ANAKINRA IMPROVES EXERCISE PEAK AEROBIC CAPACITY IN PATIENTS WITH RECENTLY DECOMPENSATED SYSTOLIC HEART FAILURE

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Objective: To evaluate the effects of anakinra on aerobic exercise capacity in patients with recently decompensated systolic HF and systemic inflammation.

Methods: We randomly assigned 60 patients with reduced left ventricular ejection fraction (<50%) and elevated C-reactive protein (CRP) levels (>2 mg/L), within 14 days of hospital discharge, to daily subcutaneous injections with anakinra 100 mg for 2 weeks, 12 weeks, or placebo in the REcently Decompensated Heart failure Anakinra Response Trial (REDHART) clinical trial (*www.clinicaltrials.gov* identifier**:** NCT01936909). Patients underwent measurement of peak oxygen consumption (VO2 [mL•kg-1•min-1]) and ventilatory efficiency (the VE/VCO2 slope) at 2, 4, 12, and 24 weeks.

Results: Treatment with anakinra did not affect peak VO2 or VE/VCO2 slope at 2 weeks. At 12 weeks, anakinra treatment for 12 weeks reduced CRP levels by >60% (P<0.01) and improved peak VO2 from 14.5 [10.5-16.6] to 16.1 [13.2-18.6] mL•kg-1•min-1 (P=0.008), whereas treatment with anakinra for 2 weeks or placebo had no significant effect on CRP levels or peak VO2. The incidence of death or readmission for HF at 24 weeks was 6%, 31%, and 30%, in the anakinra 12-week, anakinra 2-week and placebo groups, respectively.

Conclusion: Treatment with anakinra for 12 weeks improved aerobic capacity in patients with recently decompensated systolic HF, while treatment for 2 weeks did not.

C SUBUNIT OF F1/FO-ATP SYNTHASE AS TARGET FOR PREVENTING THE DETRIMENTAL EFFECT OF MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

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Objective: The pathophysiological effects of coronary heart diseases are imputable to the hurtful consequences of ischemia-reperfusion injury (IRI). The opening of a large pore in the mitochondrial membrane, namely, the mitochondrial permeability transition pore (mPTP), is widely recognized as the final step of IRI and is responsible for mitochondrial and cardiomyocyte death. We provided evidences that c subunit of the F1/FO-ATP synthase (FFAS) owns a pivotal role in mPTP formation and activity and thus we sought to test a new mPTP opening inhibitor directed against the c subunit, namely IB13, for the treatment of IRI in *ex-vivo* model of myocardial infarction.

Methods: We first synthesized and explored the potential activity of a small-molecule analogue of oligomycin A, IB13, compound that has been identified as mPTP inhibitor. After proving its ability to accumulate selectively into mitochondria and to inhibit the mPTP activity by binding the c subunit of FFAS, we tested its cardioprotective effect in an *ex-vivo* model of reperfusion injury. We isolated

rat beating hearts and placed them in a Langendorff system. The *ex-vivo* IRI protocol included 20 min stabilization of the heart, then retrograde perfusion was progressively stopped to induce 30 min of global ischemia, followed by 1 hour of reperfusion. IB13 was administered at reperfusion time during the first 10 minutes of reflow. At the end of the procedure, hearts were analyzed for cell death by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assays.
Results and Conclusions: In isolated hearts, perfusion at constant volume of IB13 10μM resulted in a decrease in coronary perfusion pressure (CPP, -17.5 ± 3.4 %) and in end-diastolic pressure (EDP, -72 ± 9.86 %) with an increased left ventricle developed pressure (LVDP, +36.4 ± 3.9 %) that mark a reduction of the diastolic stiffness, vasoconstriction and the deterioration of myocardial performance. In control conditions, 64% of cardiomyocytes were TUNEL positive, a percentage that was significantly reduced in the presence of IB13 10μM. These findings confirmed the ability of IB13 to inhibit mPTP opening *ex-vivo* and to limit the detrimental effect of myocardial IRI.

THE HUMAN AMNIOTIC FLUID STEM CELL SECRETOME AS NEW PARACRINE SOURCE TO UNLOCK ENDOGENOUS CARDIAC REGENERATION

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Objective: Cardiovascular disease is mainly related to inefficient cardioprotection, defective repair and lack of myocardial renewal. However, recent work demonstrated that the adult mammalian heart is not completely devoid of regenerative capability since harbouring an endogenous restorative program based on cardiac progenitor cell (CPC) activation and cardiomyocyte proliferation. Nevertheless, these mechanisms are not efficiently active in the adult heart when facing pathological situations, such as myocardial infarction (MI). Stem cells have been broadly scrutinized for therapeutic approach with increasing attention towards the paracrine modulatory influence of their secretome. Human amniotic fluid-derived stem cells (hAFS) showed to exert significant cardioprotective effect on rat ischemic myocardium and murine and human cardiac cells undergoing cardiotoxic injury. Here we aim at analysing more in details the hAFS secretome paracrine potential in optimizing cardiac repair and triggering cardiac regeneration.
Methods: c-KIT+ hAFS were isolated from leftover samples of II trimester amniotic fluid for prenatal screening and stimulated in vitro for 24h to enhance the release of paracrine factors in their conditioned medium (hAFS-CM) under 1% O2 preconditioning (hAFS-CMHypo) versus control condition (hAFS-CMNormo). The key properties related to cardiac repair (i.e. pro-survival and angiogenic effects) and to specific regenerative aspects (CPC and cardiomyocytes proliferation) were assessed by stimulating mouse neonatal ventricular cardiomyocytes (mNVCM), human endothelial colony forming cells (hECFC) and human CPC (hCPC) with hAFS-CM. A preclinical mouse model of MI was used for further in vivo validation.
Results: hAFS-CM exerted significant anti-apoptotic effect on mNVCM undergoing oxidative and hypoxic damage, with the hAFS-CMHypo being particularly effective under ischemic condition. hAFSCMHypo was able to induce remarkable intracellular Ca2+ signals in hECFC and to trigger the proliferation of mNVCM and several populations of hCPC. Preliminary in vivo data showed that intra-myocardial injection of hAFS-CMHypo soon after MI provided substantial cardioprotection, curbed down inflammation in the short term, while sustaining angiogenesis and noticeably counteracting remodelling after 2 weeks. Profiling of the hAFS-CMHypo over the hAFS-CM Normo

identified specific cytokines as putative molecular candidates of the regenerative effects observed.
Conclusions: These encouraging findings suggest the hAFS secretome as an appealing source of paracrine factors for the development of a future medicinal advanced product for the reactivation of an endogenous regenerative program within the heart.

INDOXYL SULPHATE ACTIVATES CARDIAC FIBROBLASTS WITH ENHANCED COLLAGEN SYNTHESIS, UPREGULATED ANGIOTENSIN-NEPRILYSIN SYSTEM, AND PARACRINE INDUCTION OF CARDIOMYOCYTE HYPERTROPHY

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Objective: Left ventricular hypertrophy (LVH) is common in chronic kidney disease (CKD). Cardiac fibroblast (Fib)-driven myocardial fibrosis is being recognized as important as cardiomyocyte hypertrophy in CKD-related LVH, but the factors triggering it are only partially known. Here, we investigated whether a role may be played by indoxyl sulphate (IS), a tryptophan metabolite that accumulates in CKD since early stages.
Methods: Neonatal mouse Fib were treated with 50 μM IS, a concentration found in moderate CKD, with or without the IS antagonist, CH-223191. Oxidative stress was evaluated by using the CellROX assay, proliferation by BrdU incorporation and flow cytometry for CFSE, α-smooth muscle actin (αSMA), selected paracrine mediators, and the neprilysin-angiotensin (Ang) axis by RT-PCR, western blotting and/or immunocytochemistry, and collagen production by RT-PCR and picrosirius red staining. After incubating neonatal mouse ventricular cardiomyocytes (mNVCM) with the conditioned medium of control or IS-treated Fib, β-myosin heavy chain (β-MHC) and atrial and B-type natriuretic peptide expression was analyzed by RT-PCR and glycolysis by enzymatic assays. Adult C57BL/6 mice were given drinking water with or without IS (1 mg/mL) for 12 weeks, after which cardiac histology and markers of Fib activation and cardiomyocyte hypertrophy were examined.
Results: IS enhanced Fib proliferation, αSMA immunopositivity and collagen expression and synthesis. Moreover, it increased the levels of TNF-α and myostatin, an emerging mediator of IS action. The genes encoding angiotensinogen, Ang-converting enzyme, and Ang receptor type 1 were also upregulated by IS, as well as the gene for neprilysin, an endopeptidase that cleaves AngII and – mostly – natriuretic peptides. All these effects were counteracted by CH-223191. Compared with control cells, mNVCM incubated with the conditioned medium of IS-primed Fib exhibited higher levels of β-MHC and heightened activity of glycolytic enzymes, suggesting cellular hypertrophy. The hearts of mice treated with IS displayed histological signs of cardiomyocyte hypertrophy and initial interstitial fibrosis. At the time of this abstract, we have also observed in the lysates of IS-exposed hearts an increase in the majority of the markers of Fib activation evaluated in vitro, as well as heightened β-MHC and natriuretic peptide expression and glycolysis.
Conclusions: IS alone may contribute to CKD-associated LVH by eliciting Fib activation with collagen production, induction of a cell-autonomous Ang system, and release of paracrine factors promoting cardiomyocyte hypertrophy.

ENDOTHELIAL DYSFUNCTION AND NUTRACEUTICAL APPROACHES: *IN VITRO* SYSTEM TO STUDY THE MODULATION OF METABOLIC MARKERS ON HUMAN AORTIC ENDOTHELIAL CELLS.

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Objective: Endothelial dysfunction (ED) includes both microvascular and macrovascular complications and is considered a hallmark in the patho-physiology of metabolic syndrome (MetS). ED is characterized by impaired endothelium-dependent relaxation, excessive cytokines production, release of inflammatory factors and oxidative stress. The aim of this work is to setup experimental strategies to study *in vitro* macrovascular endothelial damages related to MetS: the same protocols will be used to test the role of natural compounds with nutraceutical interest (Berberine (BBR) and red yeast rice (RYR)).
Methods: Endothelial complications associated to MetS were assessed treating human aortic endothelial cells (HAEC) with uric acids (UA), leptin and low density lipoproteins (LDL). We analyzed the modulation of some metabolic and biochemical markers (lipid peroxidation, cholesterol determination, ROS production) or ICAM-I and Carnitine Palmitoyltransferase 2 (CPT2) production. The *in vitro* model was used to quantitatively characterize the modulation of these markers by natural compounds usually introduced with diet (BBR and RYR).
Results: UA (6, 9 and 12 mg/dl) treatment reduced HAEC viability, increased ROS production and modified ICAM-I expression. The incubation with Leptin (100 ng/ml) increased CPT2 expression (24 hrs). Moreover, LDL (200 ng/ml) promoted lipid peroxidation and altered cholesterol production. BBR and RYR differently affected the markers. Incubation of HAEC with BBR modified the response to both UA and Leptin, and counteracted ROS production, lipid peroxidation and ICAM-1 or CPT2 expression. RYR was less effective then BBR.
Conclusions: In this work we developed a new simplified *in vitro* system suitable to evaluate ED associated to MetS. Moreover, the same system was used to test the role of natural compounds with nutraceutical applications to evaluate their possible preventive effect on ED development.

GENERATION OF DESMOPLAKIN ZEBRAFISH MODELS FOR ARRHYTHMOGENIC CARDIOMYOPATHY AS SUITABLE SYSTEMS FOR THE IDENTIFICATION OF EARLY PATHOGENIC EVENTS AND NEW THERAPEUTIC TARGETS

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Objective: Arrhythmogenic cardiomyopathy (AC) is a heritable form of cardiomyopathy characterized by fibrofatty replacement which leads to right ventricular failure, arrhythmias, and sudden cardiac death, particularly in young patients and athletes. In spite of the recent discovery of genes whose mutations cause ACs, early molecular events leading to cell death and arrhythmias remain elusive. In the present study, we evaluate *in vivo* the pathogenic mechanisms of Desmoplakin (DSP) dysfunction, linked to the AC8 form, using zebrafish as a promising model for this life-threatening arrhythmic disorder.
Purpose: The aim of the study is the generation of transient AC8 zebrafish models, using an antisense knock-down strategy, and a stable zebrafish Dsp mutant, for subsequent structural and functional characterization. In addition, by exploiting zebrafish pathway reporter lines, we aim to study cell signaling alterations potentially involved in AC8 pathogenesis. The final goal is the assessment of our zebrafish AC8 models as a suitable tool for pathway-directed drug screening.
Methods: A morpholino (MO)-based antisense strategy was used to obtain the knockdown of zebrafish *dspa* and *dspb* genes, both orthologous to human DSP. Moreover, we have analyzed a zebrafish Desmoplakin a (*dspa*) mutant line (sa13356), obtained by ENU-induced mutagenesis. AC8 zebrafish models were morphologically characterized and, subsequently, functionally tested for alterations in different signaling pathways.

Results: Knock-down of both *dspa* and *dspb* and homozygous *dspa* -/- mutant embryos show a general delay in the development, microcephaly, bradycardia, arrhythmias, pericardial effusion and altered heart rate. During early adulthood, *dspa* mutant fish exhibit mild bradycardia, cardiomegaly and peripheral effusion. TEM analysis of zebrafish tissues shows highly reduced and disorganized desmososmes, resembling "pale" desmosomes identified in endomyocardial biopsies from AC patients. Moreover, the analysis of signaling pathways detects a cardiac-specific reduction of Wnt signaling responsiveness in all AC8 models, confirming previous evidences that DSP suppression leads to a reduction of canonical Wnt signaling.
Conclusions: Our AC8 transient and stable zebrafish models are able to recapitulate some AC features, pointing to zebrafish as a suitable model for the *in vivo* screening of molecularly-targeted drugs. Moreover, confirmation of the reduction in canonical Wnt signaling due to DSP mutations suggests that this pathway could be a general mechanism involved in the pathogenesis of desmosomal-associated AC forms, and, thus, a promising target for AC therapeutic intervention.

TRP EXPRESSION SIGNATURE IN TUMOR-DERIVED ENDOTHELIAL CELLS: FUNCTIONAL ROLES IN PROSTATE CANCER ANGIOGENESIS

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Objective: TRP channels play a key role in cancer progression, modulating cell proliferation and survival, cancer invasion of surrounding tissues and angiogenesis. TRP expression could therefore characterize the prostate cancer (PCa) cell phenotype. Another well-established concept is that TRPs deeply modulate endothelial cell (EC) biology and tumor angiogenesis. However, a specific TRP expression signature of PCa angiogenesis is still lacking. Our aim was therefore to define a TRP expression signature during PCa angiogenesis providing novel therapeutic targets.
Methods: By means of a qPCR screening and Western blotting, as well as immunohistochemistry, we fully profiled the expression of all TRPs in normal ECs and tumor endothelial cells (TECs) derived from PCa, as well as from breast and renal tumors. Moreover, we characterized the role of the 'prostate specific' TRPs in the modulation of EC biological processes such as cell proliferation, motility and ability to form tubules *in vitro*, as well as *in vivo* angiogenesis.
Results: We identified four 'prostate specific' trp genes whose expression is deregulated in PCa-derived ECs compared to their healthy counterpart. We specifically characterized the role of each TRP channel in both *in vitro* and *in vivo* angiogenesis, EC proliferation and migration as well as their role in PCa cell attraction by TECs.
Conclusions: Taken together, our results propose novel molecular players to selectively target PCa progression and angiogenesis. Indeed, our expression profiling and functional data could explain the transition of prostate endothelial cells to their aggressive tumor phenotype.

CARDIAC DYSFUNCTION AFTER MYOCARDIAL INFARCTION: ROLE OF PRO-INFLAMMATORY EXTRACELLULAR VESICLES.

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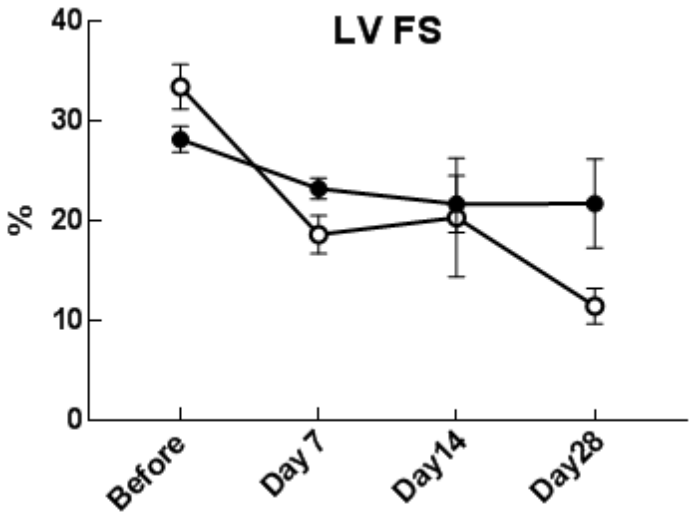
Objective: cardiac repair after myocardial infarction (MI) is a complex series of events initiated by an important inflammatory phase that serve to remove damaged cells followed by a reparative step. Recent studies have shown that extracellular vesicles (EVs) released from activated macrophages are pro-inflammatory. We asked whether EVs secreted by macrophages associated with pro-inflammatory phenotype (M1MΦ) and by macrophages associated with healing phenotype (M2MΦ) differently act in inducing cytotoxic effect on cardiomyocytes (CM).

Method: Human monocytes were isolated from buffy coats of healthy volunteers. Macrophages were obtained after culturing monocyte for about 10 days in presence of M-CSF and polarized to M1MΦ and M2MΦ by using different combinations of cytokines. After polarization, M1MΦ and M2MΦ profiles were analyzed by FACS analysis. To test cytotoxic effects of inflammatory EVs, complete or EVs-depleted conditioned medium as well as isolated EVs derived from M1MΦ and M2MΦ were added to the medium of neonatal CM and cell viability was assessed 12 hours after. To in-vivo confirm cytotoxic effect of pro-inflammatory EVs, GW4869 (specific inhibitor of EVs release) or vehicle were injected IP in rats 1 hour before the MI induction, echocardiography analysis was assessed 28 days after MI.

Results: As shown by FACS analysis M1MΦ and M2MΦ expressed specific cell antigens which are not present in naïve monocyte like CD16, CD64 (M1MΦ profile) and CD206, CD163 (M2MΦ profile). M1MΦ-derived conditioned medium was able to induce cell death in CM and the cytotoxic effect decreased with EVs-depleted medium. In-vivo left ventricular ejection fraction (EF%) was comparably reduced at 24 hrs post-MI in both, GW4869 and saline-treated group, but recovered to a greater extent in the first group than in control at 28 days post-MI.

Conclusions: EVs secreted by M1MΦ are able to induce cellular death in neonatal CM. Inhibition of EVs release during the acute phase of MI preserve heart function in an animal model of permanent LAD ligation.



CARDIO-RENAL POSITIVE EFFECTS OF DIPEPTIDYL PEPTIDASE 4 INHIBITOR SITAGLIPTIN PRESERVE DIASTOLIC FUNCTION IN A MODEL OF HEART FAILURE WITH PRESERVED EJECTION FRACTION

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Objective: Heart failure (HF) with preserved ejection fraction (HFpEF) and chronic kidney disease (CKD) often share co-morbidities like hypertension and diabetes. Moreover, renal dysfunction in HFpEF is common and is associated with increased mortality. Co-existence of heart and kidney failure is a clinical challenge, because of diagnostic and therapeutic difficulties, since many HF medications may cause, or are contraindicated in the presence of renal failure. Several studies suggesting that dipeptidyl peptidase 4 (DPP4) might be involved in the pathophysiology of heart failure prompted investigations of DPP4 inhibitors cardiovascular safety and potential benefits in HFpEF. In addition, DPP4 inhibitors have shown to delay CKD progression in experimental diabetic nephropathy. We aimed 1. to determine whether DPP4 inhibitor sitagliptin (SITA) affects the progression of HFpEF and CKD independently from the effects on glycaemia; 2.to identify mechanisms involved in the potential cardio-renal protection.
Methods: Seven-week-old Dahl salt-sensitive (Dahl/SS) rats were fed a high salt diet (8% NaCl) for 5 weeks to induce hypertension. Then, rats continued with a high salt diet and were administered with either SITA (10 mg•kg-1 by oral gavage) or vehicle for the following 8 weeks.
Results: Treatment with SITA attenuated diastolic dysfunction ameliorating hemodynamic indices. During 8 weeks of the treatment with SITA, blood pressure remained markedly elevated, with a slight, but significant reduction observed only at 19 weeks of age. Interestingly, SITA determined a reduction in DPP4 activity in the heart and kidney and an increase in GLP-1 levels in plasma. The link between high blood pressure and cardio-renal damage may involve high levels of oxidative stress and a low-grade systemic inflammation. In fact, levels of pro-inflammatory tumor necrosis factor-α, IL-6 and monocyte chemoattractant protein-1 were elevated in Dahl/SS rats but reduced by SITA treatment. SITA decreased the levels of eNOS monomer, responsible for reactive oxygen species generation, and elevated the amount of NO-producing dimeric form. The markers of oxidative and nitrosative stress were decreased. Oxidative stress and proinflammatory status observed in Dahl/SS rats contribute to activation of pro-fibrotic pathways in the myocardium and kidney. Increase of collagen deposition and activation of pro-fibrotic signalling that leads to elevated myocardial stiffness were attenuated by SITA. Moreover, a remarkable renal tubulointerstitial fibrosis and glomerulosclerosis in high salt diet-fed rats were significantly reduced with administration of SITA.
Conclusions: SITA positively modulates active relaxation and passive diastolic compliance interfering with inflammatory-related endothelial dysfunction and fibrosis associated with HFpEF. Positive cardiac outcome and simultaneous kidney protection highlight the systemic nature of HFpEF pathophysiology and the multi-organ positive effects of SITA.

SEVOFLURANE PRECONDITIONING INCREASES THE RELEASE OF CARDIOPROTECTIVE EXOSOMES FROM CORONARY ENDOTHELIAL CELLS

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Objective: Perioperative cardiac complications during cardiovascular surgery remain the major cause of morbidity and mortality. Endothelial-derived exosomes, smallest nanovesicles, increase the survival of ischemic cardiac cells. Even if the ischemic preconditioning is effective in modulating the release of cardioprotective exosomes, drugs that mimic this effect are not known yet. Sevoflurane (SEVO) preconditioning attenuates ischemia and reperfusion (I/R) injury, but the underlying mechanisms are not fully understood. Hypothesis: The preconditioning by SEVO increases the levels of cardioprotective exosomes released by coronary endothelial cells.
Methods: Murine coronary endothelial cells (MCEC) were cultured using exosome-depleted fetal bovine serum and then exposed to SEVO (0.35 mM=1 MAC) for 6h. Untreated MCEC were used as

control. Afterward, cells were exposed to acute I/R injury mimicked by exposure to 1.5 mM hydrogen peroxide (H2O2) for 1h. Cell viability and anion superoxide (O2-) production were assessed by MTT assay and dihydroethidium staining, respectively. Endothelial levels of peNOS/eNOS, an index of endothelial function, and pSTAT3/STAT3, a transcription factor linked to cardioprotection, were measured by Western blotting (WB). Endothelial exosomes (CD63- and HSP70- positive) were isolated from cell culture media of SEVO cells by serial ultracentrifugation and quantified by WB. In order to assess the exosome mediated cardioprotection, murine cardiomyocytes (HL-1) were treated for 6h with whole or exosome-depleted medium of SEVO-treated MCEC; although, untreated HL-1 were used as control. Then, acute I/R injury was induced by exposure to 1mM H2O2 for 1h.
Results: SEVO preconditioning of MCEC significantly prevents the loss of viability induced by acute oxidative stress without affecting O2-, peNOS/eNOS and pSTAT3/STAT3 levels. SEVO significantly increases the release of CD63- and HSP70- positive exosomes compared to untreated cells. After 1h exposure to H2O2, HL-1 survival reduces to 31.76% ($p<0.05$). The pre-treatment with whole conditioned medium of SEVO-MCEC increases HL-1 survival to 45.21% ($p<0.05$). Conversely, the exosomes-depleted medium of SEVO-MCEC fails in evoking protection and the HL-1 survival decreases to 13.09 % ($p<0.05$). Finally, exosomes released by SEVO-MCEC do not contain STAT3.
Conclusions: This study shows, for the first time, that SEVO induces the release of CD63- and HSP70-positive exosomes, which protect cultured cardiomyocytes against acute I/R injury. Our results fit into an emerging concept whereby the uptake of HSP70-positive exosomes increases the cellular levels of HSP70, an effector of preconditioning.

THERAPEUTIC BENEFITS OF PHOSPHODIESTERASE-5 INHIBITION IN CHRONIC HEART FAILURE: A META-ANALYSIS

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Objective: Phosphodiesterase-5 inhibitors(PDE5i) have been shown to be beneficial for patients with pulmonary arterial hypertension. However, several studies would have documented a useful effect of PDE5i even for pulmonary hypertension(PH) secondary to left-sided chronic heart failure(CHF).
Methods: We made a meta-analysis including randomized controlled trials (RCTs) which had compared PDE5i (mostly sildenafil) and placebo in CHF patients.
Results: 14 studies were included, with a total of 928 patients. In heart failure with reduced left ventricular ejection fraction(HFREF), PDE5i, compared to placebo, significantly improved the composite of death and hospitalization (OR= 0.28; 95% CI: 0.10 to 0.74). They also improved peak VO2 (difference in means[MD]: 3.76; 95% CI: 3.27 to 4.25), six-minutes walk distance (6MWD) (MD, 22.7 meters; 95% CI, 8.19 to 37.21) and pulmonary arterial systolic pressure (MD: - 11.52 mmHg; 95% CI: -15.56 to -7.49). Conversely, in CHF with preserved left ventricular ejection fraction (HFpEF), PDE5i proved not to yield any significant improvement of the investigated outcomes.
Conclusions: In HFREF, PDE5i showed beneficial effects on the composite of death and hospitalization, as well as on exercise capacity and pulmonary hemodynamics. Conversely, in HFpEF, no significant clinical, ergospirometric or hemodynamic improvement was achieved by PDE5i therapy.

RECIPROCAL REGULATION OF GRK2 AND BRADYKININ RECEPTOR STIMULATION MODULATE CA2+ INTRACELLULAR LEVEL AND PERMEABILITY IN ENDOTHELIAL CELLS

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Obective: Bradykinin (BK) is an important modulator of the cardiovascular system mainly regulating vascular homeostasis. Several endothelial mediators are under its control, through the Gq protein-coupled receptors B1 and B2, using Ca2+ as the second messenger. The G protein-coupled receptor kinase GRK2 can modulate B1 and B2 receptors through desensitization. Purpose: To verify the effects of GRK2 inhibition in regulating endothelial function in response to BK.
Methods: In bovine aortic endothelial cells (BAEC) we evaluated the GRK2 expression and subcellular localization, in response to BK (30 nM), by western blot analysis on whole, membrane, mitochondrial and cytosolic lysates. To assess modifications of GRK2 degradation pattern in response to BK, we evaluated the interaction with mdm2 by immunoprecipitation and performed an ubiquitination test. In the same cells, in response to BK and KRX-C7 (1μM), a specific HJ-loop derived peptide inhibitor of GRK2, we evaluated: Ca2+ accumulation by a fluorescent probe (Fluo4 AM), NO production by DAF-FM Diacetate, and cell permeability by in vitro permeability assay (Millipore).
Results: At 5 min, BAEC stimulation with BK induces an increase of GRK2 levels, which reverberates in several cellular compartments (Membrane, Mitochondria, and cytosol); at 15 minutes GRK2 returns to baseline levels. BK-induced GRK2 accumulation is proteasome dependent since GRK2 ubiquitination is significantly reduced post-BK stimulation and the interaction between GRK2 and mdm2, its specific E3 ligase, decreases. Consistently, the GRK2 accumulation can be prevented by proteasome inhibition (MG132). BK Causes Ca2+ cytosolic accumulation which is sensitive to GRK2 activity, as it is enhanced by inhibition of the kinase with KRXC7 (CTRL: 50,4% vs KRXC7: 72% of fluorescence intensity over basal). NO-dependent vasodilation and permeabilization are the typically acute endothelial response to BKA, and GRK2 inhibition by pre-treatment for 1 hour with KRX-C7, enhances BKA – dependent in vitro production of NO (BKA+KRX: 20 vs BKA: 10; fold of increase over basal). Moreover, also permeability of endothelial cells induced by BK is enhanced when they are treated with KRXC7 (BKA+KRX: 40% vs BKA: 24%; increase over basal).
Conclusions: BK induces GRK2 intracellular accumulation, which in turn desensitizes BK receptors. Proteasome plays a key role in this negative feedback loop, by acutely regulating GRK2 cellular levels. Inhibition of GRK2 affects endothelial response to BKA, enhancing calcium accumulation, NO production, and cell permeability.

ROLE OF SDF-1A/CXCR4 AXIS IN THE HOMING AND UPTAKE OF CARDIAC PROGENITOR CELLS DERIVED EXOSOMES BY DAMAGED CARDIOMYOCYTES

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Objective: Following ischemic injury, the myocardium releases a number of cytokines, chemokines and growth factors that influence stem cell-mediated repair. Within minutes to an hour after acute MI, the expression of stromal cell-derived factor-1α (SDF-1α) is upregulated in the heart, providing a signal to circulating stem cells that express the chemokine receptor CXCR4 for homing and engraftment. Hypoxia induced activation of the SDF-1α/CXCR4 axis recruited the anti-apoptotic kinases ERK and Akt. Whether the axis SDF-1α/CXCR4 may also modulate the effectiveness of exosomes (Exo), secreted form cardiac progenitor cells (Exo-CPC) is unknow. We therefore aimed to investigate whether this axis plays a role in the homing and uptake of Exo-CPC by damaged

Cardiomyocytes (CM). In addition, investigated whether the SDF-1α/CXCR4 axis plays a role in the cellular uptake.
Methods: CPCs producing Exo are genetically engineered to overexpress CXCR4 or null vector (pCDNA3.1). CXCR4 protein concentrations in CPCs and Exo was measured by flow cytometry and western blot analysis. To track Exo, we combine overexpression of CXCR4 with c. elegans species specific Cel-miR-39. Exo containing Cel-miR-39 and expressing CXCR4 (ExoCR4-Cel39) or control Exo (ExoCTR-Cel39) were isolated and incubated with CM to in-vitro assess internalization by CM. CM treated with various exosomes were exposed to staurosporin (1uM) for 12 hrs. For ex-vivo experiments rat hearts were excided 24 hrs after MI was induced and perfused in retrograde manner in a Langendorff system. ExoCR4-Cel39 and ExoCTR-Cel39 were added to the perfusate. Hearts were perfused for 2 hrs before their enzymatic dissociation and CM isolation.
Results: By comparing ExoCTR-Cel39 and ExoCR4-Cel39 both derived from CPC the latter showed higher tropism for CM, demonstrating the implication of the transmembrane protein CXCR4 into Exo-uptake. Calcein-AM staining showed that the number of viable cells was significantly improved in ExoCR4 staurosporin-treated CM (CM + ExoCR4 group, 98% ± 1,6) as compared to ExoCTR treated CM (CM + ExoCTR group, 81% ± 3,5). Data were confirmed ex-vivo in a Langendorff system. Cel-miR-39 levels were higher in CM from hearts perfused with ExoCR4-Cel39 -containing perfusates, as compared with ExoCTR-Cel39.
Conclusion: This study reveals a novel role of Exo derived from CPC overexpressing CXCR4 and highlights a new mechanism of intercellular mediation of progenitor cells for MI treatment.

SIGNAL TRANSDUCTION MECHANISMS OF THE CALCIUM SENSING RECEPTOR IN NEONATAL RAT CARDIAC FIBROBLASTS AND MYOCYTES: A RE-EVALUATION WITH REAL-TIME IMAGING AND ELECTROPHYSIOLOGICAL APPROACHES

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Objective: A number of reports have suggested that the extracellular Calcium-Sensing Receptor (CaR), a pleiotropic GPCR calcium sensor involved in the systemic regulation of calcium absorption/excretion, may play a role in cardiac cell physiology and pathology. Interestingly, diverse signalling pathways have been described to be activated by this receptor in cardiac cells from different experimental models. Notwithstanding the recent abrupt proliferation of literature on the role of CaR in the heart, only scattered data are available regarding the effect of CaR agonists and modulators on second messenger dynamics of spontaneously beating cardiomyocytes.
Methods: Here, by using single cell real-time imaging of calcium and cAMP levels we aimed at evaluating the impact of different CaR agonists and modulators on second messenger dynamics both in fibroblasts and spontaneously beating myocytes isolated from neonatal rat ventricles. Real time single cell imaging was performed on Ca2+ fluorophore (fluo4) -loaded or cAMP-probe - (H30) transfected cardiac cells. In parallel, the spontaneous electrical activity of beating cardiomyocytes was evaluated by conventional microelectrodes.
Results: Stimulation of fluo-4 loaded cardiac cells with the calcimimetic NPS-R, or CaR agonists such as spermine and neomycin induced clear cytosolic [Ca2+] peaks in fibroblasts, while reduced the frequency of Ca2+ oscillations in spontaneously-beating cardiomyocytes. Direct cAMP measurements in living cardiac myocytes demonstrated an apparent reduction of cAMP levels upon

CaR stimulation with NPS-R and spermine. Accordingly, acute exposure to NPS-R and spermine significantly reduced spontaneous electrical activity frequency of neonatal rat cardiomyocytes in a pertussis-toxin sensitive manner.
Conclusions: The data collected demonstrate that different physiological CaR-agonists and modulators activate the PLC/IP3 pathway in cardiac fibroblasts while decrease Ca2+ oscillation- and SEA- frequency in cardiomyocytes, via Gi mediated modulation of cAMP levels.
Thanks to a multi technical approach on living cells, here we showed for the first time and in a straightforward manner that CaR-activation exert cell specific intracellular signalling pathways in neonatal cardiac cells. Given the quite wide use of calcimimetic and calcilytics in a number of human diseases associated with CaR abnormalities the implication of such findings spans from the basic molecular cardiology field to the clinics.

EFFECTS OF SIMULATED HYPERGLYCEMIA IN VITRO ON INSULIN SIGNALING IN ENDOTHELIAL CELLS

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Objective: Emerging evidence in myocytes, hepatocytes and adipocytes indicates that hyperglycemia, a major feature of type 1 diabetes (T1DM), also plays a critical role in the development of insulin resistance and progression of type 2 DM (T2DM). Insulin regulates vascular homeostasis and endothelial function but the role of hyperglycemia in the development and progression of insulin resistance in endothelial cells remains incompletely understood. Aims: We aimed at investigating the impact of high glucose on insulin signaling in human aortic endothalial cells (HAECs). We tested the hypothesis that high glucose per se and/or through its hyperosmolar component may lead to insulin resistance by lowering the metabolic, anti-inflammatory and anti-atherogenic insulin signaling through a down-regulation of the PI3K/AKT pathway.
Methods: Serum-starved HAECs were preincubated with 5.5 mmol/L glucose (normoglycemia, NG), high glucose (HG, at 17.5, 30.5 and 50.5 mmol/L), or equimolar concentrations of the hyperosmolar control mannitol (HM) for short- (3 hours) and long-term exposures (24 hours), followed by insulin treatment (1-10-100 nmol/L) for 45 minutes. Expression of insulin receptor-α subunit (IRα), insulin receptor substrate type 1 (IRS-1), eNOS and phosphorylated isoforms of AKT, ERK1/2, and p38 were evaluated.
Results: HG, and to a lesser extent HM, increased the expression of eNOS, while decreasing the expression of AKT and its active phosphorylated isoform pAKT in a concentration-depending manner ($p<0.01$ versus NG by ANOVA, n=3 independent experiments). In long-term exposure HG, and to a lesser extent HM, increased the expression of ERK1/2 ($p<0.01$ versus NG by ANOVA, n=3), while at any time point they did not modify the expression of p38 and its active phosphorylated isoforms pERK1/2 and p-P38. In NG, IRα, pAKT, pERK1/2, p-P38 were increased in insulin treated cells. In HG or HM (17.5 and 30.5 mmol/L), insulin was not able to activate the PI3K/AKT/ eNOS pathway, as compared to the control. Insulin was able to induce the up-regulation of IRS-1, pERK1/2 and p-P38, although no changes of IRα were found ($p<0.01$ versus NG by ANOVA, n=3).
Conclusions: By decreasing the anti-inflammatory and anti-atherogenic AKT, hyperglycemia and its hyperosmolar component negatively impact insulin signaling in human macrovascular endothelial cells, even when physiological and pathophysiological insulin concentrations are added. The impairment of the PI3K/AKT/eNOS pathway after physiological insulin treatment could contribute to detrimental effects on cardiovascular homeostasis under HG conditions, and might shift toward the activation of certain mitogenic effectors, such as ERK1/2 and p38, the only ones that respond to physiological insulin treatment in HG. Such effects may be relevant for the vascular complications

of diabetes and indicate a biochemical basis explaining the progression of insulin resistance as a result of endothelial glucotoxicity in diabetes.

CHRONIC ANTIHYPERTENSIVE THERAPY WITH THIAZIDE DIURETICS IN OLDER WOMEN AND RISK OF OSTEOPOROSIS: A RECENTLY MUCH- DEBATED ASSOCIATION

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Objective: An alleged association of chronic use of thiazide diuretics with an increased risk of bone fragility fractures has been highlighted by a relatively recent prospective cohort study (Am J Med. 2016 Dec;129(12):1299-1306). However, the concept that thiazides exert a beneficial effect on osteoporosis is still a predominant view. This effect would be mediated by the decrease in renal clearance of calcium ions, a pharmacological feature recognized for a long time now to this class of drugs, as opposed to the increase in calcium urinary excretion attributed instead to loop diuretics, i.e. furosemide and similar drugs. The purpose of this retrospective study is to attempt to clarify whether regular use of thiazide diuretics as antihypertensive therapeutics is associated with a significantly increased risk of osteoporotic fractures in female patients, aged over 70 years.

Methods: In this single-centre retrospective study, we followed up a cohort of female patients with (n= 80) and without (n= 158) thiazide-induced hyponatraemia.

Results: A total of 48 osteoporotic fractures was recorded during a mean follow-up period of 59 months. By means of univariate regression analysis, an association was found between thiazide induced hyponatraemia and increased risk of vertebral fractures (Odds ratio[OR]: 7.6 95% confidence interval [CI] : 3.755 - 15.39 ; p <0,0001). Multivariate regression analysis, however, showed that age (OR: 1.54; 95%CI: 1.253 -1.894) and body mass index (OR: 0.328; 95%CI: 0.1703-0.632) were the only independent predictors of osteoporotic fractures. No association of a history of thiazide-induced hyponatraemia and risk of fracture was noticeable in the final model.

Conclusions: Because a history of thiazide-induced hyponatremia is associated with osteoporotic fracture in univariate but not multivariate analyses, a possible explanation is that confounding factors of older age and low body mass index are responsible for the apparently heightened risk of fragility fractures in hypertensive female patients with thiazide-related hyponatremia.

IMPACT OF DIFFERENT MODALITIES OF CLOPIDOGREL ADMINISTRATION ON SYSTEMIC OXIDATIVE STRESS IN PATIENTS UNDERGOING ELECTIVE PERCUTANEOUS CORONARY INTERVENTION

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Objective: Antiplatelet therapy with clopidogrel is of paramount importance in patients with coronary artery disease (CAD) undergoing percutaneous coronary intervention (PCI) to prevent thrombotic events. Coronary endothelial damage occurring during PCI leads, however, to an immediate release of reactive oxygen species and an increase in systemic oxidative stress. Aim of the present study was to assess the impact of different modalities of clopidogrel administration on systemic oxidative stress parameters in patients with CAD undergoing elective PCI.

Methods: We enrolled 28 patients; of these, 19 (67.9%) were clopidogrel-naïve and received a loading dose of 600 mg immediately before the procedure (loading group), whereas 9 patients (32.1%) were on chronic clopidogrel therapy with 75 mg daily and did not receive any further doses prior to PCI (chronic group). Peripheral venous blood samples were collected at baseline (T0), and 6 (T1) and 24 (T2) hours after the procedure. Oxidative stress was assessed by a global evaluation of derivatives of reactive oxygen metabolites (DROM) and biological antioxidant potential (BAP) using

the FREE carpe diem assay (Diacron International srl, Grosseto, Italy). DROM was expressed in Carratelli units (CARR U), whereas BAP was quantified in μmol/L.
Results: In the overall cohort, DROM increased from T0 (280.0±57.4 CARR U) to T1 (301.4±61.5 CARR U) and subsequently decreased at T2 (289.2±60.1 CARR U) (ANOVA p=0.056). A significant increase in DROM was observed at t1 in the chronic group (317.0±59.6 vs 279.4±54.7 CARR U at T0; p=0.039), whereas this was blunted in the loading group (294.0±62.6 vs. 280.3±60.1 CARR U at T0; p=0.534). Compared with T0, DROM values remained elevated also at T2 in the chronic group (316.8±68.1 CARR U; p=0.041 vs. T0), whereas in the loading group DROM values were not significantly different at T2 (276.2±52.8 CARR U; p=0.999 vs. T0). Delta DROM, defined as the difference between DROM values at T2 and T0, was -4.1±43.5 CARR U in the loading group and 37.3±41.5 CARR U, respectively (p=0.019). BAP values were in the overall cohort 2038.3±402.9 μmol/L at T0, 1958.2±294.1 μmol/L at T1, and 1985.8±313.2 μmol/L at T2 (ANOVA p=0.607). There were no significant differences in BAP values between the 3 time points in either study group.
Conclusion: PCI seems to induce a transient increase in DROM levels, but has no significant effect on BAP. Compared with a chronic clopidogrel therapy, the administration of a 600-mg clopidogrel loading dose immediately before the procedure seems to attenuate the increase in DROM levels.

CHARACTERIZATION OF ASPIRATED THROMBI IN PATIENTS WITH ST-ELEVATION MYOCARDIAL INFARCTION

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Objective: Recent studies have shown that exists an association between histological features of thrombi and major cardiovascular outcomes clinical in patients with ST-segment elevation myocardial infarction (STEMI). The aim of this study was to analyze structural features of coronary thrombi in STEMI patients and to evaluate the effects of age patients on coronary thrombus composition and routine measured laboratory parameters in acute phase of myocardial infarction.
Methods: Coronary thrombi (n=54) were removed by thromboaspiration in 123 patients presenting for primary percutaneous intervention and were analyzed by immunostaining and classified in fresh or older. Correlation between pathological characteristics of thrombi and clinical parameters were assesssed. In order to evaluate the effect of age on thrombus composition, the population was divided into two groups using a cut-off of 65 years.
Results: Older thrombi were present in 92% of patients with STEMI. Old thrombi were mainly composed of fibrin (39%), red cells (36%) and young collagen fibers (20%) and classified in lytic (n =31) and organized (n = 19). No significant histopathological differences were found between lytic and organized thrombi (p = 0.54). Group I includes young-adult patients (age <65 years, n=25) had significantly higher levels of peripheral leucocytes (p=0.005), lymphocytes (p<0.0001), monocytes (p=0.04) and platelets (p=0.007) than group II of elderly patients (age >65 years n=25). On the other hand, group II exhibited a higher neutrophil-to-lymphocyte (N/L) blood ratio (p=0.05) and a higher value of VES (p=0.0015). The percentage of old/lytic and organized thrombi did not differ between two groups patients. Only, patients with age <65 years presented significantly higher infiltration of lymphocyte (p=0.03). About to other histopathological characteristics, there were no statistically relevant differences between patients.
Conclusion: These results indicate the presence of older coronary thrombi in patients with STEMI. Patients with age <65 years have a higher infiltration of lymphocyte in thrombi compared to older patients. Moreover, a different systemic inflammatory response is found between young and elderly patients in acute phase of myocardial infarction.

REMODELING OF ATRIAL REPOLARIZATION AND ATRIAL CHAMBER DEFORMATION: A POTENTIAL LINK IN THE DEVELOPMENT OF ATRIAL FIBRILLATION?

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Objective: Electrical remodeling is a major determinant of the atrial fibrillation (AF) substrate associated or not to chronic heart disease. Action potential (AP) shortening plays a key role and results from the drastic reduction of the calcium current. Differently, repolarizing potassium currents are moderately increased or reduced, such as the inward rectifier current, IK1, or the transient outward current, Ito, respectively, or moderately altered, mainly the ultra-rapid delayed rectifier potassium current, Ikur. These results and the atrial selective expression of Kv1.5/IKur, point to this channel as a potential antiarrhythmic target. Additionally, emerging experimental evidence suggest that remodeling of atrial repolarization and Kv1.5/IKur expression may be linked to atrial dilation, a phenomenon that may precede the development of arrhythmias. In the human setting such a link is presently unexplored.

Methods: To address the specific role of Ikur in electrical remodeling, we used F17727 as a highly specific and open channel blocker of Kv1.5/IKur (IC50=1.5μM) over the other major cardiac current including Nav1.5, hERG, KVLQT1/mink (IC50 >10μM) with the exception of Kv4.3 (61% inhibition at 10μM). Efficacy of F17727 in the human setting was tested in right atrial myocytes isolated from patients in chronic AF and in sinus rhythm (SR) undergoing corrective cardiac surgery. AP recordings were performed using the perforated patch-clamp technique at different pacing rates (0.5, 1, and 2 Hz).

Results: At all rates, 10μM F17727 prolonged AP duration (APD), an effect, which was significantly more pronounced in the AF than in SR group. At 1 Hz, APD measured at 90% of repolarization was prolonged by 207.8±24.1 and 79.3±54.7ms in AF and SR group, respectively (n=5-6, $p<0.05$). AP amplitude and resting diastolic potential were not modified. To address the association between atrial deformation and remodeling of repolarization, in a selected group of patients with or without chronic atrial fibrillation, speckle tracking echocardiography was performed prior to corrective cardiac surgery. Analysis gave a range of mechanical parameters, namely atrial mechanical dispersion and global Peak Atrial Longitudinal Strain (PALS), both related to atrial deformation. For each patients, AP parameters were measured from single atrial myocytes dissociated from samples discarded after cardiac surgery. Interestingly, both mechanical dispersion and global PALS resulted linearly related with AP duration evaluated at different values of repolarization.

Conclusions: Ikur selective blockade has potential antiarrhythmic properties on the atrial AP of AF and SR patients, which is more pronounced in AF, suggesting a gain of function of IKur mediated repolarization in AF. Atrial AP duration of AF and SR patients is linearly related with atrial deformation, suggesting a potential link between atrial electrical remodeling and chamber deformation. Further investigations are necessary to test the predictive value of speckle tracking echography for atrial arrhythmogenic remodeling.

NANOSPONGE-CYCLODEXTRINS FUNCTIONALIZED WITH OXYGEN PROTECTS H9C2 CELLS FROM HYPOXIA/REOXYGENATION INJURY: IMPLICATIONS FROM AN IN VITRO MODEL

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Objective: Nanoparticle-based imaging and nanocarriers therapies have emerged as essential tools for many fields of modern medicine, in order to track the fate of cells and optimize drug delivery. Up to now, however, there are only few reports on the effect of nanocarriers of different types on oxygen delivery, even though this would be of great interest for the design of high impact therapies in several cardiovascular diseases (CVDs). In particular, Cyclodextrin Nanosponges (C-NS) can be envisioned as innovative tools to improve the delivery of oxygen in a controlled manner in CVDs.
Methods: We tested oxygenated C-NS (OX-C-NS) at different concentrations (0.2, 2 and 20 μg/ml) for their capability to reduce cell mortality during hypoxia and reoxygenation (H/R) protocols. For comparative purpose, we also tested "blank materials" (C-NS filled with nitrogen gas without oxygen) and the effects of C-NS in Normoxia. To test the effectiveness of C-NS, we used H9c2, a cardiomyoblast cell line derived from rat heart, exposed to Normoxia (5% CO2 and 21% O2) or Hypoxia (5% CO2 and 95%N2) in a Hypoxic Chamber. The cellular mortality was measured with MTT assay.
Results: In Normoxia, regardless of OX-C-NS formulation, the H9c2 cells displayed a tendency to an increased proliferation, which seemed somewhat correlated to the concentration of OX-C-NS used. The different concentration of OX-C-NS, applied before Hypoxia, induced a significant reduction of cell mortality compared to C-NS without oxygen. Also, the application of OX-C-NS at the beginning of reoxygenation induced a marked reduction of cell death.
Conclusions: OX-C-NS may induce H9c2 cell proliferation in Normoxia and may protect H9c2 from H/R injury *in vitro*. The administration of oxygen in a controlled manner during or after an ischemic event may be an innovative approach for reduction of Ischemia/Reperfusion injury, with consequent reduction of chronic CVDs. Our preliminary results, and in particular the observation of a remarkable efficacy in reoxygenation, suggest an interesting potentiality for medical application of C-NS during the treatment of myocardial infarction. Further studies are required to ascertain the protective potential of C-NS on cardiac I/R injury under *in vivo* conditions.

APELIN-INDUCED CARDIOPROTECTION INVOLVES PTEN INHIBITION BY SRC KINASE

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Objective: The cardioprotection against ischemia-reperfusion (I/R) injury consists in reduction of infarct size, limitation of myocardial contracture and improvement of post-ischemic mechanical recovery. Cardioprotection may be obtained with the administration in early reperfusion of the endogenous peptide apelin which acts with a mechanism triggered by the G-protein coupled receptor APJ and includes the PI3K-Akt-NO signaling pathway. PI3K-Akt activation is counteracted by the phosphatase and tensin homolog (PTEN), whose inhibition by Src has been suggested to be required for the effectiveness of the cardioprotective interventions. Since either oxidation or phosphorylation can inhibit PTEN, the present study aims to investigate whether apelin protective mechanism involves PTEN phosphorylation by Src.
Methods: The experiments were carried out on Langendorff-perfused rat hearts. In the control group the hearts underwent 30-min of global ischemia and 2-hours of reperfusion. In the apelin treated group, apelin-13 (0.5 μM) was infused during the first 20-min of reperfusion. In another group PP2, the specific inhibitor of Src kinase, was co-infused with apelin.
Left ventricular pressure was continuously recorded. After reperfusion infarct size was measured with nitro-blue tetrazolium technique. Western blot analysis was performed to test PTEN phosphorylation.
Results: Apelin significantly ($p<0.001$) reduced infarct size from 60±3 to 30±3% of the left ventricle taken as the risk area. The effect of apelin on infarct size was suppressed by coinfusion of PP2. The increase in diastolic pressure, taken as an index of contracture, reached about 70 mmHg during the first 10 min of reperfusion and declined to about 45 mmHg after 2 hours of reperfusion in control group. This increase was significantly ($p<0.001$) reduced by apelin so that it remained around 30 mmHg for the entire period of reperfusion. Also in this case the effect of apelin was suppressed by

PP2. In control group, left ventricle developed pressure (LVDevP) recovered to about 35% of pre-ischemic value at the end of reperfusion. If apelin was infused, this recovery reached about 70% of the pre-ischemic value at the end of apelin administration and remained almost unchanged for the entire period of reperfusion. Also in this case the effect of apelin was suppressed by PP2.
Western blot analysis revealed that apelin increased PTEN phosphorylation, an effect which was suppressed by inhibition of Src kinase with PP2.
Conclusion: myocardial protection by apelin against I/R injury includes the inhibition of PTEN by a phosphorylation induced by Src kinase.

NANOPARTICLES AT THE NEUROVASCULAR UNIT: IN VITRO AND IN VIVO STUDIES TO ASSESS THE BLOOD-BRAIN BARRIER PERMEABILITY AND FUNCTION

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Objective: The dilemma of the protection from noxious substances from the blood and the delivery of vital metabolites has always to be faced when dealing with the brain. Endothelial cells, forming the blood-brain barrier (BBB) with other cell types, regulate its trafficking. It is known that many common drugs cannot cross the BBB in appreciable concentration, decreasing the rate of success of possible available treatments for many central nervous system (CNS) diseases.
In the last decades, nanomedicine has played a pivotal role in developing strategies to deliver drugs to the CNS. In our previous studies we administered liposomes functionalized with phosphatidic acid and an ApoE-derived peptide (mApoE-PA-LIP) as a potential treatment for Alzheimer's disease (AD): their administration reduced brain beta-amyloid burden and ameliorated impaired memory in AD mice. We also evaluated the adaptability of warm microemulsion process for ligand surface modification of solid lipid nanoparticles (SLN) with ApoE to target the brain. Our *in vivo* biodistribution experiments, performed to study the influence of three different administration routes on SLN-mApoE bioavailability, showed that pulmonary administration increases the DiR-loaded SLN-mApoE bioavaibility to the brain in comparison to the intraperitoneal and intravenous ones, at the same concentrations and time points. In our ongoing experiments, we decided to further investigate the activities of NPs able to cross the BBB, independently from their administration routes. The aim of this study is to evaluate the interaction of mApoE-PA-LIP and SLN at the neurovascular unit. In light of our previous results we here assess their interactions with human cerebral microvascular cells (hCMEC/D3) as in vitro BBB model. Our *in vitro* experiments by means of both the electrophysiological approach and the simultaneous calcium imaging will disclose if any active modulation on neuronal activities does occur after *ex vivo* and *in vivo* NPs administration. The obtained results will help us to better define the safety profile and active properties of NPs specifically developed to cross the BBB and to delivery their payload to the CNS.

CATESTATIN INDUCES GLUCOSE UPTAKE AND GLUT4 TRAFFICKING IN ADULT RAT CARDIOMYOCYTES

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Obective: Catestatin is a cationic and hydrophobic peptide derived from the enzymatic cleavage of the prohormone Chromogranin-A. Initially identified as a potent endogenous nicotinic–cholinergic antagonist, Catestatin has recently been shown to act as a novel regulator of cardiac function and blood pressure and as a cardioprotective agent in both pre- and post-conditioning through Akt

dependent mechanisms. The aim of this study was to investigate the potential role of Catestatin also on cardiac metabolism modulation, particularly on cardiomyocyte glucose uptake.
Methods: Experiments were performed on isolated adult rat cardiomyocytes. Glucose uptake was assessed by fluorescent glucose incubation and confocal microscope analysis. Glut4 plasma membrane translocation was studied by immunofluorescence experiments and evaluation of peripheral/internal Glut4 staining. Furthermore, we performed immunoblot experiments to investigate the involvement of the intracellular pathway Akt/AS160 in the Catestatin dependent Glut4 trafficking.
Results: Our results show that 10nM Catestatin induces a significant increase in fluorescent glucose uptake, comparable to that exerted by 100 nM insulin which can be reverted by 100 nM Wortmannin (mean fluorescence intensity was 101.7±13.8 in control, 346±40.3 for Catestatin, 300.2±42.7 for Ins, 137.12±19.63 for Catestatin+Wm). Moreover, Catestatin stimulates Glut4 translocation to plasma membrane (peripheral/internal Glut4 staining was 0.86±0,04 in Contr, 1.23±0,08 for Cts, 1.04±0.04 for Ins, 0.84±0.02 for Cts+Wm) and phosphorylation of both Akt and AS160. All these effects were inhibited by Wortmannin.
Conclusions: On the whole, we show for the first time that Catestatin is able to modulate cardiac glucose metabolism, by inducing an increase in glucose uptake through Glut4 translocation to the plasma membrane, and that this mechanism is mediated by the Akt/AS160 intracellular pathway. Catestatin could therefore be an alternative agonist in respect to Insulin to increase glucose uptake in the heart, potentially relevant in diabetic cardiac malfunction.

CHAMAZULENE PREVENTS ROS PRODUCTION IN HUMAN DERMAL FIBROBLAST AND BOVINE AORTIC ENDOTHELIAL CELLS EXPOSED TO OXIDATIVE STRESS.

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Objective: Cells are continuously exposed to oxidative stress due to production of reactive oxygen species (ROS) that may in some conditions induce cell damage. In this study we evaluated the capability of Chamazulene, an azulene compound from chamomile essential oil, to counteract ROS production in different cell models: Human Dermal Fibroblasts (HDF) and Bovine Aortic Endothelial Cells (BAEC) cultures.
Methods: Cells viability at different concentrations of Chamazulene was evaluated through the WST-1 Assay, while ROS production acutely induced by H2O2 (500 μM) or High Glucose (4.5 g/L) treatment was quantified with 2'-7'-Diclorofluorescein Diacetate probe and cytofluorimetric assay or confocal microscopy.
Results: Our results showed a reduction in ROS production induced by Chamazulene after cell treatment with H2O2 or High Glucose, thus suggesting an *in vitro* antioxidant activity of the compound. This preliminary study shows the possible role of Chamazulene as a scavenging molecule underlining its possible use to prevent ROS production and cell damage.

HGF-MIMIC ANTIBODY ADMINISTRATION TO COUNTERACT DOXORUBICIN CARDIOTOXICITY

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Objective: Doxorubicin (Doxo) is a highly effective and widely used anti-cancer drug. Unfortunately, its use is limited by its cumulative dose-dependent cardiotoxicity (CTX). Various molecular mechanisms are involved in Doxo-mediated CTX, including DNA damage, oxidative

stress, apoptosis and dysregulation of autophagy in cardiomyocytes. The addition of cardioprotectants to chemotherapy has been proposed as a preventing strategy to reduce the CTX risk. Thus, new agents targeting the detrimental activities of Doxo are attractive candidates as cardioprotective molecules. The Hepatocyte Growth Factor (HGF) / Met receptor couple has been shown to protect from cell death, oxidative stress and excessive autophagy in cardiac cells. In a previous work, we have demonstrated that agonist anti-Met antibodies, that mimic the biological effects of HGF, mitigate the cardiac damage derived from hypoxia. In this work, we exploited the potential cardioprotective function of an HGF-mimic antibody in the context of Doxo-CTX.
Methods: Adult male C57BL/6J mice were randomized to placebo (group 1), Doxo (group 2) and Doxo+ the HGF-mimic antibody (group 3). Mice were treated with i.p. injections of PBS (group 1) or Doxo 7 mg/kg (group 2 and 3) for 3 weeks. Group 3 received the agonist antibody (5 mg/kg) the day before each cycle of chemotherapy. Body weight was measured weekly. The cardiac function was assessed by magnetic resonance imaging (MRI) at week 5 and 6 (2 and 3 weeks after cessation of chemotherapy). At sacrifice, the mice organs were weighted and the heart was examined through histological and molecular analysis.
Results: The treatment with the HGF-mimic antibody prevents the Doxo-induced cardiomyopathy in mice. In particular, MRI analysis showed that Met agonist antibody administration improves the heart systolic function through a thickening of contractile fibers, indicated by both MRI and heart weight measurement. In addition, Met receptor agonist antibody reduced the death rate and the loss of body weight and muscle volume produced by Doxo. From a molecular point of view, the presence of HGF-mimic antibody attenuated Doxo-mediated cell death mechanisms: apoptosis, excessive autophagy and mitochondrial dysfunction. In addition, the presence of antibody modulated DNA repair in response to DNA damage.
Conclusions: Altogether, these results suggest that the HGF-mimic antibody prevents some of the cardiotoxic effects mediated by Doxo. Thus, HGF-mimic can be proposed as novel therapeutic tools for cardioprotection, which will give the opportunity to develop cardio-safer anti-cancer therapies.

CROSS-TALK BETWEEN OSTEOBLASTIC DIFFERENTIATED MESENCHIMAL STEM CELLS AND ENDOTHELIAL CELLS IN CO-CULTURE.

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Objective: The aim of the study was to evaluate the biological effects of the interaction between mesenchymal stem cells (MSC) differentiated into osteoblasts and human microvascular endothelial cells (HMEC) in order to better understand the complex cross-talk mechanisms in co-cultures.
Methods: For this purpose, we used MSC derived from adipose tissue (ASC) or from human deciduous teeth pulp (SHED): these cells were induced to differentiate or not into osteoblasts by specific growth media.
Results: A first comparative analysis between the two MSC types obtained by the use of BioPlex technology revealed differences in the basal expression of cytokines, growth factors and interleukins. Furthermore, to verify the efficacy of the differentiation conditions, we evaluated cell proliferation and the expression of the main osteoblast differentiation markers (OCN, OPN, BMP-2, BSP-1, collagen type I). Then, both ASC and SHED (differentiated or not) were co-cultured with HMEC to investigate the biological effects exerted on microvascular endothelial cells, in terms of proliferation, migration and angiogenic potential *in vitro*. In addition, the paracrine factors responsible for endothelial activation by ASC and SHED were evaluated in terms of differential expression of growth factors and pro-angiogenic markers (VEGF, ANG1, HGF, IGF, PDGF, TGFb, FGF-2 and NGF).

Finally, we report that stem cells induced to differentiate into osteoblasts do favor in turn endothelial activation by simulating differentiation to bone tissue in which angiogenesis plays an key role.
Conclusions: This study suggests that easily accessible tissues such as adipose tissue and deciduous teeth dental pulp can represent, upon *in vitro* osteoblastic differentiation, an important MSC source suitable for regenerative medicine and, in particular, for autologous cell transplant.

FUNCTIONAL CHARACTERIZATION OF A NOVEL TRUNCATING MUTATION IN LAMIN A/C GENE IN A FAMILY WITH A SEVERE CARDIOMYOPATHY WITH CONDUCTION DEFECTS

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Objective: Truncating LMNA gene mutations occur in many inherited cardiomyopathy cases, but the molecular mechanisms involved in the disease they cause have not yet been systematically investigated. Here, we studied a novel frameshift LMNA variant (D243Gfs*4) identified in three members of an Italian family co-segregating with a severe form of cardiomyopathy with conduction defects.
Methods: HEK293 cells and HL1 cardiomyocytes were transiently transfected with either Lamin A or D243Gfs*4 tagged with GFP (or mCherry). D243Gfs*4 expression, cellular localization and its effects on diverse cellular mechanisms were evaluated with western blotting, laser-scanning confocal microscopy and video-imaging analysis in single cells.
Results: When expressed in HEK293 cells, GFP- (or mCherry)-tagged LMNA D243Gfs*4 colocalized with calnexin within the ER. ER mislocalization of LMNA D243Gfs*4 did not significantly induce ER stress response, abnormal Ca2+ handling and apoptosis when compared with HEK293 cells expressing another truncated mutant of LMNA (R321X) which similarly accumulates within the ER. Of note, HEK293-LMNA D243Gfs*4 cells showed a significant reduction of connexin 43 (CX43) expression level, which was completely rescued by activation of the WNT/β-catenin signaling pathway. When expressed in HL-1 cardiomyocytes, D243Gfs*4 significantly impaired the spontaneous Ca2+ oscillations recorded in these cells as result of propagation of the depolarizing waves through the gap junctions between non-transfected cells surrounding a cell harboring the mutation. Furthermore, mCh-D243Gfs*4 HL-1 cardiomyocytes showed reduced CX43-dependent Lucifer Yellow (LY) loading and propagation. Of note, activation of β-catenin rescued both LY loading and LMNA D243Gfs*4 -HL-1 cells spontaneous activity propagation.
Conclusions: Overall, the present results clearly indicate the involvement of the aberrant CX43 expression/activity as a pathogenic mechanism for the conduction defects associated to this LMNA truncating alteration.

DIABETES INDUCES SYSTEMIC MICROVASCULAR REMODELLING

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Objectives: The variability in tissue response to chronic hyperglycaemia makes it difficult to sustain an unified hypothesis on diabetes associated multiorgan failure. A significant literature exists on the functional impairment of circulating and bone marrow endothelial progenitor cells (EPCs) as a feature of diabetes. However, whether this phenomenon results in an unbalance between tissue injury and

repair is poorly described. Thus, a morphometric analysis of the microvascular network on human pancreas, heart and bone marrow in response to diabetes was carried out.
Methods: The effects of type 2 diabetes on different vascular compartments was investigated in 10 autoptic samples and 10 normoglycemic patients in which adequate histologic preparations of each organ of interest were available. Morphometric analysis of the tissue composition and the number and distribution of blood and lymphatic vascular vessels were determined by immunohistochemistry in human pancreatic, myocardial and bone marrow tissues.
Results: Capillary and venules density were significantly reduced in pancreatic insulae, whereas no changes were observed in exocrine parenchyma. However, the reduction in functional tissue as a result of diabetes tended to decrease vessels-to-pancreatic cells ratio compared to control parenchyma. Diabetes determined a significant increase in interstitial fibrosis and vascular remodeling also in the myocardium. Similarly, capillary and sinusoids density were significantly reduced in central and paratrabecular areas of diabetic bone marrow when compared to non diabetic cases ($p<0.05$). Compared to controls, lymphatic vessels were also significantly reduced in diabetic pancreas ($p<0.05$) while arteriolar density was unaffected. Interestingly, CD34pos progenitor cells were significantly reduced ($p<0.01$) in both bone marrow and pancreas of diabetic patients compared to controls.
Conclusions: Rearrangement of the blood and lymphatic network and reduction in CD34pos progenitors concur in multiple tissues with diabetes. Although we did not established whether this was a consequence or a cause of diabetes associated multiorgan damage, our approach may offer new insights on the understanding of the diabetic paradox of a tissue specific angiopathy.

NOVEL ANTI-OBESITY QUERCETIN-DERIVED Q2 PREVENTS METABOLIC DISORDERS IN RATS FED WITH HIGH-FAT DIET

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Objective: Obesity is often accompanied by an increased morbidity and mortality due to an increase of the cardiovascular disease risk factors, diabetes mellitus and dyslipidemia. Research is constantly working on protective molecules against obesity. In the present study, a novel Quercetin derivative Q2 was synthesized to overcome the poor bioavailability and low stability of Quercetin, a natural flavonoid with antioxidative and antiobesity properties.
Methods: Rats were fed (12ws) with normodiet (fat:6.2%), High Fat Diet (fat:60%), HFD+Q2 in water (500nM). Metabolic and anthropometric parameters were measured. 3T3-L1 preadipocytes were incubated with Q2 (1-25μM) and the differentiation program was evaluated by lipid accumulation through ORO staining. Gene and protein expression levels were assessed by RT-PCR and Western blot analysis.
Results: Compared to HFD, HFD+Q2 rats showed reduced body weight, abdominal obesity, dyslipidemia and improved glucose tolerance. This is associated to lower adipose and liver modifications compared to hypertrophy and steatosis observed in HFD. In 3T3-L1 cells, lipid accumulation was significantly impaired by treatment with Q2. Indeed, Q2 significantly decreased the expression of the main adipogenic markers, c/EBPα and PPARγ both at mRNA and protein level.
Conclusions: Our results indicate that Q2 markedly decreases differentiation of 3T3-L1 preadipocytes and contributes to prevent metabolic disorders as well as adipose and liver alterations typical of severe obesity induced by a HFD.

MIR-182 IS A TBX5 EFFECTOR DURING HEART DEVELOPMENT IN ZEBRAFISH

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Objective: MicroRNAs, small molecules of 22-25 nt, inhibit translation of target mRNAs and with transcription factors comprise two major layers of gene regulatory networks with strictly interconnected activities. Tbx5, a dosage sensitive gene, is a pivotal player involved in heart/limbs development and its mutations are responsible of the Holt-Oram syndrome (HOS) in human, characterized by upper limb malformations and congenital heart defects (CHD)s both in morphology and electrophysiology.

Methods: With the hypothesis that Tbx5 and miRNAs can work cooperatively trough mutual cross-regulation, we performed *miRNA-profiling* on RNA extracted from E11.5–E12.0 hearts isolated from WT and HOS mice. By a bioinformatic approach we selected the miR-182 resulted differentially expressed in HOS and able to putatively target evolutionarily conserved genes related to heart development. The miR-182 was functionally tested *in vivo* in zebrafish with experiments of transient and stable mis-expression and by *in situ* hybridization analysis.

Results: miR-182 was found to be up-regulated in HOS mouse phenotype. In line with this data, miR-182 overexpression in zebrafish embryos resulted in a dose-dependent cardiac defects. miR-182 overexpression decreases the pool of cardiac progenitor cells by reducing their proliferation rate during early stages of development, affects myocardial cell morphology and ventricular muscle fiber at 48 hpf. By digital droplet PCR analysis we observed that miR-182 overexpression determines the downregulation of some calcium channel genes which were putative miR-182 targets. In *Tg(myl7:gCaMP)* zebrafish line the miR-182 overexpression caused an alteration of calcium wave across the heart suggesting an impact of miRNA activity on calcium handling. Both transient and stable overexpression of miR-182 caused events of strong arrhythmias and a reduction of heart rate on the whole. Finally, the downregulation of miR-182 was able to partially rescue HOS phenotype in zebrafish Tbx5 knockdown embryos and in Tbx5 mutants.

Conclusion: Our approach further support the importance of microRNA regulation in HOS pathology and demonstrate that miR-182 is a conserved Tbx5 effector with implications both in heart development and functions.

UNDERSTANDING THE POOR ANGIOGENIC CAPACITY OF THE MAMMALIAN HEART

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Objective: The reason why a hypoxic tumor forms its own vasculature, mainly through the secretion of the Vascular Endothelial Growth Factor (VEGF), whereas an ischemic heart cannot, remains

obscure. In this work, we investigated whether cardiac endothelial cells (ECs) loose their capacity to proliferate soon after birth, similar to mammalian cardiomyocytes.
Methods: The effect of VEGF in embryonic and adult heart and skeletal muscle was analysed by injecting AAV (Adeno-Associated Vector)-encoded VEGF into the three organs. ECs from these organs were isolated using CD31 magnetic beads and analysed by flow cytometry, cell culture assays and RNAseq. Cells isolated from EGFP (Enhanced Green Fluorescent Protein) transgenic pups were injected into skeletal muscle and adult heart. Cancer cells were injected into adult heart and skeletal muscle.
Results: VEGF injection in the skeletal muscle and embryonic heart induced significantly more vessel formation compared to the adult heart. Therefore, we wanted to understand whether intrinsic properties of ECs or presence of some inhibitory factors within the adult heart determine the different angiogenic potential of the three organs. Flow cytometry analysis of ECs showed presence of an EC sub-population characterized by high expression levels of tip cell markers, VEGFR2 and CD105, in the embryonic/neonatal but not in the adult heart ECs. Consistently, formation of filopodia by tip cells and vessel-like tubular structures in response to VEGF was much more evident for embryonic/neonatal then for adult cardiac ECs. RNAseq data from the three EC types reveal a differential expression profile for coding genes, miRNAs and lnc-RNAs. ECs purified from the heart of EGFP transgenic pups formed capillaries and integrated into the vascular network of skeletal muscle but not of adult heart, suggesting the presence of an anti-angiogenic factor in the latter organ. Furthermore, the expression of VEGFR1 and its soluble isoform sFlt1 was significantly increased in adult compared to embryonic hearts, consistent with the role of sFlt1 in keeping cornea avascular. Finally, cancer cells injected into the heart of adult mice grew much less compared to the same number of cells injected into the skeletal muscle, possibly indicating that impaired angiogenic potential of the heart inhibited tumor growth.
Conclusions: Collectively, these results indicate that both cell-autonomous and non autonomous mechanisms halt the proliferation of ECs in the post-natal heart and pave the way to novel therapeutic opportunities to promote angiogenesis in cardiac ischemia and, possibly, to control tumor progression.

ACTIVATED FIBROBLASTS SHAPE CARDIOMYOCYTE METABOLISM TOWARDS DEPRESSED MITOCHONDRIAL OXIDATION AND ATP DEPLETION

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Objective: Cardiac fibroblasts (Fib) activated to myoFib regulate cardiomyocyte (CM) functions in a paracrine way. Here, we hypothesized that myoFib-secreted factors might affect energy metabolism of CM.
Methods: Neonatal mouse Fib were cultured in high-glucose (HG, 450 mg/dL) serumfree (SF) DMEM with or without transforming growth factor-beta (TGFβ, 1 to 10 ng/mL) for 48 hours, after which the conditioned medium was collected and α-smooth muscle actin (αSMA) expression was assessed by immunofluorescence. Next, neonatal mouse CM were incubated with: i) normal culture medium (CM-medium); ii) the whole conditioned medium of control or TGFβ-stimulated Fib (Fibmedium or myoFib-medium, respectively); iii) TGFβ alone; iv) low-glucose (LG, 100 mg/dL) SF DMEM, supplemented with oleate and palmitate (LG-FA DMEM) in concentrations so that cell viability was the same as with CM-medium; v) LG-FA DMEM added with 40-200 μg/mL Fib or myoFib-medium, as obtained after centrifugation with 3kDa cutoff tubes. After 30 hours, activity of the main glycolytic enzymes and release of lactate were evaluated by in-house assays, ATP and AMP levels by enzyme coupling method, and oxygen (O2) consumption and ATP synthesis in response to exogenous pyruvate/malate or succinate by using an amperometric electrode. Cell viability was investigated by MTT assay and apoptosis by staining for caspase 3.

Results: As expected, TGFβ dose-dependently increased αSMA immunoreactivity in Fib, indicating activation. While metabolic parameters were similar for CM incubated with CM-medium or Fib-medium, CM exposed to myoFib-medium displayed substantially reduced pyruvate/malate or succinate-driven O2 consumption and ATP synthesis, a drop in the ATP/AMP ratio, and enhanced glycolysis and lactate production. Apoptosis was very low and only non-significant changes were observed in CM treated with TGFβ alone, arguing against initiation of cell death or direct effects of TGFβ as the leading cause of this metabolic perturbation. To work in a more physiological context and to control for the amount of Fib released factors acting on CM, we repeated the experiments adding definite concentrations of Fib- or myoFib-medium to LG-FA DMEM. Again, myoFib-medium, but not Fib-medium elicited a significant decrease in O2 consumption, ATP synthesis and the ATP/AMP ratio. Interestingly, the activity of glycolytic enzymes was not significantly different between myoFib-medium and CM-medium or Fibmedium. CM viability was not modified by myoFib-medium.
Conclusions: The myoFib secretome profoundly deranges mitochondrial CM metabolism. This phenomenon may have important implications in cardiac pathology.

COMPLEXITIES AND CHALLENGES OF GENOME DIAGNOSTICS IN INHERITED ARRHYTHMIC CARDIOMYOPATHIES IN THE PADUA EXPERIENCE

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Objective: Next Generation Sequencing (NGS) unveiled a wide genetic overlap among inherited arrhythmic cardiomyopathies. Mutations in sarcomeric and desmosomal genes respectively are linked mainly to major primary cardiomyopathies such as Hypertrophic (HCM) and Arrhythmogenic Cardiomyopathy (AC). Comprehensive genetic screening of HCM and AC index cases revealed major challenges in genetic diagnosis in the clinical setting.
Materials and methods: Our cohort, including 31 HCM (9F, 22M, mean age 41±12y) and 189 AC index cases (73F, 116M, mean age 35±12y) fulfilling current clinical criteria, underwent massive parallel sequencing on a Miseq platform using a cardiovascular panel of 174 genes (TruSightCardio, Illumina).
Results: Genetic testing identified at least one pathogenic genetic variant in 24/31 (77%) HCM index cases and in 94/189 (50%) AC cases.
Seventeen of the 24 HCM cases carried one mutation in a major disease-related gene encoding for sarcomere proteins: 7 in *TTN*, 8 in *MYBPC3* and 2 in *MYH7*. Further, 7 carried mutations in minor disease-related genes: *MYPN*, *OBSCN*, *MYH6*. Whereas a genetic overlap was observed in 5/17 (29%) HCM mutation carriers: 2 *MYBPC3* and 2 *OBSCN* carriers showed an additional variant of unknown significance (VUS) in *DSC2* and/or *DSP* gene respectively, whereas 1 *TNNI3* carrier exhibited additional 2 VUS in *DSG2* and *DSP* genes.
Ninety-four of the 189 AC cases carried one or more mutations in a major disease-related gene encoding for desmosomal proteins: 15 were single variant *DSP* carriers, 7 *DSG2*, 29 *PKP2*, 7 *DSC2*, 2 *JUP* and 24 were compound or digenic heterozygous desmosomal variant carriers. Notably, 1 *PKP2* carrier had an additional VUS in *MYBPC3*, while 6 AC cases negative for variants in disease-related genes carried VUS in HCM-related genes: 4 *MYBPC3*, 1 *MYH7* and 1 *MYH6* carriers.
Conclusions: Next Generation Sequencing in genome diagnostics of inherited arrhythmic cardiomyopathies revealed the presence of a combination of sarcomeric and desmosomal variants in HCM and AC cases. Since the clinical interpretation of this genetic background remains challenging, cascade genetic screening is demanded to elucidate the clinical significance of rare genetic variants.

PI3Kγ INHIBITION PROTECTS FROM ANTHRACYCLINE-INDUCED HEART FAILURE AND REDUCES TUMOR GROWTH

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Objective: Anthracyclines, such as doxorubicin (DOX), are potent anti-cancer agents used in the treatment of solid tumors and hematological malignancies. However, their clinical use is hampered by severe cardiotoxicity which cannot be controlled by standard heart failure pharmacotherapy. In this study, we explored the potential cardioprotective effects of inhibiting PI3Kγ, a previously described cardiac maladaptive enzyme, in a murine model of cardiomyopathy induced by DOX.

Methods: Mice expressing a kinase inactive PI3Kγ (PI3Kγ kinase-dead; KD) and their wild-type counterparts (WT) were exposed to chronic DOX treatment (a cumulative dose of 12 mg/kg, 4 mg/kg i.p. at days 0, 7 and 14). Heart function was assessed by echocardiography before and 6 weeks after the first DOX injection. Cardiac remodeling and signaling transduction were studied. To mimic clinical cancer therapeutic strategy, 1×105 4T1 breast cancer cells were injected in WT and KD mice, followed by DOX treatment as above. The PI3Kγ selective inhibitor IPI145 (5 mg/kg i.p. daily for 3 weeks) was used in 4T1 xenografts, whereas AS605240 (5 mg/kg i.p. daily for 3 weeks) was used in Her2/NeuT spontaneous breast tumor model, together with DOX, to assess the potential synergic anti-cancer effect. Tumor growth was monitored up to 4 weeks.

Results: Compared to WT controls, KD mice were protected against DOX-induced contractile dysfunction (% FS WT DOX: 20.5±1.3; KD DOX: 36.6±2.2, ***P<0.001). In line with this finding, DOX-induced cardiac remodeling, including cardiac atrophy, cardiomyocyte apoptosis and collagen deposition, was significantly prevented in KD than in WT hearts. Mechanistically, the protection of KD mice was due to dampened activation of TLR-9/PI3Kγ/Akt/mTOR/Ulk-1 signaling axis in cardiomyocytes, ultimately resulting in enhanced autophagic clearance of injured mitochondria. Intriguingly, PI3Kγ inhibition also provided antitumor effects by reducing recruitment of pro-tumor M2-like macrophages. Accordingly, pharmacological blockade of PI3Kγ, with IPI145 or AS605240, synergized with DOX in limiting tumor growth, while preventing its iatrogenic cardiotoxicity, in xenografts and in models of spontaneous mammary tumor growth.

Conclusions: Altogether, these findings picture a scenario where pharmacological blockade of PI3Kγ preventing chemotherapy cardiotoxicity, and, at the same time, unleashing anticancer immunity.

IN VITRO EFFECTS OF CAFFEINE ON HUMAN UMBILICAL ARTERY AND VEIN ENDOTHELIAL CELLS

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Objective: Caffeine is purine alkaloid naturally present in coffee, tea, cola-like drinks and used as a mild stimulant in various energy beverages and foods. The physiologic effects of caffeine are clinically exploited to increase alertness, help concentration, improve mood, and limit depression.

Importantly, clinical activity has been documented to treat bronchopulmonary dysplasia syndrome and apnea in premature newborns. Beneficial effects as anti-fibrotic, anti-tumor, and antineurodegenerative compound have been recently claimed. Caffeine abuse may acutely result in toxic effects including tachycardia, vomiting, cardiac arrhythmias, seizures and death. Average coffee consumption in healthy adults is estimated 3mg/Kg/day or 200-400mg/day. Caffeine is commonly used during pregnancy in which amounts of 300mg/day was estimated. Thus, the debate on whether its consumption is irrelevant or detrimental remains open. The purpose of this study was to test, *in vitro*, the direct effect of caffeine on human umbilical artery (HUAEC) and vein (HUVEC) endothelial cells.

Methods: EC lines were exposed to 500μM, 1mM, 2mM, 5mM, 10mM, and 20mM caffeine. Proliferation and viability were measured by MTT assay after 24, 48, and 72 hours of treatment. The ability to organize into tubule-like networks on Matrigel and migration by wound healing assay were evaluated after 24 hours. Cytoskeleton organization was investigated by immunofluorescence microscopy. Western blot analysis was employed to identify potential molecular targets of caffeine activity.

Results: Caffeine inhibited EC lines proliferation and viability in a dose- and time-dependent manner. In particular, at 500μM and 1mM no significant effects were observed, while 10 mM and 20 mM caffeine concentrations were cytotoxic and were not further investigated. Inhibition of tubular formation by EC lines was more evident following increasing caffeine concentrations (2-5mM). Moreover, a dose-dependent impairment in wound healing was documented. Interestingly, this effect was more pronounced in HUAECs compared to HUVECs. Disarrayed cytoskeletal filaments were also observed in EC lines exposed to 2 and 5mM caffeine. Preliminary results from western blot analysis showed a downregulation of ROCK1/2 expression and p-FAK phosphorylation in both EC lines. Moreover, according to MTT assay, lower bcl-2 protein level was observed following caffeine exposure.

Conclusions: Viability, angiogenic and migration ability, and cytoskeletal integrity of human cordderived ECs are affected by caffeine in vitro, suggesting a potential detrimental effect of its excessive consumption during pregnancy.

SEARCHING FOR OXIDIZED CARDIOLIPIN IN LEUKOCYTES OF CARDIOPATHIC PATIENTS BY MALDI-TOF/MS

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Objective: Failure of the heart functioning and cardiovascular diseases are often associated with bioenergetic deficiency and oxidative phenomena. Cardiolipin, the lipid marker of mitochondria, plays an important role in respiratory chain and ATP synthesis. In the present study we have examined the possibility that cardiolipin oxidation occurs and is favoured in cardiopathic patients.

Methods: We have searched for oxidized cardiolipin forms in white blood cells of two groups: controls (24) and cardiopathic patients (21). The lipid analyses have been performed by direct MALDI-TOF mass spectrometry of membranes of leukocytes isolated after erythrocytes precipitation by dextran, starting from 1 ml of whole blood only.

Results: Firstly, we have induced the oxidation of standard cardiolipin in vitro and then detected oxidized cardiolipin forms by MALDI-TOF/MS. Then evidence for the presence of some oxidized cardiolipin forms has been obtained in the blood of cardiopathic patients and not in healthy controls.

Conclusions: The comparison of the two sets of analyses has showed MALDI signals attributable to oxidized cardiolipin species in the mass spectra of aged chronic heart failure patients only.

A NUTRACEUTICAL APPROACH TO PREVENT DYSMETABOLIC SYNDROME INDUCED BY HIGH FRUCTOSE DIET IN MICE AND RATS

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Objective: The global increase in type 2 diabetes prevalence is a major health issue and it will inevitably lead to increase morbidity and mortality due to cardiac complications. Diets rich in fructose produce metabolic alterations associated to dysmetabolic syndrome, hence in hyperglycemia, glucose intolerance, increased production of triglycerides and advanced glycation end-products (AGEs). AGEs are responsible for oxidative stress and inflammation. A nutraceutical strategy for reducing insulin resistance and cardiovascular inflammation could be based on the replacement of fructose with tagatose, an isomer of D-galactose and stereoisomer of D-Fructose, that has been established as GRAS (Generally Recognized as Safe) by the FAO/WHO since 2001 for use in food and beverages. The aim of this research was to assess the actions of tagatose in the prevention of metabolic syndrome and cardiovascular inflammation induced by high fructose diet in mice and rats.

Methods: Both wistar rats and C57BL/6 mice underwent 12 weeks long diet and where divided in 5 groups (30% solid and liquid fructose, 30% solid and liquid tagatose and standard diet, Ssniff Spezialdiäten GmbH, Soest, Germany). Animal weights and arterious blood pressure were monitored for the entire period as well as glycemia and insulinemia. Blood samples were collected at 6 and 12 months. At the end of the experiment heart, kidney, liver, gastrocnemius and abdominal fat were collected for biomolecular essays and histological immunostaining.

Results: Fructose fed animals showed a significant weight gain, 30% increase in fructose groups versus 20% in standard diet, versus 5% in tagatose groups. Arteriosus pressure increased in fructose fed animals (+ 10%), no variations in tagatose and standard diet. Plasmatic levels of leptin, IL6, TNFα, IL1β were significantly higher in fructose fed animals and close to normal in tagatose and standard diet as well as AGEs (HBA1c), triglycerides and LDL cholesterol. COX 2 and AGE receptor expression in tissue samples were significantly higher in fructose fed animal; livers were heavier and fat infiltrated.

Conclusions: The results of this research proved to be useful for the validation of tagatose in regulating the enzymatic pathways that lead to an increased synthesis and a reduced use of glycogen, suggesting a role of this sugar in fighting overweight and type 2 diabetes. Furthermore, our data indicate that tagatose has a high potential for technology transfer and development of innovative nutraceutical approaches for the prevention of metabolic syndrome and its cardiovascular complications.

MITOCHONDRIA FROM STRIATE MUSCLES: NOVEL TECHNIQUES TO ISOLATE AND CHARACTERISE THE SUBSARCOLEMMAL AND INTERMYOFIBRILLAR FRACTIONS

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Objective: It has been shown that striate muscle mitochondria can be classified in two fractions depending on their structure and distribution in the cell: subsarcolemmal (SSM) and interfibrillar (IFM). It has also been reported that different factors can selectively affect relative abundance of IFM and SSM. Thus, developing novel techniques to distinguish and separate these fractions is of great importance.

Methods: In order to quantify and characterize IFM and SSM, we isolated gastrocnemius and myocardium from physically trained and sedentary rats and we tested different techniques. Longitudinal and transversal sections of a striate muscle have been used for immunohistochemistry analysis. Mitochondria were labeled with anti-voltage dependent anion channel (VDAC) antibody. 8 μm thick slices were examined with LSM-800 confocal microscope.

Furthermore, mitochondria have been extracted following a modified protocol from Frezza's et al. in order to perform studies on functional organelles. This protocol allows us to obtain intact and

functional mitochondria used to perform functional assays. To analyze physical properties of extracted mitochondria, Beckman coulter CYAN flow cytometer has been used. Moreover, isolated mitochondria were processed and analyzed by 2D gel electrophoresis with 7 cm 3-10 pH strips, followed by MALDI-TOF mass spectrometry analysis.
Results: Immunohistochemistry analysis suggests equal distribution of mitochondria inside muscle fiber. Though, we find that longitudinal sections can provide better insights on 3D structure and arrangement within the fibers. Flow cytometry analysis can provide significant data. However, due to mitochondrial size, particular attention should be paid on background noise reduction. Proteomics analysis highlighted some differences between mitochondria extracted from muscles which did or did not undergo intense physical exercise.
Conclusions: Mitochondria are involved in a plethora of physiological and pathophysiological processes. Therefore, creating new tools to understand their structure in relation to the intracellular distribution is of pivotal importance.

MICRORNA-34A MODULATES VASCULAR CALCIFICATION

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Objective: Vascular calcification (VC) is associated with aging and it is a risk factor for cardiovascular and all-cause mortality. VC involves the transdifferentiation of vascular smooth muscle cells (VSMC) towards an osteochondrogenic lineage that results in calcium phosphate salts deposition in the arterial wall. It has recently emerged the important role of senescence and the senescence-associated secretory phenotype (SASP) in mediating this process. MicroRNAs are negative post-transcriptional regulators of gene expression. We already demonstrated that microRNA-34a (miR-34a) is upregulated in aged mouse aortas and that miR-34a induces VSMC senescence through the modulation of its target SIRT1, that is also a VC inhibitor, and promotes the expression of SASP factors. Since miR-34a role in VC has never been investigated, a detailed characterization of this microRNA in VC may lead to efficient therapeutic strategies against it.
Methods: VSMC were cultured in either growth medium or calcification medium which contains a pathological concentration of inorganic phosphate. VSMC were transfected with either a miR-34a mimic or a miR-34a hairpin inhibitor or the respective negative control and they were infected with a miR-34a-overexpressing lentivirus or with the control virus. Precipitated calcium was quantified by colorimetric analysis. miR-34a, SASP factors and osteoblastic markers expression was evaluated by qRT-PCR. miR-34a targets protein levels were evaluated by Western Blot analysis. Mir34a+/+ and Mir34a-/- mice were treated with vitamin D to induce soft tissue calcification.
Results: In our *in vitro* model of VSMC calcification two miR-34a targets, Axl and SIRT1, that are also known VC inhibitors, displayed decreased protein levels in calcified VSMC when compared with control VSMC grown in normal medium. We also demonstrated that miR-34a modulation in VSMC could affect Axl protein levels, as we have previously showed for SIRT1 expression. Interestingly, VSMC infected with the miR-34a-overexpressing lentivirus and undergone to the calcification protocol displayed an increased calcium deposition when compared with cells infected with the control virus. Accordingly, in the mouse model of soft tissue calcification we observed that Mir34a genetic ablation could prevent the calcification of the kidney, lung, heart and aorta. This result was also confirmed by Von Kossa staining on aortic section. Finally, the protein levels of the VC marker Sox9 were lower in the aortas of Mir34a-/- when compared with Mir34a+/+ mice.
Conclusion: miR-34a may control VC through the inhibition of specific targets.

PLATELET LYSATE-DRIVEN ENDOTHELIAL CELL REPAIR: THE MASTERMIND ROLE OF INTRACELLULAR CALCIUM

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Objective: Tissue regeneration requires precise coordination among endothelial, epithelial and mesenchymal morphogenesis. Growth factor-induced angiogenesis plays a key role in recovery from ischemic disease and organ regeneration. Recent studies show that platelet growth factors could open new horizons in the myocardial infarction treatment. Platelets have attracted much interest in this field, since they are rich in wound-healing mediators, although the cellular mechanisms involved in this platelet-induced angiogenic stage of tissue repair have not been clarified yet.
In the present study, we have explored the mechanisms of endothelial damage repair induced by a platelet lysate (PL), a medical product that has been shown to accelerate the *in vitro* wound healing of several cellular types.
Methods: *By using in vitro* scratch wound and cell migration assays, coupled to light microscope image analysis, Western immunoblotting and confocal calcium imaging, we have explored the mechanisms of endothelial damage repair induced by a platelet lysate (PL) on different endothelial cell types, including human (HuVEC, HMVEC-c) and non-human mammalian (PAOEC, bEnd5) models.
Results: Our data showed that PL accelerates wound closure in endothelial cell monolayers. A more in depth analysis showed that the effect of PL occurs through the stimulation of cell proliferation and migration that are strictly dependent on intracellular Ca2+ regulation. Therefore, we demonstrated, that PL treatment determines a significantly increase of the intracellular Ca2+ concentration due to the activation of different calcium channels predominantly present in the plasma membrane.
Conclusions: This study has demonstrated that PL activates repair mechanisms in various types of injured endothelial cell layers. These data bring scientific support to possible clinical applications of platelet derivatives in blood vessel repair, and in particular to the functionalisation of biomaterials used in tissue engineering. In fact, the implementation of platelet derivatives in biomaterials could offer innovative solutions to blood vessel engineering.

FUNCTIONAL ANCIENT GRAIN BREAD RICH IN IRON, ZINC, FLAVONOIDS AND ALPHA LIPOIC ACID ATTENUATES POST-ISCHEMIC MYOCARDIAL REMODELING IN RATS

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Objective: Single intake of iron or zinc or flavonoids or alfa lipoic acid (ALA) enhances anti-oxidant capacity and prevents cardiovascular diseases. However, their combined effects on infarcted hearts are unknown. Bread made from iron-and zinc-biofortified “Gentil Rosso”, a Tuscan ancient grain, contains higher levels of bioavailable iron (+180%), zinc (+640%), flavonoids

(+1000%) and ALA (+50%) compared to modern bread. Hypothesis: Long-term dietary intake of biofortified ancient bread prevents heart failure after acute myocardial infarction (AMI).
Methods: Adult male Wistar rats (300-320g) underwent to left coronary artery ligation (n=20) and were fed for 6 weeks with normal chow (3.347Kcal/g, 6.2% fat, 18.7% proteins, 51% carbohydrates) supplemented with functional bread (AMI+FB; n=10) or regular bread (AMI+RB; n=10). Body weight and food intake were weekly evaluated. Intraperitoneal glucose tolerance test (IPGTT) and cardiac echocardiography were performed before and after diet. LV infarct scar size, capillary and arteriolar density were evaluated in explanted hearts. Myocardial levels of interleukin (IL)-1α, -1β, -2, -10 and -4 were assessed by an Enzyme-Linked Immuno-Sorbent Assay (ELISA).
Results: At similar food intake (21±2g/day), the body weight after diet was increased by 26% in both experimental groups ($P<0.001$). Plasma glucose levels at baseline and during IPGTT were similar in both experimental groups before and after diet. After diet, the reduction of LV ejection fraction, an index of global contractility, was significantly lower in AMI+FB compared to AMI+RB rats (12±1 vs 40±5%, respectively). The LV infarct scar size of AMI+FB rats was smaller than AMI+RB animals (30.5±3 vs 42±3.3%, $P<0.001$, respectively). The myocardial capillary and arteriolar density were, respectively, 16.3 and 15.35% higher ($P<0.05$) in the LV border zone of AMI+FB than AMI+RB group. Moreover, biochemical analysis of the LV border zone indicated lower ($P<0.01$) levels of pro-(IL1β, 1α and -2) and anti-inflammatory (IL-10 and -4) cytokines in the AMI+FB rats compared to AMI+RB group.
Conclusions: The simultaneous supplementation of iron, zinc, flavonoids and ALA with functional bread prevents the onset of heart failure subsequent to AMI by inhibiting the inflammatory response.

TWO DECADES OF GENETIC TESTING IN HYPERTROPHIC CARDIOMYOPATHY IN A SINGLE CENTER: THE ADDITIVE VALUE OF EXTENDED NEXT-GENERATION SEQUENCING PANELS LIES IN THE EARLY DIAGNOSIS OF METABOLIC MIMICS

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Objective: Hypertrophic cardiomyopathy (HCM) is a relatively common inherited heart disease and one of the most frequent causes of sudden cardiac death. Following the release of genetic data from large population cohorts, the causative role of many genes has been questioned together with the usefulness of adopting extended gene panels in the diagnostic setting of HCM. By analysing genetic tests results of HCM patients, we aim at a formal diagnostic yield comparison of different sequencing methodologies and gene panels, and at the definition of a reliable core set of genes implicated in Mendelian HCM.
Methods: We computationally analysed results obtained over 19 years of genetic testing on 1198 HCM index cases with Sanger dideoxy sequencing (n=585) and next-generation sequencing (NGS, n=613). Index cases were screened on four different gene panels targeting 3, 8, 12 and 40 genes associated to HCM in literature.
Results: Our data suggest how only genes characterized by incontrovertible evidence of association to HCM or to its mimics (Fabry disease, Danon disease, Wolff-Parkinson-White syndrome and transthyretin amyloidosis) yield comparably interpretable and actionable results in the diagnostic setting, with equal results obtained with Sanger sequencing and NGS. The specific advantage of an expanded NGS gene panel is the possibility to promptly identify patients affected with HCM mimics through a “genotype-first” approach, enabling early diagnosis and prompt differential patient

management. The newly-devised disease- and gene-specific metric *diagnostic effectiveness* scored PLN as a "core" HCM gene, suggesting that this gene should be routinely screened together with those irrefutably associated to primary HCM (MYBPC3, MYH7, TNNI3, TNNT2, MYL2, MYL3, TPM1 and ACTC1) and to HCM mimics (GLA, LAMP2, PRKAG2 and TTR).
Conclusions: We show how the additive value of NGS expanded gene panels in clinical HCM genetic testing lies in the possibility to systematically screen those genes associated to HCM mimics, sometimes phenotypically indistinguishable from primary HCM, aiding a "genotype-first" vitally important early diagnosis. We underscore high actionability and interpretability of identified mutations to be limited to robustly validated sarcomeric genes, PLN and those associated to HCM mimics. As a whole, these data also support the concept that screening a large number of genes offers limited additional sensitivity in HCM, and that novel approaches to investigate the underlying complex genetic aetiological background should be developed.

HOW TO PREDICT NEW-ONSET ATRIAL FIBRILLATION IN STEMI PATIENTS TREATED BY PRIMARY PERCUTANEOUS CORONARY INTERVENTION: THE ALBO SCORE

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Objective: Biomarkers are useful to identify individuals at risk of developing new-onset atrial fibrillation (NOAF) in patients with hypertensive cardiomyopathy and heart failure. However, few data on their prognostic value in the setting of ST-Elevation Myocardial Infarction (STEMI) are available. We aimed to develop and validate a risk score, based on common clinical risk factors, to assess the incidence of NOAF during hospitalization after primary percutaneous coronary intervention (pPCI).
Methods. The risk score for NOAF occurrence during hospitalization (mean 5±6 days) was developed in a cohort of 1135 consecutive STEMI patients undergoing pPCI while was externally validated in a temporal cohort of 771 STEMI patients. Biomarkers and clinical variables significantly contributing to predicting NOAF were assessed by multivariate Cox regression analysis.
Results. Independent predictors of NOAF were age ≥ 80 years [6.97 (3.40-14.30), hazard ratio (95% confidential interval), $p < 0.001$], leukocyte count > 9.68 x 103/μL [2.65 (1.57-4.48), $p<0.001$], brain natriuretic peptide (BNP) > 80 ng/L [2.37 (1.13-4.95), $p = 0.02$] and obesity [2.07 (1.09-3.92), $p = 0.03$]. By summing the hazard ratios of these predictors we derived the ALBO risk score (Age, Leucocyte, BNP, Obesity) which yielded high C-statistics in both the derivation cohort (0.734 [0.675-0.793], $p <0.001$) and the external validation cohort (0.76 [0.688-0.831], $p<0.001$).
Conclusions. The ALBO risk score, comprising biomarkers and clinical variables that can be assessed in hospital setting, could help to identify high-risk patients for NOAF after pPCI so that a prompter action can be taken.

SIMILARITIES ON THE PERMISSIVE ROLE OF TISSUE OXYGENATION ON COMPRESSION- AND CONTRACTION- INDUCED HYPERAEMIA

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Objective: Skeletal muscle vasculature exhibits a rapid dilatation in response to mechanical stimulation (e.g., muscle compression, MC), that leads to a transient hyperemia. This characteristic, called mechano-sensitivity, is considered to play an important role in the initial phase of functional hyperemia. The compression-induced hyperaemia was recently shown to progressively decrease in spite of continuing stimulation, the extent of attenuation being correlated with the increase of tissue

oxygenation in the relevant muscles. Aim of the present human study is to test the hypothesis that contraction-induced hyperaemia shares the same dependence on prior mechanical stimulation as compression-induced hyperaemia.
Methods: In 10 healthy subjects hemodynamic changes are assessed in response to: a short mechanical compression (MC) at a supra-systolic pressure (150 mmHg) delivered to the lower leg by means of a customized pneumatic device, an electrically-stimulated contraction (ESC; pulse duration: 500 μs; frequency: 20 Hz; total train duration: 0.5 s) of the calf muscles, and a combination of both stimuli separated by 25-s of pause. Hemodynamic monitoring includes near infrared spectroscopy, detecting tissue oxygenation and blood volume in lateral gastrocnemius muscle, as well as simultaneous echo-Doppler measurement of blood flow at femoral artery.
Results: Single MC and ESC elicited comparable hyperaemic responses (41.0 ± 15.5 and 41.9 ± 12.4 ml, respectively) and transiently increased local tissue oxygenation from 65.7 ± 1.1 to 77.9 ± 4.7 % ($P<0.05$) and from 65.9 ± 1.0 to 78.7 ± 3.9 % ($P<0.05$), respectively. After the 25 s blood flow was returned to basal level while tissue oxygenation was about at its peak (ranging from 77.9 to 79.4% in the different conditions, $P<0.05$). Irrespective of whether this condition was caused by prior ESC or MC the hemodynamic response to MC or ESC delivered at this time was considerably attenuated (by 66 to 89% in the different conditions, $P<0.05$).
Conclusions: The contraction-induced hyperemia shares the same dependence on mechanical pre-conditioning that characterizes the compression-induced hyperaemia, supporting the idea of a common mechano-sensitive mechanism, inactivated by increased tissue oxygenation. This mechanism may play a role in limiting hyper-perfusion, thus preserving local homeostasis and systemic resources.

ELECTRICAL ABNORMALITIES AND SARCOLEMMAL T-TUBULAR DISARRAY OF CARDIOMYOCYTES INDUCED BY DOXORUBICIN VS. TRASTUZUMAB CHEMOTHERAPY

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Objective: The combination of monoclonal antibody Trastuzumab (TRZ) and Doxorubicin (DOXO) chemotherapy has reduced the risk for breast cancer recurrence by 50% and mortality by 30%, at the cost of an increased heart failure incidence in survivors. While the cardiotoxicity induced by combined therapy is well known, their effects on electrical and structural properties of cardiomyocytes (CMs) are poorly understood. Using an in-vivo experimental protocol, we analyzed the effect of DOXO and TRZ on CM at the single cell level.
Methods: Rats received 6 intraperitoneal injections of either DOXO (cumulative dose, 20 mg/kg), TRZ alone (cumulative dose 20 mg/kg) or saline (CTRL) over a 2-week period. Echocardiography was performed at different time points. CMs were isolated from both LV and RV free walls using a Langendorff system. Single-cell CM action potentials (APs) were recorded during steady-state pacing at 1 Hz, and quantified as AP duration at 50% (APD50) and 90% (APD90) of repolarization. To assess T-tubular (TT) disarray of sarcolemmal membranes, isolated CMs were incubated with di-3-ANEPPDHQ (20 mmol/L). Eight-bit gray-scaled images were subjected to spatial Fast Fourier Transform analysis for quantification of periodic component of pixel variance. Analyses were performed both at the end of treatment (day 12), 1 week (day 19) and 4 week (day 30) later.
Results: At day 19 LV end-systolic volume (LVESV) and LV ejection fraction (LVEF) significantly impaired in both DOXO and TRZ treated animals compared to CTRL group. The worsening of heart function persist in animals treated with DOXO, but was reverted in TRZ-treated rats at day 30. DOXO- and TRZ-treated rats did not show alterations in RV parameters. At single cell level at day 12 APDs were strongly prolonged in both LV and RV DOXO-CM compared to CTRL-CM. TRZ treatment affected APDs only at day 19 and it was evident only in LV CMs. Delayed after depolarizations (DADs) were significantly increased as well as the frequency of spontaneous

elementary calcium release events (Ca2+-sparks) in both LV and RV DOXO treated cells. The periodic component of transverse TT was decreased in DOXO-CM by 22% and 28.5% in RV and LV CMs, respectively, as compared with controls. Thus was consistent with a structural disarray of CM sarcomeres. In contrast, the periodic component of transverse TT was not affected by TRZ alone.
Conclusions: TRZ and DOXO treatments induce electrophysiological changes (i.e., ADP prolongation) but only the latter is associated with perturbation of sarcomeric structure in CMs. These changes may contribute to doxorubicin-induced arrhythmogenicity.

NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE (NAADP) GATES TWO PORE CHANNEL 1 TO MEDIATE Ca2+ RELEASE FROM ACIDIC Ca2+ SIGNALS IN HUMAN ENDOTHELIAL PROGENITOR CELLS.

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Objective: Nicotinic acid adenine dinucleotide phosphate (NAADP) is the most recently discovered Ca2+-releasing messenger, that increases the intracellular Ca2+ concentration [Ca2+]i by mobilizing the acidic Ca2+ stores of the endolysosomal (EL) system. NAADP acts by gating two pore channels 1 and 2 (TPC1-2), which release a bolus of Ca2+ to the cytoplasm that may be amplified by ryanodine receptors and/or inositol-1,4,5-trisphosphate receptors (InsP3Rs) through the mechanism of Ca2+-induced Ca2+ release. We have recently discovered that a sizeable acidic Ca2+ pool endowed with TPC1, but not TPC2, is also present in human endothelial progenitor cells (EPCs). Our preliminary data indicated that the EL Ca2+ pool could interact with the endoplasmic reticulum (ER) to shape intracellular Ca2+ signals, but we did not investigate either the contribution of TPC1 to NAADP-evoked Ca2+ signals or their involvement to physiological Ca2+ responses. Herein, we sought to assess whether the EL and ER Ca2+ stores actually interact and assessed the role of NAADP-dependent, TPC1-mediated Ca2+ release in ATP- and VEGF-induced intracellular Ca2+ signals in human EPCs.
Methods: Changes in [Ca2+]i were monitored from EPCs loaded with the Ca2+-sensitive dye, Fura-2/AM (2 μM, 30 min), by using a CCD camera.
Results: The lysosomotropic agent GPN caused a robust elevation in [Ca2+]i which did not involve extracellular Ca2+ entry. The same result was obtained when EPCs were challenged with bafilomycin A1, which collapses the pH gradient (ΔpH) necessary for Ca2+ uptake into acidic stores, or nigericin, a protonophore which also dissipates the ΔpH of acidic organelles. A careful analysis of a larger group of cells, however, disclosed that GPN did not reduce the Ca2+ response to cyclopiazonic acid (CPA), a selective inhibitor of ER Ca2+ sequestration. Likewise, CPA did not affect GPN-induced Ca2+ mobilization. Also, NAADP-evoked Ca2+ signals were fully inhibited by GPN, nigericin and genetic suppression of TPC1, while it was unaffected by CPA and 2-APB, a selective InsP3R inhibitor. Finally, VEGF-induced intracellular Ca2+ oscillations were abolished by GPN, nigericin and the genetic suppression of TPC1, while these manoeuvers did not impair ATP-induced Ca2+ signals. Of note, VEGF-induced EPC proliferation was blocked by pharmacologically and genetically interfering with NAADP-dependent Ca2+mobilization.
Conclusions: These results rule out the Ca2+-dependent interaction between the EL and ER Ca2+stores in human EPCs. Nevertheless, NAADP gates TPC1 to mediate EL-dependent Ca2+ release, thereby the proangiogenic Ca2+ response to VEGF.

COOPERATIVE EFFECTS OF Q10 AND VITAMIN D3 ON CARDIAC CELLS

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Objective: In this work, we demonstrate the cooperative effect of Q10 and vitamin D3 on H9c2 cardiac cells.
Methods: The effects of Q10 and vitamin D3 alone or added together on cell viability, nitric oxide and reactive oxygen species productions in cardiac cells have been studied. Moreover, the involvement of PI3K/Akt and ERK/MAPKs pathways leading to eNOS activation and the involvement of VDR have been also investigated. The same agents were also tested in an animal model to verify vasodilation, nitric oxide and reactive oxygen species production.
Results: Data obtained in this work demonstrate for the first time the beneficial and cooperative effect of the stimulation with Q10 and vitamin D3. Indeed, in cardiac cells Q10 and vitamin D3 added together were able to induce a nitric oxide production higher than the production induced by the substances alone. The effect on vasodilation induced by cooperative stimulation have been confirmed *in vivo* model as well.
Conclusion: The use of a combination of Q10 and vitamin D to counteract increased free radical production could be a potential method to reduce myocardial injury or effects of aging on the heart.

ACETYLCHOLINE INDUCES Ca2+ SIGNALS AND NITRIC OXIDE RELEASE FROM HUMAN BRAIN MICRVASCULAR ENDOTHELIAL CELLS.

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Objective: Basal forebrain neurons control intracortical arterioles by releasing acetylcholine (Ach), which stimulates endothelial cells (ECs) to produce the vasodilating gasotransmitter, nitric oxide (NO). Recently, we published the evidence that Ach-induced Ca2+ spikes are shaped by a complex interaction between endoplasmic reticulum (ER) Ca2+ release via InsP3 receptors (InsP3Rs) and store-operated Ca2+ entry (SOCE) and lead to NO release in mouse brain ECs (bEND5) (Zuccolo et al., Cell Calcium 2017). Herein, we sought to assess whether Ach stimulates NO production in a Ca2+-dependent manner also in a human model (hCMEC/D3).
Methods: Changes in [Ca2+]i and NO were monitored from hCMEC/D3 cells loaded with the Ca2+-sensitive dye, Fura-2/AM (2 μM, 30 min), and the NO-sensitive fluorophore, DAF/FM (4 μM, 1 h), respectively, by using a CCD camera.
Results: Ach induced a dose-dependent biphasic Ca2+ signal, which achieves its peak at 100 μM, in hCMEC/D3 cells. Ach-evoked Ca2+ response was shaped by an initial intracellular Ca2+ discharge followed by extracellular Ca2+ entry. Pharmacological manipulation indeed revealed that Ach-inducedCa2+ response in hCMEC/D3 cells involved ER Ca2+ release from InsP3Rs and Ca2+ entry through SOCE. Interestingly, as observed in bEND5 cells, the Ca2+ response to Ach was abolished by atropine, a selective antagonist of muscarinic receptors. Moreover, Ach-induced Ca2+ signal led to robust NO release, that was inhibited by L-NAME, a selective NO synthase blocker. Finally, a throughout transcriptomic analysis revealed that all the major components of the endothelial Ca2+ toolkit are expressed in hCMEC/D3 cells, including InsP3R3, STIM2 and Orai1-3.
Conclusions: Overall, these data demonstrate that Ach is able to induce NO synthesis in a Ca2+-dependent manner also in human microvascular ECs. The difference of the Ach-induced waveforms (Ca2+ oscillations vs. biphasic Ca2+ increase) depends on the different architecture of their Ca2+ signalling toolkit.

THE EFFICACY OF LATE SODIUM CURRENT BLOCKERS IN HYPERTROPHIC CARDIOMYOPATHY IS DEPENDENT ON GENOTYPE: A STUDY ON TRANSGENIC MOUSE MODELS WITH DIFFERENT MUTATIONS.

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Objective: The late-Na+current (INaL) blocker ranolazine reduced the rate of arrhythmogenic events and improved diastolic function in the myocardium of patients with hypertrophic cardiomyopathy (HCM), shortening action potentials and reducing intracellular Na+ and Ca2+ concentration. It is necessary to evaluate whether the efficacy of INaL-blockers in this disease could be influenced by different causing mutations of hypertrophic cardiomyopathy.

Methods: We evaluate the electro-mechanical abnormalities occurring in cardiomyocytes from the hearts of two transgenic HCM mouse models carrying mutations in troponin-T gene (R92Q and E163R) and test how the two different lines respond to INaL-blockers. Cells were treated with ranolazine or with GS-967.

Results: R92Q cardiomyocytes showed increased INaL, lower Ca2+ transient amplitude and elevated diastolic [Ca2+]i. Ca2+transients and INaL recorded in E163R cells were comparable to WT. Both R92Q and E163R cells showed arrhythmogenic events. In R92Q cardiomyocytes, ranolazine and GS-967 shortened action potentials, lowered intracellular [Na+], hastened Ca2+ transient kinetics, reduced diastolic Ca2+, reduced the rate of spontaneous beats and Ca2+ waves. In E163R preparations INaL-blockers did not affect action potentials nor the kinetics or amplitude of Ca2+transients, did not hasten twitch kinetics. Nevertheless, ranolazine and GS-967 reduced diastolic [Ca2+]i and lowered the occurrence of spontaneous beats and Ca 2+ waves in both models.

Conclusions: The novel INaL-blocker GS-967 exerts same effects of ranolazine, albeit at a 20-times lower concentration. Different HCM-related mutations lead to different degrees of cellular remodeling. INaL-blockers exert their full efficacy only in presence of INaL overexpression and severe Ca2+ handling abnormalities, as in R92Q mice. INaL-blockers may exert an antiarrhythmic effect even in the absence of complex EC-coupling abnormalities. Our data suggest that the efficacy of INaL-blockers in HCM patients is dependent on genotype.

CORRELATION BETWEEN HYPERTENSION AND BRAIN NEUROINFLAMMATORY FACTORS IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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Objective: Under hypertensive conditions, the brain areas linked with the blood pressure control are often exposed to severe alterations. In particular, by modifying the blood flow, hypertension is responsible for neuroinflammatory-dependent damages that result in alterations of the structural and functional properties of brain vessels.

Aim: the aim of this work was to evaluate whether the hypertensive disease could modify the expression of pivotal brain neuroinflammatory factors and anti-apoptotic signals such as caspase-1, caspase-3, NF-kB, IL-1β, NLRP3, and iNOS, that are known to be importantly involved in inflammatory pathways.

Methods: Male age-matched Spontaneously Hypertensive Rats (SHR) and normotensive controls rats (WKY), 20-22 weeks old, were used. Brain areas (amygdale, brainstem, hyppocampus, and hypothalamus) were dissected for the subsequent experimental protocols. Blood pressure was measured by the tail cuff method; in situ hybridization assay, western blott and immunohistochemistry analysis were performed.
Results: Increased mRNA levels of caspase-1, NLRP3 and IL-1β were detected in amygdalar, hypothalamic, plus brainstem areas whereas only in few hippocampal sites of SHR respect to the normotensive controls. NF-kB expression was elevated in hippocampal and hypothalamic areas of SHR, while NLRP3 only moderately increased in amygdala and brainstem sites. iNOS expression strongly increased in the brainstem and moderately in the other areas. Immunohistochemical evaluations notably and moderately increased cleaved caspase-3 cell levels in hippocampus and hypothalamus areas, respectively.
Conclusions: Hypertension correlates to the neuroinflammation of the brain areas responsible for the blood pressure control, as evident by the altered levels of key inflammatory molecules. These findings define the possibility to design therapeutic approaches able to improve brain blood flow and subsequently reduce hypertensive-dependent cerebral complications.

SILICA NANOPARTICLES ACTIVELY ENGAGE WITH MESENCHYMAL STEM CELLS IN IMPROVING CARDIAC PRO-REGENERATIVE FUNCTIONAL EFFECTS

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Objective: Transplanted stem-cells may orchestrate postischemic cardiac repair and remodelling after myocardial infarction. We evaluated whether the uptake of silica nanoparticles (SiO 2 -NPs) by human mesenchymal stem cells (hMSCs) could exert any functional effects that might ultimately impact on hMSC regenerative potential.
Methods: After SiO2-NP treatment several assays were performed: Cell adhesion and cell detachment. Focal adhesion complexes, Connexin-43 (Cx43) expression and dye transfer assay were evaluated using flow cytometry and confocal microscopy.
Results: SiO 2 -NP internalization affects the mean area as well as the maturation level of hMSC focal adhesions; these parameters were elevated in SiO 2 -NP -treated hMSCs, accounting for their increased adhesion capacity on fibronectin coated surfaces and their augmented engraftment in the myocardial tissue upon injection in infarcted isolated rat hearts. Furthermore, SiO 2 -NP treatment enhanced hMSC expression of Cx43, the building block protein of cardiac gap junctions, thus favouring their interaction with co-cultured cardiac myoblasts in an ischemia-like environment.
Conclusions: These findings provide strong evidence that SiO 2 -NPs do not only passively interact with hMSCs, but also actively engage in mediating biological effects, some of which might ultimately result in an augmented stem cell regenerative potential.

CIRCULATING LEVELS OF SOLUBLE RAGE DECLINE WITH AGING AND PREDICT AGE-ASSOCIATED CARDIAC REMODELLING

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Objective: High incidence of cardiovascular diseases in the elderly is in part attributable to cardiac remodelling associated to physiological aging. The Receptor for Advanced Glycation End-products (RAGE) is a multi-ligand membrane-bound receptor involved in many inflammatory disorders. RAGE soluble isoform (sRAGE) acts as a decoy molecule being able to block the activation of the membrane-bound protein, and its circulation levels have been found altered in several chronic and acute inflammatory diseases. The aim of this study was to determine whether sRAGE is a biomarker of aging and age-related cardiac remodelling, and evaluate the contribution of RAGE isoforms to cardiac senescence.

Methods: sRAGE levels were evaluated in the serum of healthy subjects from 20-92 years and of 1- to 22-months-old mice by ELISA. Left ventricle (LV) function and remodelling of *Rage-/-* and wild-type (wt) mice were measured by 2D-echocardiography. Immunohistochemistry determined cardiac collagen levels. Protein and gene expression were assessed by Western Blot and RT-PCR, respectively.

Results: We found a significant decrease of circulating sRAGE with age in mice. Notably, serum sRAGE negatively or positively correlates with LV dimensions or function, respectively. Interestingly, no detectable amount of any RAGE isoforms was found in murine LV, however, *Rage-/-* mice displayed a significant increase of LV volumes and diameters in diastole and systole, and a concomitant decrease in Ejection Fraction and Fractional Shortening, compared to age-matched wt animals during aging. Moreover, *Rage-/-* mice exhibited higher deposition of collagen content and heart failure marker genes expression (BNP and Ankrd1) with senescence in respect to the wt counterpart. Finally, human studies confirmed a strong inverse correlation of serum sRAGE with chronological age in healthy subjects.

Conclusions: Our results indicate that circulating sRAGE is a biomarker of healthy aging and age-related cardiac changes. The absence of RAGE in mice exacerbates cardiac remodeling with senescence. Altogether, our data suggest that, among RAGE isoforms, sRAGE may play a pivotal role in determining intrinsic heart ageing.

FIRST EVIDENCE ON THE NOVEL HYPOTHALAMIC PEPTIDE PHOENIXIN-14 AS CARDIAC MODULATOR AND CARDIOPROTECTIVE IN NORMAL AND OBESE RATS

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Objective: Phoenixin (PNX) is a peptide identified in 2013. It is mainly expressed in the hypothalamus in which it shares a distribution patter comparable to nesfatin-1, a peptide with anorexic and cardiovascular activities. It is able to cross the blood-brain barrier and this suggests that it elicits peripheral functions. Preliminary mass spectrography data indicate that PNX is present in the mammalian heart. This study aimed to quantify PNX in heart and plasma in of normo-weight and obese rats, and to evaluate if the peptide influences the myocardial function under basal condition and in the presence of ischemia/reperfusion (I/R).

Methods: By ELISA, PNX was detected in both hypothalamus and heart.

Results: In the plasma, PNX levels increased during the post-prandial phase in normal but not in obese rats. On the isolated and Langendorff perfused rat heart exogenous PNX determined negative inotropism and lusitropism. Western Blotting of cardiac extracts revealed that Erk1/2, Akt and eNOS phosphorylation increased after exposure to increasing concentrations of PNX. PNX (EC50 dose),

administered in post-conditioning, induced a better systolic recovery and a smaller infarct size with respect to hearts exposed to I/R alone. PNX-dependent cardioprotection was mediated by RISK and SAFE cascades and apoptosis inhibition. Preliminary data obtained in obese rats showed that PNX post-conditioning cardioprotection is obtained only at a concentration (1 nM) that is higher than that required in normo-weight rats.
Conclusions: This study represents a first evidence on the involvement of PNX in cardiac modulation, particularly in relation to normal and pathological conditions.

ARE THE NANOPARTICLES FRIENDS OR FOES WHEN INHALED?

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Objective: Recent applications in nanomedicine focus on nanoparticles as they are promising tools for site-specific delivery of drugs and diagnostic agents, through the possibility to functionalize their surface with target-specific ligands. Recently, we showed that among the different administration routes, pulmonary delivery is feasible not only for the local treatment of airway diseases but also for the systemic administration. Our results suggest that pulmonary administration could be exploited for delivery of nanoparticles designed for brain therapy. On the other hand a big claim rised up about the emerging evidence suggesting that living near major roads might adversely affect health. Indeed despite the mounting global effect of cardiovascular and neurodegenerative diseases, their cause remains largely unknown. Concern is growing that exposures associated with air pollution and mainly to the inhaled ambient fine particles might contribute to these pathology. We will try to outline a road map in order to disclose the inner mechanisms by which nanoparticles interact with microvascular endothelial cells thus triggering effects induced at the endothelial level further linking with systemic and site specific inflammation.

ENHANCEMENT OF CARDIAC DIFFERENTIATION OF MOUSE PLURIPOTENT STEM CELLS BY β3 ADRENOCEPTOR STIMULATION

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Objective: Identification of signalling mechanisms able to enhance cardiac differentiation of stem cells is important to envisage cardiac stem cell therapy. Previous studies have shown that β-adrenoceptors (β-Rs) stimulation through activation of β1 and β2-Rs enhances cardiomyogenesis of mouse embryonic stem cells (mES) beyond survival and proliferation of cardiac progenitor cells (CPCs). In heart failure (HF) it is established that β1 and β2-Rs have different effects on cardiomyocytes compared to β3-R. A recent clinical trial has showed that β3-Rs stimulation by mirabegron increased left ventricular ejection fraction (LVEF) in patients with severe chronic HF. Despite β3-R role in adult heart is extensively studied and its expression has been detected in mES, no data exist on β3-Rs during cardiac maturation.
Methods. mES were differentiated into the cardiogenic lineage in control conditions (Ctr) and with BRL37344 (7μM) or SR59230A (10μM), selective β3-Rs activator or inhibitor of, respectively.
Results. WB analysis showed that throughout cardiac differentiation β1-R was highly expressed in the early phase and significantly declining thereafter; by contrast, both β2 and β3 have a rather stable expression during the process. Development of spontaneous contractile activity in differentiating

mES was modulated by β3-R selective stimulation or inhibition, suggesting a possible developmental role of β-Rs in the cardiogenic process. Q-PCR analysis revealed that compared to Ctr, β3-R stimulation significantly increases expression level of cardiogenic genes, including mesodermal marker (d5: brachyury ≈+760%), precardiac (d5: Wnt3A ≈+25000%, Wnt11 ≈+20000% and β-catenin ≈+725%), first heart field (d7: Mef2C ≈+1360%, Hand1 ≈+1000%) and late cardiac markers (d7: Gata4 ≈+60%, Nkx2.5 ≈+62%, Tbx5 ≈+44%, Tbx20 ≈+150%), with negligible effects on second heart field markers. Oppositely, β3-R blockade severely reducing significantly the expression of mesodermal (d5: brachyury ≈-50%, Mesp1 ≈-96%), precardiac (d5: Wnt3A ≈-99%, Wnt11 ≈-88%), first (d7: Mef2C ≈-90%, Hand1 ≈-95%) and second (d7:hand2 ≈-99%, Isl1 ≈-84%, Tbx1 ≈-71%, Fgf10 ≈-90%) heart field and late (d7: Gata4 ≈-90%, Nkx2.5 ≈-95%, Tbx5 ≈-95%, Tbx20 ≈-95%) cardiac markers. Interestingly, pluripotency marker (d2 Oct4 ≈+193%) and neurogenic phenotype (d14 Msl1 ≈+100%) were significantly upregulated by β3-Rs blockade, while the endodermic marker Sox17 was suppressed (d14 ≈-60%), suggesting that disruption of β3-R signalling likely shifts differentiation toward the neurogenic lineage. Furthermore, measurements of EBs during the early differentiation phase revealed that β3-R stimulation produced a significant elongation of diameters (d7 from 680±20 to 917±60μM), thus suggesting an involvement of β3-Rs with signalling pathways regulating myogenic cell proliferation and/or hypertrophy. Moreover, frequency of spontaneous contraction was significantly enhanced by β3-R stimulation (d12 from 27±0.6 to 37±1.4bpm), suggesting that electrogenic mechanisms driving mES spontaneous beating may be regulated by β3-R signalling. We further explored this point by analysis of genes involved in sinoatrial node (SAN) formation (d7: Tbx3≈ 1100%, Tbx18≈+7000% and Shox2≈+4200%), development (d7 cTnI≈+5700%, Cav1.3≈+5700%) and function (d8 Hcn1≈+7000%, Hcn4≈+1300%, at day 8 Trpc1≈+88% and Trpc3≈+95%) and observed that β3-R stimulation causes a robust up-regulation. Finally, we evaluated atrial and ventricular myosin light chains (d7 mlc2a≈+3100% and mlc2v≈+280%) and confirmed a shifted toward atrial phenotype due to stimulation.

Conclusions. In conclusion, β3-Rs are functionally expressed in mES undergoing cardiogenic differentiation and mediate an important signal to enable cardiac specification of mES.

TREATMENT OF HFPEF: AN UNRESOLVED ENIGMA

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Objective: Heart failure (HF) with preserved ejection fraction (HFpEF) accounts for 50% of HF cases. While the majority of HFpEF patients do not have a recognized primary cardiac pathology, they are of advanced age, more often female and have high prevalence of non-cardiac comorbidities, such as hypertension, obesity, diabetes, chronic obstructive pulmonary disease and chronic kidney disease. In a recently proposed paradigm, HFpEF is regarded as a systemic syndrome mediated in large part by risk factors and co-morbidities, resulting in a systemic multi-organ proinflammatory state which affects the heart, leading to myocardial remodelling and dysfunction through a signalling cascade, which begins with coronary microvascular endothelial dysfunction. It subsequently involves myocardial infiltration by activated macrophages, which induce reactive interstitial fibrosis and altered communication between endothelial cells and surrounding cardiomyocytes. Current guidelines confirm that no treatment has been shown to reduce morbidity and mortality. Our recent studies aimed to determine whether the chronic administration of sitagliptin (SITA) or ranolazine (RAN) affects the course of LV dysfunction in a Dahl salt-sensitive (Dahl/SS) rat model of HFpEF. When fed high-salt diets, Dahl/SS rats develop hypertension, renal failure, insulin resistance and dyslipidemia. The development of HF by 19 weeks is not associated with a decrease in LV systolic function or an increase in LV end-diastolic diameter, which mimics the characteristics of clinically observed HFpEF. The widespread expression of dipeptidyl peptidase 4 (DPP4) in vasculature,

myocardium and immune cells raises the possibility that this protein plays a role in cardiovascular function. In particular, the finding that DPP4 activity is often associated with inflammation and cardiac remodelling points to an involvement of DPP4 in the pathophysiology of HF. Additionally, one of the potential mechanisms involved in HFpEF pathophysiology is an increase late Na+ current (*I*Na) in cardiac myocytes. RAN by selectively inhibiting late *I*Na can decrease Na+-dependent calcium accumulation and is expected to promote Ca2+ extrusion through the Na+/Ca2+ exchanger improving myocyte relaxation and diastolic tension, as shown in several preclinical and clinical settings. SITA positively modulated active relaxation and passive diastolic compliance interfering with inflammatory-related endothelial dysfunction and fibrosis associated with HFpEF. Because of a non-diabetic nature of our model and unaltered blood glucose levels, the cardioprotective action of SITA lays beyond its effect on glycaemia. Inhibition of late *I*Na by administration of RAN resulted in the improvement in diastolic function without significant changes in blood pressure. In this view, the findings presented here provide insights into the role of DPP4 inhibition and a decrease of the late *I*Na in HFpEF. Because of the pathophysiological complexity and a significant clinical heterogeneity of patients that present with an HFpEF syndrome, our results may be relevant to the specific patient subpopulation.

STEM WAVES FOR TISSUE HEALING

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Objective: We play a part in the Universe's electromagnetic vibrations and sounds. As in the Universe, in biological organisms rhythmic oscillation and synchronization of oscillatory patterns are an essential requisite for recognition and connectedness. A major result from the emergence of coherent biological rhythms is *cell polarity*. In somatic and stem cells, *cell polarity* results from and acts on the modulation of cellular ion fluxes, electric fields, and vibrational patterns of the cytoskeleton and nucleoskeleton. Cell polarity is crucial in the physiological modulation of stem cell differentiation and aging, as shown by the fact that altered cell polarization invariantly associates with disease, pathological aging and cancer. Here, we will discuss our recent findings showing that properly conveyed radioelectric fields are able to: (i) enhance the differentiating potential of mouse embryonic stem cells, (ii) induce pluripotency in human adult stem cells, promoting their differentiation into cardiac, neural, skeletal muscle and endothelial cells, (iii) afford direct reprogramming towards the same lineages in human somatic cells (dermal fibroblasts), (iiii) reverse human stem cell aging *in vitro*, (iiiii) reprogram PC12 cancer cells into dopaminergic neurons, and (iiiiii) optimize stem cell polarity.
We will also discuss the role of mechanical vibrations in the modulation of (stem) cell signaling networks and cellular expression of multilineage potential. On the whole, we would like to present an evolving picture of cells capable to perceive themselves as a rhythmic component of the universe, sensing and producing magnetic fields and sound vibrations, progressing through transition states interspaced by the emergence of ordered *images* of structure and function. We can now govern the appearance of these *images* with electromagnetic and acoustic energies. Due to their diffusive nature, we are able to target and reprogram the stem cells where they are, in all tissues of the body. This strategy promotes our natural ability for self-healing, affording a regenerative medicine without the needs for stem cell transplantation.

HIGH GLUCOSE EXPOSURE PROMOTES ACTIVATION OF PRO-INFLAMMATORY PATHWAYS IN CORD BLOOD-DERIVED CD34+ STEM CELLS

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Objective: Diabetic patients exhibit a systemic pro-inflammatory state that correlates with their increased propensity to atherosclerosis development. In addition, as consequence of hyperglycemia, they present a decreased number and dysfunctional circulating CD34+ cells that under normal physiological conditions contribute to vascular homeostasis. However, these precursors also give rise to important effector cells of the innate immune system. We hypothesized that uncontrolled hyperglycemia might skew CD34+ cells phenotype towards inflammatory cells. Purpose: We sought to evaluate whether high glucose exposure of CD34+ stem cells promotes activation of inflammatory pathways.
Methods: CD34+ cells were purified from cord blood of healthy donors and expanded in normal-glucose (NG; with 30 mM mannitol for osmotic control) or high-glucose (HG; 30 mM) serum-free medium plus cytokines (FLT3 50 ng/ml, SCF 50 ng/ml, IL3 20 ng/ml and IL6 20 ng/ml) for up to 20 days. The expression of NF-kB p65, KAT2B/PCAF, and TNFα genes was evaluated by quantitative real-time PCR. Western Blot was used to evaluate acetylation of NF-kB p65 at lysine-310.
Results: After 20 days the expression of NF-kB p65 and TNFα genes were significantly up-regulated in HG-CD34+ cells when compared to NG-CD34+ cells (t-test; $p=0.0034$ and $p\leq0.05$ vs NG respectively). Interestingly, KAT2B/PCAF gene, a histone acetyltransferase implicated in NF-kB p65 acetylation and coactivation, also resulted overexpressed (t-test; $p=0.0225$). We therefore assessed the acetylation of NF-κB p65 at level of lysine-310. This posttranslational modification is critical for nuclear stabilization and full transcriptional activity of NF-κB, responsible for the expression of inflammatory genes. The analysis revealed an increased acetylation at lysine-310 in HG-CD34+ cells.
Conclusions: These results, although preliminary, suggest that hyperglycemia through activation of inflammatory pathways might skew CD34 cells differentiation into cell lineage with pro-inflammatory properties.

VITAMIN D REPLACEMENT AMELIORATES SERUM LIPOPROTEIN FUNCTIONS, ADIPOKINE PROFILE AND SUBCLINICAL ATHEROSCLEROSIS IN PRE-MENOPAUSAL WOMEN

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Objective: Low vitamin D (vitD) has been linked to increased cardiovascular (CV) risk, but the effects of vitD supplementation on CV disease are not clarified. We evaluated the impact of vitD normalization on lipoprotein functions, such as the HDL cholesterol efflux capacity (CEC), which is inversely correlated to CV risk, the pro-atherogenic serum cholesterol loading capacity (CLC), adipokine profile and subclinical atherosclerosis in women.
Methods: Healthy premenopausal women with vitD deficiency (n=31) underwent vitD supplementation. HDL CEC and serum CLC was measured by a radioisotopic and fluorimetric assay respectively. Serum adipokines were measured by ELISA. Subclinical atherosclerosis was evaluated by flow-mediated dilation (FMD), pulse wave velocity (PWV) and augmentation index (AIx), measured with standard techniques.
Results: VitD replacement restored normal levels of serum 25-hydroxyvitamin D (25OHD). Total CEC significantly improved (+ 19.5%; $p<0.01$), with a specific increase in the ABCA1-mediated CEC (+ 70.8%; $p<0.0001$). No change was observed in aqueous diffusion nor in the ABCG1-mediated CEC. Serum CLC was significantly reduced (-13.3%; $p = 0.0026$). Serum levels of adiponectin were increased (+50.6%; $p<0.0001$) and levels of resistin were decreased (-24.3%; $p<0.0001$). HDL CEC, serum CLC and adipokine modifications were accompanied with a significant

improvement in FMD (+4%; *p<0.0001*), PWV (-4.1%: *p<0.0001*) and AIx (-16.1%; *p = 0.0015*). After vitD replacement, an inverse relationship was found linking resistin levels with the ABCA1-mediated CEC (r2 = 0.220; *p = 0.009*).
Conclusion: Our data support the use of vitD to ameliorate vascular health in vitD deficient subjects with low CV risk.

CARDIOVASCULAR EFFECTS OF MULTIPLE MIELOMA THERAPY WITH PROTEASOME INHIBITORS

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Objective: Proteasome inhibitors (PI) have now become the cornerstone of multiple myeloma pharmacological therapy. Carfilzomib is an irreversible and selective inhibitor of the chymotrypsic-like activity of the 20S proteasome; Bortezomib is a non-selective and reversible inhibitor of proteasome. The aim of our research was I) evaluate the cardiovascular effects of the administration of Carfilzomib and Bortezomib; II) explore the use of Endopat and Sphygmocor in this setting; III) hypothesize protocols for prevention of cardiovascular side effects.
Methods: We conducted an observational study on patients naïve to other chemotherapies, initiated for first-line therapy with Carfilzomib or Bortezomib. Patients should not have heart disease. We conducted serial assessments of the following parameters: aortic stiffness by Sphygmocor, endothelial function by EndoPAT system, systolic and diastolic parameters by transthoracic echocardiography, blood sampling for nt-pro-BNP and TpI evaluation (cardiotoxicity markers).
Results: We evaluated 21 patients treated with Carfilzomib and 18 patients with Bortezomib. Patients receiving Carfilzomib had a rise in pressure values (systolic blood pressure increased by 10 mmHg, diastolic pressure increased by 5 mmHg, p value <0.05). After 4 months we found alteration of diastolic function in patients treated with carfilzomib (reduction of diastolic tissue Doppler values, increasing PAPs) without modification of contractility parameters. Endothelial function and pulse wave velocity changed very rapidly only in patients treated with Carfilzomib (RHI from 1.96 to 1.47 after 1 cycle, p value <0.05, PWV from 5,6 m/sec to 7,04 m/sec, p value < 0,05). After 8 months we observed that patients with Carfilzomib had more cardiovascular adverse events requiring therapeutic modifications (need for adaptation of antihypertensive therapy, dyspnea, echocardiographic congestion, arrhythmias, Hazard Ratio for Carfilzomib vs Bortezomib: 4,9, log rank test, p value 0.026). At multivariate analysis, baseline endothelial dysfunction and arterial hypertension are correlated with the risk of events (HR 5 and 7 respectively, p value <0.05).
Conclusions: Our findings confirm the differences between the side effects of the two proteasome inhibitors even when used as first line therapy and reaffirm the need for screening of patients who will have to undergo cancer therapy, even when therapy have shown safety on the left ventricular systolic function; finally, for patients treated with proteasome inhibitors, our results suggest that, for prevention of side effects, the target of blood pressure should be lower than the limit of 140/90 mmHg, indicated for the treatment of hypertension.