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Hemodynamic effects of combined sildenafil and L-arginine during acute pulmonary embolism-induced pulmonary hypertension

Allethea R. Souza-Silva [a], Carlos A. Dias-Junior [a], Juliana A. Uzuelli [a], Heitor Moreno Jr. [b], Paulo R. Evora [c], Jose E. Tanus-Santos [a,*]

[a] Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes, 3900, Ribeirao Preto, SP, 14049-900, Brazil
[b] Department of Pharmacology, State University of Campinas, University of Sao Paulo, Av. Bandeirantes, 3900, Ribeirao Preto, SP, 14049-900, Brazil
[c] Division of Experimental Surgery, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes, 3900, Ribeirao Preto, SP, 14049-900, Brazil

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Abstract

Sildenafil attenuates acute pulmonary embolism-induced pulmonary hypertension. However, the hemodynamic effects of sildenafil in combination with other vasodilators during acute pulmonary embolism have not been examined yet. In the present study, we examined the hemodynamic effects of combined sildenafil (0.25 mg/kg, i.v.) and L-arginine (100, 200, 500, and 1000 mg/kg/h, i.v.) in an anesthetized dog model of acute pulmonary embolism. Plasma nitrite/nitrate (NO_x) and cGMP concentrations were determined using an ozone-based chemiluminescence assay and a commercial enzyme immunoassay, respectively. We found that L-arginine alone did not attenuate acute pulmonary embolism-induced pulmonary hypertension. However, significant decreases in mean pulmonary artery pressure were observed 30, 45, 60, and 75 min after the administration of sildenafil alone or after the combined administration of sildenafil and L-arginine (all $P<0.05$). No significant differences among groups were observed in the respiratory parameters. While L-arginine significantly increased NO_x concentrations, cGMP concentrations increased only when sildenafil was administered (all $P<0.05$). These results suggest that while sildenafil attenuates acute pulmonary embolism-induced pulmonary hypertension, L-arginine does not enhance the beneficial hemodynamic effects of sildenafil. In addition, these findings suggest that stimulation of NO synthesis with L-arginine during acute pulmonary embolism does not produce beneficial effects.

Keywords: Acute pulmonary embolism; L-arginine; Nitric oxide; Phosphodiesterase-5 inhibitor; Pulmonary hypertension; Sildenafil

1. Introduction

Acute pulmonary embolism is a severe disease resulting from the migration of emboli to the lungs and obstruction of pulmonary vessels (Sadosty et al., 2003). While very little progress has been made in the pharmacological management of the acute right heart failure and circulatory shock of this high-mortality condition (Layish and Tapson, 1997), accumulating experimental evidence indicates that blockade of the pulmonary vasoconstriction may have a role in the management of acute pulmonary embolism (Smulders, 2000; Stratmann and Gregory, 2003; Tanus-Santos et al., 2000a,b; Tanus-Santos and Theodorakis, 2002). Particularly, the use of drugs that selectively dilate pulmonary vessels may attenuate acute pulmonary embolism-induced pulmonary hypertension without causing systemic hypotension. In this regard, we and others have demonstrated that inhaled nitric oxide (NO) consistently attenuates the pulmonary hypertension found in different animal models of acute pulmonary embolism (Bottiger et al., 1996; Tanus-Santos et al., 1999a,b; Weimann et al., 2000). Moreover, L-arginine, which is substrate for NO synthesis, attenuated acute pulmonary embolism-induced pulmonary hypertension in an isolated rat lung model of acute pulmonary embolism through mechanisms involving increased NO synthesis (Souza-Costa et al., 2005). Taken together, these experimental data suggest that increasing NO bioavailability in the pulmonary vessels may attenuate acute pulmonary embolism-induced hypertension.

Since many of the biological effects of NO are mediated by cyclic guanosine 3′,5′-monophosphate (cGMP), which is produced after NO activates soluble guanylate cyclase, an alternative to achieve increased endogenous cGMP concentrations in

* Corresponding author. Tel.: +55 16 602 3163; fax: +55 16 633 2301.
E-mail address: tanus@fmrp.usp.br (J.E. Tanus-Santos).

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pulmonary vessels is the use of sildenafil, a specific phosphodiesterase type 5 (PDE5) inhibitor (Webb et al., 2000). Indeed, sildenafil has recently been studied as a selective pulmonary vasodilator in a dog model of acute pulmonary embolism (Dias-Junior et al., 2005a,b), thus suggesting that direct or indirect activation of the NO-cGMP pathway in the pulmonary vessels attenuates acute pulmonary embolism-induced hemodynamic derangements.

While sildenafil has been shown to attenuate acute pulmonary embolism-induced pulmonary hypertension without causing systemic hypotension (Dias-Junior et al., 2005a,b), the hemodynamic effects of sildenafil in combination with other vasodilators during acute pulmonary embolism have not been examined yet. In the present study, we hypothesized that L-arginine would enhance the beneficial hemodynamic effects of sildenafil in a dog model of acute pulmonary embolism. In addition, although L-arginine has been shown to attenuate acute pulmonary embolism-induced pulmonary hypertension when administered before lung embolization (Souza-Costa et al., 2005), no previous study has examined whether L-arginine attenuatesacute pulmonary embolism-induced hemodynamic derangements in a whole animal setting. Therefore, we have also addressed the in vivo effects of L-arginine infused intravenously during acute pulmonary embolism.

2. Material and methods

2.1. Animal model and hemodynamic measurements

The study complied with international guidelines of the European Community for the use of experimental animal and was approved by the institutional ethics committee. We used a whole animal model of acute pulmonary embolism (Dias-Junior et al., 2005a,b) to study the effect of the combination of sildenafil and L-arginine on acute pulmonary embolism-induced hemodynamic changes. Thirty-four mongrel dogs (13.9±1.7 kg) of either sex were anesthetized with ketamine (10–15 mg/kg, i.m.), xylazine (1,5 mg/kg, i.m.), and pancuronium (0.1 mg/kg, i.v.), tracheally intubated, and their lungs mechanically ventilated with room air using a volume-cycled respirator (C.F. Palmer, London, UK). The tidal volume was 15 ml/kg and the respiratory rate was adjusted to maintain a baseline physiologic arterial carbon dioxide tension. Anesthesia was maintained with an intramuscular injection of ketamine (3 mg/kg) and xylazine (0.3 mg/kg) every 30 min. Fluid-filled catheters were placed into the left femoral artery and right femoral vein for mean arterial pressure monitoring via a pressure transducer and fluid administration, respectively. A 7.5 F balloon-tipped Swan-Ganz thermodilution catheter was placed into the pulmonary artery via the left femoral vein, its correct location being confirmed by detection of the typical pressure wave of this artery. The catheter was connected to pressure transducers to allow the monitoring of mean pulmonary artery pressure, central venous pressure, and pulmonary capillary wedge pressure. The transducers were zeroed at the level of the right heart and recalibrated before each set of measurements. Thermodilution cardiac output was determined in triplicate by injecting 3 ml of saline and the results recorded on a computerized system (Monitor DC Baxter, Edwards Critical Care Vigilance, Irvine, CA). The heart rate was measured using a surface electrocardiogram (lead I). Blood samples were drawn from the femoral artery at predetermined times for blood gas analysis. Arterial oxygen tension, arterial O_2 saturation, carbon dioxide tension, and pH were determined using a blood gas analyser (Stat Profile 5 Analyser; Biomedical, Waltham, MA).

After at least 20 min for stabilization, a baseline hemodynamic evaluation was performed. Thereafter, acute pulmonary embolism was induced as previously described (Dias-Junior et al., 2005a,b). Briefly, repeated injections (every 30 s) of 300 μm microspheres (Sephadex G50; Pharmacia Fine Chemicals; Uppsala, Sweden) into the inferior vena cava over 5–10 min. The amount of microspheres infused in each dog was adjusted to induce an increase of 20 mm Hg in mean pulmonary artery pressure. Hemodynamic evaluation was performed 15 and 30 min (15E and 30E time points, respectively) after the acute pulmonary embolism was induced and the animals were randomly assigned to one of four experimental groups: (1) dogs in Sild group ($N=8$) received sildenafil 0.25 mg/kg (Pfizer, São Paulo, Brazil) infused intravenously in 15 min (Dias-Junior et al., 2005a,b); (2) dogs in Arg group ($N=7$) received L-arginine at doses of 100, 200, 500, and 1000 mg/kg/h, each dose infused intravenously for 15 min; (3) dogs in Sild+Arg group ($N=8$) received a co-infusion of sildenafil and L-arginine, which corresponded to the combined infusions given to dogs in Sild and Arg groups, (4) dogs in the Control group ($n=8$) received the same volume of saline. Finally, a group of Sham operated, non-embolized animals ($n=3$) received only saline infusions. Hemodynamic evaluations were performed every 15 min after 30E (15, 30,45, 60, and 75 time points). The cardiac index, systemic vascular resistance index, and pulmonary vascular resistance index were calculated by standard formulae. Arterial blood samples were drawn at baseline, 15E, 30E, 15, 30, 45, 60, and 75 time points for blood gas analysis and measurement of plasma nitrite/nitrate, and cGMP concentrations as described below.

2.2. Measurement of plasma nitrite/nitrate concentrations

Arterial blood samples were collected in tubes containing EDTA. After blood centrifugation at 800×g for 5 min, plasma aliquots were removed and stored at −70 °C until analyzed in duplicates for their nitrite and nitrate (NO_x) content using an ozone-based chemiluminescence assay (Castro et al., 2004; Souza-Costa et al., 2005; Wang et al., 2004). Briefly, the plasma samples were treated with a 2:1 volume of cold ethanol and centrifuged at 14,000×g for 5 min. NO_x were measured by injecting 25 μL of the supernatant in a glass purge vessel containing vanadium (III) in 1 N hydrochloric acid at 90 °C, which reduces NO_x to NO gas. A nitrogen stream was bubbled through the purge vessel containing vanadium (III), then through 1 N NaOH, and then into a NO analyzer (Sievers Model 280 NO Analyzer, Boulder, CO, USA), which detects NO released from NO_x for chemiluminescent detection.

2.3. Measurement of plasma cGMP concentrations

Arterial blood samples were collected in tubes containing EDTA for the determination of plasma cGMP levels using a commercial enzyme immunoassay (ELISA, Amersham Biosciences, Buckinghamshire, UK). The plasma was separated by centrifugation and stored at −70 °C until assayed according to the manufacturer's instructions.

2.4. Statistical analysis

All the results are expressed as means±S.E.M. One-way analysis of variance (ANOVA) for repeated measures and paired Student's two-tailed *t*-test were used to determine the changes in the hemodynamic and biochemical parameters, respectively, in each group. Comparisons among groups at each time point were analyzed by one-way ANOVA. When the ANOVA was significant, the differences were tested by the Dunnett multiple comparisons test. A probability value <0.05 was considered the minimum level of statistical significance.

3. Results

3.1. Hemodynamic and respiratory responses

Baseline hemodynamic and respiratory parameters were similar in all experimental groups (Table 1, and Figs. 1–3). The hemodynamic and respiratory data from the Sham operated animals showed no significant changes throughout the study period. Thus, in order to simplify the data interpretation, the results of Sham operated animals are not presented.

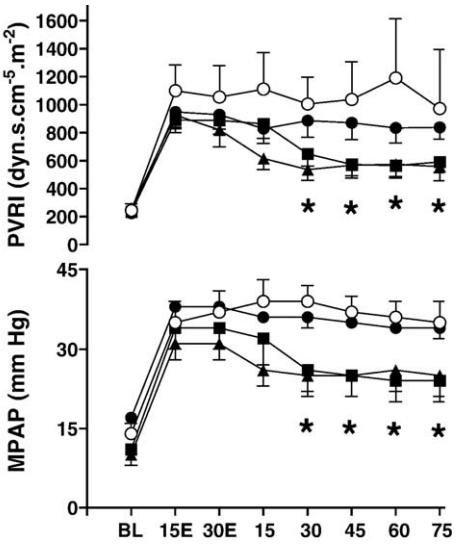

Fig. 1. Pulmonary vascular resistance index (PVRI) and mean pulmonary arterial pressure (MPAP) at baseline (BL), after 15 min (15E) and 30 min (30E) of acute pulmonary embolism, and after 15 min (15), 30 min (30), 45 min (45), 60 min (60) and 75 min (75) of L-arginine and sildenafil infusion in the Sild+Arg group (▲), or only L-arginine infusion in the Arg group (●), or only sildenafil infusion in the Sild group (■), or saline infusion in the Control group (○). Values are the mean±S.E.M. *$P<0.05$ for Sild and Sild+Arg groups versus the control group.

Stepwise injections of 300 μm microspheres into the inferior vena cava over a period of <10 min induced sustained pulmonary hypertension 30 min after the end of microspheres administration (Fig. 1) without other significant hemodynamic changes (Figs. 2 and 3). While the animals in the Control and in the Arg groups showed no further hemodynamic changes after

Table 1
Respiratory responses at baseline, after 15 min (15 E) and 30 min (30 E) of acute pulmonary embolism, and after 15 min (15), 30 min (30), 45 min (45), 60 min (60) and 75 min (75) after L-arginine and/or sildenafil infusion in the Control ($n=8$), Arg ($N=7$), Sild ($N=8$), and Sild+Arg ($N=8$) groups

Parameter	Baseline	15E	30E	15	30	45	60	75
pH (−log[H^+])								
Control	7.41±0.03 [a]	7.29±0.03	7.27±0.04	7.25±0.03	7.22±0.04	7.24±0.03	7.21±0.06	7.20±0.06
Arg	7.42±0.02[a]	7.34±0.02	7.32±0.02	7.31±0.02	7.28±0.03	7.27±0.03	7.26±0.04	7.25±0.05
Sild	7.40±0.02[a]	7.32±0.03	7.30±0.04	7.29±0.05	7.27±0.06	7.26±0.07	7.25±0.02	7.25±0.06
Sild+Arg	7.34±0.05[a]	7.22±0.05	7.24±0.05	7.18±0.05	7.17±0.05	7.15±0.05	7.14±0.04	7.14±0.07
$PaCO_2$ (mm Hg)								
Control	34±1[a]	32±2	38±3	39±3	39±1	38±2	38±3	39±3
Arg	33±1[a]	39±3	37±1	38±0	37±2	37±2	38±6	37±7
Sild	30±1[a]	34±2	35±3	36±3	38±5	38±6	39±3	39±5
Sild+Arg	36±4[a]	45±6	41±5	47±6	47±6	48±6	48±7	47±5
PaO_2 (mm Hg)								
Control	99±4[a]	67±9	52±4	52±3	49±3	50±3	50±2	47±4
Arg	91±3[a]	70±9	68±5	69±4	73±8	75±8	74±7	73±7
Sild	90±4[a]	57±4	60±4	60±4	61±5	65±5	65±6	66±1
Sild+Arg	94±10[a]	54±10	58±14	57±10	59±12	62±15	63±8	64±1
SaO_2 (mm Hg)								
Control	97±1[a]	85±4	77±4	80±3	75±3	77±3	76±2	78±4
Arg	97±1[a]	91±4	85±9	92±2	91±2	92±2	90±3	89±4
Sild	97±1[a]	85±5	86±5	85±6	84±8	85±8	86±6	85±1
Sild+Arg	93±2[a]	73±8	77±9	75±7	73±9	73±9	74±8	75±6

Values are mean±S.E.M.
pH=arterial pH; $PaCO_2$=arterial CO_2 tension; PaO_2=arterial O_2 tension; SaO_2=arterial O_2 saturation.
[a] $P<0.05$ vs. 30E.

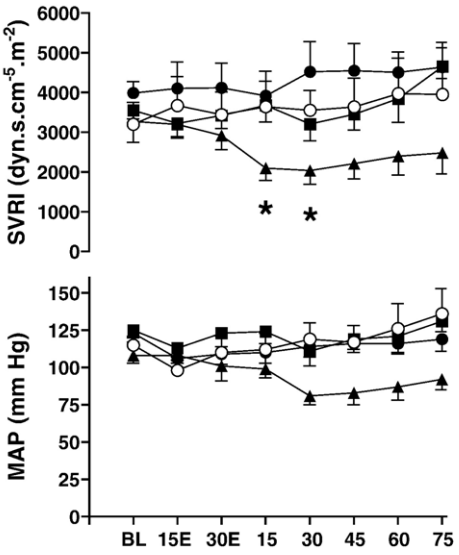

Fig. 2. Systemic vascular resistance index (SVRI) and mean arterial pressure (MAP) at baseline (BL), after 15 min (15E) and 30 min (30E) of acute pulmonary embolism, and after 15 min (15), 30 min (30), 45 min (45), 60 min (60) and 75 min (75) of L-arginine and sildenafil infusion in the Sild+Arg group (▲), or only L-arginine infusion in the Arg group (●), or only sildenafil infusion in the Sild group (■), or saline infusion in the Control group (○). Values are the mean±S.E.M. *$P<0.05$ for Sild+Arg group versus the Control group.

theacute pulmonary embolism-induced pulmonary hypertension (Figs. 1–3), significant decreases in mean pulmonary artery pressure and pulmonary vascular resistance index were observed 30, 45, 60, and 75 min after the intravenous administration of sildenafil alone (Sild group), or after the combined administration of sildenafil and L-arginine (Sild+Arg group; Fig 1; all $P<0.05$). In addition, significant reduction in systemic vascular resistance index was observed only in the Sild+Arg group 15 and 30 min after the co-administration of sildenafil and L-arginine (Fig. 2, $P<0.05$). No other significant hemodynamic changes were observed.

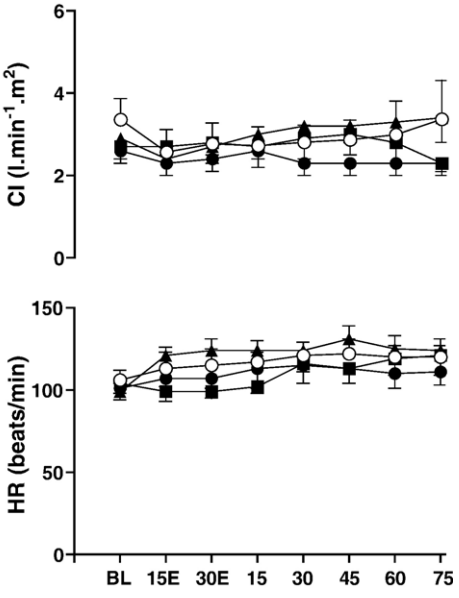

Fig. 3. Cardiac index (CI) and heart rate (HR) at baseline (BL), after 15 min (15E) and 30 min (30E) of acute pulmonary embolism, and after 15 min (15), 30 min (30), 45 min (45), 60 min (60) and 75 min (75) of L-arginine and sildenafil infusion in the Sild+Arg group (▲), or only L-arginine infusion in the Arg group (●), or only sildenafil infusion in the Sild group (■), or and saline infusion in the Control group (○). Values are the mean±S.E.M.

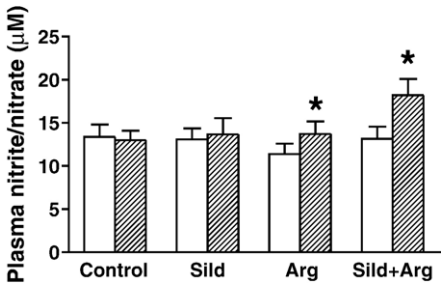

Fig. 4. Concentrations of nitrite/nitrate in plasma at baseline (open bars) and after 60 min (diagonal bars) of saline infusion in the Control group, or only sildenafil infusion in the Sild group, or only L-arginine infusion in the Arg group, or L-arginine and sildenafil infusion in the Sild+Arg group. Values are the mean±S.E.M. *$P<0.05$ versus baseline.

While lung embolization was associated with marked decreases in arterial pH, in arterial O_2 tension, and in arterial O_2 saturation, and with increases in arterial CO_2 tension (all $P<0.05$; Table 1), treatment with sildenafil, or L-arginine, or the combination of sildenafil and L-arginine produced no significant effects on the respiratory parameters (Table 1).

3.2. Plasma nitrite/nitrate concentrations

Treatment with L-arginine significantly increased plasma nitrite/nitrate concentrations in Arg and in Arg+Sild groups (both $P<0.05$). However, no significant increases in plasma nitrite/nitrate concentrations were observed in Control and Sild groups (Fig. 4).

3.3. Plasma cGMP concentrations

Treatment with sildenafil significantly increased plasma cGMP concentrations in Sild and in Arg+Sild groups (both $P<0.05$). However, no significant increases in plasma cGMP concentrations were observed in Control and Arg groups (Fig. 5).

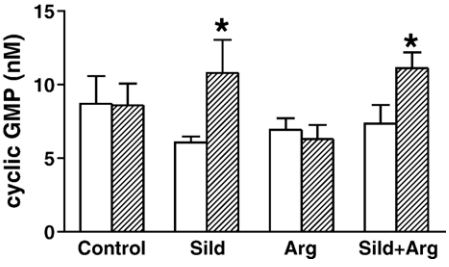

Fig. 5. Concentrations of cyclic GMP in plasma at baseline (open bars) and after 60 min (diagonal bars) of saline infusion in the Control group, or only sildenafil infusion in the Sild group, or only L-arginine infusion in the Arg group, or L-arginine and sildenafil infusion in the Sild+Arg group. Values are the mean±S.E.M. *$P<0.05$ versus baseline.

4. Discussion

The main findings of this study were that (i) intravenous administration of L-arginine (at doses of 100, 200, 500, and 1000 mg/kg h) did not attenuate acute pulmonary embolism-induced pulmonary hypertension; (ii) L-arginine did not enhance the beneficial hemodynamic effects of sildenafil; (iii) although L-arginine increased plasma nitrite/nitrate concentrations, plasma cGMP concentrations increased only when sildenafil was used to treat acute pulmonary embolism-induced pulmonary hypertension.

Acute pulmonary embolism-induced pulmonary hypertension results from the interaction of at least three main factors: the mechanical obstruction of pulmonary vessels, general pulmonary arteriolar constriction attributable to a neurogenic reflex, and the release of vasoconstrictors by activated platelets, leukocytes, and endothelial and lung cells (Barnes and Liu, 1995; Smulders, 2000). While the current treatment of acute pulmonary embolism is focused on removing the mechanical obstruction of pulmonary vessels (Sadosty et al., 2003), pharmacologic interventions focusing on the pulmonary vasoconstriction are now being considered as potentially effective in the treatment of hemodynamically unstable patients (Smulders, 2000). In this regard, pulmonary vasodilators that have been tested in animal models of acute pulmonary embolism include sildenafil, NO, prostacyclin, ketanserin, hydralazine, amrinone, isoproterenol, nitroglycerine, nitroprusside, captopril (Dias-Junior et al., 2005a,b; Smulders, 2000). While it is generally accepted that vasodilators may be effective in hemodynamically stable patients with acute pulmonary embolism, only inhaled NO or intravenous sildenafil produced selective pulmonary vasodilation during experimentally induced acute pulmonary embolism (Bottiger et al., 1996; Dias-Junior et al., 2005a,b; Smulders, 2000).

In the present study, although treatment with L-arginine increased plasma nitrite/nitrate concentrations, it produced no attenuation of acute pulmonary embolism-induced pulmonary hypertension. Consistent with these findings, L-arginine produced no effects when it was added to the lung perfusate in rat isolated perfused lung preparation before lung embolization (Souza-Costa et al., 2005). Conversely, L-arginine significantly attenuated acute pulmonary embolism-induced pulmonary hypertension when it was added to the lung perfusate before lung embolization (Souza-Costa et al., 2005). Although we have not examined the effects of L-arginine administered previously to lung embolization, we speculate that increased reactive oxygen species generation during acute pulmonary embolism (Dias-Junior et al., 2005a; Souza-Costa et al., 2005) leads to endothelial nitric oxide synthase (eNOS) uncoupling through increased oxidative stress-induced oxidation and depletion of BH_4, a cofator required for NO synthesis (Li et al., 2002). Therefore, it is possible that stimulation of dysfunctional eNOS with L-arginine administered after acute pulmonary embolism may increase superoxide/peroxynitrite formation (Dikshit et al., 1989), thus precluding L-arginine from producing beneficial effects. Supporting this hypothesis, it has recently been shown that while L-arginine decreased peroxynitrite formation when administered before myocardial ischemia/reperfusion, an increased peroxynitrite formation was observed when L-arginine was administered after reperfusion (Liang et al., 2004).

Administration of sildenafil alone significantly reduced mean pulmonary artery pressure after acute pulmonary embolism without producing significant systemic hypotension in the present study. These findings are similar to those previously reported (Dias-Junior et al., 2005a,b). The simultaneous administration of L-arginine, however, did not improve the beneficial hemodynamic effects of sildenafil. Indeed, we found a transient decrease in systemic vascular resistance index in group Sild+Arg. Although no significant reduction in mean arterial pressure was observed in this group, it is widely accepted that NO-releasing drugs can potentiate sildenafil-induced hypotension (Webb et al., 2000). Interestingly, although L-arginine increased plasma nitrite/nitrate concentrations in the present study, plasma cGMP concentrations increased only in dogs treated with sildenafil. Therefore, while L-arginine may have increased NO bioavailability, it produced no corresponding increases in cGMP concentration or beneficial hemodynamic effects. Conversely, treatment with sildenafil increased cGMP concentration and this effect was associated with hemodynamic improvement. These findings are consistent with an increased oxidative inactivation of NO by reactive oxygen species released during acute pulmonary embolism (Gryglewski et al., 1986), which may have been attenuated by antioxidant effects produced by sildenafil (Abdollahi et al., 2003a,b; Dias-Junior et al., 2005a). In addition, sildenafil may be acting through a pathway independent of the NO-PDE5-cGMP system. For example, sildenafil has been implicated in the suppression of NADPH oxidase expression and superoxide formation in porcine pulmonary artery endothelial cells (Muzaffar et al., 2005).

In conclusion, our results suggest that intravenous administration of L-arginine does not affect acute pulmonary embolism-induced pulmonary hypertension. Moreover, while sildenafil selectively attenuates acute pulmonary embolism-induced pulmonary hypertension, L-arginine does not enhance the beneficial hemodynamic effects of sildenafil. These findings suggest that stimulation of NO synthesis with L-arginine during acute pulmonary embolism does not produce beneficial effects.

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