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The role of the endothelin-1 pathway as a biomarker for donor lung assessment in clinical ex vivo lung perfusion

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BACKGROUND: Normothermic ex vivo lung perfusion (EVLP) is a preservation technique that allows reassessment of donor lungs before transplantation. We hypothesized that the endothelin-1 (ET-1) axis would be associated with donor lung performance during EVLP and recipient outcomes after transplantation.

METHODS: ET-1, Big ET-1, endothelin-converting enzyme (ECE), and nitric oxide (NO) metabolites were quantified in the perfusates of donor lungs enrolled in a clinical EVLP trial. Lungs were divided into 3 groups: (I) Control: bilateral transplantation with good early outcomes defined as absence of primary graft dysfunction (PGD) Grade 3 (PGD3) ; (II) PGD3: bilateral lung transplantation with PGD3 any time within 72 hours; and (III) Declined: lungs rejected after EVLP.

RESULTS: There were 25 lungs in Group I, 7 in Group II, and 16 in Group III. At 1 and 4 hours of EVLP, the perfusates of Declined lungs had significantly higher levels of ET-1 (3.1 ± 2.1 vs 1.8 ± 2.3 pg/ml, $p = 0.01$; 2.7 ± 2.2 vs 1.3 ± 1.1 pg/ml, $p = 0.007$) and Big ET-1 (15.8 ± 14.2 vs 7.0 ± 6.5 pg/ml, $p = 0.001$; 31.7 ± 17.4 vs 19.4 ± 9.5 pg/ml, $p = 0.007$) compared with Controls. Nitric oxide metabolite concentrations were significantly higher in Declined and PGD3 lungs than in Controls. For cases of donation after cardiac death, PGD3 and Declined lungs had higher ET-1 and Big ET-1 levels at 4 hours of perfusion compared with Controls. At this time point, Big ET-1 had excellent accuracy to distinguish PGD3 (96%) and Declined (92%) from Control lungs.

CONCLUSIONS: In donation after cardiac death lungs, perfusate ET-1 and Big ET-1 are potential predictors of lung function during EVLP and after lung transplantation. They were also associated with non-use of lungs after EVLP and thus could represent useful biomarkers to improve the accuracy of donor lungs selection.
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Ex vivo lung perfusion (EVLP) is a contemporary preservation technique that allows for repeated donor lung assessment and treatment in a normothermic metabolically active state. Clinical translation has shown that EVLP

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perfusion and assessment of high-risk donor lungs provides similar outcomes to lungs that are transplanted using conventional donation criteria, thus representing an important and safe expansion of the donor pool.[1,2]

Decisions on whether proceed with transplantation during EVLP are currently based primarily on physiologic parameters (gas exchange, airway pressures, pulmonary compliance).[3] Future directions in the field point to the development of biomarkers that would (1) improve the diagnostic accuracy by detecting the small, although clinically relevant, percentage of lungs that undergo EVLP and still end up developing severe primary graft dysfunction (PGD); (2) make the decision process more objective and easier for less experienced operators; and (3) increase the current 80% transplant conversion rate after EVLP by using injury-specific targeted therapies. Thus, according to this vision, EVLP will serve as platform for more advanced diagnosis and treatment of donor lungs.[4,5]

Endothelin-1 (ET-1) is the most abundant and best characterized 21-amino-acid peptide from the endothelin family. It is produced from cleavage of 38-amino-acid big endothelin-1 (Big ET-1) by endothelin-converting enzyme (ECE). Besides being a potent vasoactive peptide, ET-1 also functions as a mediator of acute lung injury.[6] Mechanistic studies have shown that ET-1 promotes harmful cross-talk between the endothelial and alveolar compartments by stimulating nitric oxide (NO) production, leading to impairment in alveolar fluid clearance and pulmonary edema.[7] More specifically to lung transplantation, ET-1 has been linked to PGD[8] and bronchiolitis obliterans.[9] Experimental blockade of ET-1 may have beneficial effects on early graft function.[10,11] Furthermore, in clinical lung transplantation, ET-1 messenger RNA (mRNA) levels in lung tissue biopsy specimens taken immediately before reperfusion have been shown to correlate with severe PGD.[8]

In the setting of EVLP, the ET-1 axis would be of particular interest because, unlike most mediators that tend to accumulate in the absence of hepatic or renal clearance mechanisms in the ex vivo circuit, ET-1 is mainly metabolized by the lung.[12] We hypothesized that in metabolically active high-risk donor lungs perfused with a clinical intent, the ET-1 axis is active and reflects graft performance during EVLP and after transplantation.

Methods

The cases included in this study were part of the Human Ex vivo Lung Perfusion Trial, conducted by the Toronto Lung Transplant Program to assess high-risk brain-dead donor (BDD) lungs and lungs from donation after cardiac death (DCD).[1] Indications for EVLP were (1) last partial pressure of arterial oxygen (Pao_2)/fraction of inspired oxygen (Fio_2) of <300 mm Hg; (2) pulmonary edema detected on chest X-ray or during examination of the lungs at procurement; (3) poor lung compliance during examination of the lungs at procurement; and (4) high-risk history (e.g., multiple blood transfusions, questionable history of aspiration). From February 2009 to January 2010, all DCD lungs underwent EVLP. After this period, the EVLP indications for DCD lungs were similar to the those described above or an interval between withdrawal of life-sustaining therapies and arrest >60 minutes.

Our EVLP technique has been described in detail elsewhere.[4] Briefly, donor lungs were placed on the Toronto EVLP system and perfused and ventilated for 4 to 6 hours. Hourly functional assessment included Pao_2/Fio_2 in the perfusate, peak, mean and plateau airway pressure, dynamic and static compliance, and pulmonary artery pressure. X-ray images of the lung were taken at 1 and 3 hours of EVLP. Lungs declined for transplantation presented a Pao_2/Fio_2 <400 mm Hg or $>15\%$ worsening of any of the above parameters. Additionally, there should be no worsening X-ray infiltrate and no purulent or frothy secretions on the interval bronchoscopy.

Donor lungs were divided into three groups: (1) Control—lungs accepted after EVLP, allocated for bilateral transplantation, and the recipient developed no PGD Grade 3 (PGD3) within 72 hours; (2) PGD3—lungs accepted after EVLP, allocated for bilateral transplantation, and the recipient presented PGD3 at any point within 72 hours; (3) Declined—lungs were turned down for transplantation after EVLP evaluation. PGD3 was scored according to the International Society for Heart and Lung Transplantation criteria, with a Pao_2/Fio_2 <200 and chest X-ray infiltrates within 72 hours.[13] The study did not include single-lung transplants, lobar transplants, and recipients bridged to transplant with extracorporeal life support.

Sample collection and protein analysis

Perfusate samples were collected at 1 hour and 4 hours of EVLP and stored at –80°C for later analysis. Perfusate levels of ET-1 axis proteins were measured using enzyme-linked immunosorbent assay kits (ELISA) for ET-1 (ET-1quantikine ELISA kit; R&D Systems, Minneapolis, MN), Big ET-1 (big ET-1 human ELISA kit; Enzo Life Sciences, Farmingdale, NY), and ECE (ECE ELISA kit; USCN Life Science, Houston, TX).

NO metabolite measurement

Total NO metabolite (NOx) levels were measured using a chemiluminescence analyzer (Eco Physics, Switzerland) and a liquid purge vessel system, as previously described.[14] All samples were deproteinated using Amicon Ultracel-0.5 10 K centrifugal filters (Millipore catalog UFC501096) by centrifugation at 14,500 *g* for 15 minutes. Then, sample supernatants (25 μl) were injected into the purge vessel system containing vanadium (III) chloride (0.05 mol/L in 1N hydrochloric acid) as reducing agent.[15]

Statistical analysis

EVLP physiologic parameters were compared among the different groups using 2-way analysis of variance. Categoric variables were compared with Fisher's exact test, and numeric variables were compared with the Mann-Whitney test. For biomarker comparisons, the area under the curve (AUC) was calculated from a receiver operating characteristic (ROC) curve. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc, La Jolla, CA).

Results

From September 2008 to September 2012, we performed 77 EVLPs. Perfusates were not available from 8 donors early in our experience, and 21 donors met the exclusion criteria.