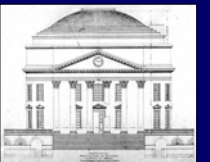




Resident Macrophages in Lung Tissue are Sufficient to Precipitate Pulmonary Reperfusion Injury

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Background

Lung ischemia reperfusion (IR) injury involves contribution from alveolar macrophages, vascular endothelial cells, circulating neutrophils, adhesion factors, free radicals, and a plethora of chemokines and cytokines. Our group and others have attempted to better understand the mediators of this process. The complexity and amplification of the various signaling cascades involved in IR injury, however, have made investigation of individual components of IR injury difficult to assess.

Previous studies from our laboratory have demonstrated that IR in isolated perfused rabbit lungs involves both circulating and resident lung cells. Other groups have demonstrated that lung IR injury requires oxygen radicals, the xanthine oxidase system, leukocyte adhesion and rolling factors, and activation of the alveolar macrophages(1,2,3). Although ischemia is clearly a factor, it has become increasingly evident that reperfusion is responsible for the majority of injury after lung transplantation (4).

Neutrophils have long been recognized as a critical component to the inflammatory cascade, yet their role in initiation of acute lung IR injury is not well understood. Evidence that neutrophils play an important role in lung IR injury has been suggested by investigators using leukocyte depletion techniques as well as antibodies directed toward adhesion molecules (4,5,6). In contrast, others have demonstrated that significant reperfusion injury can be induced without neutrophil participation (7).

Because of the discrepancies regarding the role of circulating leukocytes (recipient) and resident leukocytes (donor) in lung transplant IR injury, we sought to develop a model which eliminated the role of circulating cells all together to focus strictly on resident lung cells.



Figure 1



Figure 2

Methods

We developed a novel technique of evaluating IR in an isolated murine lung system. The advantages of this system are: 1) buffer perfusion eliminates contribution from circulating leukocytes, 2) a non-blood perfused system eliminates the need for large quantities of blood to prime the reperfusion pump; and most importantly, 3) this system allows us to explore more in-depth cellular and molecular mechanisms by utilizing transgenic mice.

Surgery:

Mice are anesthetized with ketamine and xylazine followed by tracheotomy and intubation, ventilation is maintained at 110 bpm with 40% oxygen.

Animals are heparinized and exsanguinated.

Using the isolated perfused mouse lung system (Hugo Sachs Elektronik, Germany, Figure 1), The pulmonary artery is cannulated through the right ventricular outflow tract and perfused with isotonic pH balanced buffer (Figure 2 and diagram).

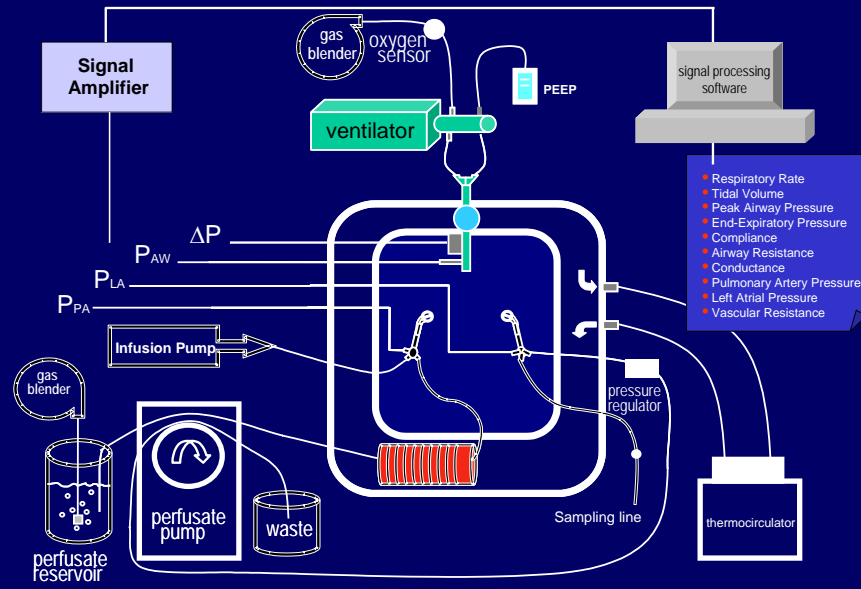
The pO₂ and pCO₂ tension was similar to mixed venous blood gas.

The pulmonary circuit is closed by left atrial cannulation.

Lungs are stabilized for 10 min and rendered ischemic for 60 min by halting perfusion and ventilating with nitrogen. Reperfusion begins with buffer-reperfusion and oxygen ventilation for 60 min.

Parameters measured include:

- Pulmonary artery pressure
- Airway resistance
- Static Compliance
- Pulmonary Edema (Lung weight Index)
- Histologic Lung Injury Score graded by pathologist based on degree of septal thickening, fibrin deposits, and pulmonary edema



Sham 120 min Reperfusion 60 min Ischemia - 60 min Reperfusion

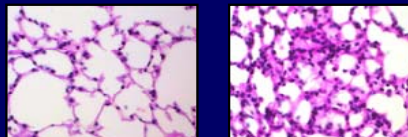


Figure 3. H&E staining showing increased injury after IR (right).

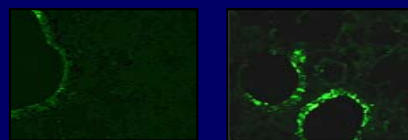
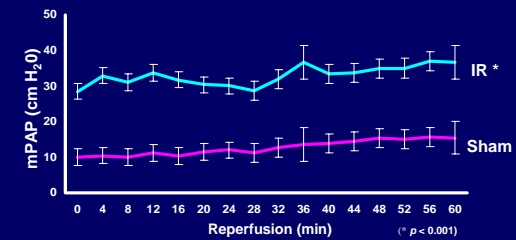


Figure 4. Immunofluorescent staining of nitrotyrosine* (a marker for oxidative injury) is markedly increased in vessels and airways after IR (right).

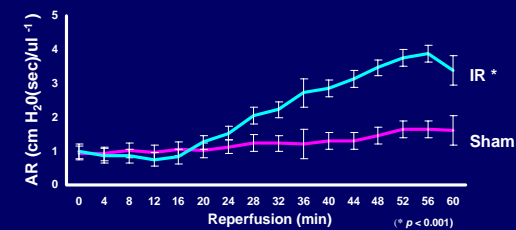
*We thank Dr. Andrew Gow, Children's Hospital of Philadelphia, for nitrotyrosine staining

Results

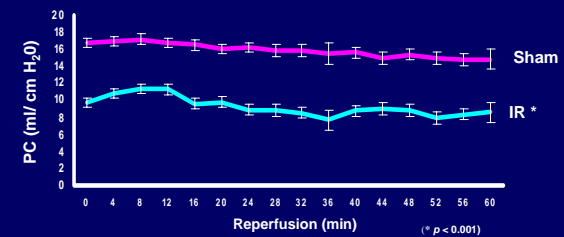
Mean Pulmonary Artery Pressure (mPAP) is significantly higher immediately upon reperfusion in IR lungs compared to Sham. mPAP remained significantly higher in IR lungs throughout 60 min of reperfusion.



Airway Resistance (AR) remained similar between groups during early reperfusion. Significant increases in AR occurred at 28 min and continued to increase throughout reperfusion in IR compared to Sham.



Pulmonary Compliance (PC) was significantly diminished throughout the entire reperfusion period in IR compared to Sham.



Summary

- Perfusion alone causes a mild decline in measured physiologic parameters over 120 minutes.
- A 60 min period of ischemia followed by 60 min reperfusion generates the classic physiologic responses seen in acute IR injury.
- Significant worsening of all measured hemodynamic parameters (PC, mPAP, and AR) occurs after IR.
- Immunofluorescent staining of nitrotyrosine (a marker for oxidative injury) is markedly greater after IR.
- IR injury is also illustrated by histology.

Conclusions

- Lung reperfusion injury can be initiated by resident lung cells (macrophages) in a non-blood perfused isolated lung system.
- The application of this novel technique will allow further investigation of specific components of pulmonary IR injury.

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