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ORIGINAL ARTICLE

Pulmonary Effects of Chronic Elevation in Microvascular Pressure Differ Between Hypertension and Myocardial Infarct Induced Heart Failure

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Background Chronic heart failure (CHF) following coronary artery ligation and myocardial infarction in the rat leads to a homeostatic reduction in surface tension with associated alveolar type II cell hyperplasia and increased surfactant content, which functionally compensates for pulmonary collagen deposition and increased tissue stiffness. To differentiate the effects on lung remodelling of the sudden rise in pulmonary microvascular pressure (Pmv) with myocardial infarction from its consequent chronic elevation, we examined a hypertensive model of CHF.

Methods Cardiopulmonary outcomes due to chronic pulmonary capillary hypertension were assessed at six and 15 weeks following abdominal aortic banding (AAB) in the rat.

Results At six weeks post-surgery, despite significantly elevated left ventricular end-diastolic pressure, myocardial hypertrophy and increased left ventricular internal circumference in AAB rats compared with sham operated controls ($p \leq 0.003$), lung weights and tissue composition remained unchanged, and lung compliance was normal. At 15 weeks post-surgery increased lung oedema was evident in AAB rats ($p = 0.002$) without decreased lung compliance or evidence of tissue remodelling.

Conclusion Despite chronically elevated Pmv, comparable to that resulting from past myocardial infarction (LVEDP > 19 mmHg), there is no evidence of pulmonary remodelling in the AAB model of CHF.

Keywords Abdominal aortic banding • Lung mechanics • Pulmonary remodelling • Rat • Pulmonary oedema

Introduction

Chronic heart failure (CHF) is a complex syndrome impacting various end-organs including pronounced effects on the lung. The major causes of CHF, myocardial infarction and hypertension, clearly differ both in the initiating event and ongoing cardiac insult. However they are not generally recognised as leading to different responses in the lung. While the acute effects of a sudden increase in pulmonary microvascular pressure (Pmv) such as increased pulmonary capillary filtration, are known, the effects of an initial rapid versus slow rise in Pmv on subsequent chronic effects have not been investigated.

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The left coronary artery ligation rat model is the most commonly used experimental model of CHF [1]. In addition, damage to the alveolocapillary barrier with leakage of surfactant protein-B from the lung to systemic circulation [2,3], as well as lung histopathology demonstrating fibrotic remodelling and epithelial damage and repair [4], are both remarkably similar in this model to that described in human CHF [5–8]. Using this model, we have demonstrated pulmonary vascular remodelling and thickening of the alveolocapillary barrier leading to decreased intrinsic bi-directional fluid flux [9] and decreased tissue compliance which is overcome by homeostatic reduction in surface tension via alveolar type II cell hyperplasia and increased surfactant content [10]. However, this model does not allow for differentiation between the long-term effects on lung remodelling of the sudden acute rise in Pmv associated with myocardial infarction, and those of chronic Pmv elevation.

Therefore, we aimed to investigate the effects on lung remodelling of gradually manifest, chronic elevation in Pmv, using a hypertensive model of CHF. We determined the cardio-pulmonary outcomes due to chronic hypertension at six and 15 weeks following abdominal aortic banding (AAB) in the rat.

Materials and Methods

Animals

Male Sprague-Dawley rats (250-300 g) were used throughout the study and were randomly allocated to either the AAB or sham operated control groups (n = 9-10 per group). The study protocol was approved by the Flinders University Animal Welfare Committee, Adelaide, Australia.

Induction of Heart Failure

CHF was induced using a modification of the AAB method of Chung and co-workers [8]. Briefly, rats were anaesthetised (5% isoflurane) and intubated before connection to a Harvard rodent ventilator. Following a midline abdominal incision the abdominal aorta was constricted at the suprarenal level by a 4.0 prolene suture tied around the aorta and a blunted 23 gauge needle, which was then withdrawn. The abdomen was closed and Metamizol (0.1 mg/kg i.p., Troy Laboratories) as well as furosemide (2 mg/kg, Sigma-Aldrich) via drinking water were administered daily for three days post-operatively. Sham animals underwent the same procedure excluding ligation of the aorta.

Cardiorespiratory Assessment

After six or 15 weeks rats were anaesthetised (thiopentone sodium, 120 mg/kg i.p.) and a polyethylene catheter passed through the right carotid artery and into the left ventricle. Systemic blood pressure, heart rate and left ventricular end-diastolic pressure (LVEDP) were monitored for 15 min. Arterial blood was sampled for blood gas-pH analysis (ABL 5, Radiometer, Copenhagen, Denmark) and for collection of plasma which was stored at -80 °C for later analysis for specific proteins, as below.

Measurement of Static Lung Compliance

The pressure-volume relationship of each lung in air was determined as previously described [10]. Briefly, following tracheal cannulation the heart and lungs were removed and airway pressure measured using an air-filled transducer (Sorenson Trans Pac, Abbott Critical Care Systems, N. Chicago, IL) and recorded (MacLab system 4, AD Instruments, Sydney, Australia) at incremental inflation and then deflation with 1 ml steps of air. Total lung capacity was defined as a pressure of 30 cmH_2O [11,12]. P-V expiration data was curve fitted as previously described [10,13].

Bronchoalveolar Lavage

Following completion of the P-V curve the lung was degassed at 0.5 atm for 60s before lavage at 2 °C with three separate 32 ml/kg body weight aliquots of saline, each instilled and withdrawn three times. The lavage was centrifuged (150 *g*, 5 min, 2 °C) to remove the cell pellet and supernatant stored at -80 °C for later analysis of total soluble protein (BioRad DC Protein Assay; BioRad Laboratories, Hercules, CA), and of specific proteins by ELISA using matched antibody pairs for keratinocyte growth factor (KGF) and transforming growth factor (TGF)-β (R&D Bioscience, Minneapolis, MN) in assays developed in our laboratory, as described previously [10,14]. The right upper lobe was then removed, weighed and lyophilized for determination of lung wet-to-dry weight ratio.

Myocardial Assessment

The heart was weighed before the right ventricle (RV) was dissected off the septum and the left ventricle (LV) transversely cut into four pieces for planimetric assessment of internal circumference.

Lung Histology

Blind histological assessment was performed on lung tissue following fixation via intratracheal instillation of Zamboni's fixative solution to an inflation pressure of 20 cmH_2O before 24-hour immersion. Fixed tissues were cut in 4 µm sections and stained with haematoxylin and eosin for morphometric assessments. Immunohistochemical identification of ATII cells was performed, as previously described [10].

Assessment of Tissue Composition

Tissue composition of freeze-dried right upper lobes was determined as previously described [10]. Briefly, lipid and protein were separated via a 2:4 propan-2-ol:chloroform mixture overnight before centrifugation. The non-aqueous fraction was removed and dried under nitrogen to obtain purified lipid. Soluble and insoluble protein fractions were separated by centrifugation and total soluble protein determined (BioRad DC Protein Assay; BioRad Laboratories, Hercules, CA). The insoluble protein pellet was dried overnight at 50 °C in a non-humidified chamber. Insoluble collagen content was assessed via determination of the hydroxyproline content of the tissue, as previously described [10]. Briefly, freeze-dried right lower lobe was hydrolysed