Universiteit
Leiden
The Netherlands

Molecular and cellular characterization of cardiac overload-induced hypertrophy and failure

Umar, S.

Citation
Umar, S. (2009, June 18). *Molecular and cellular characterization of cardiac overload-induced hypertrophy and failure*. Retrieved from https://hdl.handle.net/1887/13860

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/13860

Note: To cite this publication please use the final published version (if applicable).

Chapter 9

Summary, Conclusions, Future Perspectives and Samenvatting

Summary, Conclusions, Future Perspectives and Samenvatting

Summary of the thesis

The major objectives of research described in this thesis include investigation of molecular and cellular mechanisms of cardiac hypertrophy and failure. In a study on monocrotaline-induced pulmonary arterial hypertension in rats mesenchymal stem cell therapy was tested as a novel option to treat this disease.

Chapter 1 deals with general introduction of the thesis. It provides information about the molecular and cellular characteristics of the healthy heart, the heart with hypertrophy and the failing heart. Extracellular matrix (ECM) composition, synthesis and degradation, integrins and integrin signaling, nitric oxide synthases (NOSs) and NO are discussed in relation to normal, hypertrophic and failing myocardium. In addition, experimental models of myocardial hypertrophy and heart failure are described. Particularly, the characterization and pathology of right ventricular function in animals with monocrotaline-induced pulmonary hypertension are emphasized.

In **chapter 2** we hypothesized that pressure overload is “felt” by the myocardium through stretch-like effects imposed on integrins, the receptor by which cardiomyocytes are attached to the ECM. In the cell model of neonatal rat cardiomyocytes (NRCMs) *in vitro*, we activated the integrins by administration of a pentapeptide containing Arg-Gly-Asp (RGD) to test whether integrin stimulation leads to NRCM hypertrophy. The pro-hypertrophic effect of RGD-containing pentapeptide on NRCMs was compared with the well-known pro-hypertrophic effects of $_1$-adrenoceptor stimulation with phenylephrine. Saline-treated NRCMs were used as control. The hypertrophic response was quantified by measuring cell surface area (CSA). Phosphorylation of NO-synthase-1 (NOS1) was also assessed. CSA was increased by 38% with RGD and by 68% with PE versus control. A general NOS-inhibitor (L-NAME) inhibited RGD-induced hypertrophy completely, but had no significant effect on PE-induced hypertrophy. The L-NAME-induced inhibition of RGD-induced NRCM hypertrophy could be overcome, at least partly, by co-administration of a NO-donor, nitroprusside. Co-administration of a NO-donor had no effect on NRCMs incubated with PE + L-NAME. Ryanodine and BAPTA-AM inhibited RGD-induced hypertrophy completely but not that induced by PE, indicating the involvement of intracellular Ca^{2+} ions in the RGD-induced NRCM hypertrophy. NOS-1 phosphorylation was increased with RGD by 61%. Hence we concluded that integrin stimulation of NRCMs by RGD leads to hypertrophy. Abrogation of RGD-induced hypertrophic response upon NOS-inhibition and rescue of this hypertrophic effect by NO-donor suggest that integrin stimulation-induced hypertrophy of NRCMs depends upon NO, possibly derived from NOS1.

In **chapter 3** we demonstrated that ventricular failure may be associated with a disturbed myocardial collagen turnover. In patients with heart failure, myocardial collagen turnover can be assessed by serum concentrations of aminoterminal propeptides of type I and type III collagen (PINP and PIIINP) and carboxyterminal telopeptide of type I collagen (ICTP) that either represents measures of collagen synthesis (PINP, PIIINP) or collagen degradation (ICTP). We investigated the effects of cardiac resynchronization therapy (CRT) on myocardial collagen turnover in 64 patients with heart failure by comparing PINP, PIIINP and ICTP concentrations in serum obtained at baseline and after 6 months of CRT. We hypothesized that in patients with heart failure CRT leads to reverse ventricular remodeling, associated with a net collagen formation. Forty-six patients (72%) showed a >10% reduction in LV end-systolic volume at follow-up and were classified as responders to CRT, the other 18 patients (28%) were classified as non-responders. Responders demonstrated a mean increase of serum PINP and PIIINP during follow-up, by 42% and 12%, respectively. In non-responders, serum PINP and PIIINP remained unchanged during follow-up. At baseline, responders had significantly lower serum PINP than non-responders (by 21%). ICTP levels of responders at baseline tended to be higher than in non-responders (by 70%, p=ns), and in both groups ICTP levels did not change upon CRT. We concluded that reverse LV remodelling following CRT is associated with increased collagen synthesis rate in the first 6 months of follow-up.

It has been known for some time that monocrotaline (MCT)-induced pulmonary artery hypertension (PAH) and right ventricular (RV) failure are associated with the activation of matrix metalloproteinases (MMPs) in RV myocardium. In **chapter 4** we investigated whether NO plays any role in PAH-induced RV hypertrophy and failure. To that purpose, two doses of MCT were used that produced RV hypertrophy (RVH) only and RV hypertrophy and subsequent RV failure (RVF), respectively. In RVH and RVF, RV weight/ body weight increased by 36% and 109%, whereas RV ejection fraction decreased by 23% and 57% compared to control, respectively. A protein associated with integrins called focal adhesion kinase (FAK) became phosphorylated in RVH (2.5-fold compared to control) but slightly in RVF (1.15-fold compared to control). Phosphorylation of NOS1-P was increased in RVH (3.0-fold compared to control) and in RVF (3.3-fold compared to control). MMP-2 was highest in RVH and intermediate in RVF (3.5- and 1.8-fold compared to control, respectively). MMP-9 was elevated in RVH and RVF (2.4- and 2.9-fold compared to control, respectively). We concluded that 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.

Chapter 5 provides a comprehensive review of novel therapeutic approaches to treat PAH. PAH is a life-threatening disease with an important pulmonary component that may provide a target to direct therapy. These treatment options include a spectrum of pharmacotherapeutic agents. In addition, we discuss the emerging trends of using gene and cell therapy for the treatment of PAH. Finally, we discuss the possible applications of experimentally tested interventions for

therapeutic purposes in humans with PAH. The therapeutic agents include anti-mitogenic compounds, agents that have pro-endothelial function, agents with vasodilatory effects, compounds with pro-angiogenic effects, anti-inflammatory agents, agents with anti-oxidant effects, and agents that induce apoptosis of pulmonary artery smooth muscle cells.
Cell therapy is a novel treatment option and autologous mesenchymal stem cell (MSC) therapy is expected to be a safe and efficacious option to treat patients with PAH.

Chapter 6 describes a study on the effects of stem cell therapy on pulmonary pathology associated with MCT-induced PAH in rats. We demonstrated that MSCs from donor rats with PAH, injected i.v. into recipient rats that had MCT administration 14 days earlier, reduce pulmonary parenchymal damage, medial hypertrophy of pulmonary arterioles, and RV hypertrophy. At 28 days after MCT, rats had PAH (peak RV pressure had increased from 27.2 to 41.5 mmHg), and increased lung weight (by 73%). Lung histology revealed severe narrowing of precapillary arterioles, thickening of arteriolar walls (3.4-fold increased vs. control), thickening of alveolar septa (3.5-fold increased vs. control), and increased RV mass (by 63% vs. control). Treatment with MSCs attenuated PAH (to 30.7±4.4 mmHg), and almost normalized lung weight (21% higher than control), wall thickness of arterioles (20% higher than control), thickness of alveolar septa (9% higher than control), and RV hypertrophy (RV mass 8% higher than control). Most beneficial effects of i.v. injected MSCs were less prominent or absent in MCT-treated rats i.v. injected with skin fibroblasts. We concluded that i.v administration of MSCs from donor rats with PAH to recipient rats with PAH decreases RV peak systolic pressure, pulmonary arteriolar narrowing, alveolar septum thickening, and RV hypertrophy. These results suggest that autologous stem cell therapy may help alleviate pulmonary symptoms of patients with PAH.

In **chapter 7** the effects of MCT-induced PAH without and with cell therapy on the RV myocardium are described in more detail. At 28 days after MCT, rats had depressed LV ejection fraction (from 56 to 42%). In PAH rats treated with MSCs, RV ejection fraction was near normal (52%). In MCT-treated rats that had received i.v. skin fibroblasts, effects on RV peak systolic pressure, RVH and RV function were far less pronounced than in MCT-treated rats that had received MSCs. We concluded that i.v. administration of MSCs from donor rats with PAH to recipient rats with PAH decreases PAH, reverses RV hypertrophy, and improves RV function. These results suggest that patients with PAH may be treated successfully using autologous MSCs.

Chapter 8 presents the results of an exploratory study of the electrophysiologic properties of adult rat cardiomyocytes isolated from normal RV myocardium. We analyzed mechanisms of excitability of RV cardiomyocytes isolated from normal adult rat hearts, making use of the naturally occurring variability of excitability of these cells. We focused on the role of voltage-activated K^+ current (*Ikv*), including the transient current I_t and the sustained current *Iss*, in shaping the current-pulse evoked action potential (AP) and in generating sustained depolarizing current-induced automaticity (DIA). Simulation experiments were carried out with a

computer model of the right ventricular myocyte of the rat. The model experiments reproduced the decrease of AP-duration with an increase in I_t and revealed a DIA-mechanism based on *IcaL* deinactivation and *Iss* deactivation at depolarized potentials.
The results provide the electrophysiological baseline-properties of the adult rat right-ventricular myocytes, which can serve as the control properties of these cells if taken from rats with pulmonary hypertension. A provisional comparison of excitability properties of normal RV myocytes with those of PAH RV myocytes showed no striking differences between these two call types, e.g. DIA occurred in both groups.
Thus, to date it is still unknown whether and how this mechanism of DIA is involved in the high risk of arrhythmias in patients with heart failure.

Conclusions

1. Stimulation of integrin receptors on the cardiomyocyte membrane leads to cardiomyocyte hypertrophy by a nitric oxide-dependent mechanism.
2. Activation of signaling cascades involving the activation of FAK and NOS1 leads to the activation of MMPs in the failing right ventricular myocardium. Activated NOS1 in failing right ventricular myocardium suggests a role of (***i***) excessive NO in the development of heart failure, and (***ii***) MMPs leading to ventricular remodeling.
3. Reverse left ventricular remodeling following CRT in patients with congestive heart failure is associated with increased rate of collagen synthesis in the first 6 months of follow-up.
4. Intravenous therapy with mesenchymal stem cells from donor rats with MCT-induced PAH given to recipient rats with MCT-induced PAH reduces right ventricular hypertrophy and improves right ventricular function by improving lung pathology associated with PAH.
5. Insight in the membrane mechanism of depolarization-induced automaticity seems of importance for understanding the high risk of arrhythmias in patients with heart failure.
6. The experiments described in this thesis contribute to the knowledge about molecular and cellular characteristics of hypertrophic and failing myocardium, and may contribute to therapy of patients with PAH with autologous mesenchymal stem cells.

Future perspectives

1. Excessive NO production in the failing myocardium may be harmful and lead to nitrosylation of ryanodine receptors, rendering them dysfunctional as a consequence. More research is needed to define the exact role of (excessive) NO in the failing myocardium.
2. A variety of therapeutic modalities including drug therapy, gene therapy and cell therapy has been tested in several animal models of PAH including MCT-induced PAH in rats. Several of these therapeutic options have been shown to be effective also in PAH patients leading to improved life expectancy and a better quality of life. However, many patients still remain symptomatic despite therapy. Cell therapy is a novel treatment option, but more animal data should be collected to investigate optimal cell type, *in vitro* cell transduction, route of cell administration, and the number of cells to be injected. Autologous mesenchymal stem cell therapy is expected to be a safe and efficacious option to treat patients with PAH.