Sex-mediated response to beta-blockers in sepsis:

an experimental, randomized study

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ABSTRACT

Objectives: To investigate any gender effect of the beta-1 adrenergic blocker, landiolol, on cardiac performance and energy metabolism in septic rats, and to explore the expression of genes and proteins involved in this process.

Design: Randomized animal study.

Setting: University research laboratory.

Subjects: Male and female Wistar rats.

Interventions: One hour after cecal ligation and puncture, male and female rats were randomly allocated to the following groups: sham male, cecal ligation and puncture male, cecal ligation and puncture + landiolol male, sham female, cecal ligation and puncture female, and cecal ligation and puncture + landiolol female. Cardiac MRI was carried out 18 hours after cecal ligation and puncture to assess in vivo cardiac function. Ex vivo cardiac function measurement and 31P magnetic resonance spectroscopy were subsequently performed using an isovolumic isolated heart preparation. Finally, we assessed cardiac gene and protein expression.

Measurements and Main Results: In males, landiolol increased indexed stroke volume by reversing the indexed end-diastolic volume reduction without affecting left ventricle ejection fraction. In females, landiolol did not increase indexed stroke volume and indexed end-diastolic volume but decreased left ventricle ejection fraction. Landiolol had no effect on ex vivo cardiac function and on high-energy phosphate compounds. The effect of landiolol on the gene expression of natriuretic peptide receptor 3 and on protein expression of pAKT:AKT ratio and endothelial nitric oxide synthase was different in males and females.

Conclusions: Landiolol improved the in vivo cardiac performance of septic male rats while deleterious effects were reported in females. Expression of natriuretic peptide receptor 3,

pAKT:AKT, and endothelial nitric oxide synthase are signaling pathways to investigate to better understand the sex differences in sepsis.

Keywords: adrenergic beta-blockers; cardiac function; energy metabolism; magnetic resonance imaging; sepsis; sex differences

INTRODUCTION

The adrenergic system plays a key role in septic shock (1). However, prolonged and excessive adrenergic stimulation is cardiotoxic (2). In critically ill patients, the administration of dobutamine to maintain an elevated cardiac index is associated with increased mortality (3). In a previous animal study, we have shown that use of a pure beta-agonist (isoproterenol) led to hypertrophic cardiac failure (4). In experimental models of sepsis esmolol, a selective $beta_1$-adrenergic blocker, improved cardiac contractility, stroke volume (SV) and vascular responsiveness to norepinephrine (5–7). In patients in established septic shock, Morelli *et al* also showed that esmolol infusion reduced heart rate (HR), increased SV and decreased norepinephrine requirements (8).

Experimentally, the host response to an inflammatory insult differs according to sex (9,10). We previously showed that male mice were at higher risk of developing more severe forms of infection from *Coxiella burnetii* (11,12). In an endotoxin model, *ex vivo* cardiac performance was more impaired in males (10). Sex also affects the response to beta-blockers. In healthy volunteers, young females had improved adrenergic receptor sensitivity to beta-adrenergic, as compared with their male counterparts (13,14).

To our knowledge, in sepsis, the gender-dependent response to beta-blockers remains undetermined. A sex-based approach may however be critical in personalizing the management of patients with septic shock. Our hypothesis was that beta-blockade would prevent cardiac dysfunction in males but not in females. We designed a rat model of intra-abdominal sepsis to assess the impact of landiolol, an ultra-short half-life beta-blocker on sex.

Landiolol has a high affinity for $beta_1$ receptors, with a $beta_1$:$beta_2$ ratio of 255, compared to 33 for esmolol (15). Its half-life is 4 min, which is potentially useful in highly unstable patients.

We thus assessed the sex-dependent effects of landiolol on *in vivo* cardiac performance during sepsis using magnetic resonance imaging (MRI). We further evaluated the effects of landiolol on *ex vivo* cardiac performance, energy metabolism and oxidative stress on an isolated perfused heart preparation. Finally, we investigated the expression of candidate genes involved in various pathways and known to be involved in cardiac function to elicit potential mechanisms of action during sepsis.

MATERIALS AND METHODS

Animals and cecal ligation and puncture model

Male and female Wistar rats (9-12 weeks old, Charles River, Saint-Germain sur l'Arbresle, France) were housed for a 5-7-day acclimatization period in a temperature and light-controlled room with free access to water and food. All animal procedures were conducted in accordance with national Guidelines for Care and Use of Laboratory Animals in conformity with the 2010/63 EU directive and with the approval of the Institutional Animal Care Committee of Aix-Marseille University (APAFIS#3746-201601221813985; May 2016). The anesthetic and surgical procedure is reported in the supplemental data (Supplemental Digital Content 1, http://links.lww.com/CCM/D503).

Experimental protocol

Supplement Figure 1 summarizes the experimental protocol. One hour after cecal ligation and puncture (CLP), males and females were randomized to receive landiolol (AOP Orphan, Vienna, Austria) diluted in n-saline and infused at 0.1 mg/kg/min or n-saline (10 mL/kg/h). The

infusion volume was similar in all groups. Six groups were studied: sham male (n = 6), CLP male (n = 8), CLP + landiolol male (n = 7), sham female (n = 7), CLP female (n = 8) and CLP + landiolol female (n = 7). Eighteen hours after surgery, rats were anesthetized using 1.5-2% isoflurane. The caudal ventral artery was cannulated (24-gauge catheter) for blood pressure recording (TruWave pressure transducer, Edwards Lifesciences, Irvine, CA). Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP) were recorded. Variation in body weight between Hours 0 and 18 were measured. Cardiac MRI was performed 18 hours after CLP to assess *in vivo* cardiac function (cine-MRI) and myocardial perfusion (Arterial spin labeling, ASL MRI). *Ex vivo* cardiac function measurement and ^{31}P magnetic resonance spectroscopy (MRS) were performed at Hour 19 using isolated heart preparation. At Hour 20, hearts were frozen for subsequent biochemical analyses and quantification of gene expression.

MRI assessment of in vivo cardiac function

MRI was performed 18 hours after CLP, immediately after arterial cannulation. During MRI, inhalation anesthesia was maintained with 1.4-1.8% isoflurane in 1.5 L/min oxygen continuously delivered through a face mask. A warming pad was placed on the back of the animal and body temperature recorded by a rectal probe. All images were acquired using a Bruker Biospec 4.7T/30 imager (Bruker Biospin, Rheinstetten, Germany). Animals were placed prone on an actively decoupled surface coil (Rapid Biomedical, Rimpar, Germany) used for radiofrequency reception. Homogeneous radiofrequency excitation was achieved using a proton volume resonator (diameter 60 mm; homogeneous length 80 mm). Ventilation was monitored using a pressure sensor connected to an air-filled balloon positioned under the abdomen. The electrocardiogram (ECG) was monitored by siting two subcutaneous thoracic

electrodes and HR was recorded. An ECG and respiration trigger unit (Rapid Biomedical, Würzburg, Germany) was used to gate the MR sequences.

A gradient-echo cine-MRI sequence was used to acquire strictly perpendicular slices in two- and four-chamber long-axis orientation and short-axis orientation at mid-ventricular level (FLASH, field of view, 4×4 cm^2; slice thickness = 2 mm; matrix size = 128 × 128; TR = 5.1 ms; TE = 1.2 ms; 45 phases per cardiac trigger). Image post-processing was performed using an in-house developed program running under an IDL environment (Interactive Data Language, ITT Visual Solutions, Boulder, CO, USA). Left ventricular volumes were determined using an ellipsoid model, with the left ventricle assimilated to an ellipsoid of revolution. Endocardial and epicardial areas were manually delineated on short-axis images, with ventricular lengths determined from four-chamber long-axis views in diastole and systole, respectively. End-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV), left ventricle ejection fraction (LVEF), mean wall thicknesses in diastole (WTd) and systole (WTs), and systolic wall thickening (WTn) were calculated from the volume measurements, as described previously (16). Cardiac output (CO) was calculated as CO = HR x SV. EDV (EDVi), ESV (ESVi), SV (SVi) and cardiac output (CI) were indexed to body surface area.

In vivo myocardial perfusion was quantified using the cine-ASL technique, as previously described (17). An ECG- and respiration-gated Look–Locker gradient-echo flow-sensitive alternating inversion recovery ASL technique was used to acquire two T1 maps from a single short-axis slice placed at ventricular mid-level, one after a slice selective inversion pulse and one after a global inversion pulse. The following parameters were used: field of view = 4 × 4 cm^2; slice thickness = 3 mm; matrix size = 128 × 64; train of 50 gradient echoes; angle = 12°.

The inversion slice was chosen to be 1.7 times thicker than the excitation slice and produced with an adiabatic 4-ms-duration hyperbolic secant pulse. The number of acquired signals (N) after each inversion was adapted as a function of the heart rate to obtain inversion times up to 7.5 s, leading to a total acquisition time of approximately 25 min including both inversion series. N was typically 40 at a heart rate of 310 beats/min. The long acquisition duration after labeling was used because of the post-processing required, in which two mono-exponential fits are performed for global and slice selective inversion, respectively. Image analyses were performed using a home-made program running in an IDL environment which generated absolute myocardial blood flow (MBF) maps. MBF was determined by manually delineating regions of interest in the entire left ventricular myocardium on the corresponding MBF maps.

Isolated rat heart preparation for ex vivo cardiac function and ^{31}P MRS

At Hour 19, rats were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital sodium. Blood samples were taken through the arterial catheter and immediately centrifuged. After bilateral thoracotomy, the heart was quickly excised and mounted onto an isolated perfused heart apparatus according to the Langendorff method (4). The aorta was cannulated and the heart retrogradely perfused at constant pressure with 37°C Krebs-Henseleit solution (containing in mmol/L : NaCl 118; KCl 4.7; $MgSO_4$ 1.2; $CaCl_2$ 1.75; ethylenediaminetetraacetate 0.5; $NaHCO_3$ 25; D-Glucose 11; pH 7.4) saturated with 95% O_2 and 5% CO_2.

A latex balloon was introduced into the left ventricle to evaluate *ex vivo* cardiac function. End-diastolic pressure was set to 10 mmHg. Left ventricular developed pressure (LVDP) and HR were monitored (18). The rate-pressure product (RPP), calculated as the product of LVDP and

HR, was used as an index of cardiac function. Coronary flow was measured by timed collection of the coronary venous effluent, and indexed by weight.

The perfused rat heart was placed in a 20-mm MR sample tube and inserted into a ^{31}P probe seated in the bore of a superconducting wide-bore (89-mm) 4.7-T magnet (Oxford Instruments, Oxford, UK) interfaced with a Bruker-Nicolet WP-200 spectrometer (Bruker, Karlsruhe, Germany). The appropriate conditions for acquiring ^{31}P magnetic resonance spectra and the quantification of phosphorus metabolites have been detailed previously (4,18). Briefly, a series of 8 spectra (4 min acquisition each) were recorded to determine phosphocreatine (PCr), adenosine triphosphate (ATP), inorganic phosphate (Pi), phosphomonoesters (PME) and intracellular pH (pHi). Cardiac function and ^{31}P MR spectra were simultaneously monitored during the perfusion protocol. For the quantification of gene expression and biochemical analyses, hearts were rapidly freeze-clamped with a Wollenberger clamp precooled in liquid nitrogen and kept at –80 °C before analysis.

Ribonucleic acid (RNA) extraction and quantitative reverse transcription-polymerase chain reaction

Total RNA was isolated from the left ventricles using TRIzol Reagent according to the manufacturer's instructions (Invitrogen, Life Technologies, Carlsbad, CA, USA). The quality of RNA was confirmed on the Agilent 2100 Bioanalyzer (Agilent Technologies, Germany) using the Agilent RNA 6000 Nano chips. The concentration of RNA was determined by reading absorbance at 260/280 nm on a NanoDrop (ND-1000, Thermo Scientific). RIN (RNA Integrity Number) ranged from 1.8 to 2.0, indicating good integrity of the RNA. cDNA synthesis was carried out with superscript VILO MasterMix (Invitrogen, Thermo Fisher Scientific,

Netherlands) on 1 µg of total RNA in 20 µl of final volume. Quantitative real-time PCR (qPCR) analyses of genes and housekeeping gene using SYBR Green PCR master mix (Applied Biosystems, Life technologies, UK) were performed on the Stratagene Mx3000P (Agilent Technologies). Supplemental Table S1 summarizes the primer sequences. The expression levels of candidate genes were normalized to RPL32 and the delta CT (cycle threshold) value used for subsequent statistical analysis.

Immunoblotting and western-blot analyses

Total protein was extracted from frozen LVs in RIPA lysis buffer supplemented with PMSF and protease inhibitors cocktail at 4°C (Santa Cruz). Protein quantification was performed with Q-Bit protein assay (Thermo). Protein extracts were denatured in SDS loading buffer supplemented with DTT. Lysates of 50-100µg were then subjected to electrophoresis using the NuPAGE® Bis-Tris gels (4-12%) (Invitrogen) and were blotted to nitrocellulose membrane using the iBlot2® gel transfer system (Life Technologies) according to the manufacturer's instructions. Blots were probed with the following antibodies: actin (Millipore MAB1501, 1/5000), Akt (Cell Signaling 9272, 1/1000), endothelial nitric oxide synthase (eNOS) type III (BD Biosciences 610296, 1/1000) and phosphorylated-Akt (Ser473) (Cell Signaling 9271, 1/1000). After washing, HRP-conjugated secondary antibodies were used. Blots were treated with ECL reagent (Western Lightning Ultra, Perkin Elmer). After exposure and scanning of films, analysis was performed using ImageJ software (NIH, place). These experiments were based on the results of a prior study assessing the effects of esmolol on cardiac dysfunction in sepsis (5).

Biochemical analyses in plasma

Plasma lactate concentration was measured using an assay kit (Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). After statistical evaluation, the number of rats required was 6 per group to show a 30% increase in SV by landiolol with a power and an alpha risk of 80% and 5%, respectively. Significant differences between groups were determined using two-way analysis of variance (ANOVA) followed by Sidak post-hoc testing with Graphpad Prism software (Graphpad Prism 7.0, La Jolla, CA, USA). A p value <0.05 was considered statistically significant. Isolated heart preparation data from 6 rats (CLP male (n = 2), sham female (n = 2), CLP female (n = 2)) were not determined due to traumatic removal.

RESULTS

Effects of CLP and landiolol on weight variation and temperature after 18 hours

In both males and females, CLP was associated with weight gain (p <0.001 for both) and hypothermia (p <0.001 and p = 0.003, respectively), as compared with sham animals (Table 1). The CLP + landiolol male group had a lower weight gain (p = 0.001) and less hypothermia (p = 0.015) than untreated CLP males. In septic females, landiolol did not impact upon either weight or temperature.

Effects of CLP and landiolol on hemodynamic variables after 18 hours

Landiolol decreased HR from 404 ± 9 to 330 ± 11 b/min in septic males (p <0.001), and from 388 ± 14 to 345 ± 14 b/min in septic females (p = 0.024) (Table 1). Landiolol did not affect blood pressure in males, whereas DBP and MBP decreased in landiolol-treated females (p = 0.002 and 0.013, respectively). Plasma lactate concentrations were similar in all groups.

Effects of CLP and landiolol on in vivo cardiac function using MRI (H18)

Cardiac MRI variables are reported in Table 2 and indexed parameters in Figure 2. In males, SVi, CI and EDVi decreased after CLP (p <0.001 *vs.* sham) whereas only CI and EDVi decreased in females (respectively, p = 0.020 and p = 0.046 *vs.* sham).

In septic males, landiolol administration increased SVI, ESVi and EDVi (all p <0.05) compared with untreated animals (Table 2, Figure 2). LVEF and systolic wall thickening (sWtn) did not differ between groups. In females, landiolol increased ESVi (p < 0.001) and decreased LVEF (p <0.001) and sWtn (p = 0.009) compared to untreated animals.

Effects of CLP and landiolol on ex vivo cardiac function in the isolated heart preparation

Table 3 shows myocardial function and coronary flow in the isolated rat heart preparation at Hour 19. CLP decreased LVDP and RPP in both males and females (all p <0.05). LVDP, RPP and coronary flow index did not differ between landiolol-treated and untreated CLP groups.

^{31}P MRS measurements of myocardial energy metabolism and pHi (Hour 19)

High-energy phosphate compounds and pHi measured by ^{31}P MRS are shown in Table 4. Data represent the average of the four last spectra. PCr, ATP, PME, Pi, pHi and PCr/ATP ratio were similar in male and female groups.

Effects of CLP and landiolol on myocardial gene expression (Hour 20)

Figure 3 shows gene expression of IL-10, IL-15 and IL-1ra in the left ventricle of sham, CLP and CLP + landiolol rats. More detailed results are given in Supplemental Table S2. IL-6, TNF-alpha, and IL-1beta expression did not differ between groups. In contrast, IL-10 increased in males and females after CLP (p <0.001 and p <0.01, respectively), without any effect of landiolol. IL-15 was lower in CLP females (p = 0.01 vs sham) and landiolol reversed this effect (p = 0.032 *vs.* CLP female group). IL-1ra was higher in the CLP female group compared to sham (p = 0.016) and landiolol attenuated this effect. These variations were not found in males (Figure 3).

Genes encoding signaling proteins known for their role in cardiac function are detailed in Supplemental Table 2. Regarding the steroid receptors, expression of the glucocorticoid (NR3C1), mineralocorticoid (NR3C2), estrogen (ESR1) and beta1-adrenergic (ADRB1) receptors were similar after CLP in males and females. Landiolol had no significant impact (Supplemental Table 2). Expression of blood pressure regulators (natriuretic peptide receptor NPR1, NPR2, NPR3, endothelin-1 receptor (EDNRA), angiotensin type-1A (AT1-AR), angiotensin type-2 receptors (AT2-R), angiotensin II Receptor Associated Protein (AGTRAP)) were also determined (Supplemental Table 2). CLP decreased NPR3 transcript levels in both males and females (p < 0.001) (Figure 4). Landiolol reversed this decrease in males (p >0.05 *vs.* sham males) but not in females (p = 0.002 *vs.* sham females). CLP did not affect expression of EDNRA in males and females (Figure 4), but landiolol reduced EDNRA expression level in females (p = 0.04 *vs.* CLP females). The expression of genes encoding proteins involved in the calcium-signaling pathway was also evaluated (Supplemental Table 2). In males, no effect of CLP was

observed on transcript levels of phospholamban (PLN), ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2 (ATP2A2) and ryanodine receptor 2 (RYR2). In females, RYR2 expression was decreased after CLP with landiolol reversing this effect xs(Figure 4).

In CLP males, the AKT:pAKT ratio decreased (p = 0.04 compared with sham males) (Figure 5) and this was unaffected by landiolol. In CLP females, the AKT:pAKT ratio was similar to sham and landiolol increased this ratio (p = 0.004 compared with CLP females) (Figure 5). In males, eNOS expression was higher in CLP than in sham (p = 0.004) Landiolol attenuated this effect (Figure 5). In females, CLP and landiolol did not affect eNOS expression, as compared with sham females. As compared with males, eNOS expression was higher in sham (p = 0.028) and landiolol females (p = 0.026).

DISCUSSION

To our knowledge, this is the first comparison of response to beta-adrenergic blockers in males and females with sepsis-induced myocardial dysfunction. Our results highlight disparities between the sexes. While beta-blockers improved cardiac performance in the septic males, they were associated with a reduction in LVEF and sWtn in the septic females. A strength of our study relies on the assessment of cardiac function using MRI, providing an *in vivo* assessment of cardiac function in addition to *ex vivo* evaluation. At variance with previous models based only on the isolated perfused heart (7,19,20), the *in vivo* study incorporates heart-vessel interactions in the analysis of cardiac performance. As in septic patients, beta-blockers were associated with reduced ventriculo-arterial decoupling (21,22), underlining the clinical relevance of the model.

In males, landiolol improved SVi, probably by reversing the reduction in EDVi after CLP, but without any significant effect on LVEF, CI and Wtn. This improvement in diastolic function is in line with previous experimental studies (7,20). Using a conductance catheter in male rats undergoing CLP, Kimmoun *et al* also found improved myocardial function with beta-blockade (5). A meta-analysis has shown an association between diastolic dysfunction and mortality in septic patients (23) so this may represent an important protective effect of beta-blockade.

In contrast to septic males, septic females however maintained SVi at the level of sham females. Landiolol did not improve SVI, CI and EDVi but decreased LVEF and sWtn. Cardiac performance *ex vivo* was more impaired in males than in females after endotoxin administration (10). In our *in vivo* studies, landiolol improved cardiac performance in septic males but appeared to be deleterious in females. While not yet formally examined in septic patients, women undergoing high risk surgery did not benefit from perioperative beta-blockade, a finding at variance with men (24). With exercise, the hemodynamic response to metoprolol in women resulted in a larger reduction of HR and blood pressure (25).

In our model, septic myocardial dysfunction was more pronounced in males. In an experimental model of fluid resuscitated sepsis, Khaliq *et al*. compared the hemodynamic effects of esmolol in predicted survivors and predicted non-survivors in a fecal peritonitis model using male rats only (26). In the predicted non-survivor group, esmolol infusion was associated with an increase in SV, while CO remained stable, whereas SV and CO decreased with esmolol in predicted survivors. Thus, the underlying severity of the septic myocardial dysfunction may impact upon the response to beta-blockers. In our study landiolol did not

alter myocardial blood perfusion nor indexed coronary flow in either gender. In septic patients, coronary flow is typically normal or increased (27).

Mitochondria are the predominant generators of ATP in most cell types. Cardiomyocyte mitochondrial dysfunction is characterized by ultrastructural damage, decreased activity of electron transport chain enzyme complexes, and an increase in reactive oxygen species (ROS) production (28–30). In sepsis, mitochondrial dysfunction is associated with the degree of organ failure (31). Here, sepsis did not affect high-energy phosphate compounds and pH_i, measured by ^{31}P MRS, using an isolated perfused heart preparation. Myocardial ATP content remained unchanged while LVDP decreased. In another model of sepsis using ^{31}P MRS, energetic parameters were also stable despite alterations in cardiac function (32,33), suggesting that energetic metabolism is not limiting cardiac function in sepsis. Conversely, using biochemical analyses, Escames *et al* however found a decrease in ATP production in hearts from septic mice (34).

In our study, CLP was associated with weight gain reflecting increased capillary leak. Landiolol minimized this gain in males. In critically ill patients, a positive fluid balance is associated with worse outcomes (35,36). Morelli *et al*. showed that esmolol infusion improved the microcirculation (37). Whether this acts through restoration of phosphorylation pathways merits further study; increased AKT phosphorylation was found both by us and others (5).

There was a sex-dependent effect on expression of genes involved in the inflammatory response in our septic animals. The lack of difference in TNF, IL-1 beta and IL-6. may reflect the late timepoint (24h) of collection of cardiac tissue (38). However, in line with a previous

study, landiolol did not seem to interacting with most inflammatory pathways (39). Of note, landiolol acts predominantly on beta-1 receptors. As reported elsewhere (1), the mechanism of beta-1 receptor activation on inflammatory pathways remains unclear, and at variance with that of beta-2 receptor activation.

NPR3 has an anti-apoptosis role and protects cardiomyocytes (40). NPR3 decreased in both sexes after CLP and landiolol could reverse this effect in the septic males only. Landiolol however reduced the expression of endothelin-1 a potent vasoconstrictor with pro-inflammatory properties (41,42) in the septic females only. This could favor the occurrence of hypotension associated with landiolol use.

In our model, we found a different expression of eNOS and AKT:pAKT in males and females. Females have a reduced susceptibility to cardiac ischemic injury with NO generation playing a pivotal protective role (43,44). In a model of endotoxemia, the protection of the female heart was associated with the AKT/eNOS pathway (45). Kimmoun *et al*. also showed an increase in AKT and eNOS phosphorylation with esmolol in a model of sepsis (5). These pathways may thus be implicated in the different response to beta-blockers according to sex.

Our study has several limitations. The ultra-short half-life of landiolol (15) can have resulted in low tissue concentrations in the isolated heart. Thus, in our *ex vivo* model, the drug effect may have been attenuated. The isolated heart also removes the effects of circulating myocardial depressant factors that are implicated in sepsis-induced myocardial dysfunction so this may not necessarily reflect the in vivo situation. Another limitation is the choice of the intravenous dose of landiolol, which was infused at a fixed dose of 0.1 mg/kg/min. The effect

of landiolol is dose-dependent (20). In a previous experimental study using male Wistar rats, this dose (0.1 mg/kg/min) reduced HR without affecting blood pressure (20). In our study, landiolol decreased HR by 18% and 10% in males and females, respectively. Blood pressure remained constant in males but decreased in females. The dose could be adapted to an individual animal's response, since the pharmacokinetics of beta-blockers depends on gender and age (25). Our septic rats did not develop profoun hypotension thus the role of norepinephrine was not assessed. Future studies should assess the action of landiolol in a septic shock model to reflect its use to date in patients (8). Furthermore, we studied rats of equivalent age to young adult humans; most patients with sepsis are however post-menopausal so our results may not necessarily reflect responses in older females

CONCLUSION

Our model confirms that cardiac function was more impaired in septic males compared to their female counterparts. Landiolol ameliorated the cardiac dysfunction in males, particularly in relation to an improvement in diastolic function. Landiolol however produced deleterious effects in females. Phosphorylation of AKT and altered expression of eNOS, NPR3 and endothelin-1 receptors are potential signaling pathways that merit further investigation in order to better understand sex differences.

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