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Ligustrazine effect on lipopolysaccharide-induced pulmonary damage in rats☆

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ARTICLE INFO

Article history:
Accepted 3 February 2015

Keywords:
Ligustrazine
LPS
Pulmonary damage
Lung exudative lesion
Vascular endothelial particles

ABSTRACT

We investigated the effectiveness of ligustrazine (tetramethylpyrazine, TMP) in alleviating pulmonary damage induced by lipopolysaccharide (LPS). Twenty-four healthy male Sprague-Dawley rats were randomly divided into three groups: the blank group, LPS group, and TMP treatment group (TMP group). The LPS group was intraperitoneally injected with LPS (20 mg/kg), and the TMP group was intraperitoneally injected with LPS (20 mg/kg) and ligustrazine (80 mg/kg). Blood gas analysis, hematoxylin–eosin staining dye extravasation and diffuse alveolar damage (DAD) score, the wet/dry lung tissue (W/D) ratios, the expression of CD31+ vascular endothelial microparticles (EMPs), tumor necrosis factor alpha (TNF-α) levels, and the protein expression of Rho-associated coiled-coil-forming protein kinase (ROCK) II and Toll-like receptor 4 (TLR4) were analyzed. Compared with the blank group, the arterial partial pressure of oxygen (PaO_2) declined in both 1 and 4 h ($P < 0.01$), the W/D ratio and DAD score increased ($P < 0.01$), and the TNF-α levels in serum, CD31+ EMPs, and protein expression of ROCK II and TLR4 were significantly increased ($P < 0.01$) in the LPS group. Compared with the LPS group, PaO_2 significantly increased in the TMP group at 4 h ($P < 0.05$), while the W/D ratio and DAD score were significantly decreased in the TMP group ($P < 0.01$). TNF-α levels, CD31+ EMPs, and protein expression of ROCK II and TLR4 were significantly decreased in the TMP group compared with the LPS group ($P < 0.01$). This study demonstrated that TMP can alleviate LPS-induced pulmonary damage by attenuating pulmonary vascular permeability and CD31+ EMP levels in the plasma, reducing the release of the inflammatory mediator TNF-α and inhibiting the protein expression of ROCK II and TLR4.

☆ *Funding statement*: This study was funded by the National Natural Science Foundation of China (No. 81000024) and the Shanghai Municipal Health Bureau (No. 2012J011A). We confirm that Dr. Huiqi Wang and Dr. Yuanzhuo Chen contributed equally to this work. All the authors have participated actively in the study, and they have seen and approved the submitted manuscript.
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http://dx.doi.org/10.1016/j.burns.2015.02.010
0305-4179/

1. Introduction

Lipopolysaccharide (LPS)-induced pulmonary damage, characterized by dyspnea and hypoxemia, is the pulmonary manifestation of an acute systemic inflammatory process and an important cause of mortality in critically ill patients [1]. However, its pathogenesis has not been fully elucidated due to its complexity. Furthermore, LPS is one of the most common factors that induces pulmonary damage [2].

One feature of pulmonary damage induced by LPS is increased vascular permeability due to pulmonary microvascular endothelial damage. This is followed by enhancement of water content in the tissue space, especially in the lung interstitium and pulmonary alveoli. This results in pulmonary edema, the release of inflammatory cytokines, formation of a transparent film from an abundance of protein exudate, and pulmonary interstitial fibrosis. Multiple organ dysfunction syndrome (MODS) occurs commonly in the late stages of the disease [3]. Endothelial cells are the important targets injured by inflammatory mediators. Inflammatory factors flow with blood to distant sites to stimulate and injure endothelial cells, causing the permeability of pulmonary microvascular endothelial cells and the release of endothelial microparticles (EMPs) to increase. In the present study, CD31+ EMPs are regarded as a marker of pulmonary microvascular endothelial damage. In lung injury, the arterial partial pressure of oxygen (PaO_2), lung wet-to-dry weight (W/D) ratio, and diffuse alveolar damage (DAD) can be used as indicators of lung injury [4,5]. Tumor necrosis factor alpha (TNF-α) is one of the cytokines that can induce pulmonary permeability and edema [6]. The protein expression of Rho-associated coiled-coil-forming protein kinase (ROCK) II and TLR4 was analyzed to assess the function of TMP and explore the possible mechanisms.

The active ingredients of ligustrazine, which is named tetramethylpyrazine (TMP) according to its chemical structure, are extracted from *Ligusticum wallichii* used in traditional Chinese medicine. Pharmacological studies show that TMP has immune-regulating functions: inhibiting some cytokine expressions, suppressing inflammation, improving blood rheology, protecting vascular endothelial cells, inhibiting platelet aggregation, reducing fibrosis, and other pharmacological activities [7,8]. It has been widely used to treat cardiovascular diseases but it has rarely been applied to treat acute and chronic respiratory failure. Related research is limited and the relevant mechanism has not been reported. This study aimed to investigate whether TMP can ameliorate LPS-induced pulmonary damage and to explore the possible mechanisms responsible for the effects of TMP on LPS-induced pulmonary damage.

2. Materials and methods

2.1. Ethics statement

All animal-related procedures were approved by the Animal Care and Use Committee of The Tenth People's Hospital of Shanghai (permit number: 2011-RES1). This study was also approved by the Science and Technology Commission of Shanghai Municipality (ID: SYXK 2007-0006). The rats were kept at 18–26 °C on a 12-h light/dark cycle with free access to water and standard rat chow. They were allowed to acclimatize for a minimum of 1 week. The environment was maintained at a relative humidity of 30–70%.

2.2. Animal models

Sepsis-induced pulmonary damage rat models were made by intraperitoneal endotoxin injection. All rats (230 ± 30 g) were randomly divided into three groups: the blank group ($n = 8$), the LPS group ($n = 8$), and the TMP group ($n = 8$). The LPS and TMP experimental groups were intraperitoneally injected with LPS (20 mg/kg), and the blank group was injected with an equal amount of saline solution. After 30 min and sepsis preparation, the TMP group was intraperitoneally injected with TMP (80 mg/kg), and the blank and LPS groups received the same volume of normal saline. Thereafter, 0.5 ml of blood was collected from the internal carotid artery after 1 and 4 h, respectively. PaO_2, lung W/D ratios, and DAD scores were determined to assess whether the pulmonary damage rat model is well established.

2.3. Experimental design

All animals were randomly divided into three groups as described previously ($n = 8$, in each group): the blank group, the LPS group, and the TMP group. The LPS and TMP groups received an intraperitoneal injection of LPS. Ligustrazine was administered intraperitoneally to rats of the TMP group. Eight normal rats acted as controls. The rats in each group were monitored every hour. These rats were executed by exsanguination from the internal carotid artery at 4 h under anesthesia. We harvested lungs of all live rats at 4 h and repeated the experiment twice.

2.4. Arterial blood gas analysis

At 1 and 4 h, respectively, we collected blood from the internal carotid artery and administrated arterial blood gas analysis results, including the oxygenation index (OI) calculation.

2.5. Pulmonary function assessment by measuring lung wet/dry ratios and histopathological observation

At 1 and 4 h after sepsis-induced lung injury, the rats were exsanguinated from the internal carotid artery. The two lungs harvested from each animal were separated. The right lung was deposited for subsequent tests (immunohistochemistry staining, etc.). The left lung was homogenized and the homogenate weighed. The homogenate was centrifuged (14,000 × *g*, 10 min) and desiccated in an oven (70 °C for 24 h) to determine the dry weight. The lung wet-to-dry weight ratio (W/D) was computed from lung wet and dry weights.

Formalin-fixed lung tissues were embedded in paraffin, cut into 4-μm-thick sections, and stained with hematoxylin and eosin (HE). The tissues were observed using a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan). Pulmonary damage was scored according to the following DAD criteria [9]: (1) effusion of neutrophils to the alveolar space and the interval:

Please cite this article in press as: Wang H, et al. Ligustrazine effect on lipopolysaccharide-induced pulmonary damage in rats. Burns (2015), http://dx.doi.org/10.1016/j.burns.2015.02.010