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Purpose: Calcineurin inhibitors (CNI) as cyclosporin A and tacrolimus are the most commonly used immunosuppressive drugs following solid organ transplantation and in various autoimmune diseases. But, CNI therapies are also associated with severe side effects like arterial hypertension, atherosclerosis, insulin resistance, brain, liver and renal complications, and cardio-toxicity. However, the effects of CNI on cardiac contractile function are poorly studied. In this study, we have investigated the acute effect of CNI on cardiac contractile function and the beneficial effects mediated by PI3Kg inhibition.
Methods: The intact heart left ventricle contractility (Pressure-Volume-Conductance) was evaluated in mice 5h after intra-peritoneal injection of CNI in WT, PI3Kg KO (total deletion of PI3Kg), PI3Kg KD ("kinase dead"; lacking PI3Kg kinase function) and in WT treated with selective inhibitor of PI3Kg. Thereafter, cardiomyocytes were studied using several biochemical approaches to investigate the beta adrenergic signaling pathway including PLB activation, cAMP production and phosphodiesterase (PDE) activity. Finally a long term follow up with histological studies in cardiac tissue as well as ECG using telemetry and survival analysis was performed.
Results: We intriguingly found 5h after CNI injection an important reduction (<50%) of cardiac contractility (End-systolic elastance; Ees) that was confirmed in vitro with a down-regulation of beta-adrenergic signaling upon cardiomyocyte stimulation with CNI (4h). Interestingly, these effects were completely absent after the same treatment in transgenic mice and cardiomyocytes (PIKg KO, PIKg KD), and in mice and cardiomyocytes treated simultaneously with PI3Kg selective inhibitor. Following up these findings, we have also observed in cardiac sections (H&E, TUNEL) 15h after CNI, the presence of necrotic cells as well as myofibre break-up indicating a possible sign of ventricular fibrillation. Whereas the observed side effects of CNI were correlated with higher mortality, the group of mice simultaneously treated with PI3Kg inhibitor interestingly exhibited a better survival rate ($p < 0.05$).
Conclusion: In this study, we observed an acute cardio-depressive effect of CNI associated with sudden death and established the rationale for PI3Kg inhibition as a potential therapeutic approach.

P2293

Role of matrix metalloproteinase-2 isoforms in diabetic heart

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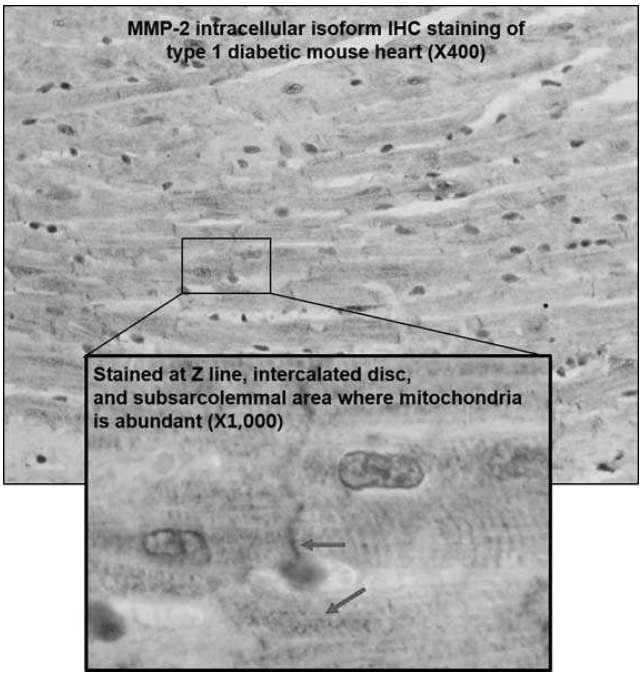
Background: Diabetic cardiomyopathy (DM CMP) is defined as cardiomyocyte damage and ventricular dysfunction in diabetic condition, which is independent of the concomitant coronary artery disease and/or hypertension. Matrix metalloproteinases (MMPs) are reported to account for pathogenesis of DM CMP by increasing myocardial extracellular collagen contents. Recently, intracellular isoforms of MMP-2 were reported. They are usually located at nucleus and mitochondria induced by oxidative stress, and reported to account for cardiac dysfunction through activating innate immunity and apoptosis at intracellular level in ischemic heart model. Purpose: We hypothesized that intracellular isoforms of MMP-2 are also induced by high glucose stimulation, where oxidative stress is also induced. Therefore, we aimed to evaluate the intracellular isoforms of MMP-2 in vivo and in vitro diabetic heart model.
Methods: Rat cardiomyoblast (H9c2 cell) was cultured with 30mM of high glucose concentration for 24 or 48hours. In vivo type 1 diabetic mouse model was made by 40mg/kg of streptozotocin intraperitoneal injection for consequent 5 days. After sacrificing mouse at 12 and 24 weeks, quantitative real-time polymerase chain reaction (qRT-PCR) of isoforms of MMP-2 and innate immunity/apoptotic markers were done as well as pathological analysis including immunohistochemical (IHC) staining. Results: Quantitative RT-PCR and immunofluorescence staining showed that there was expression of intracellular isoforms of MMP-2 in H9c2 cell compared to negative expression in control group. There was no definite histologic change of diabetic cardiomyopathy. For the IHC staining and qRT-PCR, however, there was distinct expression of intracellular isoforms of MMP-2 in diabetic mouse heart. Conclusion: Intracellular isoforms of MMP-2 were induced by high glucose stimulation in in vitro and in vivo diabetic heart model. Further evaluation of its role in diabetic cardiomyopathy should be followed.



MMP-2 intracellular isoform in tissue

P2294

The role of BNP on adipose tissue adaptations promoted by left ventricular chronic pressure overload

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Introduction and aims: The progression of chronic pressure overload (CPO) is associated to cardiac cachexia as a consequence of insufficient energy supply. Additionally, some studies demonstrate that the heart secretes cardiokines able to modulate the adipose tissue (AT) structure and function promoting adiposopathy. In this study we investigate the effects of CPO in AT during the early stages of the disease.
Methods: Wistar rats were submitted to ascending aortic banding (Ba group; 0.6mm diameter) or sham procedure (Sham group). After 8 weeks, left ventricular (LV) function and structure (echocardiography and invasive hemodynamics) was evaluated and samples (LV and AT) collected for histological and molecular evaluations. Plasma was obtained for quantification of circulating B-type natriuretic peptide (BNP). Finally, visceral AT from normal rats was incubated with the BNP plasma concentrations detected in the Sham and Ba group (0.27 and 0.47ng/ml respectively) for 24h and then collected for molecular studies.
Results: Eight-weeks of banding increased LV systolic pressure and triggered cardiac remodeling with fibrosis and cardiomyocytes' hypertrophy when compared to Sham animals. The same group was at a compensated stage of the disease with higher ejection fraction, however a stiffer myocardium was observed with increased end diastolic pressure-volume relation and passive force of isolated cardiomyocytes. Despite similar adiposity between the 2 groups, aortic constriction triggered adipocyte atrophy as well as AT increased fibrosis and dysfunction, as observed by overexpression of pro-inflammatory adipokines. The incubation of AT from normal rats with BNP confirmed that the elevated circulatory levels of this cardiokine were able to induced increased expression of pro-inflammatory adipokines by the AT.
Conclusions: We demonstrated that higher circulatory levels of BNP promoted by LV CPO are able to induce adiposopathy characterized by remodeling of the AT and overexpression of pro-inflammatory adipokines.

	Sham	Ba
LV systolic pressure (mmHg)	110 ± 3.6	153 ± 10.5 *
Heart/tibial length (g/cm)	2.3 ± 0.05	3.3 ± 0.30 *
LV cardiomyocyte cross-sectional area (μm²)	382 ± 23.6	484 ± 33.6 *
LV fibrosis (%)	4.2 ± 0.52	6.3 ± 0.94 *
LV ejection fraction (%)	78 ± 0.9	89 ± 1.9 *
LV end diastolic pressure volume relationship	0.04 ± 0.006	0.11 ± 0.031*
LV passive force at 2.2μm (mN/mm²)	3.3 ± 0.29	4.4 ± 0.57
Plasma BNP (ng/ml)	0.27 ± 0.048	0.47 ± 0.080 *
AT/tibial length (g/cm)	7.9 ± 0.88	7.5 ± 0.25
AT fibrosis (%)	7.2±0.31	8.7±0.61 *
Adipocyte CSA (μm²)	1659±103.8	1287±85.1 *
AT TNFα (AU)	0.03±0.013	0.06± 0.018 *
AT IL-1β (AU)	0.04 ± 0.01	0.28 ± 0.15 *
TNFα (AU) after incubation with BNP	26380 ± 1428	31125 ± 1455 *
IL-1β (AU) after incubation with BNP	9038 ± 678	12221 ± 1086 *

Data presented as mean± SEM. * p<0.05 vs Sham.

Table

P2295

Effects of ranolazine in a model of doxorubicin-induced left ventricle diastolic dysfunction

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Background: Doxorubicin (DOXO) is a highly effective anticancer drug but its clinical application is impeded by cardiotoxicity. Asymptomatic diastolic dysfunction can be the earliest manifestation of DOXO cardiotoxicity. Therefore, a search for therapeutic intervention that can interfere with early manifestations and possibly prevent late cardiotoxicity is warranted. Increased DOXO-dependent reactive oxygen species may explain, in part, Ca2+ and Na+ accumulation that contributes to diastolic dysfunction and development of heart failure.
Purpose: We tested whether the administration of ranolazine (RAN), an anti-anginal drug, immediately after completing DOXO therapy, can affect diastolic dysfunction and interfere with the progression of functional decline.
Methods: Fischer 344 rats received a DOXO cumulative dose of 15 mg/kg over a period of 2 weeks. After the assessment of diastolic dysfunction, the animals were administered with RAN (80 mg/kg/die) for the following 4 weeks.
Results: While diastolic and systolic function progressively deteriorated in DOXO-treated animals, treatment with RAN relieved diastolic dysfunction and prevented worsening of systolic function decreasing mortality. RAN lowered myocardial NADPH oxidase 2 expression and 3-nitrotyrosine content. A reduced NCX and Nav 1.5 expression and an increment of SERCA2 were also detected. In addition, RAN lowered DOXO-induced increased phosphorylation and oxidation of Ca2+/calmodulin-dependent protein kinase II and decreased fibrosis.
Conclusions: RAN, by modulating cardiac Ca2+ and Na+ handling proteins and oxidative stress, was effective in attenuating DOXO-induced diastolic dysfunction and prevented the progression of cardiomyopathy.

P2296

Allogeneic amniotic membrane-derived mesenchymal stem cell therapy is cardioprotective, restores myocardial function, and improves survival in a model of anthracycline-induced cardiomyopathy

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Introduction: Recent data suggests that anthracycline induced cardiomyopathy (AICM) is a stem cell disease affecting the local (myocardial) and systemic stem cell niches, which limits autologous stem cell therapy in this condition. We hypothesized that an allogeneic stem cell transplant approach using amniotic membrane-derived mesenchymal stem cells (AM-MSC) could be an effective therapy for this condition. Using a model of AICM, we evaluated the effects on ventricular systolic and diastolic function as well as survival, of AM-MSC therapy administered via intravenous route (IV), compared to administration via percutaneous contrast echocardiography-guided intramyocardial injection (IMI).
Methods: Cardiomyopathy was induced in New Zealand rabbits with IV daunorubicin (4 mg/kg, weekly for 6 weeks). One group received IV therapy with 5 million/kg AM-MSC (IV Group, n=8), 24 hours after each weekly cycle of daunorubicin. A second group received one dose of 5 million AM-MSC, 8 weeks after the first dose of anthracycline, via contrast echocardiography-guided percutaneous IMI (IMI Group, n=8). A third group received no cell therapy (AICM Group, n=8). A final group neither received daunorubicin nor cell therapy, thus constituting an age-matched Control Group (n = 8). A complete echocardiographic exam was performed at baseline and repeated every two weeks.
Results: In all groups, ventricular systolic and diastolic function was normal at baseline (Figure 1A-B). The IV group did not exhibit significant alterations of myocardial function at 8 weeks, consistent with a cardioprotective effect (Figure 1A-B). In contrast, significant alterations of both systolic and diastolic function by daunorubicin were observed in IMI and AICM groups at 8 weeks, consistent with the development AICM. Whilst ventricular systolic function significantly improved at 10 and 12 week time points in IMI group, it continued to deteriorate in AICM group. Of note, significant alterations in myocardial function were apparent in IV group after 10 weeks, but significantly less conspicuous than in AICM group, suggesting delayed-onset cardiotoxicity. Survival in rabbits from IMI and IV groups was improved (77% and 75%, respectively) compared to AICM group (22%) ($p < 0.04$).
Conclusions: The observation of early cardioprotection from AICM in IV group at 8 weeks, with later decline of LVEF from 10 weeks suggests that anthracyclines induced delayed-onset cardiotoxicity, which affected, at least in part, endogenous as well as exogenous stem cells, thus hindering long term cardioprotection. The significant recovery of LVEF at 10 and 12 weeks in the IMI group, suggest that this is an effective therapy once cardiomyopathy ensues. Further studies with an extended time course of IV administration of allogeneic AM-MSC are required to evaluate whether this therapy may confer prolonged cardioprotection from AICM, as well as to elucidate the mechanisms of delayed-onset cardiotoxicity observed in IV group.

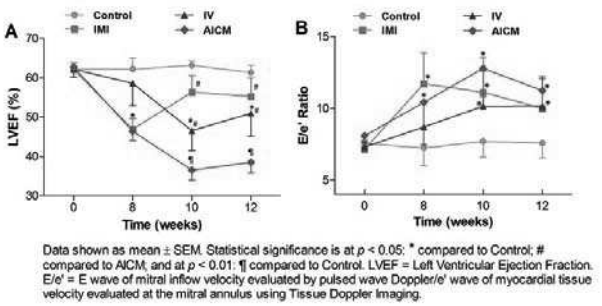

Figure 1

P2297

MicroRNA-155 promotes LPS-induced myocardial NO overproduction and amplifies cGMP-PKG signaling pathway by targeting CD47

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Purpose: Sepsis-associated cardiovascular dysfunction (SACVD) remains a leading cause of death in critically ill patients. Among several pathophysiological mechanisms, excessive NO production and signaling have been shown to negatively affect cardiac contractility and vascular reactivity. CD47, a transmembrane receptor for TSP1, mediates a potent inhibitory effect on NO-cGMP-PKG signaling pathway and its role in SACVD remains unknown.
Methods: Protocol 1: Experimental sepsis was induced using LPS injection (40mg/Kg, ip) in C57BL/6J (WT; n=40) and miR-155-/- (KO; n=40) male mice. Cardiac function was evaluated through echocardiography. Myocardial water content and microvascular permeability (Evans blue method) were assessed. Myocardial NO, cGMP levels and PKG activity were evaluated. Quantification of miR-155, CD47, NOS2, NOS3 and phospho-VASP (Ser239) were assessed by immunoblotting (this