Inflammatory Response to Pulmonary Ischemia–Reperfusion Injury

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Abstract
Lung ischemia–reperfusion (IR) injury is one of the most important complications following lung transplant and cardiopulmonary bypass. The pulmonary dysfunction following lung IR has been well documented. Recent studies have shown that ischemia and reperfusion of the lung may each play significant yet differing roles in inducing lung injury. The mechanisms of injury involving neutrophil activation, and the release of numerous inflammatory mediators and oxygen radicals also contribute to lung cellular injury, pneumocyte necrosis, and apoptosis. We herein review the current understanding of the underlying mechanism involved in lung IR injury. The biomolecular mechanisms and interactions which lead to the inflammatory response, pneumocyte necrosis, and apoptosis following lung IR therefore warrant further investigation.

Key words Cardiopulmonary bypass · Inflammation · Ischemia–reperfusion · Lung injury · Transplant

Introduction
Ischemia–reperfusion (IR) lung injury resulting in pulmonary dysfunction is a significant clinical problem following cardiac surgery and lung transplantation. Severe and potentially life-threatening graft dysfunction can occur in up to 20% of all patients after lung transplantation.1 Furthermore, the phenomenon of postoperative lung dysfunction after the use of cardiopulmonary bypass (CPB) is at least in part associated with pulmonary ischemia and reperfusion injury.2,3 The understanding behind the complex pathophysiology of IR lung injury remains incomplete. Unlike any organ in the human body, the lung possesses two blood supply networks with extensive anastamotic connections and a total of three potential sources for lung tissue oxygenation, thus making lung IR injury all the more complex and intriguing to study. Over the past two decades, the role of neutrophils, free radicals, and other inflammatory mediators in IR injury have been extensively investigated. However, these responses and mediators appear to contribute only in part to lung IR injury. We herein review and discuss the current knowledge on the physiology and inflammatory responses that are associated with lung IR injury.

Evidence for Pulmonary IR Injury
The presence of pulmonary IR injury is evident from certain physiological, biochemical, and histological changes. The physiologic changes following lung IR can grossly be divided into increased microvascular permeability, increased pulmonary vascular resistance (PVR), and gas exchange abnormalities from pulmonary edema. Microvascular permeability, as measured by the pulmonary capillary filtration coefficient, increases up to 10-fold after reperfusion in lung IR models.4–7 Such an increase in pulmonary microvascular permeability appears to have a bimodal pattern peaking at 30 min and 4 h after reperfusion.8,9 The initial phase is more dependent on activated pulmonary macrophages involving chemokines, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and monocyte chemotactant protein-1 (MCP-1),9 whereas the late phase depends more on products from activated neutrophils and TNF-α.9 In addition, the majority (52%) of the change in microvascular permeability after IR can be accounted for by the increase in postalveolar (venular) vessel permeability.5
The rise in PVR after lung IR injury (up to 3-fold normal levels), rather than pulmonary vascular permeability, has been suggested to play a critical role in inducing pulmonary edema.\(^\text{10}\) Particularly in the early phase after reperfusion.\(^\text{11-16}\) Vasconstriction from the pulmonary precapillary system was found to play an essential role in increasing PVR after lung IR.\(^\text{17}\) The resulting pulmonary edema as shown by the increase in total extravascular lung water content after ischemia\(^\text{2}\) and reperfusion\(^\text{7,14-18}\) causes poor gas exchange and lung mechanics, thus reducing arterial oxygen tension (\(\text{PaO}_2\))\(^\text{12,14-16,18}\) and elevating the alveolar–arterial gradient (\(\text{A-aDO}_2\))\(^\text{18}\) as well as the peak airway pressure.\(^\text{11}\) A remarkable increase in alveolar dead-space ventilation during the first hour after reperfusion has also been shown in patients undergoing bilateral lung transplantation.\(^\text{19}\)

Biochemical changes that reflect the presence of lung injury after IR include those directly or indirectly responsible for causing lung injury (e.g., metalloproteinase, myeloperoxidase, plasminogen activators and inhibitors), products associated with or released from the injured lung (e.g., oxidized glutathione), and the reduction of products normally synthesized by the lung (e.g., guanosine 3',5'-cyclic)phosphate (cGMP), nitric oxide (NO), \(\text{Na}^+/\text{K}^+\)-adenosine triphosphatase [ATPase]). Proteolytic enzymes released from activated PMN such as matrix metalloproteinases (MMPs) and myeloperoxidase have long been markers of lung injury. They have been shown to degrade the endothelial basal lamina, thereby increasing the microvascular permeability of lung tissue.\(^\text{2}\) Soccol et al. found significantly higher MMP-2 and MMP-9 levels in the bronchoalveolar lavage (BAL) fluid of lungs after IR, and also identified a positive correlation between the MMP levels and pulmonary microvascular permeability.\(^\text{20}\) In addition, significant increases in the myeloperoxidase activity can be detected in the lung tissue following IR.\(^\text{12}\) More recently, increased activity of plasma plasminogen activator inhibitor and a decreased activity of tissue-type plasminogen activator were detected after lung IR.\(^\text{21}\) The imbalance in the fibrinolytic activity may lead to lung injury by causing both pulmonary vascular fibrin deposition and microvascular thrombosis.\(^\text{21}\)

Glutathione, a nucleophilic scavenger of reactive metabolites and oxidizing species, is a sensitive indicator of in vivo oxidant stress and IR oxidation injury. In an animal model, higher levels of plasma and BAL oxidized glutathione have been found after lung IR in comparison to controls, thus indicating the presence of lung oxidation injury.\(^\text{22}\)

The normal pulmonary production of NO by nitric oxide synthase, which is mediated through the second messenger cGMP, is dependent on the integrity of the pulmonary vascular endothelium and lung epithelium.\(^\text{2}\) Lung tissue cGMP levels and pulmonary nitric oxide synthase were found to be significantly reduced after reperfusion in an IR lung model reflecting lung tissue injury.\(^\text{12,23}\) Lower pulmonary cGMP levels following lung IR is associated with pulmonary hypertension,\(^\text{24}\) more microvascular permeability,\(^\text{24}\) and poor lung compliance and \(\text{PaO}_2.\(^\text{25}\)

The development of pulmonary edema following lung IR is partly regulated by the \(\text{Na}^+/\text{K}^+\)-ATPase activity on the basolateral surface of alveolar epithelial cells. The integrity of \(\text{Na}^+/\text{K}^+\)-ATPase was found to be essential for the resolution of pulmonary edema.\(^\text{26}\) Following lung transplantation, Kim et al. found an initial period of up-regulation of \(\text{Na}^+/\text{K}^+\)-ATPase mRNA in alveolar cells, which was shortly followed by a decrease in the mRNA and \(\text{Na}^+/\text{K}^+\)-ATPase protein levels.\(^\text{26}\) This phenomenon was especially evident after prolonged lung ischemia during transplantation, which thus caused pulmonary edema.\(^\text{26}\)

Histologically, the initial cellular response to IR lung injury involves endothelial cellular rounding and contraction rather than cell lysis in an \textit{in vitro} model.\(^\text{27}\) A proposed mechanism for the cellular contraction involves the cellular influx of Ca\(^{2+}\) causing intracellular actin-myosin contraction.\(^\text{5}\) In addition, changes seen by light and electron microscopy in animal lung biopsy specimens obtained following lung IR and transplantation included alveolar capillary interstitial edema, hyaline membrane formation along alveolar ducts, polymorphonuclear cell (PMN) infiltration of pulmonary vessels, as well as the detachment of endothelial cells and type I pneumocytes from the basement membrane. Furthermore, increased class II major histocompatibility complex (MHC-II) antigen expression in bronchial epithelium, vascular endothelium, and cellular infiltrates in the transplanted animal lung were also detected.\(^\text{15,16,28-32}\)

**How Much of the Lung Injury is Ischemia and Reperfusion Related?**

The lung possesses three potential sources of oxygenation from the pulmonary arteries (PA), bronchial arteries (BA), and alveolar ventilation. Their relative contributions towards the delivery of oxygen to maintain lung tissue viability, including during lung IR, remains unclear. An experimental canine study has shown that depleting lung oxygenation by either stopping PA perfusion or alveolar ventilation resulted in a similar degree of lung dysfunction in terms of increased pulmonary capillary permeability and pulmonary vascular resistance.\(^\text{10}\) Moreover, maintaining alveolar ventilation with oxygen in warm ischemic (PA occluded) lungs attenuated the increase in pulmonary permeability and