Experimental model of isolated lung perfusion in rats: technique and application in lung preservation studies*

Modelo experimental de perfusão pulmonar isolada em ratos: técnica e aplicações em estudos de preservação pulmonar

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Abstract

Small animal models are particularly suitable for lung preservation studies, because they are simple and cost-effective. This brief communication focuses on the technical description of an ex vivo lung perfusion model in rats by means of a commercially available apparatus, which was the first to be installed in a thoracic surgery research laboratory in Brazil. The model and its preparation, together with its applications for lung preservation studies, are described in detail. All technical details can also be seen in a video posted on the website of the Brazilian Journal of Pulmonology.

Keywords: Lung transplantation; Reperfusion injury; Rats; Models, animal.

Resumo

Estudos de preservação pulmonar em modelos experimentais realizados em animais de pequeno porte são de realização mais simples e barata. Esta comunicação tem o enfoque de descrever tecnicamente um modelo de perfusão pulmonar ex vivo em ratos, com o uso de um equipamento disponível comercialmente que foi o primeiro a ser instalado em um laboratório de pesquisa em cirurgia torácica no Brasil. Descrevemos detalhadamente o modelo e sua preparação, assim como suas aplicações para estudos de preservação pulmonar. Os detalhes técnicos da preparação podem ser observados também em um vídeo postado no *site* do Jornal Brasileiro de Pneumologia.

Descritores: Transplante de pulmão; Traumatismo por reperfusão; Ratos; Modelos animais.

Lung transplantation is a well-established form of treatment for selected patients with terminal lung disease. Lung preservation for transplantation is of fundamental importance since it allows organs to be maintained viable for longer periods of ischemia and with adequate functional results after reperfusion. Lung preservation studies require time for development, as well as being expensive, especially if involving large animal models (e.g., dog and pig). Therefore, it is desirable that experimental small animal models, which are simple and cost-effective, be established, serving as a screening filter for new methods that will subsequently be applied to more complex models. The ex vivo lung perfusion model was designed for this purpose, initially using rabbit lungs perfused with homologous venous blood obtained by exsanguination of animals of the same species. This model made it possible to conduct studies in which temperature[1] and lung preservation solutions[2,3] were researched, establishing many of the principles that, to date, have been guiding the methods of lung preservation in clinical use. The disadvantage of this model was the limited perfusion time (approximately 10 min), since the blood that had been oxygenated by the pulmonary block no longer returned to the circuit. The preparation was modified for use in a similar system involving

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rats and subsequently improved.[4-6] Although these preparations were stable for periods of up to 5 h, they were difficult to perform and control since they used an animal placed in parallel with the perfusion circuit to deoxygenate the blood. Later, the animal was replaced with a membrane oxygenator for the blood deoxygenation.[7] This greatly simplified the system, making it stable and therefore allowing prolonged perfusions, along with assessment of hemodynamic parameters and parameters of respiratory mechanics.[8] The current ex vivo lung perfusion model allows real-time data acquisition and computer-based storage, being marketed under the name IL-2 - Isolated Perfused Rat or Guinea Pig Lung System (Harvard Apparatus, Holliston, MA, USA; Hugo Sachs Elektronik, Hugstetten, Germany; Figure 1). Its wide range of use includes studies of lung preservation for transplantation; studies of respiratory mechanics and gas exchange in models of acute lung injury; functional assessment in models of induced obstructive and vascular disease (i.e., emphysema and pulmonary hypertension); analysis of remote pulmonary effects in metabolic and endocrine diseases (i.e., diabetes); investigation of drug pulmonary metabolism; assessment of absorption and effects of drugs and inhaled agents (by interposing an inventive nebulizer provided by the manufacturer); analysis of gas transport; studies of mechanical ventilation alternatives (i.e., liquid ventilation with perfluorocarbons); etc.

The objective of this communication was to present this ex vivo lung perfusion model, which is currently used for lung preservation studies in our laboratory and is unprecedented in Brazil. Its preparation and handling steps are described in detail in order to facilitate its implementation and use by other research laboratories intending to use it in the future. All technical details can also be seen in a video posted on the website of the Brazilian Journal of Pulmonology (http://www.jornaldepneumologia.com.br/portugues/modelo_perfusao.asp).

To extract the donor heart-lung blocks, male Wistar rats (250-300 g) are anesthetized with sodium thiopental (50 mg/kg, i.p.), weighed and placed on the preparation board. A laparotomy is performed along with resection of the sternum, exposure of the cervical trachea, placement of the tracheostomy, and initiation of mechanical ventilation (room air; RR = 70 breaths/min; and positive end-expiratory pressure = 1 cmH_2O). The inferior vena cava is exposed in the retroperitoneum and used for administration of heparin (1,500 IU). The diaphragm is opened in a radial direction with great caution in order not to injure the lung, which is extremely delicate and fragile in these animals. With the pleural cavity open, the inferior pulmonary ligament is sectioned, exposing the supradiaphragmatic inferior vena cava. The heart is exposed, a right ventriculotomy is performed adjacent to the pulmonary artery, the inferior vena cava previously exposed is sectioned, and the tip of the left ventricle is sectioned longitudinally. Subsequently, antegrade lung flushing is initiated, through a cannula introduced into the pulmonary artery by the ventriculotomy. The hypothermic preservation solution is administered by gravity from a reservoir placed 10 cm above the heart, with spontaneous drainage of effluent through the left ventriculotomy. During the flushing, hyperventilation is induced in order to avoid areas of atelectasis, thereby promoting equal distribution of the solution in the parenchyma. At the end of the flushing, the trachea is ligated, with a suture below the cannula, and is sectioned with the lungs inflated. The pulmonary extraction itself is initiated in the craniocaudal direction from the superior cervical mediastinal aperture, by anterior and inferior traction on the trachea along with dissection of the posterior mediastinum. The heart-lung block is removed, placed in hypothermic solution (saline solution

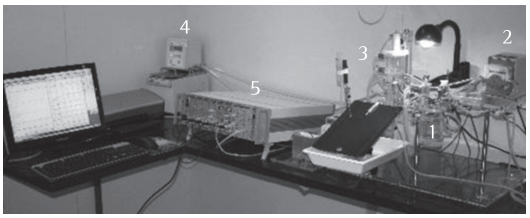

Figure 1 - IL-2 Isolated Perfused Rat or Guinea Pig Lung System. 1) negative pressure chamber; 2) connected perfusion roller pump; 3) blood reservoir and membrane deoxygenator; 4) water heater in which water circulates around the entire perfusion system to maintain temperature; and 5) data acquisition module for acquiring data on hemodynamics, respiratory mechanics, and blood gas analysis (pH, PaO_2), with transducers and a volume ventilator coupled to a personal computer.

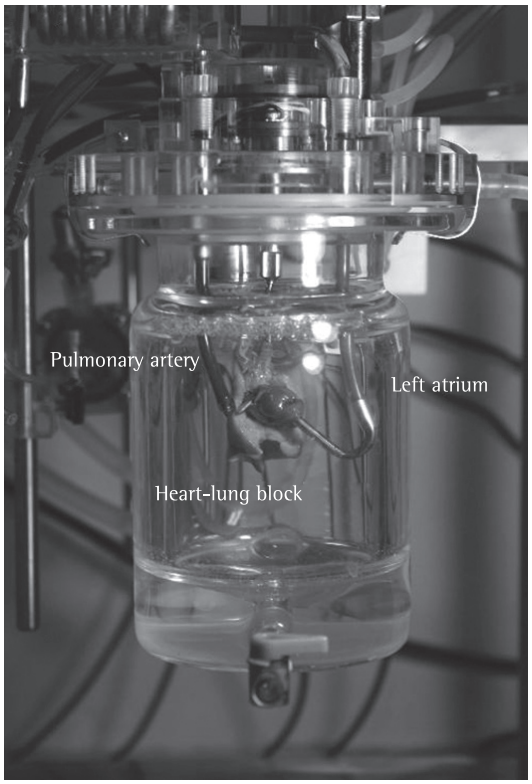

Figure 2 - Negative pressure chamber containing the heart-lung block. The heart-lung block is placed within the negative pressure chamber and connected to the ventilation outlet, being perfused through the pulmonary arterial cannula and drained through the left atrial cannula.

or preservation solution) and stored at 4°C for the designated period of ischemia.

Approximately 30-40 min before perfusion, three animals are anesthetized, ventilated, and heparinized by the same technique as described for donor preparation. The inferior vena cava is exposed, and homologous blood is obtained by venous puncture. The gas mixture (90% N_2 and 10% CO_2; at a flow rate of 200-300 mL/min) is administered continuously through a membrane deoxygenator with 0.245 m^2 of contact area (Medisulfone® D150 Hemofilter; Medica s.r.l., Medolla, Italy). Saline solution is added to the blood, at a ratio of 1:1 or in sufficient quantity to obtain a total volume of approximately 80 mL, with a hematocrit level of 15-20%. The blood is then poured into the reservoir, heated, and recirculated through the system at a low flow rate (3 mL/min) for approximately 15 min. Meanwhile, the heart-lung block is prepared. First, the trachea is again cannulated with the adapter for the ventilation system, and the cannula is installed by left ventriculotomy in the left atrium through the mitral valve and held in place by a U-shaped (4-0 nylon) suture. A nonabsorbable (3-0 cotton) suture is passed through the transverse sinus and tied with a loose knot. The block is taken to the perfusion system, and the tracheal cannula is attached to the end of the ventilator. Only then is the pulmonary artery cannulated by right ventriculotomy and is the knot of the suture previously passed through the transverse sinus, and which fixes the cannula to the artery and, at the same time, occludes the ascending aorta, tightened. At this point, the negative pressure chamber is closed, and ventilation is initiated with approximately 25% of the planned tidal volume, a RR of 60 breaths/min, an inhalation/exhalation ratio of 60%, and a breath/min rate with a 50% increase in tidal volume (Figure 2). The tidal volume is slowly increased over 10 min until 10 mL/kg of body weight is reached. Concomitantly, perfusion at a low flow rate (2 mL/min) is initiated, being slowly increased over 5-10 min until the desired flow rate (5-7 mL/min) is reached. In this phase, it is imperative that the flow rate be slowly and gradually increased concomitant with the increase in respiratory tidal volume, controlling the pressure in the pulmonary artery, which is maintained at 10-15 mmHg. This aims to heat the heart-lung block slowly and, at the same time, minimize the reperfusion injury caused to the vasculature by the mechanical stress imposed by the high flow rate in the initial phase. Once ventilation and perfusion are stabilized (10 min), data are collected every 10 min for the next 60 min. Blood samples (0.3 mL) are collected through the pulmonary arterial cannula and the left atrial cannula for blood gas analysis, as well as for determination of hematocrit and electrolyte levels. At the same time, the system provides data on respiratory dynamics and hemodynamics (tidal volume, pulmonary airflow, minimum/maximum pleural pressure, RR, compliance, resistance, conductance, and pulmonary artery pressure), as well as pH and PaO_2.

The ex vivo lung perfusion model, in addition to the fact that it can be used in studies of lung viability and of acute lung injury, has proven extremely useful for testing preservation solutions/additives and periods of ischemia in

our laboratory. The number of parameters of respiratory mechanics and gas exchange, as well as of hemodynamic parameters, provided in real time significantly facilitates the investigation and streamlines the processing of the results and of the statistical analysis, since the results can be entered directly into an electronic spreadsheet after each experiment.

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