

Sildenafil attenuates pulmonary hypertension in acute porcine endotoxaemia

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Research article

Keywords: Phosphodiesterase V inhibitor; acute lung injury; LPS; septic shock; experimental model; swine.

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Abstract

Background Pulmonary hypertension is a risk factor for morbidity and mortality in septic shock. Pulmonary hypertension increases the right ventricular afterload, which tends to reduce the right ventricular output, leading to right ventricular dysfunction. 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 is able to attenuate endotoxin-induced pulmonary hypertension in a porcine model. Further, we investigated the effects on hemodynamic and oxygenation functions. We also evaluated lung morphology and the effect on plasma cytokine and troponin response. Methods Twenty pigs in which endotoxaemia was induced through intravenous bacterial lipopolysaccharide endotoxin (LPS) infusion (4µg/kg/h) were randomly assigned to Control group (n=10) – which received saline solution; or to Sildenafil group (n=10) – which received sildenafil orally (100 mg) previous the endotoxaemia. Results LPS induced a significant pulmonary hypertension with an increase in pulmonary arterial pressure, pulmonary vascular resistance index, end-diastolic volume of the right ventricle and also a decrease in PaO₂/FiO₂. Pulmonary and systemic arterial pressures were significantly lower in the Sildenafil group and sildenafil prevented the LPS effect on the end-diastolic volume of the right ventricle. Sildenafil maintained oxygenation with superior PaO₂/FiO₂ and lower oxygen extraction rate, but had no effect on intrapulmonary shunt. All cytokines and troponin increased after LPS infusion in both groups similarly. The LPS induced lung lesions, but there were no intergroup differences in the histological scoring system. Conclusion Sildenafil attenuated endotoxin-induced pulmonary hypertension preserving right heart function and maintained oxygenation without effecting shunt fractioning. However, caution should be taken due to the potential systemic vasodilatory effect. Sildenafil did not attenuate lung lesions and did not presented an anti-inflammatory effect in a porcine model of endotoxaemia.

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Key words: Phosphodiesterase V inhibitor; acute lung injury; LPS; septic shock; experimental model; swine.

Background

Pulmonary hypertension is one of the major causes of morbidity and mortality in critically ill patients with septic shock [1]. The pathophysiology includes pulmonary hypertension, formation of extravascular lung water (EVLW), and deterioration of pulmonary gas exchange [2]. Among that, pulmonary hypertension is considered an aggravator factor [3]. Pulmonary hypertension increases the right ventricular afterload, which tends to reduce the right ventricular output, leading to right ventricular dysfunction [4]. 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) [3, 5-7]. 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 [4].

Sildenafil inhibits phosphodiesterase type V, increasing intracellular cGMP which causes hyperpolarization of smooth-muscle membranes and vascular relaxation [8]. 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 [9]. Despite these attractive attributes, very little data are available for the use of sildenafil in sepsis.

The aim of this study was to investigate the hypothesis that sildenafil is able to attenuate endotoxin-induced pulmonary hypertension in a porcine model. Further, we investigated the effects on hemodynamic, oxygenation parameters, and lung morphology. Lastly, we investigated the effect of sildenafil on the plasma cytokine levels and troponin release.

Methods

Animals

A total of 20 Large White pigs weighting 23.7 \pm 2.5kg 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 Ethics Committee of the Faculdade de Medicina da Universidade de Sao Paulo (n.262/13).

Anaesthesia and preparation

Following premedication with ketamine (5mg/kg intramuscular) and midazolam (0.25mg/kg intramuscular), a catheter was inserted into the auricular vein and anaesthesia was induced with propofol (5mg/kg intravenous). Anaesthesia was maintained by isoflurane (1.4%). The lungs were mechanically ventilated (Primus ventilator, Dräger, Lübeck, Germany) with a tidal volume of 8ml/kg, 5cmH₂O of positive end-expiratory pressure (PEEP) and respiratory rate was adjusted to maintain EtCO₂ between 35-45mmHg, on volume controlled ventilation with a FiO₂ of 40%. Pancuronium (0.1mg/kg followed 5 µg/kg/min intravenous) and normal saline (5 ml/kg/h) were administered during the experiment. Core temperature was maintained between 37° and 39°C by using a heating pad.

The right internal jugular vein was surgically exposed and a 7.5 F pulmonary artery catheter was inserted (Swan-Ganz Catheter, Baxter Healthcare Corporation; Irvine, CA, USA) into the pulmonary artery to measure cardiac output (thermodilution technique), mean pulmonary artery pressure (MPAP), and central venous pressure (CVP). Standard formulas were used to calculate the cardiac index (CI), the systemic and pulmonary vascular resistance index (SVRI and PVRI), and left and right ventricular stroke work indices (LVSWI and RVSWI). A catheter was inserted into the right femoral artery for continuous blood pressure monitoring (Pulsioath Picco PV2015L20, Pulsion Medical Systems, München, Germany) and blood gas sampling. Oxygenation data (DO_{2i}, VO_{2i}, O₂ER and Qs/Qt) were calculated using standard equations.

The right femoral vein was also cannulated for endotoxin infusion and fluid-administration.

Sildenafil dose

The dose of sildenafil was based on 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 into two groups: Control (CTL, *n*=10), and Sildenafil (SIL, *n*=10). After 30-min stabilization period, baseline parameters were collected and a single-dose of 100 mg sildenafil (Viagra, Pfizer) or saline was administered through a gastric tube. After 30-min of sildenafil/saline administration, endotoxaemia was induced by a bacterial lipopolysaccharide (LPS) endotoxin infusion at 4µg/kg/h intravenously (*Escherichia coli* O111:B4 LPS, 2.000.000 EU/mg, product number L2630, Sigma-Aldrich, St. Louis, MO, USA).

Blood sampling, hemodynamic and oxygenation measurements were performed at baseline, prior to LPS infusion (T₀), and every 30-min until 180 min of LPS infusion. A timeline for interventions and measurements is shown in Fig 1.

Animals were euthanized at the end of the experimental procedure by deepening anesthesia (5% isoflurane) and potassium chloride administration (19.1%, 10ml).

Fig 1.

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 ml of blood was withdrawn at baseline and at the end of the study (LPS180). The samples were centrifuged spun at 1200g and plasma was stored at -80°C for TNF α , IL-1 β , 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 right diaphragmatic lung were collected for histological examination and stained with hematoxylin-eosin. The histological evaluation was performed 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 [10].

Statistical analysis

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 were performed with Prism 6 for windows (GraphPad Software) and SigmaPlot 11 (Systat Software).

Results

Hemodynamic data

Heart rate and cardiac index increased significantly without differences between groups at LPS 60 and LPS90 (table 1). The MPAP and PVRI increased significantly in both groups; however the Sildenafil group showed significantly lower values compared to Control group during all the evaluation period except for PVRI at LPS90 (Fig 2). The MAP increased significantly at 30-min of LPS infusion (LPS30) in all animals, and then decreased significantly below baseline values in the Sildenafil group. The RVSWI increased significantly in all time points after LPS infusion in the control group, whereas in Sildenafil group the increase was significantly higher only at LPS30.

Table 1.

Fig 2.

Lactate and oxygenation data

There were significant increases in arterial lactate from 1.8 ± 0.6 (baseline) to 2.8 ± 0.8 mmol/l (LPS180) in the Control group and 2 ± 1.0 (baseline) to 3.25 ± 1.3 mmol/l (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 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-min of LPS infusion, in which the Sildenafil group showed lower values (table 2).

Table 2.

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, no differences were observed with time and between groups (table 3).

Table 3

Cytokines and troponin

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

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 4

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 endotoxaemia 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 were already described in other studies and are associated with massive release of thromboxane and endothelin-1 [11].

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 [12,13].

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 [14]. 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 [15,16]; 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 [17].

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. [4,18]. Right ventricle dysfunction is associated with lower cardiac output, higher norepinephrine doses, higher troponin and lactate [16]. 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 [8,19,20]. In patients with primary pulmonary hypertension the decreased in MAP is clinically insignificant, but its effect in septic patients is probably deleterious [4,21] 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), demonstrating a markedly systemic vasodilator effect in acute lung injury probably due to inflammation [22]. 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 Keinsasser et al., where sildenafil caused increases in intrapulmonary shunt in anesthetized pigs which was reflected by marked decreases in PaO_2 [8], 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 [23].

Sildenafil has demonstrated an anti-inflammatory effect in experimental models of acute lung injury and sepsis in rats [24]. We found no effect of sildenafil in cytokines. Although one reasonable explanation for these divergent findings may be the species differences, but we cannot exclude that sildenafil might have no anti-inflammatory effect in this LPS model.

Troponin has been shown to be an early indicator of pulmonary hypertension related RV dysfunction. [25]. All animals had higher troponin concentration at the end of the study. Our findings support that troponin increases in lung injury [26,27]. Elevated plasma levels of troponin are associated with poor outcomes in pulmonary hypertension and in acute lung injury [25,27,28]. Sildenafil has been shown to decrease myocardial leak of troponin in rat model of myocardial hypertrophy [29], 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. [30], 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 acute lung injury have no anti-inflammatory effect, thus cannot attenuate lung lesions.

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. Moreover, 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. Finally, this was a model of acute porcine endotoxaemia 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.

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 maintained oxygenation without effecting shunt fractioning. Finally, sildenafil did not present an anti-inflammatory effect in a porcine model of endotoxaemia. 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.

Abbreviations

CVP: central venous pressure; CTL: Control group; cGMP: guanosine cyclic monophosphate; EVLW: extravascular lung water; HR: heart rate; IC: cardiac index; LPS: lipopolysaccharide endotoxin; MPAP: mean pulmonary artery pressure; LVEDV: left ventricular end-diastolic volume; LVEF: left ventricular ejection fraction; LVESV: left ventricular end-systolic volume; LVSWI: left ventricular stroke work index; NO: nitric oxide; PEEP: positive end-expiratory pressure; PVRI: pulmonar vascular resistance index; RVEDV: right ventricular end-diastolic volume; RVSWI: right ventricular stroke work index; SIL: Sildenafil group; SVRI: systemic vascular resistance index; TNF-a: tumor necrosis factor-a

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Faculdade de Medicina da Universidade de Sao Paulo (n.262/13).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing of interest.

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Authors' contributions

Conception and design: DTF, JOCA Jr, ; Data collection: DAGK,DAO, DRRM; Data analysis: DAO, COM, RAM; Drafting the manuscript: DAGK, DAO Revision of the manuscript after critical review: DTF, JOCA Jr. All authors read and approved the final manuscript.

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Figure Legends

Fig 1. Time line for interventions and measurements.

LPS: lipopolysaccharide endotoxin

Fig 2. Mean arterial pressure, mean pulmonary arterial pressure, systemic vascular resistance index and pulmonary vascular resistance index in Control and Sildenafil group. ☒ p<0.05 vs. baseline . *p<0.05 CTL vs. SIL.

MAP: mean arterial pressure; MPAP: mean pulmonary arterial pressure; SVRI: systemic vascular resistance index; PVRI: pulmonary vascular resistance index.

Fig 3. Cytokines and cardiac troponin in Control and Sildenafil group. * denotes significant intergroup differences (P<0,05). • denotes outliers

Tables

Due to technical limitations, tables 1 through 4 are only available as a download in the supplemental files section.

Figures

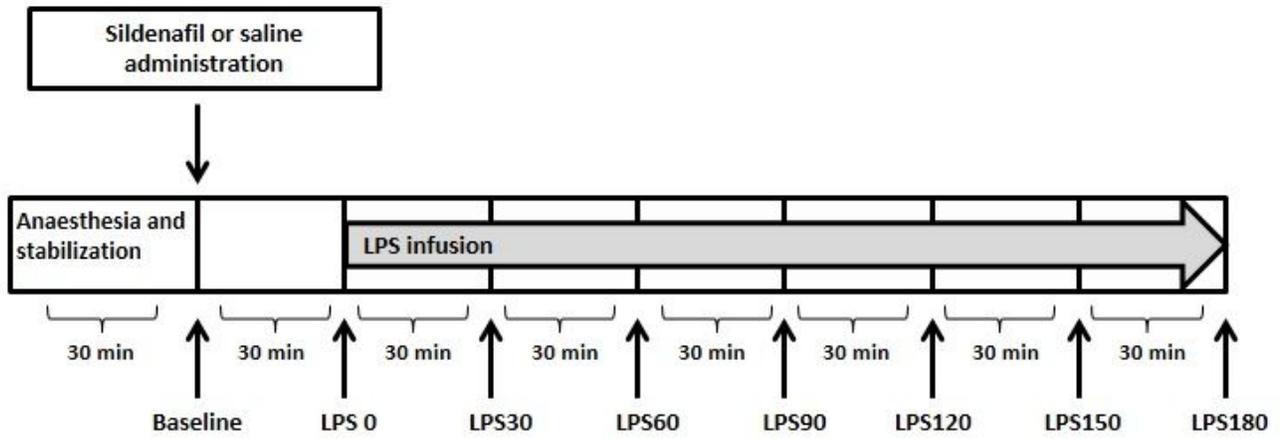


Figure 1

Fig 1. Time line for interventions and measurements. LPS: lipopolysaccharide endotoxin

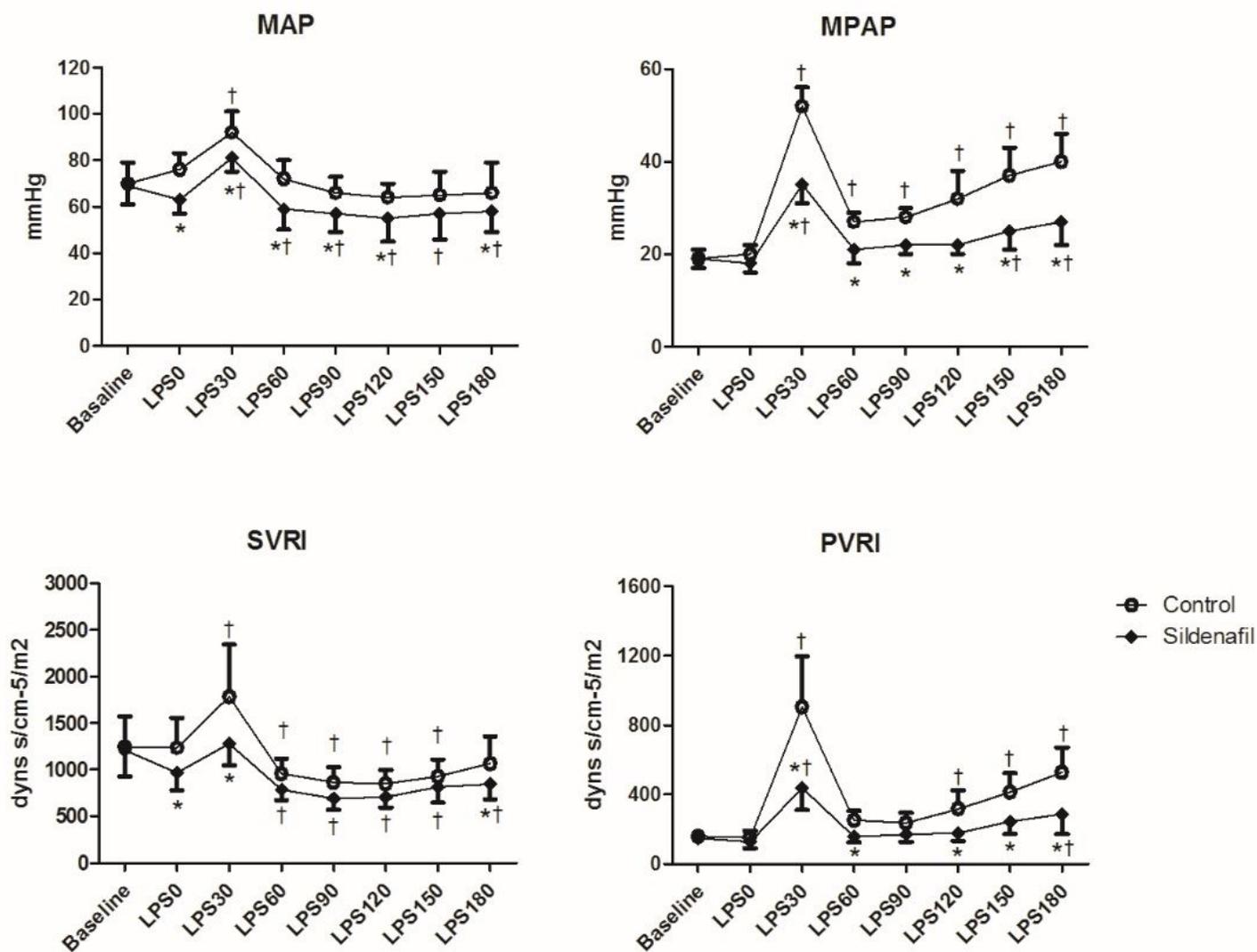


Figure 2

Fig 2. Mean arterial pressure, mean pulmonary arterial pressure, systemic vascular resistance index and pulmonary vascular resistance index in Control and Sildenafil group. † p<0.05 vs. baseline. *p<0.05 CTL vs. SIL. MAP: mean arterial pressure; MPAP: mean pulmonary arterial pressure; SVRI: systemic vascular resistance index; PVRI: pulmonary vascular resistance index.

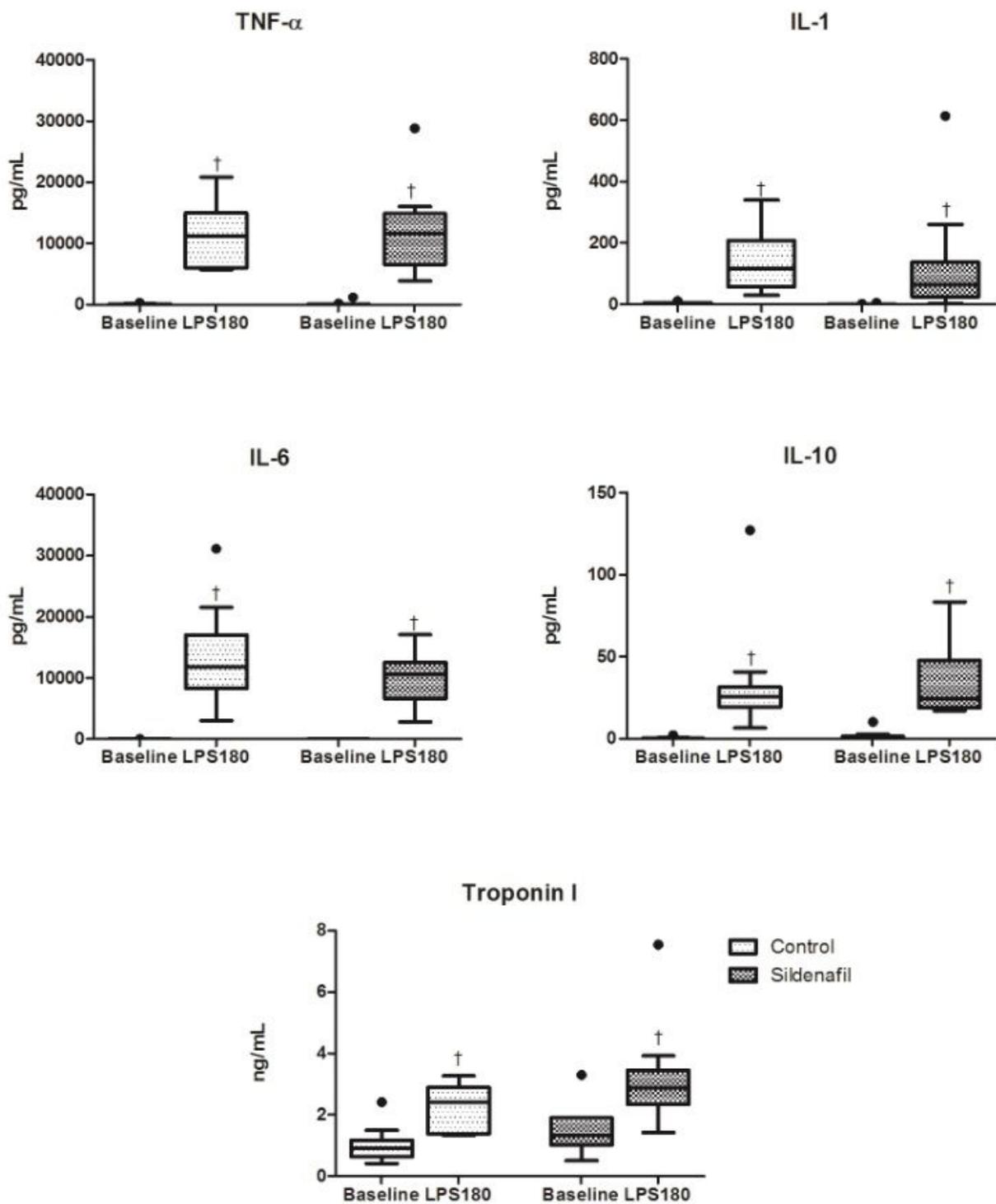


Figure 3

Fig 3. Cytokines and cardiac troponin in Control and Sildenafil group. * denotes significant intergroup differences ($P < 0,05$). • denotes outliers

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