Original Research

Characterisation of a Rat Model of Pulmonary Arterial Hypertension

Theofilos Kolettis,[1] Antonios P. Vlahos,[2] Maria Louka,[3]
Konstantinos E. Hatzistergos,[4] Giannis G. Baltogiannis,[1] Maria M. Agelaki,[1]
Agathokleia Mitsi,[1] Vassiliki Malamou-Mitsi[4]

Departments of [1]Cardiology, [2]Child Health, [3]Biological Sciences and [4]Pathology, University of Ioannina, Greece

Introduction: Pulmonary hypertension portends an adverse outcome. Animal models have improved current understanding of the complex pathophysiology of the disease, but may be technically demanding. Moreover, plexiform vascular lesions are rarely observed, limiting the extrapolation to human pathophysiology. The aim of the present study was first, to assess the feasibility of closed-chest pressure recordings, and mainly, to further characterise a new model of endothelin receptor-B deficient rats.

Methods: Jugular venous catheterisation was assessed in 15 Wistar rats. Pressure recordings via a left lateral thoracotomy and histological findings were compared in three rat groups (age 20 ± 1 weeks, weight 200-250 g): (a) wild type (n=10, group A); (b) wild type after monocrotaline injection (n=10, group B); and (c) endothelin receptor-B deficient rats (n=10, group C) after monocrotaline injection.

Results: Pressure recordings via the jugular approach were feasible in only 3 (20%) rats. Compared to group A, there was a trend (H=4.6, p=0.0962) towards increased mortality in groups B and C, due to respiratory arrest during intubation attempts. Pulmonary artery systolic pressure in group C was 24.7 ± 1.3 mmHg, higher than in group B (21.5 ± 1.2, p=0.036) or group A (11.8 ± 0.5, p<0.0001). Adverse pulmonary vascular remodelling was more prominent in group C than in group B.

Conclusions: Endothelin receptor-B deficient rats constitute a useful model of pulmonary artery hypertension after monocrotaline injection. The ease of pressure recordings via a left lateral thoracotomy may aid in the more widespread use of this model.

Address:
Theofilos M. Kolettis

Department of Cardiology, University of Ioannina 1 Stavrou Niarhou Ave. 45110 Ioannina, Greece
e-mail:
thkolet@cc.uoi.gr

The pulmonary circulation functions as a low-pressure, high-flow system. During the past decade, pulmonary arterial hypertension has been increasingly recognised as an important public health problem.[1] It is characterised by vascular remodelling, consisting of vasoconstriction and medial hypertrophy.[1,2] These changes lead to increased pulmonary artery pressure and vascular resistance and eventually to right-sided heart failure.[1,2] Pulmonary arterial hypertension can present in its idiopathic form, but it is also associated with congenital heart disease, scleroderma, human immunodeficiency virus infection, portal hypertension and anorectic drug ingestion.[3]

Despite substantial treatment advances offered by prostacyclin, endothelin receptor blockers and phosphodiesterase inhibitors, survival rates and quality of life remain poor.[4] The pathophysiology of the disease is complex and not well understood. Endothelin-1, a potent vasoconstrictor with growth promoting properties, results in endothelial dysfunction and vascular smooth muscle proliferation in the pulmonary vascular tree.[5,6] Endothelin-1 acts via two G-protein coupled receptors (A and B)[6] and holds a key role in the progression of the disease.[1] Activation of A-receptors results in vasoconstriction and smooth muscle cell proliferation, but the role of B-receptors is not

well defined and variable effects on the pulmonary vasculature have been reported.[5,6]

Animal models of pulmonary hypertension have contributed to our understanding of the underlying mechanisms and have aided in the development of therapeutic targets. The most widely used are the chronic hypoxia and the monocrotaline-induced rat models of pulmonary arterial hypertension.[7] Although pulmonary vasoconstriction increases pulmonary arterial pressure in these models, the full spectrum of pathological changes in the pulmonary vascular tree is rarely observed.[2] Thus, data from these models may not be extrapolated to human pathophysiology. Recently, the spotting lethal (sl) rat, a naturally occurring rodent model of Hirschsprung disease, has been shown to carry a deletion in the endothelin-B receptor gene that abrogates the expression of functional endothelin-B receptors.[8] Transgenic homozygous (sl/sl) colonies have been created, displaying pulmonary vasculature lacking the expression of mRNA for the endothelin-B receptor.[9] In response to hypoxia, these rats developed severe pulmonary arterial hypertension, diminished cardiac output, and increased total pulmonary vascular resistance.[9]

Despite recent advances in experimental animal models, several limitations and technical difficulties may preclude their widespread use. Thus, the purpose of the present study was twofold: first, to assess the feasibility of direct pressure recordings in the anaesthetised as well as in the conscious rat; second, to further characterise the rat model of monocrotaline-induced pulmonary hypertension in endothelin-B-deficient homozygous (sl/sl) rats.

Methods

Experimental animal population

The study was conducted in 45 rats of either sex, of similar age and weight (20 ± 1 weeks old, 200-250 g, respectively). The animals were maintained at the facilities of the University of Ioannina; they were housed in individual cages, under optimal laboratory conditions (controlled humidity, temperature and light/dark cycles) and were given water and standard rat chow *ad libitum*. Animal care was in accordance with the recommendations in the Declaration of Helsinki, as well as with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, volume 25, no 28, revised 1996). All procedures followed the institutional and national guides for the care and use of laboratory

animals and the study protocol was approved by the local state authority (Prefecture of Ioannina).

Experimental design

The study consisted of two parts. In the first part, the feasibility of internal jugular venous catheterisation was studied in 15 Wistar rats (200-250 g). The second part included pulmonary artery recordings via the right ventricular outflow tract. For the purposes of this part of the study, three groups of rats were selected: group A consisted of 10 Wistar rats, group B consisted of 10 Wistar rats after monocrotaline injection, and group C consisted of 10 endothelin-B deficient homozygous (sl/sl) rats. The latter rat colony was kindly provided by Dr. M. Yanagisawa (Howard Hughes Medical Institute, Department of Molecular Genetics, University of Texas Southwestern Medical Centre, Dallas, Texas, USA) and was bred in our animal laboratory facilities.

Monocrotaline injection

Pulmonary arterial hypertension was induced by a single monocrotaline injection, as previously described.[7] In brief, aliquots of monocrotaline (Crotaline, Sigma-Aldrich Ltd.) were prepared using HCl and NaOH. Under brief ether anaesthesia, a single injection containing 60 mg/kg was given subcutaneously to groups B (n=10) and C (n=10). All rats were inspected daily for signs of tachypnoea or reduced activity.

Haemodynamic measurements

During the first part of the study, haemodynamic measurements were performed via the internal jugular vein. Under ether anaesthesia, the animals were intubated, mechanically ventilated (ventilator model 7025, Ugo Basile, Comerio, VA, Italy) and anaesthetised with 2% isoflurane in oxygen (1-2 L/min). The right internal jugular vein was exposed and three types of catheters were tested: (a) a 4F polyvinyl catheter (n=5), (b) a 3F silicone catheter (n=5) and (c) a 4F latex catheter (n=5). Under continuous pressure recording, the catheter was advanced until a satisfactory right ventricular and/or pulmonary artery recording was obtained.

During the second part of the study, pulmonary artery measurements were performed three weeks after monocrotaline injection. The animals were anaesthetised as described above and a left lateral

thoracotomy was performed; the pectoral muscles were dissected, the heart was exposed and the pericardium was carefully removed. A 6-0 suture (Ethicon, Johnson & Johnson Medical, Inc.) was placed at the apex and slight traction was applied to provide stability. A 21G venous cannula was inserted through the right ventricular outflow tract into the main pulmonary artery. The heart was repositioned *in situ* and the catheter was connected to a pressure recording system (Dinamap, Johnson & Johnson Medical, Inc.) through a saline-filled system. Two-minute continuous recordings were performed as soon as a stable signal was obtained. Subsequently, all but 3 rats were sacrificed with potassium chloride and the heart and lungs were harvested for pathology assessment.

Ambulatory pressure recording

To monitor pulmonary artery pressure in conscious animals, three rats, one from each group, were chosen randomly. After initial pressure recordings, the catheters were filled with heparinised normal saline and the catheter shaft was sealed at its entry to the right ventricular outflow tract using BioGlue (Cryolife, Inc.). Subsequently, the free end was exteriorised to the back of the neck through a subcutaneous tunnel and sutured *in situ*. Pressure recordings were obtained every 15 minutes for 60 minutes following extubation and resumption of physical activity.

Histological studies

The specimens were preserved in phosphate-buffered 10% formalin. Lung tissues were washed, distended appropriately and dissected into small pieces. Subsequently, tissues were embedded in paraffin and cut into 5 μm slices. The sections were stained with haematoxylin-eosin and pulmonary vascular wall thickness was assessed under light microscopy.

Statistical analysis

All values are expressed as mean ± standard error. Continuous variables were compared using one-way analysis of variance, followed by post-hoc Duncan's multi-range test. Differences in mortality were compared using the non-parametric Kruskal-Wallis analysis of variance. Statistical significance was defined at an alpha level of 0.05.

Results

Animal population and mortality

Of the 15 rats included in the first part of the study, right ventricular pressure was successfully recorded in 3 (20%) animals, all with a 4F latex catheter; pressure dumping was handled with frequent saline flushes. The remaining 12 (80%) rats died during the experimental procedure. Post-mortem examination revealed perforation of the superior vena cava (n=5), the right atrium (n=3), or the inferior vena cava (n=4). Occasional catheter kinking due to vessel tortuosities was observed.

In the second part of the study, no rat died during the three-week period following monocrotaline injection. However, all animals in both monocrotaline groups (B and C) became progressively tachypnoeic with reduced physical activity; these features were more prominent in group C. During the experimental procedure, no animal from group A died spontaneously, but there was a trend (H=4.6, p=0962) towards an increased mortality in groups B and C. Specifically, 4 (40%) rats in group B and 3 (30%) rats from group C died of respiratory arrest during endotracheal intubation attempts. Thus, the final study population consisted of 10 rats (222 ± 4 g) in group A, 6 rats (224 ± 4 g) in group B and 7 rats (218 ± 2 g) in group C.

Haemodynamic measurements

There was a statistical variance (F=51.28, p<0.0001) in pulmonary artery systolic pressure, due to higher values in groups B and C compared to group A (p=0.00152 and p=0.000072, respectively). Moreover, pulmonary artery systolic pressure was significantly (p=0.036) higher in group C than in group B.

A statistical variance (F=19.05, p=0.00002) was also present in pulmonary artery diastolic pressure, due to higher values in groups B and C compared to group A (p=0.000294 and p=0.000124, respectively). However, pulmonary artery diastolic pressure was comparable (p=0.57) between the two monocrotaline groups. All values are depicted in Figure 1.

The quality of pulmonary artery pressure recordings through the externalised catheter was generally poor. Only a few signals were of good quality, precluding statistical analysis. Nonetheless, pulmonary artery pressure recordings showed a 5-10 mmHg increase in systolic pressure in conscious animals after resumption of physical activity, compared to values during anaesthesia.
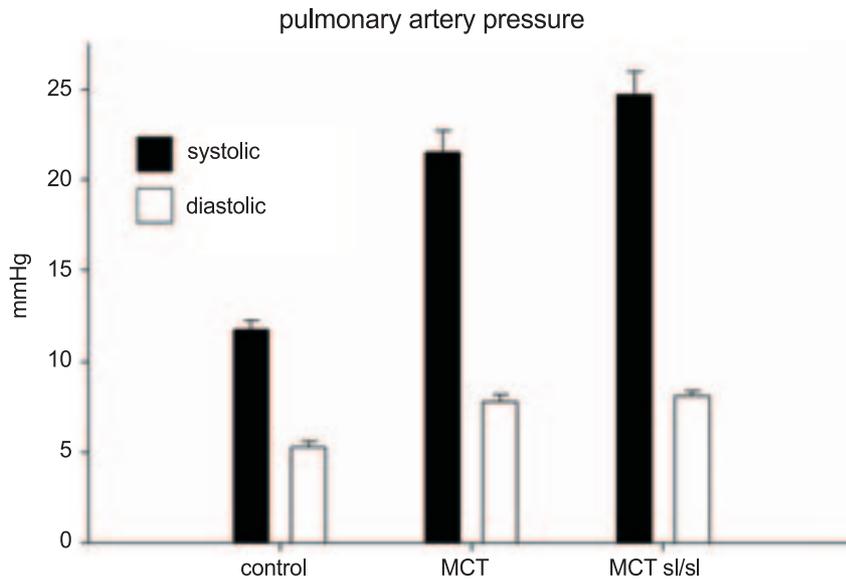
Figure 1. Diagram depicting systolic and diastolic pulmonary artery pressures in the three rat groups. MCT – wild type rats after a single monocrotaline injection, MCTsl/sl – endothelin-B receptor deficient rats after a single monocrotaline injection.

Histological assessment

Both monocrotaline groups (B and C) displayed increased vascular wall thickness due to medial hypertrophy. These features were more prominent in group C (Figure 2). However, plexiform neointimal proliferative lesions were not observed in any rat group.

Discussion

In addition to its primary form, a wide variety of diseases may lead to pulmonary arterial hypertension.[3] Once established, pulmonary hypertension portends an adverse effect on morbidity and mortality.[1-3] Despite advances in medical treatment, reversal of the disease, documented by a progressive and sustained fall in pulmonary artery pressure, is rare.[4] The diverse aetiologies and the relative inefficacy of current therapies call for more intensive research towards a better understanding of the complex pathophysiology of the disease.

In the present study, we evaluated the feasibility of a pulmonary artery pressure recording technique via the internal jugular vein in rats. This minimally invasive approach has been described previously in detail.[10] The use of a closed-chest model provides the significant advantage of pulmonary artery measurements in more physiological conditions.[10] However, in our experiments, occasional catheter kinking due to vessel tortuosities may have resulted in inaccurate
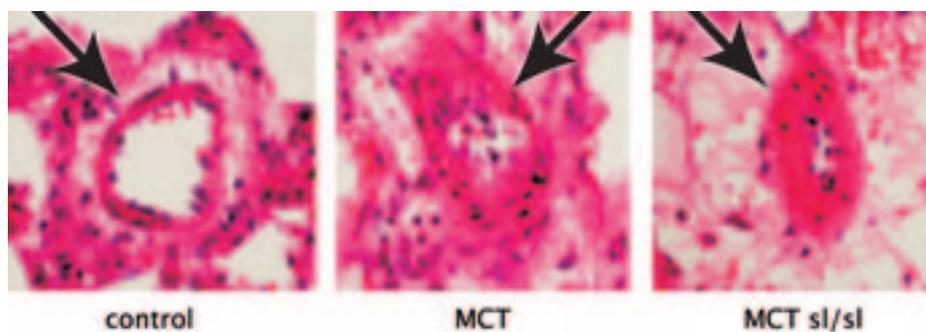
Figure 2. Characteristic examples of lung vessel histology after haematoxylin and eosin staining. Note the more prominent medial hypertrophy (black arrows) in a MCT sl/sl rat, compared to MCT or to control. Abbreviations as in figure 1.

recordings, underlining the importance of catheter selection. Moreover, the high mortality observed in our animal population indicates that the use of this model is technically demanding and a prolonged learning curve may be expected. In contrast, direct pressure measurement via a left lateral thoracotomy is more easily applicable, without substantial differences in pressure values.

A considerable strength of the present report is the study of endothelin receptor-B deficient homozygous (sl/sl) rats. If exposed to hypoxia or endothelin-1 infusion, these rats develop severe pulmonary hypertension.[11] We further characterised the haemodynamic and histological responses of these rats, three weeks after a single monocrotaline injection. We found an exaggerated haemodynamic response of homozygous (sl/sl) rats, not only compared to controls, but also compared to wild type rats under identical experimental conditions. These features, coupled with the simplicity of a single monocrotaline injection and direct pressure recordings, make this model particularly attractive for the study of various therapeutic interventions. Nonetheless, acute mortality rates as high as 30-40% should be anticipated.

Our present study further characterised the histological features of the wild type and homozygous (sl/sl) rats after monocrotaline-induced pulmonary hypertension. Both animal groups developed pulmonary vascular lesions, consisting of prominent medial hypertrophy. Although we found a greater degree of occlusion in the vessel lumen in homozygous (sl/sl) rats, we were not able to demonstrate a clear development of plexiform neointimal proliferation. These findings are in agreement with those reported by Ivy et al,[12] who showed that lungs from these rats closely resembled human histology when monocrotaline was administered in younger (6 weeks of age) but not in older (over 3 months of age) animals.[12] In keeping with these findings, monocrotaline injection in our homozygous (sl/sl) rats at a mean age of 20 weeks failed to produce the full pathological spectrum of the disease.

The reasons for the more pronounced haemodynamic and histological findings in homozygous (sl/sl) rats lacking the expression of endothelin-B receptors are not clear. In previous reports,[9] mRNA for endothelin-converting enzyme and plasma endothelin-1 levels were higher in homozygous sl/sl rats than in wild type controls, while metabolites of nitric oxide and prostacyclin were lower.[9,12] These findings suggest that the endothelin-B receptor plays a protective role in the pulmonary hypertensive response to chronic hypoxia. Further research is warranted to elucidate the pathophysiological role of endothelin-1 in pulmonary hypertension.

Conclusions

Endothelin receptor-B deficient rats constitute a useful model for the study of pulmonary artery hypertension. Monocrotaline injection should be given at an age of approximately at 6 weeks in order to achieve histology resembling human findings. Closed chest models are technically demanding and a learning curve should be anticipated. The ease of pressure recordings via a left lateral thoracotomy may aid in the more widespread use of this model.

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