 cells/mL (2 mL per well). Microglial cells were treated with lidocaine (0.2, 2, and 20 μg/mL) for 1 h and then stimulated with LPS (1 μg/mL) for 24 h, with a control group receiving 20 μg/mL lidocaine only. In a second set of experiments, microglial cells were preincubated with 10 μM SB203580 or 100 μM PDTC for 1 h, followed by LPS treatment for 24 h. The dosages of lidocaine, SB203580, and PDTC were determined according to previous studies [20,21].

2.3. Cell Counting Kit-8 assay

Cell viability was determined using the Cell Counting Kit-8 assay (CCK-8; Beyotime, Jiangsu, China) [22]. Briefly, microglial cells were plated in 96-well plates at a density of 1×10^4 cells per well in 100 μL Dulbecco Modified Eagle Medium and preincubated with various concentrations of lidocaine for 1 h, followed by LPS (1 μg/mL) treatment for 24 h. After treatment, 20 μL of CCK-8 reagent was added to each well and incubated for 2 h at 37°C. The absorbance of the solution was measured using a microplate reader (Bio-Rad Laboratories, Hercules, CA) at a test wavelength of 450 nm and a reference wavelength of 630 nm.

2.4. NO assay

NO concentrations in culture supernatants were determined using Griess reagent (Sigma–Aldrich). Briefly, microglial cells were preincubated with lidocaine (0.2, 2, and 20 μg/mL) for 1 h, followed by LPS (1 μg/mL) treatment for 24 h. The supernatants were collected and mixed with an equal volume of Griess reagent. Samples were incubated at room temperature for 10 min, and absorbance was subsequently read at 540 nm using a microplate reader.

2.5. MCP-1, PGE_2, and cytokine assays

Microglial cells were seeded in six-well plates at a density of 4×10^5 cells/mL and preincubated with lidocaine (0.2, 2, and 20 μg/mL) for 1 h, followed by LPS (1 μg/mL) treatment for 24 h. MCP-1, PGE_2, TNF-α, and IL-1β concentrations from 0.1 mL samples of culture supernatants were determined using an enzyme-linked immunosorbent assay (ELISA; R & D Systems, Minneapolis, MN) following the manufacturer's instructions and absorbance readings at 450 nm were determined on a microplate reader.

2.6. Quantitative real-time polymerase chain reaction analysis

Total RNA was extracted from microglia using Trizol (Invitrogen Corporation, Carlsbad, CA) according to the manufacturer's instructions and reverse transcribed with Moloney Murine Leukemia Virus reverse transcriptase (Promega, Madison, WI). Quantitative real-time polymerase chain reaction (qPCR) was performed using a LightCycler 2.0 Real-Time PCR System (Roche Applied Science, Indianapolis, IN). Complementary DNA was amplified using specific primers for inflammatory mediators gene expression, and the results were normalized to β-actin gene expression. The relative mean fold change of inflammatory mediators gene expression in the experimental group was calculated using the $2^{\Delta\Delta\ Ct}$ method and compared with the control group [23].