PATHOPHYSIOLOGY AND PHARMACOLOGY OF NITRIC OXIDE – CYCLIC GUANOSINE MONOPHOSPHATE SIGNALING IN DIABETIC CARDIOMYOPATHY

PhD thesis

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Introduction

Diabetes mellitus (DM) is a chronic endocrine disease characterized by higher-than-optimal blood sugar levels. It has two major forms, namely type 1 and type 2 diabetes (T1DM; T2DM) that are different in their basic pathophysiological features. T1DM develops due to the autoimmune destruction of the pancreatic β-cells, which leads to insulinopenia. In contrast, hyperinsulinemia and insulin resistance are the major drives in T2DM. Regardless of its mechanism, the altered metabolism in DM leads to the manifestation of a special cardiac disease, called diabetic cardiomyopathy. Therefore, the diabetes "epidemic" contributes not only to the increased cardiovascular mortality but also raises the economic burden of cardiac diseases. Consequently, novel therapies are urgently needed in order to treat and prevent diabetes-associated disorders.

The development of diabetic cardiomyopathy is associated with characteristic changes of the cardiac structure and function. Several key morphological and molecular features have been described in the pathophysiology of the disease. The dysfunctional metabolism in DM (disturbances in insulin signaling, diminished activity and downregulation of the glucose transporter type 4, substrate switching in the heart including reduced glucose and lactate and increased free fatty acid supply) results in increased nitrosative and oxidative stress in the cardiac tissue. Moreover, lipid overload, hyperglycemia-induced glucotoxicity, inflammation and overactivation of reactive oxygen species (ROS)-producing enzymes (e.g. nicotinamide adenine dinucleotide phosphate-oxidases) and mitochondrial dysfunction together contribute to the production of oxidative agents such as superoxide. Superoxide thereafter reacts with different molecules including nitric oxide (NO) to form the highly reactive nitrosative agent peroxynitrite. Peroxynitrite is able to bind and damage different structural elements in the cardiac tissue including enzymes, DNA and the members of the contractile apparatus. These processes together cause significant cardiac dysfunction in DM (mainly systolic dysfunction in T1DM and mainly diastolic dysfunction in T2DM).

The NO – cyclic guanosine monophosphate (cGMP) – soluble guanylate cyclase (sGC) – protein kinase G (PKG) signaling pathway has been described to exert anti-hypertrophic, anti-fibrotic and anti-apoptotic effects in various cardiovascular disorders. The activity of the pathway depends on the intracellular level of the second messenger cGMP, which is regulated by two main ways: 1) sGC is responsible for its production while 2) phosphodiesterase-5A (PDE5A) (dominantly in the heart) is responsible for the degradation of the molecule.

DM, however, through the presence of oxidative stress leads to the deterioration of the above signalling pathway. Main points of the dysregulation of cGMP signaling are the oxidative

deactivation of sGC and the overactivation of the PDE5A enzyme. Theese processes together significantly decrease the intracellular amount of cGMP thereby deactivation of PKG pathway occurs.

Novel pharmacological modulators of the NO-cGMP-sGC-PKG signaling have been developed in recent years. A promising novel class of drugs, the sGC activators are able to reactivate the dysfunctional enzyme by binding to its heme-binding subunit in oxidative stress. Cinaciguat, a member of the sGC activators, has been described to have beneficial effects in experimental myocardial infarction, myocardial ischemia/reperfusion injury, endothelial dysfunction induced by nitro-oxidative stress and in the prevention of cardiac hypertrophy. Cinaciguat has demonstrated efficacy in a proof-of-concept study in patients with acute decompensated heart failure, however, the acute intravenous use of the drug for the same indication in a phase-IIb clinical study was associated with high number of hypotension as adverse event. Therefore, the study has been prematurely terminated but the investigators concluded that rather a chronic oral application could have beneficial effects in chronic heart failure patients.

Another point of intervention in cGMP signaling is PDE5A inhibition. PDE5A inhibitors (such as vardenafil) are on-demand treatment for erectile dysfunction. The crucial role of PDE5A in myocardial remodeling has been described in PDE5A knockout mice. The protective effects of enhanced cGMP-signaling via pharmacological inhibition of PDE5A by vardenafil have been demonstrated in several cardiovascular diseases, such as acute myocardial infarction, cardiac hypertrophy, heart failure and drug-induced cardiotoxicity. The beneficial effects on heart failure patients of the PDE5A inhibitor sildenafil have been investigated in a clinical trial. However, sildenafil failed to improve exercise capacity and the clinical outcomes in advanced heart failure patients. In spite of that, PDE5A inhibition has been proposed to be of therapeutic importance in heart failure patients with DM.

Objectives

Recent literature data have drawn the attention to the importance of co-morbidities in heart failure patients as they significantly affect the mortality and morbidity. Among the different co-morbidities, DM is a major one. Although DM accounts for a large portion of mortality of heart failure patients as a contributor to different cardiovascular diseases, DM itself leads to the development of heart failure regardless of the effect on coronary artery disease or hypertension.

To date, the pharmacological therapy of multi-morbid heart failure patients is far from optimal. Therefore, co-morbidities have to be taken into account as part of personalized medicine and for that novel drugs are needed to treat patients appropriately. Enhancement of cGMP signaling in the myocardium might provide a novel and promising therapeutic option to treat diabetic heart failure.

Based on that, the aims of our present investigations were as follows:

1. Comparison of the functional (systolic and diastolic) and the structural hallmarks (oxidative stress, fibrotic and hypertrophic remodeling and apoptosis) of diabetic cardiomyopathy in animal models of T1DM and T2DM

2. Revelation of the alterations of NO-cGMP signaling in diabetic cardiomyopathy in the two types of DM

3. Explore the possible therapeutic effects of sGC activation by the novel sGC activator cinaciguat in T1DM-associated diabetic cardiomyopathy

4. Study the potential cardioprotective effects of the PDE5A inhibitor vardenafil on the development of T2DM-related diabetic cardiomyopathy

Methods

Model of T1DM

T1DM was induced in young male Sprague-Dawley (SD) rats with a single i.p. injection of streptozotocin (60 mg/kg). 72h after induction animals with a random blood glucose level >15 mmol/l were considered to be diabetic and were included into the study. Experiments were performed after 8 weeks of DM duration.

Model of T2DM

Zucker Diabetic Fatty (ZDF) rat was used as a model of T2DM. Homozygous recessive males (fa/fa) develop obesity, fasting hyperglycemia and DM due to a genetic mutation of the leptin receptor and a special diet (Purina #5008). Homozygous dominant (+/+) and heterozygous (fa/+) lean genotypes remain healthy. Seven-week old male ZDF diabetic (fa/fa) and ZDF lean (+/?) rats were used. Experiments were performed at the age of 32 weeks.

Study protocol

For comparative investigation 16-week-old SD and 32-week-old ZDF rats were used.

Cinaciguat was tested in T1DM. Groups: vehicle-treated control (Co; n=12), cinaciguat-treated control (CoCin; n=12), vehicle-treated diabetic (DiabCo; n=12) and cinaciguat-treated diabetic (DiabCin; n=10) groups. Eight-week old animals were treated vehicle (Co, DiabCo) or with cinaciguat per os (CoCin, DiabCin; 10 mg/kg/day) for eight weeks.

Vardenafil was tested in ZDF rats. Seven-week old male ZDF diabetic (fa/fa) and ZDF lean (+/?) rats were divided into four groups: vehicle-treated controls (ZDFLean; n=8), vardenafil-treated controls (ZDFLean+Vard; n=7), vehicle-treated diabetic (ZDF; n=7) and vardenafil-treated diabetic (ZDF+Vard; n=8). Rats were treated with 10 mg/kg vardenafil continuously or with vehicle (0.01 mol/l citrate buffer) orally for 25 weeks.

Biochemistry

Blood glucose and urine glucose was determined. Plasma cGMP and plasma nitrite/nitrate levels were measured.

Echocardiography

The cardiac morphology of ZDF was studied by echocardiography under 1-2% isoflurane anesthesia. B-mode images were acquired in the long-axis and in the short-axis at the mid-papillary level and left ventricular (LV) anterior, posterior wall thicknesses (LVAW; LVPW)

and LV internal diameter (LVID) were measured in end-systole and end-diastole ('s' and 'd'). The following parameters were calculated: LVmass=1.04x[(LVAWd+LVIDd+LVPWd)^3-LVIDd^3], relative wall thickness (RWT)=(LVAWd+LVPWd)/LVIDd. LVmass was normalized to the body weight (BW; kg) (LVmass index) and to the tibia length (LVmass/TL).

In vivo hemodynamic investigation

Invasive hemodynamic investigation was performed in order to characterize cardiac function in detail and to assess the effect of drug treatments. The following parameters were measured by LV pressure-volume analysis (P-V): mean arterial pressure (MAP), heart rate (HR), LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), maximal slope of systolic pressure increment (dP/dt_{max}) and diastolic pressure decrement (dP/dt_{min}), time constant of LV pressure decay (Tau_W; Weiss method), ejection fraction (EF), stroke work (SW) and cardiac output (CO), the slope of LV end-systolic P-V relationship (E_{es}; curvilinear model), preload recruitable stroke work (PRSW) and the slope of LV end-diastolic P-V relationship (EDPVR).

Force measurement in permeabilized left ventricular cardiomyocytes

Permeabilized rat LV cardiomyocytes were mounted in a mechanical apparatus to measure isometric force and sarcomere length (SL). Maximal active force (F_{max}; in the presence of a saturating Ca^{2+} concentration) and Ca^{2+}-independent passive force ($F_{passive}$; in relaxing solution) were measured at a SL 2.3 μm, while $F_{passive}$ was also registered for a range of SLs (between 1.9 μm and 2.5 μm).

Gene expression studies

From LV mRNA samples, the expression of the following genes were measured by quantitative reverse transcription polymerase chain reactions: catalase, glutathione reductase (GSR), superoxide dismutase 1 and 2 (SOD1 and 2), thioredoxin-1, atrial natriuretic factor (ANF), myosin heavy chain alpha and beta (α- and β-MHC), Bcl-2, Bcl2-associated X protein (BAX), endothelial NO synthase (eNOS), heat shock protein 70a1 (HSP70a1), matrix metallopeptidase 2 and 9 (MMP-2 and 9), tissue inhibitor of MMP 1 and 2 (TIMP-1 and 2), collagen 1a1 and 3a1 (Col1a1 and 3a1), fibronectin, phospholamban (PLB), caspase-12, c-fos, c-jun and endothelin-1 (ET-1). Gene expression data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Western blotting

Protein expression of eNOS, sGC, PDE5A, PKG, vasodilator stimulated phosphoprotein (VASP) and phosphorilated-VASP (p-VASP), MMP-2 and 9, transforming growth factor β1 (TGF-β1), PLB, p-PLB, poly (ADP-ribose) polymerase (PARP1) were investigated by western blot. GAPDH and tubulin were used as loading controls.

Histopathology and immunohistochemistry

We performed hematoxylin and eosin (HE), Masson's trichrome (MT) and Picrosirius red staining to assess cardiomyocyte hypertrophy (cardiomyocyte diameter) and fibrosis (MT score and pircosirius red area). Additionally, 3-nitrotyrosine (3-NT, marker of nitro-oxidative stress), cGMP, TGF-β1 and fibronectin immunohistochemistries were done. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed to assess DNA fragmentation and apoptosis.

Statistical analysis

Data are presented as means and standard errors of the mean (SEM). Shapiro-Wilk's normality test was used. A $p < 0.05$ was used as a criterion of significance.

Comparative investigation of T1DM and T2DM-related diabetic cardiomyopathy

An unpaired two-sided Student's *t*-test was used. To compare diabetes-induced alterations between the two models individual data of each animal was normalized to the average value of the corresponding control group.

Experiments on the effect of sGC activation in T1DM-related diabetic cardiomyopathy

Two-way analysis of variance (ANOVA) with "T1DM" and "Cinaciguat" as independent factors was carried out. Tukey HSD post hoc test was used to assess intergroup differences.

Experiments on the effect of PDE5A inhibition in T2DM-related diabetic cardiomyopathy

Two-way ANOVA with "T2DM" and "Vardenafil" as independent factors was carried out. Tukey HSD post hoc test was used to assess intergroup differences.

Drugs

Cinaciguat was suspended in 0.5% methylcellulose solution. Vardenafil was dissolved in 0.01 mol/l citrate buffer.

Results

Comparative investigation of diabetic cardiomyopathy in T1DM and T2DM

Basic characteristics

DM was associated with decreased BW and increased blood and urine glucose levels.

Nitro-oxidative stress

DM was characterized by increased nitro-oxidative stress, as indicated by the higher 3-NT immunoreactivity and upregulation of different antioxidant systems (catalase, thioredoxin-1, GSR, HSP70a1, SOD1 and SOD2) in the LV. Interestingly, the extent of nitro-oxidative stress was more pronounced in T1DM compared to T2DM.

Cardiac hypertrophy and fibrosis

Cardiac remodeling was a common hallmark of both DM models. We observed significantly increased HW/BW ratio in DM. Echocardiography findings supported cardiac hypertrophy in ZDF rats. Accordingly, cardiomyocyte diameter was increased in both types of DM indicating the presence of cardiomyocyte hypertrophy. An altered pattern of myocardial gene expression was observed including increased ANF, β/α-MHC, ET-1, c-fos and c-jun expressions. Additionally, the altered metabolism in DM led to the development of myocardial fibrosis in the heart of the animals (as shown by increased MT score and Picrosirius areas and upregulated TGF-β and fibronectin expression, and by the dysregulation of MMPs and Col1a1 and 3a1 production). Similar to nitro-oxidative stress, the pathological remodeling of the heart was more pronounced in type 1 diabetic animals when compared to ZDF rats.

DNA injury and apoptosis

The extensive nitro-oxidative stress resulted in DNA injury and apoptosis as indicated by the elevated number of TUNEL positive cardiomyocyte nuclei in the myocardium. We observed that the more pronounced oxidative stress in T1DM was associated with more prominent increase of DNA injury and apoptosis. We found that caspase-3 and PARP1 activation were elevated, while BAX/Bcl-2 ratio was unchanged.

Alterations of the NO-sGC-cGMP-PKG signaling

Significant deterioration of the NO-sGC-cGMP-PKG signaling pathway was found as myocardial cGMP levels were significantly lower and PDE5A was upregulated in the diabetic

myocardium regardless of its type. The downregulation of eNOS might have further contributed to this phenomenon in T1DM. Consequently, the activity of this pathway was diminished in both types of DM as shown by the lower p-VASP/VASP ratios. Interestingly, plasma total nitrite/nitrate levels were not different in type 2 diabetic animals.

In vivo and in vitro cardiac function

MAP was not different between diabetic and corresponding controls while HR was lower in T1DM when compared with its control group. Classical systolic parameters (EF, CO, dP/dt_{max}, LVSP, SW) and the sensitive contractility indices (E_{es}, PRSW) showed significant decrease in T1DM (Figure 1A). Despite these alterations T2DM was characterized by

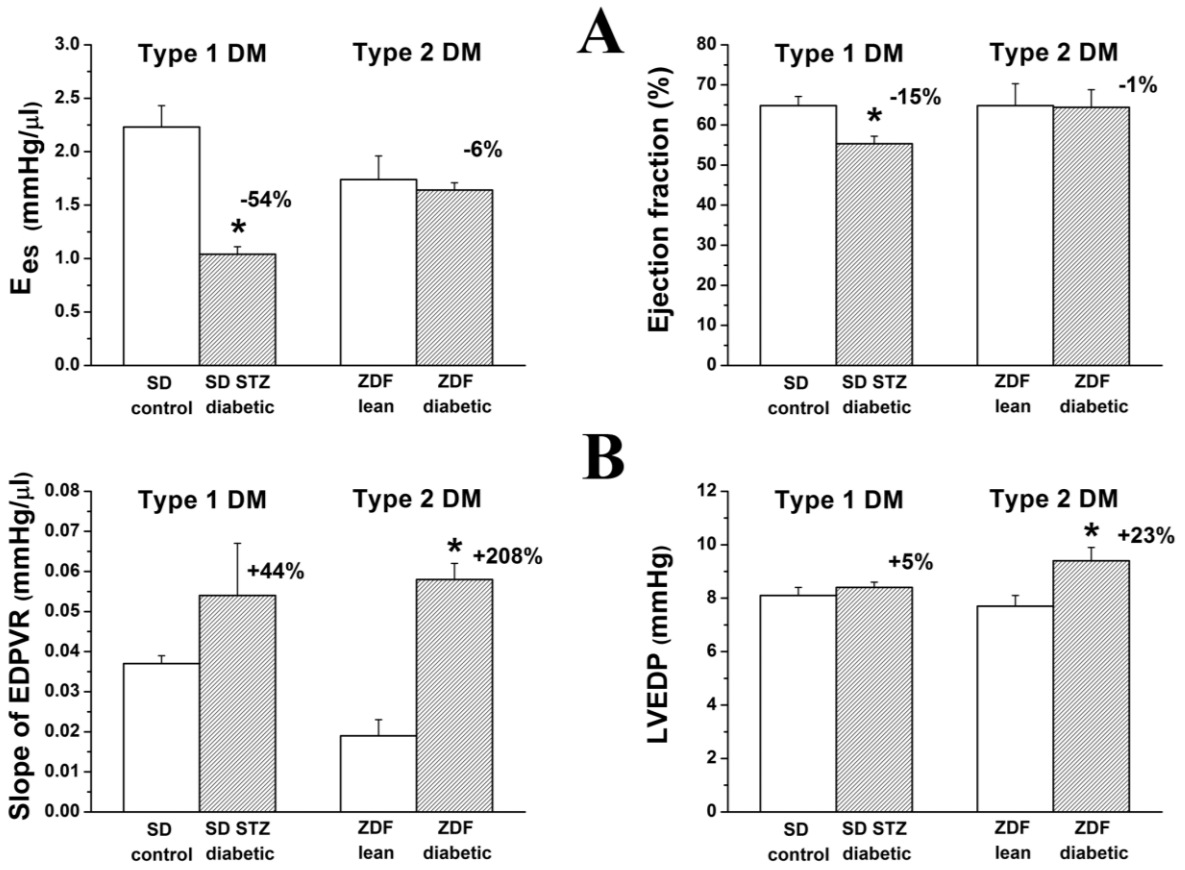

Figure 1. Cardiac dysfunction in T1DM and T2DM models
Contractility indices (A): slope of end-systolic pressure-volume relationship (E_{es}), ejection fraction (EF). Stiffness parameters (B): slope of end-diastolic pressure-volume relationship (EDPVR), slope of end-diastolic pressure (LVEDP). Groups: SD control, streptozotocin (STZ)-induced diabetic SD, Zucker Diabetic Fatty (ZDF) lean, and ZDF. Percent changes between diabetic and corresponding non-diabetic control groups are indicated in both DM models. *$p<0.05$ vs. the corresponding non-diabetic group.

preserved systolic function (unchanged systolic and contractile indices) (Figure 1A). Cardiac relaxation (Tau_W) was markedly impaired in both animal models. Significant increase of EDPVR and LVEDP was observed only in T2DM demonstrating pronounced LV diastolic stiffness (Figure 1B). In vitro force measurement of LV cardiomyocytes showed elevated $F_{passive}$ without the change of F_{max} as an indicative of cardiomyocyte stiffness at the sarcomere level.

Effects of cinaciguat on T1DM-associated cardiomyopathy

Basic characteristics

Cinaciguat had no effect on blood glucose levels and on HW, BW and HW/BW ratio.

Nitro-oxidative stress

Cinaciguat effectively reduced the DM-associated increase of 3-NT immunoreactivity and prevented the upregulation of the different antioxidant enzymes.

Cardiac hypertrophy and fibrosis

Cinaciguat had anti-fibrotic effects in T1DM, as it decreased the TGF-β1 immunoreactivity and the MT positive area of the myocardium. Cinaciguat prevented MMP dysregulation by reverting MMP/TIMP ratios to control levels. Anti-hypertrophic effects of the drug were confirmed by lower cardiomyocyte diameter and reduced ANF gene expression in T1DM.

DNA injury and apoptosis

Cinaciguat reduced DNA injury and apoptosis (less number of TUNEL positive nuclei) in DM when compared to DiabCo animals. It had no effect on BAX/Bcl2 ratio.

Alterations of the NO-sGC-cGMP-PKG signaling

Cinaciguat restored the activity of the NO-sGC-cGMP-PKG signaling [elevated plasma/cardiac cGMP levels, lower PDE5A protein expression and increased ratio of p-VASP/VASP (marker of PKG activity)] in T1DM.

In vivo cardiac function

Cinaciguat improved cardiac performance in T1DM (Figure 2.). Sytolic function was preserved as represented by improved PRSW and EF, while diastolic active relaxation and LV diastolic stiffness were also improved (shortened Tau_W and lower LVEDP and EDPVR).

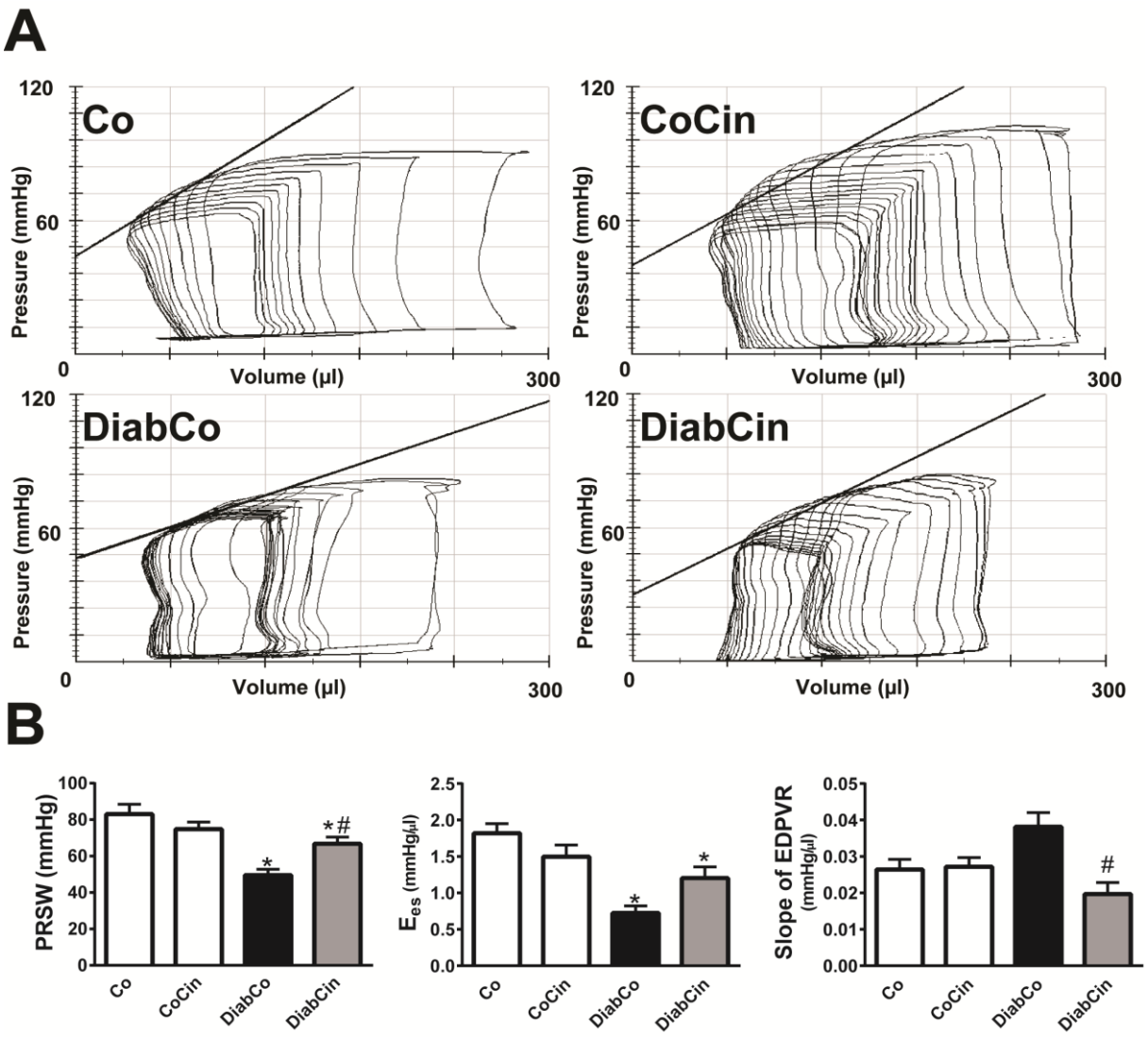

Figure 2. Effect of cinaciguat on cardiac performance in T1DM
(A) Representative pressure-volume (P-V) loops, (B) preload recruitable stroke work (PRSW), slope of LV end-systolic P-V relationship (E_{es}) and the slope of LV end-diastolic P-V relationship (EDPVR) are presented in vehicle-treated control (Co), cinaciguat-treated control (CoCin), vehicle-treated diabetic (DiabCo) and cinaciguat-treated diabetic (DiabCin) groups. *p<0.05 vs. Co, #p<0.05 vs. DiabCo

Effects of vardenafil on T2DM-associated cardiomyopathy

Basic characteristics

The PDE5A inhibitor vardenafil had no effect on BW, HW, HW/BW and on glucose levels.

Nitro-oxidative stress

Vardenafil treatment successfully prevented the T2DM-associated increase of nitro-oxidative stress (indicated by lower LV 3-NT content, lower gene expression of catalase and thioredoxin-1) in the LV of ZDF rats.

Cardiac hypertrophy and fibrosis

Application of vardenafil had anti-hypertrophic and anti-fibrotic effects on diabetic cardiomyopathy in ZDF rats. Namely, echocardiography markers of myocardial hypertrophy LVAWs and LVAWd were significantly lower in vardenafil-treated ZDF rats, while ANF gene expression and cardiomyocyte diameter/TL were also reduced in ZDF+Vard animals when compared to ZDF rats. Furthermore, vardenafil showed anti-fibrotic properties as it decreased both MT score and Picrosirius positive area and fibronectin gene expression value in ZDF rats.

DNA injury and apoptosis

The T2DM-induced elevation of TUNEL positive cardiomyocyte nuclei, cleaved caspase-3 and cleaved PARP1 levels in the LV of ZDF rats were successfully prevented by chronic application of vardenafil.

Alterations of the NO-sGC-cGMP-PKG signaling

Although vardenafil had no effect on plasma total nitrite/nitrate levels, it effectively restored the activation of the NO-sGC-cGMP-PKG signaling pathway by increasing plasma/cardiac cGMP levels, by lowering the protein amount of PDE5A and restoring the activity of PKG (restored p-VASP/VASP ratio). In spite of that, p-PLB/PLB ratio was not altered among the groups.

In vivo and in vitro cardiac function

T2DM was associated with diastolic dysfunction characterized by increased myocardial stiffness (steeper slope of EDPVR) (Figure 3A, B) and prolonged Tau_W. In contrast, systolic function was preserved (Figure 3A, C). Accordingly, in vitro cardiomyocyte function was

characterized by increased cardiomyocyte stiffness (elevated $F_{passive}$) while F_{max} did not change. Diabetic cardiomyopathy-associated severe diastolic dysfunction was effectively prevented by the application of vardenafil as the slope of EDPVR significantly improved in

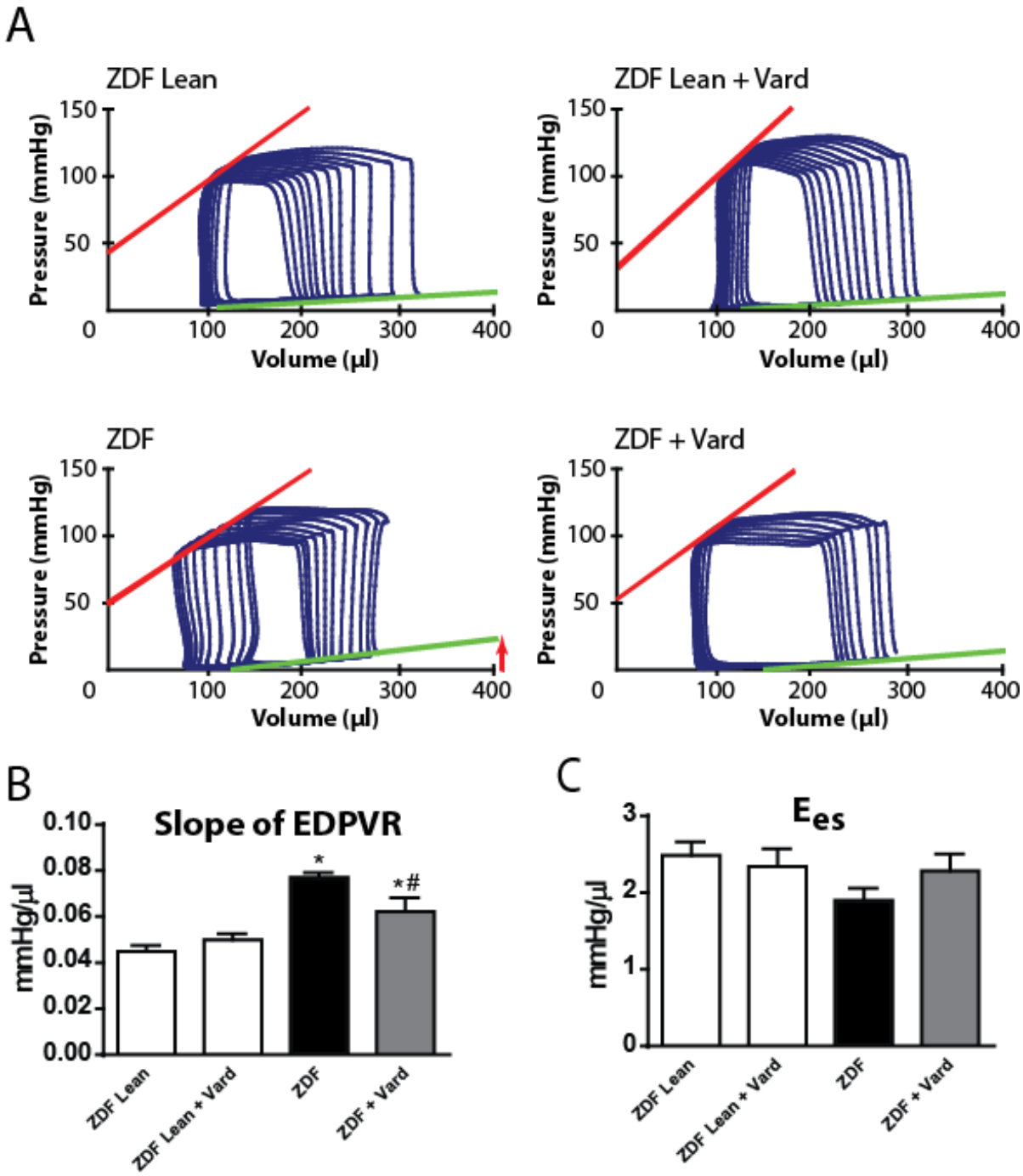

Figure 3. Effects of vardenafil on in vivo and in vitro myocardial function in T2DM
(A) Representative left ventricular (LV) pressure-volume (P-V) loops. (B) Slope of end-diastolic P-V relationship (EDPVR). (C) Slope (E_{es}) of the LV end-systolic P-V relationships. The arrow indicates the increase of EDPVR (a marker of cardiac stiffness) in ZDF. Groups: vehicle-treated controls (ZDFLean), vardenafil-treated controls (ZDFLean+Vard), vehicle-treated diabetic (ZDF) and vardenafil-treated diabetic (ZDF+Vard). *p< 0.05 vs. ZDFLean; #p< 0.05 vs. ZDF

ZDF rats (Figure 3A, B). Moreover, $F_{passive}$, a marker of cardiomyocyte stiffness, improved at different sarcomere lengths. It is notable, that the slope of EDPVR (diastolic stiffness) robustly correlated with the extent of myocardial hypertrophy and fibrosis in ZDF rats.

Conclusions

In the first project, we provided for the first time a detailed characterization and direct comparison of diabetic cardiomyopathy in T1DM and T2DM animal models. We have found that there are characteristic differences between the two types of DM in the development and properties of diabetic cardiomyopathy. Accordingly, different characteristics of myocardial dysfunction have been observed in T1DM and T2DM. T1DM was associated with overt systolic dysfunction indicated by the impairment of classical systolic parameters and sensitive, load-independent contractility indices. Moreover, active relaxation was deteriorated in type 1 diabetic animals. On the contrary, systolic function was preserved in diabetic cardiomyopathy in type 2 diabetic animals while severe diastolic dysfunction (impaired LV active relaxation, increased LV stiffness) developed. We have found an altered pattern of key pathophysiological features (nitro-oxidative stress, cardiac hypertrophy, fibrosis and cardiomyocyte apoptosis) in the diabetic heart. The NO-sGC-cGMP-PKG signaling pathway, have been shown to be severly deteriorated with the loss of its activity in the myocardium of both diabetic animal models.

The second investigation focused on the effects of the sGC activator cinaciguat in T1DM-associated diabetic cardiomyopathy. In this study, we have shown that pathological features of diabetic cardiomyopathy can be improved (nitro-oxidative stress, fibrosis, hypertrophy and apoptosis) by the application of cinaciguat, without affecting blood glucose levels. The anti-oxidative, anti-fibrotic, anti-hypertrophic and anti-apoptotic effects might have been mediated via the reactivation of the NO-sGC-cGMP-PKG axis (indicated by increased plasma and cardiac cGMP levels and restored p-VASP/VASP ratio). Consequently, a significant improvement of cardiac function in T1DM could be observed.

In the third study, effects of vardenafil treatment on the development of diabetic cardiomyopathy in T2DM have been investigated. Our study revealed the potential cardioprotective effects (anti-fibrotic, anti-oxidative, anti-apoptotic and anti-hypertrophic) of the PDE5A inhibitor vardenafil in T2DM-associated cardiomyopathy. We have shown that vardenafil, via the increase of plasma/cardiac cGMP and subsequent reactivation of the NO-sGC-cGMP-PKG pathway (restored p-VASP/VASP ratio), is able improve T2DM-associated diabetic cardiomyopathy and it preserves physiological diastolic function in ZDF rats.

Based on our results, the above modulators of the NO-sGC-cGMP-PKG pathway might become important therapeutic alternatives in the treatment of diabetic cardiomyopathy.

Bibliography of the candidate's publications

Publications related to the dissertation

Mátyás C, Németh BT, Oláh A, Török M, Ruppert M, Kellermayer D, Barta BA, Szabó G, Kökény G, Horváth EM, Beáta Bódi, Papp Z, Merkely B, Radovits T. (2017) Prevention of the development of heart failure with preserved ejection fraction (HFpEF) by the phosphodiesterase-5A inhibitor vardenafil in type-2 diabetic rats. Eur J Heart Fail, 19(3): 326-336.
IF: 6.968 (2016)

Mátyás C, Németh BT, Oláh A, Hidi L, Birtalan E, Kellermayer D, Ruppert M, Korkmaz-Icöz S, Kökény G, Horváth EM, Szabó G, Merkely B, Radovits T. (2015) The soluble guanylate cyclase activator cinaciguat prevents cardiac dysfunction in a rat model of type-1 diabetes mellitus. Cardiovasc Diabetol, 14: 145.
IF: 4.534

Radovits T, Korkmaz S, Mátyás C, Oláh A, Németh BT, Páli S, Hirschberg K, Zubarevich A, Gwanmesia PN, Li S, Loganathan S, Barnucz E, Merkely B, Szabó G. (2015) An altered pattern of myocardial histopathological and molecular changes underlies the different characteristics of type-1 and type-2 diabetic cardiac dysfunction. J Diabetes Res, 2015: 728741.
IF: 2.431

Mátyás Cs, Barta BA, Németh BT, Oláh A, Hidi L, Birtalan E, Kellermayer D, Ruppert M, Korkmaz-Icöz S, Kökény G, Horváth EM, Szabó G, Merkely B, Radovits T. (2017) The soluble guanylate cyclase activator cinaciguat prevents cardiac dysfunction in a rat model of type-1 diabetes mellitus. Cardiol Hung, 47(1): 34-45.

Mátyás Cs, Sayour AA, Korkmaz-Icöz S, Oláh A, Németh BT, Páli Sz, Hirschberg K, Zubarevich A, Gwanmesia PN, Li S, Loganathan S, Barnucz E, Merkely B, Szabó G, Radovits T. (2017) An altered pattern of myocardial histopathological and molecular changes underlies the different characteristics of type-1 and type-2 diabetic cardiac dysfunction. Cardiol Hung, 47(2): 102-111.

Publications not related to the dissertation

Varga ZV, Matyas C, Erdelyi K, Cinar R, Nieri D, Chicca A, Nemeth BT, Paloczi J, Lajtos T, Corey L, Hasko G, Gao B, Kunos G, Gertsch J, Pacher P. (2017) β-Caryophyllene protects against alcoholic steatohepatitis by attenuating inflammation and metabolic dysregulation in mice. Br J Pharmacol, doi: 10.1111/bph.13722.
IF: 5.491 (2016)

Benke K, Sayour AA, Mátyás C, Ágg B, Németh BT, Oláh A, Ruppert M, Hartyánszky I, Szabolcs Z, Radovits T, Merkely B, Szabó G. (2017) Heterotopic Abdominal Rat Heart Transplantation as a Model to Investigate Volume Dependency of Myocardial Remodeling. Transplantation, 101(3): 498-505.
IF: 3.678 (2016)

Németh BT, Mátyás C, Oláh A, Lux Á, Hidi L, Ruppert M, Kellermayer D, Kökény G, Szabó G, Merkely B, Radovits T. (2016) Cinaciguat prevents the development of pathologic hypertrophy in a rat model of left ventricular pressure overload. Sci Rep, 6: 37166.
IF: 4.259

Oláh A, Kellermayer D, Mátyás C, Németh BT, Lux Á, Szabó L, Török M, Ruppert M, Meltzer A, Sayour AA, Benke K, Hartyánszky I, Merkely B, Radovits T. (2017) Complete Reversion of Cardiac Functional Adaptation Induced by Exercise Training. Med Sci Sports Exerc, 49(3): 420-429.
IF: 4.141 (2016)

Koncsos G, Varga ZV, Baranyai T, Boengler K, Rohrbach S, Li L, Schluter KD, Schreckenberg R, Radovits T, Oláh A, Mátyás C, Lux Á, Al-Khrasani M, Komlódi T, Bukosza N, Máthé D, Deres L, Barteková M, Rajtík T, Adameová A, Szigeti K, Hamar P, Helyes Z, Tretter L, Pacher P, Merkely B, Giricz Z, Schulz R, Ferdinandy P. (2016) Diastolic dysfunction in prediabetic male rats: role of mitochondrial oxidative stress. Am J Physiol Heart Circ Physiol, 311(4): H927-943.
IF: 3.348

Ruppert M, Korkmaz-Icöz S, Li S, Németh BT, Hegedüs P, Brlecic P, Mátyás C, Zorn M, Merkely B, Karck M, Radovits T, Szabó G. (2016) Myocardial reverse remodeling after pressure unloading is associated with maintained cardiac mechanoenergetics in a rat model of left ventricular hypertrophy. Am J Physiol Heart Circ Physiol, 311(3): H592-603.
IF: 3.348

Matyas C, Varga ZV, Mukhopadhyay P, Paloczi J, Lajtos T, Erdelyi K, Nemeth BT, Nan M, Hasko G, Gao B, Pacher P. (2016) Chronic plus binge ethanol feeding induces myocardial oxidative stress, mitochondrial and cardiovascular dysfunction and steatosis. Am J Physiol Heart Circ Physiol, 310(11): H1658-1670.
IF: 3.348

Lee WS*, Erdelyi K*, Matyas C*, Mukhopadhyay P, Varga ZV, Liaudet L, Haskó G, Čiháková D, Mechoulam R, Pacher P. (2016) Cannabidiol limits Tcell-mediated chronic autoimmune myocarditis: implications to autoimmune disorders and organ transplantation. Mol Med, 22: 136-146.
IF: 3.457
*Equal contribution

Oláh A, Németh BT, Mátyás C, Hidi L, Lux Á, Ruppert M, Kellermayer D, Sayour AA, Szabo L, Torok M, Meltzer A, Gellér L, Merkely B, Radovits T. (2016) Physiological and pathological left ventricular hypertrophy of comparable degree is associated with characteristic differences of in vivo hemodynamics. Am J Physiol Heart Circ Physiol, 310(5): H587-597.
IF: 3.348

Kovacs A, Oláh A, Lux Á, Mátyás C, Németh BT, Kellermayer D, Ruppert M, Torok M, Szabo L, Meltzer A, Assabiny A, Birtalan E, Merkely B, Radovits T. (2015) Strain and strain rate by speckle tracking echocardiography correlate with pressure-volume loop derived contractility indices in a rat model of athlete's heart. Am J Physiol Heart Circ Physiol, 308(7): H743-748.
IF: 3.324

Oláh A, Németh BT, Mátyás C, Horváth EM, Hidi L, Birtalan E, Kellermayer D, Ruppert M, Merkely G, Szabó G, Merkely B, Radovits T. (2015) Cardiac effects of acute exhaustive exercise in a rat model. Int J Cardiol, 182: 258-266.
IF: 4.638

Radovits T, Oláh A, Lux Á, Németh BT, Hidi L, Birtalan E, Kellermayer D, Mátyás C, Szabó G, Merkely B. (2013) Rat model of exercise-induced cardiac hypertrophy - hemodynamic characterization using left ventricular pressure-volume analysis. Am J Physiol Heart Circ Physiol, 305(1): H124-134.
IF: 4.012

Benke K, Sayour AA, Ágg B, Radovits T, Szilveszter B, Odler B, Németh BT, Pólos M, Oláh A, Mátyás C, Ruppert M, Hartyánszky I, Maurovich-Horvat P, Merkely B, Szabolcs Z. (2016) Gene polymorphisms as risk factors for predicting the cardiovascular manifestations in Marfan syndrome. Cardiol Hung, 46(2): 76-81.

Oláh A, Németh BT, Mátyás C, Horváth EM, Hidi L, Birtalan E, Kellermayer D, Ruppert M, Gellér L, Szabó G, Merkely B, Radovits T. (2016) Cardiac effects of acute exhaustive exercise in a rat model. Cardiol Hung, 46(1): 1-9.

Oláh A, Lux Á, Németh BT, Hidi L, Birtalan E, Kellermayer D, Mátyás C, Ruppert M, Merkely G, Szabó G, Merkely B, Radovits T. (2013) Detailed hemodynamic characterization of athlete's heart – Left ventricular pressure-volume analysis. Cardiol Hung, 43(5): 224-232.

Radovits T, Mátyás C, Oláh A, Kökény G, Barnucz E, Szabó G, Merkely B. (2012) Effects of phosphodiesterase-5 inhibitor vardenafil on diabetic cardiovascular dysfunction. Cardiol Hung, 42(5): 272-279.