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The Journal of Heart and Lung Transplantation
http://www.jhltonline.org

ORIGINAL PRE-CLINICAL SCIENCE

Pulmonary microvascular lesions regress in reperfused chronic thromboembolic pulmonary hypertension

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KEYWORDS:
pulmonary endarterectomy;
microvascular disease;
pulmonary hypertension;
chronic thromboembolic pulmonary hypertension;
endothelin-1;
interleukin-6

BACKGROUND: Pulmonary microvascular disease (PMD) develops in both occluded and non-occluded territories in patients with chronic thromboembolic pulmonary hypertension (CTEPH) and may cause persistent pulmonary hypertension after pulmonary endarterectomy. Endothelin-1 (ET-1) and interleukin-6 (IL-6) are potential PMD severity biomarkers, but it remains unknown whether they are related to occluded or non-occluded territories. We assessed PMD and ET-1/IL-6 gene expression profiles in occluded and non-occluded territories with and without chronic lung reperfusion in an animal CTEPH model.

METHODS: Chronic PH was induced in 10 piglets by left pulmonary artery (PA) ligation followed by weekly embolization of right lower lobe arteries with enbucrilate tissue adhesive for 5 weeks. At Week 6, 5 of 10 animals underwent left PA reperfusion. At Week 12, animals with and without reperfusion were compared with sham animals ($n = 5$). Hemodynamics, lung morphometry and ET-1/IL-6 gene expression profiles were assessed in the left lung (LL, occluded territories) and right upper lobe (RUL, non-occluded territories).

RESULTS: At Week 12, mean PA pressure remained elevated without reperfusion (29.0 ± 2.8 vs 27.0 ± 1.1 mm Hg, $p = 0.502$), but decreased after reperfusion (30.0 ± 1.5 vs 20.5 ± 1.7 mm Hg, $p = 0.013$). Distal media thickness in the LL and RUL PAs and systemic vasculature to the LL were significantly lower in the reperfused and sham groups compared with the non-reperfused group. PMD progression was related to ET-1 and IL-6 gene expression in the RUL and to the ET-A/ET-B gene expression ratio in the LL.

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1053-2498/$ - see front matter
http://dx.doi.org/10.1016/j.healun.2014.07.005

CONCLUSIONS: PMD regressed in occluded and non-occluded territories after lung reperfusion. Changes in ET-1 and IL-6 gene expression were associated with PMD in non-occluded territories.
J Heart Lung Transplant 2015;34:457–467

Chronic thromboembolic pulmonary hypertension (CTEPH) is due to chronic pulmonary artery (PA) obstruction by organized clots persisting after pulmonary embolism.[1,2] However, in addition to proximal obstruction by clots, pulmonary microvascular disease (PMD) develops in non-occluded territories, contributing to elevate the pulmonary pressure.[3] PMD is induced by increases in blood flow[4–6] and pressure, possibly with a contribution of circulating factors,[7] resulting in non-specific pathologic lesions typical of pulmonary hypertension (PH).[5,8] In occluded territories, post-obstructive pulmonary vasculopathy (POPV) develops in response to the chronic PA occlusion. POPV manifests as increases in PA media thickness and in the systemic blood supply to the lung.[9–12] Surgical treatment of CTEPH by pulmonary endarterectomy (PEA) decreases the pulmonary arterial resistance,[13] thereby preventing progression to right heart failure.[14,15] However, in some patients, PEA is followed by persistent PH,[15] which may be due to the previous development of PMD in occluded and non-occluded territories.[5,16]

To date, there is no evidence that PMD in non-occluded territories and/or POPV can regress after PEA. In several animal models, pulmonary vasculopathy induced by high blood flow regressed after high-flow correction[4] and POPV regressed after PA reperfusion.[9,17] However, the relevance of these findings to CTEPH is limited, as none of these studies were done in reliable CTEPH models.

Endothelin-1 (ET-1), a peptide produced mainly by endothelial cells, exerts mitogenic and vasoconstricting effects on adjacent smooth muscle cells, and is involved in distal PA remodeling in patients with PH.[18] Interestingly, endothelial PA cells were found to overexpress ET-1 when exposed to increased shear stress and pulsatility, as seen in patients with PH.[19,20] Herein we hypothesized that the ET-1 gene expression profile may be chiefly increased in non-occluded vascular territories, where blood flow is higher than in occluded territories. In patients with CTEPH, serum ET-1 elevation before PEA was associated with an increased risk of persistent PH.[21]

In addition, inflammation was recently identified as a key contributor to PA remodeling in PH lungs.[22] Interleukin-6 (IL-6) is a pleiotropic cytokine that influences inflammatory reactions and is a main inducer of C-reactive protein (CRP) secretion.[23] In CTEPH patients, serum CRP levels predict the prognosis after PEA.[24] Moreover, serum IL-6 elevation correlates with the hemodynamic severity of primary PH.[25]

These data suggest a role for ET-1 and IL-6 in PMD development in CTEPH. An unresolved issue of considerable interest is whether these cytokines are differentially expressed in occluded and non-occluded territories.

We recently developed a piglet model of CTEPH modeling the hemodynamic changes due to chronic PA occlusion, with occluded and non-occluded vascular territories, in which PMD could be studied separately.[21,26]

The aims of this study were to determine whether PMD in occluded and non-occluded territories regressed after surgical lung reperfusion used to replicate PEA, and to look for differences between occluded and non-occluded territories in expression levels of IL-6, ET-1 and the two endothelin receptors, ET-A and ET-B.

Methods

All procedures were approved by our institutional animal care committee according to institutional guidelines that complied with the "Principles of Laboratory Animal Care," developed by the National Society for Medical Research, and *The Guide for the Care and Use of Laboratory Animals*, prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health.

CTEPH animal model and surgical interventions

We studied 15 male Large White piglets, each weighing between 20 and 25 kg. CTEPH was induced in 10 piglets, as described by Mercier et al,[26,27] by left PA ligation, which was followed by weekly embolization of the right lower lobe arteries with enbucrilate (Histoacryl; B. Braun, Melsungen, Germany) under fluoroscopic control, for 5 weeks. The right upper lobe (RUL) remained non-occluded, whereas all remaining right lung territories were progressively occluded. At Week 6, 5 piglets underwent surgical left PA revascularization (reperfused CTEPH group) to mimic PEA, by interposition through a left thoracotomy of a vascular prosthesis (diameter 8 mm, length 10 mm) between the pulmonary trunk and left PA. The other 5 piglets were followed up for 6 additional weeks (CTEPH group). These two groups were compared with a sham group ($n = 5$) given weekly saline injections into the PAs.

Hemodynamic assessment

Hemodynamic measurements were performed using a Swan–Ganz catheter connected to a monitor (Vigilance; Edwards Lifesciences, Irvine, CA) at baseline, Week 6 and Week 12. The total pulmonary resistance index (TPRI, in WU/m^2) was calculated by dividing mean pulmonary artery pressure (MPAP, in mm Hg) by cardiac index (CI, in liters/min/m^2). Body surface area (m^2) was computed as: weight (kg)$^{0.656}$ / 10.[28]

Lung sampling, light microscopy and vascular morphometry

At Week 12, random biopsy specimens weighing 300 to 500 mg were taken from the RUL and left lung (LL), snap-frozen in liquid nitrogen, and stored at –80°C or fixed in 4% paraformaldehyde solution instilled into the airway. For light microscopy and