Pulmonary Artery Smooth Muscle Cells Response to Vaso-active Stimulations In a Real-time 3-Dimensional Model

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INTRODUCTION: The tone of the pulmonary arteries is the summation of the activity of each smooth muscle cell (PASMC) within a vessel wall and its interaction with the endothelial cells and extracellular matrix (including collagen). There are reported phenotypic differences between PASMC in the inner & outer layers of pulmonary artery walls[1]. The response of a tissue engineered blood vessel to contractile and relaxing stimulants in-vitro is essential to predicting the response of the physiological and pathological vessels in-vivo. Previous work showed that pulmonary artery relaxation to nitric oxide is inhibited after exposure to chronic hypoxia [2]. We hypothesised that PASMC will differ in their ability to contract or relax a 3D collagen gel. Using a Culture Force Monitor (CFM) we sought to quantify the cellular response of PASMC derived from inner and outer normal and hypoxic arteries, harvested from piglet models, over 24 hours in response to contractile agonists and relaxing antagonists.

METHODS: Piglets were exposed to hypoxia (50KPa) for 3-14 days then sacrificed at day 14. Large intrapulmonary arteries were dissected and SMC derived from inner & outer layers were then cultured in DMEM/F12 medium with 10% FCS. 5 ml rectangular Collagen gels2 (rat tail collagen type I, 10x minimal essential medium, sodium hydroxide) were prepared in a sterilised silicone polymer mould and seeded with 5 million cells (passage 3-6). The gel was allowed to set with 2 A-frames (layered polyethylene mesh with a stainless steel frame) on either side and then suspended in DMEM with 10% FCS. One A-frame was connected to a fixed point in the CFM while the other is connected to a transducer. Real time contractile force generated (1 per second) and cellular response were recorded over 24 hours (at 37 ^{o}C, 5% CO^{2}). Once the cells reached tensional haemostasis an agonist (U46619) or antagonist (Sodium Nitropruside, SNP) drug was added to the system and the response was measured in real time.

RESULTS: Normal Outer PASMCs generated an immediate and tri-phasic contractile response to agonists with a mean peak force generated of 74 dynes ± SEM 24. Normal Inner PASMCs showed a similar response to the agonist (mean peak force 172 dynes ± SEM 61). In response to the antagonists, Normal Outer PASMCs relaxed to a mean of 236 dynes ± SEM 123 and Normal Inner to 153 dynes ± SEM 20. Hypoxic Outer PASMCs generated an increase in contractile force of 31.2 dynes ± 10.7 SEM and Hypoxic Inners a contractile force of 37.4 dynes ± SEM 11. Neither the Hypoxic Outer nor Inner PASMCs showed a response to SNP.

DISCUSSION AND CONCLUSIONS: We demonstrated that PASMCs derived from the normal pulmonary vessels respond to contractile and relaxing stimulants. On exposure to chronic hypoxia, the cells retained their ability to contract in response to agonists however they were unresponsive to antagonist stimulation. These findings suggest relaxing mechanisms of PASMC has been permanently altered as a response to chronic hypoxia, which will have implications in tissue engineering of blood vessels or treatment of pulmonary hypertension.

REFERENCES: [1] S.M. Hall, A. Hislop, C. Pierce et al (2000) prenatal origins of human intrapulmonary arteries. *Am J Respir Cell Mol Bio* **23**:194-203. [2] Tulloh, R.M., Hislop, A.A., Boels, P.J. et al (1997) Chronic hypoxia inhibits postnatal maturation of porcine intrapulmonary artery relaxation. *Am J Physiology* **272**, H2436-H24