

Pulmonary Macrophage Inhibition and Inhaled Nitric Oxide Attenuate Lung Ischemia-Reperfusion Injury

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Background. Lung ischemia-reperfusion injury (LIRI) is postulated to occur biphasically. Donor pulmonary macrophages mediate early injury, and neutrophil-dependent injury predominates in the later phase of LIRI. We hypothesized that the biphasic response to LIRI would be attenuated by the administration of gadolinium, a known pulmonary macrophage inhibitor, and inhaled nitric oxide (NO), a pulmonary vasodilator that also interferes with neutrophil chemotaxis.

Methods. Using our isolated, ventilated, blood-perfused rabbit lung model, study groups ($n = 10$ per group) underwent two hours of reperfusion after 18 hours of cold ischemia (4°C). Lungs received gadolinium alone, or inhaled NO in the presence or absence of macrophage inhibition with gadolinium.

Results. Compared with control animals, pulmonary macrophage inhibition with the concurrent administration of inhaled NO increased lung compliance ($p < 0.01$)

and oxygenation ($p = 0.03$), while also decreasing pulmonary artery pressure ($p < 0.01$), myeloperoxidase content by 63% ($p < 0.01$), wet to dry ratios by 23% ($p < 0.01$), and lung tissue ($p < 0.01$) and bronchoalveolar lavage tumor necrosis factor- α (TNF- α) protein levels ($p < 0.01$).

Conclusions. The severity of LIRI was most significantly reduced by the inhibition of pulmonary macrophages and the concomitant use of inhaled NO. Pulmonary macrophages, likely through the elaboration of proinflammatory cytokines such as TNF- α , not only cause early injury themselves but also prime cells such as neutrophils to injure lungs in the later stages of LIRI. The LIRI was effectively blunted by the reduction of macrophage-dependent injury by gadolinium while inhaled NO also attenuated injury by reducing pulmonary hypertension and minimizing neutrophil sequestration.

(Ann Thorac Surg 2007;84:247–53)

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Lung transplantation is commonly accepted as the treatment of choice for most etiologies of end-stage lung disease. Lung ischemia-reperfusion injury (LIRI) continues to worsen outcomes in approximately 15% to 33% of lung transplants despite advances in lung preservation, immunosuppression, and postoperative management [1, 2]. An almost eightfold increase in early mortality occurs in patients with severe LIRI compared with those without LIRI [1]. In addition to the potentially permanent damage suffered to the lung from ischemia-reperfusion (IR) injury, LIRI has been found to increase the risk of bronchiolitis obliterans [3]. As attempts are made to increase utilization of marginal donors, the incidence of LIRI is likely to increase as will the need to find effective therapeutic regimens targeting the robust inflammatory response that ultimately injures the lung [4].

Donor alveolar macrophages (AMs) have recently been implicated as key mediators in the development of LIRI [5–7]. The AMs and the proinflammatory cytokines they

release, most prominently TNF- α , play crucial roles in cell activation, trafficking, and end-organ damage [8]. Lung IR models utilizing anti-TNF- α antibody pretreatment as well as studies using TNF- α knockout mice have shown decreased leukocyte infiltration and lung injury [9, 10]. The depletion of AMs with liposomal-clodronate in an in situ buffer-perfused model reduced bronchoalveolar lavage TNF- α and monocyte chemoattractant protein-1 (MCP-1) levels, and subsequently improved IR-induced changes in pulmonary artery pressure, lung compliance, and pulmonary edema [6].

Neutrophils, in contrast to AMs, have long been implicated in the pathogenesis of IR injury. Current evidence supports a biphasic, response to lung IR, where early injury is mediated by donor AMs and late injury is neutrophil-dependent [5, 11]. We hypothesized that LIRI would be most significantly attenuated by addressing both of the currently known cellular components of injury with gadolinium chloride (GdCl_3) and inhaled nitric oxide (NO). Gadolinium chloride, a rare lanthanide earth salt, is known to inhibit AM function in various experimental models [5, 11–13]. Inhaled NO decreases IR injury through vasodilatory effects and the ability to decrease neutrophil chemotaxis and platelet aggregation [14–16]. The ability to further minimize LIRI by blunting the effects of the two known cellular mediators of LIRI,

Accepted for publication Feb 14, 2007.

Presented at the Basic Science Forum of the Fifty-third Annual Meeting of the Southern Thoracic Surgical Association, Tucson, AZ, Nov 8–11, 2006.

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Table 1. Experimental Protocol

Protocol	No.	18 Hours Cold Ischemia	Gadolinium (14 mg/kg iv) Pretreatment ^a	Inhaled NO (20 ppm) Reperfusion	L-NAME (100 μM) Reperfusion
Immediate	10	–	–	–	–
GdCl ₃ + NO	10	+	+	+	–
GdCl ₃	10	+	+	–	–
NO	10	+	–	+	–
Sham	10	+	–	–	–
L-NAME	10	+	–	–	+
L-NAME + GdCl ₃	10	+	+	–	+

^a Pretreatment: gadolinium is administered 24 hours prior to harvest.

GdCl₃ = gadolinium chloride; L-NAME = L-N^G arginine methyl ester; NO = nitric oxide.

donor AMs and neutrophils, is evaluated in our isolated-blood perfused, rabbit lung model of transplantation.

Material and Methods

Animal Care

All animals received humane care in accordance with the “Guide for Care and Use of Laboratory Animals” published by the National Institute of Health (National Institutes of Health publication No. 85-23, revised 1985). The Animal Care and Use Committee at the University of Virginia reviewed and approved the protocol for this study before experimentation.

Experimental Protocol

Seven experimental groups were compared utilizing an isolated, whole blood-perfused, ventilated rabbit lung model (Kent Scientific, Model TIS3862, Litchfield, CT). Group 1 lungs underwent reperfusion for 120 minutes immediately after lung harvest. All subsequent groups (2 through 7) were reperfused for 120 minutes after 18 hours of cold ischemia at 4°C (see Table 1 for the experimental protocol for each group). The dose and timing of GdCl₃ (Sigma Corporation, St. Louis, MO), 14 mg/kg intravenously, and 24 hours prior to harvest, respectively, were determined by previous experiments in our lab [5]. In the appropriate groups, inhaled NO (Messer Gases, Morrisville, PA) was administered at 20 ppm through a flowmeter, starting 10 minutes after the initiation of reperfusion and monitored with an electrochemical sensor (RKI Instruments, Union City, CA) just proximal to the lungs, throughout the remainder of the reperfusion period [16, 17]. When given, the potent, nonspecific inhibitor of NO, L-N^G arginine methyl ester (L-NAME) (Sigma Corporation), was added at the beginning of reperfusion to the whole blood at 100 μM based on previous experiments utilizing the isolated, rabbit lung model [18, 19].

Harvest Procedure

New Zealand white rabbits of both sexes (3.0 to 3.5 kg) were randomly assigned to seven experimental groups. Each animal was anesthetized with intramuscular ketamine (50 mg/kg) and xylazine (5 mg/kg). Tracheal intu-

bation was performed through a tracheostomy and mechanical ventilation (Model RSP1002, Kent Scientific) was instituted with room air at a respiratory rate of 30 breaths/minute. A median sternotomy and a thymectomy were then performed. The superior and inferior vena cavae were loosely encircled with ligatures and the pericardium was opened. Both the pulmonary artery (PA) and the aorta were dissected free and similarly encircled. A purse-string suture was then placed in the right ventricular outflow tract and intravenous heparin was administered (1,000 U/kg). After the PA was injected with 30 μg of prostaglandin E1, the vena cavae were ligated to initiate ischemia. The PA was then cannulated through a right ventriculotomy. After the left ventricle was vented through a left ventriculotomy and the aorta was ligated, 100 mL/kg of Perfadex (Vitrolife, Kungsbacka, Sweden) solution was infused into the PA at 30 cm H₂O pressure at 4°C. Topical cooling was achieved with cold saline solution slush. During the PA flush, the left atrium was cannulated through the left ventriculotomy with an outflow catheter. The lung-heart block was then excised. Inflated lungs were then stored at 4°C for 18 hours in groups 2 to 7 or immediately reperfused (group 1). The immediately reperfused lungs were flushed with Perfadex (Vitrolife) stored at 4°C but not topically cooled.

Reperfusion Procedure

After harvest, the lung-heart block was suspended from a force transducer and ventilation was initiated with a 95% oxygen and 5% carbon dioxide gas mixture (Model RSP1002, Kent Scientific). All groups underwent 120 minutes whole-blood perfusion. For each group, one breath of approximately 30 cm H₂O positive end expiratory pressure was given once per minute in the first five minutes of the stabilization period to eliminate atelectasis. Lungs were ventilated at a constant tidal volume of 10 cc/kg with 3 cm H₂O of positive end expiratory pressure at a rate of 30 breaths per minute. Lung-heart blocks were then connected through the PA and the outflow catheters to a venous blood reperfusion circuit. New Zealand white rabbits served as fresh venous blood donors. Blood was circulated through a pediatric oxygenator set to deoxygenate the blood and add carbon dioxide

Table 2. Measured Values at Each Time Point^a

	Group	N	15 Min	30 Min	60 Min	90 Min	120 Min	Within Group p Value		
PA pressure										
Immediate	1	10	21.9 ± 1.4 ^b	17.9 ± 1.7 ^b	17.7 ± 2.3 ^b	18.9 ± 2.4 ^b	20.4 ± 2.0 ^b	0.000	Between Groups ANOVA p value = 0.000	Interaction ANOVA p value = 0.005
GdCl ₃ + NO	2	10	24.0 ± 2.0 ^{b,d}	20.0 ± 4.2 ^{b,d}	19.5 ± 5.0 ^b	19.8 ± 5.0 ^b	22.4 ± 3.6 ^b	0.049		
GdCl ₃	3	10	26.9 ± 4.2 ^b	24.6 ± 2.8	24.1 ± 2.9	23.6 ± 3.0	27.4 ± 3.4	0.040		
NO	4	10	29.8 ± 3.1 ^d	28.4 ± 3.2 ^d	24.5 ± 2.6	23.9 ± 3.6	25.0 ± 3.7 ^b	0.000		
Sham	5	10	32.4 ± 5.6	30.0 ± 7.7	30.8 ± 7.3	31.4 ± 8.5	32.6 ± 8.5	0.933		
L-NAME	6	10	40.7 ± 2.8 ^b	40.1 ± 2.7 ^b	35.8 ± 3.0	39.1 ± 3.0	41.8 ± 2.7 ^b	0.000		
L-NAME + GdCl ₃	7	10	38.5 ± 3.4 ^b	35.2 ± 11.4	34.7 ± 10.3	35.7 ± 11.2	39.9 ± 9.5	0.693		
Pulmonary compliance										
Immediate	1	10	7.12 ± 0.11 ^b	7.11 ± 0.13 ^b	7.07 ± 0.14 ^b	6.90 ± 0.28 ^b	6.79 ± 0.28 ^b	0.000	Between Groups ANOVA p value = 0.000	Interaction ANOVA p value = 0.003
GdCl ₃ + NO	2	10	6.9 ± 0.3 ^{b,c,d}	6.73 ± 0.4 ^{b,d}	6.23 ± 0.7 ^{b,d}	6.14 ± 0.7 ^{b,d}	5.88 ± 0.64 ^{b,c}	0.002		
GdCl ₃	3	10	6.05 ± 0.43 ^c	6.07 ± 0.32	5.77 ± 0.30	5.51 ± 0.31	5.11 ± 0.20 ^c	0.000		
NO	4	10	5.80 ± 0.19 ^d	5.71 ± 0.20 ^d	5.58 ± 0.22 ^d	5.48 ± 0.18 ^d	5.28 ± 0.16	0.000		
Sham	5	10	5.53 ± 0.66	5.49 ± 0.65	5.23 ± 0.54	5.14 ± 0.55	4.94 ± 0.56	0.170		
L-NAME	6	10	5.29 ± 0.25	4.95 ± 0.12	5.05 ± 0.16	4.69 ± 0.24	4.59 ± 0.43	0.000		
L-NAME + GdCl ₃	7	10	5.31 ± 0.94	5.32 ± 1.11	5.05 ± 0.94	4.76 ± 0.92	4.64 ± 0.85	0.386		
PaO₂										
Immediate	1	10	663 ± 8.2 ^b	681 ± 5.5 ^b	680 ± 4.3 ^b	673 ± 6.4 ^b	666 ± 8.4 ^b	0.000	Between Groups ANOVA p value = 0.000	Interaction ANOVA p value = 0.089
GdCl ₃ + NO	2	10	651 ± 22.2 ^{b,d}	645 ± 27.5 ^b	633 ± 30.3	637 ± 26.9	645 ± 21.6 ^b	0.763		
GdCl ₃	3	10	636 ± 43.1	639 ± 27.6 ^b	649 ± 43.3	633 ± 39.9	614 ± 26.0	0.486		
NO	4	10	615 ± 7.0 ^d	623 ± 11.3	630 ± 13.3	624 ± 13.6	612 ± 19.5	0.035		
Sham	5	10	594 ± 63.3	598 ± 32.9	600 ± 40.6	603 ± 42.5	598 ± 41.4	0.944		
L-NAME	6	10	584 ± 51.2	587 ± 33.6	576 ± 35.8	568 ± 33.4	557 ± 36.9	0.421		
L-NAME + GdCl ₃	7	10	546 ± 57.1	551 ± 53.8 ^b	551 ± 64.3	578 ± 36.2	544 ± 57.2 ^b	0.630		

^a Data are reported at each time point as mean ± standard deviation. ^b p < 0.05 compared to sham. ^c p < 0.05 comparing GdCl₃ to GdCl₃ + NO. ^d p < 0.05 comparing NO to GdCl₃ + NO.

ANOVA = analysis of variance; GdCl₃ = gadolinium chloride; L-NAME = L-N^G arginine methyl ester; NO = nitric oxide; PA = pulmonary artery.

in order to simulate venous blood (Po₂ = 60 mm Hg/Pco₂ = 60 mm Hg). The lungs were subsequently perfused through the pulmonary artery cannula at 60 mL/minute with "venous" blood. The temperature of the lungs and perfused blood were maintained at 37°C throughout reperfusion.

Physiologic Parameters

Recordings of pulmonary physiologic parameters were collected by a dynamic data acquisition program (DASYLab, Dasytec, USA, Inc, Amherst, NH). Pulmonary venous blood samples were collected for blood gas analysis (Bayer 348 pH/Blood Gas Analyzer, Bayer Corp, E. Walpole, MA) at 15, 30, 60, 90, and 120 minutes after initiation of reperfusion.

Lung Wet to Dry Weight Ratio

Lung wet to dry weight ratios were used as a measurement of pulmonary edema. After 120 minutes reperfusion, samples of right lower lobe lung tissue were blotted and weighed immediately. These samples were then desiccated under vacuum at 55°C until a stable dry weight was achieved. The wet weight and dry weight were then used to calculate the lung wet to dry weight ratio.

Lung Tissue Myeloperoxidase

Myeloperoxidase assay (MPO) was performed on lung tissue to quantify neutrophil sequestration as previously described [20].

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed on all lungs after the reperfusion period ended. The right upper and middle lobes were isolated and lavaged with 10 mL of normal saline. The BAL fluid was then centrifuged at 1,500 g for five minutes at 4°C. The supernatant was snap frozen for subsequent cytokine analysis. The pellet from the BAL was resuspended in normal saline after the red blood cells were lysed. Cells were plated using Cytospin 3 (Shandon, Cheshire, England) and counted with a hemacytometer.

Enzyme-Linked Immunosorbent Assay (ELISA)

Lung tissue lysate was generated from a wedge of the right lower lobe. After appropriate processing, the supernatant was used for ELISA. To normalize for protein levels between samples, the protein concentration for each of the lung samples was measured using the BCA protein assay kit. (Pierce, Rockford, IL). The TNF-α ELISA assay used for lung tissue and bronchoalveolar

lavage was purchased from BD Biosciences (San Diego, CA) and performed according to directions from the manufacturer. Samples were run in triplicate.

Lung Injury Score

After tissue processing and staining for hematoxylin and eosin, lung samples were graded by a blinded pathologist. Each sample was graded with a lung injury score based on the number of neutrophils, amount of interstitial infiltrate, and the percentage of edematous alveolae involved. Each of these three categories was given a score of 0 to 3, resulting in a possible score ranging from 0 for normal lungs to 9 for the most injured lungs.

Statistics

A repeated measures analysis of variance (ANOVA) was performed with one within-subject factor (time with five levels) and one between-subject factor (group with five levels; gadolinium, L-NAME, L-NAME gadolinium, NO, and NO gadolinium). Reported *p* values are considered significant when less than 0.05. Profile plots for the interaction of group and time were generated to examine the interaction of group and time. A two-way ANOVA was performed for pairwise comparisons between specific groups and time points. The Tukey "honestly significant difference" multiple comparison analyses were performed for significant main effects. Values are expressed as the mean ± SD.

Results

Physiologic Measurements

A significant decrease in PA pressure was seen in the group receiving GdCl₃ and NO compared with sham (*p* = 0.001). The GdCl₃ and NO administration also resulted in improved pulmonary compliance and oxygenation compared with sham (*p* = 0.001 and *p* = 0.028, respectively). Pretreatment with GdCl₃ and the administration of inhaled NO resulted in overall similar oxygenation and PA

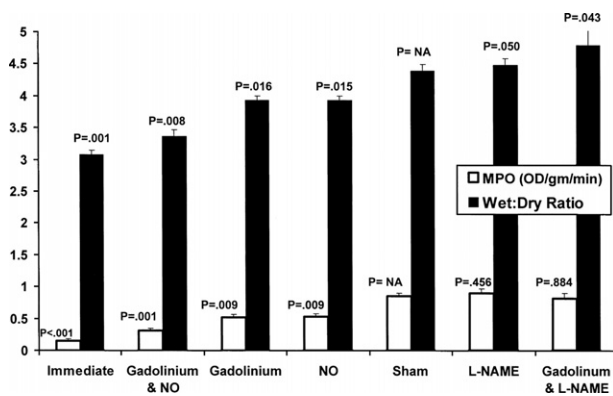


Fig 1. Lung tissue myeloperoxidase (MPO) activity and wet to dry ratios. (ANOVA [MPO], *p* = 0.000; ANOVA [wet to dry ratio], *p* = 0.000; all groups compared with sham; *n* = 10 for each group.) (ANOVA = analysis of variance; L-NAME = L-N^G arginine methyl ester; NA = not applicable; NO = nitric oxide.)

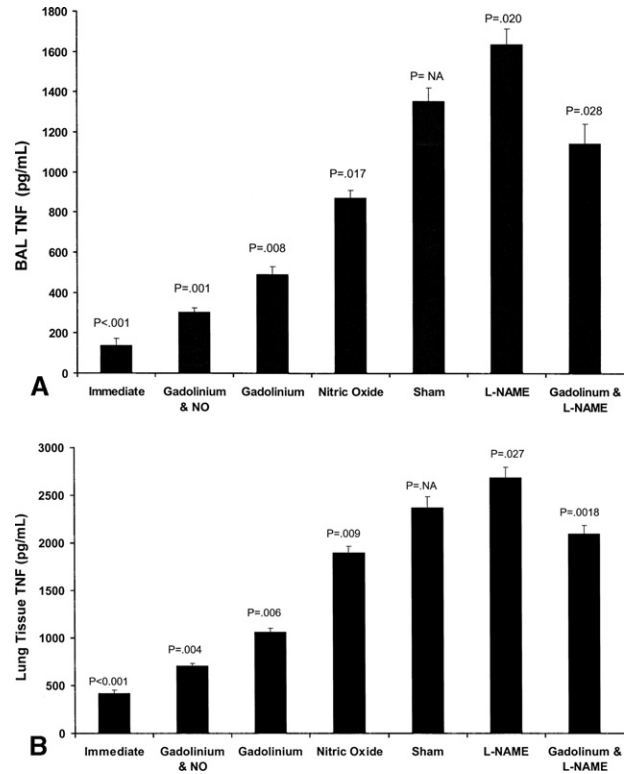


Fig 2. (A) Bronchoalveolar lavage (BAL) and (B) lung tissue tumor necrosis factor (TNF). (Analysis of variance *p* value = 0.000; all groups compared with sham; *n* = 10 for each group) (L-NAME = L-N^G arginine methyl ester; NA = not applicable; NO = nitric oxide.)

pressure curves compared with the immediately reperfused group (no cold ischemia) (*p* = 0.988 and *p* = 0.268, respectively). Measured values for PA pressure, pulmonary compliance, and oxygenation are shown in Table 2.

Myeloperoxidase Content

Using myeloperoxidase (MPO) content as an indicator of lung tissue neutrophil sequestration, GdCl₃ and inhaled NO administration resulted in the greatest decrease in MPO compared with sham (63% reduction, *p* = 0.001) (Fig 1). Macrophage inhibition alone decreased MPO content by 39% compared with group sham (*p* = 0.009). Contrary to inhaled NO, which decreased MPO content by 34% (*p* = 0.009), NO inhibition with L-NAME resulted in a 6% increase in MPO content compared with sham (*p* = 0.456).

Wet to Dry Analysis

The GdCl₃ and inhaled NO administration resulted in the greatest improvement in wet to dry ratios (marker of pulmonary edema) versus sham (*p* < 0.001) (Fig 1).

ELISA for TNF-α

The ELISA of homogenized BAL fluid and lung tissue (Figs 2A, B) evaluated the effect of macrophage inhibition and supplementation of inhaled NO on the release of

Table 3. Lung Injury Score

	Neutrophils	<i>p</i> Value ^a	Edema	<i>p</i> Value ^a	Interstitial Infiltrate	<i>p</i> Value ^a	Total Score	<i>p</i> Value ^a
Sham	1.2 ± 0.18	N/A	1.9 ± 0.10	N/A	1.7 ± 0.24	N/A	4.8 ± 0.40	N/A
GdCl ₃	0.8 ± 0.18	0.23	1.6 ± 0.27	0.30	1.4 ± 0.27	0.37	3.8 ± 1.51	0.28
GdCl ₃ + NO	0.5 ± 0.28	0.02	0.8 ± 0.18	<0.01	1.2 ± 0.18	0.07	1.7 ± 0.27	0.01

^a *p* value = groups compared with sham.

GdCl₃ = gadolinium chloride; NO = nitric oxide.

TNF- α . Macrophage inhibition was associated with a 64% and 55% reduction in lung tissue and BAL TNF- α levels, respectively ($p < 0.001$). Inhaled NO also reduced TNF- α levels compared with sham (BAL, $p = 0.017$; lung tissue,

$p = 0.009$). A 70% and 87% reduction in TNF- α levels in BAL and lung tissues, respectively, was seen in the group receiving GdCl₃ and inhaled NO compared with sham (BAL, $p = 0.001$; lung tissue, $p = 0.004$). The sole administration of L-NAME resulted in the highest increase in TNF- α content in lung tissue and BAL compared with sham (13% and 21%, respectively) (BAL, $p = 0.020$; lung tissue, $p = 0.027$).

Lung Histology and Cell Count

The lung severity score was significantly lower in lungs receiving GdCl₃ and inhaled NO compared with sham (Table 3). The GdCl₃ and inhaled NO administration (Fig 3A) also demonstrated significant decrease in neutrophils-high powered field and interstitial-alveolar edema compared with sham (Fig 3B). Cell counts and staining were also done to determine the effect of GdCl₃ pretreatment on AM cell count prior to reperfusion. The GdCl₃ pretreatment ($n = 4$) resulted in a 26% reduction in alveolar macrophages compared with pretreatment with saline ($n = 4$) ($p < 0.05$).

Comment

Donor AMs and neutrophils are considered to be the key cellular mediators in the pathogenesis of LIRI. Many studies have examined the role of inhibition of donor AMs or neutrophils in the development of LIRI. However, we have found no studies which have directed interventions at each of the known cellular components of LIRI in order to determine if additive improvements in lung function occur. In our study, macrophage inhibition and inhaled NO were able to decrease TNF- α levels and neutrophil sequestration independently of each other. Their concurrent administration resulted in the greatest decrease in TNF- α and neutrophil sequestration-infiltration and the most significant improvement in lung function. This observed synergy between AM inhibition and neutrophil attenuation translated into clinically relevant and additive improvements in oxygenation, pulmonary compliance, and pulmonary artery pressure greater than simply focusing attention to only one of the cellular mediators of injury.

Early IR-injury is thought to be mediated by donor AMs, and late injury by neutrophils. Neutrophils pose a significant threat as they release reactive oxygen species and proteases, and cause capillary plugging secondary to

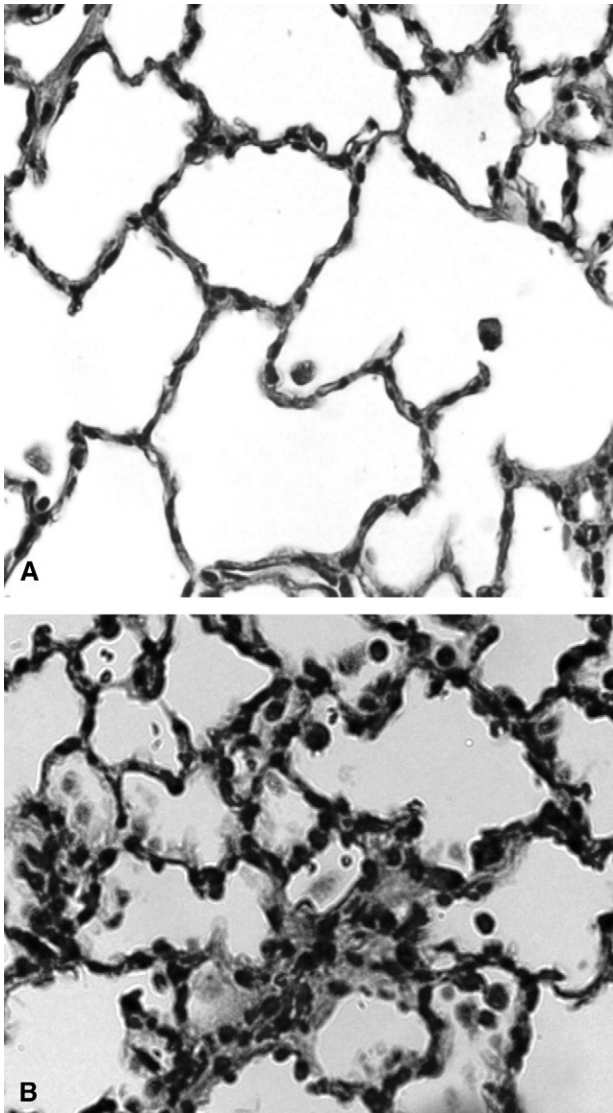


Fig 3. Representative hematoxylin and eosin section of lung tissue ($\times 40$) from lungs receiving gadolinium and inhaled NO (A) and sham lungs (B).

their less deformable conformation when activated [21]. The concept that cells other than neutrophils cause LIRI is relatively recent and has been supported by studies demonstrating significant injury despite the administration of antineutrophil antibodies or the use of leukocyte-depleting filters [7, 20]. Eppinger and colleagues [7] found that neutrophil depletion was not protective early in reperfusion (<30 minutes) but decreased injury late in reperfusion, implicating other cells responsible for early injury.

Current evidence supports that donor AMs are responsible for early, neutrophil-independent injury. Alveolar macrophage inhibition has been shown to improve early lung function and minimize cytokine and chemokine release [5, 6, 22]. The depletion of AMs in an in situ buffer-perfused lung IR model, which eliminates the contribution of circulating cells, resulted in attenuation of IR-induced lung dysfunction and down-regulation of TNF- α and MCP-1 protein expression [6]. While the respiratory burst activity of donor AMs and the release of proinflammatory cytokines and chemokines cause direct, early injury to the lung, donor AMs likely play a larger more pivotal role in the pathogenesis of LIRI. Donor AMs contribute to the recruitment and activation of target cells such as neutrophils through direct cytotoxic effects and through the release of cytokines such as TNF- α . The IR models studying the kidney [23], liver [24], and heart [25] have demonstrated the ability of TNF- α to promote neutrophil recruitment and activation, and its inhibition has accomplished the opposite. In this study, macrophage inhibition decreased TNF- α levels while also decreasing neutrophil sequestration-infiltration and ultimately improving lung function. Studies delineating the time course of TNF- α release in LIRI have shown that its early release mediates the expression of other proinflammatory cytokines [10]. In a study of renal IR injury, macrophage inhibition was protective not only by decreasing early increase in proinflammatory cytokine levels but also through the resulting decrease in leukocyte infiltration [26].

Nitric oxide, and its ability to interfere with neutrophil chemotaxis and migration to sites of inflammation, was of primary importance in this study as one of our objectives was to minimize the neutrophil-dependent hit. This characteristic of inhaled NO was supported in this study as it diminished neutrophil sequestration. In an in vivo rat model NO was also found to be a capable regulator of leukocyte-endothelial cell adhesion [27], which ultimately resulted in decreased neutrophilic injury. Nitric oxide also demonstrated the ability to significantly decrease TNF- α both independently and in conjunction with macrophage inhibition. In a study of cultured AMs by Meldrum and colleagues [15], the addition of a NO donor decreased TNF- α secretion. The NO donors have also resulted in diminished cell death signaling in cultured hepatocytes and endothelial cells [28]. Using an in vivo rabbit model of LIRI, Yamashita and colleagues [16] were able to demonstrate the ability of inhaled NO to significantly decrease apoptosis. Conversely, in our study inhibition of NO with L-NAME resulted in increased

TNF- α , neutrophil sequestration, and pulmonary edema. The benefits of AM inhibition were also lost with the concurrent use of L-NAME. Lung ischemia-reperfusion injury is commonly considered an NO-depleted state which in this study was further worsened by the administration of an NO inhibitor.

There are potential downsides to NO administration. Conflicting evidence exists on the role of NO and the formation reactive oxygen species [29, 30]. The NO administration predisposes to the formation of oxidants such as peroxynitrite. On the other hand, NO has been shown to decrease free radical injury and the cytotoxic effects of neutrophils by inhibiting superoxide production by nicotinamide adenine dinucleotide phosphate oxidase [31]. Its paradoxical role as a free radical scavenger has been associated with decreasing cytokine-induced expression of adhesion molecules [32] and improving epithelial cell damage caused by free radicals [33]. The restoration of low cyclic guanosine monophosphate levels secondary to IR by NO has also resulted in improved endothelial cell integrity and decreased capillary leak. The aforementioned benefits of NO, along with its ability to minimize IR-induced increases in pulmonary vascular resistance and to improve ventilation-perfusion mismatch and oxygenation, make NO an attractive therapeutic agent.

The mechanism of crosstalk between AMs and neutrophils is still unclear. While evidence supports the role of proinflammatory cytokines released by AMs assisting in neutrophil recruitment, the contribution of other cells in the alveolar space, such as alveolar type II cells, is uncertain. Regardless, the utility of minimizing the contribution of the AM to the development of LIRI appears to be critical. Gadolinium chloride, while commonly used experimentally, is an unlikely therapeutic agent as it must be administered 24 hours prior to organ procurement. The action of GdCl₃ is also not specific to AMs and affects monocytes and whole body macrophages, and causes abnormalities in the clotting cascade, thus limiting its clinical applicability. As AMs play an important immunologic role, it is unlikely that the complete suppression of its function is clinically reasonable. However, reduction or neutralization of the proinflammatory mediators released by AMs is clinically relevant. The therapeutic utility of inhaled NO is less debated. While many clinical studies have shown benefits to inhaled NO in the treatment of LIRI, a more aggressive treatment regimen that more precisely addresses the likely underlying bimodal, injurious response to IR is necessary for clear, irrefutable improvements.

Ultimately, the potential importance of this study does not rest in the actual interventions used. The idea that additive benefit can be achieved by targeting therapy at each of the involved cell types or the deleterious products elaborated from those cells may lead to further decreases in IR-injury. Likewise, the concept of minimizing damage from the cellular mediators of LIRI and further delineating the mechanism of interaction between these cells will likely translate into critical improvements in patient care.

Research for this study was funded by NIH RO1 HL 056093 (ILK) and NIH Training Grant 5 T32 HL007849 (ILK). The authors thank Sheila Hammond and Anthony Herring for their technical expertise and contributions and Kimberly Shockey for her statistical expertise.

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