The Heart Surgery Forum #2010-1031
14 (1), 2011 [Epub February 2011]
doi: 10.1532/HSF98.20101031

Online address: http://cardenjennings.metapress.com

Effective Pulmonary Artery Perfusion Mode during Cardiopulmonary Bypass

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ABSTRACT

Aim: Reducing lung injury during cardiopulmonary bypass (CPB) is important for patients' recovery. The present study was designed to research convenient and effective pulmonary artery perfusion mode during CPB in an animal model.

Methods: Twelve healthy mongrel dogs were randomly divided into 2 groups: a control group and a perfusion group designed to simulate clinical CPB-induced lung injury. During CPB, pulmonary artery perfusion with modified low-potassium dextran (LPD) solution was performed immediately after the initiation of ischemia and before reperfusion for 3 to 4 minutes each time, with pressure maintained at 15 to 20 mmHg; animals in the control group were not perfused. After pulmonary reperfusion, the changes in pulmonary function and tissue biochemical data were determined.

Results: Compared with the control group, lung compliance, oxygenation, and vascular resistance after reperfusion were significantly improved in the perfusion group. The malonaldehyde concentration, neutrophil sequestration ratio, and tissue water content also decreased significantly in the perfusion group.

Conclusion: The pulmonary artery perfusion mode used in this experiment could relieve CPB-induced lung injury effectively. Improving cellular tolerance to hypoxia and decreasing inflammatory reaction may be the important mechanisms. Moreover, this mode is convenient and does not interfere with the intended operations, which is promising for clinical use.

INTRODUCTION

There is increasing evidence that the combination of ischemia-reperfusion (I/R) and the normal systemic inflammatory response during cardiopulmonary bypass (CPB) leads to lung injury. The injury to the lungs is more severe than to other tissues, and postoperative pulmonary dysfunction remains a life-threatening problem for patients undergoing CPB, especially infants. Therefore, there have been intensive efforts focused on developing methods to protect the lungs during CPB. The use of pulmonary artery perfusion with a hypothermic protective solution has been shown to have protective effects against I/R injury [Halldorsson 1998; Schnickel 2006] and CPB-induced injury [Wei 2004; Gabriel 2008]. Although this technique does provide protection against I/R- and CPB-induced injuries, it can also cause damage to endothelial cells and the internal environment. Furthermore, as an additional operation, the protective perfusion is bound to increase the workload and could even interfere with the intended operations. Thus, it is important to determine a reasonable and effective perfusion protocol in the course of application of this technique.

There is currently no accepted argument about the suitable pulmonary perfusion mode during CPB. Perfusion pressure not only influences the flow rate and perfusate distribution, but also directly determines the stress being exerted on the pulmonary vasculature [Halldorsson 2000]. The perfusion pressure may more completely and accurately reflect the impact of perfusion on lung tissues. As such, it may be preferable to tightly control perfusion pressure, rather than flow rate, during perfusion. In addition to perfusion pressure, the perfusion duration and opportunity are all important aspects directly influencing the protection effect. Thus, in this experiment we defined perfusion pressure, duration, and opportunity as 3 variables of perfusion mode and tried to establish a convenient and effective perfusion protocol during CPB.

Received February 18, 2010; received in revised form July 18, 2010; accepted August 18, 2010.

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MATERIALS AND METHODS

Establishment of Animal Models and Mode of Pulmonary Perfusion

All the animal use and care protocols were reviewed and approved by the animal care committee of Shenyang Northern Hospital. Twelve healthy mongrel dogs (weighing approximately 15 kg) were shaved, fasted overnight (6 hours), and then the next day received cannulas in the right femoral artery and left ulnar vein to allow continuous monitoring of arterial blood pressure and blood gas levels and blood transfusion. After preoperative intravenous injection of atropine (0.01 mg/kg), anesthesia was induced with intravenous injection of morphine (2 to 4 mg), propofol (1.5 to 2.5 mg/kg), and pipecuronium bromide (0.1 mg/kg) and was maintained by continuous infusion of propofol (50 to 150 μg/kg per minute) with a micro pump. Mechanical ventilation (Galileo Ventilator, Hamilton Medical, Bonaduz, Switzerland) was established by oral trachea cannula with a double-lumen endotracheal catheter, which could be converted for use with either double-lung or single-lung ventilation. Volume-controlled

ventilation was used with an inspired oxygen concentration of 50% to 60%, tidal volume of 10 to 15 mL/kg, and positive end-expiratory pressure (PEEP) of 3 mmHg. All the animals received 1 g of intravenous cefazolin preoperatively.

After median sternotomy, the pericardium and both pleural spaces were entered. A 7F Swan-Ganz Flow-Directed Thermodilation Catheter (93A-131-7F, Edwards Lifesciences Corp, Irvine, California, USA) was inserted through the right internal jugular vein into the main pulmonary artery for determination of pressure and cardiac output. A catheter was placed in the left atrium for pressure measurement. The left hilar structures (left pulmonary artery, veins) and right pulmonary arteries were dissected, and umbilical tapes were placed under the hibateral pulmonary arteries. The animals were then given 3 mg/kg of heparin intravenously. CPB was established through the right atrium and ascending aorta. The flow rate was maintained at CPB 60 to 90 mL/kg per minute, and the average systemic pressure was kept at 60 to 70 mmHg, with the heart beating continuously. Dopamine or nitroprusside sodium was used intermittently to adjust the pressure. Heparin was administered according to activated coagulation time (ACT), which was kept between 400 and 500 seconds. The temperature of the nasopharynx was reduced to and kept at about 32°C.

In the control animals, the left pulmonary artery was blocked, and the tracheal cannula was adjusted for ventilation of only the right lung.

For animals in the perfusion group, an 8F DLP arterial cannula (DLP Inc., Grand Rapids, Minnesota, USA) was inserted through the main pulmonary artery incision into the left pulmonary artery in order to infuse the protective solution. The cannula was stabilized by tightening the umbilical tape under the root of the left pulmonary artery, with simultaneous blockade of the left pulmonary blood flow. The tracheal cannula was adjusted for ventilation of only the right lung. A catheter was placed distal to the tape to allow for continuous monitoring of perfusion pressure.

In the 2 groups, during the period of blockage, the roots of the left pulmonary veins were clamped, and the upper and lower pulmonary veins were incised longitudinally at about 5 mm [Strüber 2000]. After 90 minutes of ischemia, the left pulmonary artery was unclamped and reperfused, and the left mechanical ventilation was restored. Just prior to the unclamping of the left pulmonary artery, the incisions in the pulmonary veins were closed with running 5-0 Prolene sutures, and the roots of the left pulmonary veins were unclamped.

After reperfusion of the left lung, the temperature of the nasopharynx was elevated gradually to about 36°C. When the circulation was stable, the CPB flow rate was decreased gradually, and the animals were weaned from the CPB. At the same time, propofol was discontinued, and the animals were kept sedated with intermittent administration of morphine, pipecuronium bromide, or midazolam. After 4 hours of reperfusion, functional measurements were carried out and specimens were obtained for biochemical analysis. During the entire procedure, the blood gas and electrolyte concentrations were kept within normal limits. All the animals had normal hematocrit (40% ± 3%) initially, which was kept at about 30% during CPB and about 35% after CPB by use of adequate diuresis, blood transfusion, and ultrafiltration as needed.

During the 90 minutes of CPB and ischemia, the left lung was treated as follows: in the control group, there was no perfusion of the protective solution; in the perfusion group, pulmonary artery perfusion with modified low-potassium dextran (LPD) solution was performed immediately after the initiation of ischemia and before reperfusion for 3 to 4 minutes each time, with pressure maintained at 15 to 20 mmHg. The temperatures of the protective solutions were 8°C and 25°C, respectively. During perfusion, left mechanical ventilation was restored temporarily.

Preparation of the Modified LPD Solution

The pulmonary protective solution consisted of dextran-40 (40 g/L), glucose (15 g/L), insulin (3 U/L), Na_2HPO_4 (6.4 g/L), NaH_2PO_4 (0.6 g/L), NaCl (3 g/L), KCl (0.45 g/L), anisodamine (50 mg/L), L-arginine (2.0 g/L), methylprednisolone (200 mg/L), and prostaglandin E_1 (250 μg/L), with Na^+ 146 mmol/L, K^+ 6.0 mmol/L, and colloid osmotic pressure (COP) of 24 to 28 mmHg, which was slightly higher than normal plasma COP. The pH was adjusted to 7.7 to 7.8 by titrating with H_3PO_4 solution.

Measurements

Determination of Pulmonary Function. Baseline pulmonary functions (F_b) and post-reperfusion pulmonary functions (F_p) were measured before ischemia and after 4 hours of reperfusion, respectively, by transiently clamping the right pulmonary artery, adjusting ventilation for the left lung, thus diverting all blood flow and ventilation directly through the left lung. All ventilation settings were kept the same between the 2 readings. Changes in pulmonary functions (pulmonary vascular resistance [PVR], compliance, oxygenation index) are expressed as the ratio of both pulmonary function values [Halldorsson 2000], ie, $(F_b - F_p)/F_b$.

Pulmonary Vascular Resistance. PVR was calculated as follows: PVR (dyn sec cm^{-5}) = (PAP – LAP)/CO 80, where PAP represents the mean pulmonary arterial pressure measured with a pulmonary artery catheter, LAP represents the mean left atrial pressure, and CO represents cardiac output, as measured using the Swan-Ganz catheter (Edwards Lifesciences Corp) and an Edwards 9502 computer (Baxter Health-Care Corp, Edwards Division, Santa Ana, California, USA) according to thermodilution. The average of 3 measurements was used for CO [Halldorsson 2000].

Dynamic Lung Complaince. Dynamic lung compliance (C_d) represents lung compliance during breathing, which was calculated as the change in volume over the change in pressure during breathing, ie, $C_d = \Delta V/\Delta P$. Under mechanical ventilation, C_d was calculated as follows: C_d (mL/cm H_2O) = V_t/(PIP – PEEP), where V_t represents the tidal volume, PIP represents peak inspiratory pressure, and PEEP represents positive end-expiratory pressure. Animals were sedated sufficiently, spontaneous breathing was interrupted, and any secretions in the trachea were removed by suction prior to measurement.

Pulmonary Oxygenation Index. Blood samples (3 mL) were drawn from the left pulmonary vein for blood gas analysis and to obtain the oxygen partial pressure (PvO_2). The pulmonary oxygenation index, expressed as the ratio of PvO_2 and fraction of inspired oxygen (FiO_2), reflected the oxygenation of the left lung.

Analysis of Blood and Tissue Samples. ***Malonaldehyde Concentration.*** After 4 hours of reperfusion, 3 mL of left pulmonary venous blood was collected and centrifuged at 4°C. An aliquot of the resulting supernatant was taken for measurement of the malonaldehyde (MDA) concentration using the thiobarbituric acid–reactive assay and the colorimetric method [Liu 2000]. The MDA assay kit was purchased from Haoyang Biological Product Co., Ltd. (Tianjin, China), and the analysis was performed according to their instructions.

Sequestration Ratio of Neutrophils. Blood was collected from the right atrium and the left pulmonary vein after 4 hours of reperfusion, and both neutrophil granulocyte counts (C_R and C_L) were determined [Liu 2000]. The pulmonary neutrophil sequestration ratio was expressed as: $(C_R - C_L)/C_R$.

Tissue Edema. A biopsy sample was taken from the superior portion of the left upper lobe after 4 hours of reperfusion. It was weighed and then dried to a constant weight at 80°C to 85°C. Percentage of lung water was calculated using the following formula: tissue water content = (wet weight – dry weight)/wet weight 100%.

Statistical Analysis

All values were expressed as mean ± standard error. Unpaired Student *t* tests were used for comparison of variables between the 2 groups. All analyses were performed using SPSS 11.0 software for windows (SPSS Inc., Chicago, Illinois, USA) and values of $P < .05$ were considered statistically significant.

RESULTS

Changes in Pulmonary Function

All the postoperative changes in pulmonary functions were expressed as a percentage of baseline measurements (Table). After 4 hours of reperfusion, the control group exhibited significant pulmonary injury as manifested by a large change (elevation) in PVR (86.2% ± 4.26%), decreased compliance (28.3% ± 2.75% change), and decreased oxygenation index (47.2% ± 3.58% change). In contrast, compared with the control group, the perfusion group had significantly less injury, with lower PVR (28.7% change, $P < .01$) and improved compliance (10.8% change, $P < .01$) and oxygenation index (19.2% change, $P < .01$).

Changes in Biological Markers of Tissue Injury

Compared to the control group, after 4 hours of reperfusion, the MDA production and neutrophil sequestration were significantly decreased in the perfusion group (16.3 nmol/mL versus 27.5 nmol/mL, $P < .01$; 0.08 versus 0.21, $P < .01$; respectively) (Table). Although the tissue water content was not significantly different between the 2 groups, there was tendency toward improvement in the perfusion group compared with the control group.

Change Ratios of Left Pulmonary Functions and Tissue Biochemical Results after 4 Hours of Reperfusion

Variable	Control Group	Perfusion Group
Change ratio of PVR, %	86.2 ± 4.26	28.7 ± 2.56
Change ratio of lung compliance, %	28.3 ± 2.75	10.8 ± 1.07
Change ratio of oxygenation index, %	47.2 ± 3.58	19.2 ± 2.12
Concentration of MDA, nmol/mL	27.5 ± 1.76	16.3 ± 1.44
Sequestration of neutrophil	0.21 ± 0.019	0.08 ± 0.014
Tissue water content, %	85.2 ± 0.34	83.3 ± 0.30

*PVR indicates pulmonary vascular resistance; MDA, malonaldehyde. All variables except tissue water content were significantly different between groups ($P < .01$).

DISCUSSION

During cardiac surgeries, the lungs experience both I/R injury and the effects of systemic inflammation, including increased production of inflammatory cytokines, induction of peroxide, activation of the complement cascade, and production of other cytotoxic effectors, all of which contribute to vascular injury of the lungs. In addition, the increased expression of various adhesion molecules by endothelial cells promotes the adherence of neutrophils and their activation and migration to the lungs, which further aggravates lung inflammation [Boyle 1997; Verrier 1998].

We used a dog model to simulate the pulmonary damage caused by I/R injury and the systemic inflammatory reaction during CPB. In order to simplify surgical trauma and to avoid any interference with pulmonary biochemical indicators resulting from myocardial I/R, we kept the heart beating continuously during CPB. In the clinic, cardiac arrest and total CPB make the lungs almost completely ischemic. Thus, because the heart was beating continuously in our study, there was a small portion of blood flowing through the heart, which could have slightly influenced the effect of pulmonary ischemia by antidromic conduction to the left pulmonary veins. To account for the small amount of blood flow during blockage of the left pulmonary artery, we clamped the roots of the left pulmonary veins. Additionally, we incised the upper and lower pulmonary veins longitudinally to ensure perfusate flowing out maximality during perfusion and to prevent excessive retention of perfusate in the lung.

The perfusion mode used in this experiment was based on our previous experience, and was designed to assess characteristic changes that occur following pulmonary injury during CPB. First, we selected modified LPD solution as the perfusate because it could allow for a prolonged duration of pulmonary ischemia and improve pulmonary function by protecting endothelial cells and surfactant function [DeCampos 1998; Strüber 2000; Pearl 2004]. Second, we chose to initiate the perfusion just after ischemia and before reperfusion with low and normal temperatures (8°C and 25°C, respectively). The first perfusion allowed for decreased tissue metabolism and lower local deposition of blood cells, resulting in improved maintenance of the cellular tolerance to hypoxia [Liu 2000]. The second perfusion [Halldorsson 1998; Halldorsson 2000] was intended to rinse out or neutralize the production of anaerobic metabolites and enhance the resistance of endothelial cells against inflammatory reactions. Perfusion with normal (physiological) temperature perfusate helps to prevent angiospasms and adapts the vessel to the temperature after reperfusion. Third, we perfused the tissue for 3 to 4 minutes each

time rather than using continuous perfusion with oxygenated blood. Compared with ischemia-induced injury, the inflammatory reaction from non-physiological circulation and I/R might be an important factor that induces lung injury [Mills 1992]. Thus, the perfusion used in this experiment wasn't expected to completely resolve hypoxia, but was intended to elevate the tissue's tolerance to hypoxia and resistance to inflammation. In addition, 3 to 4 minutes of perfusion could help avoid tissue damage (such as pneumonedema) and any potential interference with the intended surgical procedure, which often occurs during long-time and large-dose perfusions.

Perfusion pressure not only influences the flow rate, but also is directly related to the amount of stress force being exerted on the lung. Under physiological conditions, shear stress is necessary to maintain the normal growth and development of endothelial cells [Gimbrone 2000], and proper shear stress can stimulate endothelial cells to express and release beneficial vasoactive substances [Reinhart 1994; Davis 2001]. In contrast, fluid compressive stress, stretch stress, and high shear stress can damage endothelial cells, destroy the integrity of the vasculature, stimulate inflammatory responses [Bernal-Mizrachi 2004], induce the expression of ICAM-1 [Sterpetti 1993; Nagel 1994], and cause increased neutrophil adherence, activation, and migration in the lungs during reperfusion.

From the results of this experiment, we should conclude that the pressure used in this experiment could effectively equilibrate the perfusion flow and stress forces. It could not only ensure effective perfusion flow, but also avoid exorbitant stress forces on the lung tissue.

In this experiment, this perfusion mode was convenient. Even if it were to be used in clinical cardiac surgery, there are no aspects (including preparation of perfusate, opportunity and duration of perfusion, etc.) that should present any interference with the intended operations. In the experiment, the operations of blocking and cutting open the left pulmonary veins were only based on the heart beating during CPB. Generally, the heart is arrested and opened during clinical cardiac surgery. A drainage tube is placed in the left atrium. When pulmonary perfusion is performed, perfusate can be drained from the left atrium, so the operations of blocking and cutting open the left pulmonary veins are unwanted clinically.

In conclusion, this experiment explicitly named perfusion pressure, duration, and opportunity as 3 variables of perfusion mode, and it was demonstrated that this mode could relieve CPB-induced lung injury effectively. Moreover, this mode was convenient and straightforward. It was promising for use in clinical cardiac surgery, and could not interfere with the intended operations.

However, there was limitation to the study. The range of pressures used in this experiment was limited. Further studies are needed to determine the effects of other pressures during CPB. Moreover, further research (such as modifying other aspects of the perfusion [Siepe 2008] or associating other protective measures [Goebel 2008]) should be done to obtain better results.

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