Sildenafil Citrate Alleviates Pulmonary Hypertension after Hypoxia and Reoxygenation with Cardiopulmonary Bypass

Jefferson M Lyons, MD, Jodie Y Duffy, PhD, Connie J Wagner, BS, Jeffrey M Pearl, MD, FACS

BACKGROUND: Sudden reoxygenation of hypoxic neonates undergoing cardiac operation exacerbates the systemic inflammatory response to cardiopulmonary bypass secondary to reoxygenation injury, worsening cardiopulmonary dysfunction. Reports suggest sildenafil decreases pulmonary hypertension and may affect myocardial function. Sildenafil’s efficacy for treating postbypass cardiopulmonary dysfunction remains unknown.

STUDY DESIGN: Fourteen neonatal piglets (5 to 7 kg) underwent 90 minutes of hypoxia, 60 minutes of reoxygenation with cardiopulmonary bypass, and 120 minutes of recovery. Six animals received 50 mg oral sildenafil and eight received saline at hypoxia. Data are presented as mean ± SD.

RESULTS: Sildenafil prevented the high pulmonary vascular resistance observed in controls (controls baseline 81 ± 37 dynes · s/cm$^5$ versus recovery 230 ± 93 dynes · s/cm$^5$, p = 0.004; sildenafil baseline 38 ± 17 dynes · s/cm$^5$ versus recovery 101 ± 60 dynes · s/cm$^5$, p = 0.003). Despite lower pulmonary vascular resistance after sildenafil, arterial endothelin-1 (ET-1) was increased in both groups (control baseline 1.3 ± 0.5 pg/mL versus recovery 4.5 ± 3.7 pg/mL, p = 0.01; sildenafil baseline 1.3 ± 0.3 pg/mL versus recovery 9.8 ± 4.9 pg/mL, p = 0.003). Intravenous nitric oxide (NO) levels were preserved after sildenafil treatment (sildenafil baseline 340 ± 77 nM versus recovery 394 ± 85 nM). IV NO levels in controls were decreased when compared with baseline (control baseline 364 ± 83 nM versus recovery 257 ± 97 nM, p = 0.028). Although levels of exhaled NO decreased in both groups, the sildenafil-treated animals had higher levels of exhaled NO when compared with controls at the end of recovery (0.6 ± 0.4 parts per billion versus 1.8 ± 0.9 parts per billion, respectively, p = 0.029).

CONCLUSIONS: Sildenafil alleviated pulmonary hypertension after reoxygenation with cardiopulmonary bypass. Despite increased ET-1 levels, pulmonary vascular resistance was lower with sildenafil treatment, suggesting sildenafil’s effect on the pulmonary vasculature is capable of countering vasoconstriction by ET-1. Further study into the role of sildenafil in perioperative therapy and its interactions with ET-1 are warranted. (J Am Coll Surg 2004;199:607–614. © 2004 by the American College of Surgeons)

The number of congenital heart defects requiring intervention is about 1 per 125 births per year. Definitive treatment is frequently performed in the first days of life, but significant morbidity and mortality persist. Cardiopulmonary bypass (CPB), often required for repair of many congenital defects, results in a systemic inflammatory response. Reoxygenation of hypoxic tissues at the initiation of CPB has been shown to exacerbate both pulmonary and myocardial injury. Clinical presentation of this injury is pulmonary hypertension, impaired gas exchange, and low cardiac output. Previous data have demonstrated a significant pathologic role for endothelin-1 (ET-1) in generation of pulmonary hypertension and myocardial dysfunction after both hypoxia–reoxygenation and CPB.

Traditionally, treatment of both reperfusion and...

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reoxygenation injuries has been mostly supportive, with use of ionotropes, assisted ventilation, and pulmonary vasodilators. Glucocorticoid therapy has been used to limit inflammatory response to bypass, and evidence suggests steroids improve cardiopulmonary function in the perioperative period. Avoidance of hyperoxia has also been used to minimize reoxygenation injury during and after operation. Systemic vasodilators have some benefit for treatment of pulmonary hypertension, but systemic hypotension frequently limits their use. Specific pulmonary vasodilators are also used, with inhaled nitric oxide (NO) becoming the most widely accepted. Inhaled NO is expensive, usually requires an intubated patient for accurate delivery, and is often associated with rebound pulmonary hypertension on withdrawal.

An ideal agent has yet to be found for treatment of the sequelae of CPB. This agent should decrease inflammatory response to bypass, alleviate pulmonary hypertension, preserve cardiac function, and maintain adequate tissue perfusion. It is likely that a combination of treatments is necessary to meet all criteria. One promising new agent for treatment of post-CPB pulmonary hypertension is sildenafil citrate (Viagra; Pfizer), which is widely used as a therapy for erectile dysfunction. Sildenafil is a potent and selective pulmonary vasodilator, whose mechanism of action involves inhibition of phosphodiesterase-5. The ability of sildenafil to affect pulmonary vascular resistance (PVR) with minimal systemic side effects has stimulated interest in its use to treat children with pulmonary hypertension and cardiopulmonary dysfunction after congenital heart operation. Sporadic favorable reports in animals and humans support its use. Based on these limited data, clinicians are experimenting with sildenafil as a treatment for cardiopulmonary dysfunction. There are relatively few dedicated clinical or animal studies evaluating the effectiveness of sildenafil for treating cardiopulmonary dysfunction after CPB in neonates. To evaluate the effects of sildenafil citrate on cardiopulmonary function after hypoxia and reoxygenation with CPB, we used a well-established model with which our laboratory has considerable experience.

**METHODS**

All animals received humane care in compliance with the Principles of Laboratory Animal Care by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 23 to 86, revised 1996). The Institutional Animal Care and Use Committee at Children’s Hospital Research Foundation also approved the protocol.

**Piglet model of hypoxia and reoxygenation with CPB**

Piglets weighing 5 to 7 kg were anesthetized with ketamine (22 mg/kg, IM) and acepromazine (1.1 mg/kg, IM), then intubated, and mechanically ventilated. An orogastric tube was placed. Continuous pentobarbital infusion (20 mg/kg/h), intermittent fentanyl citrate (10 µg/kg/h), and pancuronium bromide (0.1 mg/kg/h) were used throughout the experiment to maintain deep general anesthesia. Cut-down femoral arterial and venous infusion cannulas were placed. Median sternotomy exposed the heart. A Doppler flow probe (Transonic Systems, Inc) was placed around the main pulmonary artery to measure cardiac output and pressure catheters (Millar Instruments) were placed in the pulmonary artery, right ventricle, and left ventricle. A pulmonary artery infusion line was placed for mixed venous blood-gas sampling. An NO probe (Harvard Apparatus) was positioned in the left external jugular vein and sutured into place. Exhaled NO was measured through an in-line monitor (Eco Physics). Baseline hemodynamic and blood-gas measurements were taken after a 30-minute equilibration period.

Animals were made hypoxic by initiating a fraction of inspired oxygen (FiO$_2$) of 12% for 90 minutes, resulting in a systemic oxygen saturation of 65% to 70%. Hemodynamic measurements and arterial and venous blood samples were taken at 5, 15, 30, 60, and 90 minutes of hypoxia. Animals were administered fluid boluses of 5 to 10 mL/kg of 0.9% sodium chloride, as needed, to maintain a mean arterial pressure > 40 mmHg with a volume limit of 40 mL/kg. Sodium bicarbonate was given, if needed, to maintain blood pH > 7.3. Ventilator rate was

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**Abbreviations and Acronyms**

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CPB</td>
<td>cardiopulmonary bypass</td>
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<tr>
<td>ET-1</td>
<td>endothelin-1</td>
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<tr>
<td>FiO$_2$</td>
<td>fraction of inspired oxygen</td>
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<tr>
<td>GAPDH</td>
<td>glyceraldehyde-3-phosphate dehydrogenase</td>
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<tr>
<td>ICAM-1</td>
<td>intracellular adhesion molecule-1</td>
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<td>NO</td>
<td>nitric oxide</td>
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<td>ppb</td>
<td>parts per billion</td>
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<td>PVR</td>
<td>pulmonary vascular resistance</td>
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adjusted to maintain the partial pressure of carbon dioxide between 38 and 42 mmHg. The tidal volume remained at 18 ± 1 mL/kg to ensure that changes in pulmonary mechanics accurately reflected alterations in the lungs and were not secondary to ventilator manipulation. After 90 minutes of hypoxia, animals were administered heparin and placed on CPB, with a bypass circuit partial pressure of oxygen > 450 mmHg. Arterial cannulation was through the carotid artery and venous drainage was from a right atrial cannula. The aorta was not cross-clamped and no cardioplegia was given. The ventilator was placed on 100% FiO2 and ventilation rate decreased to four breaths per minute while on CPB. The CPB prime consisted of 800 mL direct-drawn whole porcine blood (Animal Biotech Industries) stabilized with sodium citrate. Ultrafiltration during CPB stabilized the hematocrit at 30% to 35%. Animals were kept normothermic with a rectal body temperature > 35°C to minimize effects of hypothermia. Flow rates on CPB were maintained at the estimated cardiac output (100 mL/kg), with a minimum mean arterial pressure of 35 mmHg.

After 1 hour, animals were removed from CPB and returned to normoxic conditions (FiO2 of 40%). Cardiopulmonary function was monitored continuously during the period after CPB. Animals were maintained under anesthesia for 2 hours after CPB and blood samples and hemodynamic measurements were obtained at 15, 30, 60, and 120 minutes of recovery. Left and right ventricular tissues in each of the animals were obtained at the terminal time point. Lung specimens were taken at baseline, end of hypoxia, and end of recovery.

Hemodynamics were monitored throughout the experiment by recording ventilator pressures, systemic arterial pressure, pulmonary artery pressures (Ponemah Physiologic Platform; Gould Systems) and cardiac output (Transonic Systems, Inc.). Respiratory function was monitored by CO2SMO Plus respiratory profile system (Novametrix). Dynamic compliance, airway resistance, end tidal carbon dioxide, and FiO2 were monitored. Alveolar–arterial gradients, ratio of partial pressure of oxygen to FiO2, and PVR were calculated for pulmonary function. Recovery values were compared with values recorded before hypoxia for each animal.

**Treatment groups**

Six piglets received 50 mg of sildenafil citrate through an orogastric tube at the initiation of hypoxia. Eight control animals received saline. The dose of sildenafil was chosen to be 50 mg orally once at the initiation of hypoxia because time to onset is approximately 15 minutes, half-life is 2 to 3 hours, and bioavailability from oral dosing is approximately 38%.14 The target is a dose of approximately 2 mg/kg in piglets weighing 5 to 7 kg. Other studies have reported favorable results with this dose.11

**Tissue protein level analyses**

Tissue samples were homogenized in 10 mmol/L 3-[N-morpholino] propane sulfonic acid buffer with protease inhibitors, centrifuged, and the supernatant frozen. Protein concentration was determined by the Bio-Rad protein assay and the samples were stored at −80°C until used. Western blots were performed with 40 μg total proteins separated on 4% to 12% acrylamide bis-tris gels (Invitrogen) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Antibodies for immunoblotting were antiporcine intracellular adhesion molecule-1 (ICAM-1), developed in our laboratory. Immunoblots were also probed with antibodies for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Chemicon International). Secondary antibodies were alkaline phosphatase-conjugated goat antirabbit or antimouse IgG. Proteins were visualized with the Western Breeze chemiluminescent detection system according to the manufacturer’s instructions (Invitrogen). Protein levels were reported as a ratio of target protein to GAPDH levels on the same immunoblot to correct for background effects.

**Myeloperoxidase activity**

Activity of myeloperoxidase, an enzyme occurring almost exclusively in neutrophils, was measured in lung tissue as a determinant of neutrophil infiltration. Frozen tissue samples (50 mg) were homogenized in 0.5% hexadecyltrimethylammonium bromide dissolved in 10 mmol/L 3-[N-morpholino] propane sulfonic acid, then centrifuged at 21,000 g for 20 minutes at 4°C. The supernatant was mixed with sodium phosphate (80 mmol/L, pH 5.5) and tetramethyl benzimide (16 mmol/L) and incubated at 25°C for 5 minutes. Hydrogen peroxide (1 mmol/L) was added and the samples incubated exactly 3 minutes at 25°C. A blank without hydrogen peroxide was also analyzed for each tissue. The reaction was stopped by the addition of 2 mol/L cold acetic acid. The optical density was measured at 650 nm on a spectrophotometer.
ET-1
Blood samples were centrifuged at 4°C and plasma frozen for later analysis. Tissue samples were homogenized in 10 mmol/L 3-[N-morpholino] propane sulfonic acid and frozen. A commercial ET-1 immunoassay kit (R&D Systems) was used to measure ET-1 protein concentration in plasma and lung tissue. Cross-reactivity with big ET-1 is 1%.

Statistical analysis
Repeated measures ANOVA were used for serial data over time and Fisher’s protected least significant difference post hoc test was used when appropriate to evaluate significant differences between individual time points. Standard ANOVA was used to make comparisons between treatment groups. A p value < 0.05 is considered significant. Statview 4.01 software was used (Abacus Concepts Inc). Data are presented as mean ± SD.

RESULTS
Pulmonary data
In control animals, PVR (Fig. 1) was increased at the end of hypoxia when compared with baseline (baseline 81 ± 37 dynes · s/cm² versus 90 minutes of hypoxia 244 ± 120 dynes · s/cm², p = 0.027) and remained elevated at the end of recovery (baseline 81 ± 37 dynes · s/cm² versus 120 minutes of recovery 230 ± 93 dynes · s/cm², p = 0.004). In the sildenafil-treated animals, PVR was unchanged from baseline at 90 minutes of hypoxia (baseline 38 ± 17 dynes · s/cm² versus 90 minutes of hypoxia 65 ± 32 dynes · s/cm²) and mildly elevated at the end of recovery when compared with baseline (baseline 38 ± 17 dynes · s/cm² versus 120 minutes of recovery 101 ± 60 dynes · s/cm², p = 0.003).

Mean pulmonary pressures in both groups were elevated at the end of hypoxia when compared with baseline values. Although mean pulmonary pressure was elevated in the sildenafil group at the end of recovery, it remained less than the control group. There were no differences in airway resistance or pulmonary compliance in either group. The partial pressure of oxygen to FiO₂ decreased during hypoxia, but rebounded to baseline levels in both groups at the end of recovery (Table 1).

Systemic hemodynamics
Sildenafil administration had no significant effect on systemic vascular resistance. Oxygen delivery was not different between groups. Sildenafil had no significant effect on cardiac output or left ventricular systolic function (LV dp/dt_max). At the end of recovery, sildenafil preserved left ventricular diastolic function (LV dp/dt_min) at baseline values (Table 1).

ET-1 levels
Plasma arterial ET-1 levels (Fig. 2) in controls showed no difference from baseline at the end of hypoxia (baseline 1.3 ± 0.5 pg/mL versus 90 minutes of hypoxia 1.6 ± 0.5 pg/mL), but were elevated at the end of recovery (baseline 1.3 ± 0.5 pg/mL versus 120 minutes of recovery 4.5 ± 3.7 pg/mL, p = 0.01). In the sildenafil-treated animals, no difference in ET-1 was observed at 90 minutes of hypoxia when compared with baseline values (sildenafil baseline 1.3 ± 0.3 pg/mL versus 90 minutes of hypoxia 3.4 ± 3.8 pg/mL), but ET-1 was elevated at the end of recovery (baseline 1.3 ± 0.3 pg/mL versus 120 minutes of recovery 9.8 ± 4.9 pg/mL, p = 0.003). No difference was noted between control animals and sildenafil-treated animals at the end of recovery (p = 0.07).

Tissue ICAM-1 and myeloperoxidase levels
Western blot analysis (Fig. 3) of lung samples revealed no differences in the concentration of ICAM-1 between treatment groups at baseline (controls 5.85 ± 1.34 versus sildenafil 4.73 ± 2.89 ICAM-1:GAPDH ratio). At
the end of recovery, sildenafil-treated animals had lower ICAM-1 protein levels compared with controls (controls 4.68 ± 1.79 versus sildenafil 2.70 ± 0.78 ICAM-1:GAPDH ratio, p = 0.05).

Lung myeloperoxidase levels in controls at baseline (0.14 ± 0.10 U/mg protein) were not significantly different from the baseline levels of sildenafil-treated animals (0.07 ± 0.04 U/mg protein). Likewise, myeloperoxidase levels in the control group at the end of recovery (0.51 ± 0.26 U/mg protein) were not different from sildenafil-treated animals (0.47 ± 0.22 U/mg protein).

**NO levels**

**IV NO**

Jugular venous blood NO (Fig. 4) in control animals, measured by an IV NO gas-permeable probe, reached a nadir at 90 minutes of hypoxia (baseline 364 ± 83 versus 90 minutes of hypoxia 195 ± 100 nM, p = 0.005) and remained depressed at the end of recovery (120 minutes of recovery 257 ± 97 nM, p = 0.028). In the sildenafil-treated animals, there were no significant changes in IV NO levels during hypoxia or recovery (baseline 340 ± 77 versus 90 minutes of hypoxia 280 ± 79 versus 120 minutes of recovery 394 ± 85 nM). IV NO levels in the sildenafil-treated animals were higher compared with control animals at the end of recovery (p = 0.045).

**Exhaled NO**

In control animals, exhaled NO declined during hypoxia (baseline 2.4 ± 0.8 versus 90 minutes of hypoxia 1.7 ± 0.8 parts per billion [ppb], p = 0.001) and continued to decline during reperfusion (120 minutes of recovery 0.6 ± 0.4 ppb, p = 0.001 versus 90 minutes of hypoxia). Exhaled NO in sildenafil-treated animals also declined steadily through hypoxia (baseline 2.8 ± 0.8 versus 90 minutes of hypoxia 2.2 ± 0.9 ppb, p < 0.001) and continued to decline.
through recovery (120 minutes of recovery 1.8 ± 0.9 ppb, p = 0.002 versus 90 minutes of hypoxia). In sildenafil-treated animals, values of exhaled NO remained markedly higher than in controls at the end of recovery (p = 0.029).

**DISCUSSION**

The main goal of this study was to evaluate ability of sildenafil citrate to attenuate cardiopulmonary dysfunction, specifically, pulmonary hypertension associated with hypoxia and reoxygenation injury. The important results from this study reveal that sildenafil citrate can attenuate pulmonary hypertension but has little effect on cardiac dysfunction associated with hypoxia and reoxygenation on CPB. Perhaps the most important finding of this study is that sildenafil markedly attenuates the rise in PVR experienced by control animals and prevents pulmonary hypertension. Lower PVR is likely a direct result of sildenafil’s mechanism of action, the inhibition of phosphodiesterase-5, and agrees with existing animal data. Increased intracellular cyclic guanosine monophosphate leads to pulmonary vasodilation, which is a result of the predominately pulmonary distribution of the phosphodiesterase-5 enzyme. Pulmonary selectivity of sildenafil was also demonstrated by lack of detrimental systemic effects. Although PVR in sildenafil-treated animals increased from baseline, it remained lower than what is considered clinically significant. The PVR in the sildenafil-treated animals correspond clinically to 1 to 1.5 Woods units, which is generally not associated with pulmonary hypertension.

Sildenafil markedly reduces PVR with minimal hemodynamic side effects. Although mean arterial pressure was slightly lower at the end of recovery when compared with baseline, the absolute difference in values was neg-
ligible and not clinically significant. Systemic vascular resistance was not statistically different. These data agree with other studies showing sildenafil has a mild depressant effect on systemic blood pressure.

Regulation of PVR falls under the control of several regulatory pathways. Perhaps the two most important are the ET-1 and NO pathways, which exert complex interdependent control over pulmonary vascular tone. Because our research group has previously shown inhaled NO to increase circulating levels of ET-1\(^9\) and sildenafil’s mechanism of action uses the NO pathway, it is not surprising that sildenafil increases circulating levels of ET-1. Despite higher ET-1 in these animals, PVR remained low after sildenafil administration. Similar to inhaled NO, it is likely that sildenafil’s effect on intracellular cyclic guanosine monophosphate is enough to overpower the vasoconstrictive signal elicited by high levels of ET-1. This finding suggests that increased levels of cyclic guanosine monophosphate contribute to the increase in ET-1.

Alternatively, the mechanism behind the increase in ET-1 may be because of NO. New data suggest that sildenafil may upregulate expression of both inducible and endothelial isoforms of NO synthase.\(^{16}\) Upregulation of these enzymes might explain the preserved levels of IV NO found in this study with sildenafil treatment. Of further interest, NO has been shown to compete with ET-1 for the endothelin receptor,\(^{17}\) which may cause signal termination and increase ET-1 levels by displacement off its receptor. NO has also been suggested to terminate the vasoconstrictive signal of ET-1 by interruption of downstream signaling processes not yet defined.\(^{18}\)

Elevated ET-1 levels have been linked to increases in the inflammatory markers monocyte chemoattractant protein-1 and ICAM-1,\(^{19,20}\) which raised concern about possible sildenafil-induced inflammation beyond that seen with CPB alone. Despite increased ET-1 with sildenafil treatment, we did not detect increased lung levels of ICAM-1. In addition, tissue myeloperoxidase, a marker of neutrophil activity, was not different from controls. It is possible that 2 hours of recovery were not enough time to allow the transcription of protein for an inflammatory response, but previous studies by our group have demonstrated changes in inflammatory mediators 2 hours after CPB.\(^5\) It is possible that sildenafil downregulates ICAM-1 through an unknown pathway. More study is required to elucidate the ICAM-1 response to sildenafil.

In conclusion, sildenafil citrate is an effective and specific pulmonary vasodilator that lacks many of the common difficulties associated with current treatments for pulmonary hypertension. In this study, sildenafil markedly reduces PVR after hypoxia and reoxygenation with CPB and does so without significantly affecting systemic hemodynamics or markers of inflammation. Sildenafil does not attenuate the myocardial dysfunction induced in this model. Further clinical study into use of sildenafil citrate for treatment of pulmonary hypertension after repair of congenital heart lesions is warranted.

**Author Contributions**

Study conception and design: Pearl

Acquisition of data: Lyons, Duffy, Wagner

Analysis and interpretation of data: Lyons, Pearl

Drafting of manuscript: Lyons

Critical revision: Duffy, Pearl

Supervision: Pearl

**REFERENCES**


