Pressure overload-induced right ventricular dysfunction and remodelling in experimental pulmonary hypertension: the right heart revisited

Peter Pokreisz1,2, Glenn Marsboom1, and Stefan Janssens1,3*

1 Center for Transgene Technology and Gene Therapy, VIB, 49, Herestraat, B-3000, Leuven, Belgium
2 Laboratory for Experimental Cardiology, KU-Leuven, Leuven, Belgium
3 Department of Cardiology, Gasthuisberg University Hospital, KU-Leuven, Leuven, Belgium

KEYWORDS
Right ventricle; Pulmonary hypertension; Remodelling; Pressure-overload

Introduction

In humans, right ventricular remodelling represents a complex set of functional and structural adaptations in response to chronic pressure or volume overload, imparted on the right heart by inborn defects (e.g. tetralogy of Fallot, Eisenmenger's and diGeorge's syndrome) or acquired diseases (e.g. pulmonary or mitral valve stenosis, chronic, hypoxic, or thrombo-embolic lung disease, left heart disease with increased pulmonary venous pressure). The ability of the right ventricle (RV) to sustain stroke volume and cardiac output in the presence of an increased pressure load will markedly determine the severity of clinical symptoms and is one of the most important determinants of survival in patients with pulmonary arterial hypertension.1,2 Some patients with increased RV afterload are capable of maintaining a near normal cardiac output for prolonged periods of time, while others experience rapid deterioration and death.3 Little is known about the underlying mechanisms that govern the transition from a pressure-overloaded hypertrophic, normocontractile RV to the dysfunctional and failing RV in various pathological conditions. Therefore, preclinical investigations exploring these pathophysiological changes in vivo as well as their underlying cellular and molecular adaptations represent a challenging field of translational cardiovascular research with significant clinical impact.4 We will hereunder review contemporary (preclinical) concepts of RV remodelling, and identify significant gaps in our understanding of critical mechanisms. At the same time we will highlight the need for translational research, specifically targeted towards unravelling key molecular events that determine RV remodelling and that may eventually offer innovative treatment strategies.

Right ventricular changes in pulmonary hypertension: homologue of left ventricle adaptation to increased afterload?

Under normal circumstances, the pulmonary circulation is a low-pressure system, with low vascular resistance...
to RV outflow. In contrast to the more muscular ellipsoid-shaped left ventricle (LV), the crescent-shaped RV has a lower volume-to-surface area ratio and because of its greater compliance, the RV can accommodate large increases in volume better than increases in pressure.\textsuperscript{5} When faced with a sudden increase in afterload, the normally thin-walled RV is incapable of generating a mean pulmonary artery pressure >40 mmHg acutely.\textsuperscript{6} In contrast, when afterload increases progressively and is sustained, significant RV adaptation occurs and enables patients to survive even with PA pressures exceeding systemic pressures in advanced disease. An increase in RV end-diastolic volume can initially improve cardiac output by the Frank-Starling mechanism and the process of adaptive myocardial hypertrophy is thought to reduce wall stress and maintain an adequate stroke volume. RV hypertrophy in animal models can be observed within 96 h of increased afterload,\textsuperscript{7} when the RV becomes more concentric and the interventricular septum flattens. However, without effective intervention to lower afterload, coronary blood flow to the RV myocardium will fall, causing RV ischaemia and cardiomyocyte apoptosis. The hypertrophied ventricle will progressively dilate, stiffen, resulting in compromised RV filling and proportionally impaired stroke volume,\textsuperscript{8} which becomes more dependent on right atrial function. Similar to what has long been recognized for left atrial function in the pressure-overloaded left heart, a comparable adaptive increase in RA contractility and distensibility (reservoir function) occurs in an attempt to maintain filling of the pressure-overloaded stiffened RV.\textsuperscript{9,10} In the end, sustained pressure overload will result in decreased cardiac output, heart failure, and deficient end-organ perfusion.\textsuperscript{1}

The concept of adaptive hypertrophy in the left heart was recently seriously challenged in mice with cardio-restricted deficiency in Gq protein signalling. Gq proteins are important intracellular mediators of prohypertrophic signal transduction pathways and mice deficient in Gq signalling maintained preserved LV function 8 weeks after thoracic constriction despite increased wall stress and blunted hypertrophy. In contrast, control mice which developed significant hypertrophy and maintained normal wall stress after 8 weeks constriction, developed LV dilatation and progressive deterioration in LV function.\textsuperscript{11} Subsequently, Kupari et al.\textsuperscript{12} challenged this same concept of adaptive LV hypertrophy in a carefully designed prospective study in patients with isolated aortic stenosis by reporting an inverse relationship between LV mass and LV ejection fraction and a higher prevalence of LV hypertrophy in patients suffering from heart failure. Whether or not the somewhat inconclusive view of a pressure-overloaded heart being better-off ‘stressed out’ (blunted hypertrophic response and preserved contractile function) than hypertrophied (with reduced contractile function), also applies to the RV is not known.

Of interest, however, are recent observations by Buermans et al.\textsuperscript{13} who observed slow-onset pulmonary hypertension (PH) and adaptive RV hypertrophy after a 30 mg/kg SC injection of monocrotaline pyrrole in rats, whereas an 80 mg/kg injection resulted in rapid development of PH accompanied by RV failure and premature death. One of the intriguing findings in this study was the observation that the degree of RV hypertrophy 14 days after injection of the low or high dose of toxin was identical despite diverging micro-array transcript profiles. These observations suggest that, at least in the monocrotaline model of toxic PH, adaptive hypertrophy does not precede maladaptive hypertrophy in RV pressure overload, but that the magnitude of the initial haemodynamic stimulus/insult determines the subsequent development of an adaptive or maladaptive cardiac phenotype. Whether or not a similar paradigm applies to other forms of PH, remains to be determined and will have significant therapeutic ramifications.

### Experimental models to study right ventricular remodelling

Right ventricular remodelling is characterized by changes in size, shape, and function of the heart and comprises atrial as well as ventricular hypertrophy and dilatation.\textsuperscript{14} A wide spectrum of animals, ranging from mice to primates, has been used to study the anatomic,\textsuperscript{15–20} pathophysiological,\textsuperscript{10,21–25} and molecular adaptations of the remodelling heart.\textsuperscript{26–31} The complex and multifaceted process of RV remodelling precludes the study of a single animal model, as being fully representative of the changes occurring in patients. Information combined from both large and small animal studies will help unravel key pathophysiological mechanisms of RV remodelling and provide the foundation for testing potential therapeutic strategies.

As for pulmonary vascular remodelling (for review see Marsboom and Janssens\textsuperscript{32}), each of the experimental models, using either physiological stimuli including hypoxia or increased or obstructed pulmonary blood flow, toxins, and drugs (e.g. monocrotaline, bleomycin), or gene targeting, shares certain characteristics with the human disease (Table 1). Whereas large animal models allow a better understanding of complex haemodynamic changes, including right ventriculo-arterial coupling and uncoupling, small rodent models and transgenic mice\textsuperscript{33} enable more detailed pharmacologic and molecular-mechanistic studies of the RV, exposed to acute or chronic pressure overload. In addition, recent studies in zebrafish (Danio rerio) have proven invaluable to better understand cardiovascular development, and to perform large-scale mutagenesis and gene expression knockdown experiments.\textsuperscript{34–37}

### Invasive techniques to evaluate functional changes in the pressure-overloaded right heart

Catheterization remains the gold standard to evaluate RV haemodynamic changes following acute and chronic pressure-overload.\textsuperscript{10,23,25,38} Conductance catheter-based measurements of the end-systolic pressure-volume relationship provides invaluable information to evaluate myocardial contractility irrespective of loading conditions and has been successfully applied for RV
contractility measurements in pigs,\textsuperscript{39,40} lambs,\textsuperscript{41} and dogs.\textsuperscript{10,42} The RV is often considered to act as a flow pump and it has been suggested that under control conditions it operates at maximum efficiency and submaximal stroke work.\textsuperscript{43} However, when acute PH is induced by injecting glass microspheres into the pulmonary vascular bed of anaesthetized pigs, the compliance of the pulmonary artery decreases progressively, thereby imposing a greater afterload upon the RV. Under these loading conditions, the RV can increase its contractility through homeometric autoregulation and in theory accommodate changes at a time when the hypertrophied RV is still capable of maintaining contractile performance and cardiac output.

Major hurdles with respect to conductance volumetry, particularly in small animals, are the difficulty of accurately and reproducibly measuring instantaneous RV volumes and defining end-systolic pressure–volume relationships on triangle-shaped RV loops. Moreover, the technology requires significant expertise and remains time-consuming, and hence is mostly used as a research tool. In rats, pressure–volume analysis of RV function has been reported following monocrotaline injection\textsuperscript{45} and pulmonary artery banding.\textsuperscript{23} In mice, LV and aortic function was described at baseline and following beta-adrenergic stimulation\textsuperscript{46–48} using a miniaturized pressure–volume catheter, but a validated pressure–volume characterization of murine RV and pulmonary artery function at baseline and after hypertrophic remodelling has not yet been reported in the literature. The small size and crescent shape of the RV and fragile structure of the murine pulmonary artery represent for now a significant experimental challenge, although not insurmountable with steady improvements in catheter technology (Figure 1C).

Alternatively, it may become possible to assess RV contractility and RV-arterial coupling without measuring actual RV volumes or modulating preload or afterload. The technique would entail a modification of a single-beat estimation of the end-systolic pressure–volume relationship, previously validated in dogs.\textsuperscript{49} Wauthy \textit{et al.}\textsuperscript{25} determined RV volume changes by integrating flow measured in the proximal pulmonary artery using an ultrasonic flow probe. They subsequently applied this technique to characterize RV adaptation to increased afterload in dogs, goats, and pigs (species with low, intermediate, and high pulmonary vascular resistance and reactivity). These experiments demonstrated that RV contractility matches increased afterload, thereby maintaining optimal or near-optimal ventriculo-arterial coupling in acute PH irrespective of absolute species-induced differences in loading. Analysis of RV-pulmonary artery coupling may also help select preferred therapeutic interventions as illustrated by the superior profile of levosimendan over a traditional ionotropic

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Physiologic stimuli} & \textbf{Stimuli} & \textbf{Animal} & \textbf{Analogy to human pathology} & \textbf{Reference} \\
\hline
Acute and chronic hypoxia & From primates to mice & Hypoxic pulmonary vasoconstriction, hypoxic PH, chronic obstructive pulmonary disease & 25, 30, 31, 38, 49, 52, 53, 58, 120 \\
\hline
Pulmonary artery constriction or banding & Dog, pig, goat, rat, sheep & Congenital heart defects, progressive pulmonary vascular remodelling & 7, 10, 23–25, 121 \\
Microspheres (synthetic spheres or fresh blood clots) & Dog, pig, rat, goat, sheep & Chronic pulmonary thrombo-embolism and development of PH & 25, 43, 122 \\
\hline
\textbf{Chemical stimuli} & Primates, dog, sheep, rat & Increased muscularization and inflammatory reaction, endothelial cell death, marked PH & 123–126 \\
\hline
\textbf{Genetic modification} & Rat & Pulmonary oedema, endothelial damage, RV hypertrophy & 127 \\
\hline
Monocrotaline & Mice, rat & Pulmonary fibrosis and hypertension & 128–130 \\
\hline
\textbf{Generation} & Pig, mice & Pulmonary vasoconstriction in sepsis & 40, 61, 131 \\
\hline
\hline
\end{tabular}
\caption{Experimental models to study right ventricular pressure-overload}
\end{table}
agent, dobutamine, in a canine model of acute right heart failure. Similarly, a potential new drug, targeting the thromboxane A2 pathway was identified in a pig model because it markedly improved RV function during endotoxic shock.

One of the challenges for future research in small animal models, is to investigate whether non-invasive Doppler or magnetic resonance imaging techniques may enable PA flow calculations and hence a similar, more detailed analysis of RV performance under acute or chronic loading conditions. Validating such measurements in a variety of transgenic models that integrate key signalling pathways of the RV remodelling process will undoubtedly advance our therapeutic abilities to better sustain RV function and improve clinical outcome, but has thus far not been reported. In the interim, however, new catheterization technology has gradually overcome technical limitations in small animal models enabling RV and PA pressure measurements in rats, guinea pigs, hamsters, and mice using fluid-filled, piezzo-electric, fibre-optic, and telemetric devices. The closed-chest, fluid-filled pressure measurement developed by Champion et al. allows to record RV, PA, and pulmonary wedge pressures in mice and has been used in pulmonary gene transfer protocols to target PH. Disadvantages of the technique include the requirement for fluoroscopic guidance, expert operator skills, and the damping artefacts of pressure waveforms. Although closed-chest measurements are theoretically preferable over open-chest approximations of physiological variables, the open-chest approach in mice, however, facilitates access of high-fidelity micro-manometer devices enabling pulmonary artery pressure monitoring during unilateral pulmonary artery or airway occlusion. Contrary to pressure monitoring catheters equipped with piezzo-electric micro-manometers, the fluid-filled catheters do not allow evaluation of pressure changes per time unit due to the low frequency response and time delay in signal recording.

Non-invasive evaluation of functional and structural changes in the pressure-overloaded right heart

Non-invasive imaging modalities, including echocardiography and magnetic resonance imaging allow serial evaluation of structural and functional changes following pressure overload. Echocardiographic evaluation of PA pressure and RV function is highly operator- and model-dependent and was successfully applied in monocrotaline-induced PH in rats. In contrast,
echocardiographic visualization of the murine right heart at baseline and following chronic hypoxia was initially established using a transoesophageal 30 MHz probe (for review see Scherrer-Crosbie et al.17). At heart rates in excess of 600 b.p.m., a high spatial resolution (<50 μm) and frame-rate acquisition are necessary for sufficient acoustic detail. Recently developed broadband annular phased-array transducers and new data acquisition techniques for two-dimensional and colour flow visualization (frame rates of 1000 frames per second) will greatly facilitate our ability to non-invasively examine the murine right heart.68,69

The three-dimensional magnetic resonance imaging technique, allows accurate and reproducible measurements of ventricular dimensions, wall thickness, and myocardial mass without relying on geometric assumptions.70 This technique is witnessing a remarkable technological development with the availability of high-field scanners (9 T and beyond) and sufficient temporal resolution to evaluate RV function in healthy mice and in mice suffering from heart failure,71 and to determine free RV wall thickness in transgenic animals.72

Finally, RV hypertrophy in association with myocardial fibrosis alters electrical coupling between cells, slows conduction, and dispersion of refractoriness, all of which predispose to arrhythmia’s, syncope, and sudden death.73 Therefore, serial electrocardiography might represent a non-invasive marker of progressive hypertrophy in RV remodelling. In rodents, and especially in mice, with particularly small body size and high heart rates, specific adaptations are necessary in electrode design and position as well as in signal amplification and noise filtering.74–78 RV enlargement correlated with a shift in the mean electrical axis of the QRS complex in the frontal plane of the electrocardiogram in monocrotaline-treated rats79 and with increased R-wave amplitude in rats exposed to chronic hypoxia.80 However, the relatively low sensitivity and high complexity of the technique are significant disadvantages limiting its translational value.

Cellular and molecular adaptations in the pressure-overloaded right heart

At the cellular level, remodelling is frequently referred to as hypertrophy of individual cardiomyocytes,14,15,18,81 although proliferation of fibroblasts,82,83 apoptotic and necrotic cell fate events,84,85 and phenotypic changes of coronary and capillary vascular cells are simultaneously involved, albeit to variable degrees.33,63,86

In vitro models using primary or immortalized cell cultures are far less complex than whole organ studies, and allow investigation of single cell expression patterns,87–89 single cell electrophysiological responses,90,91 and high-throughput

Figure 2 Integrated analysis of RV dysfunction and remodelling in pulmonary hypertension. CT, computer tomography; MRI, magnetic resonance imaging; PET, positron emission tomography; TDI, tissue Doppler imaging; TTE, transthoracic echocardiography.
screening of novel drugs. On the other hand, any simplification of such a multi-faceted process as RV remodelling must be interpreted with caution in the absence of cell-cell interactions and cellular and humoral immune surveillance. Therefore, future investigations on RV dysfunction and remodelling should integrate in vivo as well as cellular and organ-based in vitro analysis (Figure 2). This is even more pertinent in view of recent advances in developmental biology introducing a novel paradigm that recruitment and maturation of cardiac progenitor cells may perhaps also participate in myocardial remodelling in response to pressure or volume overload. This novel concept is challenging our traditional view of the heart as a terminally differentiated organ, devoid of self-renewal capacity, but we still need to carefully explore potential implications of these new insights with respect to RV remodelling.92–96

At the molecular level, mechanisms governing the transition from compensatory RV hypertrophy to failure or determining the predisposed adaptive or maladaptive RV phenotype represent a novel target for translational research. While we should try to take advantage of significant recent progress in our understanding of molecular mechanisms in LV remodelling,13,97,98 we cannot simply extrapolate them to the right counterpart as both ventricles are fundamentally different. First, the RV and LV have a different embryologic origin with distinct myocardial structures.100–103 Because embryonic gene programs are often recapitulated during hypertrophic adaptation,10,104 further elucidation of specific heart-patterning factors may help to identify new therapeutic factors and new candidate genes responsible for a maladaptive RV phenotype. In this respect, significant up-regulation of the slow β-MHC isoform has been reported in the pressure- and volume-overloaded left atrium,105 but whether these changes also occur in the right atrium remains unknown.

Second, careful examination of inborn genetic defects resulting in chronic RV overload may identify important thus far unrecognized polymorphisms and familial links. Pre- or post-natal genetic analysis of known inherited cardiac and pulmonary diseases (e.g. Fallot’s tetralogy, arrhythmogenic RV cardiomyopathy, familial PH) helped to identify candidate genes,106–110 which in turn were used in transgenic mouse models to study RV remodelling. A typical example comes from patients with severe PH who present with prostacyclin synthase deficiency in precapillary pulmonary resistance vessels. The important role of this enzyme in prostacyclin biosynthesis is clearly demonstrated in transgenic mice with lung-specific overexpression of prostacyclin synthase, who are protected against PH, thereby confirming the clinical relevance of this molecular pathway.111

Similarly, nitric oxide (NO) is an important endothelium-dependent modulator of normal pulmonary vascular tone and structure, in part via guanylate cyclase- and protein kinase G-dependent pathways. NO signalling is often impaired in cardio-pulmonary hypertensive diseases and again rodents with transgenic or gene transfer-mediated overexpression of NO synthases were found to develop less PH and RV remodelling following chronic hypoxia.52,112,113 Finally, mutations in bone morphogenic receptor type 2 (BMPR2), a member of the transforming growth factor-β receptor family, were associated with familial PH.110,114 Conversely, heterozygous BMPR2-deficient mice developed more PH and cardiopulmonary remodelling after chronic serotonin infusion or exposure to chronic hypoxia, although baseline haemodynamic and morphometric parameters were similar to wild-type littermates.115,116 Elevated levels of serotonin and mutations in its transporter (5-HTT) were also observed in patients with PH,117 whereas mice with smooth muscle cell-specific 5-HTT overexpression developed increased RV systolic pressure and hypertrophy.118 Moreover, hypoxia- or monocrotaline-induced models of PH have clearly indicated a role for enhanced PDGF signalling.119 Taken together, these observations in transgenic mice suggest important roles for TGF-beta, BMP, and PDGF signalling in the pathological remodelling of pulmonary resistance vessels, which to variable degrees can be further modulated by endothelial-derived NO, prostacyclin, or serotonin signal transduction pathways.

Thus far, the role of these signalling pathways in modulating intrinsic RV performance under different loading conditions has received little attention and represents a new and important target for translational research. To begin to study the effects of modulated myocardial NO bioavailability on the response to chronic RV pressure overload, we have generated a transgenic mouse with cardiac-restricted overexpression of endothelial NO synthase (NOS3). We have studied the effects of enhanced cardiomyocyte-restricted enhanced NO bioavailability on structural and functional adaptations of the RV to increased afterload. When NOS3-TG mice were exposed to chronic hypoxia and increased RV afterload, fractional right heart weight, and RV cardiomyocyte cross-sectional area and width were markedly reduced when compared with wild-type mice (Figure 3). These experimental data suggest that different molecular mechanisms control cardiac cell and matrix remodelling in the pressure-overloaded right heart. Clearly, complementary transcriptional profile analysis of the pressure overloaded RV and proteome analysis are required and will undoubtedly provide novel information on gene activation/silencing patterns and critical molecular interactions in RV remodelling. The availability of a wealth of transgenic mice models that have been generated to study mechanisms of LV hypertrophic remodelling should facilitate such mechanistic studies in the right heart.

Conclusions

Despite persistent limitations in fully transposing our advanced investigational technology for the remodelled left heart to its right counterpart, significant progress has been made in our understanding of the dysfunctional
right heart and interactions between pulmonary vascular loading abnormalities and RV performance. We are witnessing steady improvements in catheter technology and advanced imaging modalities, which will greatly facilitate structure–function analyses of the remodelling murine right heart. One of the major challenges will be to keep a similar pace in expanding our understanding of the fundamental cellular and molecular mechanisms that govern the adaptation of the right heart to its increased load. We need to test the effect of existing treatment algorithms for PH on intrinsic right ventricular performance, remodelling and gene expression profiles in a variety of experimental conditions. To explore novel treatment paradigms, we should take advantage of the wealth of transgenic mice models that have elucidated so many biological intricacies of the LV remodelling process, with significant therapeutic consequences. At the same time, the greater availability of pathological RV specimens and easier access to human RV biopsies should allow validation of preclinical concepts (obtained via transcriptome or proteome analysis) in patients suffering from a variety of pulmonary hypertensive disorders and RV dysfunction. Concerted translational research programmes integrating analyses in animal models of right heart failure and in patients are indispensable given the pivotal, yet often neglected, role of the RV in cardiovascular disease.

Conflict of interest: none declared.

References


The importance of RVF in pulmonary hypertension


108. Sen-Chowdhry S, Sibbitt PW, Richardson JA, Bassel-Duby R, Olson EN. BOP, a regulator of right ventricular heart development, is a direct transcriptional target of MEF2C in the developing heart. Development 2005;132:2669–2678.


