1008 Pulmonary Vascular Disease

Sunday, March 30, 2003, 9:00 a.m.-11:00 a.m.
McCormick Place, Hall A
Presentation Hour: 9:00 a.m.-10:00 a.m.

1008-120 Overexpression of 5-Lipoxygenase Induces Pulmonary Hypertension in Bone Morphogenetic Protein Receptor-2-Deficient Mice

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Background: Mutations in bone morphogenetic protein receptor-2 (BMPR2) have been associated with some familial forms of primary pulmonary hypertension (PPH). However, only about 20% of individuals with these mutations clinically manifest PPH, environmental (epigenetic) or other disease-modifying genes are likely to be important in disease pathogenesis. One such candidate disease-modifying gene could be 5-lipoxygenase (5L0), whose expression is increased in patients with PPH.

Methods: In this study, we examined the consequences of 5LO overexpression in the lungs of mice heterozygous for the mutated BMPR2 gene (+/-). Results: Early on, rats had normal pulmonary artery systolic pressures (10 ± 2 mmHg, n=5), compared to wild type mice (11 ± 2 mmHg, n=7). Delivery of a replication-deficient adenovirus expressing 5LO to the lungs of BMPRP (+/-) mice led to an increase in pulmonary artery systolic pressure (24 ± 1 mmHg, n=7 by day 12, at which time wild type mice treated with the same adenoviral vector containing 5L0 maintained normal pressures (13 ± 4 mmHg, n=7). These data show that 5-LO modifies the susceptibility of BMPR2 (+/-) mice to the development of PPH. Prior data using cultured endothelial and vascular smooth muscle cells showed that 5LO expression and BMPRP haploinsufficiency both can promote vascular cell proliferation, and that 5LO can induce endothelial and vascular dysfunction.

Conclusions: Our data in mice together with these earlier observations in cultured cells suggest a potential mechanism by which these molecular mediators support the development of PPH by promoting vascular dysfunction and cell growth.

1008-121 Glucocorticoids Suppress Pulmonary Hypertension After Cardiopulmonary Bypass by Reducing Endothelin and Neutrophil Adhesion Molecules in Neonatal Lungs

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Background: Glucocorticoids during cardiopulmonary bypass (CPB) benefit pediatric patients undergoing repair of congenital heart defects. Glucocorticoid administration during CPB has become routine therapy, but underlying mechanisms have not been fully examined. The hypothesis was that glucocorticoids could improve cardiopulmonary recovery after CPB and deep hypothermic circulatory arrest (DHCA) in part by reducing neutrophil adhesion.

Methods: Piglets (4-6 kg) were cooled on CPB with 6 °C BGA, then rewarmed and maintained for 2 hr. Methylenedisiloxane was administered both 6 hr before CPB (0 mg/kg, IM) and in CPB pump prime (30 mg/kg). Controls received no steroid treatment.

Results: Pulmonary vascular resistance (PVR) in controls increased from a baseline of 152 ± 40 to 364 ± 29 dynes x s/cm² at 2 hr of recovery (P<0.01 vs baseline). Animals receiving glucocorticoids had no increase in PVR at 2 hr of recovery (155 ± 54 dynes x s/cm²). Plasma endothelin-1 in controls increased from 1.3 ± 2.2 pg/ml at baseline to 14.2 ± 2.9 pg/ml at 2 hr of recovery (P<0.01 vs baseline), while glucocorticoid-treated animals had endothelin-1 levels of 4.7 ± 6 pg/ml (P<0.05 vs controls). Neutrophil adhesion molecule (ICAM-1) protein and mRNA in lung tissue was lower in animals receiving glucocorticoids (P<0.01, 100 nM). In contrast, mRNA and protein for the hypoxia inducible factor (HIF-1) was higher in glucocorticoid treated animals (P<0.05).

Conclusions: Glucocorticoids prevented the rise in PVR after CPB and DHCA in neonates. Improved cardiopulmonary recovery with glucocorticoid treatment correlated with lower plasma endothelin-1, as well as, reduced pulmonary ICAM-1 levels and mRNE oxidation. Reduction of neutrophil adhesion and activation by glucocorticoids contributes to alleviation of pulmonary hypertension after CPB and DHCA.

1008-122 Effects of Overexpression and Inhibition of 5-Lipoxygenase on the Development of Pulmonary Hypertension in a Rat Model

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Background: Increased expression of 5-lipoxygenase (5L0) has been demonstrated in patients with primary pulmonary hypertension. In this study, we examined the effect of overexpression of 5L0 on the development of pulmonary hypertension (PH) in a rat model.

Methods: Increased expression of 5L0 was achieved by adenovirus-mediated gene transfer, and inhibition of 5L0 was achieved by intraperitoneal injection of MK886 (an inhibitor of 5L0 activating protein) or by oral gavage with Zileuton (a specific 5L0 inhibitor).

Results: Overexpression of 5L0 did not itself lead to the development of PH in normal rats; however, overexpression of 5L0 markedly accelerated the development of PH in rats treated with monocrotaline (MCT), a compound that causes pulmonary artery endothelial dysfunction and damage. Also, both MK886 and Zileuton prevented the development of PH in rats treated with either MCT or MCT + 5L0. On day 10, rats treated with MCT + MCT + 5L0, MCT, and 5L0 showed pulmonary pressures of 15 ± 4, 25 ± 4, 18 ± 2, and 16 ± 2 mmHg, respectively (p<0.05). Rats treated with MCT + Zileuton had pulmonary pressures of 15 ± 4, 25 ± 4, 18 ± 2, and 16 ± 2 mmHg, respectively (p<0.05). These data show that 5L0 and its inflammatory mediators alone do not cause PH, but contributes to the development of PH in the setting of endothelial dysfunction or injury.

Conclusion: These data suggest that 5L0 may represent a disease-modifying gene that regulates the susceptibility of an individual to the PH phenotype.

1008-123 Role of Arginase in the Pulmonary Vascular Response to Chronic Hypoxia in the Mouse: Augmentation of Responses by In Vivo Gene Transfer

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Arginase is a bifunctional metalloenzyme that catalyzes the hydrolysis of L-arginine to form L-citrulline and urea thus regulating NOS activity by depleting the pool of L-arginine in the vascular endothelium. This enzyme has been suggested to play a role in reducing NO-mediated responses. However, the role of arginase in regulating NOS activity in the lung has not been addressed. The present study was designed to compare arginase expression, activity, and functional role in the pulmonary vascular bed of the mouse. Arginase mRNA, protein, and activity were significantly greater in mice exposed to 30 days of chronic hypoxia when compared to control mice. In contrast, while eNOS mRNA and protein were elevated in hypoxic mice, eNOS activity was lower when compared to normoxic mice. However, when assessed in the presence of ABH (a potent, selective inhibitor of arginase), NOS activity returned to levels similar to those observed in normoxic mice. In vivo expression of the human arginase gene, using a fibrovascular adenoviral vector, resulted in elevated arginase activity in the pulmonary arterial bed of the hypoxic mouse, revealed that chronic administration of ABH to hypoxic mice reduced the elevated pulmonary arterial pressure (PAP), pulmonary vascular resistance (PVR), and increase in RV free wall weight (RVF/WV) associated with hypoxia. In contrast, in vivo gene transfer of arginase, using an adenoviral vector, resulted in increased arginase expression, activity, and functional role in the pulmonary vascular bed of the hypoxic mouse. We hypothesize that arginase may function by depleting endothelial levels of L-arginine and thus reduce the NOS activity thus augmenting the hypoxic response in the mouse.

1008-124 Effects of Sildenafil on Growth and Viability of Cultured Human Pulmonary Artery Smooth Muscle Cells

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Background: The rationale for a new treatment for pulmonary arterial hypertension (PAH) should be based on the demonstration of both vasodilator and antiproliferative activities on pulmonary artery smooth-muscle cells (PASMC). Sildenafil, an orally-active inhibitor of cGMP phosphodiesterase type 5, exerts potent pulmonary vasodilator activity in PAH patients. We evaluated the effects of sildenafil on growth and viability of cultured human PASMC.

Methods: PASMC were cultured in 0.5% serum for 72 hours. Growth was stimulated by 100 ng/ml PDGF. Different concentrations of sildenafil were added to the culture (10-50-100 nM). The selective inhibition of protein kinase G (PKG) or A (PKA). Four sets of experiments were performed for each combination.

Results: 50 or 100 nM sildenafil significantly reduced PASMC proliferation as assessed by trypan blue exclusion method at baseline, 24, 48 and 72 hours. The experiments were repeated after the selective inhibition of protein kinase G (PKG) or A (PKA). Four sets of experiments were performed for each combination.

Conclusions: Sildenafil exerts a potent antiproliferative effect on PASMC stimulated by PDGF. This effect is mediated via both cGMP and CAMP dependent pathways.