EURO-COLLINS SOLUTION EXACERBATES LUNG INJURY IN THE SETTING OF HIGH-FLOW REPERFUSION

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Single-lung transplantation has been abandoned for the treatment of pulmonary hypertension by many centers because of overperfusion of the graft following implantation. Euro-Collins solution is currently used for lung preservation despite the vasoconstrictive effect of this intracellular-type solution. We hypothesized that high-flow reperfusion, alone or in combination with Euro-Collins-induced vasoconstriction, may cause lung dysfunction. Twenty-eight New Zealand White rabbit lungs were harvested and studied in an isolated, blood-perfused model of lung function after 4 hours of cold ischemia. Control lungs were preserved with 50 ml/kg cold saline solution flush and reperfused at either normal flow (60 ml/min) or high flow (120 ml/min). Experimental lungs were preserved with 50 ml/kg cold Euro-Collins solution and reperfused at normal or high flow rates. The arteriovenous oxygen gradient at the end of the 30-minute reperfusion period was significantly lower in the high-flow versus the low-flow experimental group (31.1 ± 4.2 vs 130.6 ± 41.6 mm Hg, $p < 0.05$). The pulmonary vascular resistance was increased in the high-flow groups and the experimental groups, with a statistically significant difference between low-flow experimental and control groups (64374.4 ± 5722.6 vs 37041.5 ± 2110.9 dynes $\cdot$ sec $\cdot$ cm⁻⁵, $p < 0.001$). The percentage decrease in dynamic airway compliance in the high-flow experimental group was markedly different from that in the high-flow control group ($-51\% \pm 13.3\%$ vs $-10.15\% \pm 3.4\%$, $p < 0.05$). Similarly, the wet/dry ratio of the lungs in the high-flow experimental group (13.92 ± 2.32) was significantly greater than that in the low-flow experimental group (6.27 ± 0.19 , $p < 0.01$) and than that in the high-flow control group (5.88 ± 0.23 , $p < 0.001$). These data demonstrate that high-flow reperfusion and preservation with Euro-Collins solution are deleterious to lung function, both individually and in combination, in an ex vivo rabbit lung model. Lung preservation with Euro-Collins solution may not be optimal when high-flow reperfusion is anticipated, as in the setting of unilateral lung transplantation for pulmonary hypertension. (J Thorac Cardiovasc Surg 1996;112:111-6)

Despite the acceptable results now being achieved with clinical lung transplantation, early graft dysfunction is still an important cause of morbidity and mortality.¹ The mechanism responsible for this primary graft dysfunction is not always apparent, but

it is more common in single-lung transplantation for pulmonary hypertension.^{2,3} The diversion of the entire cardiac output to the newly implanted lung results in significant pulmonary hypertension and increased microvascular hydrostatic pressure, leading to edema formation and hypoxemia. Although

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this process is potentially reversible, the resultant pulmonary insufficiency occasionally proves fatal.

Lung preservation with Euro-Collins solution (EC) remains the current clinical standard, even in the setting of unilateral lung transplantation for pulmonary hypertension. The high potassium content in this intracellular-type solution is known to induce potent pulmonary vasoconstriction.⁴ In an effort to promote an even distribution of cold EC flush throughout the graft, prostaglandin E₁ (PGE₁) is routinely administered before flush. Although this strategy has shown improvements in EC lung preservation,⁵ unfavorable comparisons with other solutions suggests that EC may not be the ideal preservation solution for the lung.⁶ Moreover, the potassium-induced increase in vascular tone seen with EC may not be completely eliminated by PGE₁ administration at reperfusion.⁷

Most studies relating to lung preservation take place in experimental models with normal reperfusion flow rates. Approaching the problem of early graft failure from a different viewpoint, we hypothesized that lung function is impaired by high-flow reperfusion, as occurs with single-lung transplantation in patients with pulmonary hypertension. Our second hypothesis was that the resultant injury is further aggravated by EC-induced vasoconstriction. These hypotheses were investigated by studying lungs after 4 hours of cold ischemia and pretreatment with PGE₁ with an isolated, blood-perfused rabbit lung model.

Material and methods

Lung-heart block harvesting. Twenty-eight New Zealand White rabbits (3.0 to 3.5 kg) were randomly assigned to four groups of seven animals each. Each rabbit was anesthetized with intramuscular ketamine (50 mg/kg) and xylazine (5 mg/kg). All animals received humane care 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 Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 85-23, revised 1985).

A tracheostomy was performed and followed by paralysis with metocurine (0.2 mg/kg). Mechanical ventilation was instituted (ventilator RSP1002; Kent Scientific Corporation, Litchfield, Conn.) with room air at a tidal volume of 12 ml/kg and a rate of 20 breaths/min. Median sternotomy and thymectomy were then performed. The superior and the inferior venae cavae were loosely encircled with ligatures and the pericardium was opened. Both the pulmonary artery (PA) and aorta were dissected free and similarly encircled. A purse-string suture was then

placed in the free wall of the right ventricle, and the rabbit was heparinized (500 U/kg). After injection of 30 μ g PGE₁ into the PA, the venae cavae were ligated and the time of ischemia onset was charted. The PA was then cannulated through the right ventricular purse-string suture, and both the right ventricular and PA ligatures were tied around the cannula. After venting of the left ventricle and ligation of the aorta, 50 ml/kg preservation solution at 4°C was infused into the PA from a height of 30 cm. Topical cooling was achieved with cold saline solution slush. During PA flush, the left atrium was cannulated through the left ventricle and a second purse-string suture was tied around the cannula. After the PA flush, the inflow and outflow cannulas were clamped. Care was taken to leave the pleurae intact until completion of the flush, to avoid parenchymal injury. The tracheostomy tube was then clamped at end-inspiration, and the lung-heart block was excised and immersed in cold normal saline solution and then stored at 4°C.

Assessment of lung function. After 4 hours of storage, the lung-heart blocks were suspended by force transducer in a warmed, humidified tissue chamber (Fig. 1). Ventilation was reestablished with 95% oxygen and 5% carbon dioxide at a tidal volume of 12 ml/kg and a rate of 20 breaths/min. The lungs were reperfused with homologous fresh whole venous blood from a main reservoir. A second venous blood reservoir was used to determine single-pass oxygenation. Blood was harvested from a single rabbit for each experiment. The inflow and outflow cannulas were then connected to the blood-filled perfusion circuit, with care taken to avoid the introduction of air bubbles. The circuit (Kent Scientific) was designed to recirculate 200 ml of warmed blood through a 270 μ m blood filter (2C7600; I.V. Systems Division, Baxter Healthcare Corp., Deerfield, Ill.) with a roller pump (7521-40; Cole-Parmer Instrument Company, Niles, Ill.) at a rate of 60 or 120 ml/min, in accordance with the experimental protocol. A 270 μ m blood filter was chosen to avoid affecting leucocyte or platelet counts. Continuous recordings of PA pressure, pulmonary venous pressure, lung weight, airway flow, and airway pressure were carried out with a dynamic data-acquisition program (Workbench PC; Strawberry Tree, Inc., Sunnydale, Calif.) on a personal computer (470A; Compaq Prolinea, Houston, Texas). This program allowed immediate calculation of pulmonary vascular resistance (PVR), tidal volume, and dynamic airway compliance. The pulmonary venous pressure was maintained within the physiologic range (4 to 8 mm Hg) by setting the appropriate height of a small outflow reservoir in the circuit. Pulmonary venous blood samples were collected for blood gas analysis (Corning 178 pH/Blood Gas Analyser; Corning Inc., Corning, N.Y.) at 10, 20, and 30 minutes after the start of reperfusion; at each sampling time, inflow from the main reservoir was interrupted and the circuit was filled with venous blood from the second inflow reservoir. A 30 ml sample of venous blood was passed through the pulmonary vasculature at each interval to ensure accurate measurement of oxygen content. Oxygen contact with exposed blood surfaces inside the reservoir containers was minimized by the continuous passive infu-

Fig. 1. Schematic of isolated ventilated and blood perfused rabbit lung model. *P*, Pressure transducer; *N*₂, nitrogen gas.

sion of 100% nitrogen. After 30 minutes of reperfusion, lung samples were taken for histologic analysis and calculation of wet/dry weight ratio after passive desiccation.

Experimental protocol. Lungs were flushed with normal saline solution or EC and reperfused at a normal (60 ml/min, S60 and EC60) or high flow rate (120 ml/min S120 and EC120; Table I). Saline solution was chosen to rinse the lungs in the control groups because of its inert endothelial effects. Data were obtained every 15 seconds and analyzed at the end of the 30-minute reperfusion period. All values are expressed as mean $\pm$ standard error of the mean.

Statistical analysis was performed by analysis of variance and Kruskal-Wallis nonparametric analysis of variance was used to compare different solutions at the same flow rate and the influence of flow rate on lungs preserved with the same solution. Differences were considered statistically significant if the *p* value was less than 0.05.

Results

There were no significant differences among groups in donor weight, total ischemic time, or hematocrit. Mean hematocrit for all groups combined was $29.53\% \pm 0.33\%$. The arteriovenous oxygen gradient at the end of the 30-minute reperfusion period was significantly lower in both high-flow groups compared with the normal flow groups

Table I. Experimental protocol and group designations

Group (<i>n</i> = 7)	Preservation solution (50 ml/kg)	Reperfusion flow (ml/min)
S60	Saline	60
S120	Saline	120
EC60	EC	60
EC120	EC	120

(S120 75.8 ± 13.0 vs S60 284.6 ± 45.6 mm Hg, *p* < 0.01; EC120 31.1 ± 4.2 vs EC60 130.6 ± 41.6 mm Hg, *p* < 0.05). The arteriovenous oxygen gradient was also lower in the EC groups than in the saline solution groups at the same flow rates, but this difference was not statistically significant (Fig. 2).

The hemodynamic data recorded during reperfusion revealed pulmonary hypertension and increased PVR in the high-flow groups (Table II). These hemodynamic derangements were especially marked in the EC120 group. Fig. 3 demonstrates a continuous tracing of PVR throughout the 30-minute reperfusion period for groups S60 and EC60. Similarly, the percentage decrease in dynamic airway compliance was significant in the EC groups. The lung water content at 30 minutes of reperfusion,

Fig. 2. Pulmonary arteriovenous (*P A-V*) oxygen gradients at 10, 20, and 30 minutes of reperfusion. Oxygenation declines in high-flow groups and EC groups during reperfusion. All values expressed as mean $\pm$ standard error of the mean (at the end of reperfusion period, $p < 0.01$ S60 vs S120; $p < 0.05$ EC60 vs EC120).

Table II. Results after 30 minutes of reperfusion

Group	PAP (mm Hg)	PVR (dynes $\cdot$ sec $\cdot$ cm $^{-5}$)	CPL change (%)	Wet/dry rate
S60	34.4 $\pm$ 1.8*	37041.5 $\pm$ 2110.9*	-4.22 $\pm$ 1.9	5.78 $\pm$ 0.1
S120	110.5 $\pm$ 11.6	68197.5 $\pm$ 7627.1	-10.15 $\pm$ 3.4	5.88 $\pm$ 0.23†
EC60	52.1 $\pm$ 4.9‡	64374.6 $\pm$ 5722.6‡	-23.9 $\pm$ 13	6.27 $\pm$ 0.19§
EC120	127.1 $\pm$ 19.9	79848.1 $\pm$ 8412.6	-51 $\pm$ 13.3¶	13.92 $\pm$ 2.23

All data are mean $\pm$ standard error of the mean. PAP, PA pressure; CPL, dynamic airway compliance.

* $p < 0.005$ (S60 vs S120).

† $p < 0.001$ (S120 vs EC120).

‡ $p < 0.001$ (EC60 vs S60).

§ $p < 0.01$ (EC60 vs EC120).

|| $p < 0.005$ (EC120 vs EC60).

¶ $p < 0.05$ (EC120 vs S120).

as expressed by wet/dry ratio, was significantly greater in the EC120 group (Table II).

Discussion

EC, an intracellular-type preservation solution, was first introduced for renal preservation. Its application to pulmonary transplantation was based on theoretic advantages, such as prevention of cell swelling, conservation of energy stores, and minimization of intracellular potassium loss.⁸ The high potassium concentration of EC depolarizes smooth-muscle cell membranes, however, causing increased vascular tone and ultimately vascular obstruction.⁹ The severe vasoconstriction during pulmonary flush prevents uniform distribution of the solution, resulting in uneven cooling and inadequate preserva-

tion.¹⁰ The administration of PGE₁ before PA flush is a routine clinical practice designed to overcome the EC-mediated vasoconstriction. This concept was based on experimental studies showing improved pulmonary preservation with PGE₁ pretreatment.⁵ Lung preservation with EC or other intracellular-type solutions has, however, led to unpredictable results in lung transplantation.^{11,12} Such varied results may be related to ineffective counteraction of EC-induced vasoconstriction. To evaluate the independent effect of a persistent increase in PVR during reperfusion, we studied lung function after a short cold storage period, thereby minimizing the confounding effects of ischemia-reperfusion injury.

Primary graft failure is of special concern in the

Fig. 3. PVR during 30 minutes in groups reperfused at normal flow. Values are expressed as mean $\pm$ standard error of the mean (at the end of reperfusion period, $p < 0.001$ S60 vs ED60).

setting of pulmonary hypertension. Because of donor shortage and high mortality rates for patients with pulmonary hypertension awaiting transplantation, unilateral lung transplantation has been advocated.¹³ In this study, impairment of lung function by high-flow reperfusion was well demonstrated. Lungs preserved with saline solution displayed a decrease in oxygenation, an increase in PVR, and pulmonary hypertension when reperfused at a high flow rate. Despite these detrimental changes, pulmonary edema formation was minimal and dynamic airway compliance was normal. The lungs preserved with EC, however, had not only impaired oxygenation, increased PVR, and pulmonary hypertension, but also severe pulmonary edema formation. This last is demonstrated by a marked decrease in airway compliance and an increased wet/dry ratio. These impairments are evidence of further compromise of lung function, probably as a result of potassium-induced pulmonary vasoconstriction not effectively counteracted by administration of PGE₁.

The PVR data shown in Fig. 3 demonstrate a severe initial increase in the PVR of the group preserved with EC and reperfused at a normal flow rate. PVR stabilized 5 to 10 minutes after a significant decrease, perhaps as the high potassium concentration was washed out during reperfusion. During this period of transient high PVR, however, substantial lung injury can occur. A possible limitation of this rabbit model may be the sensitivity of rodent pulmonary vasculature to high concentrations of potassium, although similar vasoconstrictive

effects are seen in human vasculature. Low reperfusion pressure has been shown to attenuate both coronary endothelial and left ventricular dysfunction.^{14, 15} It is conceivable that low-flow reperfusion may similarly reduce pulmonary endothelial dysfunction, pulmonary hypertension, and subsequent edema formation. To more effectively mitigate ischemia-reperfusion injury to the lung, which is characterized by an elevation in PVR and an increase in microvascular permeability,¹⁶ efforts should be directed at preserving not only parenchymal viability but also endothelial function. In addition to the vasoconstrictive properties of EC, other mechanisms resulting in cellular injury may play a role. Solutions with high potassium concentrations have not been unequivocally proved to ameliorate lung preservation; on the contrary, our study provides clear evidence that such solutions can actually be detrimental to pulmonary function.

In conclusion, high-flow reperfusion results in substantial impairment of lung function, which is further potentiated by the high potassium concentration of EC. Just as cardioprotective strategies have evolved and different methods of myocardial protection have been adapted to various clinical situations, alternative solutions and lung preservation methods should be adapted to the clinical condition in lung transplantation. A definite need for further studies with other pulmonary preservation solutions exists, especially in situations where lungs are either injured before procurement or are subjected to abnormal conditions during reperfu-

sion. Preservation of lung grafts with EC may not be optimal when high-flow reperfusion is anticipated, as in the setting of unilateral lung transplantation for pulmonary hypertension.

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