Carbon monoxide–saturated preservation solution protects lung grafts from ischemia–reperfusion injury

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Objectives: In previous work we have demonstrated that delivery of low concentrations (250 ppm) of carbon monoxide by means of inhalation to donors, recipients, or both protects transplanted lungs from ischemia–reperfusion injury (improved gas exchange, diminished intragraft and systemic inflammation, and retention of graft vascular endothelial cell ultrastructure). In this study we examined whether delivery of carbon monoxide to lung grafts in the preservation solution could protect against lung ischemia–reperfusion injury.

Methods: Orthotopic left lung transplantation was performed in syngeneic Lewis to Lewis rats. Grafts were preserved in University of Wisconsin solution with or without (control solution) carbon monoxide at 4°C for 6 hours. Carbon monoxide gas (5% or 100%) was bubbled into University of Wisconsin solution at 4°C for 5 minutes before use.

Results: In control animals, ischemia–reperfusion injury resulted in significant deterioration of graft function and was associated with a massive cellular infiltrate 2 hours after reperfusion. Grafts stored in University of Wisconsin solution with carbon monoxide (5%), however, demonstrated significantly better gas exchange and significantly reduced intragraft inflammation (reduced inflammatory mediators and cellular infiltrate). Experiments demonstrated that the protective effects afforded by 100% University of Wisconsin solution with carbon monoxide were not as potent as those of 5% University of Wisconsin solution with carbon monoxide.

Conclusions: This study demonstrates that 5% carbon monoxide as an additive to the cold flush/preservation solution can impart potent anti-inflammatory and cytoprotective effects after cold preservation and transplantation of lung grafts. Such ex vivo treatment of lung grafts with carbon monoxide can minimize concerns associated with carbon monoxide inhalation and might offer the opportunity to significantly advance the application of carbon monoxide in the clinical setting.

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Lung transplantation (LTx) outcomes have improved significantly over the last 2 decades, and as a result, LTx is a viable option for many patients with end-stage lung disease. However, although 1-year LTx survival rates have significantly improved to greater than 80%, 5-year survival rates remain poor, and both short- and long-term outcomes lag significantly below those of most other solid organ transplants (Organ Procurement and Transplantation Network).

During the procurement, transport, and transplantation process, donor lung grafts are exposed to an obligatory period of cold ischemia with subsequent warm reperfusion, leading to graft injury (ie, ischemia–reperfusion injury [IRI]). IRI is recognized as a major determinant of primary graft dysfunction, which is a major complication in clinical LTx and contributes significantly to early morbidity and mortality.^{1,2} Furthermore, the severity of IRI (and primary graft dysfunction) has been associated with the risk of having bronchiolitis obliterans syndrome years later, suggesting that the injury to lung grafts at the time of transplantation determines the fate of lung grafts or might increase susceptibility to other events that contribute to subsequent graft dysfunction.²⁻⁵ Thus it is apparent that therapeutic strategies to mitigate IRI of lung allografts

Abbreviations and Acronyms

CO	= carbon monoxide
COX	= cyclooxygenase
HO	= heme oxygenase
ICAM	= intracellular adhesion molecule
IL	= interleukin
iNOS	= inducible nitric oxide synthase
IRI	= ischemia–reperfusion injury
LTx	= lung transplantation
RT–PCR	= reverse transcription–polymerase chain reaction
TNF- α	= tumor necrosis factor α
UW	= University of Wisconsin

could have significant clinical benefit. In addition, the effect of IRI-mitigating strategies could be most profound in the setting of use of marginal donors (and perhaps donation after cardiac death). There has been an increased reliance on marginal donors to meet the growing demand for organ transplantation, and these marginal organs are more susceptible to cold IRI.^{6,7}

A number of therapeutic modalities have been attempted to attenuate IRI after organ transplantation in both experimental and clinical studies.^{8,9} Among these potential therapeutic options, the application of carbon monoxide (CO), a byproduct of the heme oxygenase (HO) system, might be one of the most promising approaches.¹⁰ Indeed, we have shown that CO delivered by means of inhalation at low concentrations to donors, recipients, or both mitigates lung IRI associated with LTx in rats.^{11,12} In these studies CO demonstrated potent anti-inflammatory and cytoprotective effects, resulting in retention of lung endothelial ultrastructure and improved graft function. Translating this strategy to the clinic can be met with significant hurdles, however, given logistical issues, concerns about exposure of health care workers to CO during prolonged inhalational therapy, and concerns regarding the adverse effects of formation of carboxyhemoglobin in recipients. Limiting CO exposure to the graft during the cold ischemic period through saturation of the perfusion and storage solution might be a more clinically applicable strategy and would minimize risk. In this study we tested the hypothesis that delivery of CO to lung grafts through saturation of the preservation solution used for flush and cold storage would prevent lung IRI. We demonstrate that flushing and storing lung allografts in preservation solution bubbled with CO significantly protects lung grafts from IRI and suggest that this approach might advance the application of CO therapy in the clinical setting of LTx.

Materials and Methods**Animals**

Inbred male Lewis (LEW, RT1^l) rats weighing 250 to 300 g were purchased from Harlan Sprague Dawley, Inc (Indianapolis, Ind),

and maintained in laminar flow cages in a specific pathogen-free animal facility at the University of Pittsburgh. Animals were fed a standard diet and provided water ad libitum. All procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee at the University of Pittsburgh and the National Research Council's "Guide for the humane care and use of laboratory animals."

CO Supplementation of Preservation Solution

For each experiment, a total of 50 mL of University of Wisconsin (UW) solution (Viaspan; Du Pont, Wilmington, Del) was vigorously bubbled at 4°C for 5 minutes before use with compressed CO gas mixed in air (5% or 100%; PRAXAIR, Danbury, Conn) under sterile condition in a fume hood.^{13,14}

Rat LTx

Orthotopic left LTx was performed in Lewis recipients from Lewis donors by using a cuff technique, as previously described.^{11,12,15} Briefly, donor rats underwent tracheotomy and were mechanically ventilated with a mixture of 100% oxygen and isoflurane, a positive end-expiratory pressure of 2 cm H₂O, a tidal volume of 10 mL/kg, and a respiratory rate of 70 breaths/min. Donor animals were heparinized (300 U), and a laparosternotomy was performed. The entire lung in the donor was isolated and excised. Lung grafts were flushed through the main pulmonary artery (20 mL, 20 cm H₂O) and stored at 4°C for 6 hours with either control UW solution or 5% or 100% CO-bubbled UW (CO-UW) solution in an inflated state with 100% oxygen. Recipient animals were intubated orotracheally and were ventilated at the same settings as donors. A left thoracotomy was performed, and the lung graft was implanted by using the cuff technique. Sham-operated animals underwent anesthesia and a thoracotomy. All recipients survived surgery and postoperative period until they were sacrificed for end points.

Experimental Groups

Three groups of animals undergoing transplantation and one group of sham-operated animals were studied. Animals in group 1 were perfused and stored with control UW solution (control UW group). In group 2 lung grafts were perfused and stored with 5% CO-UW solution (5% CO-UW group). In the third group 100% CO gas was used for bubbling of UW solution (100% CO-UW group).

Lung graft function

The function of lung grafts was assessed by determining the Po₂ (iSTAT Portable Clinical Analyzer; iSTAT Corp, East Windsor, NJ) of blood drawn from the pulmonary vein of the transplanted lung on a fraction of inspired oxygen of 1.0 during mechanical ventilation. With this technique, the resultant Po₂ value is a reflection of only graft gas exchange (sham group, n = 6; control UW group, n = 7; 5% CO-UW group, n = 6; 100% CO-UW group, n = 5).

Stain for infiltrating neutrophils and ED1⁺ macrophages

Lung graft tissues procured 2 hours after reperfusion were fixed in 10% formalin, embedded in paraffin, and sectioned at a thickness of 6 μ m. Neutrophils in transplanted and contralateral (native) lungs were stained with a naphthol AS-D chloroacetate esterase staining

kit (Sigma Diagnostics, St Louis, Mo). The macrophages were stained by means of immunohistochemistry for ED1, as described previously.¹¹ Positively stained cells were counted in a blinded fashion in 20 high-power fields ($\times 400$ magnification) per section and expressed as the number of cells per 0.1 mm^2 ($n = 5$ for each group).

SYBR Green Real-time Reverse Transcription–Polymerase Chain Reaction

The effect of CO on IRI-induced proinflammatory gene expression was assessed by means of SYBR green real-time reverse transcription–polymerase chain reaction (RT–PCR). The time point was determined based on our previous observation that maximum inflammatory mediator expression occurs 2 hours after reperfusion.¹² Total RNA was extracted from the lung grafts taken 2 hours after reperfusion with the TRIzol reagent (Life Technologies, Inc, Grand Island, NY), according to the manufacturer's instructions. The mRNA for interleukin (IL) 1β , IL-6, tumor necrosis factor α (TNF- α), inducible nitric oxide synthase (iNOS), cyclooxygenase (COX) 2, intracellular adhesion molecule (ICAM) 1, and glyceraldehyde-3-phosphate dehydrogenase were quantified in duplicate by using SYBR Green 2-step, real-time RT–PCR, as previously described.¹⁶ After removal of potentially contaminating DNA with DNase I (Life Technologies), 3 μg of total RNA from each sample was used for reverse transcription with oligo dT (Life Technologies) and Superscript II (Life Technologies) to generate first-strand cDNA. The PCR reaction mixture was prepared by using SYBR green PCR Master Mix (PE Applied Biosystems, Foster City, Calif), with the primers being designed according to the literature or published sequences. Thermal cycling conditions were 10 minutes at 95°C to activate the Amplitaq Gold DNA polymerase, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute on an ABI PRISM 7000 Sequence Detection System (PE Applied Biosystems). Expression of each gene was normalized with glyceraldehyde-3-phosphate dehydrogenase mRNA content (sham group, $n = 6$; control UW group, $n = 7$; 5% CO-UW group, $n = 6$; 100% CO-UW group, $n = 5$).

Data Analysis

Results are expressed as means $\pm$ standard error of the mean. Statistical analysis was performed by using the Student t test or analysis of variance where appropriate.

Results

Dose-dependent Solubility of CO in UW Solution

The solubility of CO in UW solution at 4°C using various CO concentrations was measured by using simplified gas chromatography with a TRI lyzer.^{13,14} Bubbling preservation solution with 5% CO for 5 minutes resulted in a CO concentration of $39.1 \pm 2.4 \mu\text{mol/L}$ in solution. The soluble CO concentration increased in a dose-dependent manner after

Figure 1. A, The solubility of carbon monoxide (CO) in University of Wisconsin solution at 4°C after 5 minutes of bubbling with different concentrations of CO gas ($n = 3$ for each concentration). CO solubility increases in a dose-dependent manner. B, Sequential analysis of CO content in University of Wisconsin solution after 5 minutes of bubbling with 100% CO gas with or without secured, air-tight lids. CO levels were maintained as long as 24 hours when the container of University of Wisconsin solution was sealed without an air phase (filled circles, $n = 3$ for each time point). However, CO concentrations decreased quickly when CO-bubbled University of Wisconsin solution was exposed to air (open circles, $n = 3$).

bubbling with 50% or 100% CO ($578.7 \pm 45.5 \mu\text{mol/L}$ and $1025.3 \pm 78.9 \mu\text{mol/L}$ CO, respectively; Figure 1, A). After bubbling with 100% CO for 5 minutes, the CO concentration in solution was maintained during the cold storage period, when the air phase was removed from the container. In contrast, when UW with CO was allowed to contact air, soluble CO in UW solution was quickly released into the air, and CO levels returned to the basal level within a few hours (Figure 1, B). These results demonstrate that once CO is bubbled into the preservation solution, the CO-bubbled solution must be kept in the tightly sealed container with a secured lid without an air layer to maintain a stable CO concentration

in solution. Subsequent experiments were performed in this fashion.

Flush and Storage of Lung Grafts in 5% CO-UW Solution Significantly Improves Graft Function

Lung graft function was assessed by determining Po_2 levels from the pulmonary veins of grafts at 2 hours after reperfusion. The Po_2 values from the graft pulmonary veins in sham-operated animals were 305.3 ± 13.1 mm Hg during mechanical ventilation. Graft function was markedly impaired 2 hours after reperfusion in grafts stored in control UW solution with Po_2 levels that were less than 30% of those seen in sham-operated animals (80.6 ± 9.4 mm Hg). Grafts flushed and stored in 5% CO-UW solution demonstrated significantly improved graft function, with graft pulmonary vein Po_2 levels of 146.7 ± 19.8 mm Hg (Figure 2, A). The Pco_2 levels in graft pulmonary vein blood were not significantly different among groups at 2 hours after reperfusion (sham group, 30.9 ± 3.1 mm Hg; control UW group, 35.3 ± 3.6 mm Hg; 5% CO-UW group, 31.8 ± 3.5 mm Hg; Figure 2, B).

5% CO-UW solution Significantly Reduces Neutrophil and Macrophage Infiltration in Lung Grafts

Neutrophil infiltration plays an important role in the inflammatory response of IRI through, among other processes, enhancing graft parenchyma damage by secreting proteolytic enzymes, such as elastases,^{17,18} and physically impairing the microcirculation. Although pulmonary neutrophils were scarce in the sham-operated animals, a number of myeloperoxidase-positive cells were noted in pulmonary grafts after cold storage in control UW solution and reperfusion, suggesting that IRI caused rapid neutrophil infiltration in the grafts. Flush and storage of grafts in CO-UW solution (5%) resulted in significantly reduced neutrophil infiltration in the grafts at 2 hours after reperfusion (Figure 3).

Macrophages activated during the IRI process initiate a cascade of events leading to the activation of the recipient inflammatory system, and play a central role in lung IRI.^{19,20} The number of ED1⁺ macrophages in control UW grafts increased to 52.7 ± 1.2 per 0.1 mm^2 2 hours after reperfusion from 17.3 ± 0.5 per 0.1 mm^2 in sham-operated animals. Lung grafts stored in CO-UW (5%) solution demonstrated significantly reduced numbers of ED1⁺ macrophages (32.9 ± 0.8 per 0.1 mm^2 ; Figure 4).

5% CO-UW Solution Significantly Reduces Expression of mRNA for Inflammatory Mediators in Lung Grafts

Lung IRI results in the rapid production and release of numerous proinflammatory mediators, as well as upregulation of adhesion molecules.^{21,22} We evaluated the effect of CO supplementation in the preservation solution on inflammatory

Figure 2. A, The pulmonary vein Po_2 level of the sham-operated animals was 305.3 ± 13.1 mm Hg. The graft pulmonary vein Po_2 level of the control University of Wisconsin (UW) group was markedly reduced (80.6 ± 9.4 mm Hg) at 2 hours after reperfusion. Lung grafts in the 5% CO-UW group showed significantly higher Po_2 levels compared with those of the control UW group at 2 hours after reperfusion (5% CO-UW group, 135.9 ± 19.8 mm Hg; * $P < .05$ vs control UW group). **B,** The Pco_2 levels of graft pulmonary veins were not significantly different between groups.

mediator expression in lung grafts after IRI with SYBR green real-time RT-PCR. In grafts stored in control UW solution, mRNA levels for the proinflammatory cytokines IL-6, IL-1 β , and TNF- α ; the stress-induced molecules iNOS and COX-2; and the adhesion molecule ICAM-1 were significantly upregulated within 2 hours after reperfusion. Flush and storage of grafts in 5% CO-UW solution significantly reduced peak levels of proinflammatory mediator mRNA by

Figure 3. Neutrophil infiltration of lung grafts 2 hours after reperfusion. Prolonged cold storage and reperfusion after transplantation resulted in massive infiltration of myeloperoxidase-positive neutrophils in control grafts. Flush and storage of lung grafts in University of Wisconsin (UW) solution bubbled with 5% carbon monoxide (CO) significantly reduced the number of neutrophils seen in the graft (* $P < .05$ vs control UW solution). (Original magnification $\times 400$). PMN, Polymorphonuclear neutrophil.

35% to 65% compared with those seen in the grafts stored in control UW solution (Figure 5).

100% CO-UW Solution Does not Provide Further Improvement in Graft Function After IRI

To evaluate whether treatment with a higher concentration of CO might provide additional graft protection compared with

5% CO, we investigated the effects of supplementation of the preservation solution with 100% CO (resulting in a 25 times higher concentration of CO in solution). Although flush and storage of grafts with UW solution bubbled with 100% CO improved graft function compared with that seen in control grafts, it did not improve graft function beyond that achieved with 5% CO-UW solution: PO_2 levels of graft pulmonary vein

Figure 4. ED1⁺ macrophage recruitment to lung grafts. The number of infiltrating alveolar ED1⁺ macrophages remarkably increased in control University of Wisconsin (UW) solution at 2 hours after reperfusion. Carbon monoxide (CO)-UW (5%) solution significantly reduced macrophage infiltration (* $P < .05$ vs control UW group). (Original magnification $\times 400$).

Figure 5. Intragraft mRNA levels of inflammatory mediators. mRNA for the proinflammatory cytokines interleukin 6 (*IL-6*), interleukin 1 β (*IL-1 β*), and tumor necrosis factor α (*TNF- α*); the stress-induced molecules inducible nitric oxide synthase (*iNOS*) and cyclooxygenase 2 (*COX-2*); and the adhesion molecule intercellular adhesion molecule 1 (*ICAM-1*) were rapidly upregulated within 2 hours after reperfusion in control animals. 5% Carbon monoxide (CO)–University of Wisconsin (UW) solution significantly reduced expression of these inflammation-related molecules (* $P < .05$ vs the control UW group).

stored in 100% CO-UW solution were 147.8 ± 15.8 mm Hg, whereas those of 5% CO-UW solution were 146.7 ± 19.8 mm Hg; (Figure 6, A).

Effects of 100% CO-UW Solution on Inflammatory Cellular Recruitment

Although 5% CO-UW solution had potent effects on the prevention of graft inflammatory cellular infiltration, the number of neutrophils in the grafts stored in 100% CO-UW solution was not different from that seen in grafts stored in control UW solution (Figure 6, B). Similarly, the number of ED1⁺ macrophages in the grafts stored in 100% CO-UW solution was 47.1 ± 1.2 per mm², which was significantly more than those seen in grafts stored in 5% CO-UW solution (Figure 6, C).

Alteration of mRNA Levels for Inflammatory Mediators With 100% CO-UW Solution

The anti-inflammatory effects of 100% CO-UW solution were also evaluated by determining mRNA expression for inflammatory mediators. As previously demonstrated, 5% CO-UW solution significantly reduced the peak levels of mRNA expression of inflammatory mediators. The effects of 100% CO-UW solution were comparable with those of 5% CO-UW solution in inhibition of iNOS, COX-2, and ICAM-1

levels. In contrast, 100% CO-UW solution did not reduce mRNA levels of IL-6, IL-1 β , and TNF- α compared with those seen in the grafts stored in control UW solution (Figure 7).

Discussion

CO has been increasingly recognized as a pleiotropic molecule with many properties, including a role in fundamental cell-signaling processes, as well as cytoprotective and anti-inflammatory properties that mitigate IRI. Although basal low levels of CO are produced endogenously in mammalian tissues through the action of the enzyme HO on heme, in the setting of oxidative stress of transplantation (as well as other scenarios), numerous strategies have been used to manipulate this system to mitigate IRI in various organ types. The use of chemical HO inducers,²³ gene therapy to overexpress HO,²⁴ and the delivery of CO directly by means of inhalation^{16,25} or the use of CO-releasing molecules^{26,27} are therapeutic strategies that have all demonstrated efficacy in experimental transplantation models of IRI. Although many or all of these therapeutic approaches might prove clinically feasible, they all create logistic difficulties, as well as (in the case of CO inhalation delivery) the potential for exposure of patients and health care workers to toxic doses of CO. To simplify delivery of CO and to avoid recipient exposure to higher doses, in the

Figure 6. The effects of a higher concentration of carbon monoxide (CO) on pulmonary graft function and cellular infiltration. **A**, Graft function, as assessed by means of graft oxygenation, was no different between grafts flushed and stored with 5% CO–University of Wisconsin (UW) solution compared with those flushed

current study we hypothesized and evaluated whether delivery of CO to lung grafts through a CO-saturated flush and perfusate solution would mitigate IRI in experimental LTx. Our data demonstrate that flush and cold storage of lung grafts in preservation solution bubbled with 5% CO diminishes IRI, resulting in significantly improved graft function and reduced graft inflammation. Notably, the therapeutic effect observed in this study is comparable with the effect we observed in previous studies in which low-dose CO (250 ppm) was delivered by means of inhalation to both lung donors and recipients (beginning before transplantation and continuing after transplantation in recipients). Another important aspect of the current study is the demonstration that CO is rapidly (over hours) released from solution if an air–solution interface exists. Thus protocols using this strategy must use airtight containers and avoid an air–solution interface.

We have previously shown that storing and flushing intestinal grafts¹³ and kidney grafts²⁸ in CO-supplemented UW solution prevents the graft deterioration associated with IRI. In our previous studies^{13,28} we applied 5% CO-UW solution (achieving a CO concentration of 40.6 $\mu\text{mol/L}$) based on the results of previous studies using CO-releasing molecules, showing that CO concentrations of 10 to 50 $\mu\text{mol/L}$ in perfusate provided the best results in protection of cardiomyocytes²⁶ and the renal vascular system²⁹ against oxidative stress. In this study we provide evidence that higher concentrations of CO in solution (achieved through bubbling UW solution with 100% CO) do not enhance the protective effects seen with 5% CO gas. This might be an important step for the clinical application of CO as an additive to the preservation solution to determine the most appropriate CO concentration in solution. Our previous work demonstrated that a CO concentration of 2.15 $\mu\text{mol/L}$ (bubbling with 0.1% CO gas) did not improve intestinal cold IRI.¹³ Based on these results, we will further optimize CO concentrations in preservation solutions in future experiments to establish an optimized, clinically applicable, effective, and safe ex vivo CO delivery method.

Although the mechanisms involved in the protection afforded by ex vivo CO delivery are not fully elucidated, we postulate one possible mechanism of the binding activity of CO with heme protein. CO possesses a strong affinity to

with 100% CO-UW solution. Both groups demonstrated improved graft function compared with that seen in control grafts. **B**, The number of infiltrating neutrophils was significantly reduced when the grafts were stored in UW solution with supplementation of 5% CO gas. However, the effects of 100% CO-UW solution were marginal in preventing neutrophil recruitment (* $P < .05$ vs control UW solution, # $P < .05$ vs 5% CO-UW solution). **C**, Although 5% CO-UW solution significantly reduced the number of ED1⁺ macrophages in the grafts, 100% CO-UW solution did not alter macrophage recruitment in the graft lung 2 hours after reperfusion (* $P < .05$ vs control UW solution, # $P < .05$ vs 5% CO-UW solution).

Figure 7. Real-time RT-PCR analysis for inflammatory mediators 2 hours after reperfusion. The upregulation of mRNA for inflammatory mediators was significantly inhibited when lung grafts were perfused and stored in University of Wisconsin (UW) solution with 5% carbon monoxide (CO). The effects of 100% CO had comparable effects in preventing interleukin 6 (IL-6), inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and intercellular adhesion molecule 1 (ICAM-1) mRNA upregulation; however, the anti-inflammatory effects of 100% CO were marginal on tumor necrosis factor α (TNF- α) and interleukin 1 β (IL-1 β) mRNA levels (* P < .05 vs control UW solution, # P < .05 vs 5% CO-UW solution).

the heme moiety of heme proteins and potentially influences the biologic behaviors of heme proteins.^{30,31} Bysani and colleagues as well as others have demonstrated that cytochrome P450, an abundant heme protein in the lung, degrades during IRI and release of heme.³²⁻³⁴ Free heme released from heme proteins, such as cytochrome P450, during IRI not only promotes peroxidation of the lipid membranes of the cells^{35,36} but also activates adhesion molecules, leading to massive cellular infiltration and increases in vascular permeability, contributing to the pathogenesis of local inflammatory processes.³⁶⁻³⁹ In addition, free heme can be a major source of iron, which participates in the generation of more detrimental hydroxyl radicals from hydrogen peroxide through the Fenton reaction.^{40,41} We have shown that 5% CO-UW solution could prevent degradation of cytochrome P450 and prevent heme/iron release during IRI in the kidney.²⁸ Our observations in this study might support the role of CO in binding heme protein and inhibiting heme/iron-derived graft injuries. Although we do not have a clear explanation why 100% CO did not prevent IRI, we postulate that high concentrations of CO might be harmful by completely inhibiting the enzymatic function of remaining cytochrome P450, altering

other critical heme proteins required to maintain cell viability, or releasing CO into the recipient circulation after transplantation. In addition, Taille and associates⁴² showed that CO modulates redox signaling by interacting with the heme moiety of NAD(P)H oxidase, the respiratory chain, or both.

In summary, our results demonstrate that the addition of CO to the preservation solution used for flush and cold storage reduces IRI in lung grafts, resulting in improved graft function and reduced inflammation. This approach is straightforward, could be easily adapted in the clinic, and could potentially have a positive effect on patient care in the early posttransplantation period and improve long-term outcomes.

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