Cardiopulmonary Bypass Is Associated With Altered Vascular Reactivity of Isolated Pulmonary Artery in a Porcine Model: Therapeutic Potential of Inhaled Tezosentan

Arnaud Mommerot, MD,*†‖ André Y. Denault, MD, PhD,‡ Jocelyn Dupuis, MD, PhD,*§ Michel Carrier, MD,*† and Louis P. Perrault, MD, PhD*†

Objective: **Whereas it is established that endothelin-1 elicits sustained deleterious effects on the cardiovascular system during cardiopulmonary bypass (CPB), presently it remains unknown whether the inhaled administration of the dual ET_A and ET_B antagonist tezosentan prevents the development of pulmonary endothelial dysfunction.**

Design: **A prospective, randomized laboratory investigation.**

Setting: **University research laboratory.**

Participants: **Landrace swine.**

Interventions: **Three groups of animals underwent a 90-minute period of full bypass followed by a 60-minute period of reperfusion. Among treated groups, one received tezosentan through inhalation prior to CPB, whereas the other one received it intravenously at weaning from CPB; the third group remained untreated. Pulmonary vascular reactivity studies, realized on a total of 285 rings, were performed in all groups, including 1 sham.**

Measurements and Main Results: **The contractility of pulmonary arteries to prostaglandin $F_{2\alpha}$ and to the thromboxane A_2 mimetic U46619 was preserved in animals submitted to CPB. By contrast, there were significant increases both in the maximal contraction to endothelin-1 and in the plasma levels of the peptide 60 minutes after reperfusion. Tezosentan administered by inhalation or intravenously did not prevent the development of pulmonary CPB-associated endothelial dysfunction. However, while hemodynamic disturbances were improved with both routes, the inhaled administration had a beneficial effect on oxygen parameters over intravenous administration.**

Conclusions: **Despite the blockade of the endothelin-1 pathway with tezosentan, the development of the pulmonary endothelial dysfunction associated with CPB still occurred. However, only the inhalation route had a significant impact on gas exchange during CPB.**

KEY WORDS: CPB, physiology/pathophysiology, endothelium, pulmonary vascular resistance/hypertension, vascular tone and reactivity

CARDIOPULMONARY BYPASS (CPB) still is warranted for the majority of cardiac surgery procedures worldwide. However, despite continuous advances in material development, it remains a potential source of complications resulting from the systemic inflammatory response induced by the contact between blood elements and artificial surfaces of the CPB circuit.[1] Lungs are one of the major targets of this complication, characterized by increased capillary permeability and pulmonary vascular resistance, a rise in alveolar-arterial oxygen gradient, and a decrease in oxygenation.[2] During the procedure, the pulmonary artery tree is perfused minimally and, when lungs are perfused again upon weaning from CPB, pulmonary endothelium experiences an ischemia-reperfusion injury. The cell activation leads to post-CPB vascular alteration[3,4] and, subsequently, to pulmonary artery hypertension and right ventricular dysfunction, which carries poor prognosis.

The authors previously have demonstrated in their swine model of CPB that pulmonary endothelium-dependent relaxations are impaired, attested by a significant decrease in the maximal relaxation following stimulation of isolated vascular rings with acetylcholine. The administration of the phosphodiesterase V inhibitor sildenafil prevented the endothelial dysfunction, supporting an impairment in the nitric oxide pathway associated with CPB.[5] This altered pathway may be the consequence of an alteration in the signaling transduction pathway through underlying smooth muscle cells or may, as seen in other models, be the result of an overwhelming contraction induced by an increase in endothelium-dependent contracting factors, such as prostanoids and endothelin-1 (ET-1).[6] ET-1 elicits potent and prolonged effects on cells of the cardiovascular system through the binding of its receptors.[7] At the vascular level, both ET_A and ET_B subtypes localized on smooth muscle cells are at the origin of a contraction. On its side, only the ET_B subtype is present on the endothelium, leading to a vasorelaxation via the release of nitric oxide and prostacyclin.

The induction and release of ET-1 in the early ischemia-reperfusion period have been suggested to be deleterious both for cardiac vessels and the myocardium,[8–10] and animal studies also suggest a potential role for ET-1 in post-CPB pulmonary artery hypertension.[11,12] Minimization of ET-1 action through a blockade of its receptors with an endothelin-receptor antagonist has been proven beneficial. In fact, decreases in pulmonary hypertension and in the incidence of post-CPB pulmonary hypertensive crises as well as improvements in pulmonary compliance and oxygenation have been demonstrated in the experimental setting.[13] Tezosentan is a dual antagonist, acting on both ET_A- and ET_B-receptor subtypes. In the clinical setting, previous phase II studies have demonstrated that tezosentan is associated with improved hemodynamic parameters, such as decreased systemic and pulmonary pressures and resistance.[14,15] In 4 phase III placebo-controlled trials (the Randomized Intravenous TeZosentan [RITZ] trials), in which a total of 1,230

*From the *Research Center, and Departments of †Cardiovascular Surgery, ‡Anesthesiology, and §Medicine, Montreal Heart Institute and Université de Montréal, Montreal, Quebec, Canada; and ‖Department of Cardiac Surgery, University Hospital of Strasbourg, Strasbourg, France.*

Address reprint request to Louis P. Perrault, MD, PhD, Department of Surgery, Montreal Heart Institute, 5000 Belanger Street, Montreal, Quebec H1T 1C8, Canada. E-mail: Louis.perrault@icm-mhi.org

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patients with acute heart failure were included, tezosentan failed to significantly improve clinical outcome despite improvement in the hemodynamic profile.[16] However, tezosentan has not been used or studied in cardiac surgery in which the endothelin system is activated during CPB. Only 1 negative clinical trial has been reported in which only the intravenous administration before CPB did not prevent right ventricular failure.[17]

The use of several agents administered through inhalation as alternative to nitric oxide in the prevention or treatment of right ventricular failure after CPB is now explored in clinical practice. The major advantages of this route over the intravenous administration are the absence of systemic hypotension and the selective delivery in the pulmonary vascular tree. The incremental benefit of inhaled administration over intravenous during CPB has been demonstrated in the authors' laboratory with sildenafil and milrinone.[5,18] In fact, the inhalation route was associated with a better oxygenation profile and better impact on vascular reactivity. However, presently it remains unknown whether the inhaled administration of tezosentan, which has a different mechanism of action from drugs currently used, would have the same advantage over intravenous administration in a CPB model; to the authors' best knowledge, there is no such information in the literature. In addition, the fact that the intravenous administration of tezosentan in the TACTICS trial did not result in a decrease in right ventricular failure during weaning from CPB supports the authors' quest for an alternative route.[17]

The relationship between CPB and both pulmonary vascular and hemodynamic alteration has been unequivocally established clinically and in experimental models. Drugs aiming at increasing the vasodilation, such as milrinone, epoprostenol and sildenafil, have been proven efficient at improving the reperfusion syndrome. However, knowledge is scarce regarding the vasocontractile environment following CPB. Indeed, it remains unknown presently whether numerous contractile pathways are impaired by the CPB procedure and whether the selective blockade of the endothelin-1 pathway with the non-selective ET_A and ET_B antagonist tezosentan may be beneficial to the development of pulmonary endothelial dysfunction and hemodynamic alterations. In addition, the inhalation route of administration has gained popularity in clinical practice as it does not have a shunt effect on the hypoxic vasoconstriction as opposed to intravenous administration. However, whether or not there are differential effects with tezosentan for both routes is unknown. Hence, in the current study, the authors' primary hypothesis was to demonstrate that tezosentan is efficient at preventing the development of pulmonary endothelial dysfunction with an advantage when given by inhalation over intravenously.

MATERIAL AND METHODS

Animal Care

Landrace swine (26 ± 0.9 kg) of either sex, 8 weeks of age, were housed on their arrival and had access to food and tap water ad libitum. All experiments were performed in compliance with recommendations of the Guidelines on the Care and Use of Laboratory Animals issued by the National Council on Animal Research and the guidelines of Animal care approved by the local animal care committee.

Study Protocol

One week after their arrival, swine were assigned randomly to 1 of the 4 following groups: (1) sham (sham, n = 10), (2) untreated cardiopulmonary bypass (untreated CPB, n = 21), (3) CPB treated with tezosentan administered by inhalation (INH, n = 3), or (4) CPB treated with tezosentan administered intravenously (IV, n = 4). To assess the therapeutic benefit of tezosentan, the selection of the dose (0.5 mg/kg) was based on a paper published by Persson et al.[19] This later demonstrated a positive effect on endotoxin-induced pulmonary hypertension and pulmonary capillary pressure when administered intravenously or by inhalation in swine. Pulmonary vascular reactivity to various vasomotor agents was determined in the entire population of all groups (Fig 1). ET-1 concentration was determined in the untreated CPB group in order to assess its fluctuation throughout the procedure and its potential impact on the alteration of the endothelial function.

Cardiopulmonary Bypass Procedure

The surgical protocol previously has been described in detail.[18] Briefly, animals were anesthetized and mechanically ventilated with a constant oxygen and air mixture (inspired fraction of O_2 = 0.66). Regular blood gas measurements allowed the ventilation parameters adjustment to be maintained within physiologic limits. After skin preparation and sterile draping, the heart was exposed through a median sternotomy. In the sham group, heparin (400 IU/kg) was administered intravenously to animals, which were kept alive and ventilated for 1 hour with 1% isoflurane. Piglets then were exsanguinated and the heart and lungs were harvested « en-bloc » in a cold modified Krebs solution (NaCl, 118.3 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; $NaHCO_3$, 25 mM; EDTA, 0.026 mM; dextrose, 11.1 mM) for vascular reactivity studies.

In the untreated CPB group, the heart rate was monitored continuously with 5 subcutaneous limb electrodes and systemic blood pressure by insertion of a femoral artery catheter. Pulmonary artery pressure, central venous pressure, and cardiac output were recorded by means of a central catheter (Swan-Ganz). Hemodynamic values were measured and blood gas samples were harvested in both atria at baseline as well as at 30 and 60 minutes after the weaning from CPB; each animal was used as its own control. After heparinization (400 IU/kg), both aorta and right atrium were cannulated; CPB was started when activated clotting time reached 400 seconds. The pump was conducted in order to achieve a complete drainage of the right side of the heart. To ensure the complete absence of blood flow through the pulmonary bed, cross-clamping of the main pulmonary arteries was performed. No aortic cross-clamping or cardioplegic arrest was performed, leaving the heart beating. After a 90-minute period of complete CPB, the pulmonary artery clamp was removed, and the mechanical ventilation was reinstituted. Swine were weaned from CPB and this was achieved without difficulties in each case. No administration of protamine was performed at the end of the procedure since the formation of complexes with heparin potentially may induce acute pulmonary artery hypertension. After the termination of CPB, animals were submitted to spontaneous pulmonary artery bed reperfusion for a 60-minute period. Sacrifice was then performed by exsanguination; heart and lungs were harvested « en-bloc » and immediately immersed in a cold modified Krebs solution for vascular reactivity studies.

For both groups receiving the administration of tezosentan, the same procedure as described for the untreated CPB group was followed. In the INH group, the bolus of tezosentan was given via the endotracheal tube through a nebulizer during the 30-minute period preceding the initiation of CPB; the conventional in-line nebulizer kit (Salter Labs, Arvin, CA) was connected to the inspiratory limb of the ventilator. In the IV group, a 10-mg bolus diluted in 20 mL of buffer