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ORIGINAL ARTICLE

Effect of Mild Hypothermia on the Expression of Toll-like Receptor 2 in Lung Tissues with Experimental Acute Lung Injury

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Objective and Design Our study aimed to determine the effect of mild hypothermia (MHT) on the expression of toll-like receptor 2 (TLR2) in lung tissue with acute lung injury. The animals were randomly divided into control, model and mild hypothermia groups.

Methods A total of 40 rats was used in the study. Acute lung injury was induced by lipopolysaccharide and MHT was maintained at 32.5∼33.0 °C using body surface ice-bag placement combined with animal thermostat system. The ratio of PaO_2/FiO_2 was recorded. The mRNA and protein expressions of TLR2 were measured by real-time polymerase chain reaction and western blotting respectively. Moreover, enzyme linked immunosorbent assay were used for the quantification of TNF-α.

Results The ratio of PaO_2/FiO_2 was increased by MHT. TLR2 and TNF-α were increased in the rat lung 1 h and 8 h in the rats with acute lung injury while they were significantly decreased by MHT. Histological examination revealed that MHT alleviated the degree of inflammation.

Conclusion Our study suggested that MHT might improve the lung function by inhibiting the inflammation via down-regulating the expressions of TLR2 in the acute injury lung tissues.

Keywords Mild hypothermia • Lipopolysaccharide • Acute lung injury • Toll-like receptor 2 • TNF-α

Introduction

Acute lung injury (ALI), characterised by refractory hypoxaemia, non-cardiogenic pulmonary oedema, low lung compliance and widespread capillary leakage, is a clinical syndrome with acute diffuse heterogeneous structural damage and decline of pulmonary functional units [1]. ALI is caused by any stimulus of local or systemic inflammation. Our previous research, and that of other groups, has demonstrated that MHT could decrease lung injury of ALI and restore the respiratory function via alleviating inflammation, reducing oxidative stress, lowering oxygen consumption, protecting the cell and delaying the decline of surfactant protein A content in pulmonary alveolus [2–5]. However, its mechanism has not been completely understood.

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Toll-like receptors (TLRs), especially TLR2, play a fundamental role in the induction and maintenance of inflammation via pathogen recognition and activation of innate immunity in lung tissue. The ligation of TLRs by pathogen-associated molecular patterns induces the signalling of the subsequent inflammatory response [6] while TLR2 seems to be tightly associated with ALI [7]. TLR2 is mainly expressed by myeloid cells and is also found in the epithelial cells from lung tissue such as bronchial and alveolar epithelial cells which participate in the lung immune response by producing inflammatory mediators and chemokines through the activation of TLR2 [8]. The expression of TLR2 in the endothelium is also sensitive to mechanical stress [9]. When the gene of TLR2 is knocked out, the mouse is highly susceptible to infection [10]. TLR2 elicits inflammatory mediator expression via producing some pro-inflammatory cytokines and chemokines to increase inflammatory cells influx and induce pulmonary oedema [11–13]. However, there have been no reports on whether MHT could alleviate ALI via down-regulating the expression of TLR2.

In the present study, we used lipopolysaccharide (LPS) to induce the experimental ALI and then used MHT to treat the ALI rats; finally the inflammation in the lung tissue with ALI was determined.

Materials and Methods

Experimental Animals

Forty healthy adult male Wistar rats (310-370 g) were included in this study. All experimental procedures and protocols were reviewed and approved by our local Animal Care and Use Committee according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (revised in 1996). All the rats were randomly divided into five groups (n = 8), the control 1 h and 8 h groups, the model 1 h and 8 h groups and the MHT 8 groups.

ALI model and MHT treatment

The rats were anesthetised with intraperitoneal injection of pentobarbital (40 mg/kg, Sigma). After anesthetisation, the right external jugular vein (for catheterisation and infusion) and the left common carotid artery (for blood collection and blood gas measurement) were separated; meanwhile, the mechanical ventilation was performed. The respiratory parameters were: respiratory rate of 80 times/min, tidal volume (V_T) of 20 ml/kg and inspiratory-to-expiratory ratio of 1: 2. For the rats in the control groups, the rats were treated by saline; for the model 1 h and 8 h groups, the ALI models were induced by injection of 200 μg/ml endotoxin lipopolysaccharide (LPS, E. coli, O55: B5, Sigma Chemical Co. L2880, USA) with 0.5 ml/kg of body weight; for the MHT 8 h group, 1 h after the treatment of LPS, the core body temperature was maintained at 32.5∼33.0 °C using body surface ice-bag placement combined with animal thermostat system and 7 h of continuous observation was performed thereafter. The animal core body temperature was continuously monitored, and the arterial blood gas analysis was performed discontinuously. If the oxygenation index (PaO_2/FiO_2) was less than 300 mmHg, it was considered as the establishment of ALI [14].

Sample Collection

The values of blood gas analyses were recorded on the baseline, 1 h and 8 h after the treatment of saline or LPS. All the animals were sacrificed at the time point. The right pulmonary lobe was taken out and stored at -80 °C for RNA and protein extraction while the left lung tissues were taken out for histological examination, fixed with 4% paraformaldehyde solution, embedded in paraffin, cut into sections, stained with haematoxylin-eosin (Sigma) and observed under optical microscope.

Histological Analysis

The lung injury was evaluated by Lung injury scores as described previously [15]. In brief, no injury = score of 0; injury in 25% of the field = score of 1; injury in 50% of the field = score of 2; injury in 75% of the field = score of 3; and injury throughout the field = score of 4. Ten random microscopic fields from each slide were analysed. The average score of the 10 slides was used to assess the severity of lung injury.

Quantitative Reverse Transcription-polymerase Chain Reaction

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used for the detection of the expression levels of TLR2. The total RNA of lung tissue was extracted using the spin column total RNA extraction kit (TIANGEN Biotech, Beijing, China). The purity and concentration of RNA were determined. Then the cDNA was synthesised using the ReverTraAce reverse transcription kit (Fermentas China Co. Ltd.) according to the manufacturer's instructions. For TLR2 primers, 5′-aaactgtgttcgtgctttctga-3′ (Sense) and 5′-ctttcttctcaatgggttccag-3′ (Antisense), and for β-action primers; 5′-cacccgcgagtacaaccttc-3′ (Sense) and 5′-cccatacccaccatcacacc-3′ (Antisense). The amplification was performed using the real-time fluorescence quantitative PCR kit with the fluorescence quantitative PCR instrument (Fermentas). The conditions were pre-denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 1 min and 55 °C for 1 min followed by a dissociation stage at 95 °C for 1 min, 55 °C for 30 sec and 95 °C for 30 sec. All samples were analysed in triplicates in three independent experiments. Reactions without cDNA were used as no template control and no RT controls were also set up to rule out genomic DNA contamination. mRNA expression was determined using the comparative Ct method.

Western Blotting

The concentration of protein of tissue homogenate was determined using BCA method. Then western blotting was performed. In brief, the protein samples underwent electrophoresis, transfer and blocking, followed by incubation with rabbit anti-TLR2 polyclonal antibody and rabbit