Lung Preservation: Current Practices

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Introduction

Certain problems related to surgical technique and immunosuppression in lung transplantation have been largely overcome, yet long-term survival has hardly changed because a persisting dilemma is the impact of so-called primary graft failure on perioperative and early mortality. Primary graft failure involves pulmonary dysfunction that is very similar to adult respiratory distress, which requires intubation and ventilation with high concentrations of oxygen and nitric oxide (NO). This situation favors lung infection, sepsis and eventual multiorgan failure in the transplanted patient. The lack of consensus in defining the clinical picture of primary graft failure prevents us from knowing the exact incidence, with estimates ranging from 11\% to 35\% depending on patient series. Furthermore, primary graft failure has been linked to an increased risk of acute rejection and to later development of bronchiolitis obliterans.

Primary graft failure may arise from a variety of factors in the donor, the recipient, or the transplanted lung itself. Failure may also arise as a result of postoperative mechanical ventilation, although it has been mainly attributed to ischemia-reperfusion injury occurring during lung preservation.

A lung graft to be transplanted must be subjected to ischemia for a certain period. Lung preservation has 2 objectives. On the one hand, the effect of ischemia must be kept to a minimum with a view to keeping the lung structurally, functionally, and biochemically intact so that function is optimal once the transplant is complete. On the other hand, the lung’s integrity must be maintained as long as possible.

The mechanism of injury that occurs through ischemia and reperfusion injury is extremely complex and has not yet been fully elucidated. Injury arises from the interaction of potent mediators and various cell types. A large number of experimental studies have contributed to our understanding of the mechanisms involved and to improved lung preservation and graft response. The impact of such studies on clinical practice, however, has been scant and at present there is no protocol available to assure lung preservation.

The purpose of this article is to review current practices in lung preservation from a clinical perspective. Preservation is based both on principles that are common to the preservation of any solid organ and to concepts that are specific to the lung.

Principles Common to the Preservation of All Solid Organs

Perfusion Solutions and Adjuvant Pharmacology

With few exceptions, lung transplant teams flush the pulmonary vascular bed with several solutions infused through the pulmonary artery (Table). The solutions serve to attenuate the effects of ischemia and their impact on later reperfusion of the pulmonary graft. The following principles must be borne in mind:

1. Prevention of cell edema. Oxygen is necessary for the cell to be able to maintain the electron transport chain needed to generate high energy phosphate links in the form of adenosine tri-phosphate (ATP), which maintains homeostasis. During ischemia, ATP depletion inhibits the activity of the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase pump, which needs the energy from ATP hydrolysis in order to be able to participate in maintaining a balanced ion gradient. Inhibition of the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase pump favors outward movement of K\textsuperscript{+}, leading to inward movement of Na\textsuperscript{+} across the cell membrane from interstitial spaces. When Na\textsuperscript{+} enters, the volume of water needed to maintain osmotic equilibrium comes with it, causing cell edema.

It is important to point out that, unlike other solid organs in which ischemia occurs in a state of anoxia, the lung needs intra-alveolar oxygen to maintain its metabolism during preservation (oxygenated ischemia). Nevertheless, the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase pump can be affected by a fall in intra-alveolar oxygen pressure and by hypothermia.
To counteract cell edema, the electrolytic composition of the preservation solution is crucial. Intracellular solutions such as modified Euro-Collins (EC) solution or the University of Wisconsin (UW) solution are rich in K⁺, thereby keeping Na⁺ from penetrating the cell. Moreover, to increase extra-cellular osmotic pressure, these solutions include substances of high molecular weight that cannot pass through the cell membrane (impermeants). The EC solution uses glucose as the impermeant, whereas the UW solution, designed to preserve abdominal organs, uses lactobionate and raffinose because glucose can pass easily through the membranes of hepatic and pancreatic cells. Since 1984, when the Stanford group introduced the clinical practice of perfusion and storage of lungs, the EC solution has been the most widely used.¹⁹ The introduction of the UW solution to clinical practice has been one of the most important events in abdominal organ implantation,²⁰ and it is also used by 13.5% of lung transplant teams.¹⁸ Few clinical trials have compared the two perfusion solutions but they seem to suggest that the UW solution is superior to the EC one.²¹,²² Randomized controlled trials have not been performed, however.

One problem intracellular preservation solutions have is their high K⁺ concentrations, as this favors vasoconstriction of the pulmonary bed and the possibility of cardiac arrest due to hyperkalemia after reperfusion. Extracellular preservation solutions with low concentrations of K⁺, widely studied in experimental settings, are now being introduced to clinical practice. Perfadex is a low-K⁺ extracellular solution whose colloid component is dextran 40, an active osmotic agent that retains water in the intravascular space, reducing erythrocyte and platelet aggregation. In addition, the low glucose content of this solution would be sufficient to maintain cell metabolism at low temperatures, a property that cannot be claimed for the EC solution, in which osmolarity that depends on the high glucose content would be partly responsible for breaking down the cell structure. Recently, a group in Munich reported that Perfadex gave results that were significantly better than those obtained with the EC solution in a clinical trial.²³ Similar conclusions have been drawn by groups in Hannover²⁴ and Toronto²⁵ although none of these trials were randomized. The Celsior solution, also extracellular and low in K⁺, was initially developed to preserve hearts and is also now being used to preserve lungs. Its composition includes impermeants such as mannitol and lactobionate, and glutamic acid is used as a nutrient. D’Armini et al²⁶ found that the Celsior solution gave results that were similar to the UW solution in a randomized controlled trial. Other authors have also reported good results for the Celsior solution.²⁷,²⁸

Currently there is a clear tendency to use low-K⁺ extracellular solutions to preserve the lung.

². Prevention of interstitial edema. Crystalloid solutions with low oncotic pressure, on the other hand, may favor the transport of water from intravascular to interstitial spaces. Interstitial edema may compromise the capillary network and make homogenous perfusion difficult during preservation, contributing to the development of ischemia-reperfusion injury. Therefore components with osmotic activities must be added to the perfusion solution to achieve an osmolarity that is similar to that of plasma (approximately 310 mOsm/L). To that end, the UW solution employs hydroxyethyl starch.

³. Prevention of acidosis. The lack of oxygen leads to anaerobic metabolism and a consequent increase of lactic acid and hydrogen ions. Normal cell activity is altered such that energy production is seriously compromised.

Even though an ischemic lung is oxygenated, to counteract a possible state of acidosis it is necessary to maintain pH as close as possible to normal using buffers such as bicarbonate and phosphate in the EC solution, sulfate and phosphate in the UW solution, or histidine in the Celsior formula. Perfadex has a pH of 5.5, which affords stability in storage for 3 years. Before use, 1 mmol/L of trometamol or tromethamine must be added to adjust the pH. The addition of 0.5-1 mmol/L of Ca²⁺ is also recommended.

⁴. Regeneration of ATP activity. Some solutions contain ATP precursors, such as adenosine in the UW solution, in order to favor the reactivation of high-energy phosphate compounds.
5. Prevention of free oxygen radical activity.

Reperfusion injury starts with a series of biochemical events occurring during ischemia, with the resulting formation of free oxygen radicals (Figure). Free radicals are unstable molecules because they contain unpaired electrons, explaining their capacity to do harm. The free oxygen radicals produced normally by a cell are removed by endogenous agents (scavengers) such as superoxide dismutase, which acts on superoxide radicals ($O_2^-$); glutathione peroxidase, which acts on hydrogen peroxide ($H_2O_2$); and tocopherol, which inhibits lipidic peroxidation.

Hypoxanthine, a nitrogen base substrate of xanthine oxidase that is generated by ATP metabolism, is responsible for the formation of uric acid and $O_2^-$. An increase or a decrease in molecular oxygen leads to the overproduction of such radicals and serious tissue injury. During hypoxia, tissue hypoxanthine levels increase; moreover, a deficit in Na$^+$/K$^+$-ATPase pump activity leads to massive Ca$^{2+}$ transport to cytoplasm, provoking activation of Ca$^{2+}$-dependent proteases charged with converting the dehydrogenase xanthine enzyme into xanthine oxidase, which is charged with the formation of $O_2^-$ radicals in the presence of oxygen during reperfusion.

Certain Ca$^{2+}$ channel blockers such as verapamil, nifedipine, or diltiazem have been used experimentally. Some preservation solutions used in clinical practice include xanthine oxidase inhibitors in their composition—allopurinol in the UW solution for example.

Unlike other solid organs, the lung is kept in a state of oxygenated ischemia, favoring the production of free oxygen radicals in reactions catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which aids in reducing NADPH to the oxidized form of NADP, oxidized form of NADPH; $O_2^-$, superoxide radical; $H_2O_2$, hydrogen peroxide; HO$^-$, hydroxyl radical; NO, nitric oxide; ONOO$^-$, peroxynitrite.

Large amounts of free iron are released to the tissues during ischemia and convert oxidized Fe$^{3+}$ ions to the reduced Fe$^{2+}$ ions, catalyzing the conversion of $H_2O_2$ to HO$^-$ radicals.

Oxidative stress, particularly that caused by the HO$^-$ and ONOO$^-$ radicals, is responsible for oxidizing sulphydryl groups, leading to lipid membrane peroxidation and serious cellular lesions, particularly in pulmonary vascular endothelium.

The addition of multiple exogenous free radical scavengers has been studied experimentally but with little clinical impact. Glutathione, a component of the UW and Celsior solutions, is a sulphydryl group antioxidant and, therefore, inhibits lipid peroxidation given that it is oxidized by $H_2O_2$, preventing HO$^-$ radicals from forming.

Endothelial cells synthesize a large number of substances responsible for vascular tone, inflammatory response, and the regulation of coagulation. The production of these substances is modulated by changes in the concentration of certain intracellular messengers.
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such as cyclic guanosine monophosphate (cGMP), cyclic adenosine monophosphate (cAMP), and cytosolic Ca\(^{2+}\). The interaction between endothelium and leukocytes, platelets, and plasma components also play a modulatory role.\(^{31}\)

Pulmonary vascular tone is the result of an equilibrium among several vasoactive mediators produced by endothelial cells. The balance can be altered by circumstances such as ischemia, hypothermia, changes in intravascular pressure, and more, with mediators that provoke vasoconstriction predominating.

NO, prostaglandin, and adenosine are among the mediators favoring vasodilation. These vasodilators also have antiinflammatory properties, given that they sequester free oxygen radicals and inhibit the adherence of neutrophils and platelet aggregation.

NO plays a key role in tissue homeostasis because it activates the guanosine triphosphate in cGMP. In turn, cGMP stimulates G proteinases that are responsible for serine and threonine phosphorylation and it also lowers the concentration of intracellular Ca\(^{2+}\), affecting vasodilation. For this reason, administering NO or one of the precursors of NO may be useful in lung transplantation.\(^{25}\) It is important to point out that NO can react with free O\(^2-\) radicals in the presence of high concentrations of oxygen and form ONOO\(^-\), which has oxidizing potential.

Authors aiming to improve lung preservation have also added sodium nitroprusside or nitroglycerin to the perfusion solution, not only for their vasodilatory effect but also because they are generators of NO.\(^{32}\)

Experimental administration of inhaled NO to the lung donor during explantation has been reported to be associated with improved function of the lung after transplant.\(^{36}\) We are not aware that this procedure has been used in clinical practice. Administering inhaled NO immediately after lung reperfusion in order to prevent reperfusion injury is controversial. Thabut et al\(^{37}\) showed that administering NO and pentoxifylline during reperfusion reduced injury; Ardehali et al.\(^{4}\) on the other hand, did not observe the same improvement. Both authors, however, were reporting results based on case histories. Nevertheless, Meade et al.\(^{39}\) did not find that NO administration during reperfusion had a prophylactic effect on reperfusion injury in a randomized controlled trial enrolling 84 patients.

Prostacyclin, an autacoid derived from arachidonic acid, stimulates G proteins in the cell membrane by way of EP2 subtype receptors, leading to activation of adenylate cyclase, which is responsible for converting ATP to cAMP, the substance that activates A subtype protein kinases charged with phosphorylation of residual threonine and serine, whose end result will be vasodilatation. In order to counterbalance vasoconstriction that is enhanced by intracellular preservation solutions, most transplant teams infuse prostaglandin E1 (PGE\(_1\)) immediately before starting perfusion of the pulmonary vascular bed. The vasodilatory action of PGE\(_1\) is due to protein kinase stimulation by way of cAMP.\(^{40}\) The role of PGE\(_1\) is still controversial, however. Some authors have defended its use in experimental settings,\(^{41}\) while others have observed no improvement in hemodynamic parameters or gas exchange in the transplanted lungs that have been so-treated.\(^{42}\) Sasaki et al\(^{43}\) observed that the vasodilatory effect of PGE\(_1\) is achieved if perfusion solutions low in K\(^+\) are used, suggesting that Ca\(^{2+}\) blockers must be added when the EC or UW solutions are used or that the usual doses of PGE\(_1\) must be increased.

Adenosine stimulates adenyly cyclase by way of G proteins on the cell membrane to increase intracellular cAMP levels and favor vasodilation. As mentioned, adenosine is a component of the UW solution.

At the other extreme are agents that enhance vasoconstriction, mainly endothelin 1, a potent vasoconstrictor whose mechanism of action is related to Ca\(^{2+}\) channels sensitive to dihydro-pyridines (Ca\(^{2+}\) channel blockers). It is also a potent proinflammatory mediator.

Lipid peroxidation will generate potent mediators such as phospholipases, specifically phospholipase A\(_2\), which mobilizes arachidonic acid and later facilitates its metabolism, generating thromboxanes and leukotrienes, which are both proinflammatory mediators and potent vaso- and bronchoconstrictors. Phospholipase A\(_2\) is also responsible for producing platelet aggregation factor, which serves as yet another mediator of inflammation and as a vasoconstrictor. Inhibitors of phospholipase A\(_2\) have been studied experimentally. In clinical practice, the ginkgolide BN52021, a derivative of Ginkgo biloba, has been used in preservation solutions, complemented by administration of the same agent to the recipient before reperfusion, and has proven useful in lung transplantation as an antagonist of platelet aggregation factor.\(^{44}\)

Endothelial cells are also largely responsible for inflammatory response. We have mentioned that vasodilatory molecules have a cytoprotective effect. Endothelin 1, on the other hand, stimulates the production of cytokines by alveolar macrophages and monocytes.\(^{45}\) A large number of pro- or antiinflammatory cytokines have been studied in the context of ischemia-reperfusion injury. A recent clinical study by Mal et al\(^{46}\) found that certain proinflammatory cytokines, such as interleukin 1\(\beta\), 6 and 8 and tumor necrosis factor alpha were significantly associated with hemodynamic failure in the early postoperative period after a lung transplant. De Perrot et al\(^{47}\) were able to demonstrate that tumor necrosis factor alpha, interferon gamma and interleukins 8, 10, 12, and 18 play important roles in lung ischemia-reperfusion injury. Concentrations of those cytokines are elevated during ischemia and, with the exception of interleukin 8, they decrease rapidly during lung reperfusion. The concentration of interleukin 8, on the other hand, increases significantly. These findings correlated with certain donor variables such as age, cause of brain death, smoking, sputum bacteriology, and
mechanical ventilation time, as well as with lung function after transplantation. The authors observed that the age of the donor correlated inversely with the concentration of interleukin 10, a cytokine with an antiinflammatory effect, a finding that could explain the fact that lungs from older donors are associated with a higher rate of postoperative mortality. Interleukin 8 concentration, on the other hand, was a factor that predicted lung function 2 hours after transplantation. Interleukin 8 is an important mediator of neutrophil chemotaxis. It is important to note that some authors have reported a high correlation between blood levels of certain cytokines and the hormone depletion that occurs with brain death. A finding of high neutrophil and interleukin concentrations in bronchoalveolar lavage fluid, as an expression of cell damage, has been closely associated with primary graft failure.41

Proinflammatory cytokines are going to be responsible for stimulating neutrophils during reperfusion. In turn, neutrophils in the presence of certain leukocyte adhesion molecules produced by endothelial cells, such as selectins and integrins, are going to be sequestered by reperfused tissue in a complex sequential process of emigration away from the vascular bed.48

Experiments have been performed with certain oligosaccharides as inhibitors of leukocyte adhesion molecules, and drugs (corticosteroids) that counteract the effect of cytokines have been applied in clinical settings. Lungs from donors who have been administered methylprednisolone have been shown to be better oxygenated before ischemia.49 Preservation solutions like UW contain methylprednisolone, and some groups have administered a bolus dose of methylprednisolone before starting to remove the lung.50

Complement activation occurs in all processes involving the inflammatory cascade. Certain complement fractions such as C3 and C5 are implicated in lesions that develop when ischemic tissue is reperfused. A natural antagonist of C3 and C5 convertases, complement receptor 1 inhibitor, has been studied in a randomized controlled trial of 59 transplanted patients; time to intubation was significantly shorter in patients who had been administered receptor 1 inhibitor before reperfusion.51

Endothelial cells play a fundamental role in coagulation. Heparin figures among the many molecules produced, but others include prothrombin and antifibrinolytic factors. The latter predominate in ischemic conditions. Experimentally, certain inhibitors of coagulation, such as C1 esterase52 and antithrombin III53 have been shown to have the ability to prevent ischemia-reperfusion injury. Although Strüder et al54 used C1 esterase to treat primary graft failure, experience is limited.

Cell damage is not confined to the endothelium. Epithelial cells, particularly type II pneumocytes, are also affected. The presence of certain mediators, such as phospholipase A2, would alter the production and accumulation of pulmonary surfactant, which is 90% lipid in composition, favoring pulmonary edema, hypoxemia, and decreased compliance, and also diminishing its immunomodulatory role in fighting inflammation and infection.56 The role of surfactant is presently one of the most innovative areas of experimentation.57 No clinical applications have been published yet for the exogenous application of surfactant to the donated lung. Although surfactant has been used to treat primary graft failure, experience is still scarce.58

As mentioned, the mechanism by which ischemia-reperfusion injury occurs is the result of interactions among potent mediators and various types of cells. The role of neutrophils in reperfusion injury has been questioned in the last decade. Steimle et al59 found that neutrophils did not have to be present for reperfusion lesions to occur in an animal model. In a later study by some of the same authors, it was found that administering antineutrophil antibodies before reperfusion had no protective effect on microvascular permeability 30 minutes after reperfusion, although later a gradual increase in the nonneutropenic control group was evident, associated with a gradual increase in myeloperoxidase.60 That study showed that ischemia-reperfusion injury becomes established in 2 phases, an early one that does not depend on neutrophils, and a later one that does.61 The donor lung contains a large number of macrophages and T lymphocytes which would be responsible for the early phase,61-64 while the late phase would be a result of recipient response expressed by the sequestering of neutrophils activated by proinflammatory mediators. All these mechanisms of response that occur during reperfusion are going to generate a second wave of free oxygen radicals that would create ischemic lesions.

Pentoxifylline, a methylxanthine used as a hemorheologic agent in the treatment of peripheral vascular diseases, has a proven inhibitory effect on activated neutrophils, and for that reason it has been used by a group of authors prior to reperfusion of the graft.37

Route of Perfusion and Temperature

Cold storage is one of the most basic strategies in organ preservation, but it interferes with many cell activities that are temperature sensitive, explaining the presence of endothelial lesions in the lung65 or the production of surfactant in relation to type II pneumocyte involvement.66 It is no less true, however, that cell metabolism decreases, favoring the viability of a lung in an ischemic state, and cold storage is therefore an essential element of preservation. The optimal temperature for preservation is unknown, however. Although early studies concluding that 10 ºC is the best temperature have recently been validated,67 4 ºC is the temperature at which lungs are infused and stored by...
most transplant groups.

Most groups also continue to infuse the solution through the pulmonary artery (anterograde approach) at a dose of 60 mL/kg. Preservation by this route is incomplete, according to experimental studies, however, because bronchial circulation is not included: the retrograde approach, through the left atrium, has been shown to be significantly better. Other studies have demonstrated less alteration of pulmonary surfactant with the retrograde approach. The addition of retrograde perfusion has also been shown to improve the function of the implanted lung. We have not found that this technique influences the development of primary graft failure, although we recommend its use given that we have sometimes observed the flushing out of thrombi that had formed in the vascular bed in spite of heparin having been administered to the donor.

The pulmonary vascular bed is highly sensitive to changes in pressure and perfusion rate, and therefore strict control of reperfusion of the implanted lung is insisted upon so that damage to alveolar and capillary structures can be prevented. This precaution is also valid when perfusing the explanted lung. Solution flushing pressures exceeding 20 mm Hg have been shown to cause pulmonary lesions. Therefore, during explantation it is important to cut at the level of the left atrial appendage or the outlet of the pulmonary vein before initiating perfusion of the pulmonary artery, so as to facilitate good drainage of the perfusion fluid and prevent hypertension in the vascular bed. Placing the bags of perfusion liquid at a height of approximately 2 m assures a good rate of perfusion at a pressure between 15 and 20 mm Hg.

**Principles Specific to Lung Preservation**

As mentioned, the state of oxygenated ischemia in which the lung is kept is a source of free oxygen radical production by way of reactions catalyzed by NADPH oxidase. The ideal oxygen concentration to use during pulmonary perfusion is currently unknown. Concentrations less than 40% have been used experimentally to achieve good lung preservation. Recently, Fukuse et al. found that the optimal inspired oxygen fraction is 5% to maintain an ideal level of metabolic activity under hypoxic storage conditions at low temperatures; higher oxygen concentrations, on the other hand, would lead to the hyperoxidation responsible for mitochondrial dysfunction due to increased lipid peroxidation. Although some groups have used an inspired oxygen fraction of 100%, most use a fraction between 30% and 40%.

The vascular bed is known to respond to atelectasis by vasoconstriction. Keeping the lungs well ventilated during perfusion facilitates pulmonary lavage. Using too high an intra-alveolar pressure can make perfusion difficult owing to the increase of capillary pressure. The problem is to know what the optimal insufflation volume is. Some experimental studies demonstrate that storing hyperinflated lungs (positive end-expiratory pressure of 30 cm H2O) significantly improves lung function parameters in the implanted lung, leading some groups to store lungs at a positive end-expiratory pressure of 25 or 35 cm H2O. Other studies, however, have demonstrated the opposite, that is, increased reperfusion edema. Meyers et al. explant lungs with the degree of insufflation determined by residual functional capacity. Our group performs lung explanation with the donor ventilated with an inspired oxygen fraction less than 40% and a volume of 12-15 mL/kg at 14-16 cycles/min, checking that atelectasis has not developed before perfusion is started. If atelectasis is observed, we use a positive end-expiratory pressure of 3-5 cm H2O before starting and later we eliminate positive pressure to prevent barotrauma as far as possible.

**Conclusions**

To achieve optimal recipient outcome after lung transplantation, proper extraction and preservation of the donor lung are necessary. In spite of the many experimental studies that have been carried out to find ways to keep a pulmonary graft in the best possible condition for the longest possible time, the impact on clinical practice has been the exception and the incidence of primary lung failure has hardly changed in the past 10 years. Current techniques for lung preservation continue to be considered less than ideal. The introduction of solutions low in K+ to clinical practice and the use of inhibitors of certain complement fractions and platelet aggregation factor may have decreased the incidence of primary graft failure, although longer experience with these products will be needed before we can tell. We hope that new approaches will be developed in the future both for preserving the lung and managing implant reaction with the aim of improving short- and long-term outcomes of transplantation.

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