

# Intratracheal Poly (ADP) Ribose Synthetase Inhibition Ameliorates Lung Ischemia Reperfusion Injury

Alexander S. Farivar, MD, Steven M. Woolley, MRCS, Charles H. Fraga, MS, Robert Thomas, BS, Andrew L. Salzman, MD, Csaba Szabo, MD, PhD, and Michael S. Mulligan, MD

Department of Surgery, Division of Cardiothoracic Surgery, University of Washington Medical Center, Seattle, Washington, and Inotek Corp, Beverly, Massachusetts

**Background.** We previously demonstrated that intravenous poly (ADP) ribose synthetase (PARS) inhibition protects against experimental lung ischemia reperfusion injury (LIRI) in an in situ, hilar occlusion model. This study determined its efficacy when administered intratracheally (IT).

**Methods.** Left lungs of rats were rendered ischemic for 90 minutes, and reperfused for up to 4 hours. Treated animals received INO-1001, a PARS inhibitor, intratracheally 30 minutes before ischemia, while controls were given IT vehicle at equivalent volumes. All groups contained at least 4 animals. Lung injury was quantitated utilizing vascular permeability to radiolabeled albumin, tissue myeloperoxidase (MPO) content, alveolar leukocyte cell counts, and arterial  $pO_2$  at 4 hours of reperfusion. Electrophoretic mobility shift assays (EMSA) assessed the nuclear translocation of NF $\kappa$ B and AP-1 in injured left lungs, while ELISAs quantitated secreted cytokine induced neutrophil chemoattractant (CINC) and MCP-1 protein in bronchoalveolar lavage fluid.

**Results.** Intratracheal PARS inhibition was 73% ( $p < 0.0001$ ) and 87% ( $p < 0.0001$ ) protective against increases in vascular permeability and alveolar leukocyte accumulation, respectively, and improved arterial  $pO_2$  ( $p < 0.0004$ ) at 4 hours of reperfusion. Myeloperoxidase (MPO) activity in treated lungs was reduced by 70% ( $p < 0.02$ ). The nuclear translocation of NF $\kappa$ B and AP-1 was attenuated at 15 minutes of reperfusion, and the secretion of CINC and MCP-1 ( $p < 0.05$ ) protein into the alveolus was diminished at 4 hours of reperfusion.

**Conclusions.** Intratracheal INO-1001 protects against experimental LIRI. The reduction in secreted chemokine protein at 4 hours of reperfusion appears to be mediated at the pretranscriptional level through attenuated NF $\kappa$ B and AP-1 activation. This route may optimize future donor organ management and improve lung recipient outcomes.

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Lung ischemia reperfusion injury (LIRI) accounts for significant morbidity and mortality after lung transplantation, affecting 15% to 25% of recipients worldwide [1]. One of the many pathophysiologic events driving this acute dysfunction is the production of nitrogen and oxygen derived free radicals, such as peroxynitrite. Peroxynitrite generates single and double stranded DNA breaks, inciting mechanisms that culminate in cellular death if the oxidative stress is overwhelming [2].

Poly (ADP) ribose synthetase (PARS) is an abundant nuclear enzyme present in most eukaryotic cells that detects nucleic acid injury and facilitates conformational DNA changes in an attempt to maintain its integrity [3]. However, subsequent to saturating DNA injury from free radicals (especially peroxynitrite), PARS is overactivated,

leading to depletion of intracellular stores of NAD<sup>+</sup> and adenosine triphosphate (ATP) [4]. Since both are involved in mitochondrial respiration and glycolysis, depletion of these factors results in deranged cellular energetics and eventual cell death [5]. Additionally, PARS has also been recently implicated in enhancing nuclear factor kappa B (NF $\kappa$ B) dependent transcriptional activation of multiple proinflammatory mediators, including tumor necrosis factor alpha (TNF- $\alpha$ ), subsequent to tissue injury [6]. The ability of PARS to modulate mechanisms that lead to cellular death as well as inflammatory injury make it an intriguing target for an intervention designed to ameliorate experimental LIRI. (Fig 1)

INO-1001 is a newer generation PARS inhibitor that is 1,000,000 times more potent than the classic inhibitor, 3-aminobenzimidazole. While the older generation of compounds scavenged free radicals in addition to inhibiting PARS, INO-1001 selectively inhibits PARS by competing

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Address reprint requests to Dr Farivar, Division of Cardiothoracic Surgery, Box 356310, University of Washington Medical Center, Seattle, WA 98195; e-mail: afarivar@u.washington.edu.

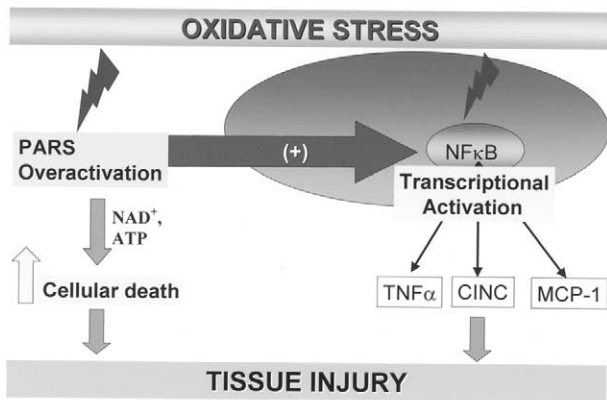
Drs Salzman and Szabo disclose that they have a financial relationship with Inotek Pharmaceuticals.

**Abbreviations and Acronyms**

- ADP = adenosine diphosphate
- AP-1 = activator protein one
- ATP = adenosine triphosphate
- BAL = bronchoalveolar lavage
- BSA = bovine serum albumin
- CINC = cytokine induced neutrophil chemoattractant
- cpm = counts per minute
- DNA = deoxyribonucleic acid
- ELISA = enzyme linked immunosorbent assay
- EMSA = electrophoretic mobility shift assay
- IT = intratracheal
- IV = intravenous
- LIRI = lung ischemia reperfusion injury
- MCP = monocyte chemoattractant protein
- MIP = macrophage inflammatory protein
- MPO = myeloperoxidase
- NFκB = nuclear factor kappa B
- PARS = poly (ADP) ribose synthetase
- PBS = phosphate buffered saline
- TNF-α = tumor necrosis factor-alpha

with NAD<sup>+</sup> at its catalytic binding site. Its half-life is 6 hours in rodents, and there are presently no known side effects.

Given that peroxynitrite is generated in this model subsequent to ischemia and reperfusion [7], and histologic lung sections of 4 hour reperfused lung demonstrate marked cellular death on dUTP nick-end labeling (TUNEL) assays, it is likely that PARS is functional in the development of lung ischemia reperfusion injury. Additionally, NFκB is critical to the transcriptional activation



*Fig 1. Mechanisms of reperfusion induced lung injury. Overwhelming oxidative stress results in PARS overactivation, which leads to depleted intracellular stores of NAD<sup>+</sup> and ATP, and subsequent cellular death from deranged cellular energetics. Additionally, PARS activation is proinflammatory, promoting NFκB dependent transcriptional activation of cytokines and chemokines. (ATP = adenosine triphosphate; CINC = cytokine induced neutrophil chemoattractant; MCP = monocyte chemoattractant protein; NFκB = nuclear factor kappa B; PARS = poly [ADP] ribose synthetase; TNFα = tumor necrosis factor alpha.)*

of multiple mediators, including macrophage inflammatory protein (MIP) 2, MIP-1α, cytokine induced neutrophil chemoattractant (CINC), and TNF-α in primary alveolar macrophages subjected to in vitro hypoxia and reoxygenation [8]. Lastly, we have previously demonstrated that intravenous PARS inhibition is markedly protective against the development of acute reperfusion injury, via an attenuation of NFκB dependent transcriptional activation of the aforementioned proinflammatory chemokines [9]. Therefore, these experiments were designed to assess the efficacy of INO-1001 when administered intratracheally (IT). Theoretically, intratracheal administration would directly affect alveolar macrophages, the cells responsible for mediating many of the early inflammatory events of LIRI [8], reduce concerns about systemic drug toxicities, and potentially optimize future donor lung experimental protocols.

**Material and Methods**

*Reagents*

INO-1001, a novel, potent PARS inhibitor, was a generous gift of Drs. Salzman and Szabo of Inotek Corporation (Beverly, MA). All other reagents were purchased from Sigma Chemical (St. Louis, MO) unless otherwise specified.

*Animal Model*

Long-Evans rats (Simonsen Labs, OR), weighing between 280 and 320 g, were used for all experiments. The University of Washington Animal Care Committee approved all experimental protocols. Animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health (National Institutes of Health Publication No. 86 to 23, revised 1985).

Animals were anesthetized with 35 mg of intraperitoneal pentobarbital, and 0.2 mg of atropine was administered intramuscularly. A 14-gauge angiocatheter was inserted into the trachea through a midline neck incision and secured with a 4-0 suture. Animals were then placed on a Harvard Rodent Ventilator (Harvard Apparatus Inc, MA) with a standardized inspired oxygen content of 60% and 2 cm H<sub>2</sub>O of positive end-expiratory pressure. Maximal peak pressures were maintained below 10 cm H<sub>2</sub>O. The animals were placed on their right side and a left anterolateral thoracotomy in the fifth intercostal space was performed. The left lung was mobilized atraumatically and the inferior pulmonary ligament divided sharply. All animals then received 50 U of heparin in saline (total volume 500 μL) via a penile vein. Five minutes after heparin administration, the left pulmonary hilum, including the left main bronchus, artery, and vein, was occluded with a noncrushing microvascular clamp. Lungs were kept moist with periodic application of warm normal saline, and the incision was covered with a plastic

Table 1. Results of Left Lung Injury Indicators

	Negative Controls	IT Vehicle Treated Positive Controls at 4 hr Reperfusion	IT INO-1001 Treated Animals at 4 hr Reperfusion	<i>p</i> value IT Vehicle vs IT INO-1001 Animals at 4 hr Reperfusion
PI ± SEM (n = # animals/group)	0.09 ± 0.008 (n = 4)	0.88 ± 0.09 (n = 6)	0.30 ± 0.007 (n = 4)	<i>p</i> < 0.001
MPO ± SEM (n = # animals/group)	0.19 ± 0.01 (n = 4)	0.86 ± 0.06 (n = 5)	0.39 ± 0.02 (n = 5)	<i>p</i> = 0.01
BAL cell counts × 10 <sup>5</sup> cells ± SEM (n = # animals/group)	8.4 ± 4 (n = 4)	165 ± 40 (n = 5)	22 ± 16 (n = 4)	<i>p</i> < 0.001

BAL = bronchoalveolar lavage; IT = intratracheal; MPO = myeloperoxidase; PI = permeability indices; SEM = standard error of mean.

wrap to minimize evaporative losses. The ischemia period was held constant at 90 minutes, after which the clamp was removed and the lung ventilated and reperfused for up to 4 hours. Animals were administered 0.5 mL of warm subcutaneous saline per hour to maintain hydration. At the end of reperfusion, a midline incision from the neck to the pubis was created for access to the chest and abdominal cavities. Blood samples were obtained from the inferior vena cava just before sacrifice, the heart-lung block was rapidly excised, and the pulmonary circulation was flushed through the main pulmonary artery with 20 mL of normal saline. The lungs were then separated from mediastinal tissues.

To determine the effects of PARS inhibition on LIRI, treated animals received 3 mg/kg of INO-1001 intratracheally thirty minutes before ischemia. INO-1001 was dissolved in 5% dextrose water (D5W) just before administration. A 150 μL solution, followed by 300 μL of air (to clear drug from dead space), was given through the tracheostomy tube with the animal in a left lateral decubitus position. Vehicle treated animals were administered D5W at equivalent volumes using the same timing regimen.

Negative controls did not undergo any surgical manipulation, while positive controls underwent the full experimental protocol including 90 minutes of ischemia followed by four hours of reperfusion. Exact numbers of animals per group for each outcome marker are shown in Table 1. Left lung injury was quantitated according to the following methods.

#### Lung Permeability Index

To quantitate reperfusion induced lung vascular injury, permeability indices (PI) were measured [7]. <sup>125</sup>I radiolabeled bovine serum albumin (BSA) was obtained from NEN Life Sciences (Boston, MA). Before using <sup>125</sup>I BSA in vivo, serial dilutions were performed to obtain an activity of approximately 800,000 counts per minute (cpm). A 1% BSA/phosphate buffered saline solution was added to make a final volume of 500 μL. Five minutes before removal of the hilar clamp, the <sup>125</sup>I BSA was intravenously injected. Immediately before sacrifice of the animals, 1 mL of blood was drawn from the inferior vena cava. The heart-lung block was then excised and flushed as described previously. The counts were quantitated in both the left lung and in the inferior vena cava blood

sample using a gamma counter. The permeability index is expressed as the ratio of the cpm in the left lung to the cpm in 1 mL of inferior vena caval blood:

$$\text{Permeability Index} = \frac{\text{left lung (cpm)}}{1 \text{ mL blood (cpm)}}$$

This ratio corrects for any variation in the systemic distribution of radioactivity and provides a reproducible measure of lung microvascular permeability.

#### Myeloperoxidase Assay

The myeloperoxidase (MPO) assay was used to quantitate lung parenchymal neutrophil accumulation [7]. Lungs for MPO analysis were harvested in a manner similar to that described above. The lungs were homogenized for sixty seconds in a solution of 0.5% hexadecyltrimethylammonium bromide and 5 mmol/L ethylenediaminetriacetate acid in 50 mmol/L potassium phosphate buffer (pH = 6.0). Samples were sonicated for 40 seconds in four ten-second bursts. The homogenized tissue was maintained on ice between all tissue processing. Samples were centrifuged at 2,300 rpm for 30 minutes at 4°C. Assay buffer was composed of 0.0005% H<sub>2</sub>O<sub>2</sub> and 0.167 mol/L O-dianisidine dihydrochloride in 100 mmol/L potassium phosphate buffer (pH = 6.0). Fifty μL of each sample was mixed with 1.45 mL of assay buffer and the change in absorbance at 460 nm over one minute was recorded.

#### Bronchoalveolar Lavage

Additional animals underwent bronchoalveolar lavage (BAL) at the time of sacrifice in order to determine alveolar leukocyte cell accumulation [7]. Using the 14-gauge angiocatheter placed for ventilation, the lungs were lavaged individually with 3 mL of sterile saline. In order to facilitate individual lung BAL analysis, the contralateral hilum was occluded. At least 80% of the instilled fluid was recovered from each lung BAL. This fluid was centrifuged (1,500 rpm × 8 minutes at 4°C) to pellet the cells. The pellet was resuspended in 10 mL of sterile water to lyse red blood cells, and this fluid was again centrifuged (1,500 rpm × 8 minutes at 4°C). The supernatant was discarded and the cells were counted using a hemacytometer (Hausser Scientific, PA).

### Arterial $pO_2$ Levels

At four hours of reperfusion, 0.2 mL of blood was drawn up from the abdominal aorta, and processed by a blood gas analyzer (Chiron Diagnostics, Emeryville, CA) for arterial  $pO_2$  levels. Two groups were studied (the vehicle treated positive controls and the INO-1001 IT animals at 4 hours of reperfusion), containing four animals per group.

### Electrophoretic Mobility Shift Assay (EMSA)

Electrophoretic mobility shift assays were performed in order to assess the nuclear translocation of proinflammatory transcription factors in left lungs [7]. Lungs were snap frozen in liquid nitrogen after flushing the pulmonary circulation with 20 mL of saline. The frozen tissue was ground to a fine powder and suspended in 4 mL of buffer containing 0.06% Nonidet P-40, 150 mmol/L NaCl, 10 mmol/L HEPES, 1 mmol/L EDTA, and 0.5 mmol/L PMSF. The solution was homogenized and centrifuged for 15 seconds (12,000 g). The pellet was discarded and the supernatant was centrifuged again for 15 seconds (12,000 g). The resultant pellet was suspended in 40  $\mu$ L of buffer containing 40 mmol/L NaCl, 20 mmol/L HEPES, 0.2 mmol/L EDTA, 1.2 mmol/L  $MgCl_2$ , 0.5 mmol/L PMSF, 0.5 mmol/L DTT, 25% glycerol, 5  $\mu$ g/mL aprotinin and 5  $\mu$ g/mL leupeptin at 4°C for 20 minutes. This solution was centrifuged for 5 minutes, the pellet discarded, and the supernatant containing the nuclear protein was stored at -70°C. Quantification of nuclear protein was performed using the bicinchoninic acid assay.

Nuclear protein (10  $\mu$ g) was incubated in a binding reaction with double stranded  $^{32}P$  end-labeled oligonucleotide containing the NF $\kappa$ B or activator protein (AP) 1 consensus binding sequence (Promega, Madison, WI). Running unlabeled oligonucleotide probe in a cold competition binding reaction assessed the specificity of each probe. The binding reaction was carried out at room temperature for 60 minutes and the proteins were resolved on a 6% nondenaturing polyacrylamide gel at 100V for 1 to 2 hours. The gels were dried and autoradiographed. Multiple samples for each timepoint were analyzed. Densitometry was performed with Image J software (Version 1.2, Silver Spring, MD) to assess relative signal intensity.

### ELISA

Sandwich ELISAs for CINC and MCP-1 have been developed in our laboratory by adding 50  $\mu$ L of a 10  $\mu$ g/mL antichemokine antibody (Peprotech, Rocky Hills, NJ) to a carbonate-coating buffer solution (pH = 9.6) [8]. The solution was plated in a 96-well (Dynex) immunoassay plate, incubated overnight at 4°C, and subsequently washed with phosphate buffered saline (PBS) containing 0.05% Tween. Nonspecific binding sites were blocked with a 1% BSA/PBS solution for 30 minutes at 37°C. Samples and standards were diluted in saline, and 50  $\mu$ L was added to each well (1 hour incubation at 37°C). A secondary biotinylated antibody (Peprotech, Rocky Hills,

NJ) specific to the epitope being studied (0.5 to 2  $\mu$ g/mL) was added to each well (1 hour incubation at 37°C). Following a 30-minute incubation with a streptavidin-horseradish-peroxidase conjugate (Pierce, Rockford, IL), the assay was developed by adding an o-phenylenediamine dihydrochloride substrate. The reaction was stopped by adding 50  $\mu$ L of 3 mol/L  $H_2SO_4$ . The linear sensitivity range of the assays have been determined and the assays show no cross reactivity with one other. Samples and standards were run in triplicate, and well-to-well variation did not exceed 5%.

### Statistical Analysis

All data are presented as mean values ( $\pm$  the standard error of the mean) unless otherwise designated. Comparisons between groups were made using a two-tailed Student's *t* test and statistical significance was defined for all tests as a *p* less than 0.05.

### Results

Results regarding left lung injury are summarized in Table 1, and include number of animals generated per group and the *p* values of IT vehicle treated positive controls versus IT INO-1001 treated animals at 4 hours of reperfusion.

#### Lung Vascular Permeability Index

Negative control, unmanipulated lungs demonstrated a permeability index of  $0.09 \pm 0.008$ , while vehicle treated, positive controls had a left lung permeability index of  $0.88 \pm 0.09$ . This represented a significant increase in permeability between these two control groups (*p* < 0.001). Intratracheal PARS inhibition decreased the PI to  $0.30 \pm 0.007$ , a statistically significant decrease (*p* < 0.001) when compared to vehicle treated positive controls. Intratracheal PARS inhibition was 73% protective against this injury indicator.

#### Myeloperoxidase Content

Negative control, unmanipulated lungs demonstrated a change in MPO content of  $0.19 \pm 0.01$ , which increased to  $0.86 \pm 0.06$  at four hours of reperfusion after IT vehicle administration. This was a significant increase in MPO content, with a *p* value of 0.008. Intratracheal INO-1001 reduced MPO content in four hour reperfused lungs to  $0.39 \pm 0.02$ , a statistically significant decrease (*p* = 0.01) which was 70% protective against lung parenchymal neutrophil accumulation.

#### Bronchoalveolar Lavage Cell Counts

When compared to unmanipulated, negative controls ( $8.4 \times 10^5$  cells), there was a significant increase in BAL cell counts ( $165 \times 10^5 \pm 40$  cells) in vehicle treated, positive controls after four hours of reperfusion (*p* < 0.005). The predominant cell type in positive control animals was the neutrophil, whereas the earlier time periods demonstrated almost exclusively alveolar macrophages. Intratracheal PARS inhibition markedly reduced

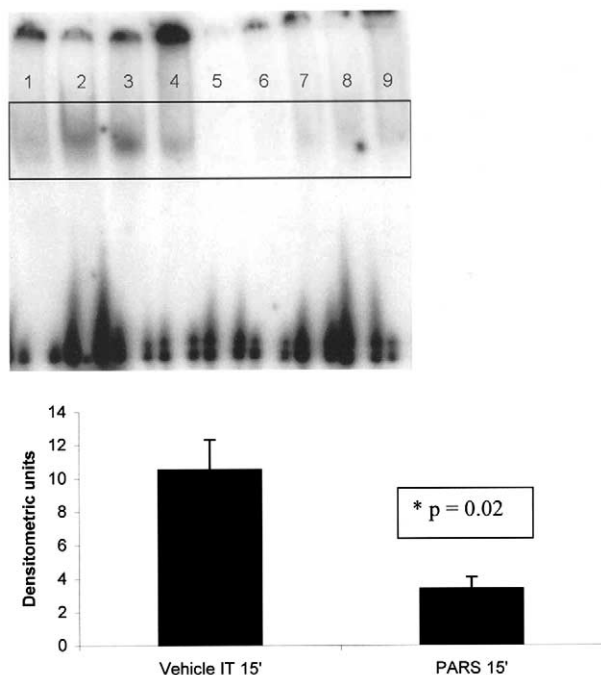


Fig 2. EMSA for NFκB nuclear translocation. Lanes 1 to 4 represent IT vehicle treated animals at 15 minutes of reperfusion, whereas lanes 6 to 9 represent IT INO-1001 treated animals at the same time point. Lane 5 is the cold competition, verifying our band as NFκB. There is a significant reduction in the nuclear translocation of NFκB after IT PARS inhibition. Relative NFκB densitometry is shown. (EMSA = electrophoretic mobility shift assay; IT = intratracheal; [NFκB] = nuclear factor kappa B; PARS = poly [ADP] ribose synthetase.)

alveolar cell counts by 87% to  $22 \times 10^5 \pm 16$  cells, which was statistically significant ( $p < 0.001$ ).

#### Arterial $pO_2$ Levels

The average arterial  $pO_2$  level of the vehicle treated, positive control animals was  $243 \pm 6$  mm Hg, while IT PARS inhibition increased arterial oxygenation to  $348 \pm 4$  mm Hg at four hours of reperfusion. The increase was statistically significant ( $p = 0.0005$ ).

#### EMSA for Proinflammatory Transcription Factor Activation

Since we have previously demonstrated in this model that there is significant nuclear translocation of NFκB and AP-1 at fifteen minutes of reperfusion [7], we used this timepoint to assess whether IT INO-1001 had any effect on the activation of these proinflammatory transcription factors. As shown on the representative EMSAs, there was significant translocation of NFκB (Fig 2) and AP-1 (Fig 3) in the IT vehicle treated animals, and IT PARS inhibition significantly reduced the translocation and activation of both transcription factors.

#### ELISA for Chemokine Protein Content in BAL

Cytokine induced neutrophil chemoattractant (CINC) protein content in the BAL effluent rose from 0.1 ng/mL

in unmanipulated lungs to  $2.11 \pm 0.21$  ng/mL in vehicle treated, positive controls at four hours of reperfusion. This increase was statistically significant ( $p = 0.02$ ). Intratracheal INO-1001 reduced the secreted CINC protein content in the BAL to  $0.725 \pm 0.05$  ng/mL, a significant decrease ( $p = 0.04$ ) relative to positive controls. Ischemia and reperfusion increased MCP-1 secretion from  $0.9 \pm 0.21$  in negative controls to  $23.4 \pm 2.8$  ng/mL in positive controls, a significant increase ( $p = 0.02$ ). Intratracheal INO-1001 reduced the secretion of this proinflammatory mediator to  $5.6 \pm 1.0$  ng/mL, which was also significant ( $p = 0.04$ ).

#### Comment

We have previously shown in this warm in situ hilar occlusion model of lung ischemia reperfusion injury that intravenous INO-1001 administration was markedly protective against the development of acute lung reperfusion injury [9]. In those studies, PARS inhibition significantly reduced levels of cellular death as assessed by TUNEL assays and caspase 3 staining relative to positive controls at four hours of reperfusion. These effects were partly mediated in the injured left lung by attenuated NFκB dependent transcriptional activation of proinflammatory mediators like MCP-1 and CINC [10].

Given that antibody blockade of individual mediators affords only partial protection against LIRI [11], we sought to delineate effector mechanisms that were synergistic with oxidant-induced, chemokine-mediated tissue injury. The activation of PARS subsequent to oxidative stress is one such mechanism, as deranged energetics due to depleted intracellular stores of  $NAD^+$  and ATP result in eventual cellular death. Additionally, PARS has also been implicated in enhancing NFκB

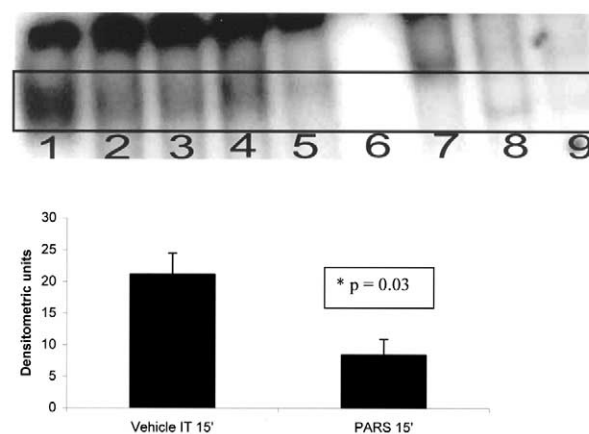


Fig 3. EMSA for AP-1 nuclear translocation. Lanes 1 to 5 represent IT vehicle treated animals at 15 minutes of reperfusion, whereas lanes 7 to 9 represent IT INO-1001 treated animals at the same time point. Lane 6 is the cold competition, verifying our band as AP-1. There is a significant reduction in the nuclear translocation of AP-1 after IT PARS inhibition. Relative densitometry is shown. (EMSA = electrophoretic mobility shift assay; IT = intratracheal; PARS = poly [ADP] ribose synthetase.)

dependent transcriptional activation of chemokine genes, providing a further proinflammatory influence on tissue injury [12]. These studies have confirmed the inflammatory contribution of PARS activation to acute lung injury as intratracheal administration of INO-1001 significantly decreased the nuclear translocation of the proinflammatory transcription factors NF $\kappa$ B and AP-1, which resulted in diminished secretion of two chemokines, CINC and MCP-1, known to mediate left lung injury in this model.

The ability to administer therapeutic agents intratracheally provides the transplant surgeon with an opportunity to selectively modulate respiratory-related inflammatory mechanisms that may ultimately contribute to lung allograft reperfusion injury. Given that lung donors usually are intraabdominal organ donors as well, intravenous administration of therapeutic compounds can have possible untoward effects on other organ systems, and their use may be met with some resistance by members of other transplant teams. Furthermore, these agents could hypothetically be given in nebulized form to the donor shortly before graft procurement.

Since we have shown that alveolar macrophages are the critical cells that orchestrate many of the early inflammatory mechanisms that ultimately result in florid lung reperfusion injury [8], including marked TNF- $\alpha$  and chemokine secretion into the alveolus, we sought to direct our therapy directly into the airway in hope of selectively modulating their early proinflammatory response. Since NF $\kappa$ B and AP-1 nuclear translocation at 15 minutes of reperfusion is required for the transcriptional activation of multiple chemokine genes [8], it is likely that the reductions in MCP-1 and CINC secretion demonstrated in these studies subsequent to IT PARS inhibition is partly mediated at the pretranscriptional level via diminished transcription factor activation.

In comparing the IV and IT routes, permeability indices and MPO content in injured left lungs were similarly protective after PARS inhibition. Intratracheal INO-1001 afforded a greater degree of protection against alveolar leukocyte sequestration, reducing cell counts by 87% compared to 73%. Both routes reduced chemokine secretion as assessed by ELISA on BAL effluent. Regarding positive control animals at four hours of reperfusion, we were able to demonstrate that IV vehicle resulted in a left lung injury that was comparable to that seen after IT vehicle administration. This was confirmed by permeability indices and the relative differences in MPO activity and alveolar cell counts between negative and positive control animals at four hours of reperfusion.

Therefore, the quoted percentages of protection against tissue injury due to PARS inhibition are justifiably comparable and statistically significant, supporting the possible future use of intratracheal agents to limit acute lung reperfusion.

In conclusion, intratracheal PARS inhibition attenuates experimental lung ischemia reperfusion injury by reducing neutrophil accumulation in lung parenchyma and alveolar spaces, and by limiting the secretion of the chemokines CINC and MCP-1 into the alveolus. Future studies utilizing IT INO-1001 are warranted in large animal, orthotopic lung transplant models, and may allow clinicians in the future to selectively modulate lung inflammatory mechanisms subsequent to reperfusion.

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