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Landiolol hydrochloride ameliorates acute lung injury in a rat model of early sepsis through the suppression of elevated levels of pulmonary endothelin-1

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ABSTRACT

Among the dysfunctions and pathologies associated with sepsis, the underlying molecular mechanisms of sepsis-induced acute lung injury (ALI) are poorly understood. Endothelin (ET)-1, a potent vasoconstrictor and pro-inflammatory peptide, is known to be involved in the pathogenesis of ALI in a rat model of sepsis. Here, we investigated whether landiolol hydrochloride, an ultra-short-acting β-blocker, plays a crucial role in ameliorating and attenuating LPS-induced ALI through modulation of the ET-1 system. Male Wistar rats at 8 weeks of age were administered with either saline or lipopolysaccharide (LPS) for three hours (3 h) and some of the LPS-administered rats were continuously treated with landiolol for 3 h. ALI was induced by LPS, including levels of both circulatory and pulmonary TNF-α and IL-6 but [PaO_2] was significantly decreased. LPS also induced a significant increase in levels of pulmonary ET-1 and ET-A receptor, but levels of ET-B receptor, which has vasodilating effects, were remarkably diminished. Further, LPS administration upregulated the pulmonary expression of HIF-1α. Finally, the treatment of LPS-administered rats with landiolol for 3 h ameliorated and prevented ALI, normalized the altered levels of pulmonary ET-1 and ET-A receptors. Landiolol also induced significant down-regulation of ET-B receptor in lung tissues in the early hours (phase) of sepsis. However, Landiolol treatment had no effect on the up-regulated inflammatory mediators (TNF-α, IL-6) in both plasma and lung tissues during sepsis, and expression of pulmonary HIF-1α also remained unchanged after landiolol treatment. Collectively, these data led us to conclude that landiolol may ameliorate sepsis-induced ALI via the pulmonary ET system.

1. Introduction

Sepsis is a critical life threatening condition with a definition that encompasses pathologic infection and physiological changes that are collectively known as systemic inflammatory response syndrome (SIRS) [1]. The mortality from sepsis and the associated complications is very high and is estimated to be about 30% [2], making it the second leading cause of death among patients admitted at non-coronary intensive care units [3]. To date, the pathogenesis of sepsis and its progression to multiple organ dysfunction syndrome (MODS) and the associated septic shock are poorly understood and thus have been the subject of investigations in the last several decades.

Acute lung injury (ALI) is a well-known and frequent complication of sepsis, and causes a significant number of sudden deaths (mortality) and morbidity [4–6]. For this reason, patients with ALI/ARDS account for a significant proportion of the intensive care unit (ICU) case load. ALI and acute respiratory distress syndrome (ARDS), the severe form of ALI, are characterized by an acute onset of severe hypoxia pulmonary infiltration [7], pulmonary hypertension, edema and deteriorated gas exchange [8]. Indeed, sepsis is a potential risk factor for ALI and ARDS [5].

To date, the molecular mechanisms underlying the pathogenesis of sepsis-induced ALI are poorly defined and understood. We do know that endothelin (ET)-1, a potent endogenous vasoconstrictor and pro-

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inflammatory peptide [9,10], is actively involved in the pathogenesis of sepsis and the sepsis-associated organ dysfunction, as well as the associated complications [11], as evidenced by data from recent studies. Specifically, plasma levels of ET-1 are elevated in various sepsis animal models, including septic patients [12–15] and a clear correlation has been observed between ET plasma levels and morbidity/mortality in septic patients. These observations implicate ET in the pathogenesis of septic shock in human [16,17]. Further, ET-1 likely has a direct role in the development and subsequent severity of ALI by increasing the pressure of pulmonary microvasculature during the first phase or hours of sepsis [18,19]. Interestingly, endothelin blocker, namely tezosentan, ameliorates pulmonary hypertension, lung edema, cardiac dysfunction, and arterial hypoxemia in an ovine model of endotoxin-induced lung injury [20]. In contrast, intravenous infusion of ET-1 causes an elevation in pulmonary artery pressure and edema [21], implying a direct role of ET-1 in the pathogenesis of lung injury.

Landiolol hydrochloride, an ultra-short-acting and highly cardio-selective beta-1 blocker, with a half-life of 4 min, has been used in treating several acute medical disorders, including arrhythmias, during heart surgery [22], acute myocardial infarction [23], acute decompensated heart failure [24], and refractory electrical storm [25]. Ultra-short-acting β-blockers, such as landiolol, can influence heart rate but exert minimal effect on cardiac function. Besides, the potential effects of landiolol on rhythm control, landiolol also plays a protective role against ALI in a rat model of lipopolysaccharide (LPS)-induced systemic inflammation, which is associated with a reduction in high mobility group box 1 (HMGB-1) [26]. Very recently, we have demonstrated that landiolol is effective in improving acute liver injury of sepsis through the modulation of TNF (tumor necrosis factor-alpha)-α [27]. In addition, we have also shown that ET-1 is highly upregulated in kidney and heart tissues in LPS-administered rats [28,29] and landiolol has inhibitory effects on the upregulated levels of ET-1 in both of these tissues. However, it is not known whether landiolol will equally exert protective effects in lung tissues during sepsis through the alteration of the ET-1 system. Indeed, in our previous study we have already reported elevated ET-1 levels in lung tissues during sepsis [30].

In the present study, we investigated whether landiolol hydrochloride, an ultra- short-acting β-blocker, can play an important role in attenuating LPS-induced ALI through the modulation of pulmonary ET-1.

2. Materials and methods

Male Wistar rats (200–250 g, 8 weeks old) were used in all experiments in the current study. Sepsis was induced by the intra-peritoneal (IP) administration of bacterial LPS from *Escherichia coli* 055: B5 (15 mg/kg), dissolved in sterile saline, as described previously [31–33]. A dose 15 mg/kg of LPS has been shown to induce morphological injures in lung [34] as shown in our dose response study.

The total number of rats used in all the experiment was 45, which was randomized into three groups, namely: Group 1 (control, n = 15), group 2 (LPS, n = 15) and group 3 (LPS + landiolol hydrochloride, n = 15). For group 1, sterile saline (2 ml/body) was administered at time 0 h and then the rats were killed after 3 h (control group). For group 2, LPS at a dose of 15 mg/kg was administered at time 0 h, and then the rats were killed after 3 h (sepsis group). In group 3, landiolol hydrochloride was administered intravenously (100 μg/kg/min) for 15 min non-stop before LPS administration (landiolol treated sepsis group). The dose for landiolol was found to be the minimal dose for normalizing the LPS-induced hyperdynamic state in the acute (early) phase (hours) of sepsis, as reported in our past study [28].

Nembutal (sodium pentobarbital, IP, 80 mg/kg body weight, routinely used in our lab) was used to kill all the rats at 3 h, at the end of the experimental protocol. Blood gas analysis was also performed in the current study. The blood samples were collected from a polypropylene tube catheter inserted into the left carotid artery for blood gas analysis, and then lung tissues were carefully harvested, snap-frozen in liquid nitrogen, and stored at −80 °C. All animals received proper care and the experimental procedures were approved by the Animal Care and Use Committee of University of Tsukuba prior to the study.

2.1. Measurements of hemodynamic parameters

Hemodynamic parameters were measured using a previously optimized methodology from our lab [27–35]. Briefly, rats were anesthetized with isoflurane inhalation (1.5%, 1 l/min) and a microtip pressure transducer catheter (SPC-320, Millar Instruments, Houston, TX, USA) was inserted into the left carotid artery at the end of the experimental protocol. Arterial blood pressure and heart rate (HR) were monitored with a pressure transducer (model SCK-590, Gould, Ohio, USA) and recorded with the use of a polygraph system (amplifier, AP-601G, Nihon Kohden, Tokyo, Japan; Tachometer, AT-601G, Nihon Kohden; and thermal-pen recorder, WT-687G, Nihon Kohden).

2.2. Echocardiography

Echocardiography was performed using a Vevo 2100 high-frequency ultrasound system (VisualSonics, Inc., Ontario, Canada), which includes an integrated rail system for consistent positioning of the ultrasound probe [36] at the end of the protocol. The fur from the chest was shaved with an electrical clipper and a gel. The animals were then connected to an electrocardiogram (ECG). An optimal parasternal long axis (LAX) cine loop (i.e. visualization of both the mitral and aortic valves, and maximum distance between the aortic valve and the cardiac apex) of >1000 frames/s was acquired using the ECG-gated kilohertz visualization technique. The probe was then rotated 90° and positioned 6 mm below the mitral annulus. Three parasternal short-axis (SAX) M-mode sequences were stored. Percent fractional shortening (% FS) was calculated in the M-mode image as FS = (EDD − ESD) / EDD, where EDD and ESD are the end-diastolic and end-systolic diameters, respectively.

2.3. Lung wet-to-dry weight ratio

Lung tissues were harvested, blotted dry and weighed in order to determine the weight of the lung in the wet state and the wet-to-dry weight ratio was calculated, as follows: the lung tissues were weighed; wrapped loosely in aluminum foil; dried in an oven at 80 °C for 24 h; and weighed again. Then the ratio lung wet-to-dry weight ratio was calculated.

2.4. Histopathology examination

After tissue harvest, the lungs were fixed in 4% buffered formalin solution, dehydrated, embedded in paraffin, and then sliced into 5-mm-thick sections to evaluate lung micro-morphological injury. After deparaffinization, tissue sections were stained using standard hematoxylin and eosin (HE) staining method.

2.5. Enzyme-linked immunosorbent assay

The concentration of each respective protein/peptide, as described below under the plasma/serum and pulmonary tissue extracts section, was determined using the following kits: serum and lung levels of tumor necrosis factor alpha (TNF-α) and ET-1; plasma levels of IL-6 (R & D Systems, Minneapolis, MN), according to the manufacturer's protocol. These kits are already validated for the measurement of rat samples in our lab.

2.6. RNA preparation and real-time quantitative polymerase chain reaction

Total RNA samples from lung tissues were isolated using the acid *guanidinium thiocyanatephenol chloroform* extraction with RNeasy (Qiagen, Tokyo, Japan). After RNA isolation, quantification and DNase I