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Resolvin D2 recovers neural injury by suppressing inflammatory mediators expression in lipopolysaccharide-induced Parkinson's disease rat model

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ABSTRACT

Activation of microglial cells have been treated as the main role in the pathogenesis of neuropathic inflammation and neurodegenerative disease, including Parkinson's disease (PD), prion disease and Alzheimer's disease (AD). Resolvin D2 (RvD2) is derived from omega-3 polyunsaturated fatty acid and performs potent anti-inflammatory and pro-resolution effects. Here we investigated the effects of intrathecal injection of RvD2 for substantia nigra pars compacta (SNpc) in vivo and primary microglia in vitro experiment on pro-inflammatory cytokine expression and NF-κB activation in Lipopolysaccharide (LPS)-induced PD rat model. The total of 30 days experimental period were used for the rats' experiment, the LPS-induced inflammation in SNpc increase the expression of NO, iNOS, TNF-α, IL-1, IL-18, IL-6, IL-1β, ROS production, the translocation of NF-κB p65, IκBα, and IKKβ expression in glial cells. After injection of RvD2, the treatment prevented development of behavioral defects and TLR4/NF-κB pathway activation. Therefore, we demonstrated a novel role of RvD2 in treatment of rat PD model and LPS activated microglia inflammation. Given the significant potency of RvD2 and well-known side effects of microglia inflammatory inhibitors, it may represent novel hotspot for treating neurodegenerative disease.

1. Introduction

Parkinson's disease (PD) is a common geriatric neurodegenerative disease, which is mainly pathologically characteristic of the progressive degeneration of nigrostriatal dopaminergic neurons in midbrain and nerve endings. Clinical symptoms include resting tremor, rigidity and bradykinesia [1]. PD incidence is only lower than that of Alzheimer's disease, although individual gene mutations have been linked to some familial PD, the vast majority of PD etiology and pathogenesis remains unclear, therefore PD treatment maintains at the symptomatic level, and there is no completely effective therapeutic measure [2,3]. McGeer et al. [4] have found activated microglial cells in mesencephalic substantia nigra pars compacta (SNpc) of PD patients, then scholars are interested in the activation of microglia as the mechanism underlying neural immune inflammation. Microglial cells are the main immune cells in the brain, and are not evenly distributed in normal brain, most dense in substantia nigra compacta of the midbrain. On one hand, activated microglia releases neurotropic factors and anti-inflammatory cytokines to play a protective role [5–7]; on the other hand, microglia activation generates a large amount of free radicals such as superoxide, intracellular reactive oxygen species, hydrogen peroxide, hydroxyl radical and cytotoxic cytokines, including tumor necrosis factor α, interleukin 1β, nitrogen monoxide and superoxide, these radicals may injury neurons [8,9].

Recently, a novel family of lipid mediators including resolvin E and resolvin D has been reported that they have obvious potency in treating neurodegenerative disorders, especially for the disease conditions associated with inflammation [10,11]. Accumulating evidences suggests that omega-3 polyunsaturated fatty acids-derived lipid mediators, such as resolvins, lipoxins, and

Abbreviations: MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-kappa B; TNF-α, tumor necrosis factor α; TLR4, toll-like receptors 4; NO, nitrogen monoxide; SNpc, substantia nigra pars compacta; LPS, lipopolysaccharide; IL-β, interleukin 1β.

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neuroprotectins produce potent anti-inflammatory and pro-resolution actions in various animal models of inflammation. In the previous research, Xu et al. [11] have reported that resolvins family shows remarkable potency in treating inflammatory pain via central and peripheral actions. Sun et al. [12] and Jin et al. [13] also have indicated that resolvins have the ability to play potent anti-inflammatory and pro-resolution role in animal models of inflammation, as well as the well-known lipoxins and aspirin-triggered lipoxins have similar actions [14–16].

In this regard, we examined whether RvD2 can attenuate LPS-induced inflammatory nerve damage in PD rat model, and further investigated the possible mechanisms involved in cultured microglia activated by LPS in vitro. Our results show that LPS did highly increase the CD11b and Iba-1marker protein expression in glial cells. Also, RvD2 attenuates LPS-induced inflammatory nerve damage in PD rat model by inhibiting the release of inflammatory cytokines and the translocation of TLR4/NF-κB pathway activation in microglial cells.

2. Materials and methods

2.1. Drugs administration

Lipopolysaccharide was obtained from Sigma-Aldrich and resolvin D2 was purchased from Cayman, USA. The RvD2 was prepared in Hanks' buffer (Wisent. bio, Nanjing, China) before it was used. The injection was performed manually within 20 s using a single injection volume of RvD2 per 25, 50 and 100 ng/kg.

2.2. Animals and treatment

200 adult male SD rats weighing 280 g–300 g were purchased from Vital River Laboratory Animals Co., Ltd. (Beijing, China) and housed with a standard 12 h on/off light cycle with food and water in their cages. All rats were randomly separated into 3 groups: (1) the control group; (2) LPS-injected group with model; (3) LPS-injected groups receiving injections with 25, 50 and 100 ng/kg for 3 days; LPS (10 mg/mL, 1.0 uL) was injected into the right part of SNpc following a previously described protocol [17,18]. After LPS injection, rats were continuously treated with RvD2 for 27 days, and the total of 30 days experimental period were used for the rats' experiment. All animal experimental protocols were performed according to the NIH of USA Guide for the care and use of laboratory animals (Publication 85-23, revised 1996).

2.3. Isolation and culture of microglial cells from neonatal rats

The isolation of rat microglial cells from 2-day-old neonatal rats according to the method of Garcia et al. [19] with certain modifications. The cerebral cortex was carefully dissected out, the meninges of cerebral cortices were cautiously separated, and cortices were minced and dissociated with 0.25% trypsin/1 mM EDTA for 20 min at 37 °C. The fragments were washed with cold D-Hank's buffer (GIBCO Corporation, Gaithersburg, MD, USA) and the meninges were gingerly removed. The re-suspended cells were then collected and seeded in uncoated culture flasks containing medium (DMEM/F12 supplemented with 10% FBS, 1×10^5 U/L streptomycin sulfate, pH 7.2, GIBCO Corporation, Gaithersburg, MD, USA) with a concentration 1×10^6/mL at 37 °C, 5% CO_2. Confluent cultures were passaged by trypsinization; microglial cells were isolated by shaking and cultured in 6-well plates, at a density of 2.5×10^5 cells/cm^2. In brief, all the cells are divided into 7 groups. The cells of RvD2 group were incubated in the initial experiments with different concentration of RvD2 (1.25 umol/L, 2.5 umol/L, 5 umol/L, 10 umol/L and 20 umol/L). The LPS group cells will only incubate in vehicle before addition of 100 ng/ml LPS under serum-free condition. Based on the control group, the RvD2+LPS groups will add 100 ng/ml LPS to the cells that have been incubated in RvD2 for 60 min.

2.4. Behavioral analysis

Apo-morphine was used to study the rotational behavior of rats. Rats were placed into cylinders that were attached to a rotameter (Columbus Instruments, Columbus, OH, USA) on the second day after the final RvD2 injection. The rats were allowed to adapt to the testing environment for 15 min and were injected hypodermically with 0.5 mg/kg apo-morphine (Sigma-Aldrich) dissolved in physiological saline. After injection for 5 min, the measurement of rotational activity began and lasted for 30 min under minimal external stimuli. The rotameter recorded the number of full clockwise and counter-clockwise turns the animals performed during the testing period. Counter-clockwise turns were counted as negative turns. Also, the clockwise turns were counted as positive turns. The net number of turns performed during the entire 30 min testing period was counted.

2.5. Tissue processing

For tissue harvest, after the rotational behavior assay, 20 rats were randomly selected from each group for morphological studies. Rats were deeply anesthetized with chloral hydrate. Frozen sections were cut into 35-um-thick sections and processed for immunohistochemistry.

2.6. Immunohistochemistry

Every fifth section of the SN (bregma −4.8 to −6.3 mm) was immunostained for detection of the microglial marker CD11b (1:500; Sigma-Aldrich). After being perforated, all the cell membranes with 0.5% Triton-X 100 and blocked with 2.5% horse serum, then sections were incubated with primary antibodies for 24 h at 4 °C. Then, the antibody was detected using an ABC Elite kit (Vector laboratories, Sigma-Aldrich) with 3,3′- diaminobenzidine (DAB) and nickel enhancement. The average optic density value in the SNpc of each CD11b-stained section was determined using an image analysis system. All sections were coded and examined blindly.

2.7. RNA isolation and quantitative real time-PCR

The total RNA extraction was performed using Trizol reagent (Gibco BRL). 1 μg of total RNA was reverse transcribed using the M-MLV-RT system (Promega). The action was carried out at 42 °C for 1 h and terminated by deactivation of the enzyme at 70 °C for 10 min. qPCR were conducted using SYBR Green (Bio-Rad) in ABI PRISM 7900HT detection systems (Applied Biosystems). Invitrogen coorporation produced all the primers for CD11b, Iba-1, TNF-α, NF-κB p65, iNOS, IL-1, IL-18, IL-6, IκBα, IKKβ, and IL-1β.

2.8. ELISA measurement

Microglial cells culture medium and rats' serum was collected for the ELISA analysis of main pro-inflammatory cytokines. For each reaction in a 96-well plate, 100 μl of medium were used, and ELISA kits (R&D) were performed according to manufacturer's protocol. The microglia were incubated with 100 ng/mL LPS for 24 h, the culture supernatants were harvested and mixed with an equal volume of Griess reagent (Sigma-Aldrich) in 96-well plates at room temperature for 20 min. Bio-Rad absorbance reader was used to measure the wavelength of 540 nm, and the nitrite (NO) concentrations were confirmed according to standard curve generated by known concentrations of sodium nitrite.

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