Regression of flow-induced pulmonary arterial vasculopathy after flow correction in piglets

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Objectives: Chronic thromboembolic pulmonary hypertension is due to partial obstruction of the pulmonary arterial bed and may resolve after pulmonary thromboendarterectomy. Persistent pulmonary hypertension, the main complication after pulmonary thromboendarterectomy, may reflect vessel alterations induced by high flow in unobstructed lung territories. The aim of this study was to determine whether correcting high flow led to reversal of the vasculopathy in piglets.

Methods: The effects of high pulmonary blood flow were investigated 5 weeks after creation of an aortopulmonary shunt (n = 10), and reversibility of vessel disease was evaluated at 1 week (n = 10) and 5 weeks after shunt closure (n = 10), compared to sham-operated animals (n = 10). Hemodynamic variables, pulmonary artery reactivity, and morphometry were recorded. We also investigated the endothelin, angiopoietin, and nitric oxide synthase pathways.

Results: High flow increased medial thickness in distal pulmonary arteries (55.6% ± 1.2% vs 35.9% ± 0.8%; $P < .0001$) owing to an increase of smooth muscle cell proliferation (proliferating cell nuclear antigen labeling). The endothelium-dependent relaxation was altered ($P < .05$). This phenomenon was associated to an overexpression of endothelin-1, endothelin-A, angiopoietin 1, angiopoietin 2, and Tie-2 ($P < .05$). After 1 week of shunt closure, all overexpressed genes returned to control values, the proliferation of smooth muscle cells stopped ,and smooth muscle cell apoptosis increased (terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling), preceding the normalization of the wall thickness hypertrophy and the pulmonary artery vasoreactivity observed at 5 weeks after shunt closure.

Conclusion: These results demonstrate that endothelin-1 and angiopoietin pathways are involved in vasculopathy development and may be important therapeutic targets for preventing persistent pulmonary hypertension after pulmonary thromboendarterectomy.

Chronic thromboembolic pulmonary hypertension (CTEPH) is a life-threatening disease with an overall 5-year survival of 10% in patients whose mean pulmonary artery (PA) pressure exceeds 50 mm Hg.[1] CTEPH is more common than previously thought and may occur in up to 3.8% of patients with acute pulmonary embolism.[2] Organized thrombotic material causing chronic vascular obstruction is the pathogenic mechanism, and pulmonary thromboendarterectomy (PTE) provides long-term hemodynamic and functional benefits and increases life expectancy.[3-5] However, PTE is associated with 5% to 23% mortality, related chiefly to reperfusion pulmonary edema and persistent pulmonary hypertension (PH) despite removal of the thrombotic material.[3-5]

Two vascular territories can be distinguished in the lung after pulmonary thromboembolism: the obstructed territory characterized by chronic ischemia and the unobstructed territory exposed to chronic high blood flow resulting from redistribution of the cardiac output.[6,7] This unobstructed territory contributes to the elevated pulmonary vascular resistance,[8] inasmuch as chronic high flow causes distal arteriopathy with vascular remodeling[9,10] similar to that found in primary PH.[11-15]

Pulmonary thromboendarterectomy in patients with high pulmonary vascular resistance is associated with high rates of mortality and postoperative persistent PH. Preoperative prostacyclin infusion, which induces PA vasodilatation and inhibits vascular remodeling, has been found to decrease post-PTE mortality in patients with preoperative pulmonary resistance values exceeding 1200 dyne · s · cm^5.[16] These findings support the hypothesis that the distal arteriopathy induced by chronic high blood flow in unobstructed territories may be the cause of persistent PH after PTE.

Knowledge of the pathobiological mechanisms underlying PH has significantly improved over the past decade. Three pathways seem to play a key role in the development of PH: endothelin, angiopoietin, and nitric oxide synthase (NOS).[17-19] The endothelial dysfunction seen in PH is associated with an imbalance between endothelium-derived

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Abbreviations and Acronyms

CTEPH = chronic thromboembolic pulmonary hypertension
eNOS = endothelial nitric oxide synthase
ET = endothelin
iNOS = inducible nitric oxide synthase
NOS = nitric oxide synthase
PA = pulmonary artery
PBS = phosphate-buffered saline
PCNA = proliferating cell nuclear antigen
PH = pulmonary hypertension
PTE = pulmonary thromboendarterectomy
SMC = smooth muscle cell
TUNEL = terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling

vasoconstrictor/mitogenic factors and vasodilatator/nonmitogenic factors. However, few studies to understand mechanisms underlying CTEPH had been published. Angiopoietin-1 expression was found to be increased in patients with CTEPH, as in various other forms of PH.[19]

We studied the effects of chronic high blood flow to the lungs in an experimental animal model of aortopulmonary shunting. We determined whether the effects of high pulmonary blood flow resolved after restoring normal blood flow to simulate the effects of PTE. We analyzed nitric oxide, endothelin, and angiopoietin pathways to understand pathophysiologic mechanisms.

MATERIALS AND METHODS

Forty Large White piglets weighing 21 ± 3 kg were used. The study 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,'' written by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (publication No.86-23, revised in 1985).

Experimental Design

The piglets were randomly allocated to four groups (n = 10 in each group). The studies were done 5 weeks after performing an aortopulmonary shunt to increase blood flow into PAs (shunt-open group), 1 week (1-week shunt-closed group) and 5 weeks (5-week shunt-closed group) after closure of this shunt, and 5 weeks after PA dissection without shunt in the sham group.

Surgical Procedures

Aortopulmonary shunt. An aortopulmonary shunt was performed as previously described by Rendas, Lennox, and Reid.[20] An 8-mm diameter polytetrafluoroethylene bypass was implanted between the ascending aorta and the main PA. Fractionated heparin was given until harvesting of the lungs or closure of the shunt to avoid postoperative occlusion of the shunt.

Aortopulmonary shunt closure. The midline sternotomy was reopened. After systemic heparinization, the aorta and main PA were partially clamped on each side of the bypass, which was divided and sutured.

Hemodynamic Measurements and Lung Harvesting

Pulmonary hemodynamics were measured before the animals were killed. Aortic blood flow was measured with a flow probe (Transonic Systems, Inc, Ithaca, NY) placed on the origin of the aorta upstream from the aortopulmonary shunt. The pulmonary and systemic flow values were measured downstream from the shunt.

Pressures and blood gases were measured by direct puncture downstream from the aortopulmonary shunt. Then, the animal was exsanguinated and the lungs rapidly removed from the chest. Patency of the shunt was assessed by direct visual inspection. Biopsy specimens weighing 300 to 500 mg from the left upper lobe were snap-frozen in liquid nitrogen and stored at –70°C or left to fix in 4% paraformaldehyde solution.

Light Microscopy and Morphometry

Fixed lung sections were processed by standard histologic techniques and embedded in paraffin. We sought to identify 30 to 40 arteries of less than 200 μm in diameter in each piglet. Medial thickness (MT) was calculated as followes:

$$\%MT = (ED - ID) \times 100/ED$$

Where ED = external diameter and ID = internal diameter.

Evaluation of In Situ PA–Smooth Muscle Cell (SMC) Death and Proliferation and Collagen Accumulation

To assess the PA–SMC proliferation, we evaluated the proliferating cell nuclear antigen (PCNA). Tissue sections were deparaffinized in xylene, followed by treatment with a graded series of alcohol washes, rehydratation in phosphate-buffered saline (PBS) (pH 7.5), and then incubated with target retrieval solution (Dako, Trappes, France) into a water bath at 90°C for 20 minutes. Endogenous peroxidase activity was blocked with H_2O_2 in PBS (3% vol/vol) for 5 minutes. Slides were then washed in PBS and incubated for 30 minutes in a protein-blocking solution consisting of PBS supplemented with 3% bovine serum albumin. The slides were subsequently incubated for 30 minutes with anti-PCNA mouse monoclonal antibody (PC-10, 1:200, Dako, Trappes, France). Antibodies were washed off and the slides were processed with the alkaline phosphatase LSAB+ system-HRP detection kit (DAKO, Carpinteria, Calif). Brown color was generated by using a diaminobenzidine substrate and nuclei were counterstained with hematoxylin.

Detection of cells undergoing apoptosis was evaluated by the ApopTag Red In Situ Apoptosis Detection Kit (Qbiogene, Illkirch, France), as specified by the manufacturer.

So that collagen accumulation could be assessed, the paraffin-embedded sections were deparaffinized and stained with Masson trichrome stain.

Assessment of PA reactivity

Pulmonary artery rings were studied as previously described.[21] Endothelin-1 (ET-1), sodium nitroprusside, acetylcholine hydrochloride, and indomethacin were purchased from Sigma Chemical Company (St Louis, Mo).

Real-Time Quantification by Polymerase Chain Reaction Assay

Real-time polymerase chain reaction assay was conducted as previously described.[12] Except for the report gene 18S, angiopoietins, and Tie-2, all the primers (ET-1, ETA, ETB, endothelial NOS [eNOS], inducible NOS [iNOS]) were specific porcine primers previously described by Rondelet and associates.[12] Primers for 18S ribonucleic acid, angiopoietins and Tie-2 had been used previously in our laboratory for human experimentation and were designed on Primer Express software (Applied Biosystems, Foster City, Calif).

Assay of Lung Phosphodiesterase 5 Activity

Phosphodiesterase 5 assays were carried out from frozen lung tissue according to established procedures.[22]