COMPARATIVE EFFECTS OF UNIVERSITY OF WISCONSIN AND EURO-COLLINS SOLUTIONS ON PULMONARY MITOCHONDRIAL FUNCTION AFTER ISCHEMIA AND REPERFUSION

OLIVIER DETRY MD,[3,6] KATTY WILLET,[2,4] BERNARD LAMBERMONT MD,[3,6]
MICHEL MEURISSE MD, PhD,[3] JOËL PINCEMAIL, PhD,[3,6] DIDIER SERTEYN VD, PhD,[7] MAURICE
LAMY MD, PhD,[5] JEAN-OLIVIER DEFRAIGNE MD, PhD,[3,6]
RAYMOND LIMET MD, PhD,[3] AND FRANCIS E. SLUSE PhD[4]

Department of Surgery and Transplantation,Universitary Hospital of Liège,
Sart-Tilman B35, B-4000 Liège, Belgium;
and Department of Bioenergetics, Institute of Chemistry,
Sart-Tilman B6C, B-4000 Liège, Belgium

Mailing Adress:
Olivier Detry, MD,
Department of Transplantation
CHU Sart-Tilman B35
B-4000 Liège, Belgium.

Footnotes:

[1] This work was supported by a F.N.R.S. grant N° 3.4545.94

[2] K W was granted by a FRIA fellowship.

[3] Department of Transplantation and Surgery, CHU Sart-Tilman B35, B-4000 Liège, Belgium.

[4] Department of Bioenergetics, Sart-Tilman B6, B-4000 Liège, Belgium

[5] Department of Anesthesiology, CHU Sart-Tilman B35, B-4000 Liège, Belgium

[6] Centre de Recherche du Département de Chirurgie, CHU Sart-Tilman B35, B-4000 Liège, Belgium.

[7] Veterinary School of Medicine, Sart-Tilman B41, B-4000 Liège, Belgium

[8] Address correspondence to: Dr. Olivier Detry, Department of Tranplantation, CHU Sart-Tilman B35, B-4000 Liège, Belgium.

Abbreviations:

ADP, adenosine diphosphate;

ANOVA, analysis of variance;

ATP, adenosine triphosphate;

CI, cold ischemia;

EC, Euro-Collins solution;

PA, pulmonary artery;

PLGD, primary lung graft dysfunction;

RC, respiratory control;

URC, uncoupled respiratory control;

UW, University of Wisconsin solution;

WR, warm reperfusion;

Abstract

Background: The aim of this study was to compare the effects of Euro-Collins and University of Wisconsin solutions on pulmonary mitochondrial function after cold ischemia and subsequent warm reperfusion.

Methods: Seventeen pigs underwent lung harvesting after classical lung flush with either University of Wisconsin or Euro-Collins solutions. The mitochondria were isolated from fresh swine lungs, from swine lungs submitted to 24 hr of cold ischemia, and 24 hr of ischemia followed by 30 min of subsequent *ex vivo* reperfusion at 37°C with Krebs-Henseleit buffer solution and air ventilation. Mitochondrial oxidative phosphorylation parameters were determined in isolated mitochondria by *in vitro* measurement of oxygen consumption rates. During reperfusion, the lung function was assessed by the pulmonary aerodynamic parameters and the pulmonary vascular resistance.

Results: Relative to controls, mitochondria submitted to cold ischemia showed an alteration in the oxido-reductase activities of the respiratory chain. However, the yield of oxidative phosphorylation was conserved. After reperfusion, pulmonary mitochondria underwent a significant worsening in the oxido-reductase activities of the respiratory chain, and a decrease in the respiratory control and the efficiency of oxidative phosphorylation. Meanwhile, the reperfused lungs showed evidence of early dysfunction, assessed by the aerodynamic parameters and pulmonary vascular resistance. In this model, there was no advantage of University of Wisconsin solution over Euro-Collins solution.

Conclusions: The mild mitochondrial alterations after cold ischemia were not sufficient to explain the limited tolerance of lung to ischemia. After reperfusion, the mitochondrial damage was more severe and could be involved in the postransplant lung dysfunction.

In recent years, pulmonary transplantation has become the treatment of choice for several end-stage lung diseases (1). However, pulmonary transplantation is still limited by the scarcity of suitable donors and the lack of a reliable prolonged method of lung graft preservation (2). Primary lung graft dysfunction (PLGD) occurs in 10% to 20% of lung transplant recipients (3), and may complicate the posttransplant course even after short ischemia duration. Clinically, PLGD results in progressive deterioration of pulmonary compliance, gas exchange, and pulmonary vascular resistance, coinciding with an increase in extravascular lung water (4). Although PLGD may be reversible, it significantly increases the pulmonary transplantation morbidity and mortality (1). The cause of PGLD is unclear, but in part PLGD may be due to lung graft injuries before, during, and after transplantation (3). Many studies investigated alternative methods of lung graft conservation, and reported some improvement of lung conservation in animal models. Despite these studies, the cellular mechanisms of damage to pulmonary cell structure and metabolism during the peritransplant period are still poorly understood. Ischemia promotes intracellular depletion of high-energy compounds. Moreover, ischemia and subsequent reperfusion may be associated with loss of mitochondrial functions and characteristics. Mitochondrial dysfunction may contribute to cell death by failure of oxidative phosphorylation with loss of mitochondrial adenosine triphosphate (ATP) synthesis leading to cellular ATP depletion (5), or by a permeability transition pore of the inner mitochondrial membrane, leading to cell death independently of cellular ATP depletion (6,7). However, the consequences of ischemia and reperfusion on pulmonary mitochondrial respiratory functions have never been investigated, and the role of mitochondria dysfunction in PLGD is not known.

The aim of the present study was to compare the effects of Euro-Collins (EC) or University of Wisconsin (UW) solutions on pulmonary mitochondrial function after cold ischemia (CI) and subsequent warm reperfusion (WR). We assessed the efficacy of the oxidative

phosphorylation of mitochondria which were isolated from fresh swine lungs, from swine lungs submitted to 24 hr of CI, and 24 hr of CI followed by 30 min of subsequent *ex vivo* reperfusion at 37°C. In order to compare the alterations of the pulmonary mitochondria and the quality of lung conservation, we evaluated the development of PGLD by monitoring the evolution of the pulmonary aerodynamic parameters and the pulmonary vascular resistance during the reperfusion.

MATERIAL AND METHODS

All animals received care in accordance with the "Guide for the Care and Use of Laboratories Animals" prepared by the National Academy of Sciences and published by the National Institute of Health (NIH Publication No. 85-23, revised 1985). The protocol for the study was approved by the Animal Care Committee at the University of Liège, Belgium.

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, Brussels, Belgium). Anesthesia 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 tracheostomy, 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 15 ml/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. Anesthesia 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 heparinization (300 U/kg), 500 µg of prostaglandin E1 (Prostin, Upjohn, 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.

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, 8 ml/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 mmole/L NaCl, 4.7 mmole/L KCl, 1.2 mmole/L $MgSO_4$, 1.2 mmole/L NaH_2P0_4, 25 mmole/L $NaHCO_3$, 2.5 mmole/L $CaCl_2$, 11.1 mmole/L 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 homogenized with a motor-driven hand-held homogenizer in the presence of SET solution (250 mmole/L sucrose, 2 mmole/L ethylene diamine tetracetic acid (EDTA), 5 mmole/L Trishydroxymethyl aminoethane, 0.5% fatty acid free bovine serum albumin (BSA)). After homogenization and filtration, the mitochondria were isolated from the solution with a standard techniques of differential centrifugations described elsewhere (8).

Mitochondrial volume measurement. Fifty microliters of mitochondrial suspension were incubated during 90 seconds with 450 μl of medium ((15 mmole/L KCl, 2 mmole/L EDTA, 5 mmole/L $MgCl_2$, 50 mmole/L Trishydroxymethyl aminoethane, 3.3 μCi tritiated water (^3H_2O)). The incubation mixture was centrifuged in a microcentrifuge (Model 3200, Eppendorf; Hamburg, Germany) during 3 min. The supernatant was decanted and acidified with $HClO_4$ (final concentration 0.7 mole/L). The mitochondrial pellet was acidified with $HClO_4$ 0.7 mole/L. The radioactivities of the mitochondrial pellets and of the supernatant solutions were determined in a liquid scintillation medium (Ready protein+; Beckman, Fullerton, CA). The mitochondrial volume (volume of the mitochondrial pellet) was calculated according to the formula:

Pellet volume (μl) = (pellet radioactivity / supernatant radioactivity) x supernatant volume (μl).

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 mmole/L KCl, 2 mmole/L EDTA, 5 mmole/L $MgCl_2$, 50 mmole/L Trishydroxymethyl aminoethane), in the presence of 1% BSA, with 5 mmole/L ketoglutarate and 5 mmole/L pyruvate as oxidizable substrates, and 2.5 mmole/L phosphate (KH_2PO_4). The concentration of added adenosine diphosphate (ADP), oligomycin and uncoupler carbonylcyanide-p-trifluoro-methoxyphenylhydrazone (FCCP) was 165 µmole/L, 16 µg/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 atom gram of oxygen consumed (ADP/O) was also determined.

Chemicals. Ketoglutarate, pyruvate, BSA and ADP were purchased from Boehringher Mannheim (Mannheim, Germany), oligomycin from Sigma (St. Louis, MO), FCCP from Du Pont de Nemours (Wilmington, DE), and ^3H_2O from Radio Chemical Center (Amersham, England). In the absence of specific indications, the other chemicals used in this study were purchased from Merck (Darmstadt, Germany).

Experimental design. The lungs were randomly flushed with EC (9 pigs) or UW solutions (8 pigs) (Table 1). 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 ischemia (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. Seven control lungs flushed with EC and 5 flushed with UW were used for mitochondrial isolation, respectively (Table 1). Five CI + WR lungs of each group were reperfused; the physiological parameters were recorded for each of these lungs. For technical reasons, one lung from each UW group had to be discarded for subsequent mitochondrial isolation (Table 1).

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.

Statistics. All data are presented as mean ± SEM. Two-way analysis of variance (ANOVA) design and corresponding F-tests were used to analyse the physiological measurement data. One-way ANOVA design and corresponding F-tests were used to analyse the mitochondrial oxygen comsumption rate data. Student t-test was used for comparison between two means.

Results of the tests are expressed by their *p*-value, and *p*-values < 0.05 were taken to be statistically significant.

RESULTS

Physiological measurements. Peak airway pressure data are represented graphically in Figure 2. The pulmonary flush with either EC (n=9) or UW (n=8) induced a significant increase in P Peak ($p<0.01$). Thirty min of WR and ventilation promoted a progressive increase of P Peak in both groups ($p<0.05$, n=5, P Peak 5 min vs. 30 min). ANOVA revealed no statistically significant effect of the kind of preservation solution (EC, UW) on the peak airway pressure ($p=0.1$). The results of the pulmonary static compliance measurements are presented in the Figure 3. The basal static compliance of the EC or UW lungs was not different ($p=0.7$). The pulmonary flush with either EC (n=9) or UW (n=8) induced a highly significant decrease in static compliance ($p<0.001$); this decrease in compliance was more severe in the lungs flushed with EC than UW ($p<0.01$). The WR and ventilation induced a significant decrease in static compliance in lungs flushed with either EC or UW ($p<0.01$, n=5, C stat 5 min vs. 30 min). During this reperfusion, the difference in static compliance between the EC and UW groups was not significant at any time point ($p>0.05$). Pulmonary vascular resistance data are presented in Figure 4. There was no statistically significant difference in pulmonary vascular resistance during reperfusion between lungs flushed with EC or UW (n=5), and no statistically significant variation was measured during the 30 min of this reperfusion (n=5). However, all the calculated pulmonary vascular resistance values were highly elevated.

Mitochondrial oxygen consumption. The mitochondrial oxygen consumption rate data are presented in Table 2. In control lungs flushed with UW, V_4 was significantly higher ($p=0.01$) and ADP/O was significantly lower ($p<0.01$) than in control lungs flushed with EC. The RC ratio was then significantly lower in the UW control lungs than in the EC control lungs ($p<0.01$). Relative

to control lungs, 24 hr of CI induced a significant decrease in V_{FCCP} (p<0.05) in lungs flushed with either EC or UW. As in control lungs, the differences in V_4, RC, and ADP/O between CI lungs flushed with EC or UW were significant after 24 hr of CI. Relative to 24 hr of CI, 30 min of WR and air ventilation after 24 hr of CI promoted a significant decrease in V3, RC ratio and ADP/O in lungs flushed with either EC or UW (p<0.01). After 24 hr of CI and 30 min of WR and air ventilation, the mitochondrial oxygen consumption rates of lungs flushed with EC or UW were not significantly different.

Transmission electron microscopy. In control lungs flushed with either EC or UW, mitochondria did not demonstrate any evidence of morphological alteration (Figure 5A and 6A). The mitochondrial outer membrane was intact, and the inner membrane presented numerous cristae. Twenty-four hours of CI promoted mitochondrial swelling, while invaginations of the inner membrane were less structured (Figure 5B and 6B). Thirty min of WR 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 (Figure 5C and 6C). These lesions seemed to be more severe in the EC lungs than in the UW lungs.

DISCUSSION

This study demonstrated that significant changes of oxidative phosphorylation occur in the pulmonary mitochondria submitted to CI, and that these alterations are exacerbated by subsequent WR with air ventilation. The lungs submitted to 30 min of WR with air ventilation after 24 hr of CI developed evidence of PLGD, assessed by elevated pulmonary artery vascular resistance, elevated peak airway pressure and decreased static compliance. In our model of *ex vivo* WR after 24 hr of CI, there was no significant advantage of UW solution over EC, regarding the protection of the mitochondrial respiratory function, and some physiological parameters including static compliance and pulmonary artery vascular resistance.

In our study, mitochondria submitted to 24 hr of CI 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}). These alterations in pulmonary mitochondrial respiratory function after CI were similar to the modifications that we observed in mitochondria isolated from rabbit kidney after CI (9). However, these results were different from the previously reported effects of CI on liver mitochondria (10), in which CI induced an increase in V_{Olig} and no modification in V_3. The mechanisms responsible for this different mitochondrial alteration in different organs remain unclear. In contrast to liver and other solid organs, deep anoxia or hypoxia may not occur during lung CI because the presence of oxygen in the airway during lung ischemia could allow slow cellular metabolism. This conservation of lung cellular metabolism during cold preservation could preserve the integrity of the cellular membranes, and the mitochondrial functions. In a rabbit model of lung preservation, there was no decrease in ATP content in lungs preserved at 4°C during 24 hr (11). The maintenance of ATP levels in the ischemic lungs may be the consequence of preserved cellular metabolism in ischemic lungs, and has been strongly related to maintenance of cell viability and membrane integrity, assessed by the trypan blue exclusion method (12).

Relative to lungs submitted to 24 hr of CI alone, lungs submitted to CI and subsequent WR 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 CI and subsequent WR 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 contrast to the preservation of pulmonary mitochondrial metabolism after 24 hr of cold preservation, the reperfused lungs showed evidence of immediate significant PLGD (after 5 min reperfusion), demonstrated by elevated pulmonary vascular resistance, elevated peak airway pressure and decreased static compliance. This discrepancy between significant PLGD and relatively preserved mitochondrial functions after 24 hr of CI suggests that the mitochondrial damage may not seem to be responsible for this PGLD. However, after 30 min of WR, the worsening of pulmonary function (pulmonary vascular resistance, peak airway pressure and static compliance) was correlated to the appearance of damage at the level of mitochondrial function.

In this study, we compared the effects of two preservation solutions, EC and UW, on mitochondrial respiratory function after CI, and after CI and subsequent reperfusion. The data demonstrated that there were no significant differences between lungs preserved with either EC or UW, regarding the mitochondrial function after 24 hr of CI with subsequent WR with air ventilation. To date, EC remains the standard lung preservation solution in clinical settings, but its efficacy is limited to 6 hours of preservation. UW proved its efficacy in intra-abdominal organs preservation (13), and is actually used by some groups as a lung preservation solution (14). However, despite some experimental and clinical reports of the UW efficacy in lung transplantation (14-17), the superiority of UW over EC in lung graft preservation has not yet been proved. In our model of swine lung CI and WR, the lungs flushed with either EC or UW showed evidence of PGLD. The PGLD intensity was similar in both groups, as demonstrated by similar

alterations of static compliance or pulmonary artery vascular resistance. However, in our model, the long CI period may be very deleterious for the lungs. This long CI may mask the superiority of one of these solutions for a shorter conservation period.

Additional findings of our study were the differences in respiratory parameters between mitochondria isolated from lungs flushed with EC or UW. Mitochondria isolated from control lungs or lungs submitted to 24 hr of CI alone after flush with EC, showed lower V_4 and higher ADP/O, compared to mitochondria isolated from lungs flushed with UW. The consequence of this lower V_4 was a higher RC ratio in the EC lungs. The differences between EC and UW disappeared after 30 min of WR. The mechanism and the significance of these findings remain unclear. The ADP/O decrease may be interpreted as an alteration in the efficiency of oxidative phosphorylation in mitochondria isolated from lungs flushed with UW, compared to lungs flushed with EC. However, these data may not suggest a better cold preservation of mitochondrial function in lungs flushed with EC because this difference is already significant between the control lungs. These data should be confirmed by future *in vitro* experiments, studying the effects of UW and EC on mitochondria from isolated pulmonary cells.

In conclusion, we applied a model of mitochondrial isolation and purification from swine lungs to determine the consequences of CI and WR on pulmonary mitochondrial respiratory functions. Pulmonary CI and WR with air ventilation induce significant alterations of mitochondrial respiratory function. However, the mild mitochondrial alterations after 24 hr of CI were not sufficient to explain the limited tolerance of lung graft to ischemia and the occurrence of PGLD after lung transplantation. After subsequent 30 min WR with air ventilation, the mitochondrial damage was more severe and could be involved in the PGLD. In our model, the UW solution showed no significant advantage over EC, regarding the preservation of these mitochondrial functions.

The authors greatly thank Mrs. C. Dessy-Doize and Mr. J. F. Bradfer, of the Electronic Microscopy Unit, Veterinary Department, University of Liège, for the transmission electron microscopy analysis, Mr. Paul Gérard, from the Department of Statistics, University of Liège, for his help in the data analysis, and Mrs. Jody Margulies MD PhD, for her kind review of the manuscript.

REFERENCES

1. Hosenpud JD, Novick RJ, Bennet LE, Keck BM, Fiol B, Daily OP. The registry of the International Society for Heart and Lung Transplantation: thirteen official report - 1996. J Heart Lung Transplant 1996; 15: 655.

2. Kirk AJ, Colquhoun IW, Dark JH. Lung preservation: a review of current practice and future directions. Ann Thorac Surg 1993; 56: 990.

3. Novick RJ, Gehman KE, Ali IS, Lee J. Lung preservation: the importance of endothelial and alveolar type II cell integrity. Ann Thorac Surg 1996; 62: 302.

4. Cooper JD, Vreim CE. Biology of lung preservation for transplantation. Am Rev Respir Dis 1992; 146: 803.

5. Aw TK, Andersson BS, Jones DP. Suppression of mitochondrial respiratory function after short-term anoxia. Am J Physiol 1987; 252: C362.

6. Bernardi P, Vassanelli S, Veronese P, Colonna R, Szabo I, Zoratti M. Modulation of the mitochondrial permeability transition pore. J Biol Chem 1992; 267: 2934.

7. Rosser BG, Gores GJ. Liver cell necrosis: cellular mechanisms and clinical implications. Gastroenterology 1995; 108: 252.

8. Labow RS, Meek E, Waghray G. Use of the University of Wisconsin solution for the preservation of cell organelle activities in rat heart and lungs. J Heart Lung Transplant 1995; 14: 66.

9. Willet K, Vaz de Macedo D, Detry O, Evens A, Pereira da Silva L, Sluse FE. Mitochondrial oxidative phosphorylation injuries occurring in situ and in vitro. Transplant Proc 1995; 27: 2827.

10. Kim S, Belzer FO, Southard JH. Loss of mitochondrial respiratory function and its suppression during cold ischemic preservation of rats livers with University of Wisconsin Solution. Hepatology 1992; 16: 742.

11. Van Raemdonck DE, Jannis NC, Rega FR, Flameng WL, Lerut TE. Delay of adenosine triphosphate depletion and hypoxanthine formation in rabbit lung after death. Ann Thorac Surg 1996; 62: 233.

12. D'Armini AM, Tom EJ, Roberts CS, Henke DC, Lemasters JJ, Egan TM. When does the lung die? time course of high energy phosphate depletion and relationship to lung viability after "death". J Surg Res 1995; 59: 468.

13. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. Transplantation 1988; 45: 673.

14. Love RB, Lopukhin SY, Welter DL, et al. Extended human lung preservation using UW solution. J Heart Lung Transplant 1997; 16: 113.

15. Aeba R, Keenan RJ, Hardesty RL, Yousem SA, Hamamoto I, Griffith BP. University of Wisconsin solution for pulmonary preservation in a rat transplant model. Ann Thorac Surg 1992; 53: 240.

16. Rinaldi M, Martinelli L, Volpato G, et al. University of Wisconsin solution provides better preservation in human lung transplantation. Transplant Proc 1995; 27: 2869.

17. Hardesty RL, Aeba R, Armitage JM, Kormos RL, Griffith BP. A clinical trial of University of Wisconsin solution for pulmonary preservation. Journal of Thoracic & Cardiovascular Surgery 1993; 105: 660.

LEGENDS

FIGURE 1. Schematic diagram of the *ex vivo* reperfusion circuit. Tv, Tidal volume; RR, respiratory rate; EEP, end-expiratory pressure; C stat, static compliance; P Peak, peak airway pressure; PAP, pulmonary artery pressure.

FIGURE 2. Peak airway pressure during the flush and the warm reperfusion. Data points represent the mean ± SEM. *, $p<0.01$ vs. basal value of the same group; +, $p<0.05$ vs. 5 min reperfusion value of the same group. CI, cold ischemia; EC, Euro-Collins solution; P Peak, peak airway pressure; UW, University of Wisconsin solution.

FIGURE 3. Static compliance during the flush and the warm reperfusion. Data points represent the mean ± SEM. *, $p<0.001$ vs. basal value of the same group; †, $p<0.01$ C stat flush EC vs. C stat flush UW; +, $p<0.01$ vs. 5 min reperfusion value of the same group. CI, cold ischemia; C stat, static compliance; EC, Euro-Collins solution; UW, University of Wisconsin solution.

FIGURE 4. Pulmonary vascular resistance during the reperfusion. Data points represent the mean ± SEM. EC, Euro-Collins solution; PVR, pulmonary vascular resistance; UW, University of Wisconsin solution.

FIGURE 5. Transmission electron micrographs of mitochondria from lungs flushed with Euro-Collins solution; (a) control lungs; (b) lungs after 24 hr of cold ischemia; (c) lungs after 24 hr of cold ischemia and 30 min of warm reperfusion with air ventilation. (magnification x 20,000).

FIGURE 6. Transmission electron micrographs of mitochondria from lungs flushed with University of Wisconsin solution; (a) control lungs; (b) lungs after 24 hr of cold ischemia; (c) lungs after 24 hr of cold ischemia and 30 min of warm reperfusion with air ventilation. (magnification x 20,000).

TABLE 1. Experimental design.

Abbreviations used in table: EC, Euro-Collins solution; UW, University of Wisconsin solution; CI, cold ischemia; WR, warm reperfusion.

TABLE 2. Mitochondrial oxidative phosphorylation data[a]

[a]Data are expressed as mean ± SEM.

[b]: V (nmole $O_2.min^{-1}.\mu l^{-1}$) = oxygen consumption, in nmole per min and per µl of mitochondria.

Abbreviations used in table: N, number of lungs used for mitochondrial preparation; n, number of measurements of mitochondrial function in the group; EC, Euro-Collins solution; UW, University of Wisconsin solution; CI, cold ischemia; WR, warm reperfusion; RC, respiratory-control ratio; URC, uncoupled respiratory-control ratio; ADP/O: number of ADP phosphorylated moles per atom gram of oxygen consumed;

†: $p<0.05$, vs. EC group at the same time point.

‡: $p<0.05$, vs. the control group, flushed with the same solution.

§: $p<0.05$,vs. CI group, flushed with the same solution.