Journal of Neuroimmunology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Minocycline decreases CD36 and increases CD44 in LPS-induced microglia

Afrouzeh Hashemi-monfared[a], Masoumeh Firouzi[a,*], Zahra Bahrami[a], Hamid Zahednasab[a], Mohammad Hossein Harirchian[b,*]

[a] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[b] Iranian Centre of Neurological Research, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Microglia
CD36
CD44
LPS
Inflammation
Cell culture

ABSTRACT

Microglia are the resident macrophages patrolling the central nervous system (CNS) to find dangerous signals and infectious agents mediating catastrophic cascades resulting in neuronal degeneration. Their morphological and biochemical properties made them enable to swift activation in response to neural insults and site-directed phagocytosis. Beside of beneficial roles in homeostasis of the brain and spinal cord, microglia can be participating in neuronal destruction and propagation of inflammation when they are unregulated or hyper-activated. A large body of research indicates that various cluster of differentiations (CDs) contribute to flame/quench the inflammatory processes occurred in immune system. In this study, we investigated the expression of CD36 and CD44 in LPS-activated primary rat microglia in response to treatment of minocycline at the levels of protein and gene using flow cytometry and real-time PCR, respectively. The results showed that minocycline decreased the expression of CD36 in cells treated with minocycline with respect to cells treated with LPS. Inversely, the expression of CD44 was increased in cells treated with minocycline in comparison to LPS-induced microglia. It seems that minocycline can modulate the expression of CDs involved in inflammatory reactions and enrich the armamentarium of therapeutic agents used for the treatment of neuroinflammatory and neurodegenerative disorders.

1. Introduction

Microglia are the guardian cells of the central nervous system (CNS) protecting the neural cells from either intrinsic or extrinsic insults (Akiyama and McGeer, 1990). They have a key role in maintaining the homeostasis of the brain and spinal cord through scavenging the CNS for plaques, eliminating of debris or unnecessary neurons, synaptic pruning and infectious agents (Schafer et al., 2012). Despite of defensive role of microglia in a normal physiological conditions, microglia can cause severe damage to neuronal cells when hyper-activated in which excessive amounts of free radicals such reactive oxygen species (ROS), nitric oxide (NO) and proinflammatory cytokines are markedly produced resulted in destruction of neurons and axonal fibers (Hartung et al., 1992). LPS is one of the exemplary molecules found in the outer membrane of gram negative bacteria that rigorously incite the immune system to resemble the inflammatory cascades occurred in pathological conditions (Chen et al., 2012). Upon the initiation of inflammation, a plenty of biological molecules are produced such as proinflammatory cytokines, free radicals and cluster of differentiations (CDs). These molecules are usually considered the representative phenomena of inflammation that enable researchers to track down the process of inflammation.

CD36, also known as platelet glycoprotein 4, is a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and oxidized low density lipoprotein (Endemann et al., 1993). It has been shown that the activation of nuclear factor kappa B (NF-kB) is directly associated with the presence of CD36 as NF-kB activation is suppressed in CD36-null mice (Kunz et al., 2008). In parallel with this, CD44 is also implicated in attenuation/promotion of inflammation (Puré and Cuff, 2001). CD44 is a cell-surface glycoprotein involved in cell-cell interaction, cell migration and metabolism of hyaluronan (Cichy and Puré, 2003). There are paradoxical reports about the role of CD44 in amplification/amelioration of inflammation urging us investigate the expression profile of this molecule in activate microglia. Despite some studies claim that CD44 plays a crucial role in recruitment of immune system to flame inflammatory process (Sebban et al., 2007), some research reports CD44 can modulate the hyper-activation of immune cells to halt the inflammatory reactions (Kawana et al., 2008b).

Minocycline is a member of tetracycline family antibiotics possessing a broader spectrum than the other members (Shapiro et al., 1997). In addition to antibacterial activity of minocycline, a great body of research shows that minocycline has neuroprotective (Tikka et al.,

* Corresponding authors.
E-mail addresses: m_firouz@ut.ac.ir (M. Firouzi), harirchm@tums.ac.ir (M.H. Harirchian).

https://doi.org/10.1016/j.jneuroim.2018.01.010
Received 5 December 2017; Received in revised form 18 December 2017; Accepted 16 January 2018

2001), anti-psychotic (Miyaoka et al., 2008) and anti-inflammatory (Hua et al., 2005) properties. Minocycline can simply cross the blood-brain barrier (BBB) and modulate the activity of microglia in neuro-degenerative disorders (Zhao et al., 2007). In this investigation, we studied the expression of CD44 and CD36 in LPS-induced microglia of newborn Wistar rats to show whether treatment with minocycline can alter the expression of these molecules in activated microglia.

2. Materials and methods

2.1. Preparation of primary murine microglia

In order to establish the primary murine microglia, ten 1-day old neonatal Wistar rats were bought from animal house of Tehran University. The protocol for the research project was approved by Tehran University of Medical Sciences (TUMS) Ethics Committee (TUMSEC). The newborn Wistar rats were anesthetized by ether and immediately decapitated. Soon after, the meninges of the brains were removed and brains were minced and dissociated with 0.25% trypsin/0.5 mM EDTA. After the cells became dissociated, they were transferred through nylon cell strainer with 70-μm pores (BD biosciences, Heidelberg, Germany). Cells were collected by centrifugation followed by resuspension in Dulbecco's modified Eagle's medium (DMEM) containing 0.584 g/l glutamine and 4.5 g/l glucose, sodium pyruvate and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) and cultured in T-75 flask in 5% CO2 at 37 °C. The culture medium was twice weekly checked and renewed. Approximately 13–16 days later, the glial cultures became completely confluent and the microglia were shaken at 240 rpm for 3 h at 37 °C. Detached microglia were centrifuged at 168 × *g* for 15 min. Cell pellets were re-suspended in warm DMEM 10:10:1 and plated at a density of 250,000 cells/cm^2. For experiments, microglial cells were re-suspended in RPMI medium supplemented with 2% FBS and 100 U/ml penicillin and 100 μg/ml streptomycin.

2.2. Validation of microglial purification

To validate the purity of microglia obtained in previous stage, the cells were stained by a specific marker called Iba-1, rabbit anti-rat/mouse Iba-1 (Abcam, Cambridge, UK), which is exclusively expressed in microglia. The staining protocol was carried out in accordance to manufacturer's instructions. In brief, the cells were then fixed with 4% formaldehyde for 10 min. After that, cells were gently washed 3 times for 5 min each with PBS, then blocked with PBST (PBS with 0.3% Triton-100) for 20 min. Then, glial cells were incubated for 60 min in dark with rabbit anti-rat Iba1 antibody at a dilution of 1:250, along with Hoechst to dye nuclei (Invitrogen H3569, dilution 1:5000). At that moment, cells were washed 3 times and the duration of each wash was 5 min. Then, the procedure continued by incubating glial cells with Alexa 568 (goat anti-rabbit, Life Tech Cat. No. A11036, dilution 1:400) for 45 min. Before imaging, cells were mounted with Fluoromount-G (Southern-Biotech, Cat. No. 0100-01). Of note, GFAP-Cy3 (Sigma C9205) at a dilution of 1:500 was used as negative control. The purity of the microglia was determined 96% (Fig. 3).

2.3. Cell culture treatment

Glial cells were pre-treated with the dose of 40 μmol/l minocycline (Sigma, St. Louis, MO, USA) 60 min at 37 °C and 5% CO_2. After that, cells were then stimulated with 10 ng/ml of LPS (Sigma, St. Louis, MO, USA) to activate microglia for 16 h at 37 and 5% CO_2.

2.4. Cell viability assay

Cell viability was evaluated by the usage of MTT (3-4-5-dimethylthiazole-2-yl-2, 5-diphenyl-tetrazolium bromide) reduction assay. Cells were incubated with MTT (0.5 mg/ml) for 24 h at 37 °C.

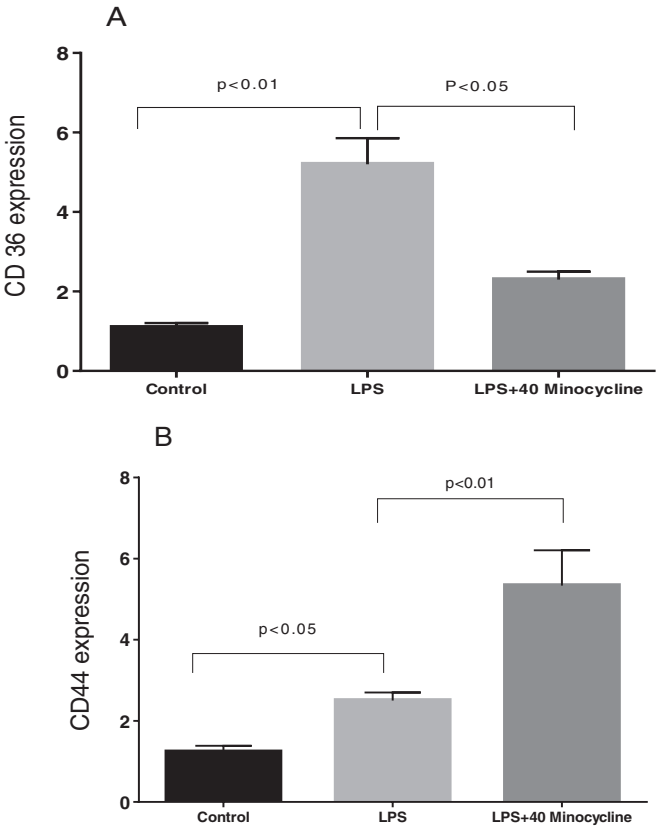

Fig. 1. The relative gene expression of CD36 and CD44 in primary cell culture of microglia. A; CD36 was increased in LPS-induced microglia and decreased when treated with minocycline. B; CD44 was increased in response to LPS and markedly intensified when microglia were treated with 40 μg/ml minocycline.

The formazan crystals in the cells were solubilized with DMSO. The level of MTT formazan was determined by measuring the absorbance at 490 nm.

2.5. Flow cytometry

Microglia were pre-incubated with 5 μg/ml fluorescein isothiocyanate CD36 (BD Biosciences, San Jose, CA) or mouse IgA as a negative control (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Hereafter, microglia were washed twice with PBS and re-suspended in 4% paraformaldehyde for fixation. The fluorescence intensity was determined by flow cytometry (Hitachi, Japan).

2.6. Real-time PCR

Total RNA was extracted using Trizol (Gibco, Grand Island, NY, USA) according to the manufacturer's protocol. Afterward, cDNA was synthesized using a reverse transcriptase kit (TaKaRa, Japan). Nearly, 20 μl of cDNA was obtained of which 1 μl was applied for each real-time PCR analysis. Real-time PCR was performed to compare CD36 mRNA in cells underwent LPS and minocycline treatments and was determined using the SYBR⁻ Premix Ex Taq™ II (Perfect Real Time, TaKaRa, Japan) and normalized to GAPDH mRNA levels. The following primers were applied for CD36 sense: 5′-CTATGCTGTATTTGAATCCGACGTT-3′ and anti-sense:5′-CCTGTGTACATTTCACTTCCTCATT-3′, CD44 sense: 5′-AGCAGCGGCTCCACC ATCGAGA-3′ and anti-sense: 5′- TCGGATCCAT GAGTCACAGTG-3′, GAPDH sense: 5′-GGAGCGAGATCCCTCCAAAAT-3′ and anti-sense: 5′-GGCTGTTGTCATACTTCTCATGG-3′.The relative fold change expression of above genes was calculated by means of the comparative C_T method with $2^{-\Delta\Delta CT}$ equation. The condition of the