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ORIGINAL ARTICLE

Activation of signaling molecules and matrix metalloproteinases in right ventricular myocardium of rats with pulmonary hypertension

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Abstract

Pulmonary hypertension induces right ventricular (RV) overload, which is transmitted to cardiomyocytes via integrins that activate intracellular messengers, including focal adhesion kinase (FAK) and neuronal nitric oxide synthase (NOS1). We investigated whether RV hypertrophy (RVH) and RV failure (RVF) were associated with activation of FAK, NOS1, and matrix metalloproteinases (MMPs).

Rats were treated without (RVC) or with a low dose of monocrotaline (30 mg/kg) to induce RVH, and with a high dose (80 mg/kg) to induce RVF. After ≈30 days, RV function was determined using a combined pressure-conductance catheter. After sacrifice, FAK, NOS1, their phosphorylated forms (FAK-P and NOS1-P), MMP-2, and MMP-9 were quantified in RV myocardium by immunohistochemistry.

In RVH and RVF, RV weight/ body weight increased by 36% and 109%, whereas RV ejection fraction decreased by 23% and 57% compared to RVC, respectively. FAK-P and FAK-P/FAK were highest in RVH (2.87 ± 0.12 and 2.52 ± 0.23 fold compared to RVC, respectively) and slightly elevated in RVF (1.76 ± 0.17 and 1.15 ± 0.13 fold compared to RVC, respectively). NOS1-P and NOS1-P/NOS1 were increased in RVH (1.63 ± 0.12 and 3.06 ± 0.80 fold compared to RVC, respectively) and RVF (2.16 ± 0.03 and 3.30 ± 0.38 fold compared to RVC, respectively). MMP-2 was highest in RVH and intermediate in RVF (3.50 ± 0.12 and 1.84 ± 0.22 fold compared to RVC, respectively). MMP-9 was elevated in RVH and RVF (2.39 ± 0.35 and 2.92 ± 0.68 fold compared to RVC, respectively).

Activation of FAK in RVH points to an integrin-dependent hypertrophic response of the myocardium. Activation of NOS1 in failing RV suggests a role of excessive NO in the development of failure and activation of MMPs leading to ventricular remodeling.

Keywords: Hypertrophy; Heart failure; Focal adhesion kinase; Neuronal nitric oxide synthase; Matrix metalloproteinase

Abbreviations: FAK, Focal adhesion kinase; nNOS, Neuronal nitric oxide synthase; NOS1, Neuronal nitric oxide synthase; NO, Nitric oxide; MMP, Matrix metalloproteinase; ECM, Extracellular matrix; SR, Sarcoplasmic reticulum; RyR, Ryanodine receptor; MCT, Monocrotaline; ROS, Reactive oxygen species; CHF, Congestive heart failure; RV, Right ventricle; LV, Left ventricle; IVS, Interventricular septum; RVC, Right ventricular control; RVH, Right ventricular hypertrophy; RVF, Right ventricular failure; PBS, Phosphate-buffered saline; LVH, Left ventricular hypertrophy; LVEF, Left ventricular ejection fraction; LVESV, Left ventricular end systolic volume; SNP, Sodium nitroprusside

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Introduction

Pulmonary hypertension is a condition that causes an overload of the right ventricle (RV). The forces of the overload are transferred to the cardiomyocytes via integrins, a family of transmembrane adhesion receptors. Integrin signaling occurs via a large array of intracellular messenger systems, including focal adhesion kinase (FAK) [25,26]. FAK is a cytoplasmic tyrosine kinase that discretely localizes to membrane regions that attach to the extracellular matrix (ECM), called focal adhesions. FAK transmits signals from the ECM via integrins to the cytoskeleton and particular cytoplasmic proteins. Stimulation of integrins and FAK leads to a hypertrophic response in cardiomyocytes [17].

We have previously shown that integrin stimulation was associated with immediate FAK phosphorylation and delayed phosphorylation of neuronal nitric oxide synthase (nNOS, NOS1) [32,33]. NOS1 is located on the sarcoplasmic reticulum (SR) of cardiomyocytes, and is considered to modify SR function via nitrosylation of the ryanodine receptor (RyR), which is the calcium release site of the SR [3,19,29,34,38]. NO and reactive oxygen species (ROS) produce peroxynitrite, known to activate matrix metalloproteinases (MMPs) [20,35] that are involved in ventricular remodeling [27]. The exact role of the NO formed in the failing myocardium is not yet fully elucidated. We hypothesize that signaling pathways involved in myocardial hypertrophy and failure are NO-dependent, including NO-dependent MMP activation leading to ventricular remodeling.

A frequently used model to study functional, structural, and molecular changes associated with compensated RV hypertrophy (RVH) and RV failure (RVF) is the rat treated with monocrotaline (MCT), a pyrrolizidine alkaloid [13,16]. MCT selectively injures the vascular endothelium of the lung and induces pulmonary vasculitis [37]. Muscularization and hypertrophy of media of pulmonary arteries lead to increased vascular resistance and increased pulmonary arterial pressure [8,22]. MCT-induced pulmonary hypertension is associated with the development of compensated RVH progressing to RVF within weeks, depending on the dose of MCT and the age of the rats [7,15,36]. Previous studies have shown selective induction of either RVH or RVF after 4 weeks of treatment with a low dose (30 mg/kg body weight) or a high dose (80 mg/kg body weight) of MCT, respectively [7,14].

In the rat model of MCT-induced pulmonary hypertension, we determined activation of FAK, NOS1, immunoreactive MMP-2, and MMP-9 in RV myocardium to compare differences with respect to their activation patterns between controls, RVH and RVF. To test whether NO stimulates expression of MMP-2 and MMP-9, neonatal rat ventricular cardiomyocyte cultures were treated without and with the NO-donor sodium-nitroprusside (SNP) for 24 h, followed by quantitative determination of immunoreactive MMP-2 and MMP-9.

Materials and methods

Animal model

All animals were treated in accordance with the national guidelines and with the approval of the Animals Experiments Committee of the Leiden University Medical Center. A total of 14 male Wistar rats (Harlan, Zeist, the Netherlands) weighing 200–250 g were randomly assigned to three groups. Animals received a single subcutaneous injection of MCT (Sigma-Aldrich, Zwijndrecht, the Netherlands) diluted in phosphate-buffered saline (PBS) in a low dose (30 mg/kg body weight, $n = 5$; RVH group) or in a high dose (80 mg/kg body weight, $n = 5$, RVF group). Control rats (RVC, $n = 4$) were injected with an equal volume of PBS.

Hemodynamics

After 4 weeks of MCT administration, RV pressure and volume signals were recorded by a combined pressure-conductance catheter. For that purpose, rats were sedated by inhalation of a mixture of isoflurane (4%) and oxygen. General anesthesia was administered by intraperitoneal (i.p.) injection of a fentanyl—fluanison–midazolam mixture in a dose of 0.25 mL/100 g body weight. The mixture consisted of two parts Hypnorm® (0.315 mg/mL fentanyl + 10 mg/mL fluanison, VetalPharma, Maarheeze, the Netherlands): one part Dormicum® (5 mg/mL midazolam, Roche, Mijdrecht, the Netherlands) and one part saline. After tracheotomy, the animals were ventilated mechanically using a pressure-controlled respirator and a mixture of air and oxygen.

After midsternal thoracotomy, a combined pressure-conductance catheter (SPR-878, Millar Instruments, Houston, TX, USA) was introduced via the apex into the RV and positioned along the long axis of the RV. The catheter was connected to a Sigma-SA signal processor (CD Leycom, Zoetermeer, the Netherlands), and RV pressures and volumes were recorded digitally. All data were acquired using Conduct-NT software (CD Leycom) at a sample rate of 2000 Hz and analyzed off-line by custom-made software. The volume signal was calibrated using an ultrasonic flow probe (Transonic Systems, Maastricht, the Netherlands) around the ascending aorta as described by [14].

Heart rate, RV stroke volume, cardiac output, RV end-diastolic volume, RV end-systolic volume, RV ejection fraction, RV end-diastolic pressure, RV peak