ID Artigo: 2879_EN
envio: 28/11/2011
Diagramador: Janaina

cubomultimidia publicações
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Ex vivo experimental model: split lung block technique*

Modelo experimental ex vivo com bloco pulmonar dividido*

Alessandro Wasum Mariani, Israel Lopes de Medeiros, Paulo Manuel Pêgo-Fernandes, Flávio Guimarães Fernandes, Fernando do Valle Unterpertinger, Lucas Matos Fernandes, Mauro Canzian, Fábio Biscegli Jatene

Abstract

Since they were first established, ex vivo models of lung reconditioning have been evaluated extensively. When rejected donor lungs are used, the great variability among the cases can hinder the progress of such studies. In order to avoid this problem, we developed a technique that consists of separating the lung block into right and left blocks and subsequently reconnecting those two blocks. This technique allows us to have one study lung and one control lung.

Keywords: Lung transplantation; Transplantation conditioning; Organ preservation; Organ preservation solutions.

Resumo

Modelos de recondicionamento pulmonar ex vivo têm sido avaliados desde sua proposição. Quando são utilizados pulmões humanos descartados para transplante, a grande variabilidade entre os casos pode limitar o desenvolvimento de alguns estudos. No intuito de reduzir esse problema, desenvolvemos uma técnica de separação do bloco pulmonar em direito e esquerdo com posterior reconexão, permitindo que um lado sirva de caso e o outro de controle.

Descritores: Transplante de pulmão; Condicionamento pré-transplante; Preservação de órgãos; Soluções para preservação de órgãos.

For patients with end-stage lung disease that is refractory to clinical treatment, lung transplantation is a well-established form of treatment that improves survival and quality of life. Updated International Society for Heart and Lung Transplantation registry data show that the curve for the total number of lung transplants per year remains on the rise, a total of 2,769 transplants having been performed worldwide in 2008.[1] However, the number of lungs considered suitable for transplantation is still lower than is that of lung transplant candidates on waiting lists. This results in a long waiting time for transplantation and significant mortality among lung transplant candidates on waiting lists. Even in lung transplant centers in developed countries, such as those in the United States, only 15-20% of the lungs available are actually used for transplantation.[2,3] Brazilian national data demonstrate an even lower rate of use of available lungs, as reported in a study using data for the state of São Paulo in 2006, which showed that only 4.9% of donor lungs were effectively transplanted.[4]

The low rate of donor lung use prompted various groups to investigate ways of increasing the number of viable organs for transplantation, with the objective of increasing the total number of transplants and reducing the waiting time for transplantation without affecting post-transplant outcomes. The most widely used strategy has been to expand and ease the criteria for donor selection. This strategy gave rise to the concept of the extended criteria donor (formerly

* Study carried out in Laboratory for Medical Research 61, Department of Thoracic Surgery, *Faculdade de Medicina da Universidade de São Paulo* – FMUSP, University of São Paulo School of Medicine – and at the Experimental Surgery Center, *Instituto do Coração, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo* – InCor/HCFMUSP, Heart Institute/University of São Paulo School of Medicine *Hospital das Clínicas* – São Paulo, Brazil.
Correspondence to: Alessandro Wasum Mariani. Avenida Dr. Enéas de Carvalho Aguiar, 44, 2º andar, bloco 2, sala 9, Cerqueira César, CEP 05403-900, São Paulo, SP, Brasil.
Tel. 55 11 3069-5248. E-mail: awmariani@gmail.com
Financial support: This study received financial support from the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, São Paulo Research Foundation).
Submitted: 30 May 2011. Accepted, after review: 25 July 2011.

known as the marginal donor). Donor organs that in the past would have been considered unsuitable for transplantation for not meeting all of the selection criteria are currently being used, including organs from donors over 55 years of age, those from smokers (smoking history, > 20 pack-years), and those showing radiographic changes. Although many studies have shown similar short-term survival rates, the use of such lungs in high-risk recipients, such as those with severe pulmonary hypertension, is associated with higher 30-day mortality rates.[5] Overall, the use of organs from extended criteria donors has not significantly reduced the number of patients on waiting lists for transplantation.

Of the studies investigating ways of effectively increasing the number of viable organs for transplantation, none piqued the interest of the scientific community as much as did the ex vivo model of lung evaluation and reconditioning proposed by Steen et al.[6] After the publication of the first results obtained with the model, various groups undertook the task of learning and improving the technique. Those researchers conducted studies aimed at developing a system to improve non-heart-beating donor lung evaluation[7] by overcoming certain technical and ethical limitations of the evaluation. To that end, they developed a ventilation/perfusion system to test the oxygenation capacity of lungs after their removal. After the first experiments in pigs, those researchers found that the system was also useful in the evaluation of donors who did not meet all of the selection criteria for organ donors but whose organs might be viable, principally those donors who met all but one criterion, namely a PaO_2/FiO_2 ratio (as assessed by arterial blood gas analysis) below 300 mmHg, as recommended in the evaluation protocol.[8] The major finding in that line of research was that the system was able to restore the oxygenation capacity of the lungs, giving rise to what is currently known by transplant groups as ex vivo lung reconditioning.

In 2009, the lung transplant group of the *Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo* (InCor/HCFMUSP, Heart Institute/University of São Paulo School of Medicine *Hospital das Clínicas*), located in the city of São Paulo, Brazil, began to work with the ex vivo lung reconditioning technique in order to study rejected donor lungs.[9] Among the numerous difficulties encountered by the group, one certainly drew our attention, namely the variability among the cases, which limited the development of certain studies because of the difficulty in comparing the harvested organs. That variability can be explained by a number of factors, including those that are inherent to the donor (such as weight, height, age, smoking history, and history of lung disease) and those that are related to brain death and ICU treatment (such as the cause of brain death, the presence of bronchial aspiration, the presence of ventilator-induced lung injury, the presence of ventilator-associated pneumonia, the presence of barotrauma, the presence of thoracic trauma, the duration of tracheal intubation, and the duration of mechanical ventilation). In order to avoid that problem, we developed a technique that consists of separating the lung block into right and left blocks and subsequently reconnecting those two blocks. This technique allows us to have one study lung and one control lung.

The objective of the present communication was to describe the abovementioned technique, which was developed in order to evaluate the left and right lungs individually but simultaneously in the ex vivo lung reperfusion system. We used lungs from brain-dead donors, as identified by the São Paulo State Department of Health Transplant Center. Their lungs had been rejected for transplantation by all lung transplant teams concerned, because the organs did not meet the selection criteria. Family members gave written informed consent for the use of the organs in the present study. The consent form was presented to the family members by the teams of the two organ procurement organizations involved in the present study, namely the HCFMUSP Organ Procurement Organization and the *Santa Casa de Misericórdia de São Paulo* Hospital Organ Procurement Organization. This precaution was taken in order to guarantee that the families of the donors were approached by a professional trained in the organ donation process and therefore avoid problems during the process. At least one more solid organ, such as a kidney or liver, was harvested, for clinical purposes, from each of the cases included in the present communication. The presence of any given difference between the right and left lungs

identified by inspection, palpation, or chest X-rays constituted an exclusion criterion. The organ procurement team harvested the lungs employing the technique that is routinely used by the lung transplant team, together with the other organ procurement teams. The solution used in order to preserve the lungs throughout the harvesting and transportation process, until their arrival at the InCor/HCFMUSP laboratory, was Perfadex® (Vitrolife, Kungsbacka, Sweden).

Immediately after the organ procurement team had arrived at the InCor/HCFMUSP laboratory (Figure 1a), the lung block was separated into right and left blocks by sectioning the left atrium, the main pulmonary artery, and the tracheal carina (Figure 1b). The separation of the lung block into right and left blocks allows each block to be submitted to a different form of preservation, among other procedures. At the end of the study period, as previously established, the left and right lungs were reconnected, by means of Y-shaped cannulae, at the trachea and pulmonary artery, the pulmonary veins remaining separated (Figures 1c and 1d). This allows the two lung blocks (study and control) to undergo reperfusion and ventilation in the ex vivo system simultaneously, with the same reperfusion solution and exactly the same ventilation parameters. This also allows us to collect samples for blood gas analysis and pulmonary artery pressure monitoring from each lung block in an independent manner, which

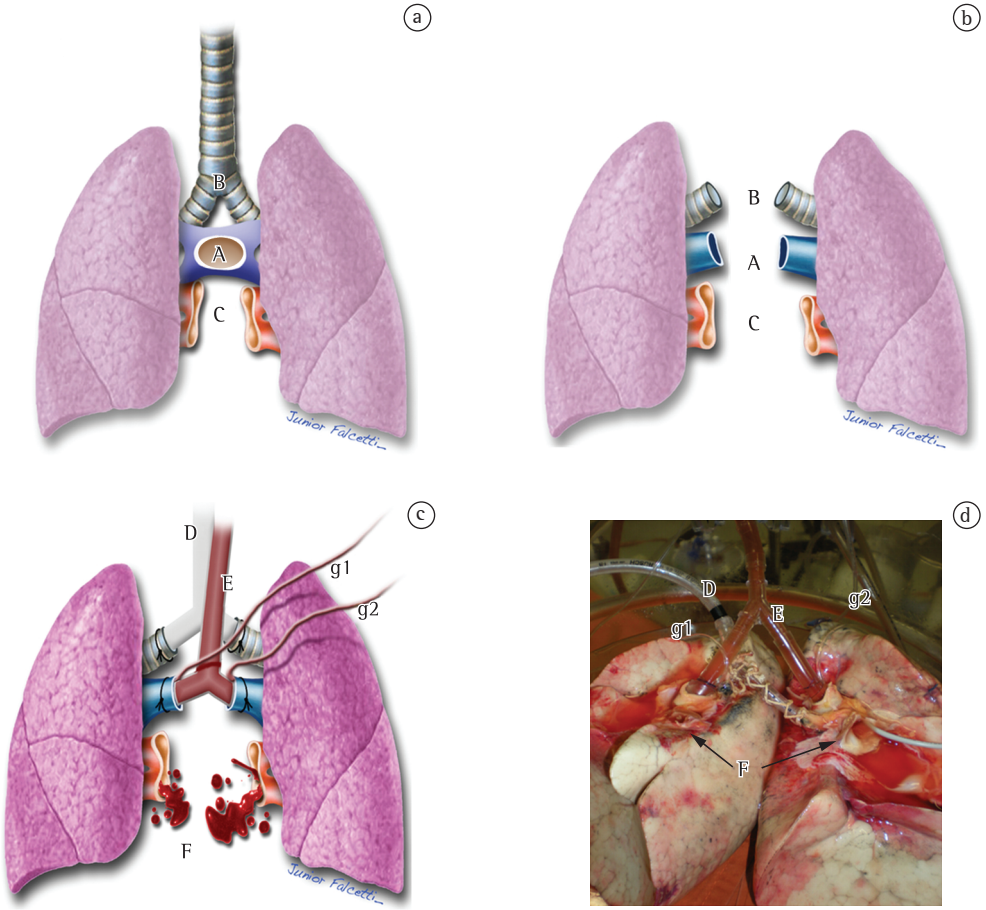

Figure 1 - Schematic illustrations and photograph demonstrating the separation and reconnection of the lung block for ventilation/perfusion in the ex vivo system. In 1a, lung block before separation. In 1b, lung block after separation. In 1c, lung block reconnected by means of Y-shaped cannulae. In 1d, photograph of the reconnected lung block. Legend: A; bronchi; B: pulmonary arteries; C: pulmonary veins; D: Y-shaped cannula connected to the bronchial stumps and the ventilator; E: Y-shaped cannula connected to the pulmonary arteries; F: separated pulmonary veins freely draining the perfusate into the containment recipient; and g1/g2: tubes to measure pulmonary artery pressure.

in turn allows us to collect functional data separately.

We employed an ex vivo system that was developed by our group and had previously been used.[10] The system comprises a containment box (Vitrolife), a centrifugal pump (Braile Biomédica, São José do Rio Preto, Brazil), a heat exchanger (Fisics Biofísica, São Paulo, Brazil), a membrane oxygenator (Braile Biomédica), and a venous reservoir.[10] The Y-shaped cannula for the pulmonary arteries had a small tube to be connected to the pressure transducer, which allowed us to monitor pulmonary artery pressure continuously. The solution returning through the pulmonary veins is free, flowing directly into the containment box, where the right and left flows are mixed and drained into the venous reservoir by the force of gravity. We chose to maintain the atrium open in order to facilitate the assembly of the system by eliminating the need for special atrial cannulae (Vitrolife). This is possible in cases of short-term perfusion (i.e., no longer than two hours); in cases of long-term perfusion, a closed system is needed in order to avoid pulmonary edema, as previously described.[11] The system is filled with 1,500 mL of Steen Solution® (Vitrolife), and we chose to use the acellular solution. In order to reduce the necessary volume of perfusate, we used oxygenators, reservoirs, and pediatric tubes (Braile Biomédica). We adjusted pH between 7.35 and 7.45 by adding trometamol (Addex-THAM®; Fresenius-Kabi AB, Uppsala, Sweden). For the perfusion of the right and left lungs, we used a maximum flow of 40% of the estimated cardiac output (calculated by a formula based on the size of the donor). That flow is sufficient for the evaluation of the block in the system and low enough to avoid pulmonary edema formation.

Three donor lungs were used in order to ensure the viability of the technique, and the data regarding those donors and their lungs are presented in Table 1. The cause of brain death was traumatic brain injury in two cases and hemorrhagic stroke in one case. A low PaO_2/FiO_2 ratio (lower than 300), as assessed by arterial blood gas analysis, was the reason why all of the lungs were considered unsuitable for transplantation, in accordance with the habitual protocol for the evaluation of donor lungs. The ages of the donors under study were 18 years, 25 years, and 52 years (mean age, 32 years). The mean time elapsed between the injection of the preservation solution and the beginning of the experiment ranged from 157 min to 201 min (mean, 184 min). The mean PaO_2, as assessed by in vivo blood gas analysis, was 233.33 mmHg. The arterial blood gas analysis performed at the end of reperfusion revealed a mean PaO_2 of 390.33 mmHg in the right lung and of 387.66 mmHg in the left lung. The model used in the present study allowed a stable reperfusion of the lungs and yielded reliable pulmonary artery pressure measurements throughout the reperfusion. The mean pulmonary artery pressure was 146.66 mmHg for the right and left pulmonary arteries. Mechanical ventilation was performed with a conventional anesthesia machine (Samurai Fuji Maximus SAT 500; K. Takaoka, São Paulo, Brazil). There were no mechanical ventilation-related problems, and the two lungs were uniformly ventilated when they were connected to the system.

The ex vivo experimental model used in combination with the split lung block technique

Table 1 - General data, blood gas analysis results, and pulmonary artery pressure measurements.

Variable	Case 1	Case 2	Case 3	Mean
Cause of donor brain death	TBI	TBI	HS	
Donor age, years	18	25	52	32
Donor gender	Male	Male	Female	
Number of days of orotracheal intubation	4.00	2.00	2.00	2.66
Lung submitted to ECMO	Left	Right	Right	
Pre-harvesting PaO_2, mmHg	216	278	206	233
System PvO_2, mmHg	91	80	97	89
Right PaO_2, mmHg	344	471	356	390
Left PaO_2, mmHg	340	459	364	388
Right pulmonary artery pressure, mmHg	170	130	140	147
Left pulmonary artery pressure, mmHg	170	120	150	147

TBI: traumatic brain injury; HS: hemorrhagic stroke; ECMO: extracorporeal membrane oxygenation; and PvO_2: mixed venous oxygen tension.

allowed us to conduct experiments involving rejected donor lungs. In addition, the split lung block technique allowed us to have one study lung and one control lung, reducing the variability among the donors.

Acknowledgments

We would like to thank Farmoterápica, Braile Biomédica, Vitrolife, the HCFMUSP Organ Procurement Organization, the *Santa Casa de Misericórdia de São Paulo* Hospital Organ Procurement Organization, and the São Paulo State Department of Health Transplant Center. The illustrations were drawn by Argemiro Falcetti Jr.

References

1. Christie JD, Edwards LB, Kucheryavaya AY, Aurora P, Dobbels F, Kirk R, et al. The Registry of the International Society for Heart and Lung Transplantation: twenty-seventh official adult lung and heart-lung transplant report--2010. J Heart Lung Transplant. 2010;29(10):1104-18.
2. Hornby K, Ross H, Keshavjee S, Rao V, Shemie SD. Non-utilization of hearts and lungs after consent for donation: a Canadian multicentre study. Can J Anaesth. 2006;53(8):831-7.
3. Punch JD, Hayes DH, LaPorte FB, McBride V, Seely MS. Organ donation and utilization in the United States, 1996-2005. Am J Transplant. 2007;7(5 Pt 2):1327-38.
4. Fernandes PM, Samano MN, Junqueira JJ, Waisberg DR, Noleto GS, Jatene FB. Lung donor profile in the State of São Paulo, Brazil, in 2006. J Bras Pneumol. 2008;34(7):497-505.
5. de Perrot M, Snell GI, Babcock WD, Meyers BF, Patterson G, Hodges TN, et al. Strategies to optimize the use of currently available lung donors. J Heart Lung Transplant. 2004;23(10):1127-34.
6. Wierup P, Haraldsson A, Nilsson F, Pierre L, Scherstén H, Silverborn M, et al. Ex vivo evaluation of nonacceptable donor lungs. Ann Thorac Surg. 2006;81(2):460-6.
7. Steen S, Liao Q, Wierup PN, Bolys R, Pierre L, Sjöberg T. Transplantation of lungs from non-heart-beating donors after functional assessment ex vivo. Ann Thorac Surg. 2003;76(1):244-52; discussion 252.
8. Ingemansson R, Eyjolfsson A, Mared L, Pierre L, Algotsson L, Ekmehag B, et al. Clinical transplantation of initially rejected donor lungs after reconditioning ex vivo. Ann Thorac Surg. 2009;87(1):255-60.
9. Pêgo-Fernandes PM, Medeiros IL, Mariani AW, Fernandes FG, Unterpertinger Fdo V, Samano MN, et al. Ex vivo lung perfusion: initial Brazilian experience. J Bras Pneumol. 2009;35(11):1107-11.
10. Pêgo-Fernandes PM, de Medeiros IL, Mariani AW, Fernandes FG, Unterpertinger FD, Samano MN, et al. Ex vivo lung perfusion: early report of Brazilian experience. Transplant Proc. 2010;42(2):440-3.
11. Cypel M, Yeung JC, Hirayama S, Rubacha M, Fischer S, Anraku M, et al. Technique for prolonged normothermic ex vivo lung perfusion. J Heart Lung Transplant. 2008;27(12):1319-25.

About the authors

Alessandro Wasum Mariani
Attending Physician. Department of Surgery, Dante Pazzanese Institute of Cardiology, São Paulo, Brazil.

Israel Lopes de Medeiros
Student in the Graduate Program in Thoracic and Cardiovascular Surgery, *Faculdade de Medicina da Universidade de São Paulo* – FMUSP, University of São Paulo School of Medicine – São Paulo, Brazil.

Paulo Manuel Pêgo-Fernandes
Associate Professor. Department of Thoracic Surgery, *Faculdade de Medicina da Universidade de São Paulo* – FMUSP, University of São Paulo School of Medicine – São Paulo, Brazil.

Flávio Guimarães Fernandes
Medical Student. *Faculdade de Medicina da Universidade de São Paulo* – FMUSP, University of São Paulo School of Medicine – São Paulo, Brazil.

Fernando do Valle Unterpertinger
Medical Student. *Faculdade de Medicina da Universidade de São Paulo* – FMUSP, University of São Paulo School of Medicine – São Paulo, Brazil.

Lucas Matos Fernandes
Resident. Lung Transplant Program, Department of Thoracic Surgery, *Faculdade de Medicina da Universidade de São Paulo* – FMUSP, University of São Paulo School of Medicine – São Paulo, Brazil.

Mauro Canzian
Attending Physician. Anatomic Pathology Laboratory, *Instituto do Coração, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo* – InCor/HCFMUSP, Heart Institute/University of São Paulo School of Medicine *Hospital das Clínicas* – São Paulo, Brazil.

Fábio Biscegli Jatene
Full Professor. Department of Thoracic Surgery, *Faculdade de Medicina da Universidade de São Paulo* – FMUSP, University of São Paulo School of Medicine – São Paulo, Brazil.