

Caspase Inhibition Improves Ischemia-Reperfusion Injury After Lung Transplantation

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Ischemia-reperfusion injury is associated with cell death in many organ systems. The role of programmed cell death (PCD) pathways and the ultimate clinical relevance of PCD in the context of lung transplantation (LTx) are unknown.

In randomized and blinded studies, rat single LTx was performed in the presence of caspase inhibitors after 'short' (6 h) and 'long' (18 h) periods of cold ischemic storage. Lung function, electron microscopic morphology, caspase 3, 8 and 9 activities and TUNEL assays were evaluated.

Endothelial cells and lymphocytes were observed undergoing apoptotic cell death with electron microscopy. Caspase activities were significantly up-regulated immediately after the initial flush and increased further during short periods of cold ischemic storage. A significant amount of apoptotic cell death was observed after LTx and reperfusion. Caspase inhibition virtually eliminated apoptotic cell death and led to improved lung function after LTx and reperfusion.

Activation of caspases during cold ischemia contributes significantly to cell death in LTx. Suppression of caspase activity appears to decrease apoptosis and improve lung function. Clearly, this needs to be investigated further with more experiments to validate the potential role of caspase inhibition as a therapeutic modality in ischemia-reperfusion-induced lung injury.

Key words: Apoptosis, caspase inhibition, ischemia-reperfusion injury, lung transplant, programmed cell death, TUNEL

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Introduction

Lung transplantation provides life-saving therapy for patients with end-stage lung disease. In the early post-transplant period, recipients are at risk for life-threatening complications from ischemia-reperfusion (IR) injury, acute rejection and infection (1). IR injury affects up to 20% of patients following LTx and can lead to increased mortality and morbidity associated with prolonged mechanical ventilation and increased lengths of stay in the intensive care unit (2).

Programmed cell death (PCD), including apoptosis, has been found to play an important role in IR injury. Apoptotic PCD following IR injury has been described in the lung (3) and other organs including the heart (4), liver (5), small intestine (6) and kidney (7). We have demonstrated that a significant percentage of cells in the transplanted human lung undergo apoptotic cell death during LTx (8). Furthermore, in animal studies, lungs transplanted after relatively short periods (6 h) of cold ischemic preservation time have minimal IR injury and good function despite exhibiting a significant amount of cell death—predominantly TUNEL (Tdt-mediated utp nick-end-labeling) positive, apoptotic, PCD. Lungs transplanted after longer cold ischemic preservation times (18 or 24 h) have severe IR injury with impaired gas exchange function. These lungs had significant numbers of TUNEL negative, necrotic, dead cells (3). Indeed, it is not known whether apoptotic PCD cell death in this setting represents a protective response (a 'quiescent' cell death for severely damaged cells) or a harmful process, which increases IR injury through death of minimally injured cells.

Programmed cell death has been classically referred to as apoptosis—a tightly regulated, energy- and substrate-dependent process involving the caspase cascade of enzymes and mitochondrial processes (9–12). In contrast, necrosis is a form of cell death that is accompanied by the loss of plasma membrane integrity and nuclear disruption. Recently, it has been hypothesized that 'apoptosis-like' and 'necrosis-like' PCD are intermediate expressions within the continuum of PCD represented by apoptosis and necrosis at each end (13–16). Figure 1 illustrates the inter-relationships between caspase pathways and differential expressions of PCD (13). The specific morphology ultimately exhibited upon cell death is dependent on the conditions that induce cell death and the substrates (e.g. ATP),

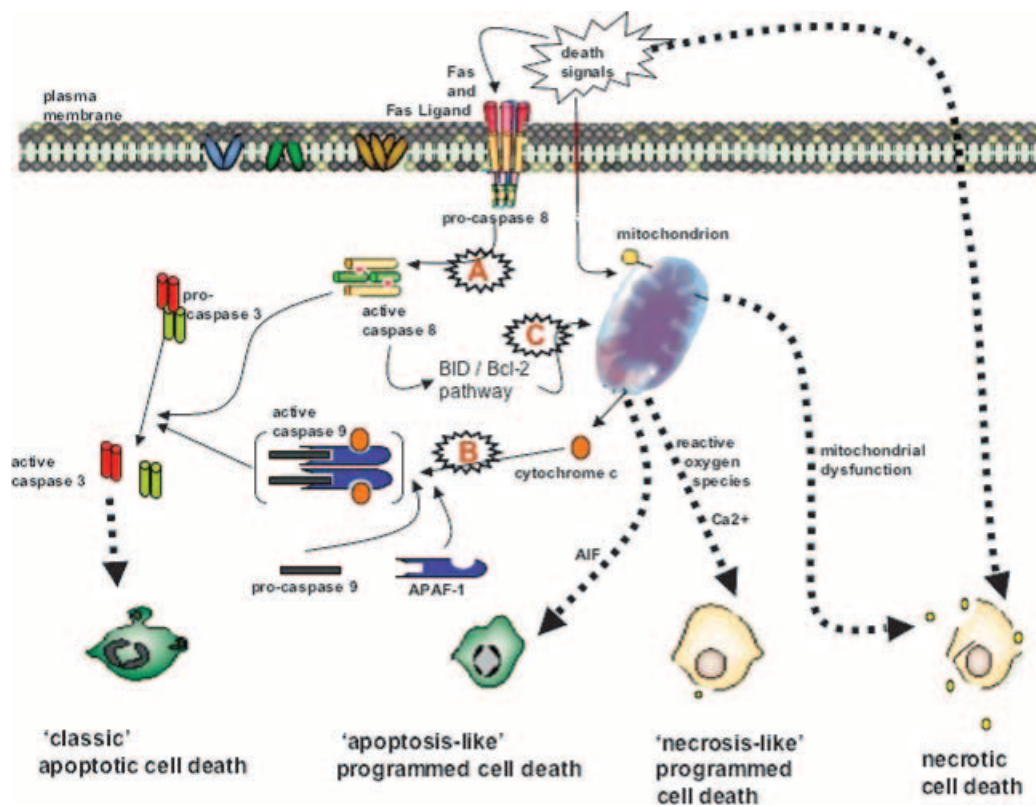


Figure 1: Patterns of programmed cell death. The continuum of programmed cell death (PCD) ranges from 'classic' apoptosis to 'apoptosis-like' PCD to 'necrosis-like' PCD to classic necrosis. 'Classic' apoptosis can be mediated through either the extrinsic pathway (A) via activation of caspase 8 and/or through the intrinsic mitochondrial pathway (B) and activation of caspase 9. The Bid/Bcl-2 pathways (C) serve as a conduit between caspase 8 and the intrinsic pathway to effect changes in the mitochondria and thereby influence patterns of programmed cell death.

which are available at the time that the cell is undergoing PCD (17).

The overall objective of this study was to examine the relationship between PCD associated with acute IR injury and lung function, in the context of LTx. We hypothesized that IR injury following LTx is a dynamic process and that PCD plays a key role in IR injury. Specifically, we sought to determine: (i) Is the apoptotic cell death seen in LTx caspase mediated? (ii) If caspases are involved, when are they activated? (iii) And finally, can PCD be inhibited and will this affect lung function post-LTx?

Materials and Methods

The protocols for all experiments involving animal surgery were approved by the Toronto General Hospital Research Institute Animal Care Committee. All animals received humane care in accordance with the Canadian Council on Animal Care (18). Male inbred Lewis rats (Charles River Inc., Montreal, Canada, 250–400g) were used for all animal experiments.

Evaluating the effects of cold flush

Twelve rats were treated in a blinded manner with a caspase inhibitor (IDN 6556 (molecular weight 569.50) in DMSO, 10 mg/kg, n = 6, Idun Pharmaceuticals, La Jolla, CA) or a corresponding volume of solvent (DMSO, n = 6, Bioshop Inc., Burlington, Canada) by intra-peritoneal (ip) injection prior to anaesthesia. Six animals (DMSO, n = 3 and IDN 6556, n = 3) underwent a pulmonary flush (20 mL of low potassium dextran (LPD, Perfadex®, Vitrolife, Gotenburg, Sweden) with 500-µg/L prostaglandin E₁ (PGE₁)) and the standard donor retrieval procedure, as previously described (3). The remaining six animals (DMSO, n = 3 and IDN 6556, n = 3) underwent an identical donor retrieval procedure, without lung flush. Lungs were then removed and subjected to TUNEL and caspase assays.

Lung transplantation experiments

Rat lung transplants (n = 23) were performed after either 6 h (n = 15) or 18 h (n = 8) of cold ischemic storage time (CIT). Both donor and recipient rats, along with the preservation solutions, were treated pre-operatively with either caspase inhibitor (IDN 6556 in DMSO, 10 mg/kg or zVAD-fmk (molecular weight 467.50) in DMSO, 5 mg/kg, Idun Pharmaceuticals, La Jolla, CA) or control solvent (DMSO) in a randomized blinded manner.

Donors: Subsequent to ip injection of caspase inhibitor or control, the animal was anesthetized and the standard flush and lung retrieval procedure was performed for the donors as previously described (3). Both lungs were

stored in 40 mL of LPD+PGE₁ and 25- μ M caspase inhibitor or control for a period of either 6 h or 18 h. At the end of cold storage, samples were taken from the right lung for TUNEL and caspase assays. The left lung was transplanted into the recipient animal.

Recipients: A modified version of the total non-microsuture technique described first by Mizuta et al. (19,20) and similar to the procedure described by Fischer et al. (3), was used for implantation of the lung after 6-h CIT. For transplants after 18-h CIT, the procedure was refined to allow independent ventilation of the transplanted lung using a second ventilator at 4 mL/kg while the native right lung remained ventilated at 6 mL/kg, as described by de Perrot et al. (21). We have found that this modification provides for more reliable and measurable ventilation of the transplanted lung. All other aspects of the transplantation procedures were identical to the 6-h group of animals. The transplanted lungs were reperfused *in vivo* for 2 h after implantation for both groups. Subsequently, lung function was assessed with blood gases, on a F_{IO₂} of 1.0, drawn from the pulmonary vein of the transplanted lung after 2 h of reperfusion. Finally, the transplanted lung was excised and samples were taken for TUNEL and caspase assays.

Caspase assays

Caspase 3, 8 and 9 activities were assayed in snap-frozen tissue samples using a modified fluorometric caspase assay (22) with substrates, DEVD-AFC, IETD-AFC and LEHD-AFC (Biosource International, Camarillo, CA), respectively. Duplicate samples of 100- μ g protein/well were incubated at 37°C for 90 min and tested in a 96-well fluorometric plate (Falcon Microtest 96, Becton Dickinson, Franklin Lakes, NJ) using a CytoFluor 4000 (PerSeptive Biosystems, Framingham, MA) with excitation at 360 \pm 40 nm and emission at 530 \pm 25 nm. Mean values from each set of duplicate samples had blanks and background values subtracted and are expressed as units/100- μ g protein.

TUNEL staining and cell counts

TUNEL studies were carried out on lung samples preserved in 10% neutral buffered formalin (EM Science, EM industries Inc., Gibbstown, NJ). Samples were embedded in paraffin, cut into 5- μ m thick tissue slices and mounted on glass slides. TUNEL was performed using the ApopTag Kit (Serologicals Corp., Norcross, GA) with minor modifications to the manufacturer's instructions (TdT enzyme was applied for 2 h at 37°C and the anti-digoxigenin-fluorescein (FITC) was applied for 40 min at room temperature) (23). The slides were counterstained with propidium iodide (PI) (Sigma Chemicals Co., St Louis, MD) for 10 min and mounted with Antifade (Serologicals).

The slides were viewed immediately using a fluorescence microscope (Nikon Eclipse, TE200, Melville, NY) and photographed using a CCD digital camera (Hamamatsu ORCA C4742-95) with a 590-nm emission filter after excitation at 510–560 nm for PI and a 520-nm emission filter after excitation at 465–495 nm for FITC (Chroma Technology Corp., Rockingham, VT). PI stains (red) all nucleated cells whether alive, necrotic or apoptotic. FITC stains (green) TUNEL positive cells. Images were collected and analyzed using Simple PCI™ (Compix Inc., Cranberry, PA) software. Slides were examined under the PI filter first to determine areas that appeared to have normal cellularity (i.e. areas of the lung without significant crush artifact). Three randomly chosen fields were photographed at high power (400 \times magnification) with the PI filter followed by the FITC filter for each slide. Using these three fields for each slide, PI positive (red) and TUNEL positive (green) cells were manually counted and represent the total number of nucleated cells and apoptotic cells, respectively. The ratio of apoptotic cells/total nucleated cells was calculated. An independent, blinded assessor performed this analysis.

Positive control slides (rat mammary tissue slides, Serologicals (23)) and negative control slides (equivalent amount of buffer was used instead of active TdT enzyme) were also subjected to the TUNEL staining procedure and confirmed the validity of the TUNEL staining.

Electron microscopy

Two additional rat lung transplant experiments were performed following the same procedure as outlined for the 6-h series, without any treatment. After 2-h reperfusion, biopsies of fresh lung tissue from the transplanted lung were obtained and prepared for electron microscopy (EM) as described previously and mounted with uranyl acetate and lead citrate stain (8). The grids were examined in a Phillips 201 electron microscope (N.V. Philips, Gloeilampenfabrieken, Eindhoven, Netherlands).

Statistical analysis

SPSS 10 (SPSS Inc., Chicago, IL) software was used for all statistical analyses. All data are expressed as mean \pm standard error of means. One-way analysis of variance (ANOVA) with Student-Newman-Keuls post hoc analyses were used to determine statistical significance in the 6-h transplant experiments as there were three groups (two treatment groups and one control group). Independent samples *t*-tests were used for the two groups (treatment vs. control groups) in the 18-h transplant experiments. All values were considered statistically significant if $p < 0.05$.

Results

Electron microscopy

Electron microscopy confirmed that cells were undergoing PCD and, furthermore, allowed determination of cell type. Biopsies from animals after 6-h CIT, transplantation, and 2-h reperfusion revealed apoptotic endothelial cells and lymphocytes (Figures 2A,B,C).

Caspase activity rises after flushing and with short periods of cold ischemic storage

Mean caspase 3 activity in lungs increased over 7-fold from baseline levels in normal lungs immediately following cold LPD+PGE₁ flush. After 6 h of cold ischemic storage, caspase 3 activity increased nearly 17-fold as compared to baseline pre-flush levels ($p < 0.0001$). By 18 h of cold ischemic storage, caspase 3 activities had declined and became almost equivalent to post-flush levels (Figure 3A). Caspases 8 and 9 activities followed a similar pattern: activity increased immediately after flush ($p < 0.0001$ for caspase 8, $p = ns$ for caspase 9), rose further after 6 h of cold ischemic storage ($p < 0.0001$ for both caspases 8 and 9) and declined by 18-h CIT (Figures 3B,C).

Systemic caspase inhibition blocks the rise in lung caspase activity

The increases seen in caspase activities were significantly reduced and in some cases, completely abolished, with caspase inhibitors. After flushing, animals treated with IDN 6556 had significantly lower caspase 3 levels compared to control animals ($p < 0.05$). At 6-h CIT, caspase 3 levels in the IDN 6556 group had declined to less than 8% of control animals ($p < 0.0001$). At 18-h CIT, caspase 3 levels were undetectable in the IDN 6556 group while the control group levels had also declined but were still present ($p < 0.03$) (Figure 3A).

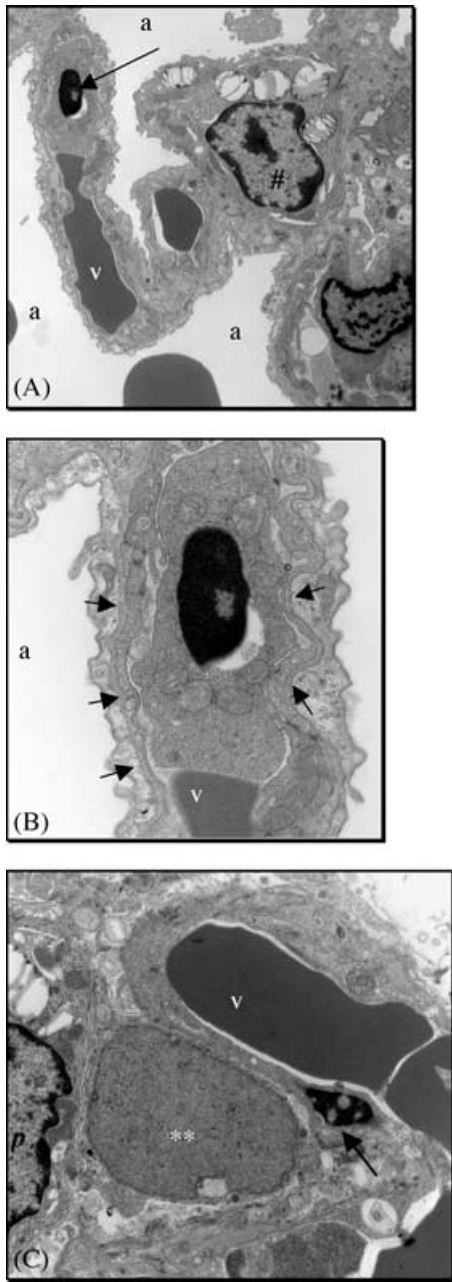


Figure 2: Electron micrographs of rat lung after 6-h CIT, transplantation and 2-h reperfusion. (Uranyl acetate and lead citrate) (A) A blood vessel (v), adjacent to alveolar space (a) containing an apoptotic cell (arrow) with typical condensed chromatin and nuclear shrinkage at a magnification of 8000 \times . # = Morphologically normal type II pneumocyte. (B) Higher magnification (25 000 \times) of apoptotic cell seen in (A). Note the contiguity of the basement membrane (arrows) around the blood vessel. The intra-vascular apoptotic cell contains no granules and few organelles and is a lymphocyte. (C) An apoptotic endothelial cell (arrow), outlines the lumen of a capillary (v) and an adjacent interstitial cell (**) undergoing necrotic cell death. A portion of a viable type II pneumocyte (p) with normal nuclear chromatin ultrastructure is also seen in this micrograph. Magnification 12 000 \times .

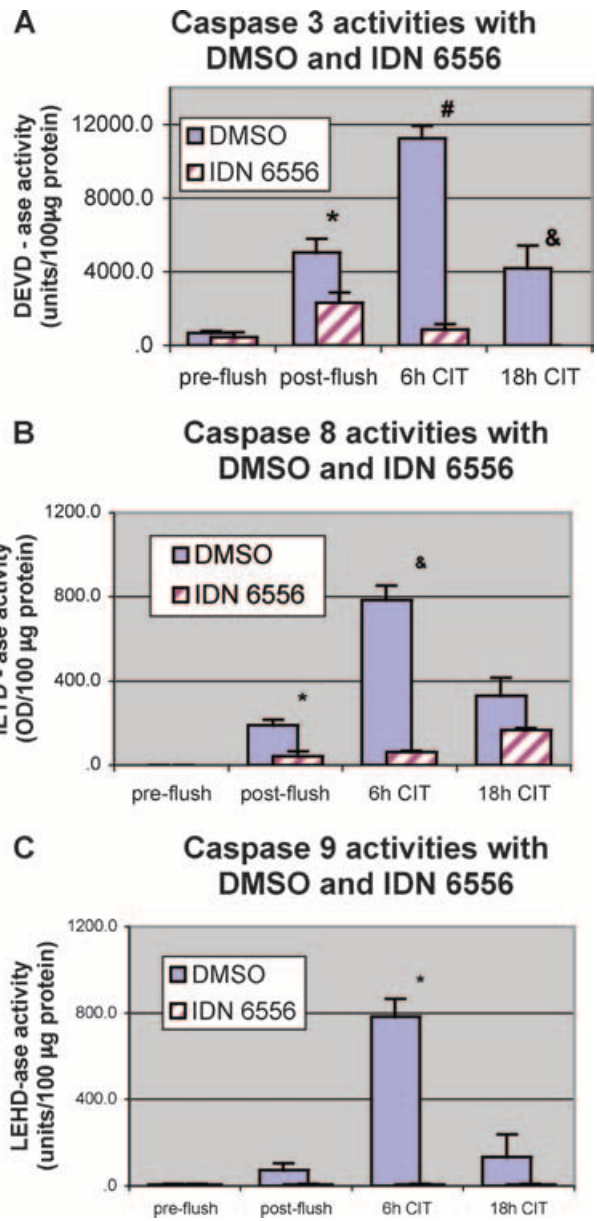


Figure 3: Caspase activities in control (DMSO) and caspase inhibitor (IDN 6556)-treated animals after cold ischemic storage. (A) Caspase 3 activities. (*, # and & represent significantly different levels of caspase 3 between control and IDN 6556 groups at each time point, $p < 0.05$, $p < 0.0001$ and $p < 0.03$, respectively) (B) Caspase 8 activities. (* and & represent significantly different levels of caspase 8 between control and IDN 6556 groups post-flush and at 6-h CIT, $p < 0.02$ and $p < 0.0001$, respectively) (C) Caspase 9 activities. (*represents significantly different levels of caspase 9 between control and IDN 6556 groups at 6-h CIT, $p < 0.0001$).

Animals administered zVAD-fmk also followed this pattern with caspase 3 activity at the 6-h CIT time point of 2569 ± 1109 , which was significantly lower than caspase 3 activity in the control group (11252 ± 668) ($p < 0.0001$). However, there was no significant difference between the zVAD-fmk group and the IDN 6556 group (871 ± 286) after 6-h CIT.

Caspase 8 activities were also inhibited with IDN 6556 and zVAD-fmk treatment. After flushing, animals treated with IDN 6556 had caspase 8 levels less than 23% of controls ($p < 0.02$). The 6-h CIT levels were only 8% of control levels in the IDN 6556 group and 13% in the zVAD-fmk group ($p < 0.0001$ comparing control group to both caspase inhibitor groups; $p = ns$ for zVAD-fmk vs. IDN 6556). Although the 18-h CIT caspase 8 levels in the IDN 6556 group were half that of control animals, this did not reach statistical significance ($p = 0.16$) (Figure 3B).

Caspase inhibition with IDN 6556 and zVAD-fmk reduced caspase 9 activities at all time points (before flush, after flush, after 6-h CIT, and after 18-h CIT) to below the detection level of the fluorometric caspase 9 assay (Figure 3C). However, the differences in caspase 9 activities between control animals and animals treated with caspase inhibitors only reached statistical significance at the 6-h CIT time point ($p < 0.0001$).

Caspase 3, 8 and 9 assays were also performed on samples of lung tissue obtained after transplantation and 2-h reperfusion. Caspase activities were very low in these samples—barely distinguishable above background levels.

TUNEL-positive cells were only found after transplantation and reperfusion following 6-h CIT

There was no evidence of cells becoming TUNEL-positive immediately following the flush with LPD+PGE₁ or after either 6-h or 18-h CIT alone—either in control or caspase inhibitor groups.

Lung sections from control animals examined after 6-h CIT transplantation and 2-h reperfusion exhibited apoptotic cells (Figures 4A,B). Both IDN 6556 and zVAD-fmk treatments effectively blocked cells from becoming TUNEL-positive (Figures 4C–F). Quantitative TUNEL assays (cell counts) of lungs after 6-h CIT transplantation and 2-h reperfusion revealed a significant reduction in apoptosis with caspase inhibition as compared to control. The baseline percentage of apoptotic cells in the control group was $3.6 \pm 0.4\%$ while the percentages of TUNEL-positive apoptotic cell in the group treated with IDN 6556 and zVAD-fmk were virtually zero: $0.1 \pm 0.1\%$ and $0.2 \pm 0.1\%$, respectively ($p < 0.05$) (Figure 5).

There were no discernible TUNEL-positive cells noted in control or caspase inhibitor (IDN 6556) groups after 18-h CIT transplantation and 2-h reperfusion.

Lung function is improved with caspase inhibition

Lung function—PO₂ of transplanted lung on a F_iO₂ of 1.0—after 18-h CIT transplantation, and 2-h reperfusion was markedly impaired in the control group but improved significantly with caspase inhibition (control: 232 ± 18 mmHg vs. IDN 6556: 368 ± 43 , $p = 0.03$) (Figure 6A). Interestingly, caspase inhibition did not have any significant effect on lung function after 6-h CIT transplantation, and 2-h reperfusion. As expected, lung function after a shorter period of CIT (6 h) was much better with PO₂ in control animals of 338 ± 94 mmHg. Under these circumstances, neither caspase inhibitor (IDN 6556: 344 ± 98 , zVAD-fmk: 284 ± 107) led to any significant improvements in oxygenation as compared to control ($p = 0.93$) (Figure 6B).

Discussion

Electron microscopy revealed endothelial cells and lymphocytes undergoing apoptotic cell death during acute IR injury associated with LTx. Our previous EM studies have also characterized type II pneumocytes undergoing PCD (8). Indeed, this work demonstrates that many different types of cells undergo PCD in the lung during acute IR injury. This exciting finding also raises new questions about potential roles of apoptotic endothelial cells and lymphocytes in bronchiolitis obliterans and chronic rejection following LTx.

Our study proves that PCD in lung transplantation is mediated via the caspase cascade of enzymes, including caspases 3, 8 and 9. The significant and dramatically increased activities of caspases 8 and 9 suggest that both the extrinsic (Fas) and intrinsic (mitochondrial) pathways of apoptotic cell death are involved. Systemic caspase inhibition blocked the rise in caspase activity in lung tissue and virtually abolished all TUNEL-positivity representative of apoptosis. This finding adds further evidence to support the assertion that PCD following LTx requires activation of caspase pathways. This is relevant, as other non-caspase-mediated forms of programmed cell death—through cathepsin B, for example—have also been shown to lead to apoptosis or apoptosis-like PCD (24). Two different pan-caspase inhibitors were used in this study. IDN 6556 and zVAD-fmk are both peptide-based inhibitors—they have similar mechanisms of action through competitive and irreversible inhibition of caspases. IDN 6556 is a relatively new agent (25) that has not been previously studied in the lungs. zVAD-fmk is a well-established pan-caspase inhibitor.

Interestingly, the initiation of cell death processes seems to occur at a much earlier stage of IR injury than was previously thought. Caspases 3 and 8 had significantly increased activities immediately after the lung flush with LPD+PGE₁. Caspase 9 activity was also increased after flushing but did not reach statistical significance. These are novel and important findings suggesting that mechanisms underlying

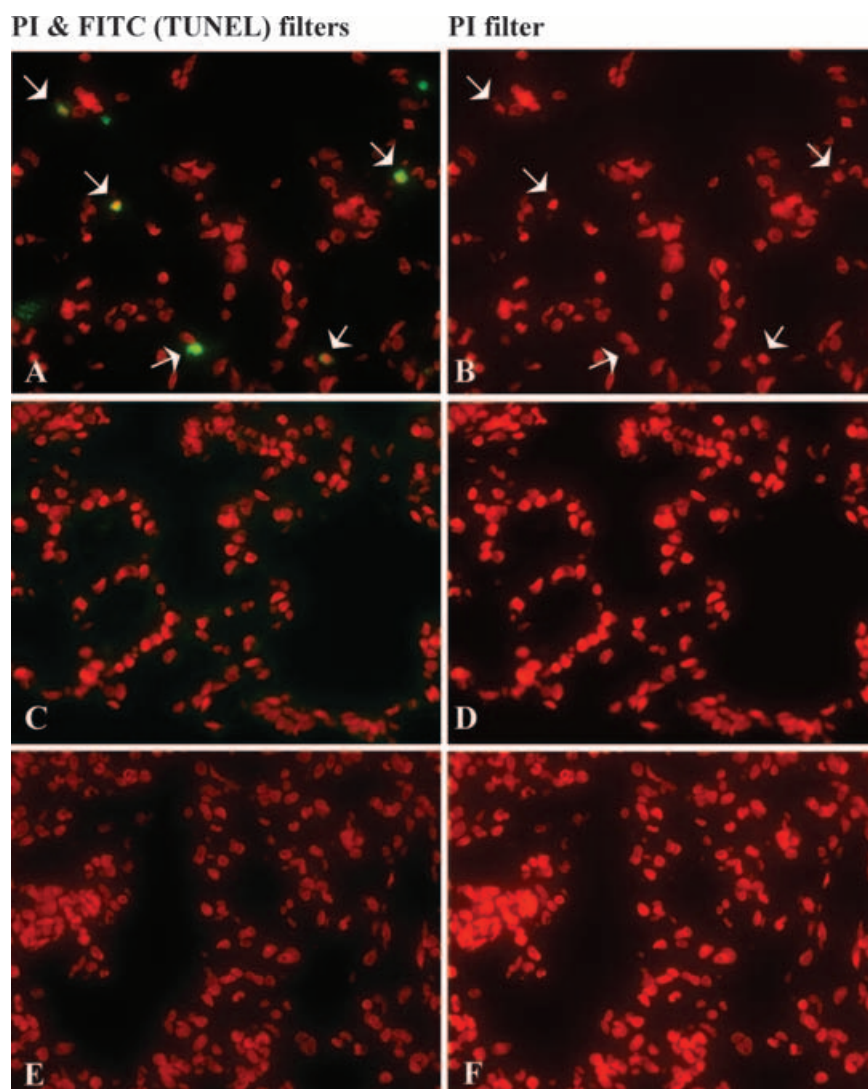


Figure 4: TUNEL staining after 6-h CIT, transplantation and 2-h reperfusion in control group (panels A, B) and caspase inhibitor groups (IDN 6556: panels C, D; zVAD-fmk: panels E, F). Magnification 400x. A, C, E PI and FITC staining: PI-positive cells (nucleated cells) are red; FITC-positive cells (TUNEL-positive) are green. Note that almost all green areas in composite image (A), (white arrows), correspond with nucleated cells in the corresponding PI image (B). Therefore, these represent true TUNEL-positive cells. B, D, F PI staining: all nucleated cells take up propidium iodide stain and appear red.

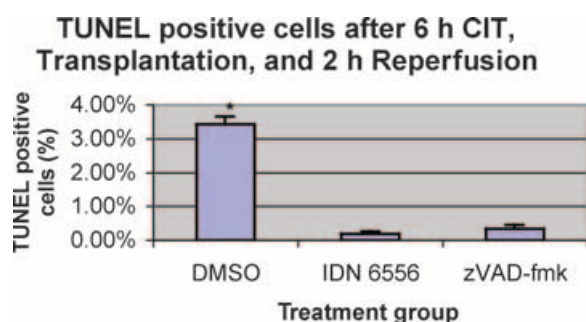


Figure 5: Percentages of cells that were TUNEL-positive after 6-h CIT, transplantation and 2-h reperfusion in control (DMSO), caspase inhibitor 1 (IDN 6556) and caspase inhibitor 2 (zVAD-fmk) groups of animals. (*p < 0.05 compared to other treatment groups).

IR injury and programmed cell death begin quite early in IR injury and continue to lead to altered cellular processes as the cold ischemic storage period progresses. These findings, though based on small numbers of animal experiments, suggest that strategies to ameliorate IR injury need to be instituted early (likely pre-flush) in the transplantation process.

The current study suggests that a smaller fraction of cells in the lung are undergoing apoptosis during IR injury as compared to our previous work (8). A number of factors may account for this apparent discrepancy. First, cameras and post-processing techniques were different between the two studies. Second, variations in staining related to sample harvesting, fixation, preparation and staining protocols may also play a part in the discrepancy between our study and previous work. Finally, cells that have undergone DNA fragmentation due to other forms of cell death including necrosis and apoptotic fragments can also stain

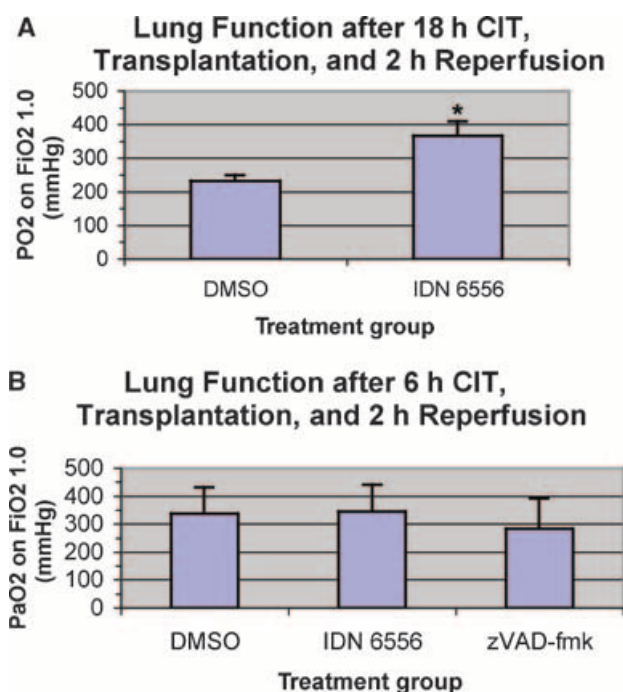


Figure 6: (A) Gas exchange function of the transplanted lung after 18-h CIT, transplantation and 2-h reperfusion. PO₂ is improved in the group treated with caspase inhibitor (IDN 6556) as compared to the control group (* $p = 0.03$). (B) Gas exchange function of the transplanted lung after 6-h CIT, transplantation, and 2-h reperfusion. PO₂ is not significantly affected in either group treated with caspase inhibitor (IDN 6556 or zVAD-fmk) as compared to the control group ($p = 0.93$).

positive by TUNEL (26) and thus contribute to error. Nevertheless, the overall patterns of cell death described in this work correlate with previous studies (3). As all specimens in this study were processed in an identical fashion, with the appropriate positive and negative controls, the findings and the comparisons between groups are indeed valid.

Caspase inhibition and lung function

Caspase inhibition appeared to improve lung function and decrease IR injury (18-h CIT group). Indeed, this novel finding serves to establish a link between PCD and clinical IR injury. However, the effects of caspase inhibition on lung function seem paradoxical—lung function was improved at the late time point (18-h CIT group) when there was no apparent apoptosis as measured by TUNEL assays, while there was little benefit at the early time point (6-h CIT group), when apoptosis is considered to be the predominant mode of cell death, and where TUNEL-positivity of the cells was effectively inhibited with caspase inhibition.

There are a number of possible explanations for these findings. First, it is well known that cold storage for 6 h prior to transplantation and reperfusion is a relatively minor ischemic stress. Gas exchange of the control (DMSO) group

is still quite good after 6 h compared to after 18 h. Thus, it is possible that the effects of caspase inhibition could not be demonstrated or assessed properly in a 6-h CIT model that inflicted a relatively smaller IR injury. With more severe IR injury (18 h), it may become apparent that caspase inhibition does indeed improve post-transplant lung function as compared to controls.

Furthermore, one of the most interesting findings of this study is that lung cells are beginning down an apoptotic path very early in the IR process—as evidenced by the dramatic rise in caspase activities after lung flush and with short periods of cold ischemic injury. However, after long periods of cold ischemic stress, there is a progressive loss of caspase activity, possibly due to enzymatic degradation or due to autolysis, and cells are unable to undergo apoptosis as measured by the TUNEL assay. Indeed, it is possible that transplantation and reperfusion after long periods of CIT lead to PCD through a form of apo-necrosis or ‘necrosis-like PCD’ (Figure 1) (13)—that is, cells that have initially been primed to undergo PCD are subsequently forced to undergo a necrotic form of PCD as caspase activities are too low to permit apoptotic PCD. This leads to severe IR injury accompanied not by TUNEL-positive apoptotic PCD but rather, with cell death that is more in keeping with necrosis (3). In the presence of caspase inhibitors, the initial up-regulation in caspase activity associated with the flush is blocked and the subsequent rise in caspase activities during the initial 6 h is almost completely abolished. Thus, the cells never actually enter the PCD sequence. It can be hypothesized that these cells are viable after 18-h CIT, transplantation and reperfusion. Thus, caspase inhibition may prevent apoptosis and thereby limit IR injury post-transplantation—lung function at the late time point would then be improved under conditions of caspase inhibition.

Although the results obtained were statistically significant, the small sample sizes represent a potential limitation to our study. Further work is needed in this area to confirm these results and further elucidate the relevant molecular pathways and the effects of caspase inhibition on cell death in this setting.

Conclusions

In this study, we have attempted to explicitly alter patterns of programmed cell death in the setting of LTx. We have demonstrated that caspases are fundamental to IR injury in LTx and that they are activated at a very early stage in the transplant process. This is a novel and important finding as it defines potential therapeutic windows to modify cell death—donor treatment and/or modifications in storage solution are probably more important than recipient treatment if one wishes to influence post-reperfusion patterns of cell death. Indeed, our work has shown that apoptotic cell death can be inhibited and this leads to reduced IR injury and improved lung function. These findings are

particularly exciting as it was previously unknown whether apoptotic cell death in this setting represented a protective response of 'quiescent' cell death or an injurious response. This raises the exciting hope that IR injury may be ameliorated and graft ischemic times may ultimately be extended in clinical LTx through modification of programmed cell death processes.

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