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Effects of different inspired oxygen fractions on sildenafil-induced pulmonary anti-hypertensive effects in a sheep model of acute pulmonary embolism

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ABSTRACT

Aims: Sildenafil is a pulmonary anti-hypertensive agent whose action could be modified by different fractions of inspired oxygen (FiO_2). We compared the effects of pure oxygen ($FiO_2 > 90\%$) or room air (21% FiO_2) on the cardiopulmonary actions of sildenafil in sheep with acute pulmonary embolism (APE).

Main methods: Forty anesthetized, mechanically ventilated sheep (34.9 ± 5.4 kg), were randomly distributed into four groups (n = 4 per group): $FiO_2 > 90\%$ without intervention; APE induced by microspheres with $FiO_2 > 90\%$, followed 30 min later by placebo (Emb_{90}); or APE followed 30 min later by intravenous sildenafil (0.7 mg/kg over 30 min) with $FiO_2 > 90\%$ (Emb + $Sild_{90}$) or 21% FiO_2 (Emb + $Sild_{21}$). Variables were recorded until 30 min after the end of treatment administration.

Key findings: Microsphere injection increased ($P < 0.05$) mean pulmonary artery pressure (MPAP) in all embolized groups (111–140% higher than that of baseline). Compared with values recorded 30 min after induction of APE (E_{30}), sildenafil induced greater decreases in MPAP in the Emb + Sil_{90} group than in the Emb + Sil_{21} group (23% and 14% lower than E_{30}, respectively). Hypotension (mean arterial pressure <60 mm Hg) Hg) was precipitated by sildenafil due to systemic vasodilation in the Emb + Sil_{21} group. Embolization lowered the PaO_2/FiO_2 ratio and increased venous admixture, but sildenafil did not alter the oxygenation impairment induced by APE.

Significance: Sildenafil induces a more consistent pulmonary anti-hypertensive effect and causes less interference with the systemic circulation with the concomitant use of pure oxygen than that with room air in the APE setting.

Introduction

The abrupt increase in pulmonary vascular resistance index (PVRI) observed in acute pulmonary embolism (APE) is caused by the mechanical obstruction of pulmonary vessels by emboli together with reactive arterial vasoconstriction (via neural reflexes and release of humoral factors), potentially leading to right ventricular failure and cardiogenic shock [18,20]. Additionally, alveolar hypoxia associated with APE may induce vasoconstriction of the pulmonary vasculature by an important autoregulatory reflex known as hypoxic pulmonary vasoconstriction, which may contribute to the elevation of pulmonary arterial pressure in the APE setting [5].

Although treatment of APE is focused on removing the mechanical obstruction, the relevance of pulmonary vasoconstriction in APE has been valued and the pharmacological blockade of this neurohumoral phenomenon has been suggested as a coadjuvant therapy of APE [20]. The usual therapeutic approaches of APE include oxygen therapy [12], however, it has been suggested that the failure of supplemental oxygen to correct arterial hypoxemia accompanying APE often reflects the existence of severe right to left shunting of venous blood through the lungs [3]. Therefore, it is of great interest to investigate the effects of vasodilators which selectively dilate the pulmonary capillaries in alveoli that are well-ventilated, thus reducing pulmonary hypertension while improving gas exchange in the presence and absence of enriched inspired oxygen fractions (FiO_2).

Sildenafil has been shown to reduce pulmonary vascular resistance and improve arterial oxygenation in patients with lung fibrosis and secondary pulmonary hypertension [11]. Interestingly, sildenafil was reported to prevent rebound pulmonary arterial hypertension following withdrawal of inhaled nitric oxide in infants [2]. Sildenafil has been

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shown to attenuate APE-induced pulmonary hypertension [9,16,21]. The main advantages presented by sildenafil for treating APE-induced hypertension include its kinetics of pulmonary vasorelaxation (rapid effect), selectivity for the pulmonary circulation (slight effects on systemic blood pressure) and its positive impact on arterial oxygenation [8,11]. Together, these data suggests that sildenafil improves the matching between ventilation and perfusion in the lungs.

Alveolar hypoxia and hypoxic pulmonary vasoconstriction in areas of low ventilation-to-perfusion (V/Q) ratios could be minimized by the use of high FiO_2 during mechanical ventilation. Because the presence of hypoxic pulmonary vasoconstriction may alter the pulmonary vasodilating actions of sildenafil, we hypothesized that the anti-hypertensive effect of sildenafil would be enhanced by the concomitant use of higher FiO_2 in mechanically ventilated sheep presenting APE. In order to confirm this hypothesis we evaluated whether the effects of sildenafil on pulmonary and systemic hemodynamics and respiratory parameters could be modified by different fractions of inspired oxygen (FiO_2). We compared the effects of pure oxygen ($FiO_2 > 90\%$) or room air (FiO_2 set at 21%) on the pulmonary antihypertensive action of sildenafil in a model of APE in anesthetized sheep.

Materials and methods

Animal

Previous approval was obtained by the Institutional Animal Care Committee (protocol number 457/2013) and guiding principles published by the European Union Directive 2010/63/EU and the ARRIVE (*Animal Research: Reporting of In Vivo Experiments*) guidelines were followed. Forty rams of the "Santa Inês" breed, weighing 34.9 ± 5.4 kg (mean ± SD) were grouped in pens (3 m^2 per animal) with free access to food and water for at least 10 days before the experiments.

Based on preliminary trials, the microembolism model resulted in mean pulmonary artery pressure (MPAP) values of 22 ± 4 mm Hg (mean ± SD) in anesthetized sheep, an n = 8 animals per group would be required for demonstrating a 30% reduction in MPAP values with a statistical power of 90%. The animals for this study were selected on the basis of a normal health status determined by physical examination performed by two veterinarians, and by packed cell volume and total plasma protein values within normal ranges to avoid animals with chronic anemia and hypoproteinemia associated with chronic gastrointestinal parasite infestation.

Instrumentation

Animals were kept with food and water for 24 and 12 h before the experiment, respectively. Five minutes after intravenous (i.v.) fentanyl (5 μg/kg) administration, anesthesia was induced and maintained with i.v. ketamine (7.5 mg/kg, bolus, followed by 20 mg/kg/h) and midazolam (0.35 mg/kg bolus, followed by 0.25 mg/kg/h) through a 14-gauge catheter placed in the jugular vein. After orotracheal intubation, volume controlled ventilation (Dräger Primus, Drägerwerk AG & Co., Lübeck, Germany) was commenced with two inspired oxygen fractions (FiO_2, as detailed below), under neuromuscular blockade produced by atracurium (0.3 mg/kg, followed by 0.5 mg/kg/h, i.v.). The tidal volume was set to 15 mL/kg with a fixed inspiration-to-expiration ratio (1:2). The minute volume ventilation (MVV) was adjusted to maintain eucapnia (arterial carbon dioxide tension, $PaCO_2$ between 35 and 45 mm Hg) by changes in the respiratory rate.

Treatment drugs (sildenafil or placebo) and fluid therapy (2 mL/kg/h of Lactated Ringer's were administered through a 20-gauge catheter placed in the cephalic vein). Mean arterial pressure (MAP) was monitored via a fluid-filled pressure transducer system (Tru Wave PX 260, Edward Lifesciences, Irvine, CA) connected to an 18-gauge femoral artery catheter implanted percutaneously.

An 8.5 Fr introducer sheath was placed in the left jugular vein for the introduction of a balloon tipped Swan Ganz thermodilution catheter (Model 131HF7 Edwards Lifesciences, Irvine, CA) into the pulmonary artery. The correct position of the catheter was confirmed by verifying the typical pressure waveforms on the screen of the monitor (AS/3, Datex Ëngstrom, Helsinki, Finland). The proximal and distal ports of the thermodilution catheter were used for monitoring mean pulmonary arterial pressure (MPAP) and central venous pressure (CVP), respectively, via fluid filled pressure transducers zeroed at the level of the heart. The distal port of the catheter was used for measuring pulmonary artery wedge pressure by temporarily insufflating the balloon with 0.7 mL of air.

Mixed-venous and arterial blood samples were collected from the pulmonary and femoral artery catheters, respectively, for temperature corrected blood gas analysis (348 pH Blood Gas Analyzer, Siemens, Halstead, UK).

Heart rate (HR) was monitored by a lead II electrocardiogram. Cardiac output (CO) was measured by the injection of 5 mL of cold (3 to 5 °C) 5% dextrose into the CVP port. For each time point CO was averaged from 3 consecutive measurements. Body surface area was measured as $BSA = 0084 * (\text{body weight kg})^{2/3}$. Cardiac index (CI), pulmonary vascular resistance index (PVRI), systemic vascular resistance index (PVRI), arterial-to-end-tidal carbon dioxide gradient [$P(a\text{-}ET)CO_2$], minute volume ventilation (MVV), PaO_2/FiO_2 ratio, and venous admixture (Qs/Qt) were calculated using standard formulae.

Group assignments

Animals were randomly assigned to one of the following groups (n = 8 per group)

1) $Sham_{90}$ group: Anesthetized sheep breathing pure oxygen ($FiO_2 > 90\%$) that did not undergo without any intervention.
2) Emb_{90} group: Anesthetized sheep breathing pure oxygen ($FiO_2 > 90\%$) that underwent APE induced by administration of 500 mg of silicone microspheres (300 μm of diameter, 10 mg/ml) divided in five bolus injections separated by 30 s intervals (Sephadex G50; Pharmacia Fine Chemicals; Uppsala, Sweden); followed 30 min later by physiological saline (placebo) administered over 30 min by a syringe pump (Pump 11 Elite, Harvard Apparatus, Holliston, MA).
3) $Emb + Sild_{90}$ group: Anesthetized sheep breathing pure oxygen ($FiO_2 > 90\%$) that underwent APE (as described before), followed 30 min later by infusion of sildenafil (0.7 mg/kg) administered over 30 min [16] by a syringe pump.
4) $Emb + Sild_{21}$ group: Anesthetized sheep breathing room air ($FiO_2 = 21\%$) that underwent APE (as described before), followed 30 min later by infusion of sildenafil (as described before).

Experimental protocol

Hemodynamic data were collected at baseline (BL), 15 and 30 min (E_{15} and E_{30}, respectively) after induction of microembolism (Emb_{90}, $Emb + Sild_{90}$, or $Emb + Sild_{21}$, respectively) or no intervention ($Sham_{90}$), 15 and 30 min (S_{15} and S_{30}, respectively) after physiological saline (Emb_{90}) or sildenafil administration ($Emb + Sild_{90}$, or $Emb + Sild_{21}$) or no intervention ($Sham_{90}$) (S_{15} and S_{30}, respectively), and 15 and 30 min after placebo or sildenafil were interrupted or no intervention (W_{15} and W_{30}). Blood gas derived data were determined at BL, E_{30}, S_{30}, and W_{30}.

After the end of data collection animals were euthanized by a lethal dose of sodium thiopental (30 mg/kg) and potassium chloride (20 mg/kg) while still anesthetized.

Statistical analysis

Using a commercially available statistical software (Prism 6.02; GraphPad, San Diego, CA), a Shapiro–Wilk test was applied to verify