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Significant reversal of cardiac upregulated endothelin-1 system in a rat model of sepsis by landiolol hydrochloride

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ABSTRACT

Aims: Landiolol hydrochloride, an ultra-short-acting highly cardio-selective β-1 blocker, has become useful for various medical problems. Recent studies have demonstrated that co-treatment with landiolol protects against acute lung injury and cardiac dysfunction in rats of lipopolysaccharide (LPS)-induced systemic inflammation, and was also associated with a significant reduction in serum levels of the inflammation mediator HMGB-1 and histological lung damage. Endothelin (ET)-1, a potent vasoconstrictor, has been implicated in pathogenesis of sepsis and sepsis-induced multiple organ dysfunction syndrome. Here, we investigated whether landiolol hydrochloride can play important roles in ameliorating LPS-induced alterations in cardiac ET system of septic rats.
Main methods: Eight-week-old male Wistar rats were administered LPS only for 3 h and the rest were treated with LPS as well as with landiolol non-stop for 3 h.
Key findings: At 3 h after LPS (only) administration, circulatory tumor necrosis factor (TNF)-α level, blood lactate concentration and percentage of fractional shortening of heart were significantly increased. In addition, LPS induced a significant expression of various components of cardiac ET-1 system compared to control. Finally, treatment of LPS-administered rats with landiolol for 3 h normalized LPS-induced blood lactate levels and cardiac functional compensatory events, without altering levels of plasma TNF-α and ET-1. Most strikingly, landiolol treatment significantly normalized various components of cardiac ET-1 signaling system in septic rat.
Significance: Taken together, these data led us to conclude that landiolol may be cardio-protective in septic rats by normalizing the expression of cardiac vasoactive peptide such as ET, without altering the circulatory levels of inflammatory cytokines.

Introduction

Sepsis is associated with tissue hypoperfusion and metabolic impairment, which may contribute to the development of the associated multiple organ failure (Singh and Evans, 2006). As an important organ system that is commonly vulnerable to sepsis or septic shock, the cardiovascular system and its dysfunction have been studied in both clinical and basic research for a long time. When sepsis-induced cardiovascular dysfunction was first described by Waisbren in 1951, it was recognized as a hyperdynamic state with full bounding pulses, flushing, fever, oliguria and hypotension (Waisbren, 1951). More recently, echocardiographic studies demonstrated impaired left ventricular systolic and diastolic dysfunction in septic patients (Poelaert et al., 1997). Cardiac dysfunction in sepsis is characterized by decreased contractility, impaired ventricular response to fluid therapy, and in some patients, ventricular dilatation (Zanotti-Cavazzoni and Hollenberg, 2009). Current data support a complex underlying physiopathology of sepsis-induced myocardial dysfunction, with a host of potential pathways leading to myocardial depression (Zanotti-Cavazzoni and Hollenberg, 2009). Cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and lysozyme C, have direct inhibitory actions on cardiomyocyte contractility (Horton et al., 2000; Kumar et al., 2007; Mink et al., 2005; Zanotti-Cavazzoni and Hollenberg, 2009). Endothelin (ET)-1 is also said to be a major factor (Zanotti-Cavazzoni and Hollenberg, 2009). ET-1 is the most potent vasoconstrictor studied so far (Yanagisawa et al., 1988), and its (ET-1) upregulation has been demonstrated within 6 h of inducing sepsis/septic shock by lipopolysaccharide (LPS) in animal models (Shindo et al., 1998). Overexpression of ET-1 contributes to an increase of inflammatory cytokines, especially TNF-α, IL-1, and IL-6, and an inflammatory cardiomyopathy that results in cardiac

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dysfunction (Yang et al., 2004). Konrad et al. reported that tazosentan, a dual endothelin type A (ET-A) and endothelin type B (ET-B) receptor antagonist improves cardiac index, stroke volume index, and left ventricular stroke work index in sepsis mouse model (Konrad et al., 2004). These findings support the involvement of ET-1 in myocardial dysfunction in sepsis. However, further investigations are needed in order to develop effective therapeutic strategies.

Landiolol, an ultra-short-acting and highly cardio-selective β-1 blocker, has become useful for various medical problems. The first study of β-blocker therapy for septic shock was published in 1969 (Berk et al., 1969). More recently, studies have demonstrated that co-treatment with landiolol protects against acute lung injury and cardiac dysfunction in a rat model of LPS-induced systemic inflammation, which was also associated with a significant reduction in serum levels of the inflammation mediator HMGB-1 and histological lung damage (Hagiwara et al., 2009). However, the effects of landiolol on the LPS-altered ET signaling system in the heart of rat are yet to be demonstrated.

In the present study, we investigated whether landiolol hydrochloride can play an important role in ameliorating the LPS-induced alteration in cardiac ET signaling system in a rat model of sepsis.

Materials and methods

Animal preparation

Male Wistar rats (200–250 g, 8 weeks old) were used in all experiments. Sepsis was induced by the intraperitoneal (IP) administration of bacterial LPS from *Escherichia coli* 055:B5 (15 mg/kg), dissolved in sterile saline. The LPS dose used in the present study has been used before to generate animal models of sepsis in our several previous studies (Jesmin et al., 2004, 2006, 2007, 2009a,b, 2011a,b; Shimojo et al., 2006a; Yamaguchi et al., 2006; Zaedi et al., 2006). The dose of LPS used in the current investigation as well as that in the previous studies produce the clinical condition that resembles sepsis in context of circulatory as well as tissue and organ dependent alteration ranging from morphological to gene expression changes (Jesmin et al., 2004, 2006, 2007, 2009a,b, 2011a,b; Shimojo et al., 2006a; Yamaguchi et al., 2006; Zaedi et al., 2006). The current LPS dose has been shown to demonstrate minimal morphological injuries in important organs prone to develop dysfunction during sepsis (Jesmin et al., 2004, 2006, 2007, 2009a,b, 2011a,b; Shimojo et al., 2006a; Yamaguchi et al., 2006; Zaedi et al., 2006). The control group received an equal volume of vehicle (sterile saline; 2 ml/body) intraperitoneally, without LPS. In every study our group conducts, we always confirm the induction and generation of sepsis model by performing a detailed assessment of inflammatory cytokines, notably levels of circulatory and tissue TNF-α, iNOS, IL-6, blood gas analysis, morphological and functional evaluation of organs likely prone to sepsis (Jesmin et al., 2004, 2006, 2007, 2009a,b, 2011a,b; Shimojo et al., 2006a; Yamaguchi et al., 2006; Zaedi et al., 2006). The different groups of animals ($n = 25$) in the present study were killed by Nembutal (sodium pentobarbital, IP, 80 mg/kg body weight) at 3 h after LPS or vehicle only. The blood samples were collected from a polyproprene tube catheter inserted into the left carotid artery for blood gas analysis, and heart tissues were harvested gently, frozen immediately in liquid nitrogen, and stored at −80 °C. All animals received care and all experimental procedures were approved by the Animal Care and Use Committee of University of Tsukuba. It should be noted that LPS (15 mg/kg, intravenous) was dissolved in normal saline administered intravenously at time 0 to different groups of rats, and then the rats were killed after 3 h. However, for the LPS + landiolol hydrochloride group, 15 min before LPS administration, landiolol hydrochloride was administered intravenously continuously for 3 h (100 μg/kg/min).

Our preliminary time course study conducted at various time points (0 h, 1 h, 3 h, 6 h, 10 h, 16 h, 24 h, $n = 13$ for each group) showed that LPS induced high levels of plasma lactate, elevated heart rate (HR) and increased percent of fractional shortening (% FS), i.e., a hyperdynamic state during the early hours of sepsis. These elevated end-points were greatly normalized significantly by landiolol as early as 3 h compared to the LPS only administered rats. This reversal of the hyperdynamic state during septic shock by landiolol resulted in maintaining low levels of essential cardiac output, systemic peripheral circulation and arterial oxygenation at all time points of sepsis, and consequently leading to an improved survival rate. Based on these facts, we chose the 3 h time point to investigate in details, the effects of landiolol on septic rat heart. On the other hand, the time duration of sepsis induction (3 h) in the present study may have little to do with the pathology in clinical sepsis response which may have a time course of days to weeks. However, the present study aimed to focus on the acute response phase of sepsis especially in context of hemodynamic alteration in experimental animal model.

Measurements of hemodynamic parameters

The 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, as described in previous study. Then arterial blood pressure and heart rate 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).

Echocardiography

Echocardiography was performed using a Vevo 2100 high-frequency ultrasound system (Visual Sonics Inc, Ontario, Canada), which includes an integrated rail system for consistent positioning of the ultrasound probe (Yang et al., 2013). The hair from the chest was removed with an electrical clipper and a hair removal gel prior to the examination. The animals were placed on a heating pad and connected to an electrocardiogram (ECG) while rectal temperature was monitored to maintain body temperature 38 ± 0.1 °C. A 35 MHz linear transducer (Visual Sonics, RMV 707, Inc, Ontario, Canada) was used for imaging. 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, i.e. at the level of the papillary muscles. Three parasternal short axis (SAX) M-mode sequences were stored. Fractional shortening (FS) was calculated in the M-mode image as $FS = (EDD - ESD)/EDD$, where EDD and ESD are end-diastolic and end-systolic diameters, respectively.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELIZA), a sensitive technique for determining circulatory and tissue protein concentration, was used to determine levels of IL-6 and TNF-α in serum and ET-1 in plasma and heart tissue extracts (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol. For ET-1 measurement, a 4.5 h solid phase ELIZA was used, and contained synthetic ET-1 and antibodies raised against synthetic ET-1. This immunoassay has been shown to accurately quantitate synthetic and naturally occurring ET-1. A monoclonal antibody specific for ET-1 was pre-coated onto a microplate. Standards and samples were pipetted into the wells and if present, ET-1 antigen was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific to ET-1 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and a color developed in proportion to the amount of ET-1 bound in the initial step. The color development was then stopped and its intensity measured. The ET-1 concentration of each sample

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