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LPS induces permeability injury in lung microvascular endothelium via AT_1 receptor

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Abstract

Lipopolysaccharide (LPS) is known to stimulate the circulation and local production of angiotensin II (Ang II). To assess whether Ang II plays a role in LPS-induced acute lung injury, rats were injected with LPS, the microvascular endothelial permeability injury was evaluated by histological changes, increased pulmonary wet/dry weight ratio, and pulmonary microvascular protein leak. Besides, increased rat pulmonary microvascular endothelial cell monolayer permeability coefficient (K_f) was measured after treatment with LPS and/or Ang II, respectively. LPS/Ang II, treatment resulted in a significant increase in K_f. Ang II cooperates with LPS to further increase K_f. Hence, LPS increases pulmonary microvascular endothelial permeability both in vitro and in vivo. Local lung Ang II was increased in response to LPS challenge, and elevated Ang II ulteriorly exacerbates LPS-induced endothelium injury. [Sar^1,Ile^8]Ang II, a selective block of Ang II type 1 (AT_1) receptors, eliminated these changes significantly. Our conclusion is that the LPS-induced lung injury may be mediated by the AT_1 receptor.
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Keywords: Lipopolysaccharide; Acute lung injury; AngiotensinII; Ang II type 1 receptor; Rat pulmonary microvascular endothelial cell; Microvascular endothelium permeability; Endothelium injury; Pulmonary edema; pulmonary wet/dry weight ratio; Monolayer permeability coefficient

Angiotensin II[1] is an important molecule controlling blood pressure and volume in the cardiovascular system. Most of the known effects of Ang II seem to be mediated via stimulation of the G protein-coupled AT_1 receptor. Historically, Ang II was only seen as a regulatory hormone that regulates blood pressure, aldosterone release, and sodium reabsorption. Now it has been shown to be involved in the regulation of inflammatory processes [1–6], being functionally associated with important transcription factors, notably nuclear factor NF-κB. In addition, it is generally accepted that locally formed Ang II could activate the cells regulating the expression of many substances, including growth factors, cytokines, chemokines, and adhesion molecules, which are involved in cell growth/apoptosis, fibrosis, and inflammation. Recently, these views have been confirmed with a novel concept: Ang II itself participates in the inflammatory response, acting as a proinflammatory mediator [7].

Acute respiratory distress syndrome (ARDS) is a severe form of lung inflammation most commonly complicating gram-negative septic shock. Endotoxin, the bacterial lipopolysaccharide (LPS), released from the bacterial cell wall is considered to be an important eliciting factor in the development of ARDS, which induces increased pulmonary endothelial permeability that

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[1] *Abbreviations used:* Ang II, angiotensin II; LPS, lipopolysaccharide; ARDS, acute respiratory distress syndrome; ALI, acute lung injury; RPMVEC, rat pulmonary microvascular endothelial cell; AT_1, Ang II type 1; ACE, angiotensin-converting enzyme; EBD, Evans blue dye; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; RAS, renin–angiotensin system; ICAM-1, intercellular adhesion molecule type 1; VCAM-1, vascular cell adhesion molecule type 1; TxA_2, thromboxane A_2.

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causes pulmonary edema and acute lung injury (ALI). However, how the mechanisms by which LPS-induced endothelial barrier dysfunction is caused are not well defined.

It has been found that the renin–angiotensin system is activated during sepsis, causing a rise in circulating renin and Ang II levels. LPS also activates Ang II in local tissues [8,9]. It is well known that the lung endothelium has metabolic functions, including the conversion of Ang I to Ang II by angiotensin-converting enzyme (ACE) [10]. Among the organs examined, the lung contained the highest Ang II activity [11]. On the basis of these observations, we believe that Ang II may play a vital role in LPS-induced ALI.

The objective of the present study was to clarify whether, during an endotoxin challenge, Ang II alters the rat pulmonary microvascular endothelium permeability, and how these responses may be modulated by an AT_1 receptor antagonist in vivo or in vitro.

Materials and methods

Reagents

LPS (*Escherichia coli* 0111:B4), Ang II, and [Sar^1,Ile^8]Ang II were all purchased from Sigma (Sigma–Aldrich, St. Louis, MO). Polyclonal antisera against the AT_1 receptor and AT_2 receptor were purchased from Santa Cruz Biotechnology (Delaware, CA, USA). Tripure Isolation Reagent was from Roche Molecular Biochemicals (Sandhofer Strasse, Mannheim, Germany). PC12W (a subline of PC12 rat pheochromocytoma cell line that expresses high levels of AT_2 receptor, but no AT_1 receptor) were purchased from Chinese Type Culture Collection (Shanghai Institute of Cell Biology, China). Evans blue dye (EBD) was purchased from Fluke (Fluke Biomedical, Carson City, Nev). Radioimmunoassay kit of Ang II was bought from Beijing Northern Biotech Institute, China. Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, trypsin, as well as penicillin, streptomycin, and amphotericin B were purchased from HyClone (HyClone Laboratories, Salt Lake City, Utah). All other reagents used were of analytical grade.

In vitro experiments

Isolation and culture of rat pulmonary microvascular endothelial cells

Isolation of microvascular endothelial cells was performed according to a modified method of Chen et al. [12]. Briefly, the fresh lungs, isolated from the sacrificed rats, were washed with 50 ml serum-free DMEM. The pleura was then carefully cut away and discarded, then outer edges of the remaining lung tissue, which did not contain large blood vessels, were harvested (by this procedure, the majority of large vessel types can be excluded), and again rinsed in serum-free DMEM. This tissue was then reduced further using scissors and inserted into the glass pellet (Costar, Cambridge, MA) and rinsed in DMEM containing 20% fetal calf serum, 100 U/ml penicillin/streptomycin, and 2.5 μg/ml amphotericin B. Tissue cultures were incubated at 37 °C in humidified air containing 5% CO_2. After 60 h, the tissue was removed and thereafter, the culture medium was replaced every 3 days, and after reaching confluence, the cells were harvested by treating with a 0.25% solution of trypsin. Cells were identified as endothelial cells by cobblestone morphology, labeling with DiI-acetylated-low-density lipoprotein, and staining with anti-human factor VIII, and used for experiments between passages 2 and 4.

RNA preparation and semi-quantitative RT-PCR assay

Total RNA was prepared from RPMVECs and P12W cells with Tripure Isolation Reagent according to the manufacturer's instructions. First-strand cDNA was generated using random-hexamer-primed reverse transcription technique on a 2.5 μg of total RNA in a 50-μl reaction volume, and all PCR procedures were performed with a 20-μl reaction volume. The primers used for RT-PCR had the following sequences: (1) rat AT_1: forward, 5′-GCCCTGGCTGATTTATGC-3′ and reverse, 5′-GGAAAGGGAACACGAAGC-3′, PCR product is 415 bp; (2) rat AT_2: forward, 5′-TGGCTTGT CTGTCCTCAT-3′ and reverse, 5′-AGACTTGGTCAC GGGTAA-3′, PCR product is 255 bp. Thermocycle is set as follows: 94 °C (2 min), 94 °C (45 s), 55 °C (45 s), 72 °C (45 s) for 30 cycles, and 5 min at 72 °C. The PCR products were stored at −20 °C before assays. The PCR amplified samples were analyzed on 2.0% agarose gels visualized by ethidium bromide stain and photographed under UV light. Quantity and quality of the included RNA were controlled by an additional PCR from the same reverse transcription samples using an internal standard β-actin. Relative intensities of bands of interest were analyzed by use of an MSF-300G Scanner (Microtek Lab) and Scan Analysis software (Biosoft), and expressed as the ratio of receptors to β-actin.

Western blotting

After the cell monolayer reaching confluence, RPMVECs were washed with ice-cold phosphate-buffered saline (PBS), they were resuspended at 10^6 cells/100 μL of ice-cold lysis buffer (50 mmol/L Tris–HCl, pH 8.0, 150 mmol/L NaCl, 1% Triton X-100, 1 mmol/L phenylmethyl sulfonyl fluoride, 0.02% sodium azide, and 1 μg/ml aprotinin), and the adherent cells were scraped off the plate into 100 μL lysis buffer/50 cm^2 culture plate surface. The cell lysates were placed on ice for 20 min, followed by centrifuge at 12,000*g* for 10 min at 4 °C. The postmitochondrial supernatant fraction was removed and

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