Società Italiana di Ricerche Cardiovascolari

Palazzo Greppi, via Sant'Antonio 10

Venerdì 23 novembre 2012

SALA NAPOLEONICA

13.00 – 13.30 Discorso di apertura del Presidente della SIRC: Prof. Germano Di Sciascio

13.30 – 14.50 SESSIONE I: CALCIUM SIGNALING
Moderatore: Prof. Francesco Moccia

13.30 – 14.20 Comunicazioni

1) **Avanzato D**, et al. "ATP-MEDIATED CALCIUM SIGNALS IN TUMOR ANGIOGENESIS: ROLES AND MECHANISMS" - Università di Torino.

2**) Bottino C**, et al. "ALTERATA ESPRESSIONE DEI CANALI DEL Ca^{2+} IN CELLULE PROGENITRICI ENDOTELIALI DI PAZIENTI AFFETTI DA CARCINOMA CELLULARE RENALE, MIELOFIBROSI IDIOPATICA E TUMORE DELLA MAMMELLA - Università di Pavia.

3) **Di Buduo C**, et al. "ADP-INDUCED STORE OPERATED CALCIUM ENTRY (SOCE) REGULATES HUMAN MEGAKARYOCYTE FUNCTION" – Università di Pavia.

4) **Dragoni S**, et al. "CANONICAL TRANSIENT RECEPTOR POTENTIAL CHANNEL 3 AS A NOVEL TARGET FOR CELL-BASED THERAPY IN ENDOTHELIAL COLONY FORMING CELLS" – Università di Pavia.

5) **Genova T**, et al. "ROLE OF TRPM8 IN THE CONTROL OF VASCULAR ENDOTHELIAL CELL MIGRATION AND ADHESION" – Università di Torino.

14.20 – 14.50 Discussione

14.50 – 16.10 SESSIONE II: STEM CELLS
Moderatore: Prof. ssa Donatella Stilli

14.50 – 15.40 Comunicazioni

1) Beltrami A. "IN VITRO ATTENUATION OF CARDIAC STEM CELL SENESCENCE ENHANCES MYOCARDIAL REPAIR" – Università di Udine

2) **Bettini M**, et al. "MECCANISMI DEL RECLUTAMENTO DELLE CELLULE STAMINALI NEL MICROCIRCOLO POSTISCHEMICO" – Università di Perugia

3) **Paccosi S**, et al. "MODULAZIONE FARMACOLOGICA DELLE INTERAZIONI FRA CELLULE MUSCOLARI LISCE VASCOLARI E CELLULE DENDRITICHE UMANE" – Università di Firenze

4) **Pennella S**, et al. "ARITMIA VENTRICOLARE DOPO SOMMINISTRAZIONE INTRAMIOCARDICA DI CELLULE STAMINALI MESENCHIMALI DERIVATE DA MIDOLLO OSSEO (CSMBs) IN UN MODELLO PRECLINICO" – Università di Modena e Reggio Emilia

5) **Vitale S**, et al. "MECCANISMI E DIAGNOSI PRECOCE DELLA CARDIOTOSSICITA' DA CHEMIOTERAPICI" – Università di Perugia

15.40 – 16.10 Discussione

16.10 – 17.30 SESSIONE III: HEART FAILURE
Moderatore: Prof. Germano Di Sciascio

16.10 – 17.00 Comunicazioni

1) **Ariano C**, et al. " NATRIURETIC PEPTIDE– GUIDED THERAPY VS. USUAL SYMPTOM-GUIDED CARE IN OUTPATIENTS WITH CHRONIC HEART FAILURE: A SYSTEMATIC REVIEW WITH META-ANALYSIS" - Cardiology Unit, Presidio Sanitario Intermedio "Elena d'Aosta", Napoli

2) **Caruso R**, et al. "ELEVATED LEVELS OF PRE-OPERATIVE INTERLEUKIN-6 AFFECT MONOCYTE ACTIVATION AND MULTI ORGAN FAILURE IN LEFT VENTRICULAR ASSIST DEVICE (LVAD) PATIENTS" - CNR Clinical Physiology Institute, Milan-Pisa

3) **De Vecchis R**, et al. "B-TYPE NATRIURETIC PEPTIDE–GUIDED VS. CONVENTIONAL CARE IN HEART FAILURE PATIENTS: A CASE-CONTROL STUDY" - Cardiology Unit, Presidio Sanitario Intermedio "Elena d'Aosta", Napoli

4) **Esposito F**, et al. "MULTIDISCIPLINARY APPROACH TO EXERCISE LIMITATION IN PATIENTS WITH HEART FAILURE: PERIPHERAL MUSCLE DYSFUNCTION AND THE BENEFITS OF SMALL MUSCLE MASS TRAINING". Università di Milano

5) **Raddino R**, et al. "VALUTAZIONE DELLA RIGIDITA' ARTERIOSA NEI PAZIENTI AFFETTI DA SCOMPENSO CARDIACO CON FRAZIONE D'EIEZIONE PRESERVATA" – Università e Spedali Civili di Brescia

17.00 – 17.30 Discussione

17.30 – 18.20 SESSIONE IV (1): MOLECULAR SIGNALING/ISCHEMIA REPERFUSION
Moderatore: Prof. ssa Isabella Tritto

17.30 – 18.00 Comunicazioni

1) **Collino M**, et al. "BENEFICIAL EFFECTS OF RELAXIN IN AN *IN VIVO* EXPERIMENTAL MODEL OF ISCHEMIC ACUTE KIDNEY INJURY" – Università di Torino

2) **Gammella E,** et al "OMEOSTASI DEL FERRO IN UN MODELLO DI POLICITEMIA" - Università di Milano

3) **Vitale S**, et al. "VIE PROTETTIVE DEL PRE- E POST-CONDIZIONAMENTO ISCHEMICO NEL MUSCOLO CREMASTERE DI RATTO" – Università di Perugia

18.00 – 18.20 Discussione

Sabato 24 novembre 2012

SALA NAPOLEONICA

09.00 – 10.20 SESSIONE IV (2) : MOLECULAR SIGNALING/ISCHEMIA REPERFUSION
Moderatore: Prof. Michele Samaja

09.00 – 09.50 Comunicazioni

1) **Bassino E**, et al. "CATESTATIN, A CHROMOGRANIN A DERIVED PEPTIDE, IN HEALTH AND DISEASE: ROLE OF PI3K/AKT PATHWAY IN THE PROTECTION AGAINST ISCHEMIA REPERFUSION INJURY" Università di Torino

2) **Caretti A**, et al. "CREATINA E D-RIBOSIO: CARDIOPROTEZIONE IN MODELLI DI IPOSSIA IN VITRO ED IN VIVO" – Università di Milano

3) **Folino A & Rastaldo R**. "APELIN13 AND MYOCARDIAL PROTECTION" – Università di Torino

4) **Leone R**, et al. "ISOLAMENTO MITOCONDRIALE E ANALISI PROTEOMICA DEL MUSCOLO CARDIACO DI RATTO IN CORSO DI INVECCHIAMENTO: VANTAGGI E CRITICITÀ" – Università di Milano

5) **Milano G**, et al. "IN VIVO INTERMITTENT HYPOXIA AS A TOOL FOR CARDIOPROTECTION" - Università di Losanna (CH)

09.50 – 10.20 Discussione

10.20 – 11.40 SESSIONE IV (3) : MOLECULAR SIGNALING/ISCHEMIA REPERFUSION
Moderatore: Prof. Gianni Losano

10.20 – 11.10 Comunicazioni

1) **Pasqua T,** et al. "CARDIAC PHYSIOPATHOLOGY: AN UPDATE ON CHROMOGRANIN A AND ITS DERIVED PEPTIDES" – Università della Calabria

2) **Pini A**, et al. "RELAXIN INHIBITS CARDIAC FIBROBLAST-MYOFIBROBLAST TRANSITION THROUGH THE UP-REGULATION OF NOTCH-1 SIGNALLING" – Università di Firenze

3) **Tagliavacca L**, et al. "IN VIVO UP-REGULATION OF THE UNFOLDED PROTEIN RESPONSE AFTER HYPOXIA" – Università di Milano

4) **Trinchera M.** "O-GLcNAc SIGNALING NEL CONDIZIONAMENTO CARDIACO E CARDIOPROTEZIONE" – Università dell'Insubria,Varese

5) **Turturici M & Roatta S**. "FENOMENI DI DILATAZIONE RAPIDA NEL MUSCOLO SCHELETRICO" – Università di Torino

11.10 – 11.40 Discussione

Sabato 24 novembre 2012

SALA FOTOGRAFI

09.00 – 09.50 SESSIONE V: ELECTROPHYSIOLOGY
Moderatore: Prof. ssa Donatella Stilli

09.00 – 09.30 Comunicazioni

1) **Bongianino R**, et al. "FUNCTIONAL AND MOLECULAR CHARACTERIZATION OF A NOVEL KCNQ1 MUTATION (T587R) THAT CAUSES LONG QT SYNDROME – IRCCS Fondazione "Salvatore Maugeri", Pavia

2) **Lodola F**, et al. "ADENO-ASSOCIATED VIRAL GENE DELIVERY OF CALSEQUESTRIN 2 PROTECTS ADULT CALSEQUESTRIN 2-R33Q KNOCK-IN MICE FROM DEVELOPING VENTRICULAR TACHYCARDIAS" – IRCCS Fondazione "Salvatore Maugeri", Pavia

3) **Merati G.** "25 ANNI DI NON LINEARITA' NELLA DINAMICA DEL RITMO CARDIACO: PROBLEMI E PROSPETTIVE" – Università di Milano

09.30 – 09.50 Discussione

09.50 – 11.00 SESSIONE VI (1) : METABOLIC SYNDROME
Moderatore: Prof. ssa Emanuela Masini

09.50 – 10.30 Comunicazioni

1) **Adorni MP**, et al. "SERUM CHOLESTEROL EFFLUX CAPACITY INVERSELY CORRELATES WITH ARTERIAL STIFFNESS IN HEALTHY SUBJECTS" – Università di Parma

2) **Carotenuto F**, et al. "OMEGA-3 FATTY ACIDS: PROSPECTS FOR A CARDIAC LIPID THERAPY" – Università di Tor Vergata, Roma

3) **Favero G**, et al. "OBESITY-ASSOCIATED VASCULAR ALTERATIONS AND MELATONIN BENEFICAL EFFECTS" – Università di Brescia

4) **Grassia G**, et al. “PLASMACYTOID DENDRITIC CELLS: KEY ANTIGEN PRESENTING CELLS IN EXPERIMENTAL ATHEROSCLEROSIS?” – Università Federico II, Napoli

10.30 – 11.00 Discussione

11.00– 12.10 SESSIONE VI (2) : METABOLIC SYNDROME
Moderatore: Prof. Tommaso Angelone

11.00 – 11.40 Comunicazioni

1) **Pederiva C**, et al. “STRATEGIE DI SCREENING IN ETA’ PEDIATRICA PER LE MALATTIE CARDIOVASCOLARI” – Università di Milano

2) **Zanotti I,** et al. “THE IMMUNOSUPPRESSANT DRUG CYCLOSPORINE A IMPAIRS THE REVERSE CHOLESTEROL TRANSPORT IN VIVO BY REDUCING STEROL FECAL EXCRETION” – Università di Parma, Università di Modena e Reggio Emilia

3) **Zimetti F**, et al. “FLOW-MEDIATED DILATION AND HDL FUNCTION IN SUBJECTS WITH HYPERALPHALIPOPROTEINEMIA: THE HYPERALPHALIPOPROTEINEMIA AND ATHEROSCLEROSIS (HALA) STUDY” – Università di Parma

4) **Zuchi C**, et al. “CARDIOVASCULAR INJURY IN THE METABOLIC SYNDROME INDUCED BY CHRONIC COLA DRINKING” – Università di Perugia

11.40 – 12.10 Discussione

ATP-MEDIATED CALCIUM SIGNALS IN TUMOR ANGIOGENESIS: ROLES AND MECHANISMS

Avanzato D, Genova T, Bernardini M, Fiorio Pla A, Munaron L.
Department of Life Sciences and Systems Biology, University of Torino.

Email: daniele.avanzato@unito.it

Changes in intracellular calcium $[Ca^{2+}]_i$ levels control critical cytosolic and nuclear events that are involved in the initiation and progression of tumor angiogenesis. Therefore, the mechanism(s) involved in agonist-induced $[Ca^{2+}]_i$ signaling is a potentially target for controlling angiogenesis and tumor growth. In this study, we used a tumor-derived endothelial cell line isolated from human breast carcinoma (BTEC) and a human microvascular endothelial cell line (HMVEC) as a normal counterpart.
We had previously reported a key role for arachidonic acid (AA)-mediated Ca^{2+} entry which is involved in the initial stages of *in vitro* tumor angiogenesis. Here we investigate $[Ca^{2+}]_i$ signals induced by extracellular Adenosine-5'-Triphosphate (ATP), a molecule that regulates short-term vascular events, and can be released during inflammation and in cancer. We show that micromolar ATP concentrations trigger different calcium signals in HMVEC and BTEC. Interestingly, 100 μM ATP response in BTECs triggers a strong and fast Ca^{2+} release from the ER, and a second sustained phase due to store-operated Ca^{2+} entry (SOCE). Biological assays were performed to compare ATP-dependent effects on HMVECs and BTECs: 100 μM ATP plays a major role in BTEC angiogenic properties because it inhibits cell proliferation, migration and *in vitro* angiogenesis. Similar results were shown for other pro-inflammatory molecules.
Our results showed that extracellular ATP is probably involved in the regulation of tumor angiogenesis. Physiopathological effects of ATP are yet to be understood and future studies will be aimed at underline differences between normal and tumor-derived ECs and how Ca^{2+} signals are related to biological effects.

ALTERATA ESPRESSIONE DEI CANALI DEL Ca^{2+} IN CELLULE PROGENITRICI ENDOTELIALI DI PAZIENTI AFFETTI DA CARCINOMA CELLULARE RENALE, MIELOFIBROSI IDIOPATICA E TUMORE DELLA MAMMELLA

Bottino C[1], Rosti V[3], Bonetti E[3], Della Porta M[5], Pedrazzoli P[4], Porta C[4], Tanzi F[2], Moccia F[2], Laforenza U[1].

[1]Dipartimento di Medicina Molecolare, Università degli Studi di Pavia, via Forlanini 6, 27100 Pavia; [2]Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani", Università degli Studi di Pavia, Pavia; [3]Laboratorio di Epidemiologia Clinica, Fondazione IRCCS Policlinico San Matteo, Pavia; [4]Medicina Oncologica IRCCS, Fondazione Policlinico San Matteo, Pavia; [5]Dipartimento di Ematologia Oncologica, Fondazione IRCCS Policlinico San Matteo, Pavia.

Email: cinzia.bottino01@universitadipavia.it

Le cellule progenitrici delle cellule endoteliali (EPC) vengono reclutate dal midollo osseo per promuovere la neovascolarizzazione tumorale e la formazione di metastasi. La comprensione dei meccanismi molecolari che guidano la proliferazione delle EPC e il processo di tubulogenesi può rivelare bersagli terapeutici per trattamenti anti-angiogenici alternativi. L'ingresso capacitativo di Ca^{2+} attraverso i canali SOC (Store Operated Channel), attivati dalla deplezione dei depositi intracellulari di Ca^{2+}, regola la crescita delle EPC nell'uomo ed è mediato dall'interazione tra il sensore del Ca^{2+} sul reticolo endoplasmatico, STIM1, e i canali del Ca^{2+} sulla membrana plasmatica, quali ORAI e TRPC.
Nel nostro studio abbiamo utilizzato tecniche di qRT-PCR, silenziamento genico e western blotting per valutare l'espressione dei SOC nelle EPC isolate dal sangue periferico di pazienti affetti da carcinoma cellulare renale (RCC-EPC), mielofibrosi idiopatica (MF-EPC) e tumore della mammella (BC-EPC) e dal sangue periferico di individui sani (N-EPC). I risultati ottenuti mostrano che nelle RCC-EPC il SOCE è aumentato a causa dell'iper-espressione di ORAI1 e STIM1. Le MF-EPC presentano uno cambiamento nelle proteine coinvolte nel meccanismo del SOCE dato dalla maggior espressione di ORAI2-3 e STIM2. Nelle BC-EPC il canale TRPC4 è maggiormente espresso e potrebbe quindi essere il principale responsabile del SOCE in queste cellule.
In conclusione, il SOCE risulta modificato (aumentato) nelle EPC di pazienti affetti da tumore e i canali di tipo SOC coinvolti differiscono nei vari tumori considerati. È possibile quindi considerare i SOC come un potenziale target terapeutico per interferire con il processo di vascolarizzazione tumorale.

ADP-INDUCED STORE OPERATED CALCIUM ENTRY (SOCE) REGULATES HUMAN MEGAKARYOCYTE FUNCTION

Di Buduo CA[1], Moccia F[2], Tanzi F[2], Cattaneo M[2], Balduini A[1,4].
[1]Biotechnology Laboratories, Department of Molecular Medicine, IRCCS San Matteo Foundation, University of Pavia, Pavia, Italy; [2]Department of Biology and Biotechnology, University of Pavia, Pavia, Italy; [3]Medicina 3, San Paolo Hospital, Department of Internal Medicine, University of Milano, Milano, Italy; [4]Department of Biomedical Engineering, Tufts University, Medford, MA, USA.

Email: christianandre.dibuduo01@universitadipavia.it

Platelets are formed and released into the bloodstream from their precursor megakaryocytes (Mks) by extending long filaments called proplatelets. However, the mechanisms that control proplatelet formation are poorly understood. In this work we investigated the role of autocrine components in regulating human Mk function. We demonstrated that in *vitro* differentiated Mks from human hematopoietic progenitors constitutively release adenosine diphosphate (ADP) which, in turn, interacts with the purinergic receptor $P2Y_{13}$ to promote proplatelet formation. Upon stimulation, the G protein coupled receptor $P2Y_{13}$ leads to phosholipase C (PLC) activation and Ca^{2+} release. Consistently, we showed that extracellular ADP binding to $P2Y_{13}$ elicit a rapid increase in $[Ca^{2+}]_i$, followed by a clear plateau, which is lowered in Ca^{2+}-free solution, thereby suggesting the involvement of store-operated Ca^{2+} entry (SOCE). Therefore, we provided the first evidence that Mks express the major candidates to mediate SOCE, STIM1 and Orai1, which were functionally activated upon either pharmacological (i.e., cyclopiazonic acid) or physiological (i.e., ADP) depletion of the intracellular Ca^{2+} pool. Conversely, the mechanism was inhibited by the PLC inhibitor, U-73122, or the specific SOCE inhibitor, BTP-2. Finally, blockage of SOCE impaired ADP-induced cytoskeleton rearrangement leading to reduced Mk adhesion and migration on extracellular matrix. These findings provide the first evidence that in human Mks ADP, upon SOCE engagement, regulate Mk functions. Overall these data open new perspectives in the evaluation the signals that control platelet release *in vivo*.

CANONICAL TRANSIENT RECEPTOR POTENTIAL CHANNEL 3 AS A NOVEL TARGET FOR CELL-BASED THERAPY IN ENDOTHELIAL COLONY FORMING CELLS

Dragoni S[1], Laforenza U[2], Bonetti E[3], Rosti V[3], Tanzi F[1], Moccia F[1].
[1]Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Italy; [2]Dipartimento di Medicina Molecolare, Università degli Studi di Pavia, via Forlanini 6, 27100 Pavia; [3]Laboratorio di Epidemiologia Clinica, Fondazione IRCCS Policlinico San Matteo, Pavia.

Email: silvia.dragoni01@universitadipavia.it

Endothelial colony forming cells (ECFCs) are endothelial progenitor cells capable of acquiring a mature endothelial phenotype and represent a suitable tool for cell-based therapy. We have shown that VEGF stimulates ECFC proliferation and tubulogenesis by causing an oscillatory increase in intracellular Ca^{2+} concentration; furthermore VEGF-induced proliferation and expansion of umbilical cord blood–derived ECFCs are faster than their peripheral counterpart. Unlike peripheral ECFCs, UCB-ECFCs express the canonical transient receptor potential channel-3 (TRPC3), that mediated diacylglycerol (DAG)-dependent Ca^{2+} entry and promotes angiogenesis in endothelial cells. This study aimed at investigating whether the higher proliferative potential of UCB-ECFCs was associated to any difference in the molecular underpinnings of their Ca^{2+} response to VEGF.
VEGF induced asynchronous Ca^{2+} oscillations in UCB-ECFCs which did not occur in the absence of external Ca^{2+}. In order to assess whether TRPC3 contributes to the onset of VEGF-elicited Ca^{2+} spikes, we exploited the membrane permeable DAG analogue, OAG. Similar to VEGF, OAG elicited a Ca^{2+} transient only in presence of extracellular Ca^{2+}. Both VEGF and OAG-induced Ca^{2+} signals were abolished by the TRPC3 blockers, flufenamic acid (FFA) and Pyr3. Ca^{2+} oscillations were inhibited by U73122, a phospholipase-C inhibitor, shortened by the SOCE inhibitor BTP-2 and switched into a monotonic transient by the SERCA inhibitor cyclopiazonic acid. Moreover FFA, BAPTA, an intracellular Ca^{2+} buffer, and BTP-2 prevented UCB-ECFC proliferation and tubulogenesis.
Future studies will have to outline whether TRPC3 overexpression in PB-ECFCs augments their proliferative rate in vitro and their regenerative potential in vivo.

ROLE OF TRPM8 IN THE CONTROL OF VASCULAR ENDOTHELIAL CELL MIGRATION AND ADHESION

Genova T[1], Gkika D[2], Bokhobza A[2], Munaron L[1], Serini G[3], Prevaskaya N[2], Fiorio Pla A[1,2].
[1]Lab. of Cellular and Molecular Angiogenesis DBIOS, University of Torino, Italy; [2]Lab. of Cell Physiology INSERM U 1003, Université de Lille 1, France; [3]Laboratory of Cell Adhesion Dynamics, IRCC, Dept. of Oncological Sciences, University of Torino School of Medicine, Candiolo (TO), Italy.

Email: tullio.genova@gmail.com

Changes in intracellular calcium $[Ca^{2+}]i$ levels control critical cytosolic and nuclear events that are involved in the initiation and progression of tumor angiogenesis in endothelial cells (ECs). Therefore, the mechanisms involved in agonist-induced $[Ca^{2+}]i$ signaling are potentially important molecular target for controlling angiogenesis and tumor growth. Several studies have shown that blood vessels in tumors differ from normal ones in their morphology, blood flow and permeability. We recently reported a key role for arachidonic acid (AA)-activated TRPV4 channel in tumor angiogenesis *in vitro*. Here we report an opposing effect of TRPM8 channel: as TRPV4, TRPM8 is differentially expressed in EC derived from human breast carcinomas (BTEC) as compared with 'normal' EC (HMVEC). However, in contrast with TRPV4 expression, TRPM8 is highly downregulated in BTEC compared with HMEC. Wound healing assays revealed a key role of TRPM8 in inhibiting cell migration of HMEC but not of BTEC. Interestingly overexpression of TRPM8 in BTEC significantly reduces cell migration while its downregulation in HMEC restores cell migration to similar levels as BTEC. TRPM8-mediated inhibition of endothelial cell migration closely correlates with its role on β1-integrin-mediated EC adhesion: again, TRPM8 activation inhibits EC adhesion while downregulating the channel reverts the effect. Moreover activation of b1-integrin completely revert TRPM8-mediated effect on EC adhesion.
Although the complete molecular mechanism remains to be clarified, the data presented clearly show that TRPM8 inhibits EC migration and adhesion by interfering with β1-integrin pathway, suggesting a balance between TRP channels in EC.

IN VITRO ATTENUATION OF CARDIAC STEM CELL SENESCENCE ENHANCES MYOCARDIAL REPAIR

Beltrami A.
Cattedra di Anatomia Patologica, Università di Udine, Udine.

Email: antonio.beltrami@uniud.it

Background. Cardiac Stem Cells (CSC) expanded *in vitro* from explanted hearts of old patients suffering from end stage heart failure (E-) are characterized, compared to those obtained from young, healthy donors (D-), by shorter telomeres, a larger fraction of cells showing: telomere induced dysfunction foci, p16 and p21 positivity, and reduced proliferation and migratory capabilities.

Aim. Assessing whether these *in vitro* alterations were associated to an attenuation of the reparative ability of E-CSC *in vivo*. Additionally, we investigated the pathways involved in E-CSC senescence and tested drugs able to reduce E-CSC senescence *in vitro* and to restore their reparative ability *in vivo*.

Methods and results. The ability of E-CSC to repair a myocardial infarction in SCID/beige immunodeficient mice was outperformed by D-CSC. E-CSC showed a trend towards a higher activity of TORC1 complex (assessed as S6K phosphorylation on Thr^{389}) and increased autophagic markers. Inhibition of TORC1 and enhancement of TORC2 signalling (assessed as phosphorylation of Akt on Ser^{473}) with a combination of 10nM rapamycin + 0.5μM resveratrol significantly reduced the fraction of senescent E-CSC *in vitro* and restored their reparative ability to D-CSC levels *in vivo*. This latter was associated with a reduction in myocyte apoptosis and senescence and with a significant increase in the tissue density of c-Kit^{+} CSC.

Conclusion. *In vitro* pretreatment of E-CSC with rapamycin and resveratrol enhances their reparative ability *in vivo* suggesting the use of such approach to enhance CSC therapy.

MECCANISMI DEL RECLUTAMENTO DELLE CELLULE STAMINALI NEL MICROCIRCOLO POSTISCHEMICO

Bettini M[1], Vitale S[1], Falzetti F[2], Di Ianni M[2], Macchia M[1], Tritto I[1], Ambrosio G[1].
[1]Cardiologia e Fisiopatologia Cardiovascolare; [2]Ematologia ed Immunologia Clinica, Università di Perugia.

Email: isabella.tritto@ospedale.perugia.it

La terapia cellulare nell'infarto miocardico acuto ha prodotto risultati promettenti negli studi pre-clinici ma piuttosto deludenti nell'uomo. In questo ambito, si conosce molto poco sui meccanismi di reclutamento nei tessuti ischemici. Nel midollo osseo, la migrazione delle HSC avviene attraverso le tre fasi utilizzate dai leucociti per raggiungere i tessuti: rolling, adesione, infiltrazione nei tessuti. Inoltre, le HSC esprimono marcatori di superficie comuni ai leucociti, e incrementano la migrazione in vitro in risposta a citochine pro-infiammatorie. Le HSC potrebbero quindi migrare nei tessuti postischemici con un comportamento simile a quello dei leucociti.

Abbiamo quindi valutato se il reclutamento delle HSC nei tessuti postischemici possa essere simile ai leucociti. Lo studio era effettuato su un modello di ischemia e riperfusione del muscolo cremastere di ratto, nel quale il comportamento delle cellule staminali all'interno di arteriole e venule era valutato tramite videomicroscopia intravitale. Il muscolo cremastere era sottoposto a 180 min di ischemia seguiti da 135 min di riperfusione. Le HSC marcate con un colorante fluorescente erano somministrato come bolo nella carotide dopo 90 min di riperfusione, e il loro comportamento monitorato per ulteriori 45 min di riperfusione. Alla fine dell'esperimento, le HSCs erano contate in tutto il muscolo cremastere; l'infiltrazione delle HSC era valutato tramite immunoistochimica e quantificata mediante real-time PCR per il recettore CD-34.

In assenza di ischemia, l'interazione fra HSCs ed endotelio era modesta, mentre dopo I/R le HSCs mostravano un notevole aumento del rolling e dell'adesione, proporzionale alla severità dell'ischemia.

Ulteriori valutazione permetteranno di definire ulteriormente le molecole di adesione coinvolte, e se altre condizioni che si verificano nella realtà dei pazienti possano modificare questo fenomeno, limitando così l'effetto della terapia cellulare.

MODULAZIONE FARMACOLOGICA DELLE INTERAZIONI FRA CELLULE MUSCOLARI LISCE VASCOLARI E CELLULE DENDRITICHE UMANE

Paccosi S[1], Musilli C[1], Romagnoli P[2], Parenti A[1,3].
[1]Dipartimento di Farmacologia; [2]Dipartimento di Anatomia, Istologia e Medicina Legale, Università degli Studi di Firenze, Università degli Studi di Firenze; [3]C.I.M.M.B.A.

Email: sara.paccosi@unifi.it

SCOPO. La formazione e la complicazione della lesione aterosclerotica sono caratterizzate da una importante componente infiammatoria ed immunitaria. Sebbene sia nota la presenza e l'attivazione di cellule dendritiche (DCs) nella neointima, il loro ruolo nella patogenesi della lesione non è ancora chiaro. Lo scopo del nostro studio è stato studiare e modulare farmacologicamente le interazioni fra DCs e cellule muscolari lisce vascolari (VSMC), in un modello in vitro di ambiente infiammatorio.

MATERIALI E METODI. DC mieloidi mature (mDCs) sono state ottenute ex vivo da precursori circolanti di donatori sani. E' stata indagata la modulazione del processo di adesione di mDCs a VSMCs stimolate con citochine infiammatorie e le possibili molecole di adesione coinvolte. Abbiamo inoltre studiato l'effetto di mDCs sull'attivazione delle VSMCs.

RISULTATI. Le mDCs aderivano alle VSMCs coronariche in maniera significativamente potenziata se le VSMCs venivano pretrattate con IFNγ e TNFα. In tale adesione erano coinvolti ICAM-1, VCAM-1 di VSMCs ed i contro recettori delle DCs, poiché in risposta a stimoli infiammatori aumentava significativamente l'mRNA di ICAM-1 e V-CAM1 e l'adesione delle mDC alle muscolari lisce diminuiva significativamente in presenza di anticorpi neutralizzanti anti-CD18 e anti-CD11c. Il pretrattamento delle VSMCs con atorvastatina e rosiglitazone riduceva significativamente l'adesione delle DCs alle muscolari stesse. I fattori solubili rilasciati da mDCs e DCs co-coltivate con VSMCs, stimolavano la migrazione di VSMCs.

CONCLUSIONI. Questi risultati dimostrano che un ambiente infiammatorio potenzia le possibili interazioni fra DCs and VSMCs, e suggeriscono un ruolo patogenetico importante delle DC nel rimodellamento vascolare.

ARITMIA VENTRICOLARE DOPO SOMMINISTRAZIONE INTRAMIOCARDICA DI CELLULE STAMINALI MESENCHIMALI DERIVATE DA MIDOLLO OSSEO (CSMBS) IN UN MODELLO PRE-CLINICO

Pennella S[1], Farinetti A[2], Lonardi R[2], Giuliani E[3], Migaldi M[4], Reggiani Bonetti L[4], Mattioli AV[1,4].
[1]Istituto Nazionale Ricerche Cardiovascolari (INRC); [2]Dipartimento di chirurgia generale e specialità chirurgiche - Università degli Studi di Modena e Reggio Emilia. [3]Dipartimento di Anestesia e Rianimazione-Policlinico di Modena; [4]Laboratori di Anatomia Patologica e Medicina Legale - Università degli Studi di Modena e Reggio Emilia; [5]Dipartimento di Scienze della Vita - Università degli Studi di Modena e Reggio Emilia.

Email: sonia.pennella@yahoo.it

Background. La terapia cellulare è una strategia terapeutica innovativa nei processi di riparazione e rigenerazione miocardica dopo infarto miocardico acuto (IMA). Uno dei problemi che a nostro parere merita di essere chiarito è la comparsa di fenomeni aritmici indotti da terapia cellulare. Lo scopo del presente studio è stato quello di valutare gli effetti pro-aritmici in seguito alla somministrazione intramiocardica di cellule staminali mesenchimali derivate da midollo osseo (CSMBs) in un modello pre-clinico di ischemia/riperfusione miocardica.

Metodi. Sono stati utilizzati 30 conigli New Zealand. E'stata indotta una ischemia/riperfusione miocardica mediante legatura temporanea dell'arteria coronaria anteriore. Le BMSCs sono state isolate, coltivate e risospese per l'iniezione. Abbiamo confrontato l'iniezione intramiocardica (i.m.) di BMSCs nell'area peri-infartuata con la somministrazione delle cellule per via endovenosa sistemica (e. v.). Un gruppo di controllo ha ricevuto una soluzione fisiologica per via i.m. per valutare l'effetto pro-aritmico della puntura. E' stato registrato il numero orario di contrazioni premature sopraventricolari (SVPC) mediante un sistema software di analisi ECG.

Risultati. Dopo l'iniezione cellulare, sono state osservate in tutti e tre i gruppi di animali un numero frequente di SVPC. E' stato registrato un aumento di SVPC nel gruppo trattato per i.m vs al gruppo trattato per e.v sia dopo 7 che 21 giorni dall'intervento vs al ctr.

Conclusione. Dall'analisi dei nostri dati emerge che: l'iniezione i.m è fonte di eventi pro-aritmici ventricolari. La somministrazione di CSMs via i.m. determina un numero di eventi pro-aritmici maggiore rispetto alla somministrazione delle cellule via e.v. e rispetto al placebo i.m.

MECCANISMI E DIAGNOSI PRECOCE DELLA CARDIOTOSSICITÀ DA CHEMIOTERAPICI

Vitale S, Barretta G, Bettini M, Macchia M, Tritto I, Ambrosio G.
Cardiologia e Fisiopatologia Cardiovascolare, Università di Perugia.

Email: isabella.tritto@ospedale.perugia.it

Un limite importante alla chemioterapia antineoplastica e' rappresentato dalla comparsa di cardiotossicità. L'ipotesi del nostro studio e' che alla base della cardiotossicità da chemioterapici vi sia una alterazione delle cellule staminali, responsabili della rigenerazione vascolare e cardiaca, che sembrano svolgere un ruolo importante nel ripristino della normale funzione vascolare e cardiaca dopo chemioterapia.

Il progetto si propone di valutare se lo sviluppo di cardiotossicità da chemioterapici sia correlato al numero e alla funzionalità delle cellule progenitrici endoteliali circolanti (EPC). Valuteremo inoltre se nuovi indici ecocardiografici di funzione cardiaca possano costituire dei marcatori precoci e sensibili di danno cardiaco, e se siano correlati alla funzione vascolare e delle EPC.

In pazienti che iniziano chemioterapia con farmaci cardiotossici, saranno misurati la funzione vascolare e cardiaca, e i livelli e funzione delle EPC circolanti. Le valutazioni saranno eseguite prima dell'inizio della chemioterapia, al termine di ciascun ciclo, e 3 e 6 mesi dopo la fine dell'ultimo ciclo, e ne sarà valutata la correlazione con lo sviluppo di cardiotossicità.

I risultati permetteranno di stabilire se la cardiotossicità sia legata alle alterazioni delle EPC; se nuove metodiche ecocardiografiche avanzate permettano l'identificazione precoce della cardiotossicità in uno stadio subclinico.

La dimostrazione di una correlazione fra cardiotossicità, funzione vascolare, e funzione delle EPC darà un contributo importante ai seguenti obiettivi:

- suggerire un meccanismo di cardiotossicità da chemioterapici

- individuare parametri ecocardiografici che possano precocemente svelare danni da cardiotossicità, permettendone la prevenzione.

- contribuire all'obiettivo di preservare non solo la funzione cardiaca, ma anche l'efficacia antineoplastica.

NATRIURETIC PEPTIDE–GUIDED THERAPY VS. USUAL SYMPTOM-GUIDED CARE IN OUTPATIENTS WITH CHRONIC HEART FAILURE: A SYSTEMATIC REVIEW WITH META-ANALYSIS

Ariano C[1,2], De Vecchis R[1], Di Biase G[2], Giasi A[1], Cioppa C[1] ,Fusco A[1], Cantatrione S[1].
[1]Cardiology Unit, Presidio Sanitario Intermedio "Elena d'Aosta", Napoli (Italy); [2]Neurorehabilitation Unit,Clinica "S.Maria del Pozzo", Somma Vesuviana (Italy).

Email: carmelariano@tiscali.it

Background. It has been asserted that serial measurements of natriuretic peptides (NP), i.e. B-type natriuretic peptide (BNP) or amino-terminal fragment of pro B-type natriuretic peptide (NT-pro BNP), may serve as an objective practical guide to modulate the intensity of drug treatment for individuals with chronic heart failure (CHF). However, considerable uncertainty remains about the alleged useful role of NP-guided therapy in this context. Therefore we decided to execute a meta-analysis of published randomized controlled trials (RCTs) to test the hypothesis that an improvement of clinical outcomes in outpatients with CHF may be achieved by adjustment of pharmacologic dosing done according to the NP determinations.

Methods. The relevant studies were collected through a search across the Pubmed database (January 1996-September 2012). For our meta-analysis parallel-group RCTs were eligible for inclusion if they met the following criteria: they enrolled patients with CHF; they randomized patients to a strategy of titrating drug therapy based on the level of a circulating NP (BNP or NT-pro BNP) compared to a parallel control group treated according to the clinical conventional criteria; and they reported all-cause mortality. In addition, it was established that each RCT to be incorporated in the evaluation should have had more than 60 participants and its follow-up should have been longer than 90 days. The primary endpoint of the meta-analysis was all-cause mortality and hospitalization, heart-failure related (combined endpoint).

Results. Among six pooled RCTs admitted to final meta-analysis (total of included patients =1775), NP-guided therapy for outpatients with CHF was shown to be associated with decreased risk of death and unscheduled hospital stays during follow up (Odds Ratio- random effect model-: 0.64 95% CI:0.43-0.95 p=0.026).

Conclusions. This meta-analysis supports the hypothesis that NP-guided therapy is superior to symptom-guided therapy for improving clinical outcomes in CHF outpatients. However, some large RCTs failed to document significant clinical improvement in terms of mortality and morbidity using NP-guided strategy; thus any attempt to clarify this still unresolved issue by means of further basic and clinical research is recommended in the future.

ELEVATED LEVELS OF PRE-OPERATIVE INTERLEUKIN-6 AFFECT MONOCYTE ACTIVATION AND MULTI ORGAN FAILURE IN LEFT VENTRICULAR ASSIST DEVICE (LVAD) PATIENTS

Caruso R[1,2], Verde A[3], Botta L[3], Milazzo F[3], Parolini M[1], Martinelli L[3], Trivella G[2], Frigerio M[3], Parodi O[1].
[1]CNR Clinical Physiology Institute, CardioThoracic and Vascular Department, Niguarda Cà Granda Hospital, Milan, Italy; [2]CNR Clinical Physiology Institute, Pisa, Italy; [3]CardioThoracic and Vascular Department, Niguarda Cà Granda Hospital, Milan, Italy.

Email: raffaele.caruso@ospedaleniguarda.it

Interleukin (IL)-6-dependent signals are proposed as crucial triggers in controlling monocyte activation, an important condition in the development of multi-organ failure (MOF) and complications in left ventricular assist device (LVAD)-patients. However it is still poorly known the relationship among pre-implant levels of IL-6 in LVAD-recipients, monocyte activation and early (1 month) outcomes. Thus thirty-seven end-stage heart failure patients undergoing LVAD implantation were enrolled. Blood and urine samples were collected preoperatively, at 3 days and 1 post-LVAD week for assessment of plasma IL-6 levels, and urine neopterin/creatinine ratio (Neo/Cr), a marker of monocyte activation. MOF was evaluated by total Sequential Organ Failure Assessment (tSOFA) score. Patients were divided in 3 groups according to tertiles of pre-implant IL-6 levels [IL-6 range (pg/mL): 0.4-3.9 in A-, 6.2-23.6 in B-, and 24.6-500.5 in C-groups]. Seven patients died because of adverse MOF during the first postoperative month (86% of died patients were in B-and C-groups). The ICU stay resulted longer in C-group [21 (13-29) days] with respect the other ones [10 (8-13) and 11 (11-16) days in A and B, respectively, $p = 0.007$]. Postoperative Neo/Cr levels related with ICU stay, and Neo/Cr showed the highest increase in C-group [121 (65-209) % with respect to pre-implant]. Postoperative tSOFA score increased only in B- and C-groups. These findings support that postoperative monocyte activation is affected by pre-implant IL-6 levels, and might contribute to poor early outcome in LVAD-patients. This study even highlights the importance of the multidisciplinary approach in the field of advanced surgery of LVAD implantation to ameliorate the outcome of implanted patients.

SensorART: A remote controlled Sensorized Artificial heart enabling patients empowerment and new therapy approaches (FP7-ICT-2009 project grant agreement 24863)

B-TYPE NATRIURETIC PEPTIDE–GUIDED VS. CONVENTIONAL CARE IN HEART FAILURE PATIENTS: A CASE-CONTROL STUDY

De Vecchis R[1], Ariano C[2], Di Biase G[2], Ciccarelli C[2], Fusco A[1], Giasi A[1], Carmela Cioppa C[1], Cantatrione S[1].
[1]Cardiology Unit, Presidio Sanitario Intermedio "Elena d'Aosta", Napoli (Italy); [2]Neurorehabilitation Unit,Clinica "S.Maria del Pozzo", Somma Vesuviana (Italy).

Email: r.de.vecchis@alice.it; carmelariano@tiscali.it

Background. Whether therapy assisted by serial determinations of serum B-type natriuretic peptide (BNP) may improve the outcome for both acute decompensated heart failure(ADHF) and chronic heart failure (CHF), is currently questioned.

Methods. A case-control study was carried out, by enrolling patients with ADHF subsequently followed up for a mean period of four months. The patients who died or were involved by relapse of cardiac complaints were assumed as cases; for every case, one patient at least alive and free from new episodes of heart failure was recruited as control. Besides, cases and controls were matched for some variables to minimize the possible confounding. The possible role of BNP-guided therapy as predictor of decreased risk of deaths or new hospitalizations, heart failure related, was explored.

Results. 18 cases and 23 controls were enrolled. A fall in BNP>60% from baseline at 5th day after admission was found to be a predictor of decreased risk of the composite endpoint "death or new hospitalization, heart failure-related" (hazard ratio= 0,1146 95% CI:0,0283 to 0,4636 p=0,0025); on the other hand, a low GFR at admission (< 60ml/min/1.73 m^2) was associated also with increased risk of the same endpoint during mid- term follow up (hazard ratio= 5,7584 95% CI: 1,0954 to 30,2710 p= 0,0397); on the contrary, BNP-guided therapy was associated with similar risk of death or CHF-related hospitalization, compared to the conventional clinical approach.

Conclusions. No substantial improvement in cardiovascular event rates was obtained in outpatients with previous ADHF, treated with BNP-guided therapy during mid- term follow up.

MULTIDISCIPLINARY APPROACH TO EXERCISE LIMITATION IN PATIENTS WITH HEART FAILURE: PERIPHERAL MUSCLE DYSFUNCTION AND THE BENEFITS OF SMALL MUSCLE MASS TRAINING

Esposito F.
Department of Biomedical Sciences for Health, University of Milan, Italy.

Email: fabio.esposito@unimi.it

Doubt still remains as to whether peripheral vascular and skeletal muscle dysfunction accompany the compromised cardiac function associated with heart failure (HF). This study sought to elucidate the mechanisms responsible for the benefits of small muscle mass exercise training in patients with HF. We studied muscle structure and oxygen (O_2) transport and metabolism at maximal cycle (whole body) and knee-extensor exercise (KE) (small muscle mass) in 6 healthy controls and 6 patients with HF who then performed 8 weeks of KE training (both legs, separately) and repeated these assessments. Pre-training cycling and KE peak leg O_2 uptake (VO_{2peak}) were ~17% and ~15% lower, respectively, in the patients compared to controls. Structurally, KE training increased quadriceps muscle capillarity and mitochondrial density by ~21 and ~25%, respectively. Functionally, despite not altering maximal cardiac output, KE training increased maximal O_2 delivery (~54%), arterial-venous O_2 (a-v O_2) difference (~10%), and muscle O_2 diffusive conductance (D_MO_2) (~39%) (assessed during KE), thereby increasing single leg VO_{2peak} by ~53%, to a level exceeding that of the untrained controls. Post-training, during maximal cycling, O_2 delivery (~40%), a-v O_2 difference (~15%), and D_MO_2 (~52%) all increased, yielding an increase in VO_{2peak} of ~40%, matching the controls. In the face of continued central limitations, clear improvements in muscle structure, peripheral convective and diffusive O_2 transport, and subsequently O_2 utilization support the efficacy of local skeletal muscle training as a powerful approach to combat exercise intolerance in HF.

VALUTAZIONE DELLA RIGIDITÀ ARTERIOSA NEI PAZIENTI AFFETTI DA SCOMPENSO CARDIACO CON FRAZIONE D' EIEZIONE PRESERVATA

Raddino R, Trichaki E, Brambilla G, Gavazzoni M, Regazzoni V, Della Pina P, Gorga E, Madureri A, Rusconi C.
Cattedra e U.O. di Cardiologia Università e Spedali Civili di Brescia.

Email: riccardo.raddino@libero.it

Introduzione. Lo scompenso cardiaco con frazione d'eiezione preservata (HFpEF) riguarda approssimativamente un terzo dei pazienti con scompenso cardiaco con una prevalenza che ammonta il 38% –54% della totalità dei casi. La rigidità arteriosa è stata riconosciuta come un fattore fisiopatologico determinante dell'ipertensione arteriosa sistolica e la PWV carotido-femorale rappresentando la rigidità aortica, riflette effettivamente ciò che causa la maggioranza degli effetti fisiopatologici sul ventricolo sinistro.

Obiettivo. Lo scopo del nostro studio è quello di valutare la rigidità arteriosa, in termini di velocità di trasmissione dell'onda del polso (PWV) nei pazienti affetti da HFpEF.

Metodi. Sono stati studiati 26 pazienti (14 femmine e 12 maschi; età media: M 62.9±8.5 F 75,1 ±4.8) con F.E conservata (50± 5%). Sono stati suddivisi in tre gruppi: Gruppo A: 11 pazienti con segni e sintomi di scompenso cardiaco e caratteristiche ecocardiografiche di HFpEF (3,4); Gruppo B: 10 pazienti con segni e sintomi di scompenso cardiaco con caratteristiche ecocardiografiche non compatibili con HFpEF; Gruppo C: 5 pazienti ipertesi con reperti ecocardiografici normali. Tutti i pazienti erano ipertesi mentre la maggior parte di essi aveva anche una storia di diabete mellito di tipo II e fibrillazione atriale.

Conclusioni. La rigidità arteriosa in termini di PWV, è superiore nei pazienti affetti da HFpEF mentre nel gruppo di pazienti con segni e sintomi di scompenso cardiaco e con reperti ecocardiografici che non rientrano nei criteri del HFpEF, la PWV è inferiore. Nel gruppo di pazienti soltanto ipertesi non è stata dimostrata alcuna variazione significativa della PWV.

Bibliografia

1. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalance and outcome of heart failure with preserved ejection fraction. *N Engl J Med* 2006;355:251–259.
2. Mottram PM, Haluska BA, Leano R, Carlier S, Case C, Marwick TH. Relation of arterial stiffness to diastolic dysfunction in hypertensive heart disease. *Heart.* 2005;91:1551-1556.
3. European Study Group on Diastolic Heart Failure. How to diagnose diastolic heart failure. *Eur Heart J* 1998;19:990–1003-
4. Left ventricular diastolic function, disfunction and failure. Cesare Rusconi, Otto M. Hess, Corrado Poggesi; 2004; *C.E.S.I. Publisher Rome*.

BENEFICIAL EFFECTS OF RELAXIN IN AN *IN VIVO* EXPERIMENTAL MODEL OF ISCHEMIC ACUTE KIDNEY INJURY.

Collino M[1], Rogazzo M[1], Pini A[2], Benetti E[1], Rosa A C[1], Fantozzi R[1],Bani D[2] , Masini E[3].
[1]Department of Drug Science and Technology, University of Turin, Turin, Italy;
[2]Department of Anatomy, Histology and Forensic Medicine, University of Florence, Florence, Italy;
[3]Department of Preclinical and Clinical Pharmacology, University of Florence, Florence, Italy

Email: massimo.collino@unito.it

Background. Since its discovery in 1926, relaxin (RLX) has long been regarded as a peptide hormone of ovarian origin involved in the periparturient widening of the pubic symphysis. More recently, growing evidence have suggested that RLX exerts a broad range of other biological effects on many organs and apparatus, including the cardiovascular system [1]. Although preclinical and clinical studies have demonstrated that RLX ameliorates impaired renal function by exerting antifibrotic and regenerative effects [2], its role in renal ischemia/reperfusion (I/R) injury, one of the most common patophysiological event leading to acute kidney injury (AKI), have never been tested.

Methods. Using a well-known rat model of 1h bilateral renal artery occlusion followed by 6 h reperfusion [3], we investigated the effects of human recombinant RLX (5 µg /Kg e.v.) given both at the beginning and after 3 h reperfusion. Serum and urinary indicators of renal injury and dysfunction were measured.

Results. Interestingly, administration of the exogenous RLX attenuated all markers of renal injury and dysfunction caused by I/R. Simultaneously, RLX administration was associated with a significant reduction in markers of leukocyte infiltration and activation, as well as markers of oxidative stress. Overall, we document here, for the first time, that RLX protects against I/R-induced renal injury and dysfunction via several different mechanisms, including inhibition of the leukocyte infiltration and activation and the related oxidative stress.

Discussion. The results of this study offer good perspectives for the clinical potential of RLX in the medical treatment of cardiovascular ischemic diseases, including AKI.

[1] Bani D, Bigazzi M. (2011). Relaxin as a cardiovascular drug: a promise kept. Curr Drug Saf.6:324-328.

[2] Samuel CS, et al., Relaxin in cardiovascular and renal disease. Kidney Int. 2006; 69:1498-1502.

[3] Collino M et al., The selective PPARgamma antagonist GW9662 reverses the protection of LPS in a model of renal ischemia-reperfusion. Kidney Int. 2005;68:529-536.

OMEOSTASI DEL FERRO IN UN MODELLO DI POLICITEMIA

Gammella E[1], Díaz V[2], Recalcati S[1], Santambrogio P[3], Vogel J[2], Gassmann M[2], Cairo G[1].
[1]Dipartimento Scienze Biomediche per la Salute, Università di Milano; [2]Institute of Veterinary Physiology, Vetsuisse Faculty, and Zurich Center for Integrative Human Physiology (ZIHP), and University of Zurich, Switzerland; [3]Dipartimento Scienze Biomediche e Tecnologiche, Istituto Scientifico San Raffaele, Milano.

Email: gaetano.cairo@unito.it

Abbiamo utilizzato topi che riescono a convivere con un'eritropoiesi cronicamente elevata causata dalla sovraespressione di eritropoietina (linea Tg6) per un duplice scopo: i) chiarire i meccanismi attivati per assicurare la quantità di ferro necessaria a mantenere un ematocrito del 80%; ii) comprendere come gli stimoli eritropoietici regolano l'espressione dell'epcidina, un peptide epatico che controlla l'omeostasi del ferro. L'epcidina, inibendo la ferroportina che esporta ferro dalle cellule, modula negativamente l'assorbimento intestinale ed il riciclo macrofagico del ferro ed è regolata dai depositi di ferro ("store regulator") e dagli stimoli eritropoietici ("erythroid regulator"). Un aumento del ferro induce l'epcidina mediante il pathway BMP6/SMAD, mentre l'incremento dell'eritropoiesi la inibisce, in modo da aumentare la disponibilità di ferro. Infatti, abbiamo trovato nei topi Tg6 un forte calo del mRNA per l'epcidina ed un aumento dell'assorbimento intestinale di ferro e dell'espressione delle proteine di trasporto DMT1 e ferroportina. Nonostante questi meccanismi compensativi, i topi Tg6 mostrano marcata carenza di ferro, sia circolante che tissutale. Per comprendere quale sia il segnale che regola l'espressione di epcidina in condizioni di eritropoiesi elevata, abbiamo aumentato la disponibilità di ferro somministrando ferro o riducendo l'eritropoiesi mediante splenectomia. Nonostante livelli di eritropoietina sempre elevati, entrambi i trattamenti aumentavano il ferro epatico e inducevano epcidina e BMP6/SMAD. Questi risultati indicano che i depositi di ferro epatico, a loro volta dipendenti dal consumo eritroide (quindi con coincidenza di "erythroid" e "store regulators"), sono il principale segnale che controlla l'espressione di epcidina in condizioni di eritropoiesi cronicamente elevata, ma efficace.

VIE PROTETTIVE DEL PRE- E POST-CONDIZIONAMENTO ISCHEMICO NEL MUSCOLO CREMASTERE DI RATTO

Vitale S[1], Bettini M[1], Marchetti S[2], Macchia M[2], Migliorati G[2], Tritto I[2], Riccardi C[2], Ambrosio G[1].
[1]Cardiologia e Fisiopatologia Cardiovascolare, [2]Farmacologia, Tossicologia e Chemioterapia, Università di Perugia.

Email: isabella.tritto@ospedali.perugia.it

Un breve periodo di ischemia/riperfusione (I/R) che si verifica prima (precondizionamento; preC) di una prolungata ischemia o all'inizio della riperfusione (postconditioning; postC) riduce le dimensioni dell'infarto. Il preC protegge anche i vasi. Scopo del nostro studio era studiare se anche il postC protegge il microcircolo postischemico, valutandone gli effetti sia sulla disfunzione microvascolare che sul reclutamento dei leucociti durante riperfusione. Abbiamo anche valutato i meccanismi della protezione microvascolare. Fondamentali per la cardioprotezione sono l'attivazione delle cascate regolatorie della cosidetta "reperfusion injury signalling kinase (RISK) pathway"), e della GSK3β, e l'inibizione dell'apoptosi; inoltre, negli effetti protettivi di preC e postC è coinvolta la generazione di ossido nitrico, che è anche il principale mediatore della vasodilatazione endoteliale. Abbiamo quindi valutato l'attivazione delle vie di trasduzione del segnale (MAPK ERK-1/2, PI3K/Akt, GSK3β, PKCε), l'espressione di eNOS, l'attivazione dell'apoptosi tramite le caspasi 3, 8, 9.

Il muscolo cremastere di ratto era sottoposto a 90 min di I e 90 min di R. Mediante videomicroscopia intravitale era monitorato il reclutamento leucocitario e alla fine dell'esperimento era valutata la riserva vasodilatante.

L'I/R induceva reclutamento dei leucociti nel tessuto, e riduzione della capacità vasodilatante. Sia il preC che il postC riducevano l'interazione leucociti-parete vasale; solo il preC era in grado di preservare la riserva vasodilatante, mentre il postC era molto meno efficace. La valutazione dei potenziali meccanismi protettivi suggerisce che sia il preC che il postC attivino la via RISK; il preC mostrava una maggiore attivazione delle caspasi rispetto al postC. Studi ulteriori sono necessari per meglio definire la correlazione delle diverse vie di protezione con l'effetto protettivo sulla funzione micro vascolare.

CATESTATIN, A CHROMOGRANIN A DERIVED PEPTIDE, IN HEALTH AND DISEASE: ROLE OF PI3K/AKT PATHWAY IN THE PROTECTION AGAINST ISCHEMIA REPERFUSION INJURY

Bassino E, Fornero S, Gallina C, Gallo MP, Levi R, Alloatti G.
Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino.

Email: giuseppe.alloatti@unito.it

Background. Catestatin (CST), a little 21-amino acid peptide derived from Chromogranin A pro-hormone, inhibits catecholamine secretion and exerts negative myocardial inotropism, acting via a nitric oxide (NO)-dependent mechanism. Although CST affects several cardiovascular parameters (e.g. reducing blood pressure and cardiac contractile force), the mechanisms underlying CST action in the heart remained elusive.

Aims. To define the mechanisms of action and the signaling pathways activated by CST in the protection against ischemia reperfusion (I/R) injury.

Methods. The protective role of CST was analyzed on isolated cardiomyocytes (cc); cell viability rate was evaluated with propidium iodide labeling, and mitochondrial membrane potential (MMP) with the fluorescent probe JC-1. The involvement of Akt, GSK3β, eNOS and phospholamban (PLN) cascade was studied by immunofluorescence and western-blot techniques. The role of PI3K-Akt pathway was also investigated by using the pharmacological blocker wortmannin (Wm).

Results. In isolated cc undergoing simulated I/R, CST increased cell viability rate by 65%; the protective effect was related to the ability to maintain intracellular calcium homeostasis and MMP, and to increase Akt^{Ser473}, $GSK3\beta^{Ser9}$, PLN^{Thr17} and $eNOS^{Ser1179}$ phosphorylation. Wm abolished CST-induced cardioprotection.

Conclusions. These results give new insights into the molecular mechanisms involved in the cardiovascular effect of CST, highlighting the PI3K pathway as the trigger and MMP preservation as the end point of its action.

Future perspectives. To characterize the upstream mechanisms involved in the activation of PI3K/Akt pathway on cc. Actually, in fact, no evidences are reported for a presence of receptors or a direct membrane interaction of CST on these cells.

CREATINA E D-RIBOSIO: CARDIOPROTEZIONE IN MODELLI DI IPOSSIA IN VITRO ED IN VIVO

Caretti A[1], Bianciardi P[2], Marini M[2], Bolotta A[2], Abruzzo PM[2], Samaja M[1].
[1]Dipartimento di Scienze della Salute-Università degli Studi di Milano, Osp. San Paolo;
[2]Dipartimento di Istologia Embriologia e Biologia Applicata-Università di Bologna.

Email: anna.caretti1@gmail.com

Background. La somministrazione combinata di creatina e D-ribosio può limitare il danno miocardico conseguente ad ipossia sostenendo il metabolismo energetico e favorendo la sintesi di ATP. E' stato dimostrato che il trattamento con creatina e ribosio riduce l'apoptosi in cardiomiociti della linea H9c2 esposti per 24 ore ad ischemia (1% di O_2 in assenza di glucosio) modulando i pathway che dipendono da AMPK, sensore dei livelli di ATP intracellulari e da AKT, coinvolto nei processi di sopravvivenza.

Obiettivo. Si vuole dimostrare che un analogo meccanismo si riscontra in un modello in vivo di ipossia cronica.

Metodi. Topi di cinque settimane esposti al 10% di O_2 per dieci giorni sono stati trattati giornalmente con la combinazione creatina-ribosio mediante oral gavage.

Risultati. Lo stress ipossico ha indotto ipertrofia del ventricolo destro e apoptosi del ventricolo sinistro quasi completamente risolti dopo il trattamento combinato con creatina e ribosio. A livello molecolare, AMPK, AKT e JNK sono stati indotti dallo stress ipossico ma, dopo il trattamento, sono ritornati a valori basali, correlando con l'effetto cardioprotettivo osservato. La vasocostrizione polmonare conseguente ad ipossia si è attenuata in presenza di creatina e ribosio come evidenziato dalla ridotta espressione dell'endotelina-1.

Conclusione. La somministrazione combinata di creatina e ribosio risulta cardioprotettiva in quanto, compensando lo squilibrio bio-energetico derivante dallo stress ipossico, è efficace sui cardiomiociti danneggiati in modo non-irreversibile in vitro ed in vivo.

APELIN13 AND MYOCARDIAL PROTECTION

Folino A, Rastaldo R.
Department of Clinical and Biological Sciences, University of Turin, Orbassano (To).

Email: anna.folino@alice.it

Apelin is an endogenous peptide with several cardiovascular activities. In particular its exogenous administration has been seen to mimic postconditioning, to improve myocardial contractility, to play an antiapoptopic role, to enhance cardiac differentiation of embryonic stem cells, to prevent cardiac hypertrophy and to promote vascular formation. Taken together all these activities seem to converge in a general protective system of the heart against various diseases. Moreover Apelin is reported to increase during ischemia together with the expression of the specific receptors APJ. These have recently been seen to be activated also by stretch responsible for cardiac hypertrophy (Scimia et al., 2012)
Apelin genes encode a 77 aminoacid preprotein with the active sequence in the COOH terminal region. Among the various fragments of the preprotein, Apelin-13 is considered the most active compound.
On Langendorff perfused rat hearts undergone 30 min of global ischemia and 2 hours of reperfusion we studied whether Apelin-13 protects myocardium against ischemia/reperfusion injury and whether the protection includes an improved postischemic mechanical recovery. We also studied whether the effect of Apelin administration was favoured by an increased expression of APJ receptors. An Apelin-13 solution was infused for 20 min either before or after ischemia to mimic pre- and postconditioning respectively.
If given before ischemia, Apelin did not produce protection even at 1 μM concentration, while it was effective at 0.5 μM concentration if given starting immediately after ischemia. The protection included reduction of the infarct size and a better mechanical recovery, the latter accompanied by an attenuation of postischemic contracture. NO-inhibition by L-NNA abolished protection. APJ expression increased significantly 15 min only after the end of ischemia, i.e. long time from the occurrence of reperfusion injury which is reported to occur mainly during the first 2 min after the end of ischemia.

Emerging problems:

1) Does endogenous Apelin-APJ system takes part in a spontaneous protection of the heart in various cardiac diseases and requirements?

2) NO-inhibition by L-NNA indicates that NO is involved in myocardial protection by Apelin. However it is not clear whether the effect is mediated by the GC-cGMP pathway or by protein S-nitrosylation as reported by Ohtani et al. (2012).

3) APJ expression increases late after the occurrence of reperfusion injury and Apelin administration. A question then arises whether Apelin activity is mediated independently of these receptors or ischemia-induced APJ intracellular trafficking involves delocalization of the APJ receptors from internal vehicles to sarcolemma.

4) APJ receptors can be also activated by fiber stretch which leads to myocardial hypertrophy via these receptors, genetic loss of APJ limits overload hypertrophy. May endogenous Apelin-Apj system be differently oriented in regulating cardiac function depending on not yet clarified conditions?

Scimia MC, Hurtado C, Ray S, Metzler S, Wei K, Wang J, Woods CE, Purcell NH, Catalucci D, Akasaka T, Bueno OF, Vlasuk GP, Kaliman P, Bodmer R, Smith LH, Ashley E, Mercola M, Brown

JH, Ruiz-Lozano P. APJ acts as a dual receptor in cardiac hypertrophy. *Nature.* 2012; 488(7411):394-8.

Ohtani H, Katoh H, Tanaka T, Saotome M, Urushida T, Satoh H, Hayashi H. Effects of nitric oxide on mitochondrial permeability transition pore and thiol-mediated responses in cardiac myocytes. *Nitric Oxide.* 2012; 26(2):95-101.

MITOCHONDRIAL ISOLATION AND PROTEOMIC ANALYSES IN RAT CARDIAC MUSCLE IN COURSE OF AGING: ADVANTAGES AND CRITICAL POINTS

Leone R[1], Viganò A[1], Fania C[1], Guelfi C[1,2].
[1]Dipartimento di scienze Biomediche per la Salute, Università degli Studi di Milano, Segrate (MI), Italy; [2]Istituto di Bioimmagini e Fisiologia Molecolare, Consiglio Nazionale delle Ricerche, Segrate (MI), Italy.

Email: roberta.leone@unimi.it

Intrinsic mitochondrial aging is one of the major risk factor for myocardial diseases onset. This degenerative process is associated to the theory of "mitochondrial aging", based on the evidence that, the accumulation of DNA and protein damages, caused by oxidative stress, negatively influences the mitochondrial function leading to cell dysfunction and finally to heart failure.

Subsarcolemmal and intermyofibrillar mitochondria were isolated by classical methodologies based on differential centrifugations and ultracentrifugation, in samples of young, old aldult and senescent rat hearts. Differential protein analyses of mitochondrial protein extracts were performed by 2D-DIGE coupled with mass spectrometry. The results suggested a discrepancy with published data on cardiac aged mitochondrial proteins, particularly at the level of the mitochondrial respiratory chain complexes. To avoid erroneous conclusions, a deep analysis of the purity and integrity of mitochondrial protein extracts was performed by immunobloting. The results indicated that mitochondrial matrix proteins were not enriched. Conversely, the mitochondrial membrane proteins were well enriched. Recently, the widely employed, classical methodology for mitochondrial isolation, was criticized because it provided information of a part of cell muscle mitochondria only, overwhelming the complexity of the tridimensional mitochondrial network.

The loss of mitochondrial matrix proteins, may explain the results obtained by differential proteome, leading to conclude that the classical isolation produces induce, inevitably, a breakup of the mitochondrial tubular network, with a consequent enrichment of membrane proteins and loss of the matrix content.

IN VIVO INTERMITTENT HYPOXIA AS A TOOL FOR CARDIOPROTECTION

Milano G[1], Rochemont V[1], Gorza L[2], Marini M[3], von Segesser LK[1], Samaja M[4].
[1]Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [2]Department of Biomedical Sciences, University of Padova, Padova, Italy; [3]Department of Histology, Embryology and Applied Biology, University of Bologna, Bologna, Italy; [4]University of Milano, Milano, Italy.

Email: giuseppina.milano@chuv.ch

Aim. We hypothesized that *in vivo* exposure to intermittent hypoxia (IH) would induce cardioprotection against I/R injury via activation of the PI3K/Akt signaling.

Materials and Methods. For exposure to IH or normoxia (N), animals (C57/BL6, 8-10 weeks old, n=8/groups) were housed in the Plexiglas chambers. IH was repeated every 6 hours for 14 day. During IH, the level of hypoxia was set to 7% O_2, with a rapid reoxygenation to 21% O_2. This event was repeated for 5 times. Animals exposed to N (Control group) received room air. Mice were subjected to occlusion of the left anterior descending coronary artery (LAD) for 30-min followed by 120-min of reperfusion. To investigate the role of PI3K/Akt signaling in IH-induced-cardioprotection, we administered the PI3K inhibitor, wortmannin (24 microg/Kg, i.p.), to the mice 10-min before ischemia. Infarct size and cardiac function were evaluated. The ventricles were frozen in liquid nitrogen for biochemical analyses of phosho-Akt and total-Akt isoforms, and the heme oxygenase-1 (HO-1) by Western Blotting.

Results. IH significantly increased both the activity of Akt and the protein expression of HO-1 compared to N group. Infarct size was markedly decreased in IH. IH led to improved myocardial performance as indicated by increased systolic pressure, dP/dt_{max} and left ventricle developed pressure. Such trend was maintained after LAD occlusion. Pharmacological inhibition of PI3K by wortmannin markedly abolished the cardioprotection induced by IH.

Conclusion. Intermittent hypoxia-induced cardioprotection in I/R injury is mediated via a PI3K/Akt pathway. Thus, the modulation of this pathway may be a viable strategy to reduce myocardial I/R injury.

CARDIAC PHYSIOPATHOLOGY: AN UPDATE ON CHROMOGRANIN A AND ITS DERIVED PEPTIDES

Pasqua T, Cerra MC, Angelone T.
Lab of Cardiovascular Physiopathology, Dept of Cell Biology, University of Calabria, 87036 Arcavacata di Rende (CS) Italy.

Email: teresapasqua@libero.it

The discovery of a cardiac production of Chromogranin A (CgA) (Steiner et al., 1990) opened a fruitful field of research whose outcome is a cardiovascular dimension for this multifunctional protein, with great relevance in cardiac physiopathology and clinics. In the rat heart, CgA is present in atrial myoendocrine (Steiner et al., 1990), and conduction cells (Weiergraber et al., 2000), co-localized with Atrial Natriuretic Peptide (ANP). In human ventricular myocardium (Pieroni et al., 2007), it co-localizes with Natriuretic Peptide type B (BNP). At the cardiac level CgA undergoes proteolytic processing, further stressing its precursor function (Glattard et al., 2006). *In vitro* functional studies indicate that the heart itself is responsive to CgA. Through the action of its amino terminal (vasostatin: VS) and catestatin (Cts) domains, CgA exerts a direct depressive, antiadrenergic and protective influence, acting as a cardiac stabilizer under normal conditions and in the presence of stress (i.e. adrenergic, endothelinergic, and ischemia/reperfusion) (Cerra et al., 2007; Cappello et al., 2007; Angelone et al., 2008a; Angelone et al., 2008b; Pagliaro et al., 2009). At the same time, through the C-terminal serpinin, CgA elicits a cardiostimulatory beta-adrenergic-like effect (Tota et al., 2012).

Recently, clinical studies suggested CgA involvement also in cardiovascular pathologies. High plasma CgA levels were found in hypertension (Takiyyuddin et al., 1995), chronic and acute heart failure (Ceconi et al., 2002), myocardial infarction (Omland et al., 2003), decompensated and hypertrophic heart (Pieroni et al., 2007), and acute coronary syndromes (Jansson et al., 2009). These alterations correlate with those of conventional cardiovascular biomarkers, such as NP (Pieroni et al., 2007), and endothelin-1 (ET-1), and have prognostic relevance, being indicative of both severity of the disease and mortality (Dieplinger et al., 2009). Accordingly, CgA plays a multifaceted role in cardiovascular homeostasis. Very recently, our studies demonstrated that full length CgA directly affects myocardial and coronary functions in normotensive and hypertensive rats. These modulations occur via endothelium-derived NO and the cGMP/PKG pathway. We also showed that intracardiac CgA processing is affected by physical (heart perfusion) and/or chemical stimuli (Isoproterenol, and ET-1). Our results pave the way to identify the spatio-temporal mechanisms which operate at cardiac level to orchestrate the activation of the sympathochromaffin/ CgA axis, including the putative intracardiac action of the CgA-derived peptides. This may expand our knowledge on the adrenergic control of the heart under normal conditions and in the presence of intense excitatory stimuli.

RELAXIN INHIBITS CARDIAC FIBROBLAST-MYOFIBROBLAST TRANSITION THROUGH THE UP-REGULATION OF NOTCH-1 SIGNALLING

Pini A[1], Nistri S[2], Sassoli C[2], Masini E[1], Bani D[2], Formigli L[2].
Depts of [1]Preclinical and Clinical Pharmacology, [2]Anatomy Histology and Forensic Medicine, University of Florence, Florence, Italy.

Email: alessandro.pini@unifi.it

The peptide hormone relaxin (RLX) has beneficial actions in the cardiovascular system and holds promise as a therapeutic option for ischemic heart disease. RLX stimulates neonatal cardiomyocyte growth and differentiation, suggesting its involvement in endogenous mechanisms of myocardial repair/regeneration. In the present study, we evaluated the effects of RLX on neonatal cardiac fibroblasts, based on the assumption that any strategies capable of attenuating fibrogenic activity of these cells may improve recruitment/expansion of the cardiac progenitors in their microenvironment. Cardiac fibroblasts were isolated from mouse neonatal heart, expanded in culture and induced to differentiate into myofibroblasts with TGF-β, in the presence or absence of RLX. RLX didn't affect fibroblast differentiation in basal culture conditions (may be a safe anti-fibrotic agent), however it was able to inhibit fibroblast-myofibroblast transition as judged by the up-regulation of α-SMA and the down-regulation of MMP-2 expression induced by TGF-β. These inhibitory effects of RLX involved the up-regulation Notch-1 pathway; in fact, Notch-1expression was significantly decreased in TGF-β-treated cardiac fibroblasts as compared to control and this reduction was prevented by the addition of RLX to TGF-β-treated cells. The endogenous Notch-1 signalling inhibition potentiated fibroblast differentiation induced by TGF-β and abrogated the inhibitory effects of RLX. These data suggest that Notch signalling may negatively regulate neonatal cardiac fibroblast-myofibroblast transition and that RLX could exert anti-fibrotic action through the up-regulation of this pathway. In conclusions these results support the role of RLX in regulating cardiac remodelling, suggesting that this hormone may be of potential therapeutic interest for cardiac

IN VIVO UP-REGULATION OF THE UNFOLDED PROTEIN RESPONSE AFTER HYPOXIA

Tagliavacca L, Caretti A, Bianciardi P, Samaja M.
Department of Medicine, Surgery and Dentistry, University of Milan, San Paolo Hospital, Milan, Italy.

Email: tagliavacca.luigina@hsr.it

Low oxygen (O_2) availability, a condition called hypoxia, has different and profound consequences in tissues and organs. Besides the hypoxia-inducible response, mammalian cells induce a coordinated cytoprotective pathway called Unfolded Protein Response (UPR). We studied the molecular basis of UPR and apoptosis in animal models exposed to different hypoxic stresses. Specifically we tested whether i) the hypoxic stress *in vivo* acts as a modifier that affect the activation of specific branches of the UPR in hepatocytes and cardiomyocites, ii) the UPR activation depends from the severity of the hypoxic stress.

We assessed the levels of several UPR markers in hypoxic animals exposed to two levels of O_2 reduction for 5 hours, a time frame that allows the induction of mRNA transcripts before the setting of adaptive mechanisms. Real-Time RT PCR and Western blotting were employed to measure the levels of expression of specific genes.

While the hepatocytes activate the apoptotic pathway mediated, in part, by CHOP and p-JNK, we could not detect an UPR-dependent apoptosis in cardiomyocytes. Moreover, severe hypoxia results in ATF4 translation, and induction of CHOP and GADD34 transcripts in liver, by contrast in the myocardium, the ATF4-CHOP-GADD34 signaling pathway is not detectably activated.

Comparison of several UPR markers in liver and myocardium enabled to underscore the ability of hepatocytes and myocites to selectively activate and fine tune the UPR signaling pathway during hypoxia *in vivo* [1].

[1] Tagliavacca L., Caretti A., Bianciardi P., Samaja M. (2012) In vivo up-regulation of the unfolded protein response after hypoxia. Biochim. Biophys. Acta, 1820 (7): 900.

O-GLCNAC *SIGNALING* NEL CONDIZIONAMENTO CARDIACO E CARDIOPROTEZIONE

Trinchera M.
Dipartimento di Medicina Clinica e Sperimentale, Università dell'Insubria, Varese.

Email: marco.trinchera@uninsubria.it

La glicosilazione di proteine citoplasmatiche e nucleari mediante O-GlcNAc (GlcNAcilazione) è una modificazione post-traduzionale molto diversa dalla glicosilazione O- ed N-*linked* che riguarda le proteine e i lipidi di membrana, poiché non implica la sintesi di un oligosaccaride ma un ciclo dinamico di singole glicosilazioni/deglicosilazioni paragonabile a quello della fosforilazione/defosforilazione che regola la funzione di molte proteine. Mentre lo stato di fosforilazione dipende da centinaia di enzimi, quello di GlcNAcilazione principalmente solo da due: polipeptide O-GlcNAc transferasi (OGT) e O-α-N-acetilglucosaminidasi (OGA); la disponibilità di UDP-GlcNAc è pure considerato un elemento regolatore.

E' noto che la GlcNAcilazione modula la funzione di alcuni fattori di trascrizione, e più recentemente sta emergendo l'ipotesi che essa correli positivamente con la sopravvivenza cellulare durante lo stress e negativamente con il danno cellulare nel sistema cardiovascolare. Ad esempio, il *knockout* generale del gene OGT è letale, mentre quello limitato al cuore non determina una immediata patologia, ma invece esacerba l'insufficenza cardiaca. Lo stato di GlcNAcilazione delle proteine è dunque in qualche modo parte del condizionamento cardiaco e della cardioprotezione?

Tale stato è facilmente valutabile tramite western blot, e sono disponibili inibitori di OGT ed OGA per validare i dati. Sarebbe interessante determinarlo in modelli *in vivo* ed *in vitro* nei quali un classico stress ischemico/ipossico segue varie forme di precondizionamento, o invece di comorbidità. Sarebbe poi necessario conoscere quali meccanismi regolano OGT e OGA, in particolare quelli genetici totalmente ignoti, identificando i promotori attivi nel miocardio e relative regioni di legame coi fattori di trascrizione.

FENOMENI DI DILATAZIONE RAPIDA NEL MUSCOLO SCHELETRICO

Turturici M, Roatta S.
Lab Fisiologia Integrativa, Dip. Neuroscienze, Università di Torino.

Email: silvestro.roatta@unito.it

Un tema che ha stimolato l'interesse di diversi gruppi di ricerca negli ultimi anni è la dilatazione rapida che si osserva nel muscolo scheletrico in risposta a stimoli di natura meccanica (es compressione esterna). Questa proprietà vascolare sembra essere una prerogativa del muscolo scheletrico, non espressa dal tessuto cutaneo (Turturici et al 2012) finalizzata ad aumentare rapidamente la perfusione all'inizio dell'attività muscolare. Si ritiene che la brusca riduzione della pressione trasmurale nella rete vascolare intramuscolare sia lo stimolo meccanico che evoca la dilatazione rapida, secondo quanto proposto originariamente da Bayliss. Va però sottolineato come la presente risposta dilatatoria abbia una latenza < 1 s, un tempo al picco di 2-4 s, si esaurisca in 20-30 s, e sia quindi molto più rapida della classica *risposta miogena*. Non è ancora chiaro il meccanismo meccano-dipendente alla base di questa risposta, né l'eventuale coinvolgimento dell'endotelio.

Nel nostro laboratorio abbiamo recentemente messo a punto un modello sperimentale *in vivo* particolarmente sensibile alla valutazione di questo fenomeno grazie alla misura del flusso sanguigno, diretto esclusivamente a tessuto muscolare, nell'arteria masseterica nel coniglio anestetizzato. Abbiamo recentemente caratterizzato la risposta iperemica a stimolazioni meccaniche di diversa natura. Il modello permette anche test farmacologici attraverso iniezione locale intra-arteriosa di sostanze.

L'importanza di questo fenomeno è legata alla sua influenza sul tono vascolare e sulle resistenze periferiche. Lo studio dei meccanismi che ne sono alla base beneficerebbe dell'integrazione con metodologie di indagine *in vitro*, su vasi isolati o cellule in coltura.

FUNCTIONAL AND MOLECULAR CHARACTERIZATION OF A NOVEL *KCNQ1* MUTATION (T587R) THAT CAUSES LONG QT SYNDROME

Bongianino R[1], De Giusti VC[1], Avelino-Cruz JE[1], Lodola F[1], Leccioli V[1], Denegri M[1], Tanzi F[4], Napolitano C[1,2], Priori SG[1,2,3].
[1]Molecular Cardiology, IRCCS Fondazione Salvatore Maugeri, Pavia, Italy; [2]Cardiovascular Genetics Program, The Leon H. Charney Division of Cardiology, University School of Medicine, New York, USA; [3]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [4]Department of Dept. of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy.

Email: rossana.bongianino@alice.it

Background. Long QT syndrome (LQTS) is an inherited arrhythmogenic disease characterized by Q-T interval prolongation and susceptibility to ventricular tachyarrhythmias associated with syncope and sudden cardiac death. LQTS-type1 arises from loss-of-function mutations in *KCNQ1* gene. The present study was designed to determine the functional and molecular characterization of a novel KCNQ1 mutation (T587R) identified in one of our patients.

Methods. We engineered the human-*KCNQ1* gene to codify the T587R mutation and a bicistronic plasmid containing both *KCNQ1*-T587R and *KNCQ1*-WT gene to mimic the heterozygous condition found in the patient. We transiently transfected the plasmids in Hek-A-cells and performed *in vitro* characterization by electrophysiology and molecular approaches.

Results. Electrophysiological analysis revealed that the *KCNQ1*-T587R mutation causes the expression of a non-functional α-subunit of the slow delayed rectifier potassium channel and the heterozygous expression showed a dominant negative effect ($p<0.05$). Molecular approaches demonstrated a significant reduction of KCNQ1-T587R in plasma-membrane and its localization in the *endoplasmatic reticulum* (ER). Since recently it was demonstrated the physical interaction between KCNQ1 and HERG that was associated with increased HERG-protein membrane localization, we co-transfected *KCNQ1*-T587R with HERG and, by western-blot analysis, we showed that *KCNQ1*-T587R is inhibiting HERG trafficking.

Conclusion. *KCNQ1*-T587R is encoding a trafficking deficient protein retained in ER. Furthermore, *KCNQ1*-T587R is inhibiting the HERG channel trafficking to the plasma-membrane suggesting an impairment of I_{Kr}. These results could contribute to the explanation of the severe clinical manifestations of the patient compared to the majority of the mild phenotype due to mutations in the C-Terminal region.

ADENO-ASSOCIATED VIRAL GENE DELIVERY OF CALSEQUESTRIN 2 PROTECTS ADULT CALSEQUESTRIN 2-R33Q KNOCK-IN MICE FROM DEVELOPING VENTRICULAR TACHYCARDIAS

Lodola F[1]**,** Denegri M[1], Avelino-Cruz JE[1], De Giusti VC[1], Curcio A[1,8], Bongianino R[1], Leccioli V[1], Boncompagni S[4], Protasi F[4], Auricchio A[5,6], Napolitano C[1,2], Priori SG[1,2,3].
[1]Molecular Cardiology, IRCCS Fondazione Salvatore Maugeri, Pavia, Italy; [2]Cardiovascular Genetics Program, The Leon H. Charney Division of Cardiology, University School of Medicine, New York, USA; [3]Department of Molecular Medicine, University of Pavia, Pavia, Italy; [4]CeSI-Center for research on Ageing & DNI-Department of Neuroscience and Imaging, University G. d'Annunzio, Chieti, Italy; [5]Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy; [6]Medical Genetics, Department of Pediactrics, "Federico II" University, Naples, Italy; [7]Department of Experimental Biomedical Sciences, University of Padova, Italy; [8]Department of Medical and Surgical Sciences, University of Magna Graecia, Catanzaro, Italy.

Email: francesco.lodola@unipv.it

Background. Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a highly lethal recessive arrhythmogenic disease associated with mutations in the calsequestrin2 (*CASQ2*) gene. We previously demonstrated that *CASQ2* gene delivery was able to rescue the arrhythmic phenotype in CASQ2-KO-mice 20 weeks after viral infection. The aim of the present study is to investigate viral gene delivery long-term effects in CASQ2-R33Q mouse model.

Methods. Newborn CPVT-mice were infected with AAV9 containing the coding sequence of WT-*CASQ2* co-expressed with GFP gene. Furthermore, we evaluated the effect 26 and 52 weeks after viral infection by *in vivo* ECG-analysis, *in vitro* electrophysiological and molecular assays.

Results. ECG-analysis demonstrated *in vivo* ventricular tachycardias after epinephrine (2 mg/Kg) administration in only 17% (n=12) of the infected-mice, while 87% (n=8) of the control CASQ2-R33Q-homozygous-mice presented an arrhythmic phenotype ($p<0.005$). Additionally, electrophysiological analysis showed that delayed after depolarization (DADs) and triggered activity (TA) were almost abolished in GFP-positive-infected-cardiomyocytes (26 weeks: DADs: 0%, TA 0%, n=18; 52 weeks: DADs: 8%, TA: 8%, n=12). Finally, western blot revealed increased CASQ2 expression in the infected-mice and immunofluorescence assay indicated its correct localization along the z-lines.

Conclusions. Viral expression of CASQ2 is a long period effective strategy able to revert the functional abnormalities of the mutant endogenous protein and prevent life-threatening arrhythmias in the CASQ2-R33Q-mice. These data suggest that despite R33Q-mice express abnormal CASQ2, the gene replacement therapy is still able to prevent arrhythmogenic mechanisms *in vitro* and ventricular tachycardias *in vivo* suggesting that CASQ2-gene transfer may become a potential therapy for recessive CPVT.

25 ANNI DI NON LINEARITA´ NELLA DINAMICA DEL RITMO CARDIACO: PROBLEMI E PROSPETTIVE

Merati G.
Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano.

Email: giampiero.merati@unimi.it

INTRODUZIONE. Da circa 25 anni la variabilità del ritmo cardiaco è sempre più utilizzata in ambito clinico nella valutazione dello stato autonomico in differenti condizioni fisiologiche e patologiche, sia cardiovascolari che di altra origine. Ad oggi, tale analisi rimane tuttavia essenzialmente basata su misure spettrali o di varianza del ritmo cardiaco, mentre i componenti complessi e non-lineari della dinamica cardiovascolare sono generalmente poco considerati, nonostante sia dimostrata la loro potenziale abilità nell'identificare disfunzioni cardiache.
OBIETTIVI. Lo scopo di questo lavoro è di fornire un contributo alla conoscenza degli stimatori di dinamica complessa nella variabilità del ritmo cardiaco, e di rappresentare lo stato dell'arte e la possibile applicazione clinica dell'utilizzo di tali indici. In particolare, verranno trattati gli stimatori che valutano l'auto-somiglianza, la complessità geometrica e la regolarità delle serie battito-battito relative agli eventi cardiaci elettrocardiografici e pressori.
MATERIALI E METODI. Verranno illustrati il significato e il metodo di misura dei più importanti parametri di dinamica non lineare applicati allo studio della complessità del segnale cardiaco, insieme ai loro possibili utilizzi in campo clinico, con esempi ottenuti dall'applicazione recente a diverse problematiche fisio- e patologiche cardiovascolari.
RISULTATI E CONCLUSIONI. Le performance dei parametri di dinamica non lineare sono generalmente assimilabili a quelle degli stimatori più comuni del dominio del tempo e delle frequenze, ma in molti casi possono fornire importanti informazioni aggiuntive allo studio del sistema cardiovascolare e del suo controllo autonomico.

SERUM CHOLESTEROL EFFLUX CAPACITY INVERSELY CORRELATES WITH ARTERIAL STIFFNESS IN HEALTHY SUBJECTS

Adorni MP[1], Favari E[1], Ronda N[1], Zimetti F[1], Salvi P[2], Borghi C[2], Bernini F[1], Cicero AFG[2].
[1]Department of Pharmacy, University of Parma; Parma; [2]Internal medicine, Aging and Kidney disease Department, University of Bologna, Bologna; the author is member of the Brisighella Heart Study group – Italy.

Email: mariapia.adorni@unipr.it

Objective. The capacity of high density lipoprotein (HDL) to induce cell cholesterol efflux is considered one of their main antiatherogenic properties. Little is known on the impact of such HDL function on vascular remodeling in healthy subjects. We investigated the relationship between serum cholesterol efflux capacity (CEC), an indicator of HDL functionality, and Pulse Wave Velocity (PWV), an indicator of arterial stiffness, in healthy subjects.
Methods and Results. Serum from 167 healthy subjects (54 males, 113 females) was used to conduct CEC measurement (aqueous diffusion and ATP binding cassette A1 (ABCA1)-dependent cholesterol efflux). Carotid-femoral PWV was measured with a high-fidelity tonometer. Both ABCA1-mediated CEC and PWV did not correlate with HDL-C levels, either as a whole group and as males and females separately. In an unadjusted model, PWV inversely correlated with ABCA1-dependent cholesterol efflux ($r= 0.183$, p-val = 0.018). No correlation was found between PWV and aqueous diffusion-dependent CEC ($r = 0.129$, p-val = 0.095). In a nested linear regression model, controlling for age, sex, body mass index, mean arterial pressure, serum low-density lipoprotein, HDL and glycated hemoglobin, PWV displays a significant negative regression on ABCA1-dependent CEC ($\beta= -0.204$, 95%CI -0.371/-0.037).
Conclusion. The finding that ABCA1-dependent CEC, but not serum HDL cholesterol level, is a significant predictor of PWV in healthy subjects points to the relevance of HDL function in vascular physiology and arterial stiffness prevention along life.

OMEGA-3 FATTY ACIDS: PROSPECTS FOR A CARDIAC LIPID THERAPY

Carotenuto F[1,2], Minieri M[3], Di Nardo P[1,2].
[1]Lab. Cardiologia Molecolare e Cellulare, Dip. Scienze Cliniche e Med. Traslazionale, Università Tor Vergata, Roma; [2]Biolink Institute, Link Campus University, Rome, Italy; [3]Dipartimento di Medicina Sperimentale e Chirurgia, Università di Roma Tor Vergata, Roma, Italy.

Email: carotenuto@med.uniroma2.it

Attention to the role of n-3 polyunsaturated fatty acids (n-3 PUFA) in human health and disease has continuously increased during recent decades. In fact, n-3 PUFA may exert beneficial effects in inhibiting the incidence and development of various diseases. These fatty acids are known for their pleiotropic effects against, among others, inflammation, platelet aggregation, hypertension, and hyperlipidemia. Different modes of action involving the eicosanoid system, the membrane fatty composition, the regulation of transcription factors, gene expression and cell signalling pathways have been proposed to substantiate n-3 PUFAs beneficial effects. In our opinion, the regulation of the apoptotic pathways represents a pivotal mechanism responsible for their beneficial effects. The induction of the apoptosis has been invoked to explain n-3 PUFA benefits on cancer disease, while its inhibition could play a crucial role in the protection exerted against neuronal and cardiovascular diseases. Our laboratory has demonstrated the ability of plant-derived n-3 PUFA alpha-linolenic acid (ALA) to exert anti-apoptotic effects in cardiomyocytes. These findings have revealed new n-3 PUFA molecular targets that can be potentially exploited to design more efficient cardiovascular drugs.

OBESITY-ASSOCIATED VASCULAR ALTERATIONS AND MELATONIN BENEFICAL EFFECTS

Favero G, Belotti F, Ferrari M, Rodella LF, Rezzani R.
Human Anatomy, Department of Clinical and Experimental Sciences, University of Brescia, Viale Europa 11, 25123 Brescia, Italy.

Email: gaia.favero@med.unibs.it

Background. Obesity is a chronic inflammatory pathology common in industrialized countries and that has a significant impact on the incidence of cardiovascular diseases. Obesity is associated with endothelial dysfunctions, greater vascular stiffness, chronic low-grade inflammation and fibrosis. These effects on vascular structure and function are mediated by inflammation and by altered adipokine secretion from fat depots that directly affect heart and blood vessels. Obesity is nowadays a growing health pandemic disease requiring an urgent intervention and recently numerous studies have investigated the prevention/treatment of obesity using naturally-occurring antioxidants. Melatonin, an endogenously produced indoleamine, is a remarkable pleiotropic molecule which functions as a highly effective antioxidant and free radical scavenger. Endogenously produced and exogenously administered melatonin has beneficial effects on the cardiovascular system and could also modulate energy metabolism.
Methods. In this study, at first, we analyzed the obesity-associated vascular dysfunctions at aorta level and then we hypothesized that melatonin administration (kindly provided by Chronolife S.r.l., Roma, Italy) can minimize and ameliorate vascular morphological changes in a mice model of obesity.
Results. We demonstrated that: 1) obesity induces fat redundant accumulation, alteration of vascular morphology, inflammation, vasocostriction and fibrosis and 2) compared with the obesity group, intake of melatonin decreases body weight and blood glucose levels, restores the correct vascular cytoarchitecture reducing also vascular inflammation and fibrosis.
Conclusion. the overall findings suggest and confirm that melatonin has no toxic effects and it should be exploit as a therapeutic tool to prevent the harmful obesity-induced cardiovascular alterations.

PLASMACYTOID DENDRITIC CELLS: KEY ANTIGEN PRESENTING CELLS IN EXPERIMENTAL ATHEROSCLEROSIS?

Grassia G[1,2], MacRitchie N[2], Ialenti A[1], Brewer[2] JM, Garside P[2], Maffia P[2,1].
[1]Department of Experimental Pharmacology, University of Naples Federico II, 80131 Naples, Italy;
[2]Institute of Infection, Immunity and Inflammation, University of Glasgow, G12 8TA, UK.

Email: gigrassi@unina.it

Plasmacytoid dendritic cells (pDCs) represent a unique subset of dendritic cells that play a pivotal role in several chronic autoimmune diseases strongly characterized by an increased risk of vascular pathology. Clinical studies have shown that pDCs are detectable in atherosclerotic plaques and others have suggested an association between reduced numbers of circulating pDCs and cardiovascular events. Recent results from mouse models are starting to define the specific role(s) of pDCs in the disease process.
We have demonstrated that continuous treatment of apoE$^{-/-}$ mice with anti-mPDCA-1 antibody caused specific depletion of pDCs in the aorta and spleen and significantly reduced atherosclerosis formation in the aortic sinus (by 46%; $P<0.001$). Depletion of pDCs also reduced macrophage (by 34%; $P<0.05$) and increased collagen content (by 41%; $P<0.05$) in aortic plaques, implying a more stable plaque phenotype. Additionally, pDC depletion reduced splenic T-cell activation and inhibited IL-12, CXCL1, MIG, IP-10 and VEGF serum levels. Interestingly, the aorta and spleen of both apoE$^{-/-}$ and C57BL/6 mice displayed similar numbers of pDCs, with similar activation status. In contrast, assessment of antigen uptake/presentation using the Eα/Y-Ae system revealed that aortic pDCs in apoE$^{-/-}$ mice were capable of presenting *in vivo* systemically administered antigens. Interactions between T cells and antigen presenting cells were mainly observed in the aortic adventitia, as imaged by multiphoton microscopy. These data support the role of pDCs as antigen presenting cells in the contest of atherosclerosis.

STRATEGIE DI SCREENING IN ETA' PEDIATRICA PER LE MALATTIE CARDIOVASCOLARI

Pederiva C, Domi A, Capra ME, Giovannini M.
Clinica Pediatrica Università degli Studi di Milano – Ospedale San Paolo.

Email: cristinapederiva@yahoo.it

L'ipercolesterolemia è uno dei principali fattori di rischio modificabili per lo sviluppo dell'aterosclerosi e della cardiovasculopatia (CVD-cardiovascular desease). La strategia proposta da American Academy of Pediatrics (2008) è uno screening selettivo.

Scopo dello studio. Individuare le famiglie ad aumentato rischio per malattie cardiovascolari.
Materiali e metodi. Ai genitori dei nuovi nati presso la Neonatologia della Clinica Pediatrica-Ospedale San Paolo di Milano è stato consegnato un questionario per valutare: 1) familiarità per CVD 2) familiarità per displipidemie 3) profilo lipidico dei genitori 4) valori di normalità della colesterolemia.
Risultati. Su 252 schede (studio ancora in corso): 126 genitori (50%) rispondono correttamente sui valori normali di colesterolemia per l'adulto (inferiori a 200 mg/dl). 73 soggetti (28,9%) conoscono il proprio quadro lipidico. 93 genitori (36,9%) affermano di avere parenti di I o II grado con CVD precoce, e nonostante la familiarità per CVD precoce, il 68,8 % di loro (64/93) non ha mai eseguito controlli per determinare il proprio quadro lipidico. 91 genitori (36,1%) affermano di avere familiarità per dislipidemie, ma 50% di loro (46/91) non conosce i propri valori lipidici.
Conclusioni. Una anamnesi familiare mirata ed accurata e' un'ottima strategia per l'individuazione dei soggetti a rischio cardiovascolare, ma presenta degli evidenti limiti, soprattutto la scarsa conoscenza del problema da parte dei genitori. Si conferma inoltre una mancata aderenza dei medici alle strategie di prevenzione di CVD: il 37% dei soggetti analizzati riferisce familiarità per CVD precoce, ma il 68% non ha mai eseguito controlli per determinare il proprio profilo lipidico. Questi dati preliminari indicano che la percentuale degli adulti con familiarità per CVD precoce e dislipidemie 'trascurata' è molto alta con conseguente ripercussione sul numero dei pazienti pediatrici a rischio, non identificati.

THE IMMUNOSUPPRESSANT DRUG CYCLOSPORINE A IMPAIRS THE REVERSE CHOLESTEROL TRANSPORT IN VIVO BY REDUCING STEROL FECAL EXCRETION

Zanotti I[1], Lusardi G[1], Greco D[1], Potì F[2], Bernini F[1].
[1]Dipartimento di Farmacia, Università di Parma; [2]Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Università di Modena e Reggio Emilia, Italia.

Email: ilaria.zanotti@unipr.it

Background and aim. We previously demonstrated that the immunosuppressive drug cyclosporine A (CsA) impairs the antiatherosclerotic process of macrophage reverse cholesterol transport in C57BL/6J mice by reducing bile and fecal excretion of neutral sterols. The objective of the present work was to investigate the mechanisms accounting for this observation.
Methods. CsA interference on the first and the last steps of RCT, cholesterol efflux from macrophages and sterol efflux in the intestinal lumen, was evaluated by radioisotope-based assays in murine peritoneal macrophages and human colon carcinoma ($CaCO_2$) cells. Hepatic expression of ATP Binding Cassette G5 (ABCG5) and G8 (ABCG8) was quantified by RT-PCR and western.
Results. Mice treated with CsA 50mg/kg/d for 7 days showed higher amount of hepatic *Abcg5* (mean of fold increase±s.d.: 2.61±0.87 vs 4.63±2.09; $p<0.05$ in vehicle and CsA-treated mice respectively) and *Abcg8* (mean of fold increase±s.d.: 5.13±2.23 vs 7.62±2,53 in vehicle and CsA-treated mice respectively) mRNA. No effect on ABCG5 protein content in the liver was apparent upon the pharmacological treatment. However, CsA significantly inhibited sterol efflux from ABCG5- and ABCG8-expressing $CaCO_2$ cells (%cpm released into cell media ±s.d: 6.49±1.42 vs 4.04±0.34; $p<0.05$ in cells untreated or treated with CsA respectively). Differently, increasing concentrations of CsA (0.1-10μM) did not affect macrophage capacity to release cholesterol to murine plasma.
Conclusions. CsA treatment in mice caused the impairment of RCT possibly through the interference with the activity of ABCG5 and ABCG8, leading to reduced excretion of sterols in the intestine.

FLOW-MEDIATED DILATION AND HDL FUNCTION IN SUBJECTS WITH HYPERALPHALIPOPROTEINEMIA: THE HYPERALPHALIPOPROTEINEMIA AND ATHEROSCLEROSIS (HALA) STUDY

Zimetti F[1], Favari E[1], Satta E[2], Giusto L[3], Vanini A[3], Tarugi P[4], Boarini S[2], Bosi C[2], Bernini F[1], Vigna GB[2].
[1]Dept. of Pharmacy, University of Parma, Parma, Italy; [2]Sect. of Internal Medicine, Gerontology and Clinical Nutrition, Department of Clinical and Experimental Medicine, University of Ferrara, Ferrara, Italy; [3]Vascular Diagnostic Unit, Medical Department, Azienda Ospedaliero-Universitaria of Ferrara, Ferrara, Italy; [4]Department of Biomedical Sciences, University of Modena & Reggio Emilia, Modena, Italy.

Email: francesca.zimetti@unipr.it

Background. We measured flow mediated dilation (FMD)**,** index of subclinical atherosclerosis, and HDL cholesterol efflux capacity (CEC) in hyperalphalipoproteinemic (HAL) and in normal subjects. 20 subjects with HDL-C >85 mg/dL and 20 with normal HDL-C levels were tested for FMD and CEC through aqueous diffusion (AD), SR-BI, ABCG1 and ABCA1.
Results. FMD did not correlate with HDLc and were comparable in both groups. PD- and SR-BI-CEC were higher in HAL subjects (6.41% ± 0.77 vs. 5.16 % ± 0.72, $p<0.001$, and 4.67% ± 0.95 vs. 2.99% ± 0.58, $p<0.001$ respectively). After normalizing efflux for HDLc levels, PD-CEC turned greater in control subjects (0.095% ± 0.021 vs. 0.067% ± 0.009, $p<0.001$), while no difference was detected in SR-BI-CEC. ABCG1-CEC was similar in both groups; after normalizing for HDLc levels, it was higher in normal subjects (0.078%±0.0058 vs 0.05%±0.0024; $p<0.001$). ABCA1-CEC was similar to ABCG1. Small HDL particles were higher in normal (19.95%±0.99 vs 16.02%±0.87; $p<0.01$) while large HDL particles were higher in HAL subjects (42.29%±1.44 vs 33.49%± 1.43; $p<0.001$).
Conclusions. AD- and SR-BI-CEC pattern relate to HDLc levels; normalization of AD-CEC caused higher CEC in control subjects: the cholesterol/phospholipid HDL ratio in HAL subjects may cause a concentration gradient weaker. ABCA1- and ABCG1-CEC are metrics of HDL functionality: normalizing for HDLc, normal subjects display higher efficiency through those pathways, probably because of higher percentage of smaller HDL; this might explain, at least in part, the lack of difference in FMD in spite of large differences in HDLc concentration between the two groups.

CARDIOVASCULAR INJURY IN THE METABOLIC SYNDROME INDUCED BY CHRONIC COLA DRINKING

Zuchi C[1], Santucci A[1], Milei J[2], Llambi HG[2], Grana DR[2], Ottaviano G[2], Paglia N[2], Macchia M[1], Tritto I[1], Ambrosio G[1].
[1]Cardiologia e Fisiopatologia Cardiovascolare, Università di Perugia, Perugia; [2]Instituto de Investigaciones Cardiológicas UBA-CONICET, Buenos Aires (Argentina).

Email: isabella.tritto@ospedale.perugia.it

A major contributor to cardiovascular morbidity is represented by the widespread and increasing prevalence of metabolic syndrome, a constellation of metabolic alterations (obesity, hypertension, raised triglycerides, diabetes, and low HDL-cholesterol) resulting in a pro-thrombotic, pro-inflammatory condition that markedly favors development of cardiovascular disease.
In this context, heart failure (HF) with preserved ejection fraction (diastolic heart failure, DHF) is a common occurrence.
Soft drinks are the leading source of added sugar worldwide, and their rising consumption has been linked to metabolic syndrome in humans. We have shown that a condition resembling the human metabolic syndrome can be obtained in rats drinking cola-like sweetened beverages.
Male Wistar rats were divided in 3 groups allowed to drink ad libitum for 6 months, either: tap water; Coca-cola, or light coke. After 6 months, in rats, chronic consumption of coke increased body weight, blood pressure, plasma glucose and tryglicerides, thus reproducing most of the features of metabolic syndrome. Furthermore, these animals showed LV dilatation and remodeling. These deleterious effects on metabolism and cardiac geometry were not seen in animals drinking light coke, thus indicating that they were largely due to the high calorie intake from sucrose in regular drink.
The results of this study offer insights in metabolic syndrome induced by soft drinks consumption, and accompanying alterations in cardiac function. Our model represents a useful tool to evaluate the effects of cola drinking on development of different components of metabolic syndrome over time, and of possible interventions.