Landiolol Hydrochloride Normalizes Diminished Levels of Cardiac Vascular Endothelial Growth Factor (VEGF) Signaling System Components in Lipopolysaccharide-Induced Sepsis Independent of Inflammatory Markers

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Abstract

Aim: Myocardial dysfunction is one of the complications associated with sepsis during its (sepsis) pathogenesis. To date, very few studies have investigated whether angiogenic factors in the heart, such as vascular endothelial growth factor (VEGF) and components of its signaling system are involved in myocardial dysfunction during the early phases of sepsis. Therefore, the present study aims to examine: 1) the expression pattern of VEGF and its signaling molecules in the rats under these (sepsis) conditions.

Method: Eight (8)-week-old male Wistar rats were administered for three hours with either LPS only once, or continuously with LPS plus landiolol.

Result: At 3 h after LPS (only) administration, circulatory levels of tumor necrosis factor (TNF)-α, IL-6, iNOS, lactate concentration and percentage of fractional shortening of the heart were significantly increased. However, levels of cardiac VEGF and its downstream signaling components were significantly down regulated. Treatment of LPS-administered rats with landiolol for 3 h normalized LPS-induced blood lactate levels, cardiac functional compensatory events, as well as VEGF and its signaling molecules, but did not alter levels of plasma TNF-α, IL-6 and iNOS.

Conclusion: Taken together, these data led us to conclude that landiolol may be cardio-protective in septic rats by normalizing coronary microcirculation through blockage of sepsis-induced decrease in expression of VEGF signaling system but independent of inflammatory cytokines.

Keywords: Heart; Landiolol hydrochloride; VEGF; Sepsis; Rat model

Introduction

Sepsis is considered as a systemic inflammatory response to infection. Due to the recent increase in the aging of the population and the wider use of immunosuppressive agents, as well as invasive procedures, the morbidity and mortality associated with sepsis has increased lately [1]. Because sepsis is associated with widespread injury of the vascular endothelia, multiple organs become dysfunctional, including cardiac failure, ARDS (acute respiratory distress syndrome), acute kidney injury and coagulation disorder. Also, since the cardiovascular system plays a key homeostatic role, sepsis-induced myocardial depression is commonly associated with increased morbidity and mortality. Although sepsis-induced myocardial depression has not yet been clearly defined, it is known to involve both the left and right sides of the heart, and is thus global or systemic [2]. Sepsis-induced cardiac dysfunction has been shown to occur very early in sepsis, including the hyperdynamic phase of septic shock, and the underlying pathogenesis may be related to mitochondrial dysfunction, as well as levels of nitric oxide, complements and cytokines [3]. However, the pathogenesis of this condition (septic myocardial depression) is so complex that there is no single effective treatment for it. Therefore, additional studies are necessary in order to improve our understanding of the disease, develop reliable diagnostic procedures, as well as effective therapeutic interventions for this disorder [4].

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen that plays an important role in neovascularization under both physiological and pathological conditions [5]. VEGF possibly exerts these biological processes through three mechanisms of action, namely by: (1) increasing blood flow to the tissue via vasodilation, (2) reducing the distance between the cells and the nearest blood vessel by stimulating angiogenesis and (3) increasing the permeability of the blood vessels to plasma, small solutes and macromolecules. VEGF is a crucial molecule in that it is up regulated in all known physiological and pathological forms of angiogenesis and is known to stimulate angiogenesis directly [6]. Although growth of new blood vessels is normally rare in the heart, chronic ischemia can stimulate VEGF synthesis, which subsequently leads to angiogenesis and coronary collateral formation [7,8]. Other functions of VEGF,
including endothelium-dependent relaxation of coronary arteries [9] and induction of microvascular permeability [10], are also well known. Further, it has also been reported that VEGF plays a cardio-protective role by inducing expression of cyclooxygenase (COX)-2 in endothelial cells [11]. Based on these collective facts, we speculate that levels of the VEGF signaling system may be altered in the heart in the early hours of sepsis, subsequently leading to the development of sepsis-induced myocardial dysfunction.

Landiolol hydrochloride, an ultra-short-acting and highly cardio-selective beta-1 blocker, with a half-life of 4 min, has recently become useful in treating tachycardia during surgery [12]. The drug has already been approved in Japan as an emergency treatment for supraventricular tachyarrhythmia conditions. Notably, recent studies have also showed that landiolol exerts protective effects on various organs during sepsis, including the heart [13], lung [14] and kidney [15]. For instance, abberant levels of vasoactive peptide, such as endothelin-1, in cardiac tissue were normalized by landiolol during the early hours of sepsis in hyperdynamic state, indicating its cardioprotective effects during this phase of sepsis [13]. Indeed, esmolol, another beta-1 adrenoreceptor blocker, was also recently found to be effective in treating septic patients. Specifically, administration of esmolol was significantly related to hemodynamic stabilization and the improvement of survival rate to 28-day during sepsis [16]. However, the exact underlying mechanisms on how these beta-1 blockers work in sepsis-induced cardiac dysfunction is to date not yet clear. For this reason, the aim of the present study is to investigate: 1) the expression pattern of VEGF and its signaling molecules in the heart during the early hours of sepsis development, 2) as well as changes in the hemodynamic parameters and 3) how landiolol alters the expression profile of cardiac VEGF and its signaling molecules associated with sepsis.

Material and Method

Animal preparation

Male Wistar rats (200-250 g, 8 weeks old) were used in all experiments in the present study. Sepsis was induced by the intraperitoneal (IP) administration of bacterial LPS from Escherichia coli 055:B5 (15 mg/kg), dissolved in sterile saline (n=15). This dose of LPS was sufficient to induce systemic inflammatory response syndrome (SIRS) [13,15,17-20]. The control group (n=15) received an equal volume of vehicle (sterile saline; 2 ml/body), without LPS. In order to investigate the specific roles of VEGF in LPS-induced SIRS, landiolol hydrochloride was co-administered with LPS (n=15). It should be noted that LPS (15 mg/kg, IP) was dissolved in normal saline and administered intravenously at time 0 to different groups of rats, and the rats were then killed after 3 hours. However, for the LPS + landiolol hydrochloride group, 15 min before LPS administration, landiolol hydrochloride was administered intravenously continuously for 3 hours (100 μg/kg/min). Some rats were recruited for survival rate study. LPS group (n=10) and LPS + landiolol group (n=10) were observed for 10 hrs and Kaplan–Meier analysis was performed to compare between these groups. The blood samples were collected from a polypropylene tube catheter inserted into the left carotid artery for blood gas analysis, and the heart tissues were harvested gently, frozen immediately in liquid nitrogen, and stored at -80°C. All animals received care that was in compliance with the institutional guidelines, and the experimental procedures were approved by the Animal Care and Use Committee of University of Tsukuba.

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=10 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 normalized 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 endotaxemic rat heart.

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 our previous studies [13,15,17-20]. Arterial blood pressure and heart rate were then monitored with a pressure transducer (model SCK-590, Gould, Ohio, USA) and recorded using a polygraph system (amplifier, AP-601 G, Nihon Kohden, Tokyo, Japan; Tachometer, AT-601 G, Nihon Kohden; and thermal pen recorder, WT-687 G, Nihon Kohden).

Echocardiography and LV pressure measurement

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. 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 at 38 ± 0.1°C. A 35 MHz linear transducer (VisualSonics, 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. Ejection fraction (EF) was calculated as EF = (EDV – ESV)/EDV, where EDV and ESV are end-systolic and end-diastolic volumes, respectively. The prolate–ellipsoid formula estimates left ventricular (LV) volumes from a single 2D parasternal long axis cine loop [21,22]. Stroke volume (SV) was calculated as EDV – ESV, where EDV and ESV are end-diastolic and end-systolic volumes derived by each of the formulas described above, or it was estimated from the pulmonary artery flow pattern [21,22]. Cardiac output (CO) was estimated as SV (acquired from the pulmonary artery flow pattern) multiplied by heart rate (acquired from the arterial pressure curve), as previously described [22].

For hemodynamic measurements, a microtip pressure transducer catheter (2 Fr, SPC-320, Millar Instruments, Houston, TX) was inserted in the left carotid artery and moved into LV of rats. LV pressure was monitored with a pressure transducer (model SCK-590; Gould, Cleveland, OH) and was recorded with the use of a polygraph system (Nihon Kohden, Tokyo, Japan), as previously described [22]. Obtained digital data were calculated to peak positive dP/dt (+dP/dt) and peak negative dP/dt (-dP/dt) (Acqknowledge Version 3.9.1.6, BIOPAC systems, Inc, CA).
Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA), a sensitive technique for determining tissue protein concentration, was used to determine levels of VEGF, iNOS, IL-6, TNF-α (R and D Systems, Minneapolis, MN) and pAkt (BioSource International, USA) in heart tissues in plasma/serum.

Western blot analysis

Cardiac tissues were homogenized with 10 vol of 20 mM Tris HCl (pH 7.4), 250 mM NaCl, 3 mM EDTA, 3 mM EGTA, 1 mM Na3VO4, 2 mM dithiothreitol, 20 mM glycophosphate, 0.6% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride, 60 μg/ml aprotinin, and 1 μg/ml leupeptin on ice using a homogenizer. The homogenate was gently rotated for 30 min at 4°C and then centrifuged at 13,000 g for 10 min at 4°C, and the protein concentration of the resulting supernatant was determined. The samples (20 μg of protein) were then subjected to heat denaturation at 96°C for 7 min with Laemmli buffer. Briefly, each sample was separated on 10% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride (Millipore, Billerica, MA) membrane. The membrane was incubated in blocking buffer with 5% skim milk in PBS (phosphate buffered saline) containing 0.1% Tween 20 for 12 h at 4°C, followed by incubation with primary antibody for 1 h at room temperature (RT). The membrane was washed with PBS containing 0.1% Tween 20 three times and incubated with horseradish peroxidase-conjugated secondary antibody, which was an anti-rabbit, anti-mouse, or anti-goat IgG (1:2,000 dilution with blocking buffer; Cell Signaling, Beverly, MA), for 1 h at RT. After washes, as described above, the levels of each molecule was determined using enhanced chemiluminescence detection reagents (Amersham Pharmacia Biotech, Piscataway, NJ) followed by exposure to a Hyper-film (Amersham Biosciences). Negligible loading/transfer variation was noted between samples. Moreover, β-actin was used as a loading control. The following antibodies, which are commercially available, were used: rabbit anti-human VEGF polyclonal antibody (Immunological Laboratories, Fujikko, Japan), Ser1177-phospho-eNOS (Santa Cruz Biotechnology).

Nitric oxide colorimetric assay

Nitric oxide (NO) was indirectly detected in cardiac tissue extracts as nitrite using a NO Colormetric Assay Kit (Roche Diagnostics, Mannheim, Germany). In this method, the nitrate present in the sample was reduced to nitrite by reduced nicotinamide adenine denucleotide phosphate in the presence of the enzyme nitrate reductase. The nitrite formed reacted with sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride to give a red-violet diazo dye. The diazo dye was measured at 550 nm, on the basis of its absorbance within the visible range.

RNA preparation and real-time quantitative polymerase chain reaction

Total RNA from cardiac tissue was isolated using RNeasy Mini Extraction Kit (Qiagen, Tokyo, Japan). After isolation, DNase I treatment and quantification, RNA was reverse transcribed to cDNA by Omniscript RT using a first-strand cDNA synthesis kit (Qiagen, Tokyo, Japan). The reaction was performed at 37°C for 60 min. The mRNA expression of target genes were analyzed by real-time quantitative PCR with TaqMan probe using an ABI Prism 7700 sequence detector (Perkin-Elmer Applied Biosystems, Foster, CA). The gene-specific primers and TaqMan probes were synthesized from Primer Express version 1.5 software (Perkin-Elmer), according to the published cDNA sequences for each gene, as previously described [21,23,24].

h compared to the control group (Figure 3B). However, hyperdynamic state induced by LPS administration was significantly normalized in LPS + landiolol group (p<0.05 vs LPS) (Figure 3B).

Expression of pro-inflammatory cytokines

Serum levels of TNF-α, IL-6 and iNOS, which are inflammatory cytokines, as determined by ELISA, were significantly increased after LPS administration by 3 h (Figure 4). However, landiolol treatment did not change levels of these molecules (Figure 4) in septic rats. Plasma VEGF level also significantly increased in LPS-only and LPS + landiolol groups compared with the control group (Figure 4).

Figure 5 shows the expression of pro-inflammatory markers (TNF-α, IL-6) in cardiac tissues under the current experimental setting and the failure of landiolol to significantly diminish their expression.

Expression of VEGF and its downstream signaling molecules

ELISA and Western blot analysis showed about 25-30% decrease in levels of VEGF protein in cardiac tissues of LPS-administered rats compared to the control group (Figure 6). This decrease in levels of VEGF protein was normalized following landiolol treatment. A similar trend in VEGF mRNA was noted (Figure 6).

Figure 7 shows a significant decrease in levels of phosphorylated Akt (pAkt), phosphorylated endothelial nitric oxide synthase (p-eNOS) and nitric oxide (NO) in cardiac tissue, which are effectors molecules downstream of VEGF, in LPS-only and LPS + landiolol groups compared with the control group (Figure 7).

Discussion

The important findings revealed by the present study are that: (1) landiolol dramatically improved the survival ratio of septic rats, as much as 80% compared to treated with LPS only, (2) landiolol normalized the hyperdynamic state of sepsis rats at 3 h, and prevented both the decrease in arterial oxygenation, as well as elevation in levels of blood lactate, which was noted within 3 h, for animals were not treated with landiolol, (3) VEGF levels were significantly down regulated within 3 h after LPS administration and treatment with landiolol attenuated this decrease in VEGF levels, (4) Akt and eNOS, the downstream effectors of VEGF significantly decreased in septic rats and landiolol normalized levels of these molecules.

The mortality rate of sepsis patients has been reported to be as much as 28.6% and the incidence has been estimated to have increased by as

<table>
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<th>Parameters</th>
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<th>LPS</th>
<th>LPS + landiolol</th>
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<td>pH</td>
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<td>7.42 ± 0.02*</td>
<td>7.42 ± 0.02*</td>
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<td>PaCO2 (torr)</td>
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<td>PaO2 (torr)</td>
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<td>85.0 ± 5.9*</td>
<td>99.9 ± 5.4†</td>
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<td>HCO3 (mmol/l)</td>
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<td>21.3 ± 1.5*</td>
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<td>Base Excess (mmol/l)</td>
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<td>-2.0 ± 0.7*</td>
<td>-3.4 ± 1.6*</td>
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<tr>
<td>Lactate (mmol/l)</td>
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<td>1.78 ± 0.17†</td>
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<td>IVST (mm)</td>
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<td>1.14 ± 0.04</td>
<td>1.12 ± 0.04</td>
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<td>LVDD (mm)</td>
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<td>5.80 ± 0.25</td>
<td>6.00 ± 0.18</td>
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<td>PWT (mm)</td>
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<td>1.12 ± 0.05</td>
<td>1.13 ± 0.05</td>
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<td>Cardiac Output (ml/min)</td>
<td>63.8 ± 6.2</td>
<td>66.1 ± 4.3</td>
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<td>Heart Rate (bpm)</td>
<td>462 ± 6</td>
<td>491 ± 8*</td>
<td>457 ± 12†</td>
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<td>LV +dP/dt</td>
<td>9828 ± 612</td>
<td>5083 ± 305*</td>
<td>9485 ± 777†</td>
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<td>LV -dP/dt</td>
<td>-9116 ± 1050</td>
<td>-4199 ± 470*</td>
<td>-8396 ± 623†</td>
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Table 1: Blood Gas Analysis, Echocardiographic Characteristics and Hemodynamic of Experimental Rats (Values are mean ± SE. Abbreviations: LPS, lipopolysaccharide; BP, blood pressure; IVST, interventricular septal thickness; PWT, posterior wall thickness; bpm, beat per minutes; LV, left ventricle. *p<0.05 vs. control; †p<0.05 vs. LPS).
In the current study, we also observed significant changes in various parameters of blood gas analysis following LPS administration, implying a successful induction of sepsis in the current experimental design (Table 1) which is consistent to our previous studies. Among these significantly altered blood gas analysis parameters, only PaO₂ and lactate concentration were significantly affected by landiolol in sepsis rats. Another similarity between our previous studies and the current model is the increase in levels of inflammatory cytokines, such as plasma TNF-α, IL-6 and iNOS [13,15,17-20] during the early hours of sepsis. While levels of cardiac TNF-α expression were significantly up regulated by 3 h following induction of sepsis here, expression of IL-6 were unchanged. Although cardiac morphological and/or functional injury/alteration data are not shown here, including inflammatory cell infiltration assessed through hematoxylin and Eosin (H & E) staining, no remarkable changes were observed in heart by 3 h post sepsis induction. These findings suggest that during the early phase (hours) of sepsis, while there was a pronounced increase in levels of systemic inflammatory cytokine, expression of local cardiac inflammatory cytokines may not have been altered drastically. Further, landiolol treatment failed to alter the elevated levels of inflammatory cytokines in sepsis animal models. In contrast to the present findings, recent studies demonstrated that co-treatment with landiolol significantly reduces serum levels of inflammatory mediator, such as HMGB-1, and histological lung damage, and protects against acute lung injury and cardiac dysfunction in a rat model of LPS-induced systemic inflammation [14]. The timing or phase of sepsis at which sampling was performed may account for the differential effects of landiolol treatment on various inflammatory cytokines in sepsis animal models. Experimental models of sepsis show a clear evidence of myocardial contractile disturbance, both in vivo and in vitro. Such disturbance is also observed in early “hyperdynamic” shock, when both aggressive volume replacement and adaptive left ventricular dilatation collectively preserve cardiac output [27]. Initially, cardiac dysfunction was considered to occur only during the “hypodynamic” phase of shock [3]. However, we now know that it occurs very early in sepsis, including the “hyperdynamic” phase of septic shock [3]. Accordingly, at 3 h after LPS administration, we found a hyperdynamic state in the current rat model of sepsis (elevated fractional shortening, hypotension). Pathologically over-enhanced cardiac function (hyperdynamic state in sepsis), which compensates systemic circulation in the early phase of sepsis, may lead to the collapse of cardiac function and systemic circulation in late phase. Treatment of LPS-administered rats with landiolol for 3 h normalized the elevated cardiac functional compensatory events, such as elevated fractional shortening in septic rats without any effect on blood pressure. Tachycardia increases cardiac workload and myocardial oxygen consumption in sepsis. In addition, shortening of diastolic relaxation time and impairment of diastolic function further affects coronary perfusion, thereby contributing to a lower ischemic threshold [28,29]. Excessive sympathetic activation also leads to catecholamine-induced cardiomyocyte toxic effects characterized by inflammation, oxidative stress, and abnormal calcium handling resulting in left ventricular dilatation, apical ballooning, myocardial stunning, apoptosis, and necrosis [29]. Taken together, these mechanisms contribute to worsening of septic myocardial dysfunction and increased mortality [29]. Reducing heart rate in hyperdynamic state induced by sepsis will decrease myocardial oxygen consumption and will improve diastolic function and coronary perfusion. Furthermore, landiolol may block adverse effects of catecholamines to other systemic organs in septic rats not only to heart, such as inflammation, oxidative stress, and abnormal calcium handling. In the present study, altered ± dP/dt and %FS were ameliorated by landiolol in rats of 3hrs after LPS administration. These are compatible with the previous data.

Figure 2C: Systolic blood pressure from the control group, 3 h LPS-administered rats, and 3 h landiolol-treated LPS-administered rats. Values are mean ± SE (n=15). *p<0.05 vs. control, #p<0.05 vs. 3 h LPS-administered rats

Figure 3: Representative echocardiography images (A) fractional shortening (FS, %); (B) from the control group, 3 h LPS-administered rats, and 3 h landiolol-treated LPS-administered rats. Values are mean ± SE (n=15). *p<0.05 vs. control, #p<0.05 vs. 3 h LPS-administered rats

much as 1.5% per year, despite efforts to control it in the United States [1]. A previous experimental sepsis model study showed that the survival ratio begins to drop at about 24h post intraperitoneal administration of 15 mg/kg of lipopolysaccharides in sepsis model of 8-week mice and dropped to less than 30% by 30h after LPS administration [25]. The results of the present study showed a survival rate of less than 30% as early as 8h post LPS administration. This difference, between the current and previous study cited here, is significant, even when we take into account the species difference, i.e., rat versus mouse. Of note, administration of landiolol improved the survival ratio of septic rats more than 80% by 10h post LPS administration. These finding indicates that landiolol might be an effective treatment option for sepsis. These current data are consistent with a previous study that demonstrated that beta1-selective antagonist (esmolol) improved survival rates in sepsis mice model [26].
The present study is the first to demonstrate that local cardiac levels of VEGF, the key angiogenic growth factor, and its downstream effecter molecules are down regulated during the early phase (hours) of sepsis, at the time when plasma levels of inflammatory cytokines are high. Under normal conditions, the local cardiac microcirculation regulates and distributes red blood cells and oxygen throughout the tissue to maintain tissue oxygen concentration [30]. However, during sepsis, distribution and regulation of local tissue oxygen delivery are compromised by decreased functional capillary density [31-34] and diminished microvascular vasoconstriction [35], despite normal or enhanced cardiac output. Thus, diminishing VEGF angiogenic signaling may not normalize the compromised coronary microcirculation in sepsis. The most important finding of the present study is the data showing that treating septic rats with landiolol restores the down regulated local levels of cardiac VEGF angiogenic signaling molecules, suggesting cardioprotective role for VEGF, which has been reported previously [11]. Further, the present data showing diminished levels of cardiac VEGF and its downstream signaling molecules level by 3 h implies that at this time point (3 h), both local angiogenesis in cardiac microcirculation and coronary dilation were prevented, subsequently leading to cardiac dysfunction in septic rat. This cardiac dysfunction was reversed by landiolol through modulation of VEGF signaling. Indeed, consistent with these current observations, beta-adrenoceptor blockage has been reported to exert beneficial survival effects in sepsis through cardioprotection and attenuating systemic inflammation [36]. Very recently, landiolol has also been reported to have organ protective effects in sepsis by normalizing the significantly elevated expression of cardiac endothelin signaling [13]. Further, administration of landiolol is also known to improve acute respiratory distress syndrome and acute kidney injury through the reduction of serum inflammation mediator, High-mobility group box 1 (HMGB-1) [14] and ameliorating the overexpression of HIF-1alpha-endothelin-1 system [15] in septic kidney, respectively.

For now, we do not have an adequate explanation for the observed reversal in the expression of local cardiac VEGF signaling by landiolol in the sepsis animal models. However, it is interesting to note that various studies under different conditions have demonstrated that the beneficial cardiac effects of β-blockers are largely mediated by VEGF. For instance, β-blockers were shown to promote angiogenesis in the mouse aortic ring assay by up regulating VEGF [37]. Also β-blockade has been shown to promote cardiac angiogenesis in heart failure via activation of VEGF signaling pathway. Further, β-blocker-induced enhancement of cardiac angiogenesis is essential for the favorable effects of this therapy on cardiac function and remodeling [38]. Lastly, β-blocker-induced enhancement of cardiac angiogenesis seems to be related to β-blocker–dependent heart rate reduction (HRR) that has
been demonstrated to enhance coronary reserve, as well as capillary and arteriolar growth in normal [39] and in the post-MI (myocardial infarction) hearts [40,41]. Thus, collectively, data from the present and previous studies indicate that adrenergic receptor blockers, including landiolol, may enhance angiogenesis through the induction of VEGF signaling in various disease conditions. In addition, consistent with these studies, cited earlier, landiolol-induced heart rate reduction during sepsis may partly explain the normalization of diminished local cardiac levels of VEGF by landiolol.

In the present study, we did not observe a direct relationship between levels of local cardiac VEGF and TNF-α in sepsis rats, even though TNF-α is capable of inducing mRNA expression of the pro-angiogenic molecules, such as VEGF and its receptors (VEGFRs) [42,43]. Instead, the present study shows an inverse pattern between the expression of TNF-α and VEGF in the heart of a septic rat. Although this inverse relationship between these two molecules cannot be adequately explained at present, we have reported similar data previously [44] using a LPS-induced lung injury model. In LPS-induced lung injury model, we demonstrated a time-dependent decrease in VEGF expression in pulmonary tissue compared to that of control rats. In contrast, pulmonary levels of TNF-α showed a significant up-regulation up to the 3 h time point and then returned to almost control levels at 6 h and 10 h after LPS administration [44]. Indeed, inverse relationships have also been described previously between TNF-α and VEGF receptors, i.e., Patterson et al. (1996) have demonstrated that TNF-α is capable of exerting significant anti-angiogenic activity by modulating the expression of VEGF receptors in cultured human vascular endothelial cells [45]. TNF-α is widely accepted as the central pro-inflammatory cytokine that mediates cellular responses to both endogenous and exogenous stimuli. Although it is responsible for significant a degree of VEGF release from a myriad of cell types, it’s (TNF-α) primary effects in high concentrations (as occurs in infection and malignancy) is anti-angiogenic [45]. Thus, high local or circulating levels of TNF-α may, in part, attenuate tissue repair by causing functional down-regulation of VEGF receptors on endothelial cells in these circumstances. But the reverse relationship between cardiac VEGF and TNF-α observed in the present study, as well as in our previous study [44], needs further investigations for more clarification.

**Conclusion**

Taken together, the present data led us to conclude that landiolol may be cardio-protective in septic rats by normalizing the expression...
of cardiac angiogenic growth factor, such as VEGF, without altering the circulatory levels of inflammatory cytokine, such as TNF-α. These findings may provide new windows of opportunities for future explorations on the use of landiolol in sepsis-induced multiple organ dysfunction syndrome.

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References


