JOURNAL OF SURGICAL RESEARCH 201 (2016) 356–363

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Relationship between mammalian target of rapamycin and autophagy in lipopolysaccharide-induced lung injury

*Ye Gao, MD, Ni Wang, MD, Lidan Liu, MD, PhD, Yiming Liu, MD, and Jin Zhang, PhD**

Department of Anesthesiology, Shengjing Affiliated Hospital, China Medical University, Shenyang, Liaoning, P.R.C.

ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form
11 November 2015
Accepted 12 November 2015
Available online 30 November 2015

Keywords:
Acute lung injury
Lipopolysaccharide
Autophagy
mTOR

ABSTRACT

Background: To investigate the relationship between the mammalian target of rapamycin (mTOR) signaling pathway and autophagy in lung tissue cells in lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice.

Materials and methods: Thirty-two male C57BL/6 mice were randomly assigned to four different treatment groups: control (C), LPS (L), rapamycin (R), and LPS + rapamycin (LR). Optical and electron microscopy were used to detect lung injury and autophagy. Tumor necrosis factor alpha, interleukin 6, and p62 in lung tissues and IgM concentrations and total protein in bronchoalveolar lavage fluid were evaluated using enzyme-linked immunosorbent assay. LC3II, LC3I, ribosomal protein p70S6 kinase1 (p70S6K1), and phosphorylation of ribosomal protein p70S6 kinase1(P-p70S6K1) in lung tissues were measured by Western blot.

Results: The L group had an increased ALI index ($P < 0.05$) compared with the C group, but there were no differences between the L and the LR groups or the C and R groups (all $P > 0.05$). According to the index of autophagy, the L, the R and C groups, the level of autophagy were in descending order ($P < 0.05$), there were no differences between the L and the LR groups ($P > 0.05$). The expression of P-p70S6K1 declined after rapamycin treatment, showing that the signaling pathway of mTOR is inhibited by rapamycin.

Conclusions: LPS could trigger the mTOR signaling pathway and autophagy of lung tissue cells in LPS-induced ALI in mice; The mTOR signaling pathway did not play a major role in lung injury and autophagy of lung tissue cells induced by LPS.

1. Introduction

The acute lung injury (ALI) and acute respiratory distress syndrome are common conditions that have few effective therapeutic approaches and high mortality rates (30%–40%) in clinical works [1,2]; thus, development of new therapeutic methods for treating ALI and related disorders are currently being pursued. The lipopolysaccharide (LPS), which is the component of cell wall of gram-negative bacteria, could induce the ALI [3,4]. Autophagy, existing in eukaryotes, could degrade the damaged or unwanted protein or organelle and maintain cellular homeostasis by recycling and reusing, playing a vital role in cell survival [5]. The autophagy can be adjusted by many cell signals, such as inflammation,

* *Corresponding author*. Department of Anesthesiology, Shengjing Affiliated Hospital, China Medical University, No. 36, SanHao Street, Shenyang, P.R.C. Tel.: 008618940259646.

E-mail addresses: zhangj_sj@163.com, zhangj_1@sj-hospital.org (J. Zhang).

0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2015.11.018

oxidative stress, endoplasmic reticulum stress and other stress responses, the absence of insulin, nutritional deficiencies, hypoxia and stimulation of other growth factors, and so forth [5,6]. In the mammalian target of rapamycin (mTOR) pathway, the mTOR is the mTOR, which can integrate a variety of extracellular signals, participate multiple signaling pathways in the body, and affect the transcription and protein synthesis, which has an important contact with cell autophagy and growth [7,8]. The p70S6K1 is a key protein downstream of the mTOR pathway and its phosphorylated state, the P-p70S6K1 is an indicator of mTOR activity [9,10].

Although the mTOR pathway activation can affect autophagy under physiological conditions [11], the relationship between the signaling pathway of mTOR and autophagy in ALI induced by LPS is as yet unclear, this study used the LPS-induced ALI mice as a model, applied the mTOR inhibitor rapamycin, and evaluated ALI by measuring tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), total protein, and the concentration of IgM in bronchoalveolar lavage (BAL) fluid [12]. We also assessed the autophagy via the ratio of LC3II and LC3I (LC3II/I), counted the mean number of autophagosomes in every electron microscopy high-magnification field, and evaluated the level of p62 [13–15], to explore the relationship between the signaling pathway of mTOR and autophagy of the lung tissue cells in LPS-induced ALI in mice and further lay the foundation for future development of the effective therapeutic approaches of ALI.

2. Materials and methods

2.1. Reagents

Rabbit anti-LC3B antibody, rabbit anti-p70S6K1 antibody, rabbit anti-phospho-p70S6K1 antibody, goat anti-rabbit IgG antibody, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). LPS was obtained from Sigma (St. Louis, MO). The enzyme-linked immunosorbent assay (ELISA) kits to test TNF-α, IL-6, p62, and total protein and IgM concentrations in BAL fluid were purchased from the company of BoYangShengWu (China).

2.2. Animals and treatment

Animals were obtained from the Laboratory Animal Center at Shengjing Hospital of China Medical University, Shenyang, China. The healthy male C57BL/6 mice (25–28g, 8–10 wk old) were used. All animals were fed at a constant temperature with access to food and water. All the animal procedures were authorized by the Shengjing Hospital of China Medical University, and all experiments were performed according to the National Institutes of Health's Guidelines for the Care and Use of Laboratory Animals.

Thirty-two male C57BL/6 mice were randomly assigned to the following four groups, with eight mice per group: They were the control (C), LPS (L), rapamycin(R), and LPS + rapamycin (LR) groups. Mice in the LR and R groups were administered intraperitoneal injections of rapamycin (4 mg/kg) every 24 h for consecutive 5 d, after which the LR group received LPS (30 mg/kg), and the R group was given the same dose of normal saline (NS; 0.015 mL/g) 1 h after the last injection of the rapamycin. Mice in the L and C groups were pretreated with NS for consecutive 5 d, then, the L group was given LPS (30 mg/kg), and the C group received the same dose of NS (0.015 mL/g). Mice were anesthetized with an intraperitoneal injection of 15% urethane (0.8 mL/kg).

After 8 h of LPS injection, we completed the sample collection immediately. The TNF-a, IL-6, p62, and the total protein and IgM concentrations in BAL fluid were measured by ELISA. The LC3II, LC3I, P-p70S6K1, and p70S6K1 in lung tissues were assessed by Western blot. In addition, we used the light and electron microscopy to evaluate the degree of lung injury and the level of autophagy.

2.3. Sample collection

Mice were euthanized 8 h after the LPS injection. An incision was made in the trachea into which a syringe needle was placed and 1-mL NS was injected into the lung tissue. Lungs were lightly massaged, and the BAL fluid was recovered. Then centrifuged (3000 r/min) the BAL fluid for 5 min, stored the supernatants at −80°C. After BAL fluid collection, the lung tissues were removed from animals and stored at −80°C for detection; Approximately 1 mm^3 of lung tissue was placed in 2.5% glutaraldehyde for electron microscopy observation; About 4 mm^3 of lung tissue was stored in 4% paraformaldehyde for optical microscopic assessment.

2.4. Optical microscopy analysis

Lung tissue samples were sectioned into 5-μm slices, which were assessed under a microscope at 400 × magnification. A specialist observed and evaluated the slices for pathologic changes to lung tissues.

2.5. Transmission electron microscopy analysis

After mice were euthanized, we took the apex of lung organization to make the samples. A sample from each mouse was collected for electron microscopy and cut into 1-μm slices. Five slices were randomly selected for observation. A blinding method was applied by the expert at 20,000 × magnification (JEM-1200EX transmission electron microscope), and five fields from each slice were randomly chosen. The number of autophagosomes observed in each field was noted, and the total number of autophagosomes was calculated per sample.

2.6. Enzyme-linked immunosorbent assay

ELISA was performed in accordance with the manufacturer's instructions. After centrifugation at 3000 rpm at 4°C, TNF-α and IL-6 levels in lung tissues were measured using ELISA kits; total protein levels and the concentration of IgM in BAL fluid were also evaluated by the detection kits.

2.7. Western blot analysis

Total protein was extracted from the lung tissues, which used the whole cell lysis buffer. Protein samples were resolved on the sodium dodecyl sulfate polyacrylamide electrophoresis gels, after which proteins were transferred to polyvinylidene