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RNase attenuates acute lung injury induced by ischemia–reperfusion in mice☆

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ABSTRACT

Treatment with ribonuclease (RNase) reportedly protects the heart after myocardial ischemia–reperfusion (I/R), but its potential effect on lung I/R injury (LIRI) is unknown. Thus, we aim to explore whether RNase treatment would relieve LIRI. Thirty-six C57BL/6J adult male wild-type mice were evenly divided into I/R + RNase (I/R + R) group, I/R group, and sham group. Lung I/R was induced by left pulmonary hilum occlusion for 1 h and reperfusion for 2 h. All mice were treated with RNase or same dosage of normal saline in advance. After I/R, blood and lung tissues were collected for analysis. The results showed that lung injury scores, wet/dry ratio, expressions of inflammatory cytokines, pulmonary apoptosis, and levels of serum extracellular RNA (exRNA), including microRNAs, were markedly elevated after I/R. However, RNase treatment significantly attenuated cytokine production in both lung tissue and serum and also suppressed pulmonary apoptosis as reflected by TUNEL staining and activated caspase-3. In addition, total serum exRNA levels in the I/R + R group had a downward trend versus the I/R group. In conclusion, the increase of circulating exRNA levels contributed to LIRI in adult mice, which could be relieved by injection of RNase during perioperative window. The potential mechanism is the decrease of serum exRNA level and the suppression of pulmonary inflammation and apoptosis.

1. Introduction

Lung ischemia–reperfusion injury (LIRI) reportedly occurs after 20% of lung transplantations [1]. The process of LIRI involves the generation of diverse inflammation-inducing substances and activation of pulmonary alveolar macrophages [2]. However, the concrete cellular and molecular signaling pathways involved in the pathologic process are still poorly studied.

Increasing evidence suggests that extracellular RNAs (exRNAs), including microRNAs (miRNAs), may have different effects in this process, such as cell differentiation, inflammatory response, and tissue damage or repair [3–4]. Different types of miRNAs released from necrotic cells, as endogenous ligands of toll-like receptors (TLRs), can stimulate the TLRs and thereby impair the vascular endothelium function [5–8]. As reported, miRNA155 could regulate the effect of progesterone on TLRs-mediated immune response, and the decrease of miRNA155 level helped to inhibit TLR-induced interleukin (IL)-6 and interferon (IFN)-β expressions [9]. Moreover, miRNA155 played positive regulatory roles in TLR3/TLR4 signaling pathways [10]. In addition, it was reported that miRNA155 and miRNA451 were highly presented in the lung [11]. Nevertheless, serum levels of exRNAs, including miRNAs and their possible associations with LIRI, have not been well characterized.

As the counter part of exRNA, RNase has been found as a potential target of novel anticancer drugs [12–13]. It is demonstrated that increased RNase1 level can decrease myocardial infarct (MI) size and preserve left ventricular systolic function, so as to improve the outcome of cardiac surgery [14]. A mouse myocardial I/R injury model showed that administration of RNase significantly reduced MI and that this process might involve inflammation and apoptosis mechanisms [15]. Meanwhile, RNase1 helped to relieve atherosclerotic plaque formation in mice [5]. In addition, a rat cerebral I/R injury model proved that RNase had a neuroprotective effect [16]. Thus, investigating the potential role of RNase treatment in LIRI is of great interest.

Therefore, we purposed to assess if RNase treatment would alleviate LIRI. We tested the changes of cytokine responses and cell apoptosis in

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vivo and measured serum levels of exRNAs including miRNAs after lung I/R. We also explored the mediators and molecular mechanisms that would be probably related to the occurrence and development of LIRI.

2. Materials and methods

2.1. Animals

Age- and weight-matched C57BL/6J wild-type (WT) mice purchased from Sichuan University (Chengdu, China) were raised in the laboratory without restriction to water or food. In order to minimize the suffering of animals, we got our protocol authorized by the Animal Ethics Committee at West China Hospital of Sichuan University, and implemented the experimental procedures under the supervision of this Committee.

2.2. Experimental protocol and lung I/R model

Thirty-six WT mice were randomly and evenly allocated to three groups: I/R + RNase (I/R + R) group, I/R group and sham group, which were treated with RNase plus I/R, saline plus I/R, and only saline, respectively. Each mouse was treated with 3 doses of RNase A (Invitrogen, USA): 30 min prior to pulmonary hilum occlusion (500 μg/100 μl, subcutaneous (SC)), right before that (200 μg/200 μl, intraperitoneal (IP)), and 2 h after that (500 μg/100 μl, SC) [15,17]. The surgical procedures of I/R can be found in a publication [18]. In brief, under anesthesia by mixture of ketamine and xylazine (IP), the mice were intubated, ventilated, and then kept at a body temperature of 36.5 °C to 37.5 °C. After that, a left 4th intercostal space thoracotomy was conducted. Then 100 U/kg heparin (iv) was injected. After 5 min, a noninvasive microvascular clip was used to occlude the left pulmonary hilum at the end of the breath for 1 h. Then we removed the clip, and the lung was reperfused for another 2 h. The sham mice underwent the same procedure, except for occluding the hilum of the left lung.

2.3. Arterial blood gas (ABG) analysis

Mice were ventilated with 40% oxygen after anesthesia. At the end of the 3 h I/R, 0.3 ml of blood was collected from the left ventricle, then ABG levels were immediately detected by blood gas analyzer.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The serum levels of nuclear factor-κB (NF-κB), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) were assessed by ELISA based on the manufacturer's instructions (R&D, USA).

2.5. Total serum RNA concentration

According to a reported method [17], the whole serum RNA was abstracted from 100 μl of serum from each group by utilizing a specialized RNA extraction kit (Promega, USA), then was detected on a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Strict RNase-free conditions were guaranteed for these trials.

2.6. Wet/dry (w/d) weight ratio

For detection of w/d, after 3 h of lung I/R, the left lung was removed and divided into two parts. Then a part of lung was immediately weighed (wet weight) and put in an oven at 70 °C for 72 h to achieve a stable dry weight. Finally, the w/d weight ratio was calculated.

2.7. Histological analysis

After fixation with 4% formaldehyde for 24 h, a part of each lower left lobe was embedded in paraffin wax, then stained with hematoxylin and eosin. With a blinded manner, a pathologist completed the histological analysis following a classic lung injury score standard [19]. The other part of each lower lobe was snap-frozen in liquid nitrogen then stored at −80 °C for the extraction of protein and total RNA.

2.8. Apoptosis analysis

The apoptosis assays including terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and activated caspase-3 were conducted according to the manufacturer's instructions (Roche, USA).

2.9. Western blot analysis

Western blot analysis was performed as described [20]. Proteins (30 μg/lane) were separated via 10% or 12% SDS–PAGE and transferred onto polyvinylidene fluoride membranes (Millipore). The membranes were treated with appropriate primary antibody (1:1000, Cell Signaling Technology, USA) against NF-κBp65 or activated caspase-3 overnight. Then the blots were incubated with anti-mouse immunoglobulin G (diluted 1:5000) for 1 h. The blot detection and intensity ratio calculation were conducted with an enhanced chemiluminescence system, with β-actin as a loading control.

2.10. Quantitative polymerase chain reaction (qPCR) analysis

qPCR was conducted on an Eppendorf PCR system. Data normalization was based on the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or U6 in each sample. The sequences for the primers in mice are as follows (from forward to reverse): 5′-GCAGCTCCTGAACCAGGGTG-3′ and 5′-CATTGGGAAGCCCCGAGGTC-3′ (NF-κBp65); 5′-CTGGGACAGTGACCTGG ACT-3′ and 5′-GCACCTCAGGGAAG AGTCTG-3′ (TNF-α); 5′-AGTTGCC TTCTTGGGACTGA-3′ and 5′-TCCACG ATTTCCCAGAGAAC-3′ (IL-6); 5′-AACTTTGGCATTGTGGAAGG-3′ and 5′-GG ATGCAGGGATGATGTTCT-3′ (GAPDH); 5′-GCGCCGCTTAATGCTAATCGTG-3′ and 5′-TGCAGGGTCCGAGG TAT-3′ (miR155); 5′-TCCGATTGAGTCATTACCAT-3′ and 5′-GTGCAGGGTCC GAGGT-3′ (miR451); 5′-CTCGCTTCGGCAGCACA −3′ and 5′-AACGCTTCAC GAATTTGCGT-3′ (U6).

2.11. Statistical analysis

Statistical analysis was calculated on IBM SPSS 19.0 software. All data were expressed as mean ± standard deviation. In order to compare with the data between groups, one-way analysis of variance (ANOVA) and Bonferroni post hoc test was performed. $P < 0.05$ was considered as significant.

3. Results

3.1. RNase treatment attenuated LIRI

After lung I/R, the lung histological analysis showed obvious interstitial edema, inflammatory cell infiltration, erythrocyte exudation, and inter-alveolar septum thickening (Fig. 1A), but RNase treatment significantly alleviated alveolar damage, neutrophil infiltration (Fig. 1A), and lung injury scores (Fig. 1B). Beyond that, I/R led to evident rise of W/D ratio, but this rise was attenuated by the RNase treatment (Fig. 1C). I/R also resulted in significant decrease of the P_aO_2/FiO_2 ratio, which was raised by RNase treatment (Fig. 1D). Thus, RNase administration could effectively reduce LIRI.