Long-term (72 hours) preservation of rat lungs

Rafael Aguiló, MD^a
 Enric Serra, MD^b
 Bernat Togores, MD^c
 Andrés de la Peña, MD^d
 Carmen Santos^d
 Alvar G. N. Agustí, MD^c

Objective: We sought to investigate whether the addition of ethanol to a preservation solution (as an antifreeze agent) might allow a reduction of the storage temperature to 0°C without causing freezing damage and improve lung function after prolonged (72 hours) ischemia.

Methods: Lungs from Sprague-Dawley rats were ventilated and perfused ex vivo at 37°C for 60 minutes in the following experimental groups: (1) the no ischemia and reperfusion (no I-R) group (n = 7), in which lungs were studied immediately after harvesting; (2) the LPD24 (n = 7) and (3) LPD72 (n = 8) groups, in which, after harvesting, lungs were flushed and immersed in low-potassium dextran solution and stored deflated at 10°C for 24 and 72 hours, respectively, until reperfusion; and (4) the TEST72 group (n = 9), in which lungs were flushed and immersed in Krebs-Henseleit buffer with added ethanol (10 mL/L) after harvesting and stored deflated at 0°C for 72 hours until reperfusion.

Results: Compared with the no I-R group, the other 3 groups had worse lung function, higher lung water content, and evidence of cell injury at reperfusion ($P < .01$). However, lung function at reperfusion (assessed on the basis of either effluent PO_2 , peak airway pressure, or mean arterial pulmonary pressure) was better ($P < .01$) in the TEST72 group than in the LPD24 or LPD72 groups. Paradoxically, lung cell structure was better preserved in the LPD24 group than in the TEST72 group (or the LPD72 group).

Conclusions: In this experimental model of rat lung ischemia-reperfusion injury, a low preservation temperature (0°C) combined with the addition of ethanol to the preservation solution improves lung function at reperfusion after 72 hours of ischemia but fails to maintain lung cell structure.

From the Serveis de Cirurgia Toràtica,^a Anatomia Patològica,^b Pneumologia,^c and Unitat d'Investigació,^d Hospital Universitari Son Dureta, Palma de Mallorca, Spain.

Supported in part by Fondo de Investigación Sanitaria (FIS 97/0914), Sociedad Española de Neumología y Cirugía Torácica (SEPAR) and ABEMAR.

Received for publication June 3, 2002; accepted for publication Aug 6, 2002.

Address for reprints: Alvar Agustí, MD, Hospital Universitari Son Dureta, Andrea Doria, 55. 07014 Palma de Mallorca, Spain (E-mail: aagusti@hsd.es).

J Thorac Cardiovasc Surg 2003;125:907-12

Copyright © 2003 by The American Association for Thoracic Surgery

0022-5223/2003 \$30.00+0

doi:10.1067/mtc.2003.149

Lung preservation for transplantation is currently performed by first flushing and immersing the lung in one of several available preservation solutions (eg, low-potassium dextran [LPD] solution) and then by maintaining it at 4°C to 10°C.¹⁻³ Because cooling decreases the metabolic rate and energy demand of the cells, this strategy protects the organ against ischemic damage^{3,4} and allows periods of safe lung ischemia of up to 6 to 8 hours.^{1,2} In theory a lower preservation temperature should allow a longer period of safe lung ischemia. However, this is not normally done because the freezing itself can damage the organ.⁵

In response to a freezing environmental temperature, some species of frogs release glucose, ketone bodies, and alcohols into the blood stream.⁶ This increases the osmotic pressure and prevents freezing at 0°C.⁶ Furthermore, because alcohols can freely cross the phospholipid bilayer of cellular membranes, their concentration is identical in the intracellular and extracellular domains. This homogeneous intracellular distribution prevents the development of electric or osmotic gradients, and

TABLE 1. Composition of the different solutions used in the study

	Krebs-Henseleit	LPD	TEST
NaCl (g/L)	6.896	6.896	6.896
KCl (g/L)	0.350	0.350	0.350
CaCl ₂ (g/L)	0.368	0.368	0.368
MgSO ₄ (g/L)	0.296	0.296	0.296
KH ₂ PO ₄ (g/L)	0.163	0.163	0.163
NaHCO ₃ (g/L)	2.092	2.092	2.092
Glucose (g/L)	1	1	1
Albumin (g/L)	2	—	2
HEPES (g/L)	3	—	3
Dextran 40 (g/L)	—	20	—
Ethanol 96° (ml/L)	—	—	10
Osmolality (mOsm/L)	310	300	490
pH	7.4	7.4	7.4

TEST, Krebs-Henseleit buffer enriched with bovine albumin, with ethanol added; HEPES, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid.

therefore water, electrolytes, or both are not pulled through membranes.⁶ On the basis of these natural observations, we hypothesized that the addition of ethanol to the preservation solution (as an antifreezing agent) might allow a further reduction of the storage temperature to 0°C without causing freezing damage and, accordingly, result in a longer preservation time. To test this hypothesis, we used an isolated, ex vivo, perfused and ventilated rat lung model of ischemia-reperfusion (I-R) lung injury.^{7,8}

Methods

Animals

We used male Sprague-Dawley rats weighing 250 to 450 g that had access to food and water ad libitum and were kept in cages at a constant temperature for 12-hour cycles of light and dark. In all cases animals received care in compliance with the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, 1996).

Design

We studied 4 experimental groups. First was the no I-R group (n = 7). Immediately after harvesting (see below), isolated lungs were mechanically ventilated and perfused with Krebs-Henseleit buffer (Table 1) enriched with 0.2 g/100 mL bovine albumin (Serva) and 0.3 g/100 mL N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (Sigma) in an organ bath for 60 minutes. Because these lungs were not submitted to ischemia at any time during the experiment, the results of this group served to establish the stability and physiology of our experimental model and to assess the severity of the I-R injury observed in the other 3 experimental groups.

The second and third groups were the LPD24 (n = 7) and LPD72 (n = 8) groups, respectively. Immediately after harvesting, isolated lungs in both groups were flushed and immersed in LPD (Table 1) and stored deflated in a precision refrigerator (model FOC225D, Velp Científica) at 10°C for 24 and 72 hours, respectively. Then lungs were ventilated and perfused in an organ bath with enriched Krebs-Henseleit buffer (Table 1) according to the

same methodology described above for the no I-R group. Because the LPD24 group resembles the preservation strategy normally used in clinical practice, comparisons with it provide information about the quality of the longer preservation strategies (72 hours).

The fourth and final group was the TEST72 group (n = 9). Immediately after harvesting, isolated lungs were flushed and immersed in the solution TEST (with ethanol; see composition in Table 1) and stored deflated at 0°C (model FOC225D, Velp Científica) for 72 hours. Then they were ventilated and perfused in an organ bath with enriched Krebs-Henseleit buffer (Table 1) according to the same methodology described above for the other 3 groups.

Methodology

Rats were anesthetized by means of intraperitoneal administration of sodium thiopental (50-60 mg/kg body weight). Lungs were isolated and harvested after catheterization of the trachea, main pulmonary artery, and left atrium, as previously described.^{7,8}

In the organ bath (Radnoti Glass Technology) lungs were pump perfused (model 7521-75, Masterflex, Radnoti Glass Technology) at a constant flow of 7 to 8 mL/min at a constant temperature (37°C; model EX-221, Radnoti Glass Technology) with enriched Krebs-Henseleit buffer (Table 1). Also, they were ventilated (model 7025, Hugo Sachs Elektronik) with a tidal volume of 2 mL, a respiratory rate of 90 breaths/min, and an end-expiratory pressure value of +3 cm H₂O by using a mixture of gases that contained 95% O₂ and 5% CO₂ (Carbueros Metálicos).

Measurements

During the 60-minute period of ex vivo perfusion and ventilation in the organ bath, samples of the pulmonary effluent flow were obtained on minute 5 to determine lactate dehydrogenase (LDH) concentrations (model CX7/DELTA, Beckmann Instruments) and every 10 minutes thereafter for Po₂ measurement (model BG3000, IZASA). Airway and pulmonary artery pressures were continuously monitored with calibrated pressure transducers (models 7/IX and 399/2, Hugo Sachs Elektronik), amplified (models TAM-A 705/1 and CFAA 677, Hugo Sachs Elektronik), and recorded into a computer by using appropriate software (Atlantis, Lakeshore Technologies) for later analysis (Pegasus, Lakeshore Technologies). From these data, we determined the peak airway pressure (PawP) and the mean pulmonary artery pressure (PAP) values on minutes 15, 30, 45, and 60 after reperfusion.

To estimate the degree of pulmonary edema at the end of the reperfusion period, we used the relationship between the wet weight of the right lung and the total body weight of the animal.^{7,8} Likewise, we assessed cell viability after reperfusion by using the trypan blue exclusion method according to previously published methodology.⁷⁻⁹

Statistical Analysis

Results are shown as means ± SEM unless stated otherwise. Results were analyzed by using a 2-way analysis of variance in which time and group were considered independent factors. A P value of less than .05 was considered statistically significant. When this occurred, differences within each experimental group through time, as well as differences between groups at the same point in time, were assessed by using 1-way analysis of variance

Figure 1. Mean $\pm$ SEM values of effluent PO_2 (A), PawP (B), and mean PAP (C) after reperfusion in the 4 experimental groups studied. The 2 groups preserved with the standard preservation solution (LPD24 and LPD72 groups) are indicated with *closed symbols*. The group not subjected to any preservation regimen (no I-R group) and the group preserved with the solution under investigation (TEST72 group) are indicated with *open symbols*. Asterisks indicate the statistical significance of differences versus the LPD24 group (* $P < .05$; ** $P < .01$). For further explanations, see text.

(followed by post hoc contrasts [least significant difference] when appropriate).

Results

Lung Function at Reperfusion

Figure 1 shows the mean $\pm$ SEM values of effluent PO_2 (Figure 1, A), PawP (Figure 1, B), and mean PAP (Figure 1, C) in the 4 experimental groups studied. Effluent PO_2 (Figure 1, A) remained at greater than 600 mm Hg throughout the experiment in the no I-R group, indicating optimal gas exchange. In contrast, it decreased significantly ($P < .01$) in the 3 experimental groups, indicating the presence of I-R injury. The severity of gas exchange impairment at reperfusion was similar in the LPD24 and LPD72 groups ($P =$ not significant) but was decreased ($P < .01$) in the TEST72 group (Figure 1, A). The analysis of PawP (Figure 1, B) yielded a very similar pattern. Hence compared with the no I-R group, all other experimental groups had higher PawP values ($P < .01$), but again, these were lower in the TEST72 group ($P < .05$; Figure 1, B). Finally, mean PAP values were indistinguishable in the no I-R and TEST72 groups (Figure 1, C). In contrast, these values were very high in the

LPD24 group ($P < .01$) and lay in between in the LPD72 group (Figure 1, C).

Overall, therefore, these results indicate that lung function at reperfusion was severely impaired in the LPD24 and LPD72 groups (vs the group not subjected to I-R) and better preserved in the TEST72 group (also vs the group not subjected to I-R, Figure 1).

Lung Structure After Reperfusion

Table 2 shows the mean $\pm$ SD values of lung water content, as assessed by the ratio of the wet weight of the right lung to the total body weight of the animal.^{7,8} Compared with the no I-R group, the other 3 experimental groups had higher values (Table 2), indicating the development of pulmonary edema at reperfusion. Differences between them were not statistically significant (Table 2), but the relationship between the wet weight of the right lung and the total body weight of the animal showed a tendency toward lower values in the TEST72 group (11 ± 6) than in the LPD24 group (18 ± 5).

LDH release is a marker of cell injury.¹⁰ Compared with the no I-R group, LDH in the pulmonary effluent flow was much greater ($P < .01$) in the remaining experimental

TABLE 2. Lung edema, effluent LDH, and cell viability (mean $\pm$ SD)

	No I-R	LPD24	LPD72	TEST72
Lung edema (Ww/Bodyw $\times 10^3$)	5 $\pm$ 2†	18 $\pm$ 5*	13 $\pm$ 2*	11 $\pm$ 6*
Effluent LDH (U/l)	25 $\pm$ 5†	392 $\pm$ 102*	749 $\pm$ 131*†	651 $\pm$ 215*†
Cell viability (%)	91.7 $\pm$ 7.8	81.9 $\pm$ 7.3	65.9 $\pm$ 29.4*	61.9 $\pm$ 17.8*†

Ww/Bodyw, Ratio between the wet weight of the right lung and total body weight of the animal.

* $P < 0.01$ versus the no I-R group;

† $P < 0.05$ versus the LPD24 group;

‡ $P < 0.01$ versus the LPD24 group.

groups (Table 2), being particularly increased in the LPD72 and TEST72 groups. Actually, LDH release in these 2 groups was greater ($P < .01$) than that seen in the LPD24 group (Table 2).

As shown in Table 2, cell viability (trypan blue exclusion method) was very high in the no I-R group (91.7% $\pm$ 7.8%), and it decreased in the LPD24 group (81.9% $\pm$ 7.3%) and, to a greater extent, in the LPD72 (65.9% $\pm$ 29.4%) and TEST72 (61.9% $\pm$ 17.8%) groups.

Overall, these results indicate that lung structure at reperfusion (assessed either on the basis of lung water content, LDH release, or trypan blue exclusion) was altered in the 3 experimental groups subjected to lung preservation (vs the group not subjected to I-R) and less with shorter preservation times (LPD24 vs LPD72 or TEST72 groups, Table 2).

Figure 2 shows the relationship between the structure and function of the lung in each of the experimental groups. This graph suggest that the severity of the I-R injury (as assessed on the basis of the efficiency of the lung to exchange oxygen) decreases in direct proportion to cell viability. Similar results (not shown) can be obtained if other functional measures of I-R injury (eg, PawP or mean PAP; Figure 1) are plotted against other variables related to the maintenance of lung cell structure (eg, lung water content or LDH release; Table 2).

Discussion

In this study we tested the hypothesis that the use of an antifreezing agent (ethanol) might prevent or minimize tissue damage caused by freezing when the preservation temperature is reduced to 0°C and, consequently, that this strategy might extend the period of safe lung ischemia up to 72 hours. Our results support this hypothesis in part because, as shown in Figure 1, lung function at reperfusion (as assessed on the basis of either effluent P_{O_2} , PawP, or mean arterial pulmonary pressure) was better after 72 hours of preservation at 0°C with ethanol than after 24 or 72 hours at 10°C with a standard preservation solution (LPD). However, we also observed that several indices of lung cell disruption (lung water content, LDH release, and trypan blue exclusion) were higher in those lungs preserved for longer periods of time (LPD72 and TEST72 groups) than in those

preserved for 24 hours only (LPD24 group, Table 2). Although this latter observation is not intuitively surprising, it is difficult to reconcile with better organ function at reperfusion (Figure 1) but clearly indicates a dissociation between the duration of ischemia and the structural derangement of the lung on the one hand and the severity of lung-function impairment at reperfusion on the other hand (Figure 2). Overall, therefore, these results raise new questions and open novel avenues for research in this field.

Potential Limitations

Before discussing the potential mechanisms and implications of these observations (see below), we think that several methodological aspects need to be specifically addressed. First, we used an ex vivo experimental model. It is well known that lung responses ex vivo might differ from in vivo conditions and that observations made ex vivo might not be reproducible if tested in vivo.⁹ Thus our results should be (by now) limited to the specific set of conditions used here.

Second, we did not use whole blood to reperfuse the lungs in the organ bath. Rather, we used an enriched Krebs-Henseleit buffer (Table 1) for the sake of experimental simplicity and to allow organ perfusion for 60 minutes without recirculation. This approach, however, does not reproduce the systemic inflammatory response that might occur in vivo and that is thought to be one of the mechanisms leading to lung injury at reperfusion.¹¹

Third, in clinical practice there is no consensus about the composition of the ideal preservation solution, the conditions of lung storage, or both.^{1,2} We decided to use the LPD24 group (preservation in LPD at 10°C during 24 hours) as a model for the standard preservation regimen because preservation solutions with a low potassium content have been reported to offer good protection of lung function at reperfusion¹²⁻¹⁶ and because the optimal temperature of lung storage in LPD has been established at around 10°C.¹⁴ Likewise, we decided to test our hypothesis after a period of 72 hours of ischemia (TEST72 group) because previous experimental studies have already reported good results with LPD after 48 hours of lung preservation.^{15,16}

Finally, we decided to use ethanol as an antifreezing

Figure 2. Relationship between the maintenance of the lung cell structure after reperfusion (as assessed on the basis of the percentage of viable cells by using the trypan blue exclusion method) and the severity of the I-R injury (as assessed on the basis of the effluent PO_2 values at the end of 60 minutes of reperfusion in the organ bath). Note that there is an inverse relationship between these factors in all groups subjected to I-R. For further explanations, see text.

agent, but we recognize that many other endogenous alcohols (eg, ketone bodies or glycerol), either alone or in combination, might have also been used for this purpose.

Interpretation of Results

The results observed in the no I-R group were those expected for normal lungs.^{7,8} Likewise, the results of the LPD24 group were similar to those previously reported,¹²⁻¹⁷ because gas exchange deteriorated rapidly during the initial 20 to 30 minutes of reperfusion and stabilized thereafter (Figure 1), indicating the presence of I-R injury. Therefore we believe that these observations support the validity, stability, and reproducibility of our experimental model.

More intriguing are the observations made on the TEST72 group (in comparison with the LPD24 and LPD72 groups). We found that the combination of ethanol and a low preservation temperature (0°C) in the TEST72 group improved lung function at reperfusion compared with that seen in the 2 groups in which this was done at 10°C in LPD (Figure 1). This effect is exactly what our working hypothesis would have predicted. However, at variance with our expectations, it can be hardly explained by an improved preservation of lung cell structure (as expected from a hypothetical antifreezing effect of ethanol combined with the preservation at 0°C) because all the indices used here to assess lung structure (eg, lung water content, LDH release, and cell viability) in the TEST72 group were indistinguish-

able from those of the LPD72 group and much worse than those of the LPD24 group (Table 2), whereas both the LPD72 group and, particularly, the LPD24 group had worse lung function at reperfusion (Figure 1). Because, to our knowledge, no previous study has investigated the hypothesis being tested here or has used our experimental design, we lack a direct reference for comparison. With this caveat in mind, however, we believe that these observations support several conclusions.

First, lung cell structure deteriorates in direct proportion to ischemic period (Table 2). This is not surprising and can be substantiated by many previous studies.¹⁻³ Furthermore, because we did not find significant differences in any of the structural indices analyzed between the LPD72 and TEST72 groups (Table 2), the process of lung cell deterioration does not seem to be influenced (in our experimental model and on the basis of the relatively crude variables used to assess lung cell structure) by the preservation temperature (10°C vs 0°C) or the presence of ethanol.

Second, several lung-function variables at reperfusion were significantly better preserved in the TEST72 group than in the LPD72 group (Figure 1). In theory the ischemic damage depends only on the intensity and duration of the ischemic insult. Because both were similar in the LPD72 and TEST72 groups, our results dissociate the duration of the ischemia (72 hours) from the severity of lung-function

derangement at reperfusion (Figure 1) and suggest that the combined use of ethanol and low preservation temperature would have contributed somehow to reduce the degree of I-R injury (Figure 1), despite failing to improve the preservation of the lung cell structure (Table 2). The mechanisms underlying this observation are currently unclear and deserve future investigation. However, they can be related to some of the endogenous mechanisms of lung protection against freezing, ischemia, or both that have been recently described, including those underlying the phenomenon of ischemic preconditioning^{18,19} and the expression of heat shock proteins.²⁰ In any case this observation indicates that the severity of lung I-R injury might not only depend on the intensity and duration of the ischemic insult but also on the capacity of the lung to sense and react to it. A better understanding of the cellular mechanisms involved in this latter pathway might open new possibilities for the treatment of lung I-R in clinical practice.

Finally, taken together, our results also suggest an inverse relationship between the maintenance of lung structure and the intensity of lung-function impairment at reperfusion (Figure 2). This is a paradoxical observation the mechanisms of which are also unclear but might well be related to the same type of endogenous protective mechanisms alluded to above, which clearly deserve further investigation.

Conclusions

Our results indicate that in an ex vivo model of rat lung I-R injury, a low preservation temperature (0°C) combined with the addition of ethanol to the preservation solution (as an antifreezing agent) improves lung function at reperfusion after 72 hours of ischemia (vs the standard preservation strategy [LPD at 10°C] for 24 or 72 hours). Yet at the same time, this strategy failed to improve several indices of lung cell structure. These observations clearly dissociate several of the factors considered (to date) key in lung preservation (eg, the intensity and duration of the ischemic insult), suggest that endogenous mechanisms might also participate in lung protection against long-term I-R lung injury, and raise many new questions and possibilities that will have to be addressed in future investigations.

References

1. Trulock EP. Lung transplantation. *Am J Respir Crit Care Med*. 1997; 155:789-818.
2. Cooper JD, Vreim CE. NHLBI workshop summary: biology of lung preservation for transplantation. *Am Rev Respir Dis*. 1992;146:803-7.
3. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. *Transplantation*. 1988;45:673-6.
4. Date H, Matsumura A, Manchester JK, et al. Evaluation of lung metabolism during successful twenty-four-hour canine lung preservation. *J Thorac Cardiovasc Surg*. 1993;105:480-91.
5. D'Alessandro AM, Southard JH, Love RB, Belzer FO. Organ preservation. *Surg Clin North Am*. 1994;74:1083-95.
6. Hochachka PW, Guppy M. Frozen frogs and frozen insects. In: Hochachka PW, ed. *Metabolic arrest and the control of biological time*. Boston: Harvard University Press; 1987. p. 120-45.
7. Shih-Wen C, Voelkel NF. The isolated lung preparation as a research tool. In: Parent RA, ed. *Comparative biology of the normal lung*. Boca Raton, Fla: CRC Press; 1992. p. 587-613.
8. Uhlig S. The isolated perfused lung. In: Uhlig S, Taylor AE, eds. *Methods in pulmonary research*. Basel, Switzerland: Birkhäuser Verlag; 1998. p. 29-55.
9. Jones DR, Becker RM, Hoffmann SC, Lemasters JJ, Egan TM. When does the lung die? Kfc, cell viability, and adenine nucleotide changes in the circulation-arrested rat lung. *J Appl Physiol*. 1997;83:247-52.
10. Drent M, Cobben NAM, Henderson RF, et al. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. *Eur Respir J*. 1996;9:1736-42.
11. Neary P, Redmond HP. Ischaemia-reperfusion injury and the systemic inflammatory response syndrome. In: Grace PA, Mathie RT, eds. *Ischaemia-reperfusion injury*. Oxford: Blackwell Science; 1999. p. 123-34.
12. Keshavjee SH, Yamazaki F, Yokomise H, et al. The role of dextran 40 and potassium in extended hypothermic lung preservation for transplantation. *J Thorac Cardiovasc Surg*. 1992;103:314-25.
13. Chien S, Zhang F, Niu W, Tseng MT, Gray L Jr. Comparison of University of Wisconsin, Euro-Collins, low-potassium dextran, and Krebs-Henseleit solutions for hypothermic lung preservation. *J Thorac Cardiovasc Surg*. 2000;119:921-30.
14. Date H, Lima O, Matsumura A, et al. In a canine model, lung preservation at 10°C is superior to that at 4°C. *J Thorac Cardiovasc Surg*. 1992;103:773-80.
15. Steen S, Kimblad PO, Sjöberg T, Lindberg L, Ingemannson R, Massa G. Safe lung preservation for twenty-four hours with Perfadex. *Ann Thorac Surg*. 1993;57:450-7.
16. King RC, Binns OA, Kaithanon RC, et al. Acellular low-potassium dextran preserves pulmonary function after 48 hours of ischemia. *Ann Thorac Surg*. 1997;64:795-800.
17. Bhabra MS, Hopkinson DN, Shaw T, et al. Critical importance of the first 10 minutes of lung graft reperfusion after hypothermic storage. *Ann Thorac Surg*. 1996;61:1631-5.
18. Sumeria MS, Yellon DM. Ischaemic preconditioning. In: Grace PA, Mathie RT, eds. *Ischaemia-reperfusion injury*. Oxford: Blackwell Science; 1999. p. 328-43.
19. Li G, Chen S, Lu E, Hu T. Protective effects of ischemic preconditioning on lung ischemia reperfusion injury: an ex-vivo rabbit study. *Thorac Cardiovasc Surg*. 1999;47:38-41.
20. Hiratsuka M, Yano M, Mora BN, Nagahiro I, Cooper JD, Patterson GA. Heat shock pre-treatment protects pulmonary isografts from subsequent ischemia-reperfusion injury. *J Heart Lung Transplant*. 1998;17:1238-46.