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EXPERIMENTAL TRIALS

Sildenafil in endotoxin-induced pulmonary hypertension: an experimental study

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Abstract

Background: Sepsis and septic shock still represent great challenges in critical care medicine. Sildenafil has been largely used in the treatment of pulmonary arterial hypertension, but its effects in sepsis are unknown. The aim of this study was to investigate the hypothesis that sildenafil can attenuate endotoxin-induced pulmonary hypertension in a porcine model of endotoxemia.

Methods: Twenty pigs were randomly assigned to Control group (n = 10), which received saline solution; or to Sildenafil group (n = 10), which received sildenafil orally (100 mg). After 30 minutes, both groups were submitted to endotoxemia with intravenous bacterial lipopolysaccharide endotoxin (LPS) infusion (4 $\mu g.kg^{-1}.h^{-1}$) for 180 minutes. We evaluated hemodynamic and oxygenation functions, and also lung histology and plasma cytokine (TNFα, IL-1β, IL6, and IL10) and troponin I response.

Results: Significant hemodynamic alterations were observed after 30 minutes of LPS continuous infusion, mainly in pulmonary arterial pressure (from Baseline 19 $\pm$ 2 mmHg to LPS30 52 $\pm$ 4 mmHg, $p < 0.05$). There was also a significant decrease in PaO_2/FiO_2 (from Baseline 411 $\pm$ 29 to LPS180 334 $\pm$ 49, $p < 0.05$). Pulmonary arterial pressure was significantly lower in the Sildenafil group (35 $\pm$ 4 mmHg at LPS30, $p < 0.05$). The Sildenafil group also presented lower values of systemic arterial pressure. Sildenafil maintained oxygenation with higher PaO_2/FiO_2 and lower oxygen extraction rate than Control group but had no effect on intrapulmonary shunt. All cytokines and troponin increased after LPS infusion in both groups similarly.

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Conclusion: Sildenafil attenuated endotoxin-induced pulmonary hypertension preserving the correct heart function without improving lung lesions or inflammation.

Introduction

Sepsis and septic shock are still one of the major causes of morbidity and mortality in critically ill patients and the pathophysiology involves a dysregulated host response to infection leading to a life-threatening organ dysfunction.[1] Acute lung injury, a frequent complication of sepsis, is characterized by pulmonary edema and atelectasis that result in impaired gas exchange and hypoxemia. Pulmonary hypertension induced by hypoxemia, microthrombi, and increased production of vasoconstrictive agents is considered an aggravator factor.[2] Pulmonary hypertension increases the right ventricular afterload, which tends to reduce the right ventricular output, leading to right ventricular dysfunction.[3] Its etiology is attributed to several factors such as pulmonary microthrombosis due to platelet activating factor release or increase of inflammatory mediators such as tumor necrosis factor-α (TNF-α) and nitric oxide (NO).[2,4] There is no consensus on the therapeutic approach of pulmonary hypertension in sepsis. The effectiveness of a pulmonary vasodilator therapy has been limited by the lack of selectivity and potency. Most pulmonary vasodilators drugs have associated systemic vasodilator action, as well as an effect on pulmonary circulation not such prominent.[3]

Sildenafil inhibits phosphodiesterase type V increasing intracellular cGMP which causes hyperpolarization of smooth-muscle membranes and vascular relaxation.[5] Sildenafil has been largely used in the treatment of pulmonary arterial hypertension, with a significant reduction in pulmonary artery pressure values, improvement of functional capacity and quality of life.[6] There are some evidences suggesting that sildenafil have anti-inflammatory effects, reducing cytokines and improving endothelial function.[7,8] Despite these attractive attributes, very little data are available for the use of sildenafil in sepsis, mostly due to its hypotensive effects which could aggravate sepsis vasoplegic hypotension.[9,10]

The aim of this study was to investigate the hypothesis that sildenafil pretreatment can attenuate LPS-induced lung injury and pulmonary hypertension in a porcine model of endotoxemia. Further, we investigated the effects on hemodynamic, oxygenation parameters, and lung morphology. Lastly, we investigated the effects of sildenafil on the plasma cytokine levels (TNFα, IL-1β, IL6, and IL10), mediators of inflammation, and on troponin I, early indicator of right ventricular dysfunction related to pulmonary hypertension.

Methods

Animals

A total of 20 Large White pigs weighting 23.7 ± 2.5 kg were obtained from a commercial laboratory pig farm (Granja RG, Suzano, Brazil). The animals were fasted overnight with free access to water and transported to the laboratory facilities on the day of the experiment. The study was approved by the Ethics Committee of the Faculdade de Medicina da Universidade de Sao Paulo (n. 262/13).

Anesthesia and preparation

Following premedication with ketamine (5 $mg.kg^{-1}$ intramuscular) and midazolam (0.25 $mg.kg^{-1}$ intramuscular), a catheter was inserted into the auricular vein and anesthesia was induced with propofol (5 $mg.kg^{-1}$ intravenous). Anesthesia was maintained by isoflurane (1.4%). The lungs were mechanically ventilated (Primus ventilator, Dräger, Lübeck, Germany) with a tidal volume of 8 $mL.kg^{-1}$, 5 cmH_2O of positive end-expiratory pressure (PEEP), and respiratory rate was adjusted to maintain $EtCO_2$ between 35–45 mmHg, on volume-controlled ventilation with a FiO_2 of 40%. Pancuronium (0.1 $mg.kg^{-1}$ bolus followed by 5 $\mu g.kg^{-1}.min^{-1}$ continuous infusion) and normal saline (5 $mL.kg^{-1}.h^{-1}$) were administered during the experiment. Core temperature was maintained between 37–39 °C by using a heating pad. The right internal jugular vein was surgically exposed and a 7.5F pulmonary artery catheter was inserted (774F75, Edwards Lifesciences, Irvine, CA, USA) into the pulmonary artery to measure cardiac output (thermodilution technique), mean pulmonary artery pressure (MPAP), and central venous pressure (CVP). Cardiac index (CI), the systemic and pulmonar vascular resistance index (SVRI and PVRI), and left and right ventricular stroke work indices (LVSWI and RVSWI) were obtained directly from cardiac monitor (Vigilance II, Edwards Lifesciences, Irvine, CA, USA). A catheter was inserted into the right femoral artery for continuous blood pressure monitoring (Pulsiocath Picco PV2015L20, Pulsion Medical Systems, München, Germany) and blood gas sampling. Oxygenation data (DO_2i, VO_2i, O_2ER, and Shunt fraction) were calculated using standard equations: $DO_2i = CaO_2 \times CI \times 10$; $VO_2i = C(a\text{-}v)O_2 \times CI \times 10$; $O_2ER = (SaO_2 - SvO2)/SaO_2 \times 100$; Shunt fraction = $[(Cc'O_2\text{-}CaO_2)/(Cc'O_2\text{-}CvO_2)] \times 100$.

The right femoral vein was also cannulated for endotoxin infusion and fluid-administration. Urinary bladder was catheterized for urinary output.

Sildenafil dose

The dose of sildenafil was based on literature[5] and pilot studies in which increasing doses of sildenafil (20, 40, 80, and 100 mg) were administered till attenuation of pulmonary hypertension induced by LPS, which was defined as a MPAP value below 40 mmHg.

Experimental protocol

The animals were previously randomized by a computer program (Random.org) in one block and allocated into two groups using consecutive numbered envelopes to be opened after animal preparation by the laboratory technician responsible for drug preparation and administration: Control (CTL, n = 10), and Sildenafil (SIL, n = 10). After a 30-minute stabilization period, baseline parameters were collected and a single-dose of 100 mg sildenafil (Viagra, Pfizer) or saline was administered through a gastric tube. All measurements were performed by a researcher unaware of group allocation. After 30 minutes of sildenafil/saline administration, endotoxemia was induced by a bacterial lipopolysaccharide (LPS) endotoxin infusion at 4 $\mu g.kg^{-1}.h^{-1}$ intravenously from LPS0 until the end of study (*Escherichia coli* O111:B4 LPS, 2.000.000 EU. mg^{-1}, product number L2630, Sigma-Aldrich, St. Louis, MO, USA).

Blood gas analysis, hemodynamic, and oxygenation measurements were performed at baseline, prior to LPS infusion (T0), and every 30 minutes until 180 minutes of LPS infusion. A timeline for interventions and measurements is shown in Figure 1.

No vasopressors or fluid bolus were administered during protocol, even if hypotension was present.

Animals were euthanized at the end of the experimental procedure by deepening anesthesia (5% isoflurane) and potassium chloride administration (19.1%, 10 mL). Immediately after killing, lung samples were collected for histopathological analysis.

Transesophageal echocardiography

The echocardiographic study was conducted by a qualified professional, using an ultrasound system with transesophageal transducer 7.5/5.0 MHz (En CHD Display, Minnesota, USA). Echocardiographic images of the heart were obtained in apical four-chamber views. We measured left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), right ventricular end-diastolic volume (RVEDV), and left ventricular ejection fraction (LVEF) was calculated by Simpson's method.

Plasma cytokines and troponin

Seven milliliters of blood was withdrawn at baseline and at the end of the study (LPS180). The samples were centrifuged spun at 1200 g and plasma was stored at -80 °C for TNFα, IL-1β, IL6 and IL10 analysis, by standard kits of sandwich enzyme immunoassay technique (Quantikine, R&D Systems, Abingdon, UK). All measurements were performed in duplicate and according to the manufacturer's instructions. The plasma troponin I was analyzed using chemiluminescence immunoassay technique employing the diagnostic set Immulite Turbo Troponin I in semi-automatic analyzer equipment (Immulite Analyzer – Diagnostic Products Corporation DPC).

Histology

Samples of the non-dependent portion of right diaphragmatic lung were collected for histology, from the same location in all animals. Hematoxylin-eosin-stained lung slices were evaluated by an experienced investigator blinded to group allocation. Ten random non-coincident fields (100x magnification) were evaluated using a scoring system (ranging from 0 to 4) for intra- and extra-alveolar hemorrhage, intra-alveolar edema, inflammatory infiltration of the interalveolar septa, and airspace, atelectasis and overinflation.[11]

Statistical analysis

Sample size calculation was based on cytokine decrease observed by Cardici et al. (2011)[12] in a rat model of sepsis treated with sildenafil.

Data are expressed as mean $\pm$ SD or median (interquartile range or minimum and maximum) for parametric and non-parametric data, respectively. Normality was tested using D'Agostino & Pearson test. For normally distributed data, a two-way ANOVA for repeated measures was applied with treatment (Sildenafil or Control) and time as fixed effects *p*-values for the effects of both treatment and time are given, and a value ≤ 0.05 was considered significant. If a significant effect due to treatment was detected, the data were further analyzed *post hoc* using the Tukey's test. For non-parametric data, Mann-Whitney test was used. All animals were included in the analysis. Statistical analysis was performed with Prism 6 for windows (GraphPad Software) and SigmaPlot 11 (Systat Sofware).

Results

Hemodynamic data

Heart rate and cardiac index increased significantly without differences between groups at LPS 60 and LPS90 (Fig. 2). The MPAP and PVRI increased significantly in both groups; however, the Sildenafil group showed significantly lower values compared to Control group ($p < 0.05$) during all the LPS infusion period except for PVRI at LPS90. The MAP increased significantly at 30-minute of LPS infusion (LPS30) in all animals, and then decreased significantly below baseline values in the Sildenafil group.

Although fluid administration was similar in both groups (5 $mL.kg^{-1}.h^{-1}$ in both groups), urinary output was significantly higher in Control group (2.7 ± 1.2 vs 1.6 ± 0.7, $p = 0.014$).

Lactate and oxygenation data

There were significant increases in arterial lactate from 1.8 ± 0.6 (baseline) to 2.8 ± 0.8 $mmol.L^{-1}$ (LPS180) in the Control group and 2 ± 1.0 (baseline) to 3.25 ± 1.3 $mmol.L^{-1}$ (LPS180) in the Sildenafil group, with no difference between groups. The DO_2I increased significantly in all animals from LPS60 with no changes in VO_2I. The SvO_2 increased significantly in both groups but was significantly higher at LPS30 in the Sildenafil group. The intrapulmonary *shunt* increased

Table 1 Oxygenation data for all animals at baseline-180 min after LPS infusion.

	Baseline	LPS0	LPS30	LPS60	LPS90	LPS120	LPS150	LPS180	RM ANOVA
DO_2 ($mL.min^{-1}.m^{-2}$)									P1 = 0.952
CTL	484 ± 85	531 ± 103	469 ± 124	667 ± 77[a]	711 ± 57[a]	702 ± 85[a]	679 ± 133[a]	643 ± 127[a]	P2 < 0.001
SIL	511 ± 102	516 ± 70	528 ± 106	629 ± 104[a]	699 ± 120[a]	683 ± 109[a]	649 ± 107[a]	656 ± 127[a]	P3 = 0.648
VO_2I ($mL.min^{-1}.m^{-2}$)									P1 = 0.027
CTL	125 ± 22	122 ± 12	129 ± 33	129 ± 19	132 ± 21	128 ± 16	126 ± 41	135 ± 23	P2 = 0.336
SIL	125 ± 26	113 ± 24	99 ± 19	114 ± 19	108 ± 17	113 ± 13	119 ± 10	127 ± 19	P3 = 0.317
ERO_2 (%)									P1 = 0.084
CTL	26 ± 4	24 ± 3	29 ± 8	20 ± 3[a]	19 ± 3[a]	18 ± 2[a]	18 ± 5[a]	22 ± 7	P2 < 0.001
SIL	25 ± 3	22 ± 6	19 ± 4[b]	19 ± 5[a]	16 ± 3[a]	17 ± 4[a]	19 ± 5[a]	20 ± 5	P3 < 0.009
Qs/Qt									P1 = 0.521
CTL	5,3 ± 1,1	6,0 ± 1,4	6,0 ± 1,8	7,4 ± 1,2[a]	8,2 ± 1,8[a]	9,3 ± 2,6[a]	9,9 ± 2,5[a]	9,9 ± 3,3[a]	P2 < 0.001
SIL	5,6 ± 0,9	6,7 ± 1,7[a]	6,8 ± 1,0	7,7 ± 2,0[a]	9,1 ± 1,8[a]	8,6 ± 1,9[a]	7,9 ± 1,5[a]	8,0 ± 1,7[a]	P3 = 0.066
PaO_2/FiO_2									P1 = 0.364
CTL	411 ± 29	401 ± 32	369 ± 71[a]	389 ± 30	383 ± 32	365 ± 42[a]	348 ± 48[a]	334 ± 49[a]	P2 < 0.001
SIL	405 ± 26	392 ± 33	410 ± 34[b]	387 ± 39	380 ± 29	380 ± 28	379 ± 25	370 ± 33[a,b]	P3 < 0.001
Lactate ($mmol.L^{-1}$)									P1 = 0.427
CTL	1.8 ± 0.6	1.7 ± 0.5	1.6 ± 0.4	1.9 ± 0.4	2.1 ± 0.5	2.4 ± 0.7[a]	2.7 ± 0.8[a]	2.8 ± 0.8[a]	P2 < 0.001
SIL	2 ± 1.0	1.9 ± 0.9	1.8 ± 0.7	2.1 ± 0.7	2.2 ± 0.7	2.5 ± 0.7[a]	2.9 ± 1.1[a]	3.2 ± 1.3[a]	P3 = 0.942
SvO_2 (%)									P1 = 0.070
CTL	73.1 ± 3.9	75.7 ± 3.3	70.4 ± 8.2	79.5 ± 2.9[a]	80.4 ± 3.2[a]	80.4 ± 2.5[a]	80.4 ± 5.7[a]	76.6 ± 7.2[a]	P2 < 0.001
SIL	74.8 ± 3.1	77.0 ± 6.2	80.1 ± 4.5[b]	80.5 ± 4.8[a]	83.5 ± 2.6[a]	82.1 ± 3.8[a]	80.1 ± 4.6	79.0 ± 5.3	P3 < 0.008

CTL, Control group; SIL, Sildenafil group; DO_2I, oxygen delivery index; VO_2I, oxygen consumption index; ERO_2, oxygen extraction rate; Qs/Qt, intrapulmonary *shunt*; SvO_2, mixed venous oxygen saturation; RM ANOVA, Repeated measures ANOVA; P1, Group effect; P2, P Time effect; P3, interaction Group x Time.
Measurements are given as mean ± SD.
[a] $p < 0.05$ vs. baseline.
[b] $p < 0.05$ CTL vs. SIL.

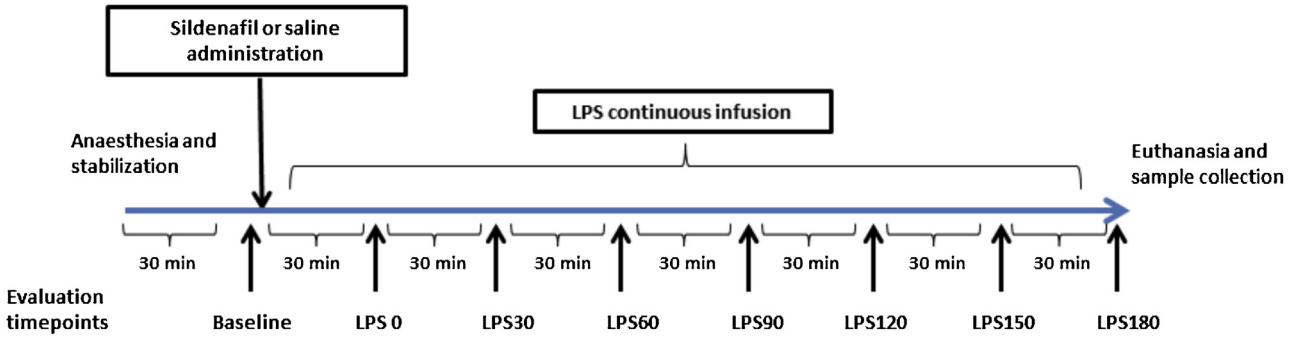

Figure 1 Timeline for interventions and measurements. LPS, lipopolysaccharide endotoxin.

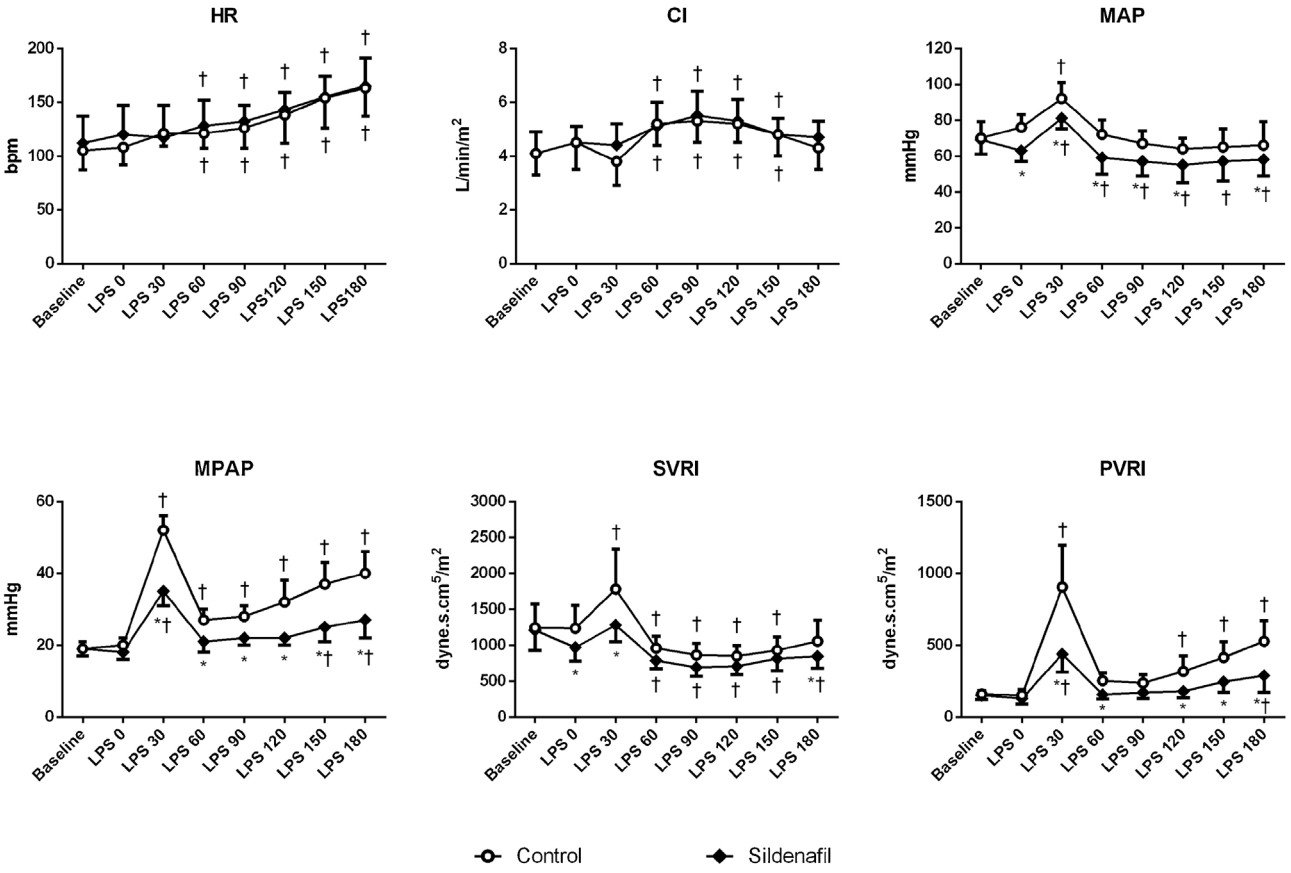

Figure 2 Hemodynamic variables in Control and Sildenafil group. HR, heart rate; CI, cardiac index; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; SVRI, systemic vascular resistance index; PVRI, pulmonary vascular resistance index.
† $p < 0.05$ vs. baseline.
* $p < 0.05$ CTL vs. SIL.

significantly over time in both groups, with no difference between groups. The PaO_2/FiO_2 decreased significantly in the Control group at LPS30, LPS 120, LPS 150 and LPS180 and was significantly lower than the Sildenafil group at LPS30 and LPS180. The O_2ER decreased in all animals from LPS60 to LPS150 with difference between groups at 30-minute of LPS infusion, in which the Sildenafil group showed lower values (Table 1).

Transesophageal echocardiography

There were no significant differences in the RVEDV in the Sildenafil group, but there were significant increases in the Control group with *post hoc* analyses identifying differences between groups at LPS30, LPS60, LPS120, and LPS180. LVEDV and LVESV decreased at LPS180 in both groups without differences between groups. For left ventricular ejection fraction,

Table 2 Echocardiography variables for all animals at baseline-180 min after LPS infusion.

	Baseline	LPS0	LPS30	LPS60	LPS120	LPS180	RM ANOVA
LV EF (%)							P1 = 0.719
CTL	62.6 ± 4.9	64.5 ± 6.5	61.6 ± 11.9	66.6 ± 7.5	62.9 ± 6.6	67.0 ± 6.4	P2 = 0.279
SIL	61.2 ± 3.2	63.8 ± 8.4	63.9 ± 8.5	66.1 ± 4.2	66.3 ± 4.8	63.0 ± 7.3	P3 = 0.391
LV EDV (mL)							P1 = 0.541
CTL	25.5 ± 6.1	26.6 ± 4.5	23.2 ± 4.5	27.6 ± 4.8	24.3 ± 5.8	21.4 ± 5.9[a]	P2 < 0.001
SIL	21.9 ± 4.8	22.0 ± 4.3	23.7 ± 6.5	21.4 ± 6.1	20.0 ± 2.7	17.4 ± 3.2[a]	P3 = 0.059
LV ESV (mL)							P1 = 0.512
CTL	9.8 ± 3.3	8.8 ± 2.5	9.0 ± 4.1	9.8 ± 3.7	9.2 ± 3.2	7.2 ± 2.8[a]	P2 = 0.002
SIL	8.4 ± 1.9	7.9 ± 2.1	8.0 ± 1.9	7.0 ± 1.4	6.8 ± 1.0	6.3 ± 1.6[a]	P3 = 0.691
RV EDV (mL)							P1 < 0.001
CTL	9.9 ± 2.1	10.7 ± 2.2	20.1 ± 3.3[a]	13.9 ± 3.6[a]	13.4 ± 3.0	13.6 ± 1.9[a]	P2 < 0.001
SIL	10.5 ± 1.7	10.7 ± 3.6	11.8 ± 4.4[b]	10.5 ± 3.6[b]	10.8 ± 2.3[b]	8.0 ± 1.7[b]	P3 < 0.001

CTL, Control group; SIL, Sildenafil group; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; RVEDV, right ventricular end-diastolic volume; RM ANOVA, Repeated measures ANOVA; P1, Group effect; P2, P Time effect; P3, interaction Group x Time.
Measurements are given as mean ± SD.
[a] $p < 0.05$ vs. baseline.
[b] $p < 0.05$ CTL vs. SIL.

Table 3 Histological scores in Control and Sildenafil group. Median (min-max).

	Control	Sildenafil	*p*-value
Atelectasis	1 (3–0)	2 (3–0)	> 0.05
Overinsuflation	12 (10–12)	12 (12–12)	> 0.05
Inflammation	10 (7–12)	9.5 (7–12)	> 0.05
Edema	0 (0–0)	0 (0–0)	> 0.05
Hemorrhage	3 (0–6)	3.5 (0–6)	> 0.05

no differences were observed with time and between groups (Table 2).

Cytokines and troponin

Plasma TNFα, IL-1β, IL6 and IL10 increased significantly with time in both groups, with no differences between groups. Troponin I concentration also increased in all animals, again without difference between groups (Fig. 3).

Histology

Histological evaluation revealed a predominance of intense mononuclear infiltrates with thickening of the alveolar septum, overinflation, disrupted alveolar septum, congestion, and areas of atelectasis in both groups. There were no differences in the histological scoring system between groups (Table 3).

Discussion

The major findings of this study are that in an experimental model of septic shock, (a) sildenafil attenuated endotoxin-induced pulmonary hypertension preserving right heart function, (b) sildenafil decreased systemic blood pressure, (c) sildenafil-maintained oxygenation without effect in shunt fractioning, and (d) sildenafil did not influence lung morphology, plasma cytokines, and troponin.

The endotoxemia was successfully induced by LPS infusion, with significant increase in pulmonary arterial pressure, tachycardia, serum cytokines, and cardiac troponin. A hyperdynamic state was observed after 30 minutes of LPS infusion, with a marked increase in pulmonary and systemic blood pressure. These LPS infusion effects on PAP were already described in other studies and are associated with the great presence of macrophages in porcine lungs and massive release of thromboxane and endothelin-1.[13] The LPS infusion in our protocol was based on Lipcsey et al. (2008) investigation,[14] who described an endotoxemia model of hypoperfusion and organic dysfunction. One possible explanation for the absence of marked hypotension/hypoperfusion, aside low dose of LPS, could be the anesthetic protocol employed in this study. We used isoflurane for anesthesia maintenance. In contrast, Lipcsey used sodium pentobarbital well-known agent to provoke more hemodynamic instability. To corroborate this fact, Schaefer et al. (1987) showed the different hemodynamic effect comparing several anesthetic agents in an endotoxic model in rats.[15]

Higher doses of LPS could be used to promote hypotension and decreased cardiac output, but also require vasoactive drugs and fluids resuscitation to prevent high mortality. Also, in some studies, LPS infusion was titrated to avoid excessive PAP increase and consequent right ventricle failure.[16] We choose a LPS dose to promote lung injury and pulmonary hypertension with mild effect on blood pressure to preserve hemodynamics during sildenafil effects. Otherwise, the animals would present excessive hemodynamic instability needing vasoactive drugs and fluid resuscitation, which would confound the sildenafil effects.

Although the LPS did not promote acute lung injury according to the Berlin definition, LPS-induced histologic lung lesions similar to acute lung injury, with marked mononuclear infiltrates in lung tissues, over inflation, and atelectasis.[17,18]

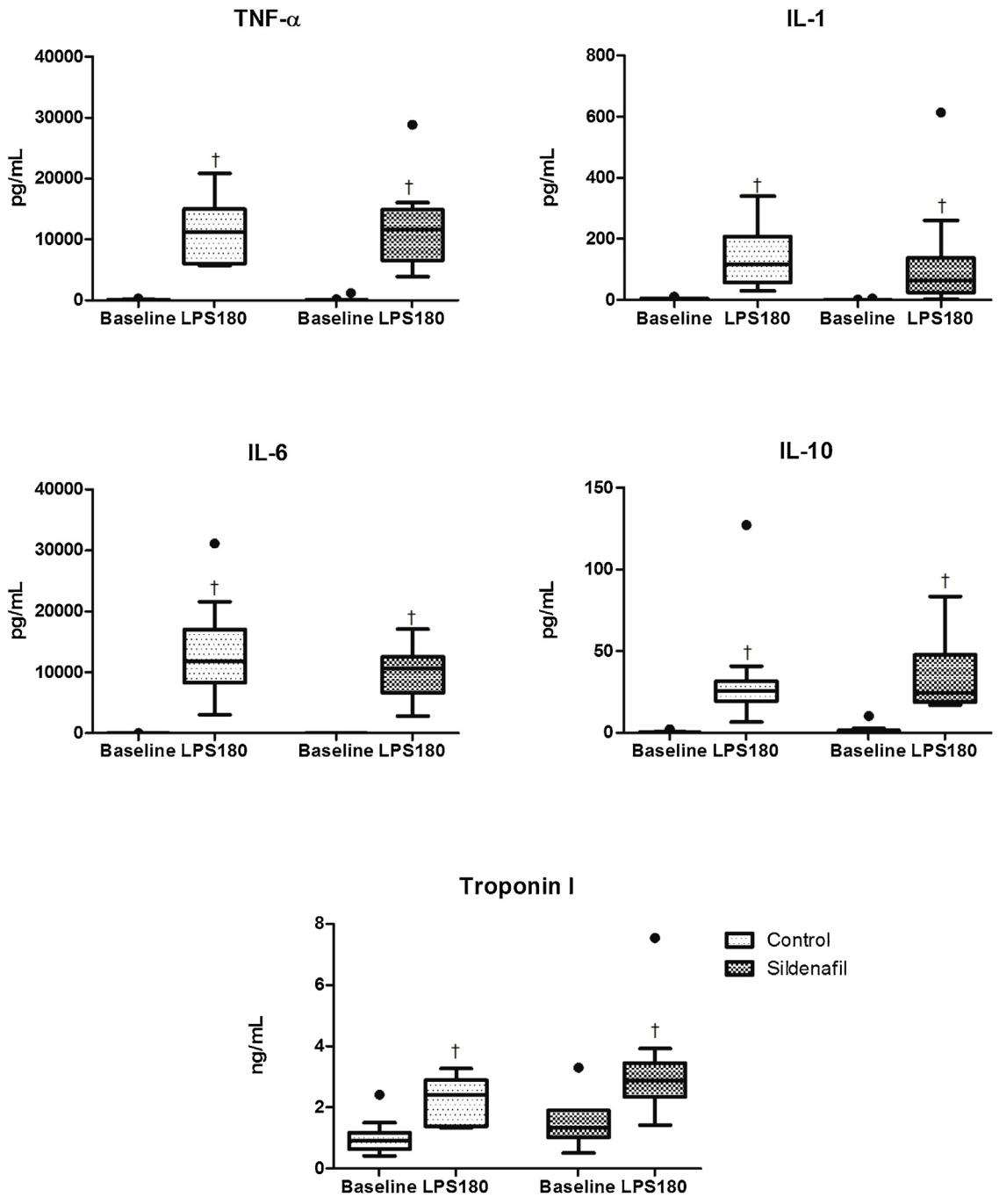

Figure 3 Cytokines and cardiac troponin in Control and Sildenafil group. * denotes significant intergroup differences ($p < 0.05$). • denotes outliers.

Sildenafil decreased pulmonary vascular resistance, pulmonary artery pressure, and RVSWI, decreasing afterload. Furthermore, animals that received sildenafil did not show changes in the end-diastolic volume of the right ventricle avoiding acute right heart dilation. The RV distention at end-diastole is a component of critical care echocardiography to diagnose RV failure as a potential cause of shock.[19] Sepsis-related myocardial dysfunction is not limited to only LV; the RV is also affected being present in about 30% of patients with severe sepsis[20]; by speckle tracking echocardiography, right ventricle dysfunction was detected in 72% of patients with severe sepsis or septic shock and was associated with high mortality.[21]

Several mechanisms lead to sepsis-related cardiac RV dysfunction. Anatomical characteristics and hypoxia due to low perfusion make it difficult to compensate RV afterload increases as we observe in acute pulmonary injury increased pulmonary vascular resistance.[3,22] Right ventricle dysfunction is associated with lower cardiac output, higher norepinephrine doses, higher troponin and lactate.[17] Therefore, sildenafil may play a role in attenuating the severity

of septic shock, avoiding RV dysfunction, but more studies are necessary.

The sildenafil effect in systemic arterial pressure has been previously reported and is dose-related.[5,10,23] In patients with primary pulmonary hypertension the decreased in MAP is clinically insignificant, but its effect in septic patients is probably deleterious[3,9] without a vasoactive support. In a porcine model of meconium-induced lung injury sildenafil reduced MAP even at the lowest dose used (0.4 $mg.kg^{-1}$), demonstrating a markedly systemic vasodilator effect in acute lung injury probably due to inflammation.[24] The decrease in MAP observed in this study might be explained by the high dose used or the presence of a significant systemic inflammation.

The DO_2I increased after LPS infusion in both groups as consequence of increase in cardiac index, resulting in greater supply of oxygen to tissues. In contrast to the report by Kleinsasser et al., where sildenafil caused increases in intrapulmonary shunt in anesthetized pigs which was reflected by marked decreases in PaO_2,[5] we found that sildenafil-maintained oxygenation without effect in shunt fractioning. In patients with pulmonary hypertension associated to lung fibrosis, sildenafil has shown to cause selective pulmonary vasodilation on well ventilated areas and improve gas exchange. It was proposed that sildenafil could amplify pulmonary vasoregulatory mechanisms, enhancing pulmonary nitric oxide effects.[25]

Sildenafil has demonstrated an anti-inflammatory effect in experimental models of acute lung injury and sepsis in rats.[12] We found no effect of sildenafil in cytokines in this porcine LPS model. Species differences, LPS dosage, and the short period of observation could explain the lack of differences in cytokines concentrations.

Troponin has been shown to be an early indicator of pulmonary hypertension related RV dysfunction.[26] All animals had higher troponin concentration at the end of the study. Our findings support that troponin increases in lung injury.[28] Elevated plasma levels of troponin are associated with poor outcomes in pulmonary hypertension and in acute lung injury.[26,27] Sildenafil has been shown to decreases myocardial leak of troponin in rat model of myocardial hypertrophy,[28] but this association in clinical settings of pulmonary hypertension is unknown. Although sildenafil was unable to reduce this cardiac marker in our study, further studies are necessary to evaluate this association.

In contrast to the report by Kiss et al.,[29] in which sildenafil presented a protective effect on lung morphology in monocrotaline (MCT)-induced rat pulmonary arterial hypertension model, we found no attenuation of endotoxin-induced lung lesions. One explanation for these divergent findings may be that sildenafil in this model of acute lung injury has no anti-inflammatory effect, thus cannot attenuate lung lesions. In a model of inhaled LPS airway injury model, sildenafil had shown no anti-inflammatory effects.[30]

There are limitations in our study. Because of the lack of information on the pharmacokinetics and pharmacodynamics of sildenafil in porcine model, the measurement of serum levels of sildenafil would be valuable. The oral administration prior to LPS infusion could be a limitation. Administration of sildenafil orally can cause differences in serum concentration and the injectable form would be more suitable for this purposed, but it is not available commercially. The chosen dose (100 mg) and time of administration were established based on Kleinsasser et al. (2001)[5] and our pilot study (25, 50 and 100 mg) data. Both in our preliminary study and Kleinsasser's, it was observed a decrease in MAP and MPAP 30 minutes after oral administration of sildenafil. Based on these findings we decide to administer sildenafil 30 minutes before LPS infusion to match sildenafil action onset and LPS endotoxemia. We believe that the same behavior would be observed in human patients, but additional studies are necessary to confirm our hypothesis. Finally, this was a model of acute porcine endotoxemia induced by bacterial LPS, and hence cannot be extrapolated to longer-term outcomes nor to clinical practices as it may not accurately reflect human septic shock physiopathology. Pigs show very important increase in pulmonary resistance in response to endotoxin, explained by the great presence of macrophages in porcine lung and the massive release of thromboxane A2.[12] Sample size and evaluation time could also be accounted for these negative results. However, considering the limitations of the study, it was possible to achieve the objective proposed to evaluate the effects of sildenafil in experimental endotoxemic shock in swine model, whose results may encourage future research with sildenafil in sepsis.

Conclusion

Sildenafil attenuated endotoxin-induced pulmonary hypertension, preserving right ventricle function and maintaining oxygenation without effecting shunt fractioning. However, sildenafil did not present an anti-inflammatory effect and did not attenuate lung lesions in this porcine model of endotoxemia. These data reinforce that sildenafil might be useful in patients with septic shock and cardiovascular and respiratory complications. However, more data are needed to determine the risk-benefit ratio of this drug in clinical practice.

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Conflicts of interest

The authors declare no conflicts of interest.

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