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Enhanced mtDNA Repair Resuscitates Transplantable Lungs Donated After Circulatory Death

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Abstract

Background: Transplantation of lungs procured following donation after circulatory death (DCD) is challenging because post-mortem metabolic degradation may engender susceptibility to ischemia-reperfusion (IR) injury. Since oxidative mtDNA damage has been linked to endothelial barrier disruption in other models of IR injury, here we used a fusion protein construct targeting the DNA repair glycosylase OGG1 to mitochondria (mt-OGG1) to determine if enhanced repair of mtDNA damage attenuates endothelial barrier dysfunction after IR injury in a rat model of lung procurement after DCD.

Materials and Methods: Lungs excised from donor rats 1h after cardiac death were cold-stored for 2h after which they were perfused *ex vivo* in the absence and presence of mt-OGG1 or an inactive mt-OGG1 mutant. Lung endothelial barrier function and mtDNA integrity were determined during and at the end of perfusion, respectively.

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Author Contributions: Y.B.T. executed experiments, analyzed data, and contributed to writing the manuscript. V.M.P. and O.M.G. conducted analyses of mtDNA damage and DAMPs. M.S.M. performed statistical analyses. J.D.S., T.N.M., and T.M.B. contributed to conception of the study, design of the experiments and writing of the manuscript. G.L.W. oversaw production of reagents, including the mtDNA repair proteins, and contributed to writing the manuscript. M.N.G. conceived of the study, contributed to data analysis, preparation of figures, and writing the manuscript.

Author Disclosure Statement: M.N.G. and G.L.W. are co-founders and part-owners of Exscien Corp., the start-up biotech company that produced mitochondrial targeted OGG1 and mutant OGG1 fusion proteins used in this research. The remaining authors have no relevant disclosures.

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Competing Interests

M.N.G. and G.L.W. are co-founders and part-owners of Exscien Corp, the start-up biotech company producing mitochondrial targeted OGG1 and mutant OGG1 fusion proteins used in this research. The remaining authors report no Competing Interests.

Results and Conclusions: Mitochondria-targeted OGG1 attenuated indices of lung endothelial dysfunction incurred after a 1h post-mortem period. Oxidative lung tissue mtDNA damage as well as accumulation of pro-inflammatory mtDNA fragments in lung perfusate, but not nuclear DNA fragments, also were reduced by mitochondria-targeted OGG1. A repair-deficient mt-OGG1 mutant failed to protect lungs from the adverse effects of DCD procurement. Collectively, these findings suggest that endothelial barrier dysfunction in lungs procured after DCD is driven by mtDNA damage and point to strategies to enhance mtDNA repair in concert with EVLP as a means of alleviating DCD-related lung IR injury.

Keywords

Lung IR injury; mtDNA damage; lung transplant; mtDNA DAMPs

Introduction

Lung transplantation is the only treatment option for end-stage lung diseases. However, the discrepancy between the number of donor lungs available and the number of lungs needed continues to grow as new indications for lung transplantation emerge and improved outcomes combine to expand of the number of patients who could benefit. In addition to the damage incurred from mechanisms of death and modes of treatment, pre-mortem factors such as infection, pulmonary edema, and vascular thrombosis contribute to the low utilization rate of donor lungs. Only 10–15% of lungs procured are judged clinically suitable for transplantation due to a subjective but rigorous selection process based on physician experience and physiological parameters.¹

Despite efforts to optimize clinical outcomes, recipients continue to be at risk due to post-transplantation complications such as Primary Graft Dysfunction (PGD)², which occurs in up to 20% of lung transplant patients. DELETED SENTENCE Persuasive evidence suggests that oxidant stress from ischemia-reperfusion (IR) injury contributes to the evolution of PGD.^{2–4} The target molecule(s) linking IR-related oxidant stress to PGD and associated activation of inflammatory cascades has not been thoroughly elucidated. Multiple observations, however, suggest that the mitochondrial genome may serve such a sentinel function. For example, unlike the nuclear genome, mtDNA is highly susceptible to oxidant-induced damage and mutations.^{5, 6} The mitochondrial genome encodes some of the proteins required for oxidative phosphorylation-dependent ATP production and for oxygen radical-dependent mitochondrial signaling; thus, disruption of mitochondrial transcription engenders a bioenergetic crisis with attendant cytotoxicity and altered cell signaling.^{7–11} Finally, oxidative mtDNA damage may lead to fragmentation of the molecule into mtDNA damage associated molecular patterns (DAMPs)^{12, 13} that via several nucleic acid receptors¹⁴ activate the innate immune response with the potential to propagate injury to local or distant cellular targets.^{12, 15, 16}

Donation after circulatory death (DCD) represents an underutilized source of transplantable lungs.¹⁷ The practice has been adopted clinically, but post-mortem lungs continue to comprise a small percentage of the lungs transplanted¹⁸ in part because of concerns that post-mortem degradation of physiologic function will jeopardize transplant outcomes.^{19–21}

The introduction of *ex vivo* lung perfusion (EVLP), which provides the ability to monitor and improve physiological performances of marginal lungs seems particularly well-suited for establishing the utility and possible reconditioning of lungs obtained in the setting of DCD.^{22, 23} The procedure also offers a means to selectively apply pharmacological therapy to donor lungs without the requirement of drug administration to the transplant recipient.²⁴ Given the potential role for mtDNA damage in IR injury, here we used a rat model of EVLP after DCD procurement to test the interrelated hypotheses that oxidative mtDNA damage is a determinant of the vulnerability of DCD lungs to IR injury and that pharmacologic enhancement of mtDNA repair using a fusion protein construct targeting 8-oxoguanine DNA glycosylase-1 (OGG1) to mitochondria improves resistance of DCD-derived lungs to IR injury.

Materials and Methods

Experimental protocol:

The experimental protocol is summarized in FIGURE 1. Experiments involving rats were carried out under a protocol approved by the Institutional Animal Care and Use Committee in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory animals. In brief, three groups of male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were anesthetized with an intraperitoneal injection of pentobarbital (50 mg) after which the animals were anticoagulated with an intra-cardiac injection of heparin sulfate (500 U). Animals were then euthanized with ascending doses (50 mg increments) of intraperitoneal injections of pentobarbital. One group served as control and, after complete cessation of cardiac activity the heart and lungs were immediately removed *en bloc*, placed in sterile plastic bags, and stored in a refrigerator at 4°C for 120 min. Subsequently, they were subjected to *ex vivo* lung perfusion. For the other two groups, the hearts and lungs were treated identically to controls except that during *ex vivo* perfusion, when perfusate temperature reached 30°C, mt-OGG1 or mutant OGG1 was added to the perfusion medium to achieve a final concentration of 10 ug/ml. At termination of lung perfusion, perfusate was reserved for isolation of cell free DNA for quantification of mtDNA and nuclear DAMPs. Tissue was harvested from the left lung for determination of oxidative mtDNA damage, and the right lung was reserved for determination of the wet-to-dry weight ratio. The methods for *ex vivo* lung perfusion, physiologic evaluation and assessment of mt and nuclear DAMP abundance and mtDNA integrity are described below.

Isolated lung perfusion:

Isolated lungs were perfused as described previously.²⁵ In brief, after procurement, lungs were ventilated at 60 breaths / minute with a gas mixture of 21% O₂, 5% CO₂ in N₂ using 5 cmH₂O positive end expiratory pressure, 2.5 mL tidal volume and with a Harvard rodent ventilator (Harvard Apparatus). The pulmonary artery and left ventricle were cannulated and the pulmonary circulation was flushed in antegrade and retrograde directions with physiological salt solution (in mM: NaCl, 119.0; KCl, 4.7; MgSO₄•7H₂O, 1.2; NaHCO₃, 22.6; KH₂PO₄, 1.2; Glucose, 5.5; and CaCl₂•2H₂O, 2.2) containing 4% albumin. After flushing, the vasculature was clamped, the lungs were inflated to approximately 60% total lung capacity, and the entire preparation stored at 4°C for 2h. Subsequently, lungs were

mounted in a warmed, humidified chamber and perfusion with warmed (37°C) physiological salt solution (composition noted above) was gradually increased to a rate of 0.04 ml/min/g body weight. Ventilation with the gas mixture, also noted above, was instituted when the lung perfusate reached 30°C. Lung weight and pulmonary arterial and venous pressures were recorded in real time with force displacement (Grass FT, West Warwick, RI) and pressure transducers (Cobe, Lakewood, CO), respectively, on a polygraph (Model 7F Grass, West Warwick, RI). Vascular filtration coefficients (K_f) were calculated at 60 ($K_f(1)$) and 120 ($K_f(2)$) minutes after the onset of perfusion as described previously.¹²

Wet to dry lung weight ratio:

In some experiments, the right lung was isolated and weighed immediately after termination of perfusion. The lung was then allowed to dry in a clean environment at room temperature for one week and the weight was reassessed. The weight of the wet lung was divided by the weight of the dry lung to calculate the wet to dry weight ratio as an index of total lung water.

Wild type and mutant OGG1 recombinant fusion proteins:

The mt-targeted OGG1 fusion protein was provided by Exscien Corp and produced according to previously described methods.²⁶ The protein construct consisted of a TAT sequence to facilitate cellular entry, a mitochondrial targeting sequence to promote mitochondrial uptake, and the mammalian DNA glycosylase, OGG1, or a DNA repair-inactive OGG1 mutant.^{25, 26} Two other constituents of the fusion proteins were a histidine tail, required to isolate the protein from culture medium, and a hemagglutinin tag for immunodetection. Both proteins were produced in the proprietary *Clear Coli* BL21(DE3)® strain of *E. coli* (Research Corporation Technologies, Tucson, AZ), genetically engineered by the manufacturer to express biologically-inactive endotoxin. Cellular uptake and mitochondrial localization of the fusion protein have been confirmed on previous occasions.^{25–27}

Determination of DNA abundance in perfusion medium:

Upon termination of perfusion, 15 mL of perfusate was collected and centrifuged at 10,000 X *g* at 4°C for 10 minutes to remove intact mitochondria and other cellular debris. Ten mL of supernatant was transferred into a sterile vial and stored at –20°C until further processing. After samples were thawed, DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) as per manufacturer's protocol. The extracted DNA was stored at –20°C. Also using previously-described methods¹², quantitative real time polymerase chain reaction (PCR) was carried out on an iCycler iQ5 (Bio-Rad, Hercules, CA) using USB VeriQuest Fast SYBR Green qPCR Master Mix with Fluorescein Kit (Afflymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol. PCR primers were designed with Beacon Designer 8.2 software (PREMIER Biosoft International, Palo Alto, CA) and were as follows: D-loop region of the mitochondrial genome, forward 5'-AGGCATCTGGTTCTTACTTCA-3', reverse 5'-TTGACGGCTATGTTGAGGAA-3', and nuclear 28S RNA, forward 5'-GATTCCCACTGTCCCTACC-3', reverse 5'-ACCTCTCATGTCTCTTACC-3'. Standard curves were generated using precisely quantified amounts of total rat lung DNA.

Quantitative Southern blot analysis of oxidative mtDNA damage:

Southern blot analysis was performed as previously reported.²⁸ Briefly, the left lung was snap-frozen in liquid nitrogen upon termination of perfusion and stored at 80°C. Purified DNA samples were isolated from powdered snap-frozen lung tissue using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA), after which the samples were digested with restriction enzymes *Pvu*MI and *Aha*II overnight at 37°C. To reveal oxidative damage, DNA samples were treated with formamidopyrimidine glycosylase (Fpg; New England Biolabs, Beverly, MA), which cleaves the strand at sites of 8-oxoguanine (8-oxoG) and other closely related oxidative base damage products. Fpg treated and untreated samples were incubated with 0.1N NaOH for 15 min at 37°C, and resolved in 0.6% agarose alkaline gel. After electrophoresis, the DNA was transferred to a nylon membrane (Sigma, St. Louis, MO) and hybridized with a PCR-generated DIG (digoxigenin 11 dUTP) -labeled probe (Sigma, St. Louis, MO) to a 13.6 kb sequence of mtDNA prepared as described above. Hybridization bands were detected with a Gel Logic 1500 Imaging System (Kodak, Rochester, NY) and densitometry was performed to quantify hybridization intensities in the Fpg-treated and untreated bands. The degree to which hybridization intensity is reduced by Fpg treatment reflects the presence and extent of oxidative DNA damage.

Statistical analyses:

Data were collected from rats classified into five different groups with each group receiving a different treatment. One-way analyses of variance with unequal group sizes was performed to compare mean outcomes. Post hoc analysis was performed using Tukey's HSD for pairwise comparisons.²⁹ Ninety-five % confidence intervals were constructed for differences in mean levels for ten pairs of groups. P values <0.05 were considered to denote statistically significant differences. Statistical analysis was conducted using JMP v14.2.0 analytical software (SAS Inc., Cary, NC). Data are displayed as scatter plots, with each point representing a single observation, and include bars reflecting mean values $\pm$ 95% confidence limits.

Results

Mitochondrial-targeted OGG1 protects lung endothelial barrier function from injury incurred by DCD procurement:

Initial experiments assessed lung endothelial barrier function in terms of the K_f at two time points, 60 K_f (1) and 120 minutes K_f (2) after initiating *ex vivo* perfusion. Paired *t*-tests applied to K_f (1) and K_f (2) values performed for each lung, regardless of experimental group failed to detect significant differences between the two time points (data not shown). Accordingly, K_f (1) and K_f (2) values for each lung were pooled for display (denoted as " K_f ") and for subsequent statistical analyses.

To determine if the post-mortem period impacted lung endothelial barrier function, K_f values were compared between lungs procured immediately or 60 min after cessation of cardiac activity. As shown in FIGURE 2, while mt-OGG1 fusion protein added to the perfusate of control lungs (0 post-mortem period) reduced K_f in comparison to control preparations not treated with the fusion protein, addition of mt-OGG1 inhibited the rise K_f attributed to the

post-mortem storage period in comparison to both un-treated and mt-OGG1-treated controls. In contrast, mutant OGG1 fusion protein failed to attenuate the rise in K_f increase associated with the post-mortem period.

Pulmonary edema formation was assessed in terms of changes in the lung wet-to-dry weight ratio. FIGURE 2 shows that the post-mortem period was accompanied by an increase in the wet-to-dry weight ratio relative to lungs excised immediately after cessation of donor cardiac activity. Importantly, mt-OGG1 administered during EVLP restored post-mortem wet-to-dry weight ratios to levels comparable to rats with no post-mortem period. Mutant OGG1 fusion protein failed to suppress edema formation in lungs from post-mortem rats. Similar to its effects on baseline K_f values, there was a non-significant tendency for mt-OGG1 fusion protein to reduce wet-to-dry lung ratio in rat lungs procured without a post-mortem period.

Mitochondrial-targeted OGG1 selectively reduces post-mortem-induced accumulation of mtDNA DAMPs in perfusate:

The amount of mtDNA DAMPs in the perfusate was determined using quantitative PCR to assess the concentration of a 200 bp fragment of the D-loop region. As shown in FIGURE 3, the post-mortem period was accompanied by increased mtDNA DAMP concentration in the perfusate. Addition of mt-OGG1 fusion protein reduced the mtDNA DAMP concentration in the post-mortem lung perfusate to control levels. Mutant OGG1 failed to reduce the amount of mtDNA accumulating as a result of the post-mortem period. Finally, mt-OGG1 added to the medium perfusing control rat lungs significantly reduced the concentration of mtDNA fragments in comparison to control lungs perfused without OGG1.

Nuclear DNA fragments in perfusion medium were quantified using PCR analysis of a sequence encoding 28S ribosomal RNA. Also as shown in FIGURE 3, relative to control the post-mortem period was accompanied by increased nuclear DNA concentration in the perfusate. Notably, however, addition of mt-OGG1 fusion protein to the perfusate failed to suppress the increase in 28S DNA abundance in post-mortem lungs, in contrast to its inhibitory effect on the rise in mtDNA D-loop fragments.

Mitochondrial-targeted OGG1 reduces mtDNA damage in lung tissue:

Changes in the equilibrium density of oxidative mtDNA base damage were calculated from hybridization intensities derived from quantitative Southern blot analyses of Fpg-detectable lesions in whole lung tissue. In this analysis, a decrease in the fraction of intact mtDNA is indicative of increased oxidative base damage. As shown in FIGURE 4, the extent of base damage in cold-stored lungs was elevated by the post-mortem period relative to cold-storage without DCD procurement. Addition of mt-OGG1 during EVLP in lungs harvested after a post-mortem period significantly inhibited mtDNA damage, while addition of mt-OGG1 during EVLP in lungs harvested immediately after cardiac death failed to impact lung tissue mtDNA integrity. Mutant OGG1 fusion protein did not reduce mtDNA damage levels in DCD, cold-stored lungs.

Discussion

Lung transplantation as a means to treat advanced lung disease is limited by the paucity of donor lungs available. While DCD lungs are an underutilized resource, enthusiasm remains somewhat muted due to concerns that post-mortem degradation and physiological changes lead to subpar lungs and unacceptable transplant outcomes.³⁰ Since its clinical introduction by Cypel et al. in 2011,³¹ EVLP has partially alleviated the shortage of donor lungs by providing an extended window of time for evaluation and possible physiologic improvement of lungs which may have otherwise been rejected for transplantation.^{22, 23} Although EVLP allows for monitoring the physiologic performance of excised lungs, an additional benefit may lie in its ability to apply pharmacologic interventions exclusively to the donor lung without need for administration to the recipient.^{20, 21}

Ischemia-reperfusion injury is marked by increased in oxidant generation, which leads to degradation of physiologic integrity by multiple signaling pathways.³² Mitochondrial dysfunction, ostensibly induced by oxidant stress, and its prevention by mitochondrially-targeted anti-oxidants³³ and intact mitochondrial transfer³⁴ has been demonstrated in IR injury models,⁴ but the specific molecular target(s) within the organelle are unknown. Mitochondrial DNA is interesting in this regard due to its well-documented sensitivity to oxidant-mediated damage^{5, 6} and findings that transgenic modulation of mtDNA repair efficiency exerts coordinate effects on oxidant-induced mtDNA damage and cytotoxicity.^{35–37} Consistent with the concept that mtDNA may be a key sentinel molecule in which damage activates downstream cellular responses, recombinant fusion proteins targeting either OGG1 or the bacterial DNA glycosylase Endo III to mitochondria prevent oxidant-induced cytotoxicity and suppresses organ dysfunction in perfused rat lungs, myocardial infarction in rats, stroke in rats, hyperoxia-induced dysmorphogenesis in newborn rat lungs, ventilator-induced lung injury in mice, pneumonia-related acute respiratory distress syndrome (ARDS) and multiple organ system failure (MOSF) in rats.^{27–33} Pharmacological enhancement of mtDNA repair also suppresses release of pro-inflammatory mtDNA DAMPs and propagation of mtDNA damage culminating in activation of Toll-like Receptor 9 to initiate a regenerative cycle of mtDNA damage and DAMP release culminating in ARDS and MOSF.^{12, 38}

We examined the postulated sentinel function of mtDNA damage in the context of physiologic degradation of lungs produced after DCD, reasoning that this particular mode of lung donation could benefit from a targeted pharmacologic strategy to improve organ quality. Evidence supporting involvement of mtDNA damage in lung physiologic degradation after DCD obtained in the present study is the association between physiologic abnormalities, oxidative base damage in the mitochondrial genome, and accumulation of mtDNA fragments in lung perfusate. Using the K_f as a quantitative indicator of endothelial barrier properties, we found that a 1h period of no cardiac activity followed by lung removal, cold storage and perfusion was accompanied by increases in endothelial permeability that exceeded increases displayed by lungs removed immediately from the donor. This finding was also reflected in lung wet-to-dry weight ratios, which indicate the severity of pulmonary edema formation. Oxidative base damage in mtDNA also was enhanced in cold stored lungs obtained after DCD as evidenced by the greater decrease in the fraction of intact mtDNA

after oxidized base excision and strand cleavage with Fpg relative to the magnitude of intact mtDNA from lung procured in the absence of DCD. Finally, accumulation of mtDNA DAMPs in the perfusion medium was increased after a 1h post-mortem period. Nuclear DNA fragments also were elevated in medium perfusing lungs obtained after DCD, but as described subsequently, this increase was unaffected by enhanced mtDNA repair.

In direct support of the contention that mtDNA damage serves as a molecular sentinel driving physiologic degradation of the lungs after DCD, we found that addition of mt-OGG1 fusion protein to the perfusion medium during EVLP restored both endothelial barrier integrity and the level of oxidative mtDNA damage level to values not significantly different from lungs harvested immediately after death of the donor. Mitochondrial-targeted OGG1 also reduced the rise in perfusate concentration of mtDNA DAMPs, but not nuclear DNA fragments, observed after DCD procurement. The failure of the fusion protein to diminish perfusate nuclear DNA fragments originating from the 28S RNA nuclear sequence attests to the specificity of the repair protein construct for the mitochondrial genome, which has been reported previously^{25, 38}. Additional evidence that oxidative mtDNA damage triggered lung endothelial barrier dysfunction after DCD is derived from the finding that mutant OGG1, devoid of DNA glycosylase activity, failed to suppress changes in barrier function, mtDNA damage or mtDNA DAMP accumulation in perfusate.

Earlier reports showed that mtDNA DAMPs are released into the perfusion medium of bacteria-challenged lungs as a consequence of oxidative damage to the mitochondrial genome. Mitochondrial DNA DAMPs so released appear to engage a feed-forward pathway involving the production of additional mtDNA damage leading to regenerative mtDNA DAMP production that culminates in edema formation. Interruption of the postulated pathway by blocking end-organ receptors for mtDNA DAMPs, specifically TLR9, or degrading mtDNA DAMPs with DNase1 or suppressing oxidative mtDNA damage using the mitochondrial-targeted DNA repair enzyme protein all suppressed the degradation of endothelial barrier function evoked in bacteria-challenged rat lungs.³⁸ To this, the present results imply that IR injury in the setting of DCD lung perfusion may involve a similar pathway of mtDNA damage and mtDNA DAMP release. If true, this points to several new pharmacologic targets to preserve integrity of lungs procured after DCD, including blockade of TLR9, increased mtDNA DAMP degradation with DNase, or repair of oxidative mtDNA to forestall regenerative mtDNA DAMP release, each of which could be applied during EVLP.

While the present observations are consistent with the idea that repair of the oxidatively-damaged mitochondrial genome underlies the protective actions of mitochondrial-targeted OGG1, other mechanisms also should be considered. For example, mtDNA fragment are but one of several DAMPs originating from the organelle¹⁴. Based on the present observations, the possibility cannot be excluded that protection of mtDNA integrity prevents release of other mitochondria-derived DAMPs which serve as proximate mediators of IR-related tissue damage. Another potential mechanism is supported by observations by Boldogh and coworkers, who demonstrated that OGG1 forms a complex with free 8-oxoG, ostensibly liberated by the OGG1-dependent glycosylase activity directed at 8-oxoG in the duplex DNA. The OGG1/8-oxoG complex so formed serves as a guanine nucleotide exchange

factor activating proinflammatory *Ras* signaling pathways.^{39–41} Mitochondrial-targeted OGG1 fusion protein could thus exert protective effects in DCD-mediated IR injury by interrupting *Ras*-mediated signaling through functioning as a storage sink for free 8-oxoG. Arguing against such a mechanism is the present finding that mutant OGG1 fusion protein, which differs at the glycosylase active site but not at the site binding free 8-oxoG, failed to demonstrate protective benefits similar to mt-OGG1. Another unresolved issue related to the mechanism of fusion protein action relates to its cellular target(s). While over-expression of mitochondrial-targeted OGG1 protects cultured pulmonary vascular endothelial cells from oxidant-mediated mtDNA damage and cytotoxicity³⁵, and although endothelial barrier properties are the principle determinant of Kf, communication between lung cell populations is known to govern endothelial permeability after various insults.^{42, 43} Identification of the lung cell populations harboring oxidative mtDNA damage and targeted by OGG1 in the setting of DCD-related IR injury will require different strategies than those described herein.

In summary, results of the present study support the hypotheses that damage to the mitochondrial genome plays an important role in the pathophysiology of endothelial barrier degradation in DCD lungs subjected to EVLP. Further, since the current study used a rat model wherein cessation of cardiac activity was preceded by anticoagulation and followed by a post-mortem period of warm ischemia, our observations may be relevant to the commonly-encountered clinical situation where a significant period of time elapses between the in-hospital expiration of a donor and lung procurement. In this setting, protection against oxidative mtDNA damage during EVLP has the potential to increase the rate of conversion of DCD lungs to transplantable status.

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FIGURE 1:
Schematic time-line of experimental protocol. See text for details.

FIGURE 2:

Scatter plots showing vascular permeability coefficients (K_f) (TOP Panel) and lung wet-to-dry weight ratios (BOTTOM Panel) measured in lungs perfused without a post-mortem period (0 PM) or after 60 min post mortem (60 PM) in the absence and presence of 10 μ g/ml OGG1 (+OGG1) or repair-deficient mutant OGG1 (+mOGG1) added to the perfusate reservoir at the start of perfusion. Each point represents a single experiment. Bars represent means $\pm$ 95% confidence interval. P values are indicated under horizontal brackets denoting specific pairwise comparisons.

FIGURE 3:

Concentrations of a 200 bp sequence of the mtDNA D-loop region (TOP Panel) and a 200 bp sequence of the nuclear gene encoding 28S rRNA (BOTTOM Panel) were quantified in perfusate at termination of perfusion without a post-mortem period (0 PM) and after 60 min post mortem (60 PM) in the absence and presence of 10 µg/ml OGG1 (+OGG1) or repair-deficient mutant OGG1 (+mOGG1) added to the perfusate reservoir at the start of perfusion. Each point represents a single experiment. Bars represent means $\pm$ 95% confidence interval. *P* values are indicated under horizontal brackets denoting specific pairwise comparisons.

FIGURE 4:

Oxidative base damage in a 10.6 kb sequence of the mtDNA coding region was quantified in rat lung tissue harvested at termination of perfusion without a post-mortem period (0 PM) or after 60 min post mortem (60 PM) in the absence and presence of 10 μ g/ml OGG1 (+OGG1) or repair-deficient mutant OGG1 (+mOGG1) added to the perfusate reservoir at the start of perfusion. Each point represents a single experiment. Bars represent means $\pm$ 95% confidence interval. *P* values are indicated under horizontal brackets denoting specific pairwise comparisons.