PORCINE MODEL OF SEPSIS-INDUCED SYSTEMIC INFLAMMATION AND ACUTE LUNG INJURY: CAN INJURED LUNGS BE RECOVERED WITH EVLP ALONE?

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Abstract

Background. The major challenge for lung transplantation (LTx) is shortage of organ donors and quality of lung grafts. To expand lung donor pool, normothermic ex-vivo lung perfusion (EVLP) has emerged as a platform for reconditioning of marginal donor lungs. This study was performed to develop a stable and reproducible large animal model of LPS-induced early phase of systemic inflammation and acute lung injury (ALI). The injured lungs were reperfused in the EVLP platform to assess the efficacy of reconditioning and recovering.

Methods. Healthy pigs (~58Kg; n=6) were anesthetized and surgically prepared for measurement of hemodynamic and lung function. After infusion of lipopolysaccharide (LPS; 20 µg/kg) for 1 hr, followed by 90-min response period, the lungs were harvested and kept on ice for 2hrs of cold ischemia, then reperfused for 4hrs with EVLP (Vivoline LS1; Lund protocol). Pulmonary function as well as inflammatory profile and markers of edema formation were investigated, both *in-vivo* (2.5hrs) and during EVLP (4hrs). Pro- and counter-inflammatory cytokines were assayed in pig blood and in perfusate samples, which were collected before, and every 30 min after starting LPS and EVLP.

Results. LPS infusion resulted in a major hemodynamic instability, characterized by a marked pulmonary hypertension (PAPm = 52 mmHg at 25 min post-infusion), followed by an increase in systemic pressure and heart rate. This was associated with an increase in the levels of TNFα by 30 min, an increase in IL-10 by 1hr and IL-6 by 2hrs, and no change in IL-1β. Ex-vivo assessment of injured lungs showed a pulmonary dysfunction characterized by gas exchange deterioration and edema formation (52% lung weight gain, compared to 4% in the control group) despite 4hrs of EVLP reconditioning. The inflammatory profile showed a stable but elevated TNFα levels (1293±81 pg/ml), and continuous production of interleukins during EVLP, mostly IL-6 (from 314±199 to 5779±1091 pg/ml). In the control-perfused lungs, the levels of TNFα are less than 100 pg/ml and no IL-6 was detected.

Conclusion. A large animal model of LPS-induced systemic inflammation and ALI has been developed. The EVLP platform, in combination with anti-inflammatory and targeted therapies, could be promising strategies to recover the injured lungs.

Introduction & Rational

1. **Challenges in Lung Transplantation: shortage of organ donors and quality of lung grafts are major limiting factors (1). Approximately 80% of lungs offered for transplantation are deemed unsuitable for transplantation (2).**

   Consequences: unnecessary deaths on the waiting list…

2. **Opportunities: Reconditioning of marginal donor lungs before transplantation with promising emerging platform: Normothermic ex-vivo lung perfusion (EVLP) (1, 3).**

   Potential benefits: expanding lung donor pool…

3. **Acute lung injury (ALI) secondary to sepsis: a major healthcare problem that affects millions of people each year and is associated with substantial mortality (4).**

   Lungs affected with acute sepsis are not eligible for transplantation (5).

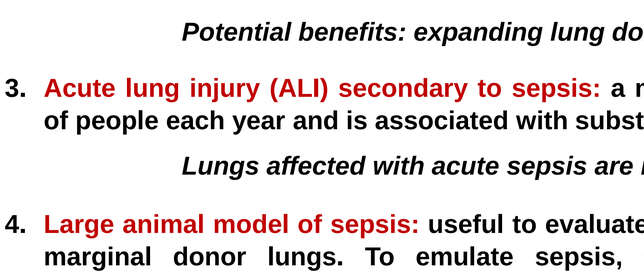
4. **Large animal model of sepsis: useful to evaluate the potential of EVLP platform to recondition marginal donor lungs. To emulate sepsis, endotoxemia was induced by intravenous administration of LPS (cell membrane component of Gram negative bacteria).**

Study Aims

1. **Develop a stable and reproducible large animal model of LPS-induced early phase of systemic inflammation and acute lung injury (ALI) that mimics acute sepsis in humans**
2. **Use the EVLP platform to recondition the injured lungs from these marginal donors and to evaluate lung function before and after reconditioning**

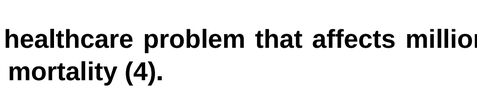
Methods

LPS-induced ALI Protocol



1- Hemodynamic and Respiratory Parameters Recording

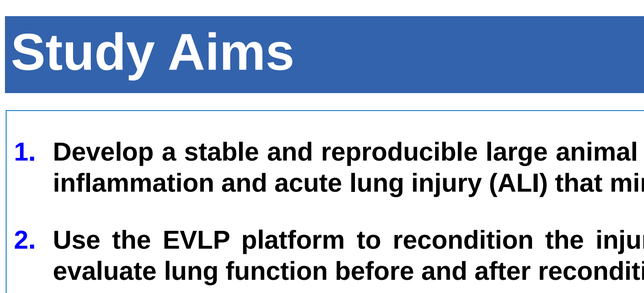
2- Blood Sampling before Lung Procurement:



3- Profiling Systemic Inflammation Markers

Porcine TNFα, IL-6, IL-10, IL-1β and CRP were analyzed in plasma using kits from DuoSet® ELISA Development System (R&D Systems Inc., MN, USA).

Ex-vivo Lung Perfusion Reconditioning Protocol



1- Hemodynamic and Respiratory Parameters Recording

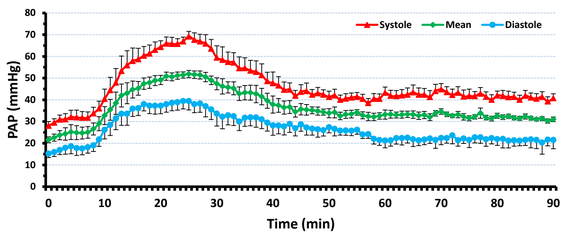
2- Perfusate Sampling:

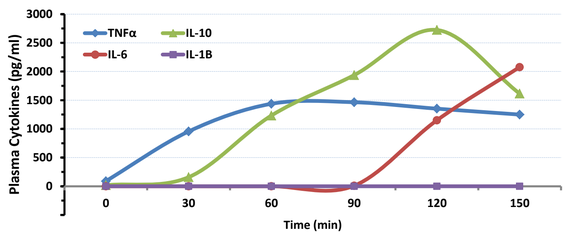
3- Profiling Lung Inflammation Markers

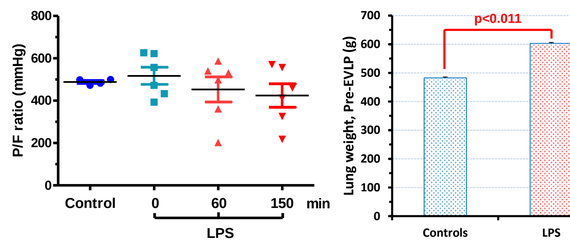
Porcine TNFα, IL-6, IL-10, IL-1β and CRP were analyzed in perfusates and BALs using kits from DuoSet® ELISA Development System (R&D Systems Inc., MN, USA).

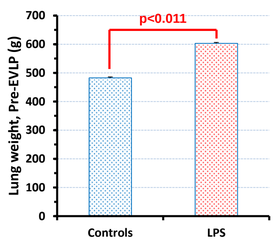
Results

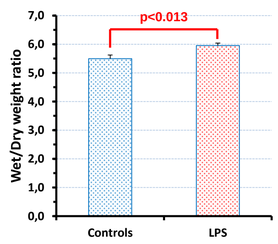
LPS-Induced Acute Lung Injury Model

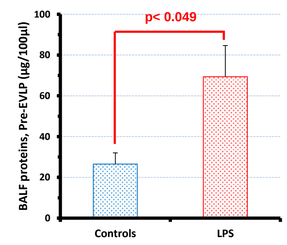






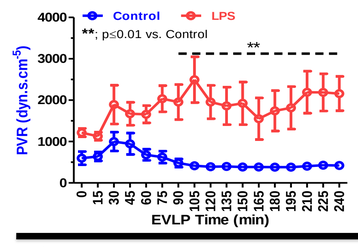


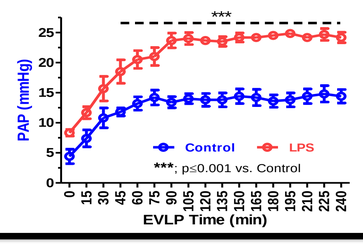


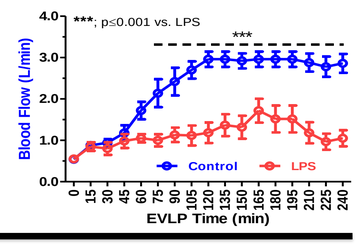


Ex-vivo Lung Perfusion Reconditioning

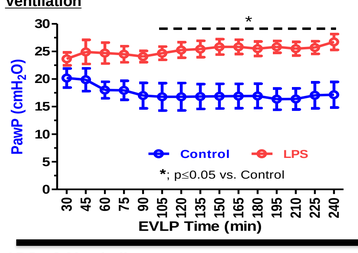
Hemodynamics

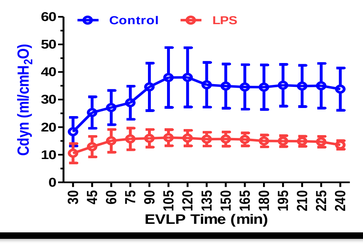


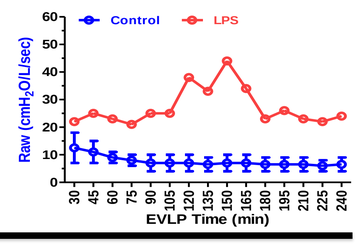




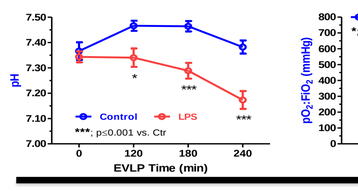
Ventilation

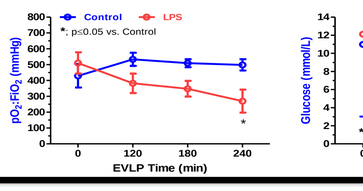


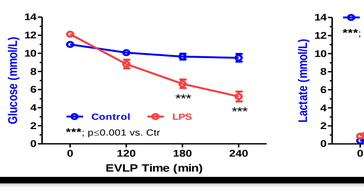


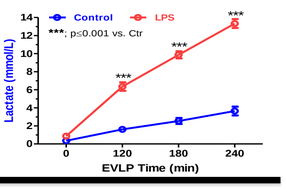


ABGs & Metabolism

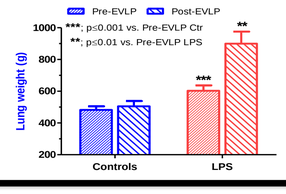


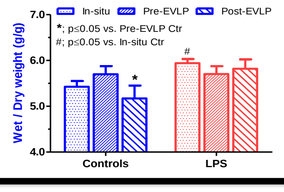




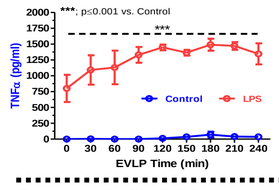


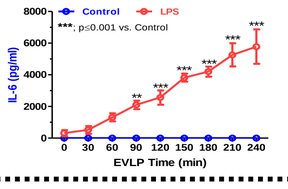
Lung Injury Markers

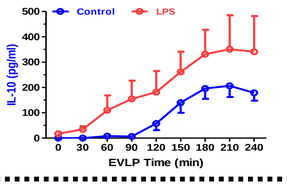


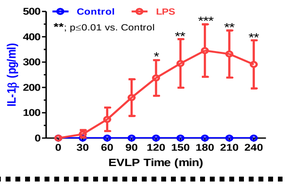


Inflammatory Profile: Perfusate

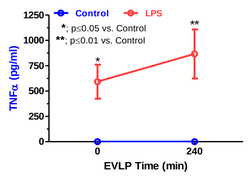


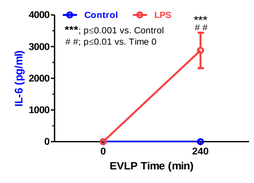


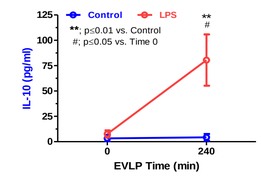


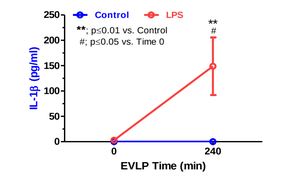


Inflammatory Profile: BALF









Conclusion

- **The porcine acute lung injury model is able to mimic the cascade of inflammatory responses common to sepsis-induced ALI in humans**
- **The lung function from these porcine marginal donors reconditioned in EVLP platform was not completely recovered**
- **The EVLP platform, in combination with anti-inflammatory and targeted therapies, provides promising strategies to rehabilitate marginal donor lungs**

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