Pulmonary Pharmacology & Therapeutics 26 (2013) 661–665

Contents lists available at SciVerse ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

Down-regulated expression of AQP5 on lung in rat DIC model induced by LPS and its effect on the development of pulmonary edema

Yingyu Jin [a,1], Guangzhe Yu [b,1], Peng Peng [a], Yanfen Zhang [a], Xiaomin Xin [a,*]

[a] Department of Laboratory Diagnosis, the First Clinical College of Harbin Medical University, Harbin Heilongjiang, China
[b] Department of Emergency Surgery, the First Clinical College of Harbin Medical University, Harbin Heilongjiang, China

ARTICLE INFO

Article history:
Received 11 October 2012
Received in revised form
13 March 2013
Accepted 18 March 2013

Keywords:
Disseminated intravascular coagulation (DIC)
Lipopolysaccharide (LPS)
Lung edema
Aquaporin 5 (AQP5)

ABSTRACT

Disseminated intravascular coagulation (DIC) is an acquired syndrome characterized by the widespread activation of coagulation, which leads to failure of multiple organs in the body. DIC of rat with lipopolysaccharide (LPS) is associated with subsequent pulmonary edema. Lung tissue is highly water permeable and expresses several aquaporins (AQPs). We therefore explored whether AQP5 involved in the pathogenesis of LPS-induced lung edema. The rats were intravenously infused with LPS (30 mg/kg) for 4 h, 6 h, 8 h, 10 h, and 12 h to induce DIC. Platelets count (PLT), D-Dimer (DD), fibrinogen (FIB), prothrombin time (PT), and activated partial thromboplastin time (APTT) were determined. Real-time quantitative PCR and Western blot were used to analyze the mRNA and protein expression of AQP5. Lung samples were stained with hematoxylin-eosin and lung wet/dry weight (W/D) ratios were measured. Here, we demonstrated that PLT and FIB values were significant decreased, the values for DD, PT, and APTT were marked increased, microthrombus was observed in lung specimens, and simultaneously with the AQP5 showed down-regulated expression following LPS infused from 4 h to 12 h. However, histopathological changes such as pulmonary edema and the increased lung W/D weight ratio were observed after LPS infused from 6 h to 12 h. These results indicated that the decreased expression of AQP5 maybe induce liquid transport obstacles between alveolar and capillary, and provides the report of AQP5 gene regulation, revealing the pathogenesis of pulmonary edema in DIC model of rat.

1. Introduction

Disseminated intravascular coagulation (DIC) is a clinical syndrome with high mortality caused by activation of systemic intravascular coagulation. The basic pathological mechanism of DIC includes the spread of microvascular thrombosis, which prevents adequate blood supply to organs and leads to cell edema, multiple organ failure and death [1,2]. Lung injury is the most common complications of DIC, despite extensive investigations into new strategies for treatment, the morbidity and mortality of DIC-induced lung injury in critically ill patients remains very high [3].

The endotoxin lipopolysaccharide (LPS), the major constituent of the cell wall of Gram-negative bacteria, is known to induce the production of several inflammatory and chemotactic cytokines, which has been thought to be a cause of the widespread cellular activation observed in patients with Gram-negative septic shock [4,5], and intravenous infusion of LPS has gained wide acceptance as an experimental model of DIC [6,7]. LPS triggers the development of DIC via the tissue factor-dependent pathway of coagulation resulting in massive thrombin generation and fibrin polymerization.

Aquaporins (AQPs), the family of water channels, play a fundamental role in transmembrane water movement in microorganisms, plants, and animal tissues. AQPs are required for normal secretory and absorptive functions of many tissues, including the eye, lung, salivary gland, sweat glands, and kidney and provide essential systemic regulation of water homeostasis in whole organisms [8,9]. To date, 13 members are known to belong to this channel family in mammalians, they are designated AQP0-12 [10]. Four aquaporins are expressed in the lung: AQP1 in microvascular endothelia, AQP3 in large airways, AQP4 in large- and small-airway epithelia [11–13], and AQP5 is expressed at high level in type I pneumocytes in the distal lung but moderately in epithelia from upper airway [14], and important for regulating airspace-capillary osmotic water permeability [15]. The cellular colocalization of

* Corresponding author. Laboratory diagnosis, the First Clinical College of Harbin Medical University, 23 Youzheng Street, Harbin Heilongjiang 150001, China. Tel.: +86 45185555415; fax: +86 45153642891.
E-mail address: xinxiaomin0451@yahoo.com.cn (X. Xin).
[1] The authors contributed equally to this work and should be considered as co-first authors.

1094-5539/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.pupt.2013.03.013

AQP1 and AQP5 in the distal lung, the observed decrease in permeability in AQP1 knockout mice, and the permeability measurements of alveolar type I cells support a role for AQP1 and AQP5 in the maintenance of water homeostasis in the lung. The expression of AQPs is altered in various models of lung infection [16], inflammation [17,18], acute and chronic injury [19,20], and fibrosis [21], indicating a role in these pathological conditions.

The recent outbreak of DIC emphasizes the lethal outcomes associated with lung injury. Acute lung injury is a clinical syndrome and characteristic of pulmonary edema, and its serious phase is the acute respiratory distress syndrome. Before, it is generally believed that pulmonary edema is mainly caused by pulmonary microvascular endothelial cells (LMECs) damage, and inadequate understanding the role of AQPs in the pathological conditions in the lung. Therefore, it is important to understand the new pathogenesis of lung injury and find beneficial strategies for its treatment. In the current study, we assessed the expression of AQP5 in the DIC rats resulting from intravascular infused with LPS. This study is the report of the regulation of AQP5 gene expression in a model of DIC and provides the demonstration of the decreased expression of AQP5 maybe induce the development of pulmonary edema.

2. Materials and methods

2.1. Animals

Male Wistar rats, 7–10 weeks old and weighing 180–220 g, were obtained from the Experiment Animals Center of the First Hospital of Harbin Medical University (Harbin, China). The animals were allowed food and tap water ad libitum. All animal experiments were conducted according to the ethical guidelines of the Animal Care and Use Committee at Harbin Medical University.

2.2. LPS-induced DIC in rats

Wistar rats were divided randomly into seven groups: the saline group ($n = 50$), the LPS 4 h group ($n = 10$), LPS 6 h group ($n = 10$), LPS 8 h group ($n = 10$), LPS 10 h group ($n = 10$), and LPS 12 h group ($n = 10$). In the LPS group, rats were continuously infused with LPS (30 mg/kg, dissolved in 10 ml sterile saline; *Escherichia coli* 055: B5 lipopolysaccharide B; Difco Laboratory, USA) via the tail vein for up to 4 h, and the infusion was performed with a pump. In the saline group, rats were received saline instead of LPS in the same manner and served as control.

The rats were anesthetized with sodium pentobarbital (30 mg/kg) once or multiple times to make animals under anesthetic for the duration (up to 12 h) and fixed on a board at an angle of 45° in a supine position. After sterilization, a mid-line incision was performed in the abdomen to isolate the abdominal aorta for blood collection. After surgical preparation, saline and LPS were respectively infused by tail vein.

2.3. Blood coagulation and fibrinolysis activity analysis

Blood were collected from the abdominal aorta of rats under anesthesia into the 4% sodium citrated tubes after LPS or saline infused at 4 h, 6 h, 8 h, 10 h, and 12 h. Rat plasma was prepared by centrifugation at 3000× *g* for 10 min and frozen at −70 °C before use. Plasma PLT, DD, FIB, PT, and APTT were measured by CA-1500 Auto Coagulative Analyzer (Sysmex, Japan).

2.4. Histopathologic examination

After treatment with saline, or with LPS at 4 h, 6 h, 8 h, 10 h, and 12 h, rats were euthanized and half of the right lung were harvested and fixed in 10% formalin buffer for 24 h at 4 °C, embedded in paraffin. A serried of microsections (4 μm) was stained with hematoxylin-eosin (H&E) and observed by light microscopy.

2.5. Wet/dry weight ratios

After treatment with saline or LPS at 4 h, 6 h, 8 h, 10 h, and 12 h, rats were euthanized, half of the right lung was then removed and the wet weight was determined. Lung tissue was placed in an oven at 55 °C for 24 h to obtain the dry weight. The ratio of the wet lung to the dry lung was calculated to assess tissue edema.

2.6. RNA preparation, reverse transcription, and qPCR

Total RNA was extracted from rat lung tissue using Trizol reagent (Invitrogen) based on the manufacturer's protocol. RNA concentrations were determined via spectrophotometry and confirmed with agarose gel electrophoresis. The amount of 1 μg of total RNA per sample was reverse-transcribed into cDNA with a Superscript II Reverse Transcriptase kit (Promega). Real-time quantitative PCR was carried out in the Applied Biosystems PRISM 7300 real-time PCR using Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen). All the real-time qPCR primers used are listed in Table 1.

2.7. Protein preparation and Western blotting

The lung tissues were harvested and frozen in liquid nitrogen before use. Samples were homogenized in cold RIPA lysis buffer (150 mM NaCl, 25 mM Tris–HCl pH 7.6, 1% NP-40, 1% sodium deoxycholate, 1% SDS, and 1% protease inhibitor cocktail) and lysed 30 min on ice.

Total lysate proteins were resolved using 15% SDS-PAGE and transferred to a PVDF-membrane (Millipore, Germany). Membrane was blocked with blocking solution (5% nonfat dry milk in TBS-T) at room temperature for 2 h and then incubated overnight at 4 °C with rabbit anti-rat AQP5 antibody (1: 500, Chemicon). After washing in TBS-T, the membrane was incubated with goat anti-rabbit IgG conjugated to horseradish peroxidase (1: 3000, Pierce) at room temperature for 2 h, and then washed in TBS-T. Immunoblots were visualized on photographic films using Enhanced Chemical Luminescence reagents (Pierce), following the method provided by the supplier. For the loading control, anti-β-actin monoclonal antibody (1: 5000, Sigma) was used.

2.8. Statistical analysis

The analyses were carried out using SPSS 13.0 for Windows software. Data was presented as mean ± standard error, comparison among multiple groups used analysis of variance (ANOVA), and further comparison between the two groups used least significant difference procedure (LSD). A value of $P < 0.05$ was considered statistically significant.

Table 1
Primers used in qPCR analysis.

Primer	Sequence (5′–3′)
AQP-5 F	TGGTGGGTGCCATTGCTG
AQP-5 R	GCCTTGCCTGGTGTTGTG
β-actin F	TGACGTGGACATCCGCAAAG
β-actin R	CTGGAAGGTGGACAGCGAGG