High-flow endobronchial cooled humidified air protects non-heart-beating donor rat lungs against warm ischemia

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Objective: Lungs from non-heart-beating donors for transplantation require protection against warm ischemic damage. Minimally invasive techniques are required to reduce organ damage during the warm ischemic period because invasive surgical procedures are often not feasible at this time. This study investigated the preservative effect of high-flow endobronchial cooled humidified air during warm ischemia in non-heart-beating donor rat lungs.

Methods: Fourteen animals were divided into a Cooling group ($n = 7$), which received cooled air/saline spray during a 2-hour warm ischemic period, and a Control group ($n = 7$), which received no cooling. After ischemia the lungs were perfused on an isolated lung perfusion apparatus.

Results: Endobronchial temperatures in the Cooling and Control groups were 8°C and 36°C at 10 minutes, and 5°C and 35°C at 20 minutes, respectively ($P < .0001$). Lung core and surface temperatures in the Cooling group were also lower than those in the corresponding Control group ($P < .0001$). After reperfusion, pulmonary arterial pressure ($P = .003$) and peak airway pressure ($P = .002$) were lower in the Cooling group than in the Control group. Higher pulmonary venous PO_2 ($P = .02$), higher adenosine triphosphate levels ($P = .01$), and lower wet/dry lung weight ratio ($P = .003$) were seen in the Cooling group compared with the Control group.

Conclusions: High-flow endobronchial cooled humidified air can decrease lung temperature and improve post-ischemic pulmonary function and adenosine triphosphate levels in non-heart-beating donor lungs.

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Rapid cooling of perfused organs by an in situ flush with cold crystalloid fluid is the mainstay of effective solid-organ preservation before transplantation from brain-dead donors.¹ However, in the non-heart-beating donor (NHBD) there is always a delay between the circulatory arrest and the initiation of cold in situ flush, especially in Maastricht NHBD Category I (dead on arrival) and Category II (unsuccessful resuscitation) donors.² Obtaining family consent and organizing organ retrieval for organ donation require considerable time, thus increasing warm ischemic time. During this time organs require protection against warm ischemic damage, and for this, minimally invasive techniques are required because the preparatory processes for NHBD preclude open surgical procedures. Topical cooling with cold crystalloid solution flush through the pleural cavity by chest tubes was developed to reduce warm ischemic damage and is effective.³⁻⁶ However, this technique still requires surgical intervention and may result in inadequate bronchial preservation with subsequent impaired healing of the bronchial anastomosis, previously claimed as a weakness of NHBD lung transplantation.⁷

Cooling the NHBD lungs in situ with cold air represents a noninvasive means of reducing the lung temperature through the bronchial tree with no incision in

Abbreviations and Acronyms

ATP	= adenosine triphosphate
NHBD	= non-heart-beating donor
PAP	= pulmonary arterial pressure
PIP	= peak inspiratory pressure

the body. Cooling the lungs with cold air from a ventilator has been investigated in animal models.^{8,9} In studies in rabbits, Van Raemdonck and colleagues⁸ reduced the temperature of the inspired air to 4°C by using a coil of copper tubing submerged in ice. In studies in dogs, Egan⁹ reduced the temperature of air by adding a coil immersed in dry ice to the ventilator circuit. Both groups succeeded in achieving hypothermic ventilation; however, both were unsuccessful in substantially decreasing the core and surface temperature of the lungs.^{8,9} Egan concluded that this was because of the disparity between the specific heat of air and tissue (composed mainly of water).⁹ Moreover, cooled air, especially dry air, delivered through a ventilator is rapidly rewarmed in the bronchial tree.¹⁰

Although these previous techniques were ineffective, this approach is still attractive because it is a noninvasive and simple technique that can be initiated by paramedical and/or junior medical staff before the initiation of topical cooling, and may confer the benefit of improved bronchial preservation in addition to that provided by topical cooling.

The aim of this study was to investigate the preservative effect of a high continuous flow of cooled air with saline spray in NHBD lungs during the warm ischemic period. We hypothesized that a high continuous flow of cooled air could decrease the temperature of the lungs more efficiently than cooled air delivered by a ventilator because a high continuous flow of cooled air can rapidly displace any rewarmed air in the bronchial tree. We also hypothesized that cold saline spray could overcome the disparity between the specific heat of air (endobronchial gas) and water (bronchial tissue).

Material and Methods**Study Design and Groups**

A total of 16 animals were used in this study. Two animals were used for pilot experiments to determine the optimal flow rate of the air and infusion rate of saline. The remaining 14 animals were divided into 2 groups. The Cooling group (n = 7) received cooled air/saline spray, and the Control group (n = 7) received no cooling during the 2-hour warm ischemic period. All animals received humane care in accordance with the National Health and Medical Research Council of Australia Code of Practice for the Care and Use of Animals for Scientific Purposes under approval from the Alfred Medical Research and Education Precinct Animal Ethics Committee.

Animal Preparation and Induction of Cardiac Arrest

Male Sprague-Dawley rats weighing 320 to 350 g were anesthetized with 60 mg/kg of intraperitoneal pentobarbitone sodium (Nembutal, Merial, Australia). A tracheotomy was performed to enable occlusive cannulation with a 16-gauge intravenous catheter (Insyte, Becton Dickinson, Franklin Lakes, NJ). Animals were ventilated with 100% oxygen at 60 breaths/min using a Harvard rodent ventilator model 683 (Harvard Apparatus Co, Holliston, Mass), a tidal volume of 2.2 mL, and a positive end-expiratory pressure of 2 cm H₂O. A midline sternotomy and upper midline laparotomy were performed. Temperature-monitoring probes (Mon-a-Therm, Mallinckrodt Medical, Inc, St Louis, Mo) were placed in the interlobar fissure between the right upper and lower lobes to measure lung surface temperature, inserted into the mediastinal lobe parenchyma to measure lung core temperature, and into the right main bronchus through the trachea to measure endobronchial temperature. A rectal thermometer was used to measure body core temperature. Baseline temperature measurements were obtained 5 minutes after closure of the sternotomy wound. A dose of 600 U of heparin sodium was injected intrahepatically, the animal was sacrificed with pentobarbital (intrahepatic injection, 120 mg/kg), and the 16-gauge tracheal cannula was removed. The cadaver was wrapped in a paper napkin and placed on a warming blanket at 38°C to simulate the non-intervention, warm ischemic period. Temperature measurements were recorded every 10 minutes throughout the 2-hour period of circulatory arrest.

Cooled Air/Saline Spray System for Preservation

A nonocclusive 20-gauge intravenous catheter (Optiva, Critikon, Tampa, Fla) was inserted into the trachea. Room air was cooled using a metal coil immersed in dry ice (Figure 1). This coil was connected to the tracheal cannula. The connecting tube was 25 cm long and insulated with fabric tape and aluminum foil to avoid rewarming the cooled air. A continuous endobronchial spray of saline (1.2 mL/h) was generated using a 30-gauge needle inserted into the tracheal cannula near its tip. A fine polyethylene tube (PE50, Clay Adams, Parsippany, NJ) was threaded down to the distal bronchus for monitoring endobronchial pressure.

Isolated Lung Perfusion

After the 2-hour preservation period, the heart and lungs were removed en bloc and weighed. The right mediastinal lobe was explanted, immersed in liquid nitrogen, and stored at -80°C for adenosine triphosphate (ATP) assay. The pulmonary artery was cannulated with a 20-gauge catheter connected to an isolated lung perfusion circuit as previously described by Fisher.¹¹ The left atrium was opened widely to vent the perfusate. The trachea was recannulated with an occlusive 16-gauge catheter, and the heart-lung block was suspended in a temperature-controlled chamber. The lungs were ventilated with 100% oxygen at 60 breaths/min, a tidal volume of 2.2 mL, and a positive end-expiratory pressure of 2 cm H₂O. The perfusate was HEPES buffer containing (grams/liter) 7.52 NaCl, 0.78 KCl, 0.37 CaCl₂, 0.15 MgSO₄·7H₂O, 0.18 NaH₂PO₄·H₂O, 2.60 HEPES, 1.80 D-glucose, 0.28 pyruvate, and 4% bovine serum albumin.

Figure 1. Delivery system for cooled air with saline spray.

The perfusate was deoxygenated by bubbling with nitrogen gas to achieve P_{O_2} near 60 mm Hg. The perfusate pH was adjusted to 7.40 by adding dilute HCl or NaOH as necessary. The perfusate temperature was maintained at 38°C with a water-jacketed reservoir and circuit, and the perfusate was pumped into the pulmonary artery by a peristaltic pump, vented through the left atrial appendage and not recirculated. The pump flow was 5 mL/min for the first 5 minutes after reperfusion and 10 mL/min for the next 25 minutes. Pulmonary arterial pressure (PAP) and peak inspiratory pressure (PIP) were measured continuously throughout the 30-minute reperfusion period with pressure transducers (MLA845 Physical Pressure Transducer, AD Instruments Pty Ltd, Castle Hill, NSW, Australia) positioned at the level of the hilum of the lungs. The oxygen partial pressure of the effluent perfusate taken from the left atrium was measured 10 and 30 minutes after reperfusion. Blood gas measurements were made using a blood gas analyzer (OPTI CCA, Osmetech Inc, Roswell, Ga). At the end of the experiment, wet and dry weights of the lungs were measured to calculate the wet-to-dry weight ratio.

Adenosine Triphosphate Assay

Frozen lung tissue was ground into a fine powder under liquid nitrogen using a mortar and pestle. The tissues were deproteinized with 0.6 M perchloric acid. The tissue suspension was then centrifuged at 6200 rpm for 10 minutes at 4°C, and the acid was neutralized with 3M K_2CO_3 . After neutralization, the samples were centrifuged again at 6200 rpm for 10 minutes at 4°C, and the supernatant was used for biochemical assay. ATP content was determined by luciferase-driven bioluminescence measurement using the ATP Bioluminescence Assay Kit CLS II (Roche, Mannheim, Germany), which can detect extremely low concentrations of ATP. The ATP concentration was then standardized

to the quantity of protein in the supernatant as assessed with a Bicinchoninic Acid Protein Assay Kit (Sigma, St Louis, Mo). Data were normalized to total protein, and the cellular ATP level was expressed in nanomoles per microgram of protein.

Statistical Analysis

All data were expressed as mean $\pm$ standard error of the mean for parametric variables or as median and interquartile range for nonparametric variables. Comparisons between the 2 experimental groups were made using unpaired *t* test or 2-way analysis of variance with repeated measurements for parametric variables and Mann-Whitney *U* test for nonparametric variables. Analysis was performed using Statview 5.0 software package (SAS Institute Inc, Cary, NC).

Results

Pilot Experiments

The pilot experiments demonstrated that a flow rate of 300 mL/min of the cooled air/saline spray achieved a temperature of 4°C at the level of the right main bronchus with an endobronchial pressure less than 15 mm Hg. A continuous saline infusion at a rate of 1.2 mL/h was the minimum injection rate without allowing obstruction of the tip of the delivery needle because of freezing. On the basis of the results of the pilot experiments, an air flow rate of 300 mL/min and a saline infusion rate of 1.2 mL/h were used throughout this study.

Temperature

Mean body core temperatures were 37.6°C $\pm$ 0.2°C at the commencement of cooling, 32.7°C $\pm$ 0.3°C after 1 hour of

Figure 2. Temperature results during warm ischemia. *P* values given are derived from repeated-measures analysis of variance. **A**, Endobronchial temperature after cardiac arrest. **B**, Lung core temperature after cardiac arrest. **C**, Lung surface temperature after cardiac arrest.

cooling, and $30.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ after 2 hours of cooling, with no significant difference between the 2 groups ($P = .07$).

Endobronchial Temperature

The endobronchial temperatures are shown in Figure 2, A. The temperature in the Cooling group rapidly de-

Figure 3. Pressures during reperfusion. *P* values given are derived from repeated-measures analysis of variance. **A**, PAP after reperfusion. **B**, Peak inspiratory pressure after reperfusion.

creased from 37°C to 8°C within 10 minutes and to 5°C within 20 minutes. These values were significantly lower than those in the Control group ($P < .0001$).

Lung Core and Surface Temperature

Both lung core (Figure 2, B) and surface (Figure 2, C) temperatures were significantly lower in the Cooling group than in the Control group ($P < .0001$).

Lung Function Results

Pulmonary Arterial Pressure

PAP steadily increased in the Control group throughout the reperfusion period. The pressure in the Cooling group remained low and was significantly less than that in the Control group ($P = .003$) (Figure 3, A).

Figure 4. Pulmonary venous effluent oxygen tension 30 minutes after reperfusion. Values given are median, range, and interquartile range.

Peak Inspiratory Pressure

PIP increased steadily during the reperfusion period in the Control group, whereas it remained close to baseline levels in the Cooling group. The difference between the 2 groups was significant ($P = .002$) (Figure 3, B).

Oxygenation

The PO_2 in the pulmonary venous effluent 30 minutes after reperfusion in the Cooling group (median = 400, interquartile range, 352-469) was greater than that in the Control group (median = 272, interquartile range, 209-301) ($P = .02$) (Figure 4).

Adenosine Triphosphate Assay

The ATP level of the lung after 120 minutes of ischemia and before reperfusion was significantly higher in the Cooling group than in the Control group ($P = .01$) (Table 1).

Wet Weight of Explanted Heart-Lung Block

There was no significant difference in the weight of the heart-lung block before reperfusion between the Cooling and Control groups ($P = .08$) (Table 1).

Wet-to-Dry Lung Weight Ratio After Reperfusion

The wet-to-dry lung weight ratio in the Cooling group was lower than that in the Control group ($P = .003$) (Table 1).

Discussion

Organs from NHBDs for transplantation need to be protected against warm ischemic damage. Minimally invasive techniques to minimize warm ischemia before initiation of topical cooling are required because invasive surgical procedures are often not feasible during the immediate post-cardiac arrest period. This study, using a rat model of

TABLE 1. Lung adenosine triphosphate content and heart-lung weight after ischemia and lung wet/dry ratio after reperfusion

	Cooling (n = 7)	Control (n = 7)	P value
After ischemia			
Lung ATP level (nmol/ μ g protein)	24.7 $\pm$ 3.3	12.3 $\pm$ 3.3	.01
Wet weight of heart-lung block (g)	3.3 $\pm$ 0.1	3.0 $\pm$ 0.1	.08
After reperfusion			
Lung wet/dry ratio	5.0 $\pm$ 0.5	7.9 $\pm$ 0.6	.003

ATP, Adenosine triphosphate.

cardiac arrest (non-heart-beating) donation, demonstrates that compared with conventional management, the use of cooled air with saline spray decreased lung temperature resulting in improved lung function and maintenance of higher lung ATP levels.

Hypothermia suppresses the metabolic rate during the warm ischemic period and improves ischemic tolerance.^{1,12,13} In the Cooling group the endobronchial temperature decreased rapidly to less than 10°C within the first 10 minutes. In the Cooling group the lung core and surface temperature also decreased from 37°C to 24°C within 2 hours after cardiac arrest, whereas these corresponding temperatures in the Control group remained at approximately 32°C for 2 hours after death. The reported effective temperature for lung preservation varies from 4°C to 25°C.^{4,5,8,12-18} The optimum temperature for prolonged storage is reported to be in the range of 7°C to 10°C¹²⁻¹⁵; however, the optimum temperature for NHBD in situ lung preservation is still unknown. In a rat experimental model of NHBD, Wierup and colleagues¹⁶ from Steen's group¹⁷ reported that ischemic NHBD lungs topically cooled to 25°C achieved results similar to non-ischemic control lungs. In a porcine experimental model of NHBD, Steen and colleagues decreased endobronchial temperature from 37°C to 17°C at 1 hour and to 15°C at 2 hours after initiation of topical cooling. In clinical NHBD lung transplantation, the same investigators decreased endobronchial temperature to 18°C after 3 hours of topical cooling.³ In the present study in a rat model of NHBD, the use of high-flow cooled air/saline spray decreased both lung core and surface temperatures by 9°C after 1 hour and by 13°C after 2 hours of circulatory arrest. Use of this technique resulted in improved lung function and maintenance of higher ATP levels in the Cooling group compared with the Control group. The protection against ischemic damage afforded by hypothermia is mediated mainly by metabolic suppression. The degree of suppression is described by the temperature coefficient, or Q_{10} . The Q_{10} is a well-established physiologic variable defined as the ratio of

metabolic rates at temperatures 10°C apart.¹⁹⁻²¹ The Q_{10} in dogs and other animals is reported to be more than 2.2.^{19,20} The Q_{10} in humans (brain tissue) is reported to be 2.3.²¹ Although the Q_{10} in different organs may be different, the use of high-flow cooled air/saline spray by decreasing lung temperature by 10°C or more would reduce the metabolic rate by at least one half and thus markedly reduce the degree of ischemic damage.

Other factors, such as inhaled oxygen, may have contributed to the highly effective lung preservation achieved in this study. A continuous high flow of room air can inflate the lungs and deliver added oxygen. Van Raemdonck and colleagues¹² described in the rabbit that both postmortem inflation and ventilation with room air contributed to improved maintenance of lung ATP levels compared with deflated lungs. D'Armini and colleagues¹³ described the association of oxygen ventilation with maintenance of ATP level and pulmonary cell viability compared with nitrogen ventilation or no ventilation.¹³ These studies suggest that even in the absence of ventilation or inflation, the continued supply of oxygen (even at the oxygen concentration of room air), is able to maintain aerobic metabolism and prevent warm ischemic damage.

The saline spray also seems to produce a beneficial effect. Although some of the saline spray fluid may have remained in the endobronchial and alveolar spaces, no deleterious effects, such as higher wet-to-dry lung weight ratio after reperfusion, lower oxygenation, or higher PAP and PIP, were seen in the Cooling group. On the contrary, the saline spray will have played a role in cooling the bronchial tree directly and prevented drying of the bronchial endothelium, which is one of the well-described disadvantages of the high air flow.

The ability of high-flow endobronchial cooled humidified air to contribute to rapid endobronchial cooling was one of the important findings of this study. Van Raemdonck and colleagues⁸ previously described the effect in rabbits of different cooling techniques on endobronchial temperature measured by a temperature probe placed in the lower lobe bronchus. One- and 2-hour postmortem endobronchial temperatures were approximately 28°C and 26°C by cooled air ventilation, 24°C and 17°C by topical cooling, and 6°C and 5°C by pulmonary flush, respectively. Wierup and colleagues and Steen and colleagues also achieved 25°C (rat model),¹⁶ 15°C (porcine model),¹⁷ and 18°C (human)³ by topical cooling.

Although there were some differences between our current study and previous studies in animal and human models (rat vs rabbit vs porcine vs human) and the level of bronchus where the temperatures were measured (right main bronchus vs lower lobe bronchus), in our study the high-flow cooled air/saline spray decreased endobronchial temperature (<10°C within the first 10 minutes) faster than topical

lung cooling reported in previous studies and at a rate similar to the pulmonary flush in the study by Van Raemdonck and colleagues. This might contribute to better bronchial preservation and healing after NHBD lung transplantation and avoid the airway problems noted in an NHBD animal study by Binns and colleagues.⁷

The other beneficial effect of this technique is that it can be simply set up in an emergency department or intensive care unit and performed without requirement of surgical intervention; therefore, this can be initiated by nurses and junior medical staff soon after donor death. This is beneficial for NHBDs especially in Maastricht NHBD Category I (dead on arrival) and Category II (unsuccessful resuscitation). For further research there is the possibility of improving non-interventional cooling before the initiation of topical cooling by the use of this endobronchial cooling technique (cooling from inside) combined with wearing an ice-cooling jacket (cooling from the outside).²² There is also the possibility of further improvement of preservation with the use of aerolized prostacyclin analogue iloprost²³ or inhaled nitric oxide²⁴ as an adjunct to endobronchial cooling.

Conclusion

High-flow cooled air with saline spray during the warm ischemic period of NHBD decreases endobronchial, lung core, and lung surface temperatures resulting in improved pulmonary function and superior maintenance of pulmonary ATP levels. Although further investigation using larger animal transplant models is necessary to determine the significance, feasibility, and efficacy of endobronchial high-flow cooled air/saline spray during the warm ischemic period, the potential clinical application of this technique as an adjunct to intrapleural topical cooling in human NHBD lungs is considerable.

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