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Optimal ex vivo lung perfusion techniques with oxygenated perfusate

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BACKGROUND: Accumulating evidence supports an increasing role of ex vivo lung perfusion (EVLP) in clinical lung transplantation. However, EVLP has adverse effects on the quality of lung grafts, which have rarely been discussed. Careful optimization of current EVLP protocols might improve outcomes. This study examined effects of different levels of oxygenation of the perfusate circulated through the lungs during EVLP and the impact on post-transplant functional outcomes.

METHODS: We compared results of 4 different oxygenation levels in the perfusate during EVLP: 6% oxygen (O_2), 40% O_2, 60% O_2, and 100% O_2. We evaluated lung function, compliance, and vascular resistance and levels of glucose and other markers in the perfusate. After EVLP, lung grafts were transplanted, and post-transplant outcomes were compared.

RESULTS: Lungs perfused with 40% O_2 on EVLP had the lowest glucose consumption compared with the other perfusates. Lungs treated with 40% O_2 or 60% O_2 exhibited significantly less inflammation, as indicated by reduced pro-inflammatory cytokine messenger RNA levels compared with lungs perfused with 6% O_2 or 100% O_2. Significantly more oxidative damage was noted after 4 hours of EVLP perfused with 100% O_2. After transplantation, lungs perfused with 40% O_2 during EVLP had the best post-transplant functional outcomes.

CONCLUSIONS: Optimization of O_2 levels in the perfusate during EVLP improved outcomes in this rat model. Deoxygenated perfusate, the current standard during EVLP, exhibited significantly more inflammation with compromised cellular metabolic activity and compromised post-transplant outcomes.
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Accumulating evidence supports the increasing role of ex vivo lung perfusion (EVLP) in clinical lung transplantation, and expectations are high that EVLP will overcome some limitations inherent to current lung transplantation protocols.[1] EVLP has a long history as an experimental model, but the more recent focus on EVLP is attributed to its value as a tool for the reassessment of donor lungs that might otherwise be rejected for transplantation.[2] However, EVLP is not a "magic bullet."[3–8] Further research is essential to refine our understanding of both the advantages and the disadvantages of EVLP and to optimize EVLP to improve lung transplant outcomes.

The Toronto EVLP method is the most widely used EVLP protocol and the most referenced method in clinical and research articles to date.[9] A notable characteristic of the Toronto EVLP protocol is that the lungs are perfused with a deoxygenated solution (6% oxygen [O_2]) and ventilated with full oxygenation (fraction of inspired oxygen of 100% at evaluation) during EVLP.[9] In contrast, during extracorporeal

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membrane oxygenation (ECMO), fully oxygenated perfusion (100% O_2) is used to treat severe acute lung injuries such as primary graft dysfunction after lung transplantation.[10,11] We initiated the current study to examine the effects of oxygenation of the perfusate used for EVLP on lung function during EVLP and outcomes after transplantation in hopes of improving lung transplant outcomes after EVLP.

Methods

Animals

Inbred male Lewis (RT-1[l]) rats weighing 250 to 300 g were purchased (Harlan Sprague Dawley, Indianapolis, IN). Animals were maintained in laminar flow cages in a specific pathogen-free animal facility at the University of Pittsburgh and given a standard diet and water ad libitum. All procedures were performed according to the guidelines of the Institutional Animal Care and Use Committee at the University of Pittsburgh and the National Research Council *Guide for the Humane Care and Use of Laboratory Animals*.

Ex vivo lung perfusion in rats

EVLP was performed using a commercially available rodent EVLP system (IL-2 Isolated Perfused Rat or Guinea Pig Lung System; Harvard Apparatus, Holliston, MA) as described previously.[12] The rats were anesthetized, underwent tracheotomy and mechanical ventilation with 100% O_2, and were given 300 IU intravenous heparin before procurement of heart-lung blocks. Heart-lung blocks were flushed with cold low-potassium dextran solution (Perfadex; XVIVO Perfusion AB, Göteborg, Sweden) and stored in Perfadex at 4°C for 1 hour. The trachea, pulmonary artery, and left atrium were cannulated during cold storage, and the heart-lung blocks were placed on the EVLP system for 4 hours. During EVLP, the lungs were ventilated with air at 37°C and perfused with Steen solution (XVIVO Perfusion AB) supplemented with 50 mg of methylprednisolone (SOLU-MEDROL; Pfizer Inc., New York NY) and 50 mg of cephalosporin (Cefazolin; APP Pharmaceuticals, LLC, Schaumburg, IL). Perfusion was started at 10% of the target flow, and the flow rate was gradually increased for the first hour to the target flow rate—calculated as 20% of cardiac output (75 ml/min/250 g donor body weight). Deoxygenated perfusate with 6% O_2, 8% carbon dioxide, and balanced nitrogen was administered during the first hour, and then the O_2 level of sweep gas through oxygenator was set at 6% O_2, 40% O_2, 60% O_2, or 100% O_2 for the remaining duration of lung maintenance on EVLP. The O_2 percentage of the sweep gas through oxygenator was monitored (Vascular Technology, Inc., Nashua, NH) with a margin of error of ±1.0%. Partial O_2 tension in the perfusate in the pulmonary artery was measured for each sweep gas setting (Table 1). Pulmonary artery pressure, peak airway pressure, and airway flow were monitored continuously. Dynamic lung compliance and pulmonary vascular resistance (PVR) were analyzed. Every hour during EVLP, the perfused lung was ventilated with 100% O_2, and the perfusate was deoxygenated with mixed gas (6% O_2, 8% carbon dioxide, and 76% nitrogen) for 5 minutes, and then the perfusate was sampled for pulmonary oxygenation and electrolyte analysis to assess lung function without the confounding effects of oxygenated perfusion. Sham-operated animals underwent anesthesia, tracheotomy, and mechanical ventilation with 100% O_2. The lungs of the sham animals were then immediately removed for analysis.

Table 1 Oxygen Concentration in Sweep Gas and Oxygen Content of Perfusate

Percentage of oxygen in sweep gas through oxygenator	Perfusate oxygenation levels in pulmonary artery (mm Hg)
6	76.5 ± 2.86
40	206 ± 8.05
60	328 ± 25.1
100	524 ± 12.5

Rat orthotopic lung transplantation

After EVLP for 4 hours, the lungs were pre-cooled with 4°C Steen solution on the EVLP system and stored at 4°C for 1 hour before transplantation. Orthotopic lung transplantation was performed using the cuff method as previously described.[12,13] The lungs after EVLP were split into left and right; left lungs were used for transplantation, and right lungs were used for subsequent assays as post-EVLP/pre-transplant samples. At 2 hours after reperfusion, the naïve lung was clamped, 100% O_2 was administered for 5 minutes through a ventilator, and recipient's blood was sampled from the graft pulmonary vein for blood gas analysis.

Determination of malondialdehyde levels in perfusate

Malondialdehyde levels in perfusate were measured using a spectrophotometric assay (BIOXYTECH MDA-586 Assay Kit; OXIS Health Products, Inc., Portland, OR) according to manufacturer's instructions.

Mitochondrial enzyme activity assay

Mitochondria were isolated from the lung tissue after 4 hours of EVLP using a mitochondria isolation kit (Thermo Fisher Scientific, Rockford, IL), and the enzymatic activity of mitochondrial complexes I and II was detected using microplate assays (Abcam, Inc., Cambridge, MA) as previously described.[4]

Measurement of adenosine triphosphate levels in lung grafts

Adenosine triphosphate (ATP) level in the lung graft tissue after 4 hours of EVLP was quantified using the ENLITEN ATP luciferin/luciferase bioluminescence assay system (Promega Corporation, Madison, WI) and a 1420 VICTOR multi-label counter (PerkinElmer Inc., Waltham, MA), as previously described.[4]

Real-time reverse transcription polymerase chain reaction

Messenger RNA (mRNA) levels for interleukin (IL)-6, IL-1β, tumor necrosis factor (TNF)-α, and glyceraldehyde-3-phosphate dehydrogenase in graft tissue after 4 hours of EVLP were assessed by SYBR-Green, 2-step, real-time reverse transcription polymerase chain reaction as previously described.[3] Also, IL-6, TNF-α, and