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ISCHEMIA AND REPERFUSION OF LUNG GRAFTS

Consequences of the decrease in the respiratory function of pulmonary mitochondria after normothermic and hypothermic ischaemia on the procurement of lung grafts from non-heart beating donors

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Publications

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Transplant Proc 1997; 29: 2338-2339.

2 "Comparative effects of University of Wisconsin and Euro-Collins solutions on pulmonary mitochondrial function after ischemia and reperfusion."
DETRY O., WILLET K, LAMBERMONT B, MEURISSE M, PINCEMAIL J, SERTEYN D, LAMY M, DEFRAIGNE JO, LIMET R, SLUSE FE
Transplantation 1998; 65, 161-166.

3 " Antioxidant status after cold ischemia of rabbit lung."
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4 "Effects of cold and warm ischemia on the mitochondrial oxidative phosphorylation of swine lungs."
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Book Chapter

1 "Conservation pulmonaire et donneurs à cœur arrêté"
DETRY O., WILLET K, MEURISSE M, SLUSE FE, LIMET R, DEFRAIGNE JO
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Presentations

1 **3rd Meeting of the Belgian Transplantation Society**
March 2, 1996, Gent, (Belgium)
"Mitochondrial function impairing after lung ischemia."
DETRY O., WILLET K, LAMBERMONT B, DEFRAIGNE JO, MEURISSE M, LIMET R, SLUSE FE

2 **15th Meeting of the American Society of Transplant Physicians**
May 27, 1996, Dallas, (USA)
"Mitochondrial function impairing after lung ischemia."
DETRY O., WILLET K, LAMBERMONT B, DEFRAIGNE JO, MEURISSE M, LIMET R, SLUZE FE

3 **9th European Bioenergetics Conference**
August 17-22, 1996, Louvain (Belgium)
"Comparative effects of ischemia on the mitochondrial function in kidney and lung."

WILLET K, DETRY O., DEGOTTE M, LAMBERMONT B, LIMET R, LAMY M, SLUZE FE

4 8th Biennal Meeting of the International Society for Free Radical Research
October 1-5, 1996, Barcelona (Spain)
" Effects of ischemia and reperfusion on the mitochondrial function of pig lungs."
WILLET K, DETRY O., DEGOTTE M, LAMBERMONT B, LIMET R, LAMY M, DEFRAIGNE JO, SLUZE FE

5 1er Congrès Francophone de Recherche Clinique et Biologique en Transplantation
December 5, 1996, Paris (France)
" Influence du temps d'ischémie chaude ou froide sur la fonction respiratoire de mitochondries pulmonaires."
WILLET K, DETRY O, LAMBERMONT B, DEFRAIGNE JO, MEURISSE M, LIMET R, SLUSE FE

6 4th Meeting of the Belgian Transplantation Society
March 1, 1997, Brussels, (Belgium)
"Comparative effects of University of Wisconsin and Euro-Collins solutions on pulmonary mitochondrial function after ischemia/reperfusion."
DETRY O., WILLET K, LAMBERMONT B., DEFRAIGNE J.O., SLUSE F.E., LIMET R.

7 17th Meeting of the American Society of Transplant Physicians
May 12, 1997, Chicago, (USA)
"In lung graft, 45 min of warm ischemia may be as deleterious as 48 hours of cold ischemia."
WILLET K, DETRY O., LAMBERMONT B, DEFRAIGNE JO, MEURISSE M, LIMET R, SLUZE FE

8 23rd Meeting of the American Society of Transplant Surgeons
May 16, 1997, Chicago, (USA)
"Comparative effects of University of Wisconsin and Euro-Collins solutions on pulmonary mitochondrial function after ischemia/reperfusion."
DETRY O., WILLET K, LAMBERMONT B, DEFRAIGNE JO, MEURISSE M, LIMET R, SLUZE FE

9 International Transplant Congresses
July 8, 1997, Washington, (USA)
"Influence of warm ischemia duration on the lung procurement from non-heart beating donors."
WILLET K, DETRY O., LAMBERMONT B, DEFRAIGNE JO, MEURISSE M, LIMET R, SLUZE FE

10 5th Meeting of the Belgian Transplantation Society
March 7, 1998, Brussels, (Belgium)
"Effects of in vitro anoxia/reoxygenation on the respiratory function of lung mitochondria."
WILLET K, DETRY O., LAMBERMONT B, DEFRAIGNE JO, MEURISSE M, , SLUSE FE

Chapter 1

Introduction

At the beginning of a new millennium, organ transplantation has revolutionised the life of many patients suffering from hepatic, cardiac, pulmonary, renal, or pancreatic insufficiency. The results improved so much that organ transplantation is not an experimental and/or heroic procedure anymore; on the contrary, the results are generally excellent and reproduced in the same manner by the majority of the teams. Moreover, the success of transplantation created a new major problem of public health: the lack of organ donors is so deep that many transplant candidates die without profiting from an intervention that could have offered them a life of quality for many years.

In recent years, pulmonary transplantation has become the treatment of choice for several end-stage lung diseases {583}. However, pulmonary transplantation is in part limited by the scarcity of suitable donors and by the lack of reliable prolonged methods of lung graft preservation {390}. The pulmonary graft need/offer ratio may be estimated at 2/1 {661}. For this reason, many patients registered on waiting list die before a pulmonary graft could be available {688}. This lack of pulmonary grafts can be partly explained by lung donor selection criteria, that are more severe than for the other organs; it is estimated that only 10 to 25% of the organ donors can be lung donors {624,680}. Moreover, the pulmonary grafts are particularly sensitive to ischaemia and reperfusion injury: primary lung graft dysfunction (PLGD) occurs in 10% to 20% of lung tranplant recipients {624}, and may complicate the posttransplant course even after short period of ischaemia. Clinically, PLGD results in a progressive deterioration of pulmonary compliance, gas exchange, and pulmonary vascular resistance, coinciding with an increase in extravascular lung water {654}. Although PLGD may be reversible, it significantly increases the pulmonary transplantation morbidity and mortality {583}. Cold ischaemia of 6 to 8 hours remains the maximum tolerable limit for most of the pulmonary transplantation groups {666}. An increase in the duration of the maximum ischaemia would allow a better distribution of the pulmonary grafts, while making possible a better allocation of the pulmonary grafts and, therefore, an increase in the number of procedures. Therefore, one way of increasing the pulmonary graft pool could be to improve the methods of lung conservation

At the biochemical and cellular level, the lesions induced by the ischaemia and the reperfusion of the transplanted organs have been studied since many years. The importance of the metabolic disorders induced by ischaemia varies according to the considered organ and species, but also according to the ischaemia duration and temperature. However some cellular phenomena are constant. Ischaemia is accompanied

by an early decrease in the cellular energy load in the form of a decrease in the cytoplasmic adenosine triphosphate (ATP) contents secondary to a reduction in the ATP production in the mitochondria. In the seconds following ischaemia, glycolysis becomes the only source of cellular production of ATP, leading to an accumulation of lactic acid and a reduction in the cellular pH. Moreover this fall of cellular ATP has a marked effect on the cellular function, since many cellular systems enzymatic require ATP for their function, and in particular the membrane ionic pumps, which promote a modification of the intracellular ionic contents. The cell becomes permeable to sodium and chlorine ions, which reach the cytosol, and at the same time potassium moves towards extra-cellular spaces. Cellular ischaemia is also accompanied by an increase in the cellular calcium contents. The clear intracellular profit of aqueous solution induces cellular oedema. Moreover, at reperfusion, the tissue reoxygenation can worsen certain lesions, by formation of activated forms of oxygen (hydrogen peroxide) and of free radicals (anion superoxyde, radical hydroxyl). These activated forms of oxygen can be produced at the time of the tissue reoxygenation by xanthine oxidase (converted by proteolysis of the cytosolic xanthine deshydrogenase at the time of ischaemia), by mitochondrial respiratory chain, by self-oxidation of some molecules (thiols, hydroquinones, cathecolamins, flavins, haemoglobin), by metabolic pathways of arachidonic acid and prostaglandins, and also by activated polymorphonuclear cells attracted by adhesion protein formation on the vascular endothelium. There are however some endogenous, enzymatic tissue protectors as superoxyde dismutase, catalase, glutathione peroxidase, or not enzymatic, as vitamin E, vitamin C, reduced glutathione, or others.

The lungs are organs whose sensitivity to the phenomena appearing at the time of ischaemia and reperfusion is well known. It was been shown that the lungs are particularly rich in xanthine oxidase {642}. A great number of studies on pulmonary ischaemia and reperfusion also demonstrated that the formation of activated products of oxygen holds an important role in the pulmonary lesions appearing after reperfusion {390}. Protective agents against the activated oxygenated forms, such as superoxyde dismutase {99}, catalase {707}, dimethylurea {98}, desferrioxamine {77}, glutathione {17} or allopurinol {77} showed a protective effect on the pulmonary function after ischaemia and reperfusion in animal models {390}.

The aim of this research was to further increase the knowledge in the tissular and cellular alterations appearing during pulmonary ischaemia and reperfusion, in order to minimise the occurrence of PLGD after pulmonary transplantation and in order to increase the tolerable length of cold ischaemia before pulmonary reimplantation. The clinical application of this research might be an increase in the pulmonary graft pool and in the results of pulmonary transplantation. In a preliminary study in a rabbit model of lung ischaemia presented in the Chapter 2 of this summary, we studied the modification

in the tissue contents in vitamins E and C, two important protectors against the lesions appearing at the time of tissue ischaemia and reperfusion. In a second step summarised in Chapter 3, we investigated the effects of hypothermic ischaemia and normothermic reperfusion on the oxidative phosphorylation of isolated pulmonary mitochondria, that play a key role in the cellular energetic metabolism. As rabbit lungs do not contain a sufficient number of mitochondria (unpublished results) we developed a model of swine pulmonary ischaemia and reperfusion that also allowed studies of pulmonary functional parameters. In this model, we also compared two preservation solutions commonly used in pulmonary transplantation, the Euro-Collins solution (EC) and the University of Wisconsin solution (UW), and we analysed the characteristics of the lungs flushed with these solutions in our model of assessment of physiologic and mitochondrial function. In a third part of this work presented in Chapter 4, we studied the effects of normothermic ischaemia on the oxidative phosphorylation of isolated pulmonary mitochondria, in order to determine the tolerable duration of cardiac arrest before procurement of lung grafts from non-heart beating donors, that are another way to increase the pulmonary donor pool.

All animals (rabbits and pigs) received human care and the experiments were performed in compliance with *The Principles of Laboratory Animal Care* formulated by the National society for Medical Research and the *Guide for the Care and use of Laboratory Animals* prepared by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Chapter 2

Antioxidant status after pulmonary cold ischaemia

The purpose of this study was to confirm that lung antioxidants are affected by cold ischaemia and, to demonstrate free radical production in the isolated rabbit lung reperfused after cold preservation. In this part of the study, we used a surgical model in the rabbit.

Material and Methods

Heart-lung block harvesting: New Zealand white rabbits weighing 2.5 to 3 kg were premedicated with intraperitoneal injection of azaperonum (Stressnil, Janssen-Cilag, Berchem, Belgium), anesthezied with intravenous sodium pentobarbital (Nembutal; Sanofi, Brussel, Belgium) and sufentanyl (Sufenta, Janssen-Cilag) and paralysed with intravenous pancuronium bromide (Pavulon, Organon-Teknika, Boxtel, The Netherlands). Each animal was intubated with a n°6 endotracheal tube through a cervical tracheostomy, and the tubing was connected to a volume-cycled respirator. The lungs were ventilated with room air (respiratory rate: 40 breaths/min, tidal volume: 40 ml, positive end-respiratory pressure: 0 cm H2O). The chest was opened through a median sternotomy, and the thymus was resected. The ascending aorta and the pulmonary artery were dissected and loosely encircled with individual ligatures. Heparin (500 UI/kg) was then infused. The pulmonary artery was cannulated with a catheter through the right ventricular outflow tract for subsequent flushing of the lungs. The pulmonary artery ligature was tied to secure the catheter in place. The aorta was also ligated around a catheter that was used to exsanguinate the animal. The lungs were flushed with 500 ml of cold (4° C) Euro-Collins (EC) solution injected in the main pulmonary artery at a height of 60 cm while ventilation was continued. The effluent was drained through the aortic catheter. For additional cooling, ice-cold normal saline was poured into the chest As soon as the flush was completed, the trachea and the oesophagus were divided together proximal to the cervical tracheotomy, and blunt dissection with a finger continued downward along the posterior mediastinum. The distal oesophagus and the descending aorta were transected at the diaphragm. Then the heart-lung block was removed. The lungs were continuously ventilated during this procedure and immediately tested under normal saline for air leaks. The trachea was then clamped with lungs inflated at end tidal volume, and the endotracheal tube was removed. The right pulmonary artery and the right main-stem bronchus were then ligated, isolating the left lung. Approximately one quarter of the lung was immediately frozen in liquid nitrogen and kept at – 196°C for measurements of antioxidant molecules at basal value. The remaining heart-lung bloc was then stored in cold EC solution for 3, 6 and 12 hours and ultimately frozen until antioxidant assay. One gram of lung tissue was homogenised in 9 ml of saline solution 0.9% and, after centrifugation, vitamin E (Vit E), free glutathione (free GSH), and protein-bound glutathione (PB-SH) were determined in the supernatant as previously described (4). Ascorbic acid (1g lung tissue in 3 ml saline 0.9%) was detected as ascorbyl free radical (AFR) by electron spin resonance (ESR) spectroscopy (Brücker ESP 300) after dimethylsulfoxide (DMSO) reaction. Relative radical concentrations (in arbitrary units, AU) were determined by the measurement of line intensities on ESR spectra recorded with identical spectrometer settings (frequency: 9.75 GHZ; center field: 3480 G; power: 20 mW; amplitude: 2 G; gain: $2x10^6$; time constant: 36 ms; sweep width: 100 G).

Reperfusion: After 3 hour storage in cold EC solution, the lower part of the trachea was clamped to prevent lung deflation and a endotracheal tube was then put into the trachea for subsequent ventilation. The heart-lung block was mounted into a Plexiglas plastic box, which was humidified and temperated at 37°C. The endotracheal cannula was connected to the same ventilator and the right lung was ventilated with 50% oxygen-50%

room air. During ventilation, the lung was reperfused through the main PA cannula with a Krebs-Henseleit bicarbonated buffer solution (pH 7.4) containing ascorbic acid 1mM injected by a roller pump at a constant flow of 40 ml/min. Effluent samples were collected from the pulmonary veins at 1, 5, 10 and 20 minutes of reperfusion and mixed with DMSO for ESR ascorbyl free radical determination as marker of free radical production (5).

Results and Discussion

The results of this study were published in addendum 1. We demonstrated significant changes in the antioxidant defences of the lung after cold ischaemia. If 3 hours of cold storage did not significantly affect the antioxidant defences of the lung, increased cold ischaemia time up to 6 and 12 hours resulted in a significant decrease in both vitamins C and E concentrations, suggesting the presence of an oxidative stress. In contrast, concentrations of SH groups increased. This mobilisation may account for the acquisition of an adaptive tolerance to oxidative stress, as it has been reported in lungs of rats exposed to ozone. From these data, synergetic interactions between antioxidants could be suggested. Vitamin C acts as first defence as shown by a significant decrease observed after 6 hours of cold ischaemia. However, a complete restoration of this hydrophilic antioxidant was observed and can be related to a significant mobilisation in SH groups that are able to regenerate vitamin C from its oxidised form. Moreover, it is well admitted from *in vitro* experiments that vitamin C can rapidly reduce vitamin E radicals in membranes to regenerate vitamin E. This could explain why vitamin E depletion only appeared 6 hours after vitamin C has decreased.

At the contrary, the ESR experiments failed to demonstrate any increased ascorbic acid oxidation (as marker of oxidative stress) in lungs reperfused after 3 hours of cold storage in EC solution, when compared to lungs not submitted to cold ischaemia. In other ESR experiments (data not shown), we were also unable to detect any spin adduct of oxygenated free radical production in pulmonary vein effluent when 5, 5'-dimethyl-pyrroline-N-oxide (DMPO) was added as spin trap agent in the perfusion medium instead of ascorbic acid.

In conclusion, this study suggested that the inflated lung stored in cold EC solution for periods not exceeding clinical practice (6 hours) presents a relatively good tolerance to oxidative stress occurring during cold ischaemia and might therefore be protected against free radical production generated during reperfusion.

Chapter 3

Effects of cold ischaemia and normothermic reperfusion on pulmonary physiological parameters and on mitochondrial respiratory function.

Comparison between University of Wisconsin and Euro-Collins solutions on mitochondrial function after ischaemia and reperfusion

If the endothelium is a crucial zone in organ transplantation, in lungs, the anatomical and functional integrity of the alveole, zone of exchange between the alveolar air and the blood of the pulmonary capillaries, is at least as important as the preservation of the endothelium {624}. The pulmonary alveolar epithelium is composed of 2 cellular types, the type I pneumatocytes, lengthened cells which cover most of alveolar surface, and the type II pneumatocytes, cuboid cells responsible for the surfactant secretion {682}. By decreasing the surface stress on the level of the interface air/blood, the surfactant protects the lung from oedema in physiological and pathological condition. In a normal human lung, 20% of the alveolar cells are of type II pneumatocytes. In spite of this reduced number, these cells are crucial for pulmonary function. The conservation of the ultrastructure of type II pneumatocytes seems to be important in the early postoperative function after pulmonary transplantation in humans {695}. Moreover, it was shown that the quality of surfactant decreases after conservation and reperfusion of the lungs, and that the in vitro function of surfactant continuously decreases with the prolongation of the duration of ischaemia {321}. Moreover, surfactant administration before pulmonary reperfusion improves the postoperative function of lung grafts in the rat {230}. It thus seems that the study of alveolar deteriorations and the function of the type II pneumatocytes occurring at the time of ischaemia and reperfusion may be a way of promising research in the improvement of the methods of pulmonary graft conservation.

The mitochondria play a key role in cellular energy metabolism: they are the principal site of the cellular breathing, which is coupled to adenosine triphosphate (ATP) synthesis in a process called the oxidative phosphorylation (OxPhos). OxPhos occurs in the respiratory chain, that is a multienzymatic complex within the internal mitochondrial membrane. The respiratory chain is the place of oxidoreduction reactions whose outcome is the oxidation of the NADH (reduced nicotinamid adenin dinucleotid) and the FADH2

(reduced flavin adenin dinucleotid) by oxygen, and these reactions are coupled to ATP synthesis via the formation of a transmembranar potential.

The mitochondrial consequences of ischaemia may be variable {684}. Among others, during ischaemia, the oxygen deficit induces a decrease in the mitochondrial and cytosolic ATP contents. If ischaemia is prolonged, the ATP level, and thus the cellular energy level, become insufficient to ensure the fundamental cellular needs; cellular homeostasis is then compromised. The cytosolic contents in sodium, calcium and inorganic phosphate increase, and may induce mitochondrial dysfonctions such as respiration uncoupling, oedema or increase in the permeability of the mitochondrial membranes. Moreover, the mitochondria may be also involved in the genesis of lesions appearing at the time of the reperfusion. At reperfusion, the mitochondria can produce activated oxygen species, including free radicals, at the level of the respiratory chain {685}. This increased production of activated oxygen species can exceed the cellular defence mechanisms. When the lesions induced to OxPhos at ischaemia and reperfusion are moderate, they can be reversible and the cell survives. On the other hand, if these lesions are severe, they can be irreversible. The appearance of mitochondrial membrane permeability causes an uncoupling of OxPhos. The mitochondria are then unable to synthesise ATP, and in this case the cellular metabolism is irremediably disturbed, resulting in cell death.

In the lung, the mitochondria are present in every kind of parenchymatous cells, namely in the cells of the alveolar epithelium (type I and II pneumatocytes), in the endothelial, bronchial, interstitial cells, and in the macrophages {683}. In the alveolar septum, the mitochondria concentration is higher in the type II pneumatocytes than in the endothelial cells or the type I pneumatocytes. The volume of alveolar pulmonary tissue (alveolar cells and canaliculi) corresponds to approximately 90% of pulmonary volume, and approximately 20% of the cells of this tissue are composed of type II pneumatocytes. One can estimate that approximately 50% of the mitochondria isolated from a lung are mitochondria coming from this type II pneumatocytes {683}. We insisted above on the importance of the functional integrity of these type II pneumatocytes in the pulmonary function after transplantation. It thus appeared interesting to us to study the OxPhos mitochondrial function in a model of pulmonary cold ischaemia and reperfusion, in order to evaluate the role of mitochondrial dysfunction in PLGD. In this model we induced a cold ischaemia of 24 or 48 hours after flush with Euro-Collins (EC) solution, and thereafter we reperfused the lungs during 30 minutes in normothermia, inducing a measurable lung

graft nonfunction. We then analysed the OxPhos function in mitochondria isolated from these lungs.

Most of the transplanted kidneys, livers or hearts are currently simply flushed with a cold solution of conservation, the nature of this solution varying according to organs and practices. The advantages of this flush are numerous: (a) the technique is simple and does not require complicated or expensive material; (b) the technique is fast and allows a fast cooling of the organs; (c) the composition of the flushing liquid is easily modifiable according to the needs or the advance of research; (d) the noxious blood components are eliminated (fibrin, complement, platelets, white blood cells).

Historically, great quantities of solutions were used in experimental pulmonary transplantation. Before 1986, none had however shown a sufficient effectiveness to allow its use in routine during pulmonary harvesting. In 1986, Starkey published the success of the use of heart-lung grafts in a primate model, using a hypothermic flush of the pulmonary artery with a modified solution of EC (60 mL/kg) {203}. In 1987, Baldwin published the first clinical use of this technique in heart-lung transplantation {672}. At that time, the modified EC solution became the standard solution for the majority of the pulmonary transplantation centres. The EC solution is still currently used in 77% of the centres, half of them adding magnesium {666}. However, when the pulmonary vessels are exposed to a hypothermic and hyperkaliemic solution such as EC, they undergo an intense vasoconstriction and a net increase in pulmonary vascular resistance. It has been proven that administration of prostaglandin E1 (North America) or I2 (Europe), both promoting an intense pulmonary vasodilatation and bronchodilatation, has real beneficial effects, by improving the distribution of the perfusion liquid, by inhibiting platelet aggregation, and even by limiting the production of oxygen free radicals {37}.

In transplantation of abdominal organs the solution developed by Belzer at the University of Wisconsin (UW) made it possible to significantly prolong the duration of ischaemia compatible with a good function of the transplanted organ {641}. This solution of conservation was also tested for the pulmonary preservation in animals and in humans {390,474,598}. However there is no demonstration yet of UW superiority on EC in clinical pulmonary transplantation. The UW solution is however used by approximately 13% of the centres {666,593,598}.

In a further step of our research, we compared EC and UW, and we analysed the characteristics of the lungs flushed with these solutions in our model allowing assessment of physiologic and mitochondrial functions.

Material and Methods

Lung harvest. Seventeen Pure Pietrain pigs of either gender (weight 20-30 kg) were premedicated by an intramuscular injection of azaperonum (80 mg; Stressnil, Janssen-Cilag, Berchem, Belgium) and intraperitoneal injection of sodium pentobarbital (20 mg/kg; Nembutal; Sanofi, Brussel, Belgium). Anaesthesia was induced by an intravenous bolus of sodium pentobarbital (5 mg/kg) and sufentanyl (0.5 μg/kg; Sufenta, Janssen-Cilag). Each animal was intubated with a n°6 endotracheal tube through a cervical tracheotomy, and the tubing was connected to a volume-controlled ventilator (PLV 100, Life Care, Lafayette, CO). The lungs were ventilated with room air (respiratory rate 20/min, tidal volume 15ml/kg, End-Expiratory Pressure 0 cm H_2O). During the procedure, an end-tidal CO_2 monitor was used to assess the adequacy of ventilation (Capnomac, Datex, Helsinki, Finland). This monitor was electrically connected to a computer (386dx33, Interlan, Liège, Belgium), and direct aerodynamic measurements were analysed and recorded by a data acquisition program (Codas, DATA Q Instruments Inc, Akron, OH). Intravenous pancuronium bromide (0.1 mg/kg; Pavulon, Organon-Teknika, Boxtel, The Netherlands) was used as a muscle relaxant. Anaesthesia was maintained by an intravenous bolus of sodium pentobarbital (1 mg/kg), as required, and by a continuous infusion of sufentanyl (0.5 μg/kg/h). After median sternotomy, the pericardium was opened, and the pulmonary artery (PA) was dissected and encircled with a ligature. The superior and inferior venae cavae were isolated. Both pleurae were opened, and the right and left azygos veins were ligated and divided. Following systemic heparinisation (300 U/kg), 500 μg of prostaglandin E1 (Prostin, Upjhon, Puurs, Belgium) were injected intravenously. Both venae cavae were ligated, and the PA was immediately cannulated by a high flow tubing through the right ventricular outflow tract, and the ligature was tied around the PA to secure the cannula. After opening of the left atrial appendage, the lungs were flushed by gravity at 50 cm H_2O with 2 L of either cold (4°C) standard EC (Fresenius AG, Wilrijk, Belgium) or cold (4°C) UW (ViaSpan, DuPont, Paris, France). The ventilation was continued during the flush, and topical pulmonary cooling was facilitated by the flow of the effluent in both pleura. Upon completion of the flush, the ventilation was discontinued, and the heart-lung block was excised. After one final mechanical ventilation, the trachea was clamped at end-inspiration and ligated. The heart-lung block was then stored inflated in either cold EC or cold UW during 24 or 48 hours.

Lung reperfusion. After 24 hr of CI, the left pulmonary hilum was dissected. The PA, pulmonary vein and main bronchus were ligated and divided, and the left lung was resected. The left atrial appendage was closed with a running suture. The left ventricle was cannulated through the aorta, and the cannula was secured by a purse-string suture. The trachea was reopened and cannulated with a n°6 endotracheal tube. A ligature was securely tied around the trachea. The heart-lung block was then inserted in a Plexiglas chamber, which was heated at 37°C and humidified (Figure 1). The endotracheal cannula was connected to the same volume controlled ventilator (PLV 100); the right lung was reventilated with room air (respiratory rate, 20/min; tidal volume, 8ml/kg; end-expiratory pressure, 0 cm H_2O). At the time of reventilation, the lung was reperfused in closed circuit through the main PA cannula with a Krebs-Henseleit bicarbonated buffer solution (pH 7.3, 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM NaH_2P0_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_4$, 11.1 mM Glucose) at 37°C for 30 min. This solution was injected by a roller pump (Type 914421, Jostra AB, Lund, Sweden) at a constant flow of 300 ml/min, perfused through the right lung, and drained by gravity through the aortic cannula. During the reperfusion, the end-tidal CO_2 monitor (Capnomac) was used to assess direct aerodynamic measurements. A tip pressure catheter (Sentron, Roden, The Netherlands) was connected to the PA cannula and was used to continuously monitored the PA reperfusion pressure. All signals were electrically transmitted to the computer (386dx33, Interlan) and recorded by the data acquisition software (Codas).

Physiological measurements. Direct aerodynamic parameters, including inspiratory and expiratory air flow rates, as well as airway pressure, were analysed by the end-tidal CO_2 monitor (Capnomac) during the lung harvesting and the *ex vivo* reperfusion. Signals were transmitted to the computer (386dx33) and recorded by the data acquisition software (Codas). Peak inspiratory pressure (P Peak, cm H_20) and pulmonary static

compliance (C stat, ml/cm H_20) were used to evaluate the extent of the pulmonary injury. C stat was calculated by the following equation:

C stat = Tv / (P- EEP)

where Tv = tidal volume; P = airway pressure at the end of the inspiratory period; EEP = airway pressure at the end of the expiratory time.

A tip pressure catheter (Sentron) was connected to the PA cannula and was used to monitored the PA pressure during the lung reperfusion. The signals were transmitted to the computer (386dx33) and recorded by the data acquisition software (Codas). During reperfusion, pulmonary vascular resistance (PVR: dyne.sec/cm^5) was calculated every 5 min according to the following equation:

PVR = MPAP / Q

where MPAP = mean PA pressure; Q = reperfusion flow rate (300 ml/min).

Mitochondrial isolation. The lungs were trimmed, and pieces were homogenised with a motor-driven hand-held homogeniser in the presence of SET solution (250 mmol/L sucrose, 2 mmol/L ethylen diamine tetracetic acid (EDTA), 5 mmol/L Trishydroxymethyl aminoethan, 0.5% fatty acid free bovine serum albumin (BSA)). After homogenisation and filtration, the mitochondria were isolated from the solution with a standard techniques of differential centrifugations described elsewhere {592}. Mitochondrial volume was determined by accessible volume to tritium water.

Mitochondrial oxygen consumption measurement. Respiratory parameters were determined at 25°C in isolated mitochondria with a Gilson oxygraph (Gilson Medical Electronics, Middleton, WI) by in vitro measurement of oxygen consumption rates in a medium at pH 7.4 (15 mmol/L KCl, 2 mmol/L EDTA, 5 mmol/L $MgCl_2$, 50 mmol/L Trishydroxymethyl aminoethan), in the presence of 1% BSA, with 5 mmol/L ketoglutarate and 5 mmol/L pyruvate as oxidizable substrates, and 2.5 mmol/L phosphate (KH_2PO_4). The concentration of added adenosine diphosphate (ADP), oligomycin and uncoupler carbonylcyanide-p-trifluoro-methoxyphenylhydrazone (FCCP) was 165 μmole/L, 16μg/ml and 5 μmole/L, respectively. The measured functional parameters of mitochondria were : the respiration rates in the presence of externally added ADP (V_3) or in its absence (V_4), which was used to calculate the respiratory control (RC) given by the ratio V_3/V_4; the respiration rates when ATP synthase is blocked by oligomycin (V_{Olig}) or in the presence of the uncoupler FCCP (V_{FCCP}); the uncoupled respiratory control (URC) given by the ratio V_{FCCP}/V_{Olig}. The yield of oxidative phosphorylation, i.e. the number of moles of ADP phosporylated by Atg of oxygen consumed (ADP/O) was also determined.

Transmission electron microscopy. Three tissue blocks of 2 mm^3 volume were prepared from a single slice taken from the midportion of the lung and were immersed in 2.5% phosphate-buffered glutaraldehyde for fixation. After rinsing, the blocks were postfixed in 1% osmium tetroxide followed by dehydration in a graded alcohol series and embedded in EM Bed812 resins (Epon 812 substitute) (EMS, Fort Washington, PE). Ultra-thin sections were cut and mounted on copper grids, stained with uranyl acetate and lead citrate. Sections were examined in a Zeiss 912 electron microscope 80KV (Carl Zeiss, Göttingen, Germany). Mitochondria photographs were taken on Kodak Electron Microscope film (Estar Thick base 4489, Kodak, Hemel Hempstead, England) and prints were made on Ilford Multigrade IV paper (Ilford, Mobberley, England). Ultrastructural changes of the mitochondria were examined, and compared.

Experimental design. The lungs were randomly flushed with EC or UW solutions. The lungs from the same heart-lung block were randomly assigned to one of the groups, and the same heart-lung block never gave both lungs to the same group. For anatomical

reasons, the lung used in the case of reperfusion was always the right lung. The mitochondria were isolated from the lungs immediately after harvesting (control lungs), or submitted to 24 hr of cold ischaemia (CI lungs), or to 24 hr of CI with subsequent 30 min of WR (CI + WR lungs). Each lung was used for only one mitochondria isolation and preparation, but the same preparation may have been used for several mitochondrial oxygen consumption measurements. Mitochondria were also isolated after 48 hours of cold ischaemia from lungs flushed with EC.

Results

The results of this study were published in part in papers presented in addendum 2 and 3.

Physiological measurements. In this part of the study we demonstrated that the pulmonary flush with either EC or UW induced a significant increase in P Peak and a decrease in static compliance ($p<0.01$). Thirty min of warm reperfusion and ventilation after 24 hr of cold ischaemia promoted a progressive increase of P Peak and induced a significant decrease in static compliance in both groups ($p<0.05$). ANOVA revealed no statistically significant superiority of a preservation solution (EC, UW). Pulmonary vascular resistance data showed no statistically significant during reperfusion between lungs flushed with EC or UW, and no statistically significant variation was measured during the 30 min of this reperfusion. However, all the calculated pulmonary vascular resistance values were highly elevated. These results, with the high values of P peak and the low compliance, demonstrated that, in this model, the 24 hours of cold ischaemia and the 30 minutes of normothermic reperfusion induced a deep primary lung graft dysfunction.

Mitochondrial oxygen consumption: Mitochondria submitted to 24 hr of cold ischaemia developed significant decrease in V_3 and V_{FCCP}. This decrease in V_3 and V_{FCCP} may be explained by an alteration in the oxido-reductase activities of the mitochondrial respiratory chain, or by a decrease in the rate of substrate import by specific translocator. However, the yield of oxidative phosphorylation was conserved, as shown by the stable ADP/O ratio. Moreover, there was no uncoupling between ATP synthesis and oxygen consumption (no increase in V_4 and V_{Olig}) and no alteration in the mitochondrial membrane permeability (stable V_{Olig}). Relative to lungs submitted to 24 hr of cold ischaemia alone, lungs submitted to cold ischaemia and subsequent warm reperfusion with air ventilation underwent a significant worsening in the oxido-reductase activities of the mitochondrial respiratory chain, demonstrated by a further significant decrease in V_3.

The respiratory control was also decreased. Moreover, a significant ADP/O decrease demonstrated an alteration in the efficiency of oxidative phosphorylation. However, the mitochondria isolated from lungs submitted to cold ischaemia and subsequent warm reperfusion with air ventilation were not uncoupled (no increase in V_{Olig} and V_4). These results demonstrated that despite a fall in the oxidative phosphorylation efficiency, ATP synthesis was preserved in these mitochondria. In the comparison of EC and UW on mitochondrial respiratory function after cold ischaemia, and after cold ischaemia and subsequent reperfusion. The data demonstrated that there were no significant differences between lungs preserved with either EC or UW. After 48 hours of cold ischaemia, a more important reduction in V3 without change of VFCCP indicated a reduction in the availability of ADP or a less activity of the F1F0ATP synthase. If the origin of this phenomenon is the ADP, that can be only by one deterioration of its transportor, since the ADP is provided to the preparation in the same quantities. Moreover, after 48 hours, we observed a reduction in ADP/O, without increase in V4 or VOlig. That can be explained by the fact that the second day of cold ischaemia induced a deterioration of the F1F0ATP synthase with increase in the permeability of the F1F0ATP synthase to the protons (H+ slip), without anomaly of the permeability of the internal mitochondrial membrane. Thus 48 hours of cold ischaemia induced significant and multiple lesions of the mitochondrial respiratory chain.

Transmission electron microscopy. In control lungs flushed with either EC or UW, mitochondria did not demonstrate any evidence of morphological alteration. The mitochondrial outer membrane was intact, and the inner membrane presented numerous cristae. Twenty-four hours of cold ischaemia promoted mitochondrial swelling, while invaginations of the inner membrane were less structured. Thirty min of warm reperfusion with Krebs-Henseleit bicarbonated buffer solution and air ventilation promoted significant alterations of the mitochondria, as evidenced by electron dense matrix and loss of general structure. These lesions seem to be more severe in the EC lungs than in the UW lungs.

Discussion

This porcine model of pulmonary harvesting, ischaemia and reperfusion narrowly copies the techniques used in clinical pulmonary transplantation {666}. We chose to flush the lungs with EC or UW through the pulmonary artery after intravenous prostaglandin E1 injection. This method of preservation is simple, fast, and reproducible. We chose to

impose a 24 hours cold ischaemia to the lungs, incompatible with a correct function after reperfusion, in order to be certain development of a PLGD.

In this model, we quantified the pulmonary lesions by hemodynamic and aerodynamic measurements. These methods were validated by other teams {76, 159}. They have the advantage of estimating the pulmonary lesions in real time. In vitro rather in vivo reperfusion eliminates the variables such as compliance of the rib cage, the dynamics of the pleural cavity, or the postoperative pain induced in the receiver, variables that can modify the measured physiological parameters. On the other hand, ventilating the lungs in an active way, with a positive pressure produced by the respirator, could be responsible for an important variation of what could be met clinically after patient extubation since the positive respiratory pressure induced in the alveoles could limit to a certain extent the tissue oedema at reperfusion. However during the 30 minutes after reperfusion of the pulmonary graft, the patient remains always dependent on the respirator, this one inducing a positive pressure in the air routes of the graft, such as in our model of 30 minutes reperfusion.

The selected model also has the advantage of its simplicity. We reperfused the lungs with a Krebs-Henseleit bicarbonate buffer solution. This solution is frequently used in experimental reperfusion. It is however certain that a reperfusion with blood would have copied in a more faithful way the phenomena accompanying lung reperfusion during transplantation. Blood has the advantage of being a buffer solution, to contain free radicals scavengers limiting the lesions related to the radical formation at the time of the reperfusion, as well as macromolecules limiting the oedema development at reperfusion. On the other hand the use of this solution of Krebs-Henseleit bicarbonate eliminates some disadvantages of blood: in order to obtain a sufficient blood volume in open circuit, one or more "donor" animals would have being sacrificed. In a closed circuit with less circulating volume (as in our model), blood should be "deoxygenated". Moreover the use of this solution eliminates all the immunological factors accompanying the reperfusion. It also makes it possible to limit the presence of haemoglobin in the pulmonary parenchyma, haemoglobin interfering with measurements of mitochondrial function.

During pulmonary flush we observed a significant increase in the inspiratory pressure as well as a decrease in pulmonary compliance. It is well shown that EC solution, rich in potassium, and cooled at 4°C, induces smooth muscle contraction in the vascular and bronchial arborisation of the lungs {37}. That induces an increase in pulmonary vascular resistance at the time of the flush that makes necessary the prostaglandin

perfusion before the flush, under penalty of impossibility of correct rinsing and cooling of the organ. That also induces the modifications of the aerodynamic parameters that we observed at the time of this study. At the time of the reperfusion we observed an aggravation of the studied physiological parameters. Indeed, the reperfused lungs developed a progressive and significant increase in inspiratory pressures, and a reduction in static compliance. These modifications, added to very high vascular resistances, are the objective reflections of the PLGD {664,678}, also marked by tissue oedema observed after the reperfusion (but not objectified or measured at the time of this study).

In summary, we developed a simple and reproducible porcine model of pulmonary harvesting, ischaemia and reperfusion which makes it possible to objectify the development of a PLGD. However, if the measured mitochondrial lesions in this study are real and significant, especially after 48 hours of cold ischaemia or 24 hours of cold ischaemia and 30 minutes of normothermic reperfusion, it seems that these lesions cannot entirely explain the importance of the non-function of the pulmonary grafts. These mitochondrial lesions must be integrated among all the induced cellular alterations at the time of ischaemia-reperfusion {642}.

In this model, we could not observe significant difference between the lungs flushed with EC or UW, and this as well after cold ischaemia as after normothermic reperfusion. That could be secondary to the fact that there is no advantage of a solution on the other in pulmonary preservation. This explanation corroborates the current data of the literature, since there is no formal demonstration of the superiority of UW on EC by clinical, randomised, multicentric study. However our results could be related to the fact that our model of cold ischaemia of long duration (24 hours) induces too deep tissue lesions. A difference in quality of preservation between UW and EC would be perhaps better appreciated by a study of a cold ischaemia of shorter duration, 8 or 12 hours for example.

Chapter 4

Effects of normothermic ischaemia on the pulmonary mitochondrial function

Pulmonary transplantation became the treatment of choice of a great number of end stage pulmonary disease, but the number of pulmonary transplantations has remained insufficient and limited for a few years by the lack of available pulmonary grafts {665}. One estimates the need/offer ratio of pulmonary transplants at approximately 2/1 {661}. A way of increasing this number of pulmonary grafts could possibly be the use of in non-heart beating donors (NHBD) {659}. At present, the organ donors are patients whose brain died, but whose heart beats, of which organs other than the brain are perfused, and whose artificial ventilation allows blood oxygenation and thus the persistence of a correct function of the various organs. These quite precise conditions are rare and the donors are thus very few. Certain authors proposed to increase the number of organs available for transplantation by the use of donors whose heart would have stopped {698}. These conditions impose to the transplanted organ an additional normothermic ischaemia, which extends between the moment of the cardiac arrest and the moment when the organ is cooled and protected by the conservation solution. Four categories of NHBD were identified and defined at the time of a congress in Maastricht {627}. The patients of category 1 are patients of whom the number is most important, namely patients brutally deceased and who are not reanimated for often obvious reasons. For example these patients are victims of car accidents or had committed a successful suicide, who die on the spot and who generally are not led to the hospital but to the mortuary. Category 2 includes patients whose reanimation is tried but fails. Often these patients arrive intubated and ventilated at the emergencies of the hospitals, where the reanimation is continued during a certain time then stopped. Category 3 includes patients who die the intensive care unit after withdraw of any respiratory support, like the patients in extremely advanced coma, without hope of recovery, but who are not organ donors because all brain death criteria are not met. Category 4 includes a marginal number of patients in brain death and whose heart stops whereas they are planned for a donation of organs.

The NHBD are currently used successfully by several teams, mostly for the harvesting of renal grafts, which tolerate a relatively long normothermic ischaemia and

whose function can be temporarily replaced by dialysis in the event of tubular acute necrosis that is often reversible after transplantation. The use of NHBD increased the number of renal grafts of 35% in Maastricht, whose transplantation team is pioneer in this matter {698}. With regard to the other organs, the use of NHBD remains anecdotal but possible for the liver and the pancreas {660}. Many authors studied successfully the possibility of using pulmonary grafts from NHBD in animal models {223, 262, 589, 19, 584, 588, 586, 623, 641, 658, 585, 626, 694}. At least one lung was harvested from a NHBD and transplanted successfully {660}, and historically the first pulmonary transplantation was carried out with a graft taken from a NHBD, deceased of myocardial infarction {675}.

It can appear paradoxical to propose the use of lungs harvested from NHBD, since the lung is an organ very sensitive to the lesions appearing during the harvesting, the ischaemia and/or the reperfusion. For all the other organs used in transplantation, the period extending between cardiac arrest and cooling by the flush of a cold conservation solution is crucial, since the cellular metabolism continues in normothermy with quickly disappearing oxygen reserves (normothermic ischaemia). The lung is different from the other organs by the fact that, after cardiac arrest, it persists a direct contact between certain pulmonary cells and oxygen in ambient air on the level of the alveoles. This oxygen could allow the persistence of an aerobic metabolism after cardiac arrest and thus of a cellular homeostasis allowing the tolerance of a prolonged circulatory stop, even in normothermy. These characteristics make that the NHBD could be a source of pulmonary graft donors.

It is appeared interesting to us to study the function of oxidative phosphorylation of mitochondria isolated from NHBD close to what could occur in clinical harvesting, comparable to the category 3 donors.

Material and Method

Surgical Model: The pigs were premedicated, placed on a heating operating table, anesthetised and ventilated according to our model described above. After median sternotomy, the pulmonary artery and both venae cavae were isolated and encircled with a ligature. Both pleurae were opened, and both azygos veins were ligated and divided. Following systemic heparinisation (300 U/kg), 500 μg of prostaglandin E1 (Prostin, Upjohn, Puurs, Belgium) were injected intravenously. After heparinisation and prostaglandin E1 injection, the pigs were euthanasied with intravenous injection of 100 mg/kg of sodium pentobarbital. The ventilation was discontinued and the tracheal tube was disconnected from the respirator. The start of the normothermic or warm ischaemia (WI) period was defined by the onset of ventricular fibrillation. The pig core body temperature was maintained between 35 °C and 37°C with the heating operating table. After 30 or 45 min of WI, the ventilation was restarted. Both venae cavae were ligated, and the pulmonary artery was immediately cannulated by a high flow tubing through the right ventricular outflow tract, and the ligature was tied around the pulmonary artery

to secure the cannula. After opening of the left atrial appendage, the lungs were flushed by gravity at 50 cm H_2O with 2 L of cold (4°C) standard Euro-Collins solution (EC) (Fresenius AG, Wilrijk, Belgium). The ventilation was continued during the flush, and topical pulmonary cooling was facilitated by the flow of the effluent in both pleura. Upon completion of the flush, the ventilation was discontinued, and the heart-lung block was excised. After one final mechanical ventilation, the trachea was clamped at end-inspiration and ligated. The heart-lung block was then stored inflated in cold EC.

Mitochondrial function: The mitochondria were isolated from the lungs immediately after the harvesting (30 min or 45 min WI), or after 24 hr or 48 hr of CI (combination of 30 or 45 min of WI and CI). The mitochondrial respiratory parameters were measured in the same way that for the mitochondria isolated after cold ischaemia alone and/or reperfusion (Chapter 3), and the results of the different groups were compared.

Results

The results of this study were published in the papers presented in addendum 3 and 4.

Compared to the mitochondria of the control group, 30 minutes of normothermic ischaemia did not induce significant modification of the mitochondrial function. After 45 minutes, the mitochondria presented a significant reduction in V3 ($p<0,005$), CR and ADP/O ($p<0,05$). On the other hand, VFCCP, V4, VOlig and CRD were not modified by 45 minutes of warm ischaemia. The reduction in CR ($p<0,05$) was caused by a reduction in V3 rather than a modification of V4. Compared to 24 or 48 hours of cold ischaemia alone, the combination of 30 minutes of normothermic ischaemia and of 24 or 48 hours of cold ischaemia did not deteriorate the function of mitochondrial OxPhos, except for an improvement of VFCCP ($p<0,05$) after 24 hours of cold ischaemia and of ADP/O ratio after 48 hours.

Discussion

In this study, we measured the evolution of the mitochondrial pulmonary phosphorylation function after 30 or 45 minutes of warm ischaemia, or rather of arrest of pulmonary blood circulation under conditions of normothermy. Thus, 30 minutes of warm ischaemia did not induce significant mitochondrial alterations. After 45 minutes of warm ischaemia, mitochondrial oxidative phosphorylation dysfunction appears, related to a deterioration of the ATP synthase function. The absence of modification of VFCCP indicates that there is no reduction in the function of the oxido-reductases or availability of substrate. Moreover, the addition of 24 hours cold ischaemia after 30 or 45 minutes warm ischaemia does not only worsen the mitochondrial function compared to cold ischaemia alone, but could even have a moderated protective effect. These results could be a biological and metabolic support to preconditioning.

This study shows that mitochondria isolated from lungs after 30 minutes of warm ischaemia do not appear to present of deterioration of their OxPhos function, by comparison with control mitochondria. After 45 minutes of warm ischaemia however, the mitochondria are significantly faded. Moreover combination of 30 minutes of warm ischaemia and 24 or 48 hours cold ischaemia does not seem to induce mitochondrial lesions more severe than that induced by a cold ischaemia alone. These results can be important for the clinical use of pulmonary grafts harvested from NHBD. To date the principal limiting factor to pulmonary transplantation remains the low number of organ donors whose lungs are usable for transplantation. The use of NHBD would increase in a very significant way the number of available organs, like that was proven in renal transplantation {699}. The NHBD were proposed as lung donors, and experimental results seem to indicate that lung transplantation after one limited period of warm ischaemia is possible {694}. The results of this study suggest that the lungs subjected to 45 minutes of warm ischaemia do not seem suitable for transplantation, from the bioenergetic point of view at least. On the other hand after 30 minutes of warm ischaemia pulmonary mitochondria seem to keep a normal function, comparable with controls. Our model can be to compare with Category 3 defined by Kootstra and al {627}, namely the donors dying in intensive care units by stop of support, also called " controlled NHBD ". In this category, the duration of warm ischaemia can be limited to 30 minutes by a fast surgery, making these lungs usable for transplantation. However our model does not completely copy category 3 of the NHBD, since we chose to inject heparin and prostaglandins before cardiac arrest, which could not be ethically justified in NHBD. In these donors indeed, it is ethically impossible to administrate any medication with protective aiming of the organs before brain death, defined generally by 10 minutes of cardiac arrest. We chose to inject these medications in general circulation before inducing the heart failure to compare under the same experimental conditions the effects of warm and cold ischaemia on mitochondrial OxPhos. However as heparin and the prostaglandins could theoretically have an effect (positive or negative) on the mitochondrial function, they were thus injected before the circulatory stop under the various experimental conditions.

This study tends to prove that the lungs seem to be less sensitive to normothermic ischaemia than other organs. This difference can partly be explained by the presence of air (and thus of oxygen) in the pulmonary airways, allowing a certain degree of aerobic metabolism after heart arrest. The safeguarding of this metabolism can allow the conservation of the mitochondrial functions, at least partly. The presence of ATP and its persistence in pulmonary tissue after ischaemia could be the consequence of this residual

aerobic metabolism, and to be partly responsible for the relative good tolerance of the lungs to warm ischaemia. This presence of ATP was related to the presence of oxygen in the airways {587,588}, and the cellular viability and the integrity of the membranes were correlated with ATP. In our model the persistence of air, and thus of oxygen in the airways, seem to allow a conservation of the mitochondrial function during 30 minutes. In a model of pulmonary normothermic ischaemia in the rabbit, the pulmonary tolerance to ischaemia was limited to 1 hour, the lung being preserved without inflation {694}. This tolerance to normothermic ischaemia could also be improved by pulmonary ventilation after the cardiac arrest, or by the conservation of the lungs in inflation {697,696}.

Another explanation of the results of this study could be a particular resistance of the mitochondria pulmonary, which will be studied in a model of study of the effect of the ischaemia of the mitochondria in vitro, after isolation.

In conclusion, in this model, 30 minutes of warm ischaemia does not deteriorate the function of OxPhos of the pulmonary mitochondria. On the other hand 45 minutes seem to cause severe mitochondrial lesions. This 30 minutes tolerance to normothermic ischaemia could at least allow the use of the pulmonary grafts harvesting from NHBD, from the bioenergetic point of view.

Chapter 5

Summary and Perspectives

The lungs are organs whose sensitivity to ischaemia and reperfusion is well known. In a rabbit model of lung ischaemia, we showed that the cold ischaemia longer than 6 hours is accompanied by a significant reduction in tissue contents in vitamins E and C, two important protectors against the lesions appearing at the time of ischaemia and the reperfusion {708}. Moreover, lungs are different from the other transplanted organs by the importance of a fragile structure, the alveole, zone of exchange between the alveolar air and the capillary blood. It was shown that among the alveolar cells, the type II pneumatocytes, secreting the surfactant, is of primary importance for the post-transplant function. It was shown that the quality of surfactant decreases after conservation and reperfusion of the lungs, and that, in vitro, the effectiveness of surfactant continuously decreases with the prolongation of the duration of ischaemia {321}. Moreover the administration of surfactant before the pulmonary reperfusion improves the postoperative function of the grafts in the rat {230}. It is known that, if all the pulmonary cells contain mitochondria, more than 50% of the mitochondria isolated from lungs come from type II pneumatocytes {683}. We chose to study the mitochondrial respiratory function of these important alveolar cells for the pulmonary function after ischaemia and reperfusion.

In this work, we developed a porcine model of ischaemia (hypo- and/or normothermic) and of normothermic reperfusion. This reperfusion was accompanied by a postoperative non-function, objectified by aerodynamic and hemodynamic parameters, as by the appearance of pulmonary oedema. This non-function was observed after 24 hours a hypothermic ischaemia, which is incompatible with a normal function of the pulmonary grafts in clinical transplantation. The reperfusion with a solution of Krebs-Henseleit bicarbonate remove any immunological artefact and any influence of the circulating blood cells in the pulmonary lesions appearing at the time of the reperfusion. On the other hand, this solution is different from blood to a significant degree, by not containing protectors against the production of free radicals at reperfusion. It is thus possible that our model exacerbates this production of free radicals, more especially as the lung is a tissue particularly rich in polymorphonuclear cells.

In this model, we could show that after 24 hours of cold ischaemia the mitochondria underwent a moderate deterioration of the oxidoreductases but without decrease in the

effectiveness of oxidative phosphorylation, decrease that could be demonstrated after 48 hours of cold ischaemia. These lesions are comparable to the mitochondrial lesions that we had observed after cold ischaemia of rabbit kidney {646}. After reperfusion, the mitochondrial lesions are more severe, with a decrease in the effectiveness of oxidative phosphorylation. Concerning normothermic ischaemia, the first 30 minutes did not cause significant mitochondrial lesions. These results at least corroborate the literature data on the relative good pulmonary tolerance to normothermic ischaemia, explained in theory by the persistence of oxygen in the airways and thus of the persistence of aerobic metabolism in spite of the circulatory arrest. For the other organs, the circulatory arrest implies anoxia, that is not really the case of lung, as oxygen is present in airways. Pulmonary transplantation could thus profit from an increase in graft pool available by harvesting lung graft from non-heart beating donors. After 45 minutes of normothermic ischaemia, mitochondrial oxidative phosphorylation dysfunction appears, related to a significant deterioration in the ATP synthase function. These results confirm that the cellular metabolism is then disturbed by the appearance of cellular anoxia because of the progressive consumption of oxygen present in the alveoles, or of the substrates necessary to the cellular metabolism.

The description and the discussion of the importance of these mitochondrial alterations in the genesis of lung graft dysfunction after transplantation must be integrated with the very broad framework of the disturbances appearing at the time of tissue ischaemia and reperfusion. From our study it comes out that hypothermia at 4°C protects effectively the pulmonary mitochondrial function since significant deteriorations do not appear before 24 hours of hypothermic ischaemia. To determine if mitochondrial deteriorations appearing after normothermic reperfusion are the cause or the consequence of the non-function of the lung appears difficult. On the other hand the lesions appearing after 45 minutes of normothermic ischaemia deserve in an unquestionable way a later study, with evaluation of the mitochondrial function after circulatory arrest in normothermy (30 and 45 minutes) and normothermic reperfusion, and with evaluation of the mitochondrial function after 30 and 45 minutes (or more) of circulatory arrest normothermic but continuation of pulmonary ventilation, or conservation of the lungs in inflation with air or pure oxygen. Our mitochondrial data should also be compared with a study of the pulmonary function by a model of transplantation with survival of the receiver pig.

Addenda

Addendum 1:

"Antioxidant status after cold ischemia of rabbit lung."

PINCEMAIL J, KOHL P, DETRY O., LAMBERMONT B, MEURISSE M, LIMET R, DEFRAIGNE JO

Transplant Proc 2000; 32: 484-485.

Addendum 2:

"Comparative effects of University of Wisconsin and Euro-Collins solutions on pulmonary mitochondrial function after ischemia and reperfusion."

DETRY O., WILLET K, LAMBERMONT B, MEURISSE M, PINCEMAIL J, SERTEYN D, LAMY M, DEFRAIGNE JO, LIMET R, SLUSE FE

Transplantation 1998; 65, 161-166.

Addendum 3:

"Effects of cold and warm ischemia on the mitochondrial oxidative phosphorylation of swine lungs."

WILLET K, DETRY O., LAMBERMONT B, MEURISSE M, DEFRAIGNE JO, SLUSE-GOFFART C, SLUSE FE

Transplantation 2000; 69: 582-587.

Addendum 4:

"Consequences of cold and warm ischemia on pulmonary mitochondrial respiratory function."

DETRY O., WILLET K, LAMBERMONT B, DEFRAIGNE JO, MEURISSE M, LIMET R, SLUSE F

Transplant Proc 1997; 29: 2338-2339.

Addendum 5:

"Conservation pulmonaire et donneurs à cœur arrêté
DETRY O., WILLET K, MEURISSE M, SLUSE FE, LIMET R, DEFRAIGNE JO
In : Les Journées de la Pitié 2000, Pavie Eds, Paris. (In Press)