SIRC

Società Italiana di Ricerche Cardiovascolari

2nd Workshop on
New Roads in Cardiovascular Research

Pisa, 13 ottobre 2014
Aula Magna Scuola Superiore Sant'Anna,
Piazza Martiri della Libertà 33

Lunedì 13 ottobre 2014

AULA MAGNA

8.15 – 8.30 CERIMONIA DI APERTURA con i saluti del Presidente della SIRC, Prof. Pasquale Pagliaro, e del Direttore dell'Istituto di Scienze della Vita, della Scuola Superiore Sant'Anna, Prof. Mario Enrico Pè

8.30 – 9.30 SESSIONE I: CARDIAC DRUGS AND CARDIOTOXICITY

Moderatori: Proff. Federico Quaini e Carlo Gabriele Tocchetti

8.30 – 9.00 Comunicazioni

1) **Ameri P**, et al. "TESTOSTERONE, BUT NOT 17B-ESTRADIOL, ANTAGONIZES DOXORUBICIN-INDUCED SENESCENCE OF CARDIOMYOCYTES" - Università di Genova.

2) **Gervasi A**, et al. "STRUCTURAL CHARACTERIZATION OF THE HEART IN TRASTUZUMAB OR TDM-1 TREATED BALB/C NUDE MICE CARRYING XENOTRANSPLANTED HUMAN ADENOCARCINOMA**"** - Università di Parma.

3) **Rebuzzini P**, et al. "ARSENIC TRIOXIDE ALTERS THE SARCOMERE ORGANISATION AND CONTRACTILE PROPERTIES OF CARDIOMYOCYTES DIFFERENTIATED FROM MOUSE EMBRYONIC STEM CELLS" – Università di Pavia.

9.00 – 9.30 Discussione

9.30 – 10.45 SESSIONE II: ANGIOGENESIS AND MOLECULAR BASES OF CARDIOVASCULAR DISEASES

Moderatori: Proff. Francesco Moccia e Claudia Penna.

9.30 – 10.10 Comunicazioni

1) **Giovannelli G**, "HYPEROSMOLARITY-ENHANCED COX-2 EXPRESSION CONTRIBUTES TO HIGH GLUCOSE-INDUCED MICROANGIOPATHY" – Università "G. D'Annunzio" di Chieti

2) **Coppini R**, et al. "MYOCARDIAL DYSFUNCTION IN HYPERTROPHIC CARDIOMYOPATHY: PRIMARY EFFECTS OF SARCOMERIC MUTATIONS VERSUS SECONDARY CARDIOMYOCYTE REMODELING" – Università di Firenze

3) **Squarzanti DF**, et al. "EFFECTS OF VITAMIN D ON CULTURED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS UNDERGOING OXIDATIVE STRESS" – Università del Piemonte Orientale

4) **Collini M**, et al. "FLUORESCENCE CROSS-CORRELATION SPECTROSCOPY METHODS FOR IN-VIVO BLOOD VELOCIMETRY" – Università di Milano-Bicocca

10.10 – 10.45 Discussione

10.45 – 11.00 BREAK

11.00 – 11.20 Key lecture: Prof. Pasquale Pagliaro

"OXIDATIVE/NITROSATIVE SIGNALING AND CARDIOPROTECTION"

11.20 – 13.20 SESSIONE III: GENETIC AND PHARMACOLOGICAL MODULATION OF CARDIOVASCULAR DISEASES

Moderatori: Proff. Tommaso Angelone, Vincenzo Lionetti, Fabio Mangiacapra, Laura Sartiani

11.30 – 13.00 Comunicazioni

1) **Brancaccio M**, et al. "OVEREXPRESSION OF THE MUSCLE SPECIFIC CHAPERONE MELUSIN DELAYS HEART FAILURE AND MORTALITY IN A MOUSE MODEL OF EMERY DREYFUS CARDIOMYOPATHY" – Università di Torino

2) **Cantafio P,** et al "CATESTATIN IMPROVES THE FRANK-STARLING RESPONSE IN NORMOTENSIVE AND HYPERTENSIVE RAT HEARTS" - Università della Calabria

3) **Casieri V**, et al. "PARKIN IS INVOLVED IN THE CARDIOPROTECTIVE EFFECT OF DIETARY PASTA INTAKE ENRICHED WITH BARLEY (1-3)BETA-D-GLUCAN ON CARDIAC ISCHEMIA REPERFUSION INJURY IN MICE" – Scuola Superiore Sant'Anna di Pisa

4) **Folino A**, et al "TRANSACTIVATION OF EGF RECEPTORS IS INVOLVED IN APELIN-INDUCED PROTECTION AGAINST ISCHEMIA-REPERFUSION INJURY", Università di Torino

5) **Marino F**, et al "PREVENTIVE AND THERAPEUTIC EFFECT OF STAT3 OR COMPLEMENT C3 DOWN-REGULATION IN TWO DIFFERENT MODELS OF AUTOIMMUNE MYOCARDITIS", Università di Torino

6) **Morano M**, et al "NRG-1 RECEPTOR ERBB3, AND NOT ERBB2 AND ERBB4, IS UP-REGULATED AFTER EX-VIVO MYOCARDIAL INFARCTION, WITH OR WITHOUT POST-CONDITIONING PROTECTION", Università di Torino

7) **Mazzoni L**, et al "RANOLAZINE REDUCES ARRHYTHMOGENICITY IN TRANSGENIC MOUSE MODELS OF HYPERTROPHIC CARDIOMYOPATHY", Università di Firenze

8) **Scavello F**, et al "CHRONIC CATESTATIN TREATMENT IMPROVES METABOLIC AND CARDIOVASCULAR COMPLICATIONS IN DIET-INDUCED OBESITY IN RATS", Università della Calabria

13.00 – 13.30 Discussione

13.30 – 14.15 Pranzo e visione dei poster

14.15 – 15.45 POSTER SESSION

Moderatori: Proff. Vincenzo Lionetti, Francesco Moccia, Pasquale Pagliaro, Federico Quaini

1) **Ariano C**, et al "METHOTREXATE AT LOW DOSES REDUCES THE RISK OF CARDIOVASCULAR MAJOR EVENTS AMONG PATIENTS WITH CHRONIC INFLAMMATORY DISEASES: RESULTS FROM A META-ANALYSIS OF OBSERVATIONAL STUDIES", Presidio Sanitario Intermedio "Elena d'Aosta", Napoli

2) **Barisione C**, et al "INDOXYL SULFATE PRIMES MONOCYTE DIFFERENTIATION INTO PRO-FIBROTIC M2 MACROPHAGES: CLINICAL IMPLICATIONS IN ABDOMINAL AORTIC ANEURYSM", Università di Genova

3) **De Vecchis R**, et al "INTRAVENOUS DIURETICS VS. ISOLATED ULTRAFILTRATION FOR ACUTE DECOMPENSATED HEART FAILURE: A SYSTEMATIC REVIEW WITH METAANALYSIS", Presidio Sanitario Intermedio "Elena d'Aosta", Napoli

4) **Mancardi D**, et al "HYDROGEN SULFIDE AND CYSTATHIONINE Γ-LYASE IN THE FAILING HEART", Università di Torino

5) **Martelli C**, et al "NON-INVASIVE OPTICAL IMAGING PROCEDURES FOR THE IN VIVO EVALUATION OF CELL POPULATIONS AND MOLECULAR PROCESSES", Università di Milano

6) **Mattioli**, et al "PLATELET ACTIVATION IN EXTRACORPOREAL CIRCULATION: EFFECTS OF UNFRACTIONATED HEPARIN ON DAMAGES INDUCED BY BIO-INCOMPATIBILITY", Università di Modena e Reggio Emilia

7) **Neri T**, et al "PARTICULATE MATTER INDUCES THE EXPRESSION OF PROCOAGULANT MICROPARTICLES BY HUMAN MONONUCLEAR CELLS", Università di Pisa

8) **Paccosi S**, et al "INFLAMMATORY MOLECULES AFFECT THE INTERACTION BETWEEN HUMAN DENDRITIC CELLS AND VASCULAR SMOOTH MUSCLE CELLS", Università di Firenze

9) **Roatta S**, et al "MECHANO-SENSITIVE RAPID DILATATION IN SKELETAL MUSCLE: A NOVEL TYPE OF VASCULAR REACTIVITY", Università di Torino

10) **Tullio F**, et al "HAS TFR2 BETA ISOFORM A ROLE IN CARDIAC IRON METABOLISM AND CARDIOPROTECTION", Università di Torino

15.45 – 16.15 Key note lecture: Prof. Federico Quaini

"STEM CELLS AND CARDIAC REGENERATION"

16.15 – 17.45 SESSIONE IV: STEM/PROGENITOR CELLS

Moderatori: Proff. Rosalinda Madonna e Francesco Moccia

16.15 – 17.15 Comunicazioni

1) **Allievi L**, et al "HYPERGLICEMIC 'MEMORY' AFFECTS COMMITMENT OF $CD34^+$ CORD BLOOD-DERIVED STEM CELLS INTO FUNCTIONAL ENDOTHELIAL PROGENITOR CELLS", IRCCS Centro Cardiologico Monzino, Milano

2) **Falco A**, et al "DYNAMIC SUSPENSION CULTURE OF CARDIAC PROGENITORS TO DEVELOP INJECTABLE SCAFFOLDS FOR CARDIAC TISSUE ENGINEERING", Università di Parma

3) **Gallina G**, et al "HUMAN MESENCHYMAL STEM CELLS LABELED WITH FLUORESCENT SILICA NANOPARTICLES APPEAR TO HAVE A DIFFERENTIAL MIGRATORY BEHAVIOR IN AN *EX -VIVO* RAT MODEL OF MYOCARDIAL INFARCTION", Università di Torino

4) **Poletto V**, et al "THE INTRACELLULAR Ca^{2+} TOOLKIT REGULATES VEGF-MEDIATED GENE EXPRESSION IN NORMAL, BUT NOT TUMORAL, HUMAN CIRCULATING ENDOTHELIAL PROGENITOR CELLS", IRCCS Policlinico San Matteo, Pavia

5) **Spinelli V**, et al "MOLECULAR AND FUNCTIONAL ROLE OF REACTIVE OXYGEN SPECIES (ROS) DURING CARDIAC DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS (MESC)", Università di Firenze

6) **Teberino MA**, et al "TRANSPLANTATION OF ADIPOSE TISSUE MESENCHYMAL CELLS CONJUGATED WITH PLGA MICROSPHERES FOSTER C-KIT+ PROGENITOR CELLS AND PROMOTES REVASCULARIZATION AND TISSUE REPAIR THROUGH PARACRINE SIGNALING IN A MURINE MODEL OF ACUTE MYOCARDIAL INFARCTION", Università "Gabriele D'Annunzio" di Chieti

17.15 – 17.45 Discussione

18.00 – 19.00 Assemblea dei Soci della Società Italiana di Ricerche Cardiovascolari

TESTOSTERONE, BUT NOT 17B-ESTRADIOL, ANTAGONIZES DOXORUBICIN-INDUCED SENESCENCE OF CARDIOMYOCYTES

Altieri P[1], Bollini S[2], Barisione C[1], Canepa M[1], Tocchetti CG[3], Bezante GP[1], Spallarossa P[1], Brunelli C[1], Ameri P[1]
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Chronic anthracycline cardiotoxicity is less common in males than in females. Here, we hypothesized that this gender difference might be at least in part secondary to distinct activities of sex hormones on cardiomyocyte senescence, which is thought to be central to the development of anthracycline long-term cardiomyopathy. Neonatal murine cardiomyocytes and H9c2 cardiomyoblasts were treated with doxorubicin alone or in combination with testosterone or 17β-estradiol, the main androgen and estrogen, respectively. As previously reported, a single 3-h pulsed exposure to doxorubicin resulted in accumulation of p53, down-regulation of telomere binding factor 2 (TRF2) and, ultimately, extensive senescence of cardiomyocytes. Of note, senescence remained significantly more frequent in treated than untreated cells up to 21 days after incubation with doxorubicin. Testosterone counteracted both immediate and delayed senescence elicited by doxorubicin, while 17β-estradiol had no effect. At the molecular level, testosterone caused phosphorylation of AKT and prevented the changes in p53 and TRF2 triggered by doxorubicin. Pre-treatment with the androgen receptor (AR) antagonist, flutamide, and the phosphatidylinositol 3 (PI3) kinase inhibitor, LY294002, abrogated the reduction in senescence, as well as AKT activation and normalization of p53 and TRF2 levels, attained by testosterone. We conclude that testosterone, but not 17β-estradiol, protects against doxorubicin-induced senescence of cardiomyocyte by modulating p53 and TRF2 via the AR-PI3K-AKT pathway. This is a potential mechanism by which males are less prone to chronic anthracycline cardiotoxicity than females.

STRUCTURAL CHARACTERIZATION OF THE HEART IN TRASTUZUMAB OR TDM-1 TREATED BALB/C NUDE MICE CARRYING XENOTRANSPLANTED HUMAN ADENOCARCINOMA

Gervasi A[1], Schirripa ML[1], Madeddu D[2], Frati C[2], Graiani G[3], Ricci P[1], Cretella D[2], Saccani F[2], Alfieri R[2], Lagrasta C[1], Quaini F[2]
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Trastuzumab (TSZ) is a potent anticancer monoclonal antibody against HER2, a human epidermal growth factor receptor. Both short- and long-term cardiotoxicity is a key safety issue with the use of TSZ. Trastuzumab emtansine (a microtubule polymerization inhibitor, T-DM1) is a novel HER2 directed antibody–drug conjugate effective against several cancers able to overcome TSZ resistance. No data are available on the cardiotoxic profile of T-DM1. Thus, we investigated drug efficacy and cardiac damages of TSZ and T-DM1 in BALB/c nude mice with tumour xenografts generated by subcutaneous injection of $8x10^6$ Calu-3 (human adenocarcinoma cell line). Mice carrying stable xenografted tumours were randomized in three groups to receive once a week: Control - saline solution 1ml/kg; TSZ- 15 mg/kg; T-DM1- 15mg/kg. After three weeks mice were sacrificed, tumour nodules and hearts were excised and myocardial sections were stained with Masson's trichrome to evaluate the presence of fibrosis and immunolabeled with smooth muscle actin (α-sma) for the distribution and density of arterioles. Finally, apoptosis was assessed by the TUNEL assay. Results indicated that both drugs increased myocardial fibrosis and induced cardiomyocyte apoptosis. However, in the presence of a superior anti-tumour activity, T-DM1 slightly attenuated myocardial collagen deposition and increased arteriolar density compared to TSZ. Thus, antibody mediated HER-2 inhibition is associated with myocardial fibrosis, apoptosis and redistribution of arterioles. The identification of specific factors implicated in adverse cardiovascular effects by different anti-cancer agents is essential to find effective strategies able to prevent cardiotoxicity.

ARSENIC TRIOXIDE ALTERS THE SARCOMERE ORGANISATION AND CONTRACTILE PROPERTIES OF CARDIOMYOCYTES DIFFERENTIATED FROM MOUSE EMBRYONIC STEM CELLS

Rebuzzini P[1], Cebral E[2], Mazzini G[3], Fassina L[4,5], Redi CE[1,6], Zuccotti M[7], Garagna S[1,5]

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Arsenic, a widely diffused environmental toxicant, exerts toxic effects on the cardiovascular system and on cardiomyocytes. However, the knowledge of its effects during the process of cardiomyocytes differentiation is meagre. Here, we differentiated mouse embryonic stem cells into cardiomyocytes in the presence of 0.1, 0.5 or 1.0 µM As_2O_3. At 1.0 µM As_2O_3 concentration, when compared to controls, treated samples show: 1) reduced size in diameter of embryoid bodies; 2) decreased expression of *Brachyury*, *Nkx2.5* and *Tnnc1* and increased *Gata-4*; 3) inefficient and delayed acquisition of the beating capacity, 4) smaller beating areas; 5) reduction of the beat frequency, contractility, contraction force and kinetic energy (kinematics and dynamics properties); 6) cardiomyocytes with disorganized and disoriented sarcomeres (immunofluorescence with α-actinin and TroponinT antibodies) and 7) altered syncytial organisation, as evidenced by more rarefied Connexin 43 foci distribution. At 0.1 µM and at 0.5 µM As_2O_3 concentration, although neither the timing of acquisition of the beating capacity nor the pattern of expression of cardiac-specific genes are not or less affected and the cardiac junctions and the sarcomeres in the syncytia are less frequently disorganised, the kinematics and dynamics properties of the beating syncytia are significantly altered. Overall, these results indicate that the presence of As_2O_3 throughout differentiation leads to the formation of morphologically and functionally altered cardiomyocytes. The magnitude of the damage depends on the concentration: whilst at the highest dose the differentiation process is altered since its early stages, at the lowest doses the effects are evident only in differentiated cardiomyocytes.

HYPEROSMOLARITY-ENHANCED COX-2 EXPRESSION CONTRIBUTES TO HIGH GLUCOSE-INDUCED MICROANGIOPATHY

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Aim/hypothesis: Diabetic hyperglycemia increases plasma osmolarity, leading to adaptive cellular responses. Cycloxygenase-2 (COX-2) plays a role in angiogenesis and plaque stability. We tested the hypothesis that glucose-induced hyperosmolarity promotes angiogenesis through activation of COX-2 expression.
Methods: Human aortic endothelial cells (HAEC) and dermal microvascular endothelial cells (HMVEC) were incubated with 5.5 mmol/L glucose (normoglycemia), high glucose (HG, at 12.5, 25 and 45 mmol/L), or equimolar concentrations of the hyperosmolar control mannitol (HM).
Results: Both HG and HM increased the expression of the water channel aquaporin-1 (AQP1) and of COX-2. HG and HM for 1 h increased the nuclear accumulation of Tonicity enhancer binding protein (TonEBP) and its binding to Tonicity enhancer element at electrophoretic mobility shift assay. HG and HM induced endothelial migration at a fluorimetric assay, and tubulization in Matrigel. Small interfering RNAs to AQP1 and to TonEBP both reverted the inducing effects of HG and HM on COX-2 expression, as well as angiogenic activities. Finally, compared with age- and sex-matched C57/BL6 control mice (N=5 wild type, WT), the retina of *Ins2 Akita* diabetic mice (N=5, male, 1 year-old mice) showed higher vascular density as visualized with CD31 staining (Figure, panel A-B; legend: ONL, outer nuclear layers; OPL, outer plexiform layers; INL, inner nuclear layers; IPL, inner plexiform layers), and increased expression of AQP1 and COX-2 (panel C-D) (** $p<0.01$ by ANOVA and t-test)**.**
Conclusion: By activating AQP1 and TonEBP, hyperosmolarity caused by HG or HM induces COX-2 expression and angiogenesis, which may be relevant for microvascular complications of diabetes.

MYOCARDIAL DYSFUNCTION IN HYPERTROPHIC CARDIOMYOPATHY: PRIMARY EFFECTS OF SARCOMERIC MUTATIONS VERSUS SECONDARY CARDIOMYOCYTE REMODELING?

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Introduction: In HCM human tissue, primary alterations at the level of the sarcomeres are associated with secondary changes of Ca^{2+} handling and membrane EP, leading to a pro-arrhythmogenic phenotype and alteration of relaxation. The relative contribution of primary vs. secondary alterations is still unknown.
Methods: We aim to study these changes in intact trabeculae, single cardiomyocytes and skinned preparations from the ventricles of transgenic mouse models carrying HCM-related mutations of cTnT (R92Q, E163R).
Results: Compared to WT, R92Q trabeculae and cells showed: (i) preserved peak isometric twitch tension at low inotropic level with reduced contractile reserve; (ii) slower Ca^{2+} transient kinetics, elevated diastolic [Ca^{2+}] and prolonged relaxation, associated with reduced SERCA function; (iii) frequent Ca^{2+} waves, after-contractions or spontaneous beats during pauses, which increased in response to isoproterenol. In E163R vs. WT trabeculae and cells, force and Ca^{2+} transient amplitude were preserved in all conditions. Interestingly, the kinetics of force development and relaxation was prolonged, despite Ca^{2+} transient kinetics was faster and SERCA function unchanged. Nonetheless, E163R myocardium showed increased arrhythmogenic activity. Further, E163R myofibrils showed a prolongation of the overall relaxation, with incomplete inactivation in the absence of Ca^{2+}. Energy cost of contraction, as well as myofilaments Ca^{2+} sensitivity, were increased in E163R vs. WT skinned trabeculae.
Conclusions: In R92Q hearts, secondary changes of EC-coupling and membrane EP appear to be a major contributor to the observed mechanical dysfunction and arrhythmogeneicity. In E163R instead, impairment of myofilament function appear to be the leading element determining mechanical and electrical abnormalities.

EFFECTS OF VITAMIN D ON CULTURED HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS UNDERGOING OXIDATIVE STRESS

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Recently, vitamin D (VD) has known an increasing importance in many cellular functions of several tissues and organs other than bone. In particular, VD showed important beneficial effects in cardiovascular system. Although the relationship among VD, endothelium and cardiovascular disease is well established, little is known about the antioxidant effect of VD.
This research was carried on in order to study the intracellular pathways activated by VD in cultured human umbilical vein endothelial cells undergoing to oxidative stress.
Nitric oxide (NO) production, cell viability, reactive oxygen species, mitochondrial permeability transition pore, membrane potential and caspase-3 activity were measured during oxidative stress induced by administration of 200µM hydrogen peroxide for 20 minutes. Experiments were repeated in presence of specific VDR ligand ZK191784.
Pre-treatment with VD alone or in combination with ZK191784 is able to reduce the apoptosis-related gene expression, involving both intrinsic and extrinsic pathways.
At the same time it has been shown the activation of pro-autophagic Beclin 1 and the phosphorylation of ERK1/2 and Akt, indicating a modulation between apoptosis and autophagy. Moreover VD alone or in combination with ZK191784 is able to prevent the loss of mitochondrial potential and the consequent cytochrome C release and caspase activation.
The present study shows for the first time that VD may prevent endothelial cell death through modulation of interplay between apoptosis and autophagy. This effect is obtained by inhibiting superoxide anion generation, maintaining mitochondria function and cell viability, activating survival kinases (ERK and Akt) and inducing NO production. This work adds new information to the debate on the benefits of VD supplementation.

FLUORESCENCE CROSS-CORRELATION SPECTROSCOPY METHODS FOR IN-VIVO BLOOD VELOCIMETRY

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Biomedical issues in vasculogenesis require methods to follow hemodynamics with high spatial and time resolution. Fluorescence cross-correlation spectroscopy meets these needs when coupled to scanning or wide field microscopy.
In this work, two complementary experimental setups are employed for the study of the vascular system of Zebrafish embryos, genetically modified to have dsRed-expressing erythrocytes. A multispot EMCCD based spectrometer allows to map the blood speed across the section of the Zebrafish main vein and artery, bringing into evidence differences in the blood shear rate in these two vessels, also in dependence on the distance from the fish heart (1). On the other hand, a confocal setup equipped with a resonant scanning head allows to acquire temporal line scans that, analyzed at 30 ms time lapses, reveal the pulsatile motion in the dorsal aorta giving an estimate of the cardiac output. Measures taken at different days after fertilization of the embryos show a peak in the angiogenic activity at 3dpf. Typical blood flow speeds detected are in the range of 0.2 to 2 mm/s.
When the Zebrafish embryos are treated with 2,3-bdm, an inhibitor of the heart contractile function, a clear decrease in the flow speed is found, thus showing that the used setups are able to detect changes in the flow conditions and that can be applied to study the effect of pathologies on the blood flux.

[1] Pozzi P., Sironi L., D'Alfonso L., Bouzin M., Collini M., Chirico G., Pallavicini P., Cotelli F., Foglia E.A. EM-CCD based Fluorescence Cross-Correlation Spectroscopy for Blood Velocimetry on Zebrafish embryos. J. Biomed. Opt., 2014 in press.

OVEREXPRESSION OF THE MUSCLE SPECIFIC CHAPERONE MELUSIN DELAYS HEART FAILURE AND MORTALITY IN A MOUSE MODEL OF EMERY DREYFUS CARDIOMYOPATHY

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Purpose. Familial cardiomyopathies are caused by genetic mutations that induce accumulation of misfolded proteins with consequent cardiomyocyte death and maladaptive cardiac remodelling. Molecular chaperones are a family of proteins devoted to prevent accumulation of misfolded proteins by promoting either their refolding or degradation via the ubiquitin–proteasome or the autophagosome systems and can thus represent a potential mean to treat familial cardiomyopathies.
Methods. Melusin is a muscle specific chaperone protein whose overexpression effectively prevents maladaptive cardiac remodeling and heart failure both in pressure overload and myocardial infarct mouse models. In this study we use in vivo gene delivery with cardiotropic adeno associated virus 9 vector to induce to increase melusin expression in the heart of mice carrying H222P Lamin A mutation mimicking human Emery-Dreifuss familial cardiomyopathy. Mutant male mice develop spontaneous dilated cardiomyopathy from 4 months of age and die within 10-12 months of age.
Results. Homozygous mutant mice were injected with AAV9-Melusin (I.V. injection of 10^{12} viral particles/mouse) either at 1 month of age before development of the cardiomyopathy or at 4 months when cardiomyopathy was already present to test for both preventive and therapeutic activity. Cardiac function was monitored by echocardiography for the following months. While untreated mice progressively developed left ventricle dilation and reduced contractility (FS%), mice injected with AAV9-Melusin retained physiological level of contractility and were protected toward LV dilation. Ten months after the treatment, 40% of mutant mice died, while 100% of melusin-treated mice were alive. A second group of mutant male mice was injected with AAV9-melusin at 4 months of age when the cardiomyopathy was already detectable. Interestingly melusin prevented the deterioration of contractility in the following 6 months and significantly reduced mortality.
Conclusions These data indicate that melusin chaperone overexpression can effectively delay the onset of Emery-Dreifuss cardiomyopathy as well as arrest the progression of the already established pathology.

CATESTATIN IMPROVES THE FRANK-STARLING RESPONSE IN NORMOTENSIVE AND HYPERTENSIVE RAT HEARTS

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Catestatin (CST:hCgA352-372), a 21-amino-acid derivate of chromogranin A (CgA) displays hypotensive/vasodilatory properties and counteracts excessive systemic and/or intra-cardiac excitatory stimuli (e.g., catecholamines and endothelin-1). Produced also by the myocardium, CST affects the heart by modulating inotropy, lusitropy and coronary tone through a NO-dependent mechanism. Mechanical stretch is an important physiological and pathological stimulus in the heart. The interaction between CST and the Frank-Starling response is unknown. The present study aimed to investigate the role of CST on the regulation of the Frank-Starling response in both normotensive (WKY) and hypertensive (SHR) rat hearts. The heterometric response was studied by using a Langendorff isolated heart setup with a liquid-filled balloon inside the left ventricle used to measure contractile parameters. Functional changes were analysed on normotensive WKY hearts and on the heart of 18-month-old SHR which resemble human chronic heart failure (CHF). Compared to normotensive hearts, the aged hypertensive heart showed a reduced contractility at a given level of preload. Hearts of both rat lines showed an improved Frank-Starling responses in the presence of CST. This improvement is particularly evident in SHR, with respect to WKY hearts. The positive Frank-Starling response in both hypertensive and normotensive herts is mediated by the EE/NOS/NO/cGMP/PKG pathway, as revealed by the application of specific inhibitors and by the evaluation of the expression of phosphorylated eNOS, nNOS, and PLN. Notably, CST-dependent positive Frank-Starling response is paralleled by S-nitrosylation of L-type calcium channels. Our data suggest that CST, acting as a physiological activator of NO, modulates the stretch-induced intrinsic regulation of the heart and, particularly in the decompensated SHR heart.

PARKIN IS INVOLVED IN THE CARDIOPROTECTIVE EFFECT OF DIETARY INTAKE OF PASTA ENRICHED WITH BARLEY (1-3)BETA-D-GLUCAN ON CARDIAC ISCHEMIA/REPERFUSION INJURY IN MICE

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Purpose. Parkin, an E3 ubiquitin ligase, exerts cardioprotecion by triggering mitophagy. The noninvasive induction of myocardial parkin is a desirable achievement. (1-3) β-D-glucan protects against cardiac ischemia/reperfusion injury, yet mechanisms are unclear. We tested whether long-term intake of pasta enriched with 3% w/v barley (1-3) β-D-glucan induces cardioprotection through increasing parkin expression.
Methods. Adult male C57BL/6 mice were fed for 5 weeks with a low-fat diet supplemented with pasta enriched with barley (1-3) β-D-glucan (3g/100mg) (β-D-glucan, n=15) or regular pasta (control, n=15). Food intake, glucose tolerance test and cardiac function were weekly assessed. At 5-week of diet, mice underwent to 30' of cardiac ischemia followed reperfusion (60') and infarct size/area at risk was assessed. Myocardial parkin and anion superoxide (O2-) level were measured respectively by western blot and dihydroethidium staining. To evaluate mitophagy, we examined the mitochondria JC1 staining in β-D-glucan-treated HL1 cardiomyocytes exposed to 100uM H2O2 for 30'.
Results. At similar food intake, 3% w/v (1-3) β-D-glucan did not affect glucose metabolism and function of normal heart. Myocardial parkin level was increased by 85±2% ($P<0.00001$) in β-D-glucan mice compared to control. At the end of reperfusion, β-D-glucan mice survived 50±2% more than control ($P<0.01$). Infarct size/area at risk and myocardial O2- load were reduced respectively by 62±5% ($P<0.001$) and 35±4% ($P<0.0001$) in β-D-glucan mice. In vitro, JC1 staining green was increased in treated HL1($P<0.001$) that showed higher parkin level and resistance to oxidative stress.
Conclusions. Long-term dietary intake of β-D-glucan-enriched pasta reduces infarct size through increasing parkin-dependent mitophagy in cardiomyocytes.

TRANSACTIVATION OF EGF RECEPTORS IS INVOLVED IN APELIN-INDUCED PROTECTION AGAINST ISCHEMIA-REPERFUSION INJURY

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Apelin is an endogenous peptide that protects the hearts against ischemia-reperfusion (I/R) injury when given exogenously at onset of reperfusion. Binding to the G protein-coupled receptor (GPCR) APJ, apelin mimics postconditioning via PI3K-NO cascade.
Since GPCR may involve transactivation of EGFRs, we want to investigate whether this transactivation is involved in apelin cardioprotection and whether it take place via activation of Src kinase or via metalloproteinase (MMP)-mediated release of heparin-binding EGF-like growth factor (HB-EGF).
Langendorff-perfused rat hearts underwent I/R (30 min of global ischemia and 120 min of reperfusion). Apelin-13 (0.5uM) was infused for 20 min from the onset of reperfusion with or without the inhibition of Src kinase (PP2, 2μM) or MMP (GM6001, 0.5μM). To evaluate myocardial injury, both infarct size and mechanical recovery after I/R were measured.
Apelin reduced infarct size from 60±3% to 30±-3% ($p<0.001$) of the left ventricle. Separated inhibition with PP2 or GM6001 totally abolished the limitation of infarct size which remained 60±4% and 58±4% respectively. While an increase from 46±3 to 4±1 mmHg in diastolic pressure at the end of reperfusion revealed the occurrence of contracture. It was attenuated to 37±9 mmHg ($p<0,001$) by apelin. This reduction was suppressed by the inhibitors.
The results showed that both pathways to transactivation of EGFR (Src kinase and MMP) are involved in PI3K activation in apelin-induced cardioprotection. These results highlight that the suppression of one of these pathways is sufficient to completely abolish the protection.

PREVENTIVE AND THERAPEUTIC EFFECT OF STAT3 OR COMPLEMENT C3 DOWN-REGULATION IN TWO DIFFERENT MODELS OF AUTOIMMUNE MYOCARDITIS

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Myocarditis (MC) often correlates with heart auto-immunity and can progress to dilated cardiomyopathy (DCM), but the factors determining disease resolution versus progression are incompletely understood. Both IL-6 and IL-17 play prominent roles in the development of MC, triggered in mice by immunization with an alpha-myosin peptide (Experimental Auto-immune Myocarditis, EAM).
We showed that pharmacological inhibition of STAT3, the main mediator of IL-6 signalling and of Th17 cell differentiation, can prevent EAM development, decreasing liver production of the complement component C3, and can also act therapeutically preventing DCM progression (Camporeale, Marino et al., EMBO Mol. Med. 2013).
The expression of a constitutively active form of STAT3 is also sufficient to trigger the onset of immune-mediated myocarditis, involving enhanced C3 production and IL-6 signalling amplification in the liver, which can be prevented both by IL-6R neutralization and by *cobra venum factor*-mediated complement depletion. Moreover, hepatotropic nanoparticles carrying STAT3 or C3-targeting siRNAs can also partly rescue myocarditis development. Experiments in progress aim to assess the preventive/therapeutic effects of this siRNA-mediated liver interference approach in EAM.
Our findings appear to be relevant to disease pathogenesis in humans, since MC patients display elevated circulating IL-6 and C3 levels and activated heart STAT3. Thus, aberrant IL-6/STAT3-mediated induction of liver acute phase response genes including C3, which can occur as a consequence of pre-existing inflammatory conditions, might represent an important predictive factor determining MC clinical outcome, and that liver-specific C3 and/or STAT3 interference may represent a potential therapeutic approach.

NRG-1 RECEPTOR ERBB3, AND NOT ERBB2 AND ERBB4, IS UP-REGULATED AFTER EX-VIVO MYOCARDIAL INFARCTION, WITH OR WITHOUT POST-CONDITIONING PROTECTION

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Neuregulin-1 (Nrg-1) is a growth factor produced by endothelial cells, necessary for a correct cardiac development. In adult heart Nrg-1 mediates different cellular processes including cell survival and proliferation as well as glucose uptake on cardiomyocytes that express ErbB receptors. For its role, Nrg-1 has been proposed as a potential drug for heart failure, and numerous *in vivo* studies demonstrated that Nrg-1 treatment improves myocardial function and survival in ischemic model throught PI3K/AKT pathway. Nevertheless, it is still not clear which ErbB receptor is activated by Nrg-1 to exert its beneficial effects in post-ischemic phase. Most of the studies were focused on the pathways activated by ErbB2 and ErbB4, although few years ago it has been shown that also ErbB3 is expressed by adult cardiomyocytes.
To better understand the involvement of the different ErbB receptors in heart failure, we performed a pilot analysis of ErbB expression on post-ischemic heart. We used the *ex vivo* Langendorff model to induce ischemia/reperfusion (I/R) injury in rat heart and we analysed the mRNA expression of Nrg-1 and ErbBs after two hours from injury by quantitative real-time PCR. We observed an increase of ErbB3 transcription in I/R hearts compared to the sham hearts, whereas no changes occur for ErbB2 and ErbB4, and Nrg-1 is barely detectable. Interestingly, ErbB3 up-regulation was also observed in infarcted hearts subjected to post-conditioning, a protective procedure that can decrease infarct size.
Altogether, our results demonstrate that Nrg-1 receptor ErbB3, and not ErbB2 and ErbB4, is upregulated in post-ischemic myocardium suggesting that heart protection exerted by Nrg-1 might be mainly mediated by ErbB3-related signaling possibly through the PI3K-AKT pathway.

RANOLAZINE REDUCES ARRHYTHMOGENEICITY IN TRANSGENIC MOUSE MODELS OF HYPERTROPHIC CARDIOMYOPATHY

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Introduction: Ca2+ handling abnormalities are a main determinant of electro-mechanical dysfunction in human hypertrophic cardiomyopathy (HCM). The late-Na+ current blocker ranolazine reduced the rate of arrhythmogenic events in HCM human myocardium.
Methods: We characterize pro-arrhythmogenic changes in E-C coupling that occur in cardiomyocytes and intact trabeculae from cTnT mutant mouse models of HCM (R92Q and E163R) and test the effects of ranolazine.
Results: Compared to wild type (WT), R92Q cardiomyocytes and trabeculae showed (i) prolonged action potentials associated with ionic current remodeling; (ii) slower rate of decay of Ca2+ transients and prolonged muscle relaxation; (iii) elevated diastolic [Ca2+]i; (iv) spontaneous Ca2+ waves, premature Ca2+ transients and after-contractions during pauses.
In E163R vs. WT trabeculae and cells, the kinetics of force development and relaxation was prolonged, despite Ca2+ transient kinetics was not slower. Additionally, E163R myocardium showed increased rate of spontaneous arrhythmogenic activity.
In R92Q preparations, ranolazine (10μM): hastened Ca2+ transient kinetics and reduced diastolic Ca2+ and reduced the rate of spontaneous beats and Ca2+ waves.
In E163R trabeculae and cells ranolazine did not affect the kinetics of Ca2+ transients. Nonetheless, the drug reduced the occurrence of spontaneous Ca2+ and contractile activity.
None of these effects of ranolazine was observed in WT.
Discussion: The beneficial effects of ranolazine on R92Q myocardium are likely mediated by the consequences of late Na+ current inhibition. Instead, in E163R myocardium they are likely mediated by mechanisms other that INaL inhibition (i.e. reduction of myofilaments Ca2+ sensitivity or RyR2 inhibition).

CHRONIC CATESTATIN TREATMENT IMPROVES METABOLIC AND CARDIOVASCULAR COMPLICATIONS IN DIET-INDUCED OBESITY IN RATS

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Catestatin (CST), a Chromogranin A-derived peptide, is a pleiotropic hormone with important effects on blood pressure, heart contractility and relaxation, heart rate variability, baroreflex sensitivity and cardioprotection against ischemia/reperfusion (I/R) damages. It also reduces fat mass in obese Chromogranin A knock-out (*Chga*-KO) mice. These effects were due to increased lipolysis, lipid mobilization and fatty acid oxidation through CST action on α2-AR and leptin receptor. Considering that metabolic disorder-dependent obesity is a cardiovascular risk factor, we evaluated whether CST maintains its cardioprotective properties also in the presence of high fat diet (HFD)-dependent obesity. Male Sprague Dowley rats were fed with different diets and then they were chronically treated with CST. One group was given a normolipidic diet (ND; 18% kcal fat, 24% kcal protein, 58% kcal carbohydrate) for 16 wk; another group was given a HFD (42% kcal fat, 15,2% kcal protein, 42,7% kcal carbohydrate) for 16 wk; the remaining group was given a HDF for 16 wk followed by supplementation with CST (5 μg/g of body weight intraperitoneally, for 12 days). After treatment, the cardiac performance was evaluated on isolated and Langendorff perfused hearts. Compared with ND rats, HFD rats showed higher body weight and abdominal obesity, dyslipidemia, impaired glucose tolerance and a reduced protection against I/R myocardial injuries. These alterations were reduced by CST administration. In particular, in HFD rat, CST increased the protection against I/R myocardial injuries by reducing infarct size and LDH level, and by activating RISK and SAFE pathways. CST treatment also reduced inflammation pathways, i.e. NF-kB, and iNOS. Our results suggest CST as a protection factor which attenuates HFD-associated cardiac damage through prevention of HFD-induced lipid accumulation, inflammation and apoptosis.

METHOTREXATE AT LOW DOSES REDUCES THE RISK OF CARDIOVASCULAR MAJOR EVENTS AMONG PATIENTS WITH CHRONIC INFLAMMATORY DISEASES: RESULTS FROM A META-ANALYSIS OF OBSERVATIONAL STUDIES

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Introduction. Considerable attention has recently been focused on methotrexate whose anti-inflammatory properties have been assumed to be helpful in reducing the risk of cardiovascular diseases (CVD) among patients with chronic inflammatory diseases as well as among patients with persistent inflammatory responses (increased levels of C-reactive protein).
To assess the association between use of methotrexate and possible decreased CVD risk, we conducted a systematic review and meta-analysis.
Methods. For inclusion in the meta-analysis , the selected studies should have included adults receiving methotrexate, with duration of follow up of ≥ 3 months; besides, they should have reported the estimates of hard cardiovascular endpoints ; furthermore, they should have assessed cardiovascular outcomes of drugs' habitual users or new users compared with patients with the same disease that had never used this drug.
Results. A total of 8 observational studies were included. The pooled odds ratio was 0.79 (95% CI = 0.7 - 0.87). When stratified meta-analysis models were assessed, the pooled OR was 0.65 (95% CI = 0.60 - 0.74) for studies that compared methotrexate initiators vs. non-initiators; furthermore, OR was 0.62 (CI = 0.56 - 0.72) in studies that adjusted for concomitant medication use.
Conclusions. Methotrexate use is associated with lower CVD risk among patients with chronic inflammation (i.e., mostly patients with rheumatoid arthritis in the assessed studies). Since this drug doesn't possess any documented effect against other CVD risk factors, it is plausible that methotrexate may potentially reduce cardiovascular risk mainly through its anti-inflammatory properties.

INDOXYL SULFATE PRIMES MONOCYTE DIFFERENTIATION INTO PRO-FIBROTIC M2 MACROPHAGES. CLINICAL IMPLICATIONS IN ABDOMINAL AORTIC ANEURYSM

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The cardiorenal syndromes (CRS) are defined by heart and kidney dysfunction, with mutual detrimental feedback; the association of even mild chronic kidney disease (CKD) with cardiovascular (CV) events is still underestimated.
In patients with abdominal aortic aneurysm (AAA), we previously found a frequent history of mild CKD and augmented CD16+monocytes.
Increased Uremic toxins (UT) plasma levels are CV risk factors in CRS patients. The UT Indoxyl-3-sulphate (IS), a ligand of Aryl hydrocarbon Receptor (AhR), accumulates early during CKD and is associated to overall mortality and CV diseases.
In this study, we found that IS plasma levels are higher in AAA patients than in age-matched controls ($p = 0.0017$) and correlate with CD14+CD16+ monocytes ($p = 0.0028$; $r = 0.3454$). We therefore investigated the effect of IS at the concentrations found in AAA patients (1, 10, 20 microM) on monocyte-macrophage differentiation.
Using a monocyte cell line (THP-1) we demonstrated that IS slowers cell cycle progression, modulates the AhR-Nrf2/HO-1 cross-talk through upregulation of AhR Repressor (AhRR) and HO-1, increases migration rate and CD163 expression. Macrophages derived from IS-primed monocytes overexpress components of the AhR/AhRR and Nrf2/HO1 axes and features of both classical (MCP-1, COX2) and alternative immunity (MMP-9 downregulation; PPARg, TIMP-1 and TGF-b overexpression). Thus, a moderate increase of IS can skew monocyte differentiation towards macrophages with low-inflammatory, profibrotic potential.
In conclusion, our results suggest that the uremic toxin IS may be involved in the pathogenesis of AAA and provide a mechanistic link between this condition and the CRS.

INTRAVENOUS DIURETICS VS. ISOLATED ULTRAFILTRATION FOR ACUTE DECOMPENSATED HEART FAILURE: A SYSTEMATIC REVIEW WITH METAANALYSIS

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Intravenous (IV) diuretics are usually employed for acutely decompensated heart failure(ADHF), but they have adverse effects, including electrolyte imbalance, hypotension, activation of neurohormones and iatrogenic cardiorenal syndrome. Isolated ultrafiltration (IUF) is an alternative method to remove sodium and water. We made a systematic review with meta-analysis to compare IUF and IV diuretics in the ADHF setting. Studies were searched for across the PubMed and Ovid databases (January 1990- December 2013). Only randomized controlled trials (RCTs) comparing IUF vs. IV diuretics in ADHF were considered. Efficacy and safety outcomes were extracted and a meta-analysis was subsequently made. Six studies involving 477 participants were included in the qualitative analysis. However the meta-analysis was limited to three studies, due to marked dissimilarity between efficacy end-points in the between- study comparison. IUF was superior to IV diuretics for 48-h fluid removal [weighted mean difference (WMD) = 1.20 L, 95% CI: 0.73-1.67 L p<0.001] and 48-h weight loss (WMD = 1.77 kg , 95% CI : 1.18 - 2.36 kg p <0.001).The proportion of patients with meaningful (>0.3 mg/dL) rise in serum creatinine at 48 hours was similar in IUF and IV diuretics groups (OR = 1.33, 95% CI: 0.81 - 2.16 p = 0.26).Greater fluid and weight losses were detected with IUF compared to IV diuretics, whereas no significant differences emerged for rise in serum creatinine . However, these conclusions arise from small number of studies involving few patients. Further comparisons between RCTs with larger sample sizes are needed in the future.

HYDROGEN SULFIDE AND CYSTATHIONINE Γ-LYASE IN THE FAILING HEART

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Hydrogen sulfide has been demonstrated to exert several biological functions of pivotal importance both in physiological and pathophysiological conditions. In turn, H2S has been related to the regulation of oxygen sensing, maintenance of normal arterial pressure, response to ischemic insult, and regulation of antioxidant defenses. It appears now clear that its high diffusion rate leads to the targeting of ion channels, intracellular proteins, and mitochondria. Ion channels have been shown to be regulated by exogenous H2S while a large number of proteins undergo a S-Sulfhydration post-translational modification. While in the heart H2S is beneficial against ischemia/reperfusion injury both as a pre- and postconditioning agent it is not clear whether it has a direct effect against the development of heart failure. We investigated the role of H2S and its producing enzyme (CSE) in an in vivo transgenic model to address a putative direct effect on the heart. CSE-KO mice were treated with Captopril and compared to control and non-treated CSE-KO in term of development of impaired left ventricular function. Moreover, CSE expression and transcript levels were addresses in a cardiomyocytes cell line under hypoxic conditions. In term of congestive heart failure reduction, the main beneficial effect seems to be mediated by the significant lowering of arterial blood pressure as proven by experiments in CSE-KO mice treated with anti-hypertensive drugs. On the other hand, H2S producing enzyme CSE is poorly expressed in the myocardium under physiological conditions while its RNA is easily detectable and modulated upon hypoxic conditions.

NON-INVASIVE OPTICAL IMAGING PROCEDURES FOR THE IN VIVO EVALUATION OF CELL POPULATIONS AND MOLECULAR PROCESSES

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Optical imaging techniques can be used to non-invasive image cells and molecular processes in living organisms. Two main procedures have been developed for cell imaging: direct labelling is based on cell tagging with fluorescent probes, whereas indirect labelling is based on cell genetic engineering with fluorescent or bioluminescent reporter genes whose proteic product can be in vivo visualized. To study molecular processes (neo-angiogenesis, inflammation, hypoxia, etc.), specific fluorescent or chemioluminescent probes, which localize proportionally with the specific process, are commercially available. In this context, endothelial progenitor cells (EPCs), were engineered with a lentiviral construct carrying the luciferase reporter gene under control of a constitutive promoter. Engineered EPCs-Luc were intramuscularly injected in a mouse model of hind limb ischemia, and their survival was monitored over time by Bioluminescence Imaging. Cell persistence was demonstrated up to sacrifice (30 days) in hind limb ischemia group, whereas cells disappeared in few days in the control group. Another mouse model was used to assess inflammation and angiogenesis during time after positioning of a scaffold for tissue regeneration under femoral artery. Inflammation Probe and Integrisense680 were used, demonstrating a reduction of inflammation concomitant to surgical recovery, and a specific angiogenic rate in relation to different scaffold compositions. Fluorescent and Bioluminescent signals were anatomically located by integrating functional images with corresponding CT scans using the IVIS Spectrum/CT instrument allowing also 3D reconstructions. In conclusion, optical imaging is a reliable technique to follow the fate of labelled cells and to evaluate molecular processes in specific rodent models.

PLATELET ACTIVATION IN EXTRACORPOREAL CIRCULATION: EFFECTS OF UNFRACTIONATED HEPARIN ON DAMAGES INDUCED BY BIO-INCOMPATIBILITY

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Background. Interaction with biomaterials of non-endothelial surfaces of cardiopulmonary bypass circuit caused platelets activation.The aim of study was to evaluate the effects of unfractionated heparin (UH) on platelet activation induced by extracorporeal circulation (ECC).
Methods. Analysis were performed on peripheral human blood (PB) obtained from 10 healthy adult blood donors.Markers were evaluated after in-vitro and after ECC stimulation, samples with and without UF were compared. In-vitro stimulation was tested on 10 ml of PB, at 37°C for 2 hours in Orbital Shaker SSL1 stirrer at flat speed. ECC stimulation was carried out on 100 ml of PB circulated 2 hours/37°C at a rate of 1.70 l/min.We tested Annexin V (marker of platelet activation) and CD41a (marker of platelet aggregation). Samples were analyzed on a flow cytometer.
Results. We compared samples with UH and samples without (control=ctr) after in-vitro and ECC stimulation.We observed a greater positivity to Annexin V after ECC compared to in-vitro (25.9 vs 8.7%; $p<0.01$) in ctr samples. Similarly, CD41a+ was greater after ECC than in-vitro stimulation (68 vs 28.3%; $p<0.01$).In samples with UH, Annexin V was greater after ECC than in-vitro stimulation (25 vs 9%; $p<0.001$). A similar result was observed for CD41a (66 vs 23%; $p<0.01$). ECC induced a platelet activation compared to in-vitro stimulation. Addition of UH led to a reduction of platelet activation in vitro, and to a more marked reduction after ECC.
Conclusions. ECC act on platelet activation.The addition of UH in the blood and in the circuit reduced activation, suggesting a positive action of the drug on the observed effects induced by biomaterials.

PARTICULATE MATTER INDUCES THE EXPRESSION OF PROCOAGULANT MICROPARTICLES BY HUMAN MONONUCLEAR CELLS

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Background: Cell derived microparticles (MP), ranging in size between 0.05 and 1 μm, are phospholipid vesicles involved in blood coagulation and inflammation. Cell activation and apoptosis can lead to MP formation; while the mechanisms that lead to MP formation are not completely elucidated, intracellular calcium mobilization is known to be involved. Particulate matter (PM) is a mixture of solid and liquid particles suspended in the air, originated from a variety of sources. Exposure to PM has been associated with increased cardiopulmonary morbidity and mortality. The observation that PM induces cytosolic calcium mobilization is consistent with the hypothesis that PM induces the generation of MP.
Aim: To investigate whether PM induces the generation of procoagulant MP by human mononuclear cells (HMC)
Methods: HMC were incubated with PM (SRM1648a) obtained from the National Institute of Standards and Technology (U.S. Department of Commerce, USA). MP were enumerated through a prothrombinase assay that measures phosphatidylserine (PS) concentration; MP-associated tissue factor activity was assessed by a one-stage clotting assay in the sedimented MP after high speed centrifugation (100000xg for 75 min).
Results: PM treatment induces a dose-dependent increase in MP generation and an increase in MP-associated tissue factor activity.
Conclusions: PM-mediated generation of procoagulant MP might help understand the correlation between exposure to airborne pollution and cardiopulmonary diseases.

INFLAMMATORY MOLECULES AFFECT THE INTERACTION BETWEEN HUMAN DENDRITIC CELLS AND VASCULAR SMOOTH MUSCLE CELLS

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Despite inflammatory and immune mechanisms participating to vascular remodeling, and dendritic cells (DCs) driving immune and non-immune tissue injury response, the interactions between DCs and vascular smooth muscle cells (SMCs) possibly relevant to vascular pathology are still unclear. This study has investigated the interaction of human coronary SMCs and human DCs in vitro, with regard to influence exerted by obesity, insulin-resistance and inflammation. Monocyte-derived DCs (Mo-DCs) obtained from obese (BMI>30) and type 2 diabetic patients were studied by electron microscopy, immunohistochemistry and CFSE dilution assay. Mo-DCs from patients displayed higher ability than those of healthy controls to adhere to coronary SMCs, possibly due to an increase in cell adhesion molecules including integrins. When Mo-DC, isolated from healthy subjects, were let to adhere to inflammatory cytokine-stimulated coronary SMCs, an increased cell adhesion was measured. This effect was counteracted by pre-treating SMCs with either a statin or a PPAR-gamma agonist.
These findings suggest that an inflammatory environment in the vascular wall may stimulate the interaction between DCs and VSMCs and give rise to a vicious circle perpetuating and even worsening local inflammation and causing or complicating atherosclerosis.

MECHANO-SENSITIVE RAPID DILATATION IN SKELETAL MUSCLE: A NOVEL TYPE OF VASCULAR REACTIVITY

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Recent experiments have shown that a transient rapid dilatation (RD) can be evoked in skeletal muscle in response to different short-lasting mechanical stimuli such as compression of the muscle, occlusion of the supplying artery and muscle contraction. Given that all these stimuli produce a transient decrease in transmural pressure (TP) in the musculo-vascular network, the ensuing dilatory response was hypothesized to result from the *myogenic response.* However, we here present evidence that both rapid increase and decrease in TP are adequate stimuli to evoke the RD.
The investigation was carried out on a consolidated experimental model: the anesthetized rabbit in which muscle blood flow is continuously monitored by ultrasound nano-probes perivascularly implanted around the masseteric artery. Custom-made systems were employed to deliver repeatable compressive (50-100 mmHg, lasting 1-100 s) and depressurizing stimuli (10-40 mmHg; 1-30 s). Results showed that both short-lasting compressive and depressurizing stimuli evoke a marked hyperemic response revealing RD. Moreover RDs are consistently evoked both at the onset and at release of both sustained compressive and depressurizing stimuli.
The results unequivocally prove that the mechanically induced RD in skeletal muscle can be elicited by both rapid increases and decreases in TP of the musculo-vascular network and thus cannot be explained by the classical myogenic response (which would rather constrict blood vessels in response to increased TP). Further studies are needed to address endothelial and smooth-muscle mechanisms underlying RD as well as the potential clinical interest in assessing vascular function through this novel vascular reactivity to mechanical stimuli.

HAS TFR2 BETA ISOFORM A ROLE IN CARDIAC IRON METABOLISM AND CARDIOPROTECTION?

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Together with liver and bone marrow, heart is an organ in which iron metabolism must be tightly regulated. In fact iron overload associated diseases, like hemoglobinopathy and hemochromatosis, can present signs of heart failure. Moreover, iron fluctuations during heart ischemia have been associated with hypoxia injury damage (Berhenstein et al, 2008). Transferrin receptor 2 (Tfr2) is a transmembrane protein that is mutated in hemochromatosis type 3. TFR2 is a gene involved in iron metabolism transcribed in two main isoforms, the full length (Tfr2 alpha) and a shorter form (Tfr2 beta). The function of the latter form is correlated to iron efflux from splenic reticuloendothelial cells via iron exporter Ferroportin 1 (Roetto et al, Blood 2010). It was previously documented that Tfr2 beta is highly transcribed in heart (Kawabata et al, 1999).
In order to understand whether Tfr2 beta has some role in cardiac iron metabolism we induced global ischemia reperfusion (I/R) in hearts of wild-type (WT) and Tfr2 beta null mice (TFR2 KI and TFR2 LCKO-KI) (Roetto et al, 2010).
Production of reactive oxygen species (ROS) is a major responsible of I/R injury (Tullio et al, Basic Res Cardiol 2013). During I/R, cardiac iron is subjected to rapid variations and the iron amount present during reperfusion could greatly influence ROS generation and modulate the reperfusion associated injury entity. Significant protection from reperfusion damage has been experimentally obtained through the ischemic preconditioning (IPC) procedure before I/R, consisting in very short cycles (a few minutes) of ischemia/perfusion before the long lasting infarcting ischemia (Murry et al, 1986). It has been reported by Chevion and coworkers that a selective and significant L-ferritin (FtL) increase induced by IPC maneuvers could acts as scavenger of the mobilized iron during the subsequent I/R infarcting phase (Chevion et al, 2008). The authors concluded that the small amount of iron released during IPC could be the signal for FtL increase.
In our study, *cardiac iron content* was evaluated through standard methodology in hearts from WT and Tfr2 beta null (KI and LCKO-KI) animals. No significant differences were found in Tfr2 mice hearts *vs* WT.
Hearts harvested from the three groups of mice underwent the following I/R protocol: 30-min ischemia and 60-min reperfusion. Myocardial infarct area was evaluated by nitroblue-tetrazolium staining. Total infarct size, expressed as a percentage of left ventricle (LV) mass, was 58±3% in hearts of WT group. A significant smaller infarct size was observed in hearts of Tfr2 KI and LCKO-KI (37±6% and 36 ±6% of LV mass, respectively) ($p<0.002$ *vs* WT).
Ferritin subunits protein analysis in Tfr2 beta null mice before I/R suggested that, while FtH amount remains constant, FtL was significantly increased compared to WT hearts, a scenario similar to that seen in preconditioned WT animals. Transcriptional analysis of the main iron genes and proteins behavior in Tfr2 targeted hearts will help clarifying the underlying molecular mechanisms.
In our study Tfr2 beta isoform seems to be involved in cardiac iron metabolism and Tfr2 silencing results to have a protective effect on I/R induced damage. It is likely that Tfr2 downregulation and FtL upregulation positively affect iron-dependent signals that are involved in both I/R injury and preconditioning cardioprotection.

HYPERGLICEMIC 'MEMORY' AFFECTS COMMITMENT OF CD34$^+$ CORD BLOOD-DERIVED STEM CELLS INTO FUNCTIONAL ENDOTHELIAL PROGENITOR CELLS

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Background: The reduced number and function of endothelial progenitor cells (EPCs) in type diabetes mellitus (DM), is likely responsible of vascular complications observed in diabetic patients. These complications persist even in subjects with good long-term glycemic control, a phenomenon termed "hyperglycemic memory", suggesting that EPCs dysfunction persists even after glycaemia normalization. The present work is aimed at verifying whether exposure to pathologic glucose concentrations affected human cord blood-derived CD34$^+$ cells function and commitment into functional EPCs and whether these effects persisted even after glucose normalization.
Results: Cord blood-derived CD34$^+$ cells were expanded up to 30 days in hyperglycemia (30 mM glucose, HG) or normoglycemia (NG) conditions. While the cells showed the same proliferation kinetics during the initial 10 days, a remarkable impairment of expansion was observed after 20 and 30 days in HG-CD34$^+$ *vs* NG-CD34$^+$ cells. Flow cytometry analysis revealed a significant reduction of CXCR4 expression and migration toward SDF-1 (CXCR4 ligand) after 20 days in HG-CD34$^+$ *vs* NG-CD34$^+$. Colony forming units-endothelial cell (CFU-EC) assay showed a lower ability to form CFU-EC in HG-CD34$^+$ *vs* NG-CD34$^+$ and interestingly, this defect was maintained even after relief from hyperglycemia (exHG-CD34$^+$). To note, CXCR4 expression and migration ability toward SDF-1 gradient was also significantly reduced in HG-CD34$^+$ and exHG-CD34$^+$-derived EPCs *vs* NG-CD34$^+$-derived EPCs.
Conclusions: These data show that HG negatively affects the commitment of cord blood derived-CD34$^+$ cells into functional EPCs. This effect persists even after normoglycaemia restoration providing the first evidence of "hyperglycemic memory" establishment in CD34$^+$ cells *in vitro*.

DYNAMIC SUSPENSION CULTURE OF CARDIAC PROGENITORS TO DEVELOP INJECTABLE SCAFFOLDS FOR CARDIAC TISSUE ENGINEERING

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Cardiovascular diseases are the leading cause of mortality in the industrialized countries. Following injury, compensatory mechanisms are spontaneously activated to maintain cardiac function although unable to efficiently repair the damaged tissue. The limitation of current therapeutic strategies requires alternative approaches represented by regenerative medicine and tissue engineering. The structural and functional complexity of the myocardium necessitates new 3D dynamic approaches to develop efficient in vitro cardiac constructs.
We tested a bioreactor which allows to culture cells in 3D suspension and, by guaranteeing spatial freedom and improving mass transfer and cell exposure to nutrients and oxygen, to promote cell-cell interaction and growth. To document the potential of dynamic culture for tissue engineering, Cardiac Progenitor Cells were isolated from adult Enhanced Green Fluorescence Protein rat hearts ($EGFP^{pos}$ CPCs). CPC were seeded on injectable hydrogel microspheres (IHM) and cultured in the Hydrogel Dynamic Culture Device (HDCD) designed on FP7 European project BIOSCENT. The effects of HDCD was evaluated in terms of cell viability and growth as well as scaffold integration and immunophenotypic changes. Cell-seeded on IHM in static condition were used as control. Preliminary analysis showed a high efficiency of dynamic suspension culture on CPC growth and adherence to microspheres. Importantly, microsphere dimensions and functionalization with growth factors relevant for cardiac repair significantly influenced CPC biological properties.
Our results propose the development of standardized protocols to test dynamic suspension cell culture device for the generation of engineered functional cardiac scaffolds and to model *in vitro* both physiologic and pathologic processes.

HUMAN MESENCHYMAL STEM CELLS LABELED WITH FLUORESCENT SILICA NANOPARTICLES APPEAR TO HAVE A DIFFERENTIAL MIGRATORY BEHAVIOR IN AN *EX -VIVO* RAT MODEL OF MYOCARDIAL INFARCTION

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INTRODUCTION. Heart diseases are still the major pathologies associated with a fatal outcome. Recent advances have been made in myocardial regenerative medicine and among different adopted techniques, stem cell therapy is one of the most promising. Adult Mesenchymal Stem Cells (MSCs) are good candidates and have already been used in clinical trials with hopeful results. In this scenario, it is of great interest to develop safe methods for the long term tracking of MSCs in animal models, in order to understand their fate and the precise mechanisms through which these cells produce their beneficial effects in the heart. For this purpose we labeled human bone marrow MSCs (hMSCs) with fluorescent Silica Nanoparticles (IRIS Dots, overnight treatment, 50µg/mL concentration) and followed their localization after injection into *ex vivo* rat hearts subjected to left ventricular infarction. **RESULTS.** Nanoparticles prevalently localized inside lysosomes, were not cytotoxic and did not alter cell adhesion and proliferation. Ctrl-hMSCs injected into the apex of infarcted hearts were found both dispersed in the left ventricle wall and clustered near its lumen, whereas greater aggregates of IRIS Dots-hMSCs were localized inside the injured left ventricle. *In vitro* analysis of cell migration indicated that IRIS Dots-hMSCs displayed a differential migratory behavior respect to Ctrl-hMSCs. **CONCLUSIONS.** IRIS Dots labeling is suitable for tracking hMSCs inside heart tissue and results in a differential migratory phenotype that seems to enhance hMSCs localization inside the infarcted heart. Further studies will be aimed at confirming these results and at elucidating the involved intracellular signaling cascades.

THE INTRACELLULAR CA^{2+} TOOLKIT REGULATES VEGF-MEDIATED GENE EXPRESSION IN NORMAL, BUT NOT TUMORAL, HUMAN CIRCULATING ENDOTHELIAL PROGENITOR CELLS

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Adult angiogenesis controls both physiological and pathological processes, such as post-ischemia vascular regeneration or cancer-associated neo-angiogenesis. Endothelial progenitor cells (EPCs) contributes to both processes following their mobilization into peripheral blood and their recruitment into sites of active neovessel formation. We have previously shown that VEGF controls EPC proliferation and *in vitro* tubulogenesis by inducing oscillations in intracellular Ca^{2+} concentration. VEGF-induced Ca^{2+} transients are shaped by the concerted interaction between Ca^{2+} release from inositol-1,4,5-trisphosphate receptors and store-operated Ca^{2+} entry (SOCE) and promote IκB prosphorylation, thereby enabling the nuclear translocation of NF-κB. The present investigation was endeavoured to assess whether and how the Ca^{2+} spiking response to VEGF controls gene expression in human circulating endothelial colony forming cells (ECFCs) isolated from both healthy donors and patients affected by renal cellular carcinoma (RCC; RCC-ECFCs). VEGF causes the expression of both genes and proteins involved in cell adhesion (E-Selectin and VCAM-1) and in the breakdown of extra-cellular matrix (MMP-9), while it does not affect cyclin D1 and Bcl-2. VEGF-induced gene expression was hindered by BAPTA, which chelates intracellular Ca^{2+}, BTP2, which blocks SOCE, and thymoquinone, a NF-κB inhibitor. Consistently, immunocytochemistry revealed that VEGF-evoked nuclear translocation of NF-κB-p65 is prevented by BAPTA, BTP2 and thymoquinone. Notably, VEGF failed to evoke both Ca^{2+} oscillations and gene expression in RCC-ECFCs albeit VEGFR-2 levels in these cells are unaltered. These results suggest that, while enhancing VEGF-induced Ca^{2+} signalling in EPCs might be a proper strategy to enhance their regenerative potential *in vivo*, VEGFR-2 is not functional in the endothelial progenitors of RCC patients.

MOLECULAR AND FUNCTIONAL ROLE OF REACTIVE OXYGEN SPECIES (ROS) DURING CARDIAC DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS (MESC)

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Purpose: Cardiomyocytes derived from mESC are a promising cell source for the regeneration of damaged heart. Despite recent progress on the identification of myogenic factors, little is known about the involvement of ROS during *in vitro* cardiac differentiation of mES. Importantly, increasing evidences suggest the participation of ROS as an important trigger for the differentiation and maturation of ESC toward the cardiac phenotype. ROS are generated from different sources including NADPH oxidase, nitric oxide synthase (NOS) isoforms and c-GMP dependent protein kinase I (PKG-I). In particular, no many data exist regarding the role of NO/cyclic GMP/PKG-I inhibition and/or up-regulation during cardiac maturation.
Material and Methods: CGR8 mESC were differentiated in hanging drops to form embryoid bodies (EBs, days 0-7). EBs were plated to observe spontaneous beating and collected at different time points (d0-d21) for transcriptional (RT-PCR, Q-PCR) and protein (WB) analysis.
Results: Q-PCR analysis shows that during cardiac maturation of EBs (d5-d21), cardiac transcription factors (mNkx2.5, mMef2c and mGata4) are progressively up-regulated, while stem cell transcription factors (mOct4 and mNanog) are down-regulated compared to mESC (d0). Moreover, during the same time window, gene expression of mNos3, mPrkg1, GuCy1b increases peaking at day 5-8, a key developmental period in *in vitro* cardiogenic process.
Conclusions: The observed upregulation of NO/cyclic GMP/PKG-I pathway strongly suggests a potential role of these enzymes to trigger cardiac differentiation. Next studies will evaluate the cardiomyogenic role of this pathway and its developmental modifications in the modulation of myocytes calcium handling and electrical properties.

TRANSPLANTATION OF ADIPOSE TISSUE MESENCHYMAL CELLS CONJUGATED WITH PLGA MICROSPHERES FOSTER C-KIT+ PROGENITOR CELLS AND PROMOTES REVASCULARIZATION AND TISSUE REPAIR THROUGH PARACRINE SIGNALING INA MURINE MODEL OF ACUTE MYOCARDIAL INFARCTION

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Rationale: The engraftment and survival of transplanted stem cells may be improved by combining such cells with scaffolds to delay apoptosis and enhance their regenerative properties.
Objectives: We examined whether poly (lactide-co-glycolide) (PLGA) microspheres (PAM) functionalized with VEGF enhance survival of Adipose tissue-derived mesenchymal stromal cells (AT-MSCs). We compared the therapeutic efficacy of transplanted AT-MSCs conjugated with VEGF-PAM with injection of conditioned medium from AT-MSCs in a murine model of acute myocardial infarction (AMI).
Methods: Twelve month-old male C57BL/6 mice underwent coronary artery ligation (Lig), followed by randomization into 6 groups (n=5/group): I. Sham operation, II. AMI control (saline PBS 20 µL), III. AMI followed by intramyocardial injection with AT-MSCs only ($2.5x10^5$ cells/20 µL), or IV. concentrated medium from AT-MSCs (CM, 20 µL), or AT-MSCs ($2.5x10^5$ cells/20 µL) conjugated with empty microspheres (V) or VEGF-PAM (VI).
Results: AT-MSCs conjugated with VEGF-PAM inhibited H_2O_2-induced apoptosis , decreased the area of fibrosis (Figure A and C) and increased myogenesis, arteriogenesis (Figure B), number of cardiac-resident c-Kit positive cells (Figure A and D, $p<0.05$ vs PBS+lig) and myocardial fractional shortening (FS) when transplanted into the infarcted hearts of C57 mice (%FS: I. 40±11, II. 15±3**, III. 29±7°, IV. 33±7°°, V. 20±1, VI. 34±7°°) (**$p<0.01$ vs sham; °$p<0.05$ and °°$p<0.01$ vs Lig+PBS; anova test). All such effects, however, were fully paralleled by the injection of CM ($p<0.01$ vs PBS+lig).
Conclusions: AT-MSCs conjugated with VEGF-PAM exert a paracrine effect, that may have therapeutic applications to enhance survival of AT-MSCs and regenerative capacity of the heart.