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Suppression of neuroinflammation by matrix metalloproteinase-8 inhibitor in aged normal and LRRK2 G2019S Parkinson's disease model mice challenged with lipopolysaccharide

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ABSTRACT

Microglial priming is caused by aging and neurodegenerative diseases, and is characterized by an exaggerated microglial inflammatory response to secondary and sub-threshold challenges. In the present study, we examined the effects of the matrix metalloproteinase-8 (MMP-8) inhibitor (M8I) on the brain of aged normal and leucine-rich repeat kinase 2 (LRRK2) G2019S Parkinson's disease (PD) model mice systemically stimulated with lipopolysaccharide (LPS). The results indicated that Iba-1 positive microglia and GFAP-positive astrocytes, which were increased by LPS, significantly decreased by M8I in aged normal and PD model mice. M8I also decreased the expression of pro-inflammatory markers in the hippocampus and midbrain of aged normal and PD model mice challenged with LPS, while it also improved the motor coordination of aged normal mice after LPS challenge in rotor rod test and the general crossing locomotor activities of LPS-treated LRRK2G2019S PD mice after LPS challenge in open field test. To assess the effects of M8I in an *in vitro* priming model, BV2 microglia were pretreated with macrophage colony-stimulating factor (CSF)-1 or interleukin (IL)-34, and subsequently stimulated with LPS or polyinosinic-polycytidylic acid (poly[I:C]). M8I inhibited the LPS- or poly(I:C)-induced production of the tumor necrosis factor-α and nitric oxide, alone or in combination with CSF-1 or IL-34. Collectively, the data suggested that M8I has a therapeutic potential in treating neurodegenerative diseases that are aggravated by systemic inflammation.

1. Introduction

Microglia, which are resident immune cells in the central nervous system, actively sense the microenvironment of the brain and respond to recover tissue homeostasis. Under physiological conditions, microglia play an important role in the brain's development and function [1,2]. Microglia are activated by various stressors and brain injuries, and are involved in neuronal recovery [2]. However, exaggerated microglial activation is closely related with the occurrence and progression of various neurodegenerative diseases and neuropsychiatric disorders [3]. Systemic inflammation also leads to microglial activation, and plays a role in the progression of neurodegenerative diseases [4,5].

Microglia proliferate and are activated in response to neuronal damage and abnormal protein accumulation such as those observed in the process of aging and neurodegeneration. This process is referred to as microglial priming and the phenotypes of primed microglia are observed in aging, neurodegenerative diseases, and traumatic brain injury [5–7]. Priming renders microglia susceptible to secondary inflammatory signals, and leads to exaggerated inflammatory responses [8]. In the brains of elderly individuals with and without Alzheimer's disease, the secondary

Abbreviations: CSF, macrophage colony-stimulating factor; EMSA, electrophoretic mobility shift assay; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium binding adaptor molecule 1; IL, interleukin; LPS, lipopolysaccharide; LRRK2, Leucine-rich repeat kinase 2; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NO, nitric oxide; PD, Parkinson's disease; poly(I:C), polyinosinic-polycytidylic acid; TNF, tumor necrosis factor.

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signal usually occurs by systemic inflammation, which is associated with rapid cognitive decline and aggravation of the symptoms [8,9]. Therefore, inhibiting the systemic inflammation or blocking the inflammatory signals from the periphery to the CNS has been suggested as a potential strategy for delaying the progression of neurodegenerative diseases [7–9].

Our group recently reported that several matrix metalloproteinases (MMPs) are upregulated under neuroinflammatory conditions and play a role as proinflammatory mediators in the brain [10–12]. In particular, we reported that MMP-8 plays a pivotal role in neuroinflammation by modulating the processing of tumor necrosis factor alpha (TNF-α) [11]. Treatment with the MMP-8 inhibitor (M8I) or MMP-8 short-hairpin RNA (shRNA) inhibited microglial activation both *in vivo* and *in vitro* under neuroinflammatory conditions. Furthermore, we demonstrated that MMP-8 is a novel pathogenic factor in focal cerebral ischemia [12], as evidenced by its increased expression, while its inhibition mitigated the infarct volume and microglial activation [12].

In the present study, systemic inflammation conditions were induced in aged normal and LRRK2 G2019S mice by peripheral injection of lipopolysaccharide (LPS). We examined the effects of M8I on neuroinflammation, neuronal cell death, and motor control behaviors. M8I not only suppressed microglial/astrocytic activation and the expression of several inflammatory markers but also improved the locomotor activity in aged normal and LRRK2 G2019S mice challenged with LPS. We further demonstrated the anti-inflammatory effects of M8I in priming model *in vitro* systems.

2. Materials and methods

2.1. Reagents

LPS (*Escherichia coli* serotype 055:B5) and polyinosinic-polycytidylic acid (poly[I:C]) were obtained from Sigma-Aldrich (St. Louis, MO). The M8I ([3*R*]-2-[4-phenyl] phenylsulfonyl-1,2,3,4-tetrahydroisoquinoline-3-hydroxamic acid) was synthesized through a custom synthesis service from Sehan Lab (Seongnam, Korea) as reported previously [13]. All reagents used for cell culture were purchased from Welgene (Gyeongsan, Korea). The antibodies against the ionized calcium binding adaptor molecule 1 (Iba1) and glial fibrillary acidic protein (GFAP) were purchased from Wako (Osaka, Japan) and Millipore (Bedford, MA), respectively. Recombinant colony-stimulating factor 1 (CSF-1) and interleukin 34 (IL-34) proteins were purchased from Biolegend (San Diego, CA). The reagents/enzymes for reverse transcription polymerase chain reaction (RT-PCR) were purchased from Promega (Madison, WI).

2.2. Microglial cell culture

The immortalized mouse BV2 microglial cell line [14] was grown and maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, streptomycin (10 μg/mL), and penicillin (10 U/mL) at 37 °C under 5% CO_2.

2.3. Experimental animals

Leucine-rich repeat kinase 2 (LRRK2) is associated with the pathology of autosomal dominant and late-onset PD. LRRK2 G2019S mutations have been reported to increase the kinase activity and alpha-synuclein inclusion bodies [15]. We used LRRK2 G2019S mutant mice at 16–22 months of age as the aged PD model. Age-matched non-transgenic littermates were also used as the aged normal control mice. LRRK2 G2019S mutant mice (FVB/N-Tg 1Cjli/J, Jackson Laboratory, Bar Harbor, ME) were purchased and housed under a 12-h light-dark cycle with free access to food and water. Normal FVB mice at the age of 10 weeks (Orient Bio Inc., Seongnam, Korea) were used as young controls. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Hanyang University (HY-IACUC-12-018), and were carried out in accordance with the guidelines of the National Institute of Heath's Guide for the Care and Use of Laboratory Animals.

2.4. LPS-induced inflammation and administration of M8I

The overall experimental scheme is summarized in Fig. 1A. LPS (0.5 mg/kg) was administered intraperitoneally (i.p.) to induce neuroinflammation in aged normal and LRRK2 G2019S PD mice, as previously described [11]. M8I (5 mg/kg) was dissolved in vehicle solution (normal saline containing 1% dimethylsulfoxide) and administered daily (i.p) for 4 days before the LPS treatment. All mice were tested in the rotor rod and open field tests before and after M8I or LPS injection. Subsequently, mice were transcardially perfused with phosphate-buffered saline (PBS) for immunohistochemical and gene expression analyses. Half-brains were post-fixed in 4% formaldehyde for 24 h at 4 °C. Subsequently, brains were incubated in 30% sucrose solution for 2 days until they sunk, and then stored in PBS solution containing 0.03% sodium azide at 4 °C. The other half-brains were used for fresh tissue preparation after the dissection of specific brain regions for biochemical gene expression analysis.

2.5. Immunohistochemistry

After post-fixation, the brains were cut into 30-μm thick coronal sections with a freezing microtome (Thermo Scientific sliding microtome, HM 430, Waldorf, Germany). Four different sections were selected for immunohistochemical staining and each section was pre-activated with a PBS solution at room temperature. Sections were incubated for 1 h in a blocking solution containing 10% normal goat serum (Vector Laboratories, Burlingame, CA) with 0.1% Triton X-100 to reduce the nonspecific binding. After blocking, sections were incubated for 3 h with the polyclonal primary antibodies Iba1 (1:300; Wako, Osaka, Japan), or GFAP (1:500; Millipore, Bedford, MA). Subsequently, sections were incubated with the secondary antibodies, HRP-conjugated anti-rabbit IgG (1:500; Vector Laboratories) or HRP-conjugated anti-mouse IgG (1:500; Vector Laboratories) for 1 h at room temperature followed by washing with PBS. Then, the peroxidase reaction was performed using 3,3′-diaminobenzidine tetrahydrochloride (DAB, Vector Laboratories) for 5 min. The sections were mounted on 0.3% gelatin-coated slides, dehydrated, and cover-slipped for microscopic image analysis (Carl Zeiss microscope, Axio observer, Oberkochen, Germany). The quantification of Iba1-and GFAP-positive cells was carried out as previously described [16].

2.6. Behavioral tests

All experimental mice were subjected to the rotor rod and open field tests to evaluate motor coordination. The rotor rod test was performed to assess the motor function and balancing as previously described [17]. Each mouse was placed on the rod that was rotating at a fixed speed. The mice had to walk on the rotating rod at a 6-rpm speed for a maximum of 60 sec, and the time latency to fall off the rod was measured for each mouse. The mice were subjected to four consecutive trials. The open field test was performed to assess the general locomotor activity. Mice were placed in the center of a clear acryl box (27.5 × 27.5 cm^2) that was divided into 25 squares (5.5 × 5.5 cm^2 each) with black lines at the bottom of the box. The movement of mice was counted and recorded over three trials (4 min/trial) with no inter-trial intervals. The numbers of

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