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Lung Ischemia Reperfusion Injury: A Bench-to-Bedside Review

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Abstract

Lung ischemia reperfusion injury (LIRI) is a pathologic process occurring when oxygen supply to the lung has been compromised followed by a period of reperfusion. The disruption of oxygen supply can occur either via limited blood flow or decreased ventilation termed *anoxic ischemia* and *ventilated ischemia*, respectively. When reperfusion occurs, blood flow and oxygen are reintroduced to the ischemic lung parenchyma, facilitating a toxic environment through the creation of reactive oxygen species, activation of the immune and coagulation systems, endothelial dysfunction, and apoptotic cell death. This review will focus on the mechanisms of LIRI, the current supportive treatments used, and the many therapies currently under research for prevention and treatment of LIRI.

Keywords

ischemic reperfusion injury, reperfusion injury, thoracic surgery, critical care, coagulopathy, intensive care unit, ischemia, nitric oxide, no-reflow, postoperative care

Introduction

Ischemia reperfusion injury is a multifaceted pathological process that complicates the perioperative management of patients undergoing any procedure that disrupts organ perfusion. Organ ischemia begins with an imbalance between metabolic supply and demand and ends with tissue hypoxia, ultimately leading to cellular damage or death. While the ultimate treatment is restoration of adequate organ perfusion, this often exacerbates tissue injury as reperfusion carries inflammatory cells/mediators and reactive oxygen species that promote further injury. This review will focus specifically on lung ischemia reperfusion injury (LIRI).

Compared with other organs, lungs are uniquely resistant to ischemia due to the availability of oxygen from both alveolar gas exchange as well as oxygen delivery from the blood via a dual circulatory system. The later is composed of the pulmonary and bronchial arteries.[1,2] Unlike the pulmonary arteries, which deliver the entire cardiac output in the form of deoxygenated blood, the bronchial arteries branch from the ascending aorta and deliver oxygenated blood (approximately 1% of the cardiac output).[1] However, any situation that impairs alveolar oxygenation or pulmonary blood blow will ultimately result in some form of pulmonary ischemia. While the incidence of LIRI varies depending on the cause and diagnostic/inclusion criteria used by studies, it is generally believed that roughly 15% of patients undergoing lung transplantation will experience some form of graft dysfunction secondary to reperfusion injury.[2] Many more patients with chronic pulmonary emboli will suffer significant LIRI following pulmonary artery thromboendarterectomy or those undergoing cardiopulmonary bypass. The consequences of LIRI are not restricted to the lungs, unfortunately. In many cases, this local inflammatory response leads to systemic inflammatory reactions typically resulting in multisystem organ dysfunction, which not infrequently results in death.

Clinical Causes of Lung Ischemia Reperfusion Injury

Lung ischemia can occur in any situation where oxygen supply fails to meet the metabolic demands of the pulmonary parenchyma due to limited blood flow and/or

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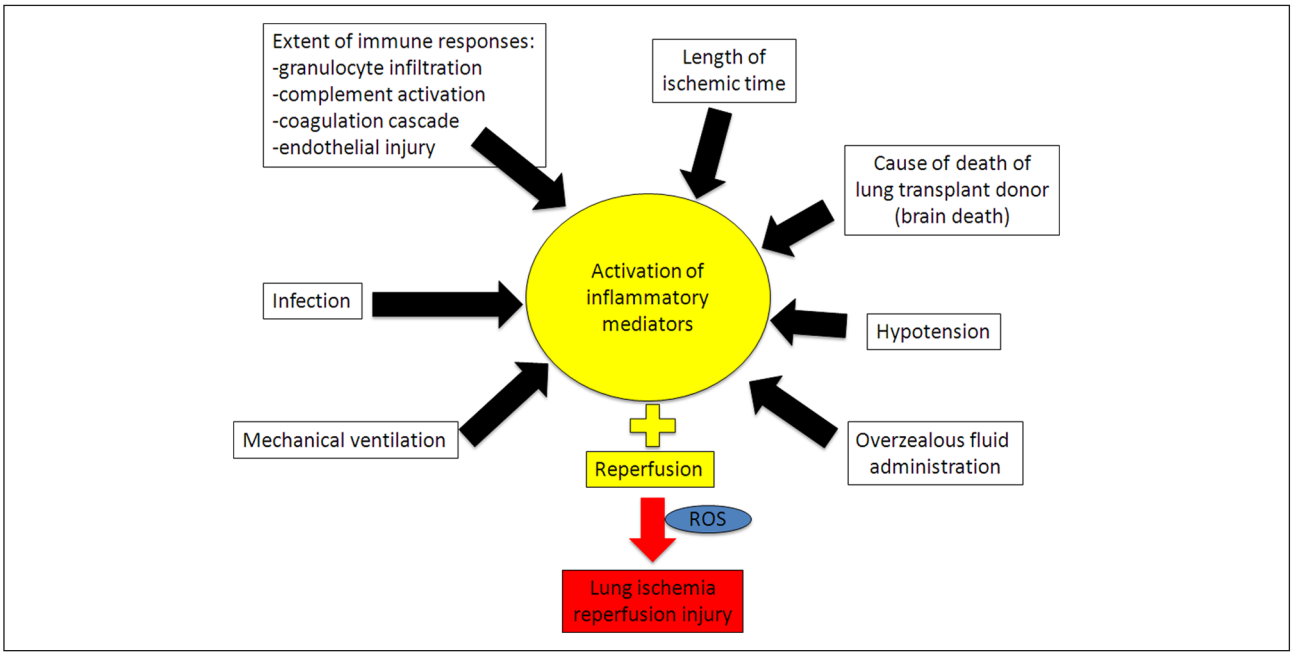

Figure 1. Factors possibly associated with lung injury ischemia reperfusion injury.
Abbreviation: ROS, reactive oxygen species.

decreased ventilation. Due to the by-products of cellular ischemia discussed in detail below, reperfusion is hindered. Additionally, reperfusion counterintuitively leads to the production of toxic molecules that actually further impair perfusion (Figure 1). Clinically there are 2 common scenarios of LIRI. When blood supply is interrupted but ventilation continues, diffusion of oxygen through the alveolar tissue continues creating *ventilated ischemia*. Examples of this include thrombotic situations such as pulmonary artery embolic phenomena, primary pulmonary hypertension, or acute chest syndrome in sickle cell patients.[3,4] When the interruption of blood supply is corrected by pulmonary thromboendarterectomy, for example, reperfusion ensues with injury following. The second common scenario is complete cessation of blood flow and ventilation, known as *anoxic ischemia*. Examples of anoxic ischemia include the cold ischemia time occurring in lung transplantation and cardiopulmonary bypass (although the lung does receive some oxygenated blood through the bronchial arteries during bypass).[5]

Physiologic Manifestations of Lung Ischemia Reperfusion Injury

Clinically, LIRI causes increased pulmonary vascular resistance and increased vascular permeability. This often leads to varying levels of noncardiogenic pulmonary edema with pulmonary wedge pressures typically less than 18 mm Hg. Oxygenation is affected as ventilation/perfusion (V/Q) mismatch follows pulmonary edema. On chest x-ray, LIRI presents as a continuum based on severity ranging from mild infiltrates to diffuse opacifications similar to an acute respiratory distress syndrome (ARDS) picture (Figure 2). Peak airway pressures increase making adequate ventilation difficult. Depending on Pao_2/Fio_2 ratios, the patient may also be classified into categories of lung injury ranging from acute lung injury (ALI) to ARDS. However, clinical diagnostic criteria for LIRI is primarily one of exclusion as currently no specific diagnostic criteria exist.[6]

Cellular Mechanisms Implicated in Ischemia Reperfusion Injury

On a cellular level, ischemia leads to 5 main processes that result in regional injury, and if left unabated can eventually cause multisystem organ failure.[7] Microscopically, LIRI appears as alveoli with marked perivascular edema, focal interstitial and intraalveolar leukocyte infiltration along with proteinous exudate (Figure 3). The 5 main cellular mechanisms implicated in LIRI include sterile immunity (innate and adaptive), complement activation, activation of coagulation, activation of cell death pathways, and, finally, endothelial dysfunction[7] (Table 1, Figure 4).

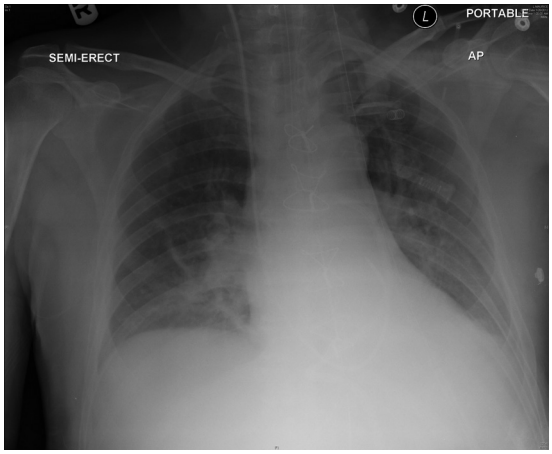

Figure 2. A chest x-ray from a patient with lung ischemia reperfusion injury (LIRI) following a pulmonary artery thromboendarterectomy.
The chest x-ray of a patient with LIRI is nondescript and similar to other lung processes, but will rarely be normal. This chest x-ray demonstrates mild pulmonary vascular congestion with a central hazy opacification, likely atelectasis, and a small left pleural effusion.

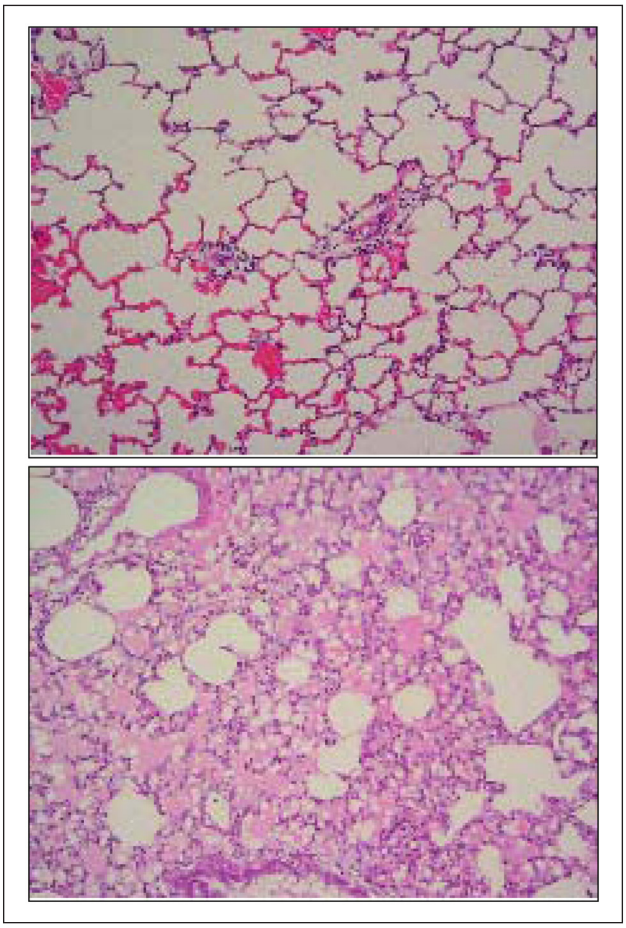

Figure 3. Light micrographs of rat lung tissue suffering from ischemia reperfusion injury.
The top image shows near normal lung with minimal inflammatory cell infiltration and mild perivascular edema. In contrast, the bottom image shows severe lung ischemia reperfusion injury with marked perivascular edema, focal interstitial and intraalveolar leukocyte infiltration, and proteinous exudate.
Reprinted from Lu HL, Chiang CH. Combined therapy of pentastarch, dexamethasone, and dibutyryl-cAMP or β_2-agonist attenuates ischaemia/reperfusion injury of rat lung. *Injury*. 2008;39(9):1062-1070. With permission from Elsevier.

Sterile Immunity

Sterile immunity involves activation of the locoregional immune response that parallels that seen with microbial invasion.[8] This inflammatory immune response, consisting of both innate and adaptive immunity, occurs in areas of sterile cell death or injury and leads to ligand activation of both the innate and adaptive immune responses.[7]

Innate immune response. The innate immune response is a complex system of host defense mechanisms that centers on pattern recognition receptors, such as Toll-like receptors (TLRs).[7-9] Various TLRs have been implicated as mediators of organ ischemia[10-12] and lung reperfusion injury[13] in animal models. During cellular injury or death, intracellular ligands are released, activating various signaling pathways such as NF-κB, mitogen-activated protein kinase (MAPK), and type-I interferon, which ultimately induce the production of pro-inflammatory cytokines and chemokines.[8] Similarly, extracellular damage-associated molecular patterns or DAMPs play an intricate role in sterile immunity and include products released from necrotic tissue/cells,[14,15] proteins released during proteolytic cascades activated by ischemia and reperfusion injury, and break down products from destruction of cellular and extracellular matrix.[15]

DAMPs, such as adenosine, either activate the immune response or attenuate immune responses, with the latter preserving cellular integrity during ischemia.[16,17] Once the immune response is activated, areas of ischemia become infiltrated with inflammatory cells, such as granulocytes.[7] Transcriptional mechanisms become activated, leading to granulopoesis that, depending on the levels, either enhance tissue recovery or worsen tissue injury.[18] The latter is commonly seen in areas of reperfusion where granulocyte margination becomes unregulated and leads to uncontrolled locoregional inflammation.[18] Other cells, such as dendritic cells and T regulatory cells, produce interleukin-10 (IL-10), an anti-inflammatory cytokine that decreases levels of tumor necrosis factor-α (TNF-α), interleukin-6, and reactive oxygen species.[7,19]

ROS, derived from H_2O_2, NADPH oxidase, and other enzymatic reactions are toxic molecules that lead to

Table 1. The 5 Cellular Mechanisms Implicated in Lung Ischemia Reperfusion Injury.

Sterile Immunity	Complement Activation	Activation of Coagulation	Activation of Cell Death	Endothelial Dysfunction
Innate immunity	Plasma and membrane bound proteins create enzymatic reactions	Platelet activation and aggregation	DAMP molecules activate sterile immune response	Increased vascular permeability results in pulmonary edema
Centers on pro-inflammatory mediators	Results in pathogen recognition and opsonization	Leads to microvascular vasoconstriction	Apoptosis causes release of ATP	Activation of complement and coagulation systems
Areas of ischemia infiltrate with granulocytes	Amplifies immune response	Thrombus formation	Autophagy ensues as protective mechanism	Imbalance of vasoconstricting and vasodilating factors
Adaptive immunity			HIF upregulates autophagy genes	"No re-flow" phenomenon
Activation of T- cells via antigen dependent and independent pathways				
B-cell antibody mediated destruction				

Abbreviations: DAMP, damage-associated molecular pattern; ATP, adenosine triphosphate; HIF, hypoxia-inducible factor.

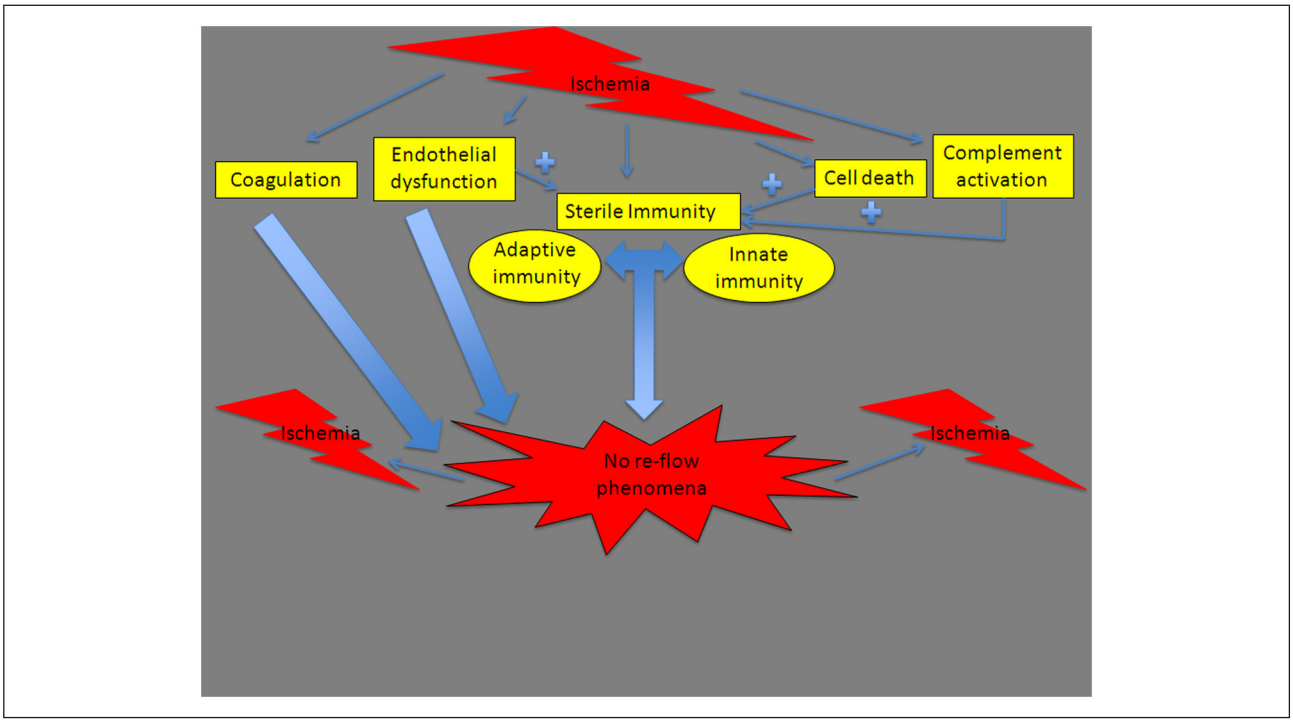

Figure 4. Cellular and molecular mechanisms involved in lung ischemic injury.
Ischemia leads to 5 main cellular processes: coagulation system activation, endothelial dysfunction, sterile immunity activation (both adaptive and innate immunities), cellular death, and complement activation. Activation of these cascades eventually leads to more ischemia due to processes detailed in the text. The "+" indicates upregulation or activation of sterile immunity.

tissue damage,[20] especially oxidative DNA damage via poly ADP-ribose polymerase 1 (PARP-1).[21] Superoxide particles are also generated as ischemia injured mitochondria are unable to efficiently transfer electrons to molecular oxygen.[22] Currently, areas of research for therapeutic interventions aim to alter the sterile immune response induced by ischemia and reperfusion such that intracellular mechanisms remain inactivated (NF-κB,

MAPK)[23-25] and extracellular receptors are antagonized (adenosine, TLRs).[26]

Adaptive immunity. Similar to innate immunity, the adaptive immune response is also activated during ischemia and reperfusion. Although not entirely understood, activation of T-cells during ischemia and reperfusion occurs in both antigen-dependent and antigen-independent pathways.[7,20] During cellular injury and death, danger signals are produced and ultimately lead to presentation of cellular antigens, promoting cellular immunity against the ischemic organ.[20] Murine models for ischemic stroke have demonstrated that IL-17 produced by γδ T-cells significantly contributes to ischemic injury, such that depletion of γδ T-cells mitigated ischemia reperfusion injury.[27] These findings suggest that pharmacotherapies suppressing γδ T-cells and upregulating T-regulatory cells may prove to be effective targets in preventing and treating ischemic reperfusion injury.

Similar to T-cell activation, the humoral immune system is also activated during ischemia. Ischemic tissues express neoepitopes, resulting in B-cell activation and antibody-mediated destruction.[28] During reperfusion, these natural antibodies not only result in complement mediated destruction of ischemic tissue but also recruit other inflammatory cells and collectively result in further tissue injury.[28-30]

Complement Activation

The complement system is a triad of enzymatic pathways (classical, alternative, and lectin pathways) involving plasma and membrane bound proteins that lead to pathogen recognition and opsonization.[30,31] Acting as a bridge between the innate and adaptive immune responses, complement exacerbates tissue injury through direct tissue destruction as well as indirectly via amplification of the immune response and recruitment of other inflammatory cells.[30,31] Human studies involving inhibition of the complement system during acute myocardial infarctions have demonstrated decreased ischemic damage; however, decreased mortality has yet to be proven.[30,32-34]

Coagulation

During ischemia and reperfusion, tissue injury and cellular damage produces and exposes many proteins and factors that initiate platelet aggregation and coagulation.[35-37] As previously described, ischemia induces activation of multiple inflammatory mediators and cascades, leading to localized endothelial injury and dysfunction.[37] During endothelial injury, selectin and integrin proteins become upregulated, fostering platelet and leukocyte activation.[38,39] Activated platelets adhere to the pulmonary endothelium and initiate microvascular constriction, which not only promotes further ischemic injury but also promotes thrombus formation.[37]

Murine models of ischemia reperfusion injury have demonstrated that interfering with integrin-dependent platelet activation and aggregation confers protection from ischemia and infarction during mesenteric arteriole injury.[35] Other murine models looking at thrombus formation during ischemia reperfusion have demonstrated a beneficial role of fibrin D-fragments, such as peptide $B\beta^{15-42}$ in decreasing inflammation and infarct size during myocardial ischemia.[35] These findings have been corroborated in human proof of concept studies where fibrin-derived peptides given as an adjunct to percutaneous coronary interventions in patients with acute ST-segment elevation myocardial infarctions resulted in decreased infarction size 5 days after the initial event.[40] Collectively, these studies have shown that preventing thrombus formation in areas of injured endothelium will not only attenuate the risk of further ischemic injury but also promote microvascular circulation.

Activation of Cell Death Pathways

The final stimulus of the inflammatory response during ischemia and reperfusion is triggered directly by cellular death or indirectly through cellular death pathways.[7] In areas of infarction, the necrotic cells release damage-associated molecular pattern (DAMP) molecules that activate the sterile immune response, whereas programmed cell death (apoptosis) initiates release of ATP, which attracts phagocytes.[14] Collectively, these actions result in recruitment of inflammatory cells to areas of ischemia and reperfusion.

The final cell death pathway involved in ischemia and reperfusion involves the protective mechanism of autophagy. Activated during states of sublethal stress, autophagy is a preservation pathway that recycles cellular debris, damaged organelles, and macromolecular cellular components to provide viable cells with nutrients.[14] Autophagy becomes activated during states of hypoxia and ischemia, as evidenced by the upregulation of hypoxia-inducible factor (HIF), which in turn upregulates autophagic genes.[7] HIF also plays a major role in the expression of vascular endothelial growth factor (VEGF), a major player in angiogenesis.[41] Swine models of myocardial ischemia have demonstrated that inducers of the autophagic response result in attenuation of ischemia reperfusion injury.[7]

Endothelial Dysfunction

As previously stated, the intense inflammatory response elicited by ischemia reperfusion injury results in endothelial dysfunction as evidenced by increased vascular permeability, activation of complement and coagulation factors, adherence of inflammatory cells, and finally an imbalance between vasodilating and vasoconstricting factors.[7] Within the lungs, increased permeability of the

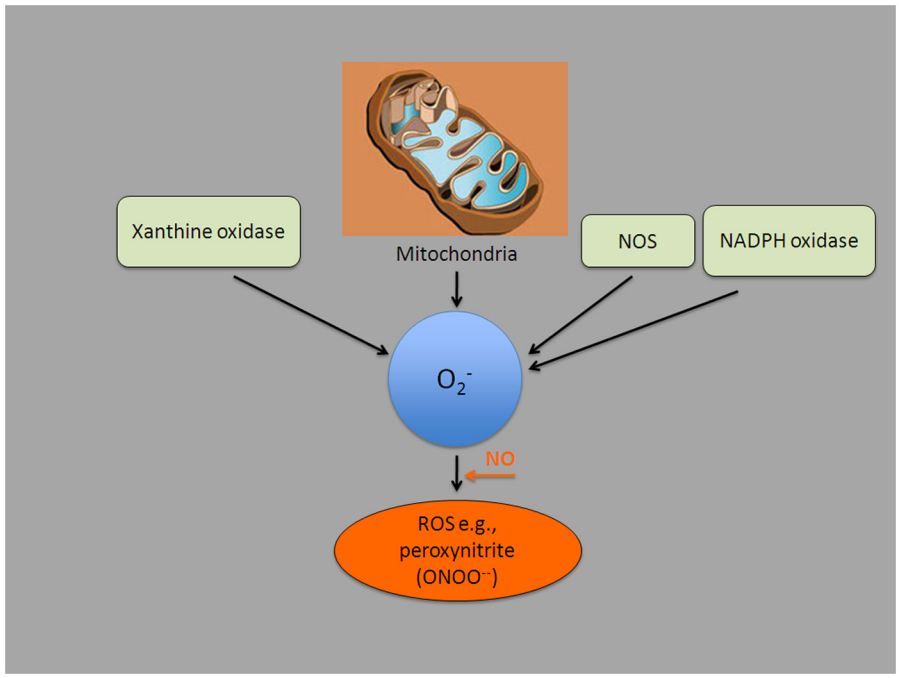

Figure 5. Main sources of reactive oxygen species (ROS).
Abbreviations: NOS, nitric oxide synthase; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; NO, nitric oxide.

alveolar–capillary barrier results in pulmonary edema. During the ischemic phase, oxygen sources become depleted, leading to decreased oxidative phosphorylation with loss of ATP-dependent cellular pumps.[41] As a result, calcium, sodium, and water enter the cell, contributing to cellular dysfunction.[41]

Within the endothelium, ischemic conditions result in transcriptional reprogramming, with an upregulation of leukocyte adhesion molecules, cytokines, and other pro-inflammatory proteins.[41] An imbalance between vasoactive mediators occurs as endothelin and thromboxane A_2 are upregulated while nitric oxide synthase and prostacyclin are downregulated.[41] During the reperfusion phase, a "no re-flow phenomenon" can occur. Leukocyte, platelet, and complement activation leads to impaired vascular relaxation and ultimately decreased microvascular flow despite reperfusion.[7,16,41] This phenomenon may be responsible for delayed graft function or continued organ dysfunction despite adequate reperfusion.[7,41]

Molecular Mechanisms of Lung Ischemia Reperfusion Injury

The formation of reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and the most unstable and reactive, hydroxyl radicals, appears to play a major role in the lung injury that occurs after ischemia reperfusion (Figure 5). Under normal circumstances the body has compensatory antioxidant mechanisms to mitigate the effects of ROS. However, following reperfusion there is a large burst of ROS, which overwhelm the body's protective measures and can lead to cell injury and death.[2,42-46] There are thought to be several key pathways through which the ROS are formed. Xanthine oxidase–dependent formation, NADPH-dependent formation, and NOS-dependent (nitric oxide synthase) formation of ROS will be discussed.[2,43,47-50]

Xanthine Oxidase

Under anoxic conditions, intracellular stores of ATP are degraded such that the final by-product is hypoxanthine.[43,48] In normal physiological states, this is metabolized by xanthine dehydrogenase.[43,48] During anoxia, xanthine dehydrogenase is converted to xanthine oxidase, which then breaks down hypoxanthine into xanthine, H_2O_2, and ROS.[43,48] These by-products have been shown to play a major role in reperfusion injury in numerous organs, including the kidneys, liver, heart, intestines, and other models of ischemia reperfusion.[45,51] However, the true role of xanthine oxidase during pulmonary reperfusion injury remains a subject of debate.

Studies performed in rabbit lungs have shown that pretreatment with the xanthine oxidase inhibitor allopurinol was protective against superoxide formation following anoxia/reoxygenation.[44,49] Allison et al showed that pretreatment with allopurinol in isolated dog lungs blocked hypoxia-reoxygenation-induced increase in pulmonary vascular permeability.[52] Similarly, rat models of LIRI have demonstrated that xanthine oxidase inhibitors significantly decreased LIRI as evidenced by decreased pulmonary vascular resistance (PVR), decreased albumin accumulation, and a marked reduction in endothelial and epithelial injury.[53]

However, other investigators have presented conflicting evidence. Jurmann et al performed a study indicating that while the damage does seem to be, at least in part, related to ROS (as shown by a significant decrease in postischemic PVR with administration of superoxide and hydrogen peroxide scavengers), the administration of intravenous allopurinol had no significant effect.[54] Other studies have found that xanthine oxidase is activated in anoxia reperfusion lung injury models, whereas NADPH oxidase is activated in ventilated ischemic models.[55] Furthermore, it has been also been shown that the level of ATP in the lung tissue does not significantly decrease in continuously ventilated lungs, which is a key part of the xanthine oxidase pathway.[56] Additionally, Kinnula et al showed that xanthine oxidase activity in normal human lungs is low and that it is not released into the blood stream during heart-lung transplantation, leading them to conclude that it is an unlikely source of postoperative complications in this patient population.[57]

NADPH-Mediated ROS Production

As previously stated, it is known that ROS are produced in ventilated ischemia; however, without anoxia there is no depletion of ATP,[56,58] and therefore other mechanisms of ROS production that do not rely on this condition must exist. One such mechanism with significant support is NADPH oxidase. NADPH oxidase is found in high concentrations in phagocytic cells, such as neutrophils and macrophages, but has also been found on the membranes of endothelial cells, including those of the pulmonary vasculature.[48,59,60] When activated, NADPH produces superoxide anion through electron transfer to NADPH to oxygen.[61]

The cessation of blood flow through the pulmonary vasculature results in an abrupt stop in shear stress to the vascular endothelial cells. This causes membrane depolarization through inactivation of ATP-sensitive potassium channels,[62] which leads to activation of NADPH oxidase. The increased production of ROS causes activation of NFκB and AP-1.[63] The presumed physiological reasoning for this reaction is to promote vasodilatation and angiogenesis.

The importance of NADPH oxidase in nonanoxic ischemia/reperfusion injury has been demonstrated through numerous studies. The formation of ROS in animal models of ventilated ischemia has been markedly reduced through the use of NADPH inhibitors such as diphenyliodonium[48,59,62] and apocynin[61,64] as well as in gp91^{phox} knockout mice.[48,58,62]

NOS-Dependent ROS/RNS Production

Nitric oxide synthase (NOS) is an enzyme that metabolizes the amino acid arginine to create citrulline and nitric oxide (NO). NO is a gaseous molecule with an unpaired electron that has numerous physiological effects, such as activating guanylate cyclase to increase production of cGMP and promote vascular smooth muscle relaxation, attenuating platelet aggregation and adhesion, and aiding in host defense and microbial killing.[65]

When NO reacts with a superoxide anion (O_2^-), a molecule found in high concentrations during reperfusion, reactive nitrogen species (RNS) are formed, the most common of which are peroxynitrite anions ($ONOO^-$).[47,66,67] Peroxynitrite, a selective oxidizer, is a stable anion, which affords it greater opportunity to diffuse through a cell and find a target for its effects. It has been shown to cause damage through multiple pathways including lipid peroxidation, inhibition of mitochondrial respiratory enzyme complexes, inactivating sodium transport in type II pneumocytes, and inactivating pulmonary surfactant.[66,68] The importance of peroxynitrite and RNS in lung ischemia/reperfusion injury has been shown both indirectly, through inhibition of nitric oxide synthesis,[68] as well as more directly in rats treated with FP-15, an iron-containing metalloporphyrin that acts as a peroxynitrite decomposition catalyst. Rats treated with FP-15 had decreased lung vascular permeability, decreased tissue myeloperoxidase content, and decreased bronchoalveolar lavage leukocyte concentration.[66] Eppinger et al found worsened lung injury resulting from initiation of inhaled NO (iNO) therapy at the start of reperfusion was a result of RNS, supported by the fact that it was avoided by either pretreatment with superoxide dismutase or by waiting 10 minutes before initiating iNO treatment, after the initial burse of superoxide production had passed.[69]

Treatment of LIRI

The current focus for management of LIRI is supportive therapy. To date, no specific medical management has been proven to specifically prevent LIRI, nor has any

Table 2. Strategies currently being used to prevent and/or ameliorate the effects of lung ischemia reperfusion injury.

Current Strategies for Possible Prevention and/or Amelioration of Lung Ischemia Reperfusion Injury:
- Lung protective ventilation
- Appropriate fluid management
- Optimize organ preservation in lung transplantation:
---improved preservation solutions
---maintain donor lung inflation ≤ 50%
---temperature management
---optimize oxygenation
- Minimize ventilated and/or anoxic ischemic time
- Inhaled nitric oxide (remains controversial)
- Inhaled prostaglandins (not proven effective in humans)

single medical intervention been proven to be the sole treatment for LIRI. Management of patients with LIRI is similar to the management of patients with ARDS.[70] For patients requiring mechanical ventilation, therapeutic goals include minimizing further lung injury from barotrauma and volutrauma with lung protective ventilation as defined by low tidal volumes to minimize plateau airway pressures (<30 cm H_2O) and institution of PEEP to maintain opening of the alveoli and distal airways.[70,71] The key is adequate oxygenation, which may require permissive respiratory acidosis provided that pulmonary hemodynamics are not worsened by hypercapnia via increases in PVR.

Maintenance of systemic and pulmonary perfusion is also imperative to mitigate further injury from ischemia or malperfusion. Given that endothelial dysfunction and capillary leak is common following LIRI, fluid management should be done cautiously as aggressive fluid resuscitation may worsen pulmonary edema. Many clinicians attempt to maintain oxygen delivery and perfusion pressure with low-dose systemic vasopressors in order to minimize fluids.[72] Many centers use iNO in cases of LIRI due to the beneficial properties of pulmonary vasodilation with minimal to no systemic effects, decreases in PVR, support of right ventricular cardiac output, and improvements in V/Q matching. However, it must be noted that it has been shown that iNO may, in fact, lead to the initiation of LIRI.[69] Inhaled prostaglandins are a less expensive alternative to iNO commonly used in LIRI despite the fact that human studies are lacking. They also provide direct pulmonary vasodilation with concomitant beneficial effects of this, but may lead to systemic hypotension. Similarly to iNO, prostaglandins inhibit platelet aggregation,[73] which is believed to be beneficial in LIRI. In settings of inadequate oxygenation leading to severe hypoxemia, institution of extracorporeal membrane oxygenation (ECMO) may be required.[70]

Potential Therapies and Areas of Research in Prevention of LIRI

Preconditioning

The concept of "preconditioning" involves exposing an organ (typically heart or lung) to an insult prior to an ischemia reperfusion insult. This in turn is thought to provide organ protection via resistance to ischemia reperfusion injury. In the case of LIRI, various methods of preconditioning have been shown to have positive effects, including exposing the lung to short periods of ischemia (ischemic preconditioning),[74-76] exposure to elevated temperatures (hyperthermic preconditioning),[77,78] and use of pharmacological agents such as iNO or 3-nitroproprionate, an inhibitor of mitochondrial complex II (chemical preconditioning).[79,80]

The exact mechanism by which preconditioning confers its protective benefits remains to be elucidated. One group of proteins likely involved is heat shock proteins (HSP). HSP are believed to be produced by cells in times of stress, such as hyperthermia, ischemia, and hypoxia and to confer cellular protection.[81] Indeed, increased levels of multiple heat shock proteins have been identified in studies of hyperthermic preconditioning.[77,78] While HSP likely also plays a role in ischemic preconditioning, numerous other molecules have also been implicated including nuclear factor-κB,[82] ATP-sensitive potassium channels,[83] and A_1 adenosine receptors,[84] indicating that the overall mechanism is more complex and multifactorial.

Therapeutic Gases

Nitric oxide. Nitric oxide, an endogenously derived molecule synthesized by NOS, is involved in numerous homeostatic pathways, with its primary role in cyclic GMP mediated vasodilation of vascular smooth muscle.[39] NOSs are categorized as either inducible or constitutively

Table 3. Strategies under investigation for treatment of lung ischemia reperfusion injury. (iNO = inhaled nitric oxide; PPAR-γ = peroxisome proliferator activated receptor-γ).

Strategies Under Investigation to Prevent Lung Ischemia Reperfusion Injury:
- **Therapeutic gases** (carbon monoxide, hydrogen, hydrogen sulfide)
- **Complement inhibitor**: Complement Receptor-1
---inhibits both C3 and C5 convertases of the classical and alternative pathways
---decreased lung injury and time to extubation after lung transplant in humans[118]
- **Inhibition of sterile immunity**
---depletion of alveolar macrophages[66]
---antibody-mediated inhibition of Interleukin-1 and TNFα[119]
- **Inhibition of Activated Protein-1 (AP-1)**
---activated in both ischemia and reperfusion
---responsible for upregulation of proinflammatory cytokines
---inhibition of c-Jun Kinase (JNK) leads to down regulation of AP-1 resulting in attenuated lung injury in rats[120, 121]
- **Preconditioning**
---ischemic preconditioning[75]
---hyperthermic preconditioning[77]
---chemical preconditioning (iNO[80] and 3-nitroproprionate[79])
- **Thiazolidinediones** (oral hypoglycemic agents)
---PPAR-γ exerts anti-inflammatory and anti-apoptotic effects through inhibiting both AP-1 and NF-kB pathways[122]
---preischemic activation of PPAR-γ by thiazolidinediones decreased microvascular permeability, pulmonary edema and levels of inflammatory cytokines such as TNF- α in rat models of LIRI[123]
- **Surfactant administration**
- **Xanthine oxidase inhibitors**[53] (e.g. allopurinol[52])
- **NADPH inhibitors** (diphenyliodonium[59], apocynin[61])
- **NOS inhibitors**
- **Peroxynitrite decompensation catalysts** (FP-15)
- **N-Acetylcysteine (NAC)**
---precursor of antioxidant, glutathione; free radical scavenger[124, 125]
- **Hyperbaric oxygen therapy**
- **Novel gene therapy**

expressed. The former is primarily found in macrophages and epithelial and endothelial cells, whereas the latter is found either in endothelium or neuronal tissues.[43] In areas of ischemia, nitric oxide decreases both platelet aggregation and leukocyte adherence to injured endothelium, scavenges ROS, and promotes normal vascular permeability.[39] Nitric oxide also aids in regenerating injured tissue.[39] In areas of ischemia, however, endothelial dysfunction leads to decreased nitric oxide synthesis and release.[39] During ischemia and reperfusion, nitric oxide production decreases as toxic oxidant production and conversion of free O_2^- H_2O_2 dramatically increases.[39] Due to this expected decrease in nitric oxide production, many studies have analyzed the role of prophylactic nitric oxide therapy in ischemia reperfusion injury following lung transplants. While adequately powered, randomized, double-blind, placebo-controlled studies are lacking, there appears to be no significant difference in immediate oxygenation, early extubation, intensive care unit length of stay, or 30-day mortality in lung transplant patients treated with iNO 10 minutes after reperfusion.[43,85,86]

Ardehali et al corroborated these findings in 28 lung transplant patients who received 20 ppm of iNO on reperfusion. As with previous studies, while prophylactic use of nitric oxide did improve oxygenation and pulmonary artery pressures in patients who developed reperfusion ischemia injury, iNO did not prevent the development of reperfusion ischemia injury.[87] In a prospective, randomized controlled study involving 30 double lung transplant recipients, Perrin et al illustrated that prophylactic use of iNO failed to decrease pulmonary edema formation and resolution after pulmonary reperfusion.[88] Perhaps even more important are the possible detrimental effects of iNO given either during ischemia or early reperfusion. Nitric

oxide can interact with superoxide anions to form peroxynitrous acid, which not only increases the release of the potent vasoconstrictor endothelin-1 but also damages the structural integrity of alveolar cells and impairs surfactant production by type II pneumocytes.[43,69] Based on these findings, some experts argue that while there does appear to be a role for iNO in patients with LIRI, prophylactic use does not appear to change outcomes.[43,86] Nonetheless, when the diagnosis of LIRI has been made, many clinicians use iNO for the beneficial effects including reduction of PVR, support of right ventricular function, and improvements in V/Q mismatch, which can be clinically effective in aiding recovery. However, large, randomized controlled studies are still needed to more clearly define the role of nitric oxide in patients with LIRI.

Hydrogen. Ohsawa et al were the first to demonstrate the therapeutic antioxidant role of hydrogen therapy in a murine model of cerebral ischemic reperfusion injury.[89] Since that time, numerous studies have investigated the anti-oxidative, anti-inflammatory, and anti-apoptotic effects of hydrogen therapy in models of sepsis, multiple organ dysfunction syndrome, organ transplantation, ALI, and ischemia reperfusion injury.[90] Using 2% hydrogen gas, it was illustrated that through hydroxyl radical scavenging, hydrogen gas is able to protect against cellular injury during ischemia reperfusion.[89] Similarily, Xie et al also demonstrated a therapeutic role for hydrogen gas in attenuating pulmonary cell apoptosis and inflammation with decreased sterile immune response and cytokine and chemokine production in murine models of ALI.[90] These findings have also been illustrated in rat models of lung injury where infusions of hydrogen-enriched saline decreased membrane peroxidation, neutrophil infiltration cytokine and chemokine production, and NF-κB activation after ischemia and reperfusion.[91] Studies addressing the safety of hydrogen therapy have shown that humans who drank hydrogen-enriched water had acceptable yet decreased levels of total bilirubin, alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transferase.[92] However, subjects also experienced loose stools, diarrhea, heartburn, and headaches.[92]

Further studies are still needed to elucidate the exact mechanisms by which hydrogen gas protects against ischemia and reperfusion. While randomized control studies using hydrogen gas therapy have yet to be done, there appears to a future role for hydrogen therapy in managing ALI, whether it be from sepsis, hyperoxia, ventilator-induced, or ischemia and reperfusion.

Hydrogen sulfide. Hydrogen sulfide (H_2S), a known environmental toxin, is widely known for its role in inhibition of mitochondrial oxidation via blockade of cytochrome oxidase.[93] Endogenous H_2S is synthesized from L-cysteine and readily permeates cell membranes to exert its various biological effects.[93] H_2S activates potassium-dependent ATP channels, leading to vasodilation and ischemic preconditioning.[93] In murine models, H_2S is known to produce a hibernating-like sate where mice pretreated with 150 ppm of H_2S were able to survive several hours at 5% Fio_2, whereas untreated mice died within 20 minutes when exposed to only 5% Fio_2.[94-96] These studies have demonstrated the protective effects of H_2S during hypoxic stress. The inflammatory response in models of H_2S remains controversial as some studies have demonstrated pro-inflammatory effects whiles others have confirmed the anti-inflammatory effects of H_2S.[93] Like the other therapeutic gases, H_2S plays a role in radical scavenging, mitigating apoptosis and upregulation of heme-oxygensase 1.[93] Murine models of LIRI have demonstrated that pretreatment with H_2S not only decreases histological pulmonary ischemia reperfusion injury and pulmonary edema but also improves overall lung compliance.[97] While these findings highlight a promising future for H_2S in the treatment of pulmonary ischemia reperfusion injury, more studies are needed to more clearly define its anti-inflammatory effects, further elucidate its molecular mechanisms, and characterize its therapeutic index before its clinical application can be investigated.

Carbon monoxide. Low-concentration carbon monoxide (15-250 ppm) has proven to be efficacious in animal models involving not only ischemia reperfusion injury but also shock and organ transplantation.[98] Endogenous carbon monoxide, which is produced from heme degradation via heme oxygenase enzymes, exerts its therapeutic effects through transcriptional upregulation of various stress response pathways.[99] The anti-inflammatory effects of CO are mediated through upregulation of potent anti-inflammatory cytokines such as transcription growth factor-β (TGF-β) and IL-10, whereas the pro-inflammatory cytokines TNF-α and IL-1 are inhibited via CO-induced modulation of p38 MAPK pathways.[98,99] CO also causes upregulation of hypoxia inducible factor-1α (HIF-1α), a major player in apoptosis, autophagy, and cellular responses against hypoxia.[98] While animal models have demonstrated a beneficial role for CO, a randomized, double-blind, placebo-controlled study by Mayr et al involving healthy subjects who were subjected to 500 ppm of CO for 1 hour followed by infusion of lipopolysaccharide failed to demonstrated any anti-inflammatory of CO.[100] Additionally, pharmacokinetic, pharmacodynamic, and toxicological studies in both animals and humans are lacking. While therapeutic CO seems promising in animal models of ischemia reperfusion injury, more studies are needed to more clearly define the role of therapeutic CO.

Surfactant Therapy

Pulmonary surfactant, a mixture of lipids and proteins, is produced by type 2 alveolar cells (AE2) and functions to decrease the surface tension at the air–liquid interface within the alveoli.[101,102] Normally, surfactant stores are categorized as either intracellular or intra-alveolar, with the latter group being responsible for alveolar patency and prevention of atelectasis.[101] LIRI results in both cellular injury and structural damage of alveolar cells, such that surfactant production becomes dysfunctional and fosters further lung injury and pulmonary edema.[103] Dysfunctional surfactant production is not unique to ischemia reperfusion injury and is commonly seen in ARDS and other models of lung injury.[104,105] Rat models of LIRI after double lung transplantation have shown that pretreatment of donor lungs with intravenous surfactant resulted in better oxygenation and pulmonary compliance and decreased pulmonary vascular resistance when compared with controls and groups where only the recipients received surfactant treatment.[106] Canine models of LIRI after single lung transplantation with prolonged graft preservation have demonstrated that combined treatment of both the donor (aerosolized surfactant) and recipient (endobronchial surfactant) with exogenous surfactant resulted in decreased lung injury as evidenced by improved pulmonary function and oxygenation.[105] More important, they illustrated that combined surfactant therapy as opposed to single therapy (treatment of either donor or recipient) was superior in terms of improved lung function and decreased ischemia reperfusion injury.[105] Struber et al were the first to report the successful use of 24-hour continuous nebulized exogenous surfactant in 5 of 6 patients with documented ischemia reperfusion injury.[107] These findings were corroborated by Kermeen et al, where 6 patients with severe ischemia reperfusion injury after transplantation similarly demonstrated significant resolution of symptoms within 24 hours after endobronchial instillation of exogenous surfactant.[108] Based on evidence from neonatal studies of ARDS and previous studies using aerosolized surfactant, bronchial instillation of surfactant more adequately delivers exogenous surfactant.[105,107,108] In comparing natural versus synthetic surfactant, natural surfactants proved to be superior in terms of pulmonary function, improved oxygenation, and decreased lung injury when administered during reperfusion when compared with synthetic surfactants.[101] Possible mechanisms for surfactant-mediated treatment of LIRI involves both replacement of active surfactant in addition to decreased apoptosis, increased anti-inflammatory cytokines, and increased production of endogenous nitric oxide.[109] While 2 small studies have demonstrated a therapeutic role for surfactant therapy in managing LIRI, larger, randomized controlled studies are needed to further elucidate optimal dosing, timing of treatment, and routes of delivery.

Prostaglandins

The use of prostaglandin E1 (PGE1) has proven to be beneficial in improving graft function when used in preservative solutions.[110] When administered during reperfusion, PGE1 results in a cyclic adenosine monophosphate–mediated inhibition of NF-κB transcription pathways and anti-apoptotic effects. PGE1 also mitigates inflammation during LIRI by creating an anti-inflammatory environment through upregulating IL-10 and anti-inflammatory cytokines while also downregulation of pro-inflammatory cytokines.[110] Collectively, this results in improved lung function in rat models of LIRI.[110] These findings were also corroborated in dog models of lung transplantation where improved oxygenation and decreased reperfusion edema were observed in groups treated with PGE1 on implantation of the donor lung.[111] While intraoperative use of aerosolized PGE1 have shown efficacy in improving pulmonary hemodynamics and oxygenation with minimal systemic hemodynamic instability when used during lung transplantation, human studies specifically investigating the use of PGE1 to prevent or to treat LIRI are lacking.[112,113]

Hyperbaric Oxygen Therapy

Hyperbaric oxygen (HBO_2) therapy is a novel technique that uses 100% fractional inspired oxygen in combination with increased atmospheric pressure to create a state of oxidative stress, activating multiple transduction pathways and protective mechanisms.[114] While this environment of oxidative stress does lead to the production of reactive oxygen and reactive nitrogen species, the levels produced are protective rather than toxic, as they result in the activation of protective signaling pathways and anti-oxidant pathways.[114] This results in arterial partial pressure of oxygen levels >2000 mm Hg with tissue levels of 200 to 400 mm Hg.[114,115] HBO_2 therapy not only impairs β_2-integrin-mediated adherence of neutrophils to injured endothelium but also impairs production of pro-inflammatory cytokines by the macrophage–monocyte system.[114,116] This results in decreased concentration of neutrophils in the tissue following reperfusion.[117] Potential risks include central nervous system oxygen toxicity presenting as seizures, reversible myopia, and development of nuclear cataracts with prolonged HBO_2 treatment exceeding 150 to 200 hours.[114] While the use of HBO_2 therapy has been studied in neuronal, intestinal, kidney, liver, and muscle models of ischemia reperfusion

injury, studies involving lung models of injury are lacking. Studies demonstrating the safety, efficacy, and optimal dosing regimens are needed to more clearly define the role of HBO_2 in management of LIRI.

Conclusions

Lung ischemia reperfusion injury is a multifaceted disease process with the inciting event of an imbalance between metabolic supply and demand. The by-product of this is tissue hypoxia, cellular damage, and often cell death. While the incidence and severity of LIRI varies depending on the cause, it is generally believed that roughly 15% of patients undergoing lung transplantation will experience some form of graft dysfunction secondary to reperfusion injury.[2] Many more patients undergoing pulmonary artery thromboendarterectomy, receiving cardiopulmonary bypass, or recovering from sickle cell pulmonary crisis will also have some form of LIRI. Due to cellular and molecular mechanisms, including sterile immunity (innate and adaptive), complement activation, activation of coagulation, activation of cell death pathways, and endothelial dysfunction, lung parenchyma is damaged. Through reperfusion, ROS are formed instigating a cascade of cellular detrimental effects.

With no specific diagnostic criteria, the diagnosis of LIRI is one of exclusion following an ischemia reperfusion insult. Currently, there is no proven preventative drug or measure for LIRI, nor are there proven treatments for LIRI. Until randomized controlled trials showing benefits of therapies in humans are completed, the cornerstone of management for LIRI is supportive with aims of preventing further lung injury.

Declaration of Conflicting Interests

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