ORIGINAL RESEARCH ARTICLE

Combined Intravenous Sildenafil and L-Arginine Administration in a Porcine Animal Model: Hemodynamic Safety Profile and Effects on Coronary Blood Flow

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Abstract

Background Endothelial dysfunction in the nitric oxide-cyclic guanosine monophosphate pathway is a potential contributor to perioperative myocardial ischemia. The nitric oxide precursor, L-arginine, and the cyclic guanosine monophosphate degradation blocker, sildenafil, have vasodilatory effects under high dosage.

Objective This study examined the hemodynamic safety and effect profiles of the combined administration of L-arginine and sildenafil using an in-vivo pig model.

Methods Hemodynamic safety including mean arterial pressure, central venous pressure, heart rate, coronary vascular resistance, and systemic vascular resistance, as well as effect profiles including cardiac output and left anterior descending blood flow were measured in ten female swine after administrations of L-arginine, sildenafil, as well as combined L-arginine and sildenafil. Measurements were compared using repeated-measures analysis of variance and linear mixed models.

Results The combination of L-arginine and sildenafil produced a significant dose-dependent increase in left anterior descending flow and cardiac output. In contrast, mean arterial pressure, heart rate, central venous pressure, coronary vascular resistance, and systemic vascular resistance did not show any significant changes. No significant change in serum osmolality was observed after administrations of L-arginine.

Conclusions The combined intravenous administration of sildenafil and L-arginine in a porcine animal model was safe, well tolerated, and had at least additive effects on left anterior descending artery blood flow. Simultaneous application of both drugs might have dose-sparing effects leading to desired coronary effects at lower and safer sildenafil and L-arginine plasma concentrations. Hyperosmolality was only a minor factor in L-arginine hemodynamic effects.

Key Points

Combined application of sildenafil and L-arginine was found to be safe and well tolerated in a large animal model.

Combined application of sildenafil and L-arginine had at least additive effects on left anterior descending artery blood flow in a large animal model.

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1 Introduction

Endothelial cells help control local vascular tone through the formation of nitric oxide (NO), which mediates vascular smooth muscle relaxation [1–3]. In patients with atherosclerotic coronary arteries, basal secretion of NO is lower and NO synthetic activity in response to mediators is disrupted [4–6]. Therefore, impaired endothelium-dependent coronary blood flow (CBF) is a potential contributor to perioperative myocardial ischemia [7].

L-arginine is the biological precursor for NO formation [2]. L-arginine is converted into NO and L-citrulline by NO synthase (NOS), allowing L-citrulline to be used as a marker for NO production. Nitric oxide stimulates the conversion of guanosine-5′-triphosphate to cyclic guanosine monophosphate in vascular smooth muscle cells resulting in vasodilatation [2]. In clinical trials, L-arginine has been shown to improve vascular function, reduce paradoxical vasospasm [2, 8–10], and vasodilate coronary arteries [11]. Unfortunately, the L-arginine dose required to achieve its reported effects has been high, limiting clinical applications of L-arginine [8, 11]. Therefore, compounds that may have L-arginine dose-sparing effects by amplifying its vascular effects were studied [12]. The selective inhibitor of phosphodiesterase type 5, sildenafil citrate, elevates cyclic guanosine monophosphate by blocking its degradation and has been found to improve endothelium-mediated relaxation [13], with vasodilatory effects on the pulmonary [14] and coronary vasculature [15]. In an in vitro study using the porcine internal mammary artery, our group demonstrated that sildenafil administered concurrently with L-arginine had synergistic vasodilatory effects [12]. This finding suggests that the combination of direct (sildenafil) and indirect (L-arginine) activation of the NO-cyclic guanosine monophosphate axis may provide clinical benefit of an increased CBF at lower doses of L-arginine.

A safe and prophylactic increase in endothelium-dependent CBF while preventing vasospasm and ischemia may be particularly relevant in individuals undergoing open-heart surgery as they are often in a tenuous hemodynamic status. No previous study has, however, examined in a large animal model reflecting the acute setting of open heart surgery whether concurrent intravenous sildenafil and L-arginine administration is beneficial by increasing coronary artery blood flow [12] or deleterious because of excessive hypotension, as it has been demonstrated for the combination of sildenafil with NO donors (e.g., nitroglycerin) [16, 17]. Therefore, the overall aim of this study was to explore whether concurrent sildenafil and L-arginine administration would safely increase NO-dependent coronary vascular resistance (CVR) and CBF in a large animal model, justifying interventional trials

in patients undergoing myocardial revascularization. We hypothesized that the concurrent intravascular administration of sildenafil will synergistically amplify the effects of L-arginine on CBF and CVR without negative effects on the overall hemodynamic safety. We tested our hypothesis in an in-vivo porcine model, with primary outcomes including CBF, CVR, and the overall hemodynamic safety profile.

2 Methods

2.1 Surgical Preparation

After the study protocols were submitted to and approved by the Institutional Review Board of the University of California, San Francisco, ten adult domestic female swine (mean weight: 70.5 ± 2.6 kg) were sedated with ketamine (25 mg/kg intramuscularly). Mask induction with isoflurane was followed by intubation with an endotracheal tube (internal diameter of 6.0 mm and 45 cm long). The animals were oxygenated with 100% oxygen (fraction of inspired oxygen of 1.0) and ventilated to maintain a physiologic arterial carbon dioxide tension. Pancuronium (0.1 mg/kg) was administered to induce paralysis and isoflurane was used to maintain anesthesia. An angiocathether (20 gauge) was placed in an ear vein for intravenous (IV) fluid administration. Prophylactic antibiotics (cefazolin 1 g intravenously prior to incision) were given. A left thoracotomy (T4–5) was performed under sterile conditions. Transit time flow probes were placed on the ascending aorta (model 28A; Transonic Systems Inc., Ithaca, NY, USA) and the proximal left anterior descending (LAD) coronary artery (3 mm, model 3S; Transonic Systems Inc.). The flow probe cables were tunneled to the animal's back. The thoracotomy was closed and air evacuated with a chest tube. Postoperative analgesia was provided with ketorolac (30 mg, one time after surgery) and multiple intramuscular doses of buprenorphine (0.3–0.6 mg, every 8 h).

2.2 Hemodynamic Measurements

To minimize the impact of surgical preparation on data collected, the measurements of systemic hemodynamics and CBF were measured via flow probes located in the ascending aorta and LAD at least 2 days after the surgery. After following the same anesthesia and surgical preparation as for a thoracotomy, the animal's neck was opened using a sterile technique, and angiocatheters (16 gauge) were placed in the left and right internal jugular veins for central venous pressure (CVP) monitoring and for drug and fluid infusions. An additional angiocatheter (20 gauge) was placed in the common carotid artery for blood sampling and pressure

measurement. After the neck was closed, a suprapubic bladder catheter was placed via a sterile technique for urinary drainage. Arterial pressure and CVP were continuously measured with disposable pressure transducers (Transpac® IV; Abbott Critical Care Systems, North Chicago, IL, USA), which were zeroed in accordance with the level of the right heart and calibrated before each set of measurements using a mercury manometer. The cardiac output (CO) and the LAD blood flow were continuously measured with the two Transonics® Transit Time Flow meters (Model 206 with (-R) Pressure Option; Transonic Systems Inc.), which were also calibrated prior to use. The animal's electrocardiogram was monitored (Electronics for Medicine Monitor Model OM). Coronary vascular resistance and systemic vascular resistance (SVR) were calculated using the following formulas:

$$CVR_{LAD} = 80 \times \frac{Diastolic\ blood\ pressure - CVP}{LAD\ blood\ flow} (dynes \cdot s \cdot cm^{-5})$$

$$SVR = 80 \times \frac{Mean\ arterial\ pressure - CVP}{CO} (dynes \cdot s \cdot cm^{-5})$$

Coronary vascular resistance was only calculated during diastole secondary to the zero-flow state in the LAD during systole. Because placing a flow probe circumferentially around the left main coronary artery is technically challenging owing to segment length, measurement of CBF was performed at the LAD vascular bed. This served as a surrogate for the entire coronary circulation. Further, CVP was used instead of left ventricular end-diastolic pressure to calculate CVR to avoid placing a piezoelectric or Millar catheter in the left ventricle. Data were recorded at 100 Hz using WinDaq data acquisition software (Transonics Systems Inc.).

The safety profile of L-arginine and sildenafil was assessed by looking for clinically and/or statistically significant changes in either mean arterial pressure (MAP) or SVR. We were concerned that the combination of the drugs might cause profound vasodilation resulting in hemodynamic instability or vasodilatory shock.

2.3 Experimental Protocol

To limit vasodilatory effects of inhalational anesthesia on the arterial vasculature, fentanyl (2 mg) and diazepam (20 mg) were administered as a slow IV bolus followed by continuous fentanyl (50 µg/kg/h) and diazepam (250 µg/kg/h) IV infusions. Isoflurane was discontinued. Pancuronium (0.05 mg/kg) was administered every 1–2 h to maintain paralysis. A 30-min period was allotted for the elimination of volatile agents as well as equilibration of fentanyl and diazepam. After baseline measurements of hemodynamic variables, CO, and the LAD blood flow, the animals were administered the study drugs (see Fig. 1 for protocol overview).

2.3.1 L-Arginine Hydrochloride (SigmaUltra; Sigma Chemical Company, St. Louis, MO, USA)

Five boluses of 60 mL of 10% L-arginine (6 g) were injected every 5 min, until the animal had received a total of 30 g of L-arginine (300 mL of 10% solution). To monitor L-arginine serum concentrations, blood samples were taken at baseline (0 min) and then at 5-min intervals following each bolus (at 5, 10, 15, 20, and 25 min). Thirty-five minutes after the last bolus, a final blood sample was taken. A total of 2 h was allowed for elimination of L-arginine.
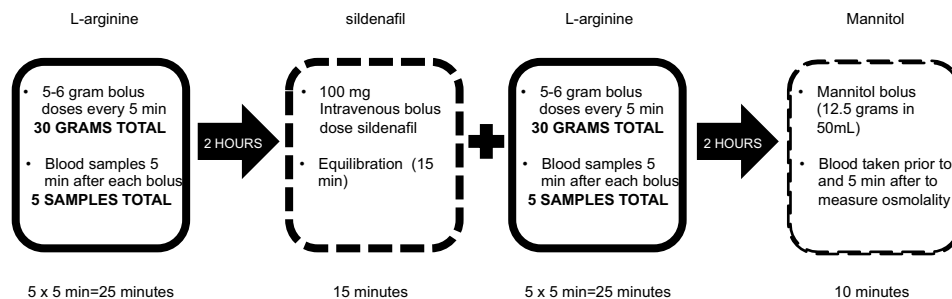

Fig. 1 Flow chart of L-arginine, sildenafil, and mannitol infusion and blood sample collection during the experiment. L-Arginine hydrochloride was injected every 5 min for five times followed by a 2-h elimination period. Blood samples were taken at baseline (0 min) and then at 5-min intervals after each bolus (at 5, 10, 15, 20 and 25 min) as well as 35 min after the last bolus. Sildenafil was then administered intravenously for 1 min and equilibrated for 15 min. After this 15-min equilibration, L-arginine hydrochloride was again injected every 5 min for five times followed by a 2-h elimination period. Blood samples were taken as described when L-arginine was administered alone. Finally, a bolus of mannitol (12.5 g in 50 mL) was administered after 5 min of baseline data collection to assess the degree of CO changes in respect to osmolality. A blood sample was taken prior to and 5 min after the administration of mannitol. Please refer to Sect. 2 for detailed information about doses for each drug

2.3.2 Sildenafil Citrate (Viagra®; Pfizer Pharmaceuticals, New York, NY, USA)

A bolus of 100 mL of 0.1% sildenafil solution (100 mg) was administered intravenously over 1 min, followed by a 15-min equilibration period. The IV solution of sildenafil was established by grounding one 100-mg tablet with mortar and pestle, dissolving it in 100 mL of sterile saline, and filtering said solution creating a 1-mg/mL concentration.

2.3.3 Sildenafil Plus ʟ-Arginine

After the 15-min equilibration period of sildenafil citrate, five 60-mL boluses of 10% ʟ-arginine (6 g) were injected intravenously in 5-min intervals, until the animal received a total of 30 g of ʟ-arginine (300 mL of 10% solution). To monitor ʟ-arginine serum concentrations, blood samples were taken at baseline (0 min) and then 5 min after each bolus (at 5, 10, 15, 20, and 25 min). Thirty-five minutes after the last bolus, a final blood sample was taken. A total of 2 h was allowed for elimination of ʟ-arginine.

2.3.4 Mannitol (250 mg/mL; Fujisawa USA, Deerfield, IL, USA)

To assess the degree of CO changes in respect to osmolality, a bolus of mannitol (12.5 g in 50 mL) was administered after 5 min of baseline data collection. A blood sample was taken prior to and 5 min after the administration of mannitol.

2.4 Laboratory Analysis

Blood samples were collected in serum separator tubes and immediately placed on ice. After the tubes were centrifuged for 20 min, the serum was removed and then stored in cryovials at − 80 °C. Serum osmolality was measured using an osmometer (Advanced Instruments; Needleham Heights, MA, USA) by the Veterinary Medical Teaching Hospital Diagnostic Laboratories (Davis, CA, USA). ʟ-Arginine and ʟ-citrulline concentrations were quantified by the Protein Structure Laboratory at the University of California, Davis (Davis, CA, USA) with an ion-exchange chromatographer (Beckman 6300 amino acid analyzer; Beckman Coulter, Fullerton, CA, USA). ʟ-Arginine is converted by NOS into ʟ-citrulline and NO, allowing ʟ-citrulline to be a marker for NO production.

2.5 Statistical Analysis

Repeated-measures analysis of variance for multiple comparisons was used to test for differences in plasma concentrations of drugs. Plasma concentrations at each dose were compared to those at the baseline control dose, using Dunnett's adjustment, and to those at the prior dose level, using Tukey–Kramer adjustment. The linear relationship between plasma concentrations of ʟ-arginine, a biological precursor for NO conversion, and ʟ-citrulline, a marker of NO production, was assessed at each dose using Pearson product-moment correlation coefficient. Finally, a linear mixed model of repeated measures with a compound symmetry covariance model and a random intercept was used to assess differences in the effect at each dose and baseline hemodynamics when ʟ-arginine was administered alone and with sildenafil. Denominator degrees of freedom was calculated via the Kenward–Roger procedure. Residuals were calculated and examined to confirm the assumptions of normal, constant, and independent residuals. All results are reported as sample means (or least-squares means) ± standard error. A p value < 0.05 was taken as the significant level for all statistical analyses. All statistical tests were performed with SAS 9.4 (SAS Institute, Cary, NC, USA).

3 Results

3.1 Infusion of ʟ-Arginine Hydrochloride Alone

3.1.1 Blood Concentrations of ʟ-Citrulline and ʟ-Arginine

The average baseline plasma concentration of ʟ-arginine was 160.8 ± 10.9 μM ($n = 10$, Table 1). There was a significant increase in the plasma concentration compared with each previous dose of ʟ-arginine for the first three boluses, but not with a further addition of ʟ-arginine (boluses 4 and 5) (Table 1) based on repeated-measures analysis of variance with Tukey–Kramer's adjustment. When compared with the baseline with Dunnett's adjustment, however, plasma concentration of ʟ-arginine was significantly higher after each of the five boluses (12- to 37-fold increases, $p < 0.001$). Thirty-five minutes after dose 5, ʟ-arginine plasma concentrations decreased from 5922.1 to 2592.7 μM ($p < 0.001$), remaining significantly elevated compared with baseline ($p < 0.001$).

The average baseline plasma concentration of ʟ-citrulline was 53.2 ± 4.1 μM ($n = 10$, Table 1). The mean ʟ-citrulline plasma concentration did not show a significant increase following ʟ-arginine administration when compared to baseline or each previous dose based on repeated-measures analysis of variance (Table 1). However, the ʟ-citrulline plasma concentration correlated positively and significantly with ʟ-arginine plasma concentrations at baseline, and at bolus #1, #2, #4, and #5 (Table 1). ʟ-Citrulline reached the highest mean concentration at dose 4 (61.5 ± 6.7 μM). Thirty-five minutes after the last ʟ-arginine bolus, the ʟ-citrulline plasma concentration remained elevated at 60.5 ± 7.1 μM, but not significantly different compared with baseline levels ($p = 0.2452$).

Table 1 Average serum L-arginine and L-citrulline concentrations after each dose of L-arginine and correlation between L-citrulline and L-arginine at each dose

L-Arginine bolus (no sildenafil)	L-Arginine (μM)	L-Citrulline (μM)	Correlation between L-arginine and L-citrulline
Baseline	160.8 ± 10.9	53.2 ± 4.1	0.822##
Bolus #1	1987.0 ± 116.8***,†††	54.7 ± 5.2	0.817##
Bolus #2	3139.2 ± 129.6***,†††	53.2 ± 4.2	0.676#
Bolus #3	4143.3 ± 205.4***,††	54.9 ± 4.4	0.571
Bolus #4	5232.7 ± 364.6***	61.5 ± 6.7	0.808##
Bolus #5	5922.1 ± 307.4***	60.2 ± 5.9	0.72#
30 min post	2592.7 ± 268.9***,†††	60.5 ± 7.1	0.622

Data are expressed as sample mean ± standard error

min minutes

*Repeated-measures analysis of variance with Dunnett's adjustment was used to test for changes in concentration relative to baseline levels, $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$

†Repeated-measures analysis of variance with Tukey–Kramer adjustment was used to test for changes in concentration relative to each previous level, $†p \leq 0.05$; $††p \leq 0.01$; $†††p \leq 0.001$

#Pearson correlation was used to test for a linear relationship between L-arginine and L-citrulline at each dose level, $#p \leq 0.05$; $##p \leq 0.01$; $###p \leq 0.001$

3.1.2 Stable Hemodynamics and Increased Coronary Blood Flow

The baseline LAD flow was consistent with previously published values [18]. Based on linear mixed models of repeated measures, mean MAP and HR were not significantly changed by the L-arginine administration when compared to the baseline values (103.4 ± 5.7 mmHg vs 100.7 ± 5.5 mmHg and 105.9 ± 5.7 beats/min vs 106.0 ± 5.6 beats/min, respectively; Table 2 and Fig. 2, $n = 10$/dose). This was accompanied by significant and dose-dependent increases in LAD flow, CO, and CVP (Fig. 2). After the fifth bolus, LAD flow, CO, and CVP remained significantly higher than their baseline levels ($p < 0.001$, $p < 0.001$, and $p = 0.004$, respectively, Table 2).

Finally, there was a significant dose-dependent decreases in CVR and SVR (Fig. 2). After the fifth and final bolus, CVR and SVR remained significantly lower than their baseline scores ($p < 0.001$ and $p = 0.005$, respectively, Table 2).

3.2 Infusion of Sildenafil Alone

The hemodynamic effects after the IV administration of 100 mg of sildenafil citrate over time (15 min) were characterized by generalized vasodilatation (decrease in CVR and SVR, $p < 0.001$ for both) and a significant reduction in MAP ($p < 0.001$) that persisted during the 15-min equilibrium after infusion (Fig. 3) based on linear mixed models of repeated measures. There was a significant increase in

Table 2 Hemodynamic effects of L-arginine alone and of L-arginine plus sildenafil

Hemodynamic measures	L-Arginine alone			L-Arginine plus sildenafil		
	Pre	End effect	Difference	Pre	End effect	Difference
CO (L/min)	7.8 ± 0.6	8.9 ± 0.5***	1.1 ± 0.4	9.9 ± 0.8	11.1 ± 1.1**	1.2 ± 0.4
LAD flow (mL/min)	52.3 ± 5.3	66.7 ± 6.6***	14.4 ± 3.6	65.8 ± 6.1	84.7 ± 9.5***	18.9 ± 4.5
MAP (mmHg)	100.7 ± 5.5	103.4 ± 5.7	2.7 ± 3.8	87.3 ± 4.7	96.4 ± 5.3	9.1 ± 3.4
CVP (mmHg)	10.3 ± 0.7	11.4 ± 0.9***	1.1 ± 0.3	9.5 ± 0.9	10.0 ± 1.0	0.6 ± 0.4
HR (beats/min)	106.0 ± 5.6	105.9 ± 5.7	− 0.1 ± 4.9	142.4 ± 9.1	148.7 ± 8.4	6.3 ± 8.9
CVR (dynes·s·cm^{-5})	119.2 ± 6.2	89.1 ± 4.7***	− 30.1 ± 3.0	77.2 ± 7.9	70.9 ± 9.8	− 6.3 ± 5.2
SVR (dynes·sec·cm^{-5})	939.1 ± 36.1	825.0 ± 39.3**	− 114.1 ± 49.6	656.1 ± 50.2	668.8 ± 70.3	12.7 ± 30.6

Data are expressed as sample mean ± standard error

The baseline values in L-arginine plus sildenafil were measured after sildenafil was given, but before L-arginine administration

CO cardiac output, *CVP* central venous pressure, *CVR* coronary vascular resistance, *H*, heart rate, *LAD* left anterior descending artery, *MAP* mean arterial pressure, *SVR* systemic vascular resistance

*A linear mixed model of repeated measures was used to assess the difference between the value at baseline (pre) and after the last bolus of L-arginine (end effect), $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$
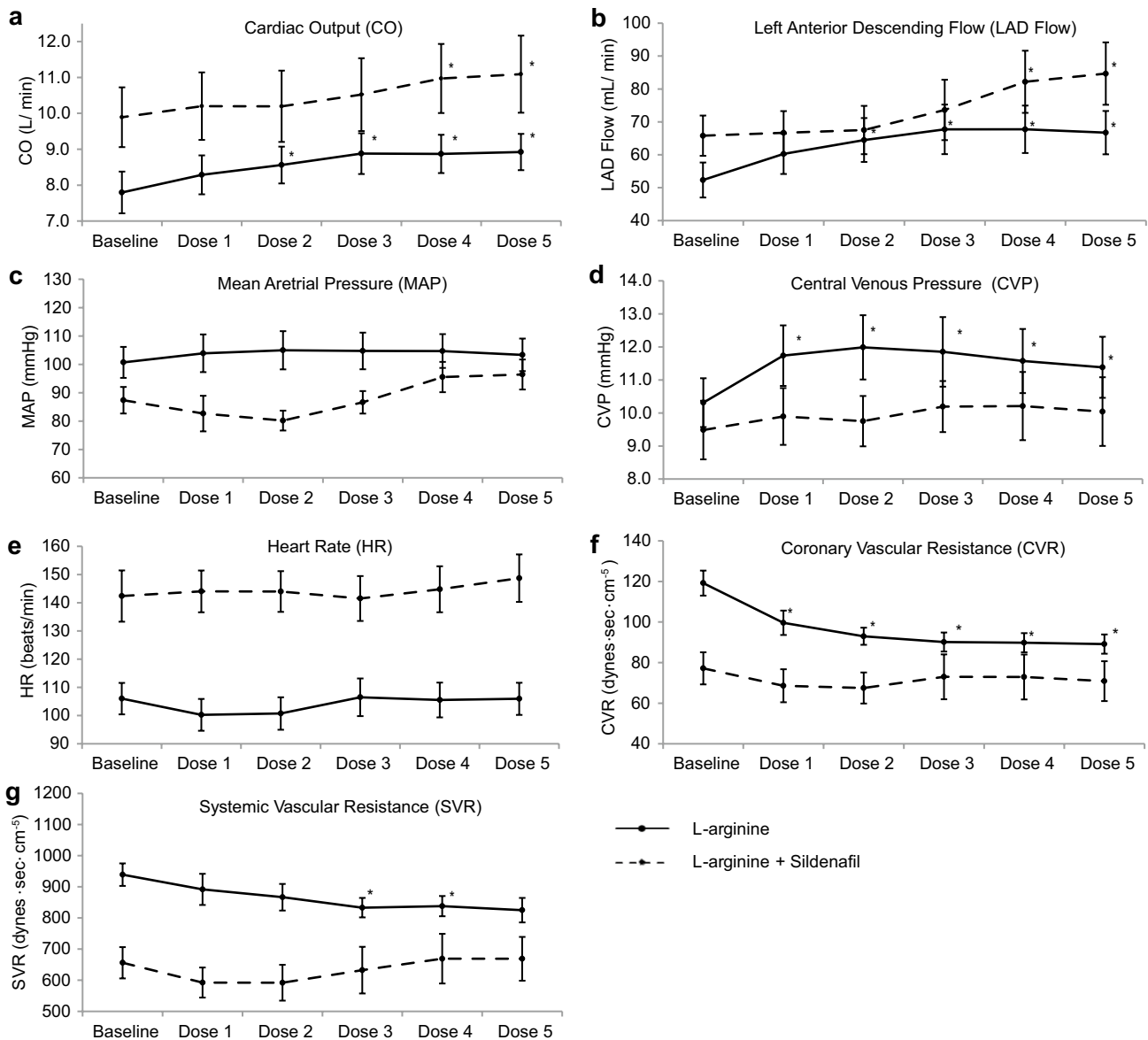
Fig. 2 Mean concentration response curves of hemodynamic safety and effects after L-arginine vs L-arginine plus sildenafil infusions. The mean hemodynamic safety and effects of L-arginine alone (solid lines) and with sildenafil (dashed lines) are compared. **a** Cardiac output (CO). **b** Left anterior descending (LAD) flow. **c** Mean arterial pressure (MAP). **d** Central venous pressure (CVP). **e** Heart rate (HR). **f** Coronary vascular resistance (CVR). **g** Systemic vascular resistance (SVR). Data are expressed as sample mean ± standard error (SE). *Indicates a significant difference of at least $p < 0.05$ compared with baseline levels based on the linear mixed models of repeated measures

HR during the first 5 min accompanied by a significant increase in LAD artery blood flow and CO compared to their baseline levels. When HR returned to its baseline level, LAD flow and CO decreased rapidly, temporarily undercutting the baseline values. In fact, between the 5th and 12th minute, there was a significant decrease in LAD artery blood flow followed by a slow return to its baseline (Fig. 3). Central venous pressure showed a significant reduction between the third and ninth minute followed by a slow baseline recovery.

3.3 Combined Administration of Sildenafil and L-Arginine

Based on the linear mixed models of repeated measures that assessed the difference between the values at baseline (measured after sildenafil was given, but before L-arginine administration) and after the last bolus of L-arginine (end effect), MAP, CVP, HR, CVR, and SVR were not significantly affected by the sildenafil and L-arginine administration (96.4 ± 5.3 mmHg vs 87.3 ± 4.7 mmHg;
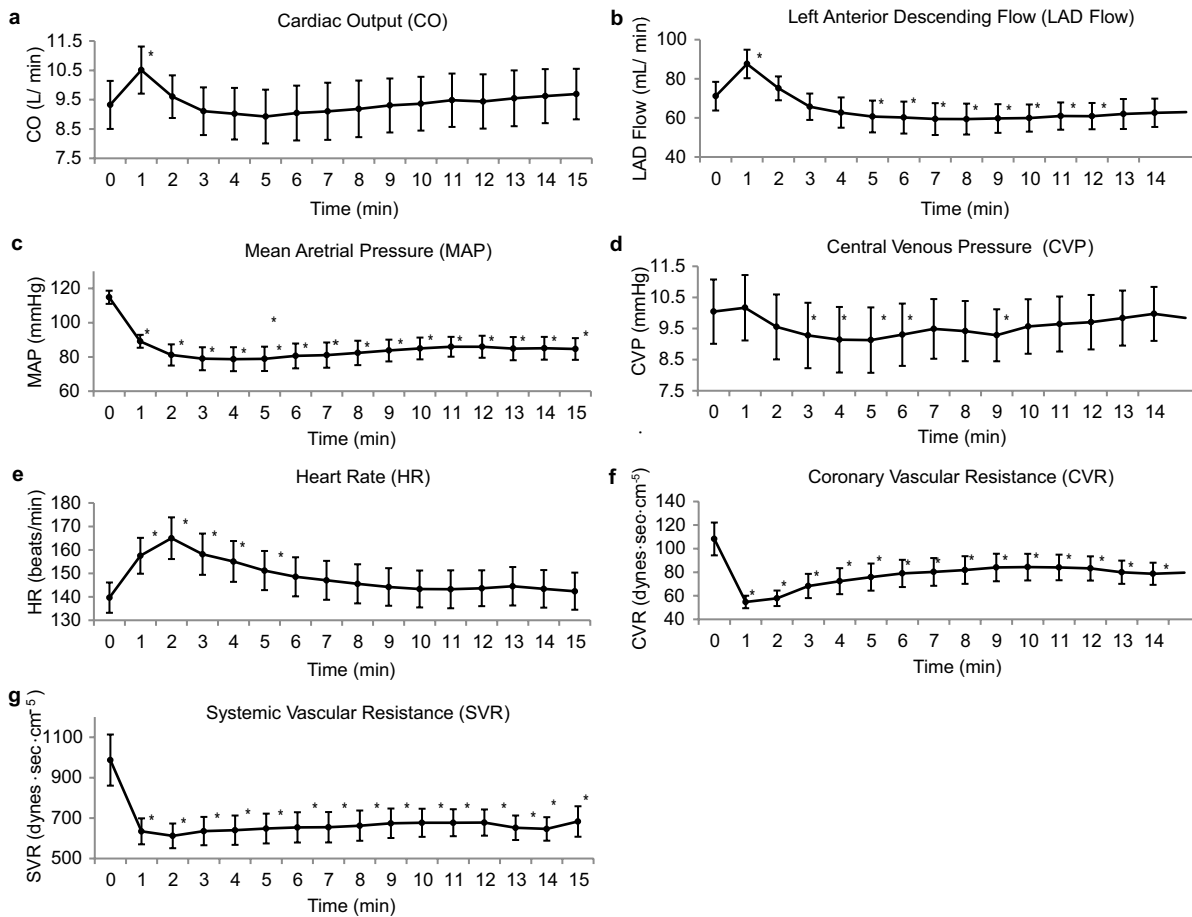
Fig. 3 Hemodynamic safety and effects of sildenafil over time (15 min). The mean trajectory of hemodynamic safety and effects of sildenafil over 15 min was shown. **a** Cardiac output (CO). **b** Left anterior descending (LAD) flow. **c** Mean arterial pressure (MAP). **d** Central venous pressure (CVP). **e** Heart rate (HR). **f** Coronary vascular resistance (CVR). **g** Systemic vascular resistance (SVR). Data are expressed as sample mean ± standard error (SE). *Indicates a significant difference of at least $p < 0.05$ compared with the baseline levels based on the linear mixed models of repeated measures

10.0 ± 1.0 mmHg vs 9.5 ± 0.9 mmHg; 148.7 ± 8.4 beats/min vs 142.4 ± 9.1 beats/min, 70.9 ± 9.8 dynes·s·cm^{-5} vs 77.2 ± 7.9 dynes·sec·cm^{-5}, and 668.8 ± 70.3 dynes·s·cm^{-5} vs 656.1 ± 50.2 dynes·s·cm^{-5}, respectively; Table 2, $n = 10$/dose). In contrast, the end effect of LAD flow and CO were significantly higher compared with the baseline values (84.7 ± 9.5 mL/min vs 65.8 ± 6.1 mL/min, $p < 0.0001$; 11.1 ± 1.1 L/min vs 9.9 ± 0.8 L/min, $p = 0.0057$, respectively, Table 2, $n = 10$/dose).

Comparisons of the LAD flow between L-arginine administration and L-arginine plus sildenafil combined administration demonstrated that each bolus of L-arginine with the presence of sildenafil increased the LAD flow, reaching significance at bolus #4 (82.2 ± 8.1 mL/min, $p = 0.0087$) and bolus #5 (84.7 ± 8.1 mL/min, $p = 0.0012$, Table 3). The magnitude of the combined drug effect was at least additive as both L-arginine and sildenafil are significantly and positively

Table 3 Effects of L-arginine alone vs L-arginine plus sildenafil on left anterior descending artery (LAD) flow (mL/min)

Bolus	L-Arginine alone	L-Arginine plus sildenafil
Baseline	52.3 ± 6.6	65.8 ± 8.1*
Bolus #1	60.3 ± 6.6	66.7 ± 8.1
Bolus #2	64.5 ± 6.6	67.5 ± 8.1
Bolus #3	67.7 ± 6.6	73.6 ± 8.1
Bolus #4	67.7 ± 6.6	82.2 ± 8.1**,††
Bolus #5	66.7 ± 6.6	84.7 ± 8.1**,††

Data are expressed as least-squares mean ± standard error

*A linear mixed model of repeated measures was used to assess the difference in LAD flow between L-arginine alone vs L-arginine plus sildenafil, *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

†A linear mixed model of repeated measures was used to assess the difference in LAD flow between the final LAD flow in the L-arginine group (after bolus #5, 66.7 ± 6.6 mL/min) vs each bolus in the L-arginine plus sildenafil group, †$p \leq 0.05$; ††$p \leq 0.01$; †††$p \leq 0.001$

associated with LAD flow (both $p < 0.0001$ for the last dose of L-arginine and sildenafil, respectively).

3.4 Mannitol

The change in serum osmolality after 12.5 g of mannitol (8.1 ± 24.3 mOsmol/kg sample) did not significantly differ from that after 30 g of L-arginine (7.7 ± 25.0 mOsmol/kg sample). However, L-arginine was associated with a significantly higher LAD artery blood flow when compared with mannitol (14.4 ± 0.4 mL/min vs 5.5 ± 0.2 mL/min, respectively, $p < 0.01$, data not shown).

4 Discussion

The salient finding of our study is that combined IV administration of sildenafil and L-arginine in a porcine animal model is safe, well tolerated, and has at least additive effects on the LAD artery blood flow and CO (Tables 2, 3; Fig. 2). None of the animals had any adverse hemodynamic changes requiring intervention (Tables 2, 3; Fig. 2). The results appear to support our hypothesis that simultaneous application of both drugs may have dose-sparing effects. This finding is significant as patients undergoing open-heart surgery are often in a tenuous hemodynamic status. Co-administration of L-arginine and sildenafil may allow a safe and prophylactic usage that increases endothelium-dependent CBF while preventing vasospasm and ischemia.

4.1 Concurrent Sildenafil and L-Arginine Infusion

Following our study proving the concept of synergistic vasodilatory effect of sildenafil and L-arginine in an in-vitro porcine internal mammary artery model [12], our group focused on investigating the combination effect in an in-vivo porcine model. No previous study has examined the effects of concurrent IV sildenafil plus L-arginine administration on coronary circulation in a large animal model reflecting the acute setting of open-heart surgery. The current study supports and expands our prior results by demonstrating that L-arginine in the presence of sildenafil induces significant and concentration-dependent increases in LAD artery blood flow (Fig. 2; Table 3), while it is safe and well tolerated (Fig. 2; Table 2). Importantly from a clinical perspective, MAP, CVP, CVR, SVR, and HR remained relatively stable. It is well established that CBF is dependent on multiple factors, including BP, HR, and CVR, for example. The increase in CBF was likely a combination of the small decrease in CVR combined with the small increase in MAP.

The effect of sildenafil alone on LAD flow was comparable to the effect measured after the fifth L-arginine dose administered alone (Table 2). Boluses of L-arginine in the presence of sildenafil increased significantly the blood flow (at bolus #4 and #5) showing the additive action of the drugs. This study did not examine the time course of offset of the effects of L-arginine or sildenafil. The decreases in CVR persisted throughout the infusions. We waited 2 h for them to resolve prior to subsequent tests. A further study would be needed to give the exact time course of offset of the effects.

Synergistic effects of sildenafil and L-arginine have already been described in a rabbit corpus cavernosum [19] and in pulmonary vasculature [14, 20, 21]. Life-threatening pulmonary hypertension in pregnancy was treated successfully with concomitant sildenafil plus L-arginine use [20]. The combination of sildenafil with L-arginine, both at lower doses than when administered alone, may have important clinical applications, as both drugs have side effects [16, 17, 22, 23]. A reduction in the amount of sildenafil and L-arginine doses may therefore lower the risk of adverse events.

For a medication to be clinically useful, the hemodynamic effects must be controllable. This study demonstrated that there were small but statistically significant decreases in SVR with L-arginine that were not large enough to cause clinically significant hemodynamic problems. Combinations of L-arginine and sildenafil did not have statistically significant changes in either MAP or SVR providing a safe hemodynamic profile.

4.2 L-Arginine Hydrochloride Infusion (Alone)

Our results support the current view of hemodynamic changes with an IV infusion of L-arginine alone, including peripheral vasodilatation, concentration-dependent decreases in SVR and MAP, an increase in CO, as well as mitigating change in HR [24–27]. Similar to our study, Bode-Boger et al. examined the pharmacokinetic-pharmacodynamic relationship of L-arginine after a single IV infusion of 30 g, and found a significant dose-dependent decrease in blood pressure and total vascular resistance [27].

Our previous work demonstrated that the effects of L-arginine on coronary circulation cannot be predicted from systemic vascular response [11]. Consequently, we used transit time flow meters to quantitatively measure blood flow and resistance of the LAD artery. We found both coronary vasodilatation and an increase in LAD artery blood flow when an IV infusion of L-arginine was administered. Both effects were significant and concentration dependent. The increase in LAD artery blood flow was nearly twice as strong as the changes in CO. This is consistent with our previous findings showing that L-arginine has more selective vasodilatory effects on coronary vasculature than systemic vasculature [11]. Moreover, quantitative human coronary angiography studies demonstrated that both IV and intracoronary L-arginine infusions induce dilatation of coronary arteries

[28–31]. Finally, L-arginine has been shown to restore the acetylcholine-induced increase in blood flow in patients with impaired endothelium-dependent dilatation of coronary microcirculation, such as in hypocholesteremia [10]. Consistent with the endothelium-dependent NO-mediated mechanism of a vascular tone model, L-NAME, a NO synthesis inhibitor, produced a reduction in LAD artery blood flow in a rat animal model [32]. The effects were then reversed with an IV administration of L-arginine [32]. The stimulation of the L-arginine-NOS-NO pathway is regarded as the dominant mechanism behind the action of L-arginine [31, 33].

L-Arginine is converted by NOS into L-citrulline and NO, making L-citrulline a marker of the production of NO. We measured L-arginine and L-citrulline concentrations from a radial artery sample. The L-citrulline plasma concentration was significantly correlated with the L-arginine plasma concentration at each dose suggesting that L-arginine was converted into L-citrulline while additionally producing NO. However, the L-citrulline plasma concentration did not show a correlation with the L-arginine plasma concentration nor with hemodynamic clinical effects among doses, despite the close temporal pattern between the L-arginine plasma concentration and hemodynamic effects [27, 34, 35]. This discrepancy may be due to the saturation of other L-arginine metabolic pathways, such as L-arginine metabolism by the hepatic urea cycle [34].

4.3 Mannitol Infusion

Hypertonic ionic (saline) and nonionic (glucose) solutions have been reported to reduce coronary vascular resistance in direct relation to hyperosmolality [36]. In our study, the administration of 30 g of L-arginine led to an increase in osmolality similar to the subsequent mannitol infusion. However, the administration of L-arginine was associated with a significantly higher increase in LAD artery blood flow than the mannitol infusion (14.4 mL/min vs 5.5 mL/min, $p < 0.01$). The change in CBF after an L-arginine infusion was 2.6 times the change expected for a similar change in osmolality; this suggests that hyperosmolality is only a minor factor in L-arginine hemodynamic effects [11].

4.4 Sildenafil Citrate Infusion

Studies examining the sildenafil effects on systemic, pulmonary, and myocardial hemodynamics used an oral route or slow IV administration [37–43]. Unlike other vasoactive drugs, there is a paucity of data on the hemodynamic effects of a rapid IV bolus administration of sildenafil. In the present study, we administered sildenafil intravenously as a bolus of 100 mg over 1 min. The hemodynamic response 1–2 min following the injection was characterized by significant arterial vasodilatation (reduced SVR and MAP) and tachycardia,

which led to a short yet significant increase in CO. The coronary circulation mirrored this with a significant decrease in CVR while causing a significant increase in LAD artery blood flow despite the markedly decreased MAP. This was followed by rapid normalization of CO, a slow return of HR back to baseline, and relatively unchanged vasodilatation. There was a decrease in LAD artery blood flow despite a stable MAP. The results appear to support the reported effects of sildenafil, characterized by a vasodilatation-induced reduction in blood pressure [37]. In fact, the peak reduction in blood pressure has been shown to positively correlate with peak sildenafil plasma concentrations [37, 44] followed by a slow return to baseline levels. Phillips et al., using a 100-mg sildenafil oral dose, demonstrated an increase in sympathetic nerve activity selective for a vascular sympathetic drive [43]. The sildenafil oral dose of 100 mg was found to have equivalent peak plasma concentrations when compared to an IV infusion of 40 mg over 40–60 min [37]. In our study, we used a 100-mg sildenafil IV dose infused over 1 min. This higher amount is likely associated with our 2.5 times higher peak plasma concentrations than reported in the study by Phillips et al. [43].

Our findings suggest that even large IV sildenafil doses are well tolerated and not associated with major adverse hemodynamic events. However, our results also suggest that sildenafil administration is associated with tachycardia, possibly owing to the described increase in sympathetic activity [43]. Considering that many patients who may benefit from sildenafil are characterized by ischemic heart disease, a slower administration over a prolonged period should be considered. Jackson et al. described that IV infusion of 40 mg over 60 min was associated with no adverse events [37].

4.5 Limitations

The use of an animal model is an important step in determining the safety profile of the combined use of L-arginine and sildenafil; however, this study has limitations.

There are several limitations to our measurement of CBF and CVR:

- These measurements were performed only at the LAD vascular distribution; *they were surrogates for the whole coronary circulation.* This was done because to measure total coronary vascular resistance, one would need to measure total CBF, which would require a flow probe on the left main coronary artery and the right coronary artery. Placing a probe on the left main coronary artery is technically challenging. Thus, the decision was made to measure CVR of the LAD vascular bed.
- We placed a catheter to measure CVP. We did not place a piezoelectric catheter into the left ventricle to measure

left ventricular diastolic pressure. Typically, CVR would be $CVR = [(\text{aortic diastolic pressure} - \text{left ventricular diastolic pressure})/\text{total CBF}] \times 80$. In a healthy pig, because the ventricular function is not impaired, the CVP provides information on right ventricular end-diastolic pressure, which parallels changes in left ventricular end-diastolic pressure. Central venous pressure was used as a surrogate of left ventricular diastolic pressure to avoid having to place a left ventricular piezoelectric or Millar catheter in the left ventricle.

- Coronary blood flow to the left ventricle occurs during diastole. The pressure gradient between the aorta and left ventricle is zero during systole resulting in zero CBF in the LAD during systole. Coronary vascular resistance was only calculated during diastole secondary to the zero-flow state in the LAD during systole.

One unexpected finding was the change in heart rate from 106 ± 5.6 beats/min to 140 ± 6.5 beats/min before sildenafil infusion (Fig. 3e). The elevated heart rate persisted throughout the sildenafil and L-arginine post-sildenafil infusions. Pancuronium, which was given to the animals multiple times throughout the study, is known to increase HR in a dose-dependent manner [45]. Possible dehydration and late effects of L-arginine may also contribute to the increase in HR. Another limitation was that L-arginine lowered SVR in the pigs in this study, but it has had little effect in post-bypass patients [11]. One explanation could be that there is already a low SVR in post-bypass patients, thus the addition of L-arginine has minimal effects. Another reason is that the pig vasculature in the current study was not dilated prior to L-arginine infusion. Administration of L-arginine could therefore lead to vasodilatory response and lower SVR. Along the same lines, the difference in the degree of atherosclerosis between patients undergoing open heart revascularization and the large animals used in this study, further limits the translation of the results to humans. Finally, the long-term safety issues were not assessed in this study.

5 Conclusions

This study tested the in-vivo response of coronary circulation to concomitant administration of L-arginine and sildenafil in a large animal model. We found that the simultaneous administration of sildenafil and L-arginine was safe and well tolerated in healthy pigs while having additive effects on LAD artery blood flow and CO. The findings support our hypothesis that simultaneous application of both drugs may have dose-sparing effects and additional studies should be performed to optimize desired coronary effects at lower and safer drug plasma concentrations. Co-administration of L-arginine and sildenafil may allow a safe and prophylactic usage that increases endothelium-dependent CBF while preventing vasospasm and ischemia.

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