Biomedicine & Pharmacotherapy 79 (2016) 176–187

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

The protective effects of bone marrow-derived mesenchymal stem cell (BMSC) on LPS-induced acute lung injury via TLR3-mediated IFNs, MAPK and NF-κB signaling pathways

Jingcai Wang[a], Ying Qin[b,*], Xiuju Mi[a]

[a] Department of Pediatrics, People's Hospital of Liaocheng, Shandong 252000, China
[b] General Hospital of Jinan Iron and Steel Group Co., Ltd., Shandong 252000, China

ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form 16 February 2016
Accepted 16 February 2016

Keywords:
BMSC
LPS
Acute lung injury
TLR3
Inflammation

ABSTRACT

The study attempted to clarify the protective role of bone marrow-derived mesenchymal stem cell (BMSC) transplantation on LPS-induced acute lung injury (ALI) of rats. BMSC were obtained from bone marrow of rat, cultured and proliferated in vitro. Rats of ALI were established through lipopolysaccharide (LPS) administration. Male rats were allocated to control group, ALI group and BMSC, transplantation group. Rats were sacrificed after BMSC injection after 12 h, 24 h and 48 h. Here we investigated the role of BMSC in LPS-induced alveolar macrophages to further demonstrate the mechanism of BMSC to lung injury. TLR3, a member of Toll-like receptor family, has been found in macrophages and the cell surface. In our study, first BMSC successfully reversed LPS-induced lung injury by hematoxylin-eosin (H&E) staining, ameliorated apoptosis via TUNEL and flow cytometer analysis, as well as improved cell structure. And then, western blot, quantitative real-time PCR, immunohistochemistry and immunofluorescence analysis were used to confirm that TLR3 was significantly down-regulated for BMSC treatment. Subsequently, TRIF and RIP1, down-streaming signals of TLR3, were inhibited greatly, leading to TRAF3, MAPK as well as NF-κB inactivity. Our results indicated that BMSC transplantation group displayed inhibitory effects on interferon (IFNs) levels via TLR3 in LPS-induced ALI and preventive effects on inflammation response via TLR3-regualted MAPK and NF-κB signaling pathway in LPS-induced lung injury. The present study indicated that BMSC could display protective effects on LPS-induced ALI and provide an experimental basis for clinical therapy.

1. Introduction

Acute lung injury (ALI) still remains a prominent source of mortality and morbidity among seriously ill patients. Although the specific mechanisms revealing the development and progression of ALI remain unclear, inflammatory response is considered as major reasons [1,2]. Responding to inflammatory stimulation, the primary sources of cytokines in lungs are macrophages, which play an important role in the pathogenesis of lung injury [3,4]. Recently, advanced studies in stem cell investigation hold a promise for the treatment and prevention of chronic debilitating diseases. Bone marrow stromal cells, known as BMSCs, have been reported to differentiate into a number of tissue cell types [5–7]. In addition, a variety of researches have demonstrated that BMSCs treatment can improve monocrotaline, bleomycin, endotoxin, or lipopolysaccharide (LPS)-induced lung injury [8–10]. BMSCs are able to release a wide range of potent mediators, such as keratinocyte growth factor, vascular endothelial growth factor, IL-10, hepatocyte growth factor and so on, which is likely to regulate the protective properties of BMSCs in the lung tissue [11].

TLR3, as a member of the Toll-like receptor family, has been found to recognize endogenous dsRNA from dying cells and double-stranded RNA from viruses [12,13]. Expression of TLR3 has been observed in endosomal compartments, such as dendritic cells and macrophages, or on the surfaces of cell [14]. Binding to TLR3 and its ligand results in conformational changes of TLR3 cytoplasmic tail, which is followed by accumulation of TIR domain containing adaptor inducing IFN-β (TRIF), and subsequently activation of the mitogen activated protein (MAP) kinase pathway, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) family of transcription factors, and the IFN regulatory factor (IRF) family of transcription factors, which then result in interferon (IFN) and cytokines of inflammatory production. And also, studies have demonstrated that the initiation of

* Corresponding author.
E-mail address: tanying252000@126.com (Y. Qin).

http://dx.doi.org/10.1016/j.biopha.2016.02.037
0753-3322/

macrophage inflammatory processes is mainly regulated by signaling proteins of MAPK and NF-κB [15–18].

However, it is still unclear whether BMSC could perform its effects of regulating the activation of TLR3 to improve inflammation response via IFNs, MAPK and NF-κB signaling pathway in LPS-induced lung injury. We are aimed to study the potential efficacy of transplanted bone marrow-derived mesenchymal stem cells in treating and repairing the ALI in animal models.

2. Mathods and materials

2.1. Animals

60 male Sprague-Dawley rats, weighing 180–200 g, were obtained from Animal Center, Tianjin, China, for the experiments. All protocols were approved by the Animal Care Committees of Tianjin University. All animal studies were conducted according to the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences [19]. Rats were randomized divided to five groups: control group (Con), LPS-induced ALI group (LPS) with 80 μg/kg lipopolysaccharide (Sigma, USA) by intraperitoneal injection, BMSC transplantation groups (LPSB) with a 3×10^7/kg BMSC transfusion dissolved in 0.5 ml normal saline via tail vein injection, which were subsequently allocated into 3 subgroups after 12 h, 24 h and 48 h of BMSC injection and chose the most effective subgroup for the following studies.

2.2. Cells and treatment

BMSC were separated from rat bone marrow as a described protocol [20]. NR8383 cell line were purchased from ATCC and cultured in Hans F–12 K medium, containing 20% heat-inactivated fetal bovine serum and 1% penicillin–streptomycin (Gibco). The medium was changed routinely. BMSC and NR8383 were co-cultured at different ratios.

2.3. Apoptosis analysis

LPS-induced apoptosis in NR8383 co-cultured with BMSCs was measured by a flow cytometer with Annexin V-FITC/PI assay. DNA damage was investigated with TdT-mediated dUTP Nick-End Labeling (TUNEL) staining in dying cells [21].

2.4. ELISA measurement

After the last administration and extracting the blood, inflammatory cytokines were investigated using ELISA kits according to the manufacturer's instructions (R&D system, USA).

2.5. Histological examination

The collections of lung samples were subjected to hematoxylin-eosin (H&E) staining and were examined for tissues injury by light microscopy. Immunofluorescence analysis of samples was performed to evaluate the levels of LPS-induced lung injury. Briefly, samples were fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4). After dehydration, thin sections were strictly evaluated under light microscopy. The H&E staining sections were performed by Heilongjiang biotechnology, Co., Ltd. (Heilongjiang, China). Samples also were subjected to immunohistochemical staining according to R&D introduction and performed by Heilongjiang biotechnology, Co., Ltd. (Heilongjiang, China).

2.6. Transmission electron microscope observation

Apical tissues were cut into small pieces, fixed in 2.5% glutaraldehyde and osmium tetroxide. Tissues were rinsed with PBS, and embedded after dehydration of ethonal and acetone. Samples were cut into thin slices with uranium acetate-lead citrate double staining for observation.

2.7. Western blot analysis

Tissues were lysed in ice-cold RIPA lysis-buffer. Lysates were subjected to centrifugation (10,000 g) at 4 °C for 20 min and the supernatants were used for quantification of the total protein concentration by using an BCA Protein Assay Kit (Beyotime Biotechnology, Haimen, China) according to the manufacturer's protocol. Same amount of total protein were clapped into10% or 12% SDS-PAGE followed by immunoblotting using the following antibodies displayed in Table 1. Western blot bands were observed using GE Healthcare ECL Western Blotting Analysis System and exposed to x-ray film of Kodak.

2.8. Quantitative real-time PCR (q-PCR)

Total RNA was isolated from tissue using the total RNA isolation system (Roboklon, Berlin, Germany) kits. Briefly, 2 μg of total RNA was reverse transcribed using the M-MLV-RT system (Promega). The system was performed at 42 °C for 1 h and terminated by deactivation of the enzyme at 70 °C for 10 min. PCR were administrated using SYBR Green kits (Bio-Rad) in ABI PRISM 7900HT detection systems (Applied Biosystems). Invitrogen Corporation produced all sequences of primers (Table 2) for qPCR. The thermal cycling conditions were as follows: pre-denaturation at 95 °C for 5 min, denaturation at 95 °C for 10 s, annealing at 58 °C for 15 s, and extension at 72 °C for 20 s. Gene expression levels were standardized to housekeeping genes (GAPDH) and expressed as a fold of control.

Table 1
Primary antibodies used in the study.

Primary antibodies	Dilution ratio	Corporation
TLR3	1:1000	Abcam
TRIF	1:1000	Abcam
TRAF3	1:1000	Abcam
IRF3	1:1000	Cell Signaling Technology
ISRE3	1:500	Cell Signaling Technology
IFN-β	1:500	Santa cruz
RIP1	1:1000	Cell Signaling Technology
TAK1	1:1000	Santa cruz
TAB1	1:500	Santa cruz
p-JNK	1:1000	Cell Signaling Technology
JNK	1:1000	Cell Signaling Technology
p-ERK	1:1000	Cell Signaling Technology
ERK	1:1000	Cell Signaling Technology
p-P38	1:1000	Cell Signaling Technology
P38	1:1000	Cell Signaling Technology
AFT2	1:500	Santa cruz
c-Jun	1:1000	Abcam
API	1:500	Santa cruz
NEMO	1:500	Santa cruz
IKK-α	1:1000	Cell Signaling Technology
IκB-α	1:1000	Cell Signaling Technology
p-NF-κB	1:1000	Cell Signaling Technology
NF-κB	1:1000	Cell Signaling Technology
IL-1β	1:1000	Abcam
IL-18	1:1000	Abcam
TNF-α	1:500	Abcam
GAPDH	1:1000	Abcam