Sotto l'Alto Patronato del Presidente della Repubblica

Con il Patrocinio di:

Alma Mater Studiorum - Università degli Studi di Bologna

Azienda Unità Sanitaria Locale di Imola

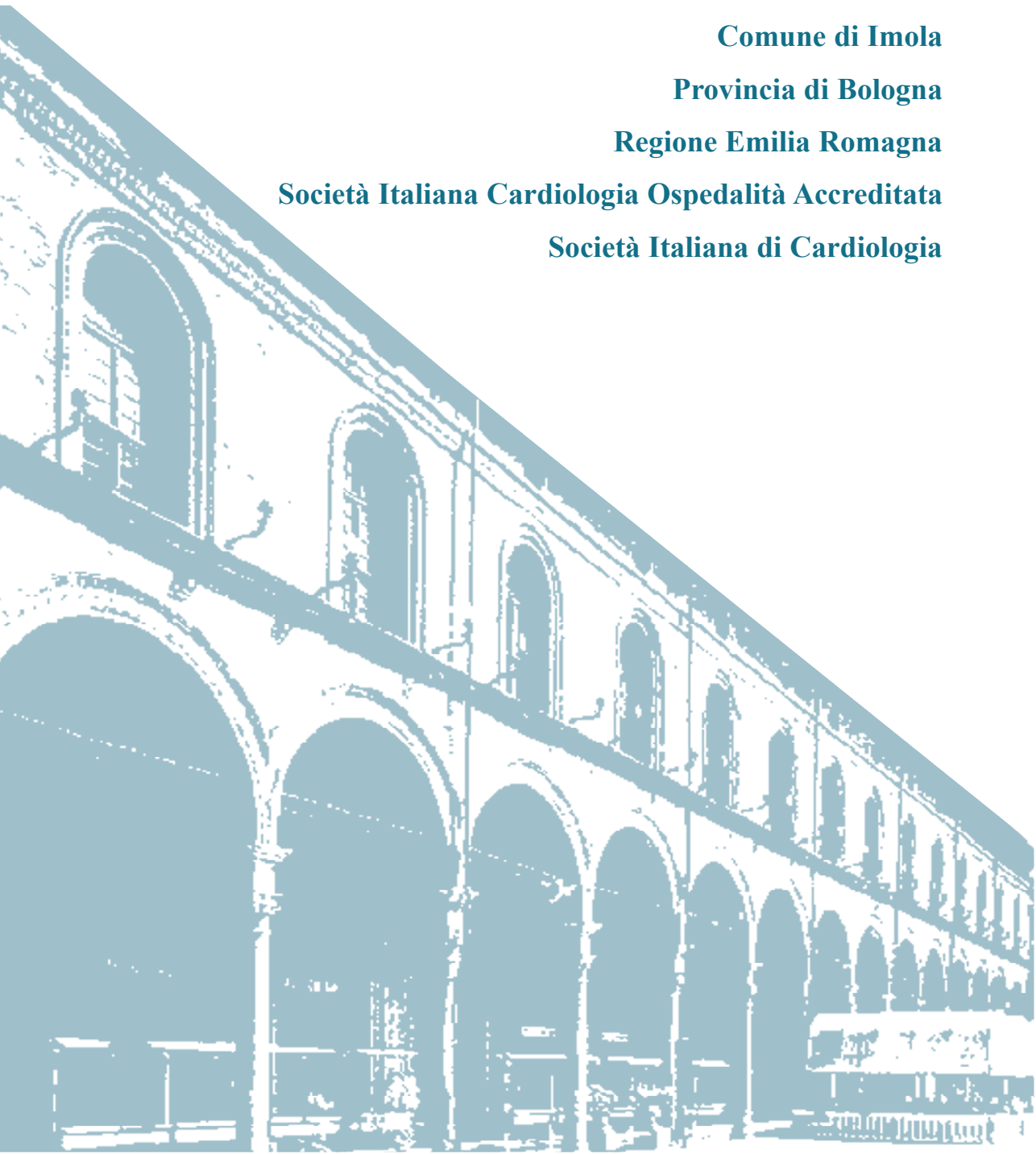
Comune di Imola

Provincia di Bologna

Regione Emilia Romagna

Società Italiana Cardiologia Ospedalità Accreditata

Società Italiana di Cardiologia



COMITATO

Presidente
GERMANO DI SCIASCIO (Roma)

Presidente Onorario
CLAUDIO M. CALDARERA (Bologna)

Direttivo SIRC e Comitato Scientifico
M. C. Cerra (Arcavacata di Rende - CS)
G. Di Sciascio (Roma)
G. Losano (Torino)
E. Masini (Firenze)
P. Pagliaro (Torino)
M. Samaja (Milano)
A. Stella (Bologna)
D. Stilli (Parma)
F. Tanzi (Pavia)
E. Tomao (Roma)
I. Tritto (Perugia)

Segreteria Organizzativa

AIM Group - AIM Congress
Sede di Roma
Via Flaminia 1068 - 00189 Roma
Tel. 06 33053.1 - Fax 06 23325623
E-mail : sirc2008@aimgroup.it
www.aimgroup.eu/2008/sirc

INDICE

RINGRAZIAMENTI

La Società Italiana di Ricerche Cardiovascolari
e l'Istituto Nazionale per le Ricerche Cardiovascolari
ringraziano vivamente:

COMUNE DI IMOLA

FONDAZIONE CASSA DI RISPARMIO DI IMOLA

per aver contribuito alla realizzazione della manifestazione.

INFORMAZIONI GENERALI

SEDE DEL CONGRESSO
Fondazione Cassa di Risparmio di Imola - Palazzo Sersanti, Piazza Matteotti, 8
40026 Imola (BO) - Tel. 0542 26606 - Fax 0542 26999.

SEGRETERIA ORGANIZZATIVA
La Segreteria Organizzativa è a disposizione dei partecipanti in sede congressuale a partire dalle ore 14.00 di giovedì 9 ottobre e per tutta la durata del Congresso.

ISCRIZIONI
Quote di iscrizione (IVA 20% inclusa)

Soci SIRC	€ 120,00
Non Soci	€ 144,00
Specializzandi, Dottorandi, Assegnisti, Borsisti*	€ 60,00

* *Una dichiarazione dell'Istituto o del Dipartimento deve attestare l'iscrizione dell'interessato alla Scuola o la qualifica specificata.*

La quota di iscrizione include la partecipazione ai lavori scientifici, la cartella congressuale, l'attestato di partecipazione, l'attestato ECM, il programma con atti, i coffee break e le colazioni di lavoro previsti nel programma.

Modalità di Pagamento
In sede congressuale il pagamento dell'iscrizione è possibile esclusivamente in contanti o tramite assegno bancario o circolare.
Per ogni iscrizione verrà rilasciata regolare fattura, si prega quindi di indicare il proprio codice fiscale e/o partita IVA.

BADGE
A ciascun partecipante regolarmente iscritto viene consegnato un badge nominativo che dovrà essere sempre esibito per poter accedere alle sessioni scientifiche. Il colore del badge è così differenziato:
Partecipanti: **Trasparente**
Relatori e Moderatori: **Rosso**

CERIMONIA INAUGURALE
La Cerimonia Inaugurale del Congresso ha luogo giovedì 9 ottobre alle ore 16.00 presso la Sala Sersanti. Seguirà un Cocktail di Benvenuto alle ore 17.30 presso la Sala Transatlantico del Palazzo Sersanti.

COFFEE BREAK E COLAZIONE DI LAVORO
Per tutti i partecipanti regolarmente iscritti al Congresso sono previsti un coffee break nei giorni di venerdì 10 e sabato 11 ottobre 2008 ed una colazione di lavoro a buffet nella giornata di venerdì 10 ottobre.

ATTESTATO DI PARTECIPAZIONE
L'attestato di partecipazione viene rilasciato a tutti i partecipanti regolarmente iscritti che ne faranno richiesta presso il desk della Segreteria al termine dei lavori congressuali.

BAR E GUARDAROBA
All'interno del Palazzo Sersanti è presente un bar a pagamento.
Un servizio di guardaroba gratuito, che osserva gli orari dei lavori scientifici, è a disposizione dei singoli partecipanti.

I lavori scientifici si svolgono presso la Sala Sersanti.

COMUNICAZIONI LIBERE
Il tempo a disposizione per le comunicazioni orali è di 12 minuti (8 per la presentazione, inclusa la proiezione di immagini e 4 per la discussione).
Per ogni comunicazione orale è necessaria l'iscrizione al Congresso di almeno uno degli autori.
Le Sessioni di comunicazioni sono previste nei seguenti giorni:

Venerdì 10 ottobre
ore 9.30 - 10.20 Elettrofisiologia Cardiaca
ore 11.05 - 12.20 Disfunzione Endoteliale
ore 15.00 - 16.40 Stress Ossidativo

Sabato 11 ottobre
ore 9.00 - 10.15 Terapia Cellulare nella Rigenerazione Miocardica

POSTER
L'esposizione dei poster ha luogo presso il Foyer del Palazzo Sersanti come segue:

Gruppo A dalle ore 17.30 di giovedì 9 ottobre alle ore 14.30 di venerdì 10 ottobre
Montaggio: giovedì 9 ottobre ore 14.00 - 16.00
Smontaggio: venerdì 10 ottobre ore 14.30.

Gruppo B dalle ore 15.00 di venerdì 10 ottobre alle ore 11.30 di sabato 11 ottobre
Montaggio: venerdì 10 ottobre ore 15.00
Smontaggio: sabato 11 ottobre ore 12.00.

Le misure dei pannelli per l'affissione dei poster sono cm. 70 (base) x cm. 100 (altezza).
Il materiale per il montaggio dei poster è disponibile presso il desk della Segreteria del Congresso.
L'affissione del poster è subordinata al pagamento della quota di iscrizione di almeno uno degli autori del lavoro.

Discussione Poster
La discussione dei poster avrà luogo alla presenza di due Moderatori nell'area stessa dell'esposizione accanto a ciascun poster con almeno uno degli autori presente.

Gruppo A: giovedì 9 ottobre dalle ore 17.30 alle 19.00 e venerdì 10 ottobre dalle ore 12.20 alle ore 14.30.

Gruppo B: venerdì 10 ottobre dalle ore 18.00 alle 19.00 e sabato 11 ottobre dalle ore 10.30 alle ore 11.30.

PROIEZIONI
In sala è prevista la proiezione di immagini da Personal computer. Non è consentito l'utilizzo del proprio computer.
I Relatori sono pregati di presentare prima dell'inizio della sessione i relativi file su CD-Rom o pen drive direttamente al tecnico in aula.

PREMIO A.M. VALSALVA

La Fondazione Cassa di Risparmio di Imola ha messo a disposizione un premio da destinare ad un giovane ricercatore italiano di età non superiore ai 45 anni.
La premiazione si tiene giovedì 9 ottobre dalle ore 16.30 alle 17.30 presso la Sala Sersanti.

PREMIAZIONE YOUNG INVESTIGATOR

I due migliori contributi scientifici (sia orali che poster) presentati al Congresso saranno premiati sabato 11 ottobre dalle ore 11.30 alle 12.00.

CREDITI ECM

La Commissione Nazionale per la Formazione Continua in Medicina del Ministero della Salute ha accreditato questo Congresso al Programma ECM, assegnando **n. 8 crediti formativi.** L'accreditamento è stato effettuato per tutta la durata del Congresso.

Per conseguire i crediti è necessario:

- ritirare al momento della registrazione, unitamente alla borsa congressuale, la cartellina contenente la scheda recapiti partecipanti, la scheda di valutazione ed il questionario di apprendimento;
- partecipare nella misura del 100% ai lavori scientifici;
- compilare i suddetti moduli e riconsegnarli presso la Segreteria al termine del congresso.

I Relatori del Congresso avranno diritto a n. 2 crediti formativi per ogni ora di docenza, indipendentemente dai crediti attribuiti al Congresso.

RELATORI E MODERATORI

R. ANTOLINI (Trento)

C. M. CALDARERA (Bologna)

M. C. CERRA (Arcavacata di Rende - CS)

R. DE CATERINA (Chieti)

D. DI FRANCESCO (Milano)

F. DI LISA (Padova)

G. DI SCIASCIO (Roma)

G. LOSANO (Torino)

C. MUSCARI (Bologna)

E. MUSSO (Parma)

P. PAGLIARO (Torino)

P. PUDDU (Bologna)

A. PARINI (Tolosa, Francia)

I. TRITTO (Perugia)

PROGRAMMA SCIENTIFICO

GIOVEDÌ 9 OTTOBRE

14.00 - 16.00 Registrazione
Esposizione Poster (Gruppo A)

16.00 - 16.30 Inaugurazione del Congressso
Presidente SIRC e Saluto delle Autorità

16.30 - 17.30 **SCIENTIFIC KEYNOTE**
Lettura A.M. Valsalva e Conferimento Premio A.M. Valsalva
Moderatori: ***G. Di Sciascio*** (Roma) - ***C. Muscari*** (Bologna)

17.30 - 19.00 **Cocktail "Wine and Cheese" e Sessione Poster (Gruppo A)**

VENERDÌ 10 OTTOBRE

8.15 - 9.00 Registrazione

9.00 - 10.20 Sessione I
ELETTROFISIOLOGIA CARDIACA
Moderatori: ***R. Antolini*** (Trento) - ***G. Losano*** (Torino)

9.00 - 9.30 **Lettura**
La corrente "funny": generazione e controllo del ritmo cardiaco
D. Di Francesco (Milano)

9.30 - 10.20 Comunicazioni libere

Modulazione delle correnti ioniche nei canali del calcio di tipo-l (CAV1.2) nativi e clonati di un analogo funzionale del diltiazem
P. Ioan, R. Budriesi, M.P. Ugenti, I. Bodi, A. Schwartz, A. Chiarini

Molecular and cellular remodeling in familiar hypertrophic cardiomyopathy: a role for 5HT2 receptors
F. Stillitano, R. Coppini, L. Sartiani, S. Suffredini, J. Olivotto, F. Cecchi, A. Mugelli, E. Cerbai

Effetti acuti dell'angiotensina II sull'elettrofisiologia del miocardio ventricolare: effetto aritmogeno acuto dell'attivazione tissutale del sistema renina angiotensina
P. Ferrero, R. Jabr, A. Kontogeorgis, M. Turner, Ch. Fry, N.S. Peters

Wavelet analysis of instantaneous heart rate in patients with acute myocardial infarction undergoing primary coronary angioplasty
C. Molinari, E. Grossini, C. Anchisi, A. Brunori, M. Invernizzi, A.S. Bongo, G. Vacca

10.20 - 10.35 **Coffee-break**

10.35 - 12:20 Sessione II
DISFUNZIONE ENDOTELIALE
Moderatori: ***G. Di Sciascio*** (Roma) - ***I. Tritto*** (Perugia)

10.35 - 11.05 **Lettura**
La disfunzione endoteliale nel diabetico
R. De Caterina (Chieti)

11.05 - 12.20 Comunicazioni libere

Myointimal cells, rather than dendritic cells or mast cells, are major sources of TNFalpha and NO in atheromata
S. Bacci, L. Pieri, P. Romagnoli

L'urotensina II esercita effetti pro-aterotrombotici in cellule endoteliali coronariche umane in coltura
G. Petrillo, P. Cirillo, M. Pacileo, L. Sasso, A. Paglia, V. Di Palma, P. Maietta, L.G. D'Ascoli, L. Brevetti, M. Chiariello

High insulin impairs PI3-kinase/AKT/nitric oxide mediated insulin signaling in human endothelial cells
R. Madonna, R. De Caterina

NA+/H+ exchanger 1- and aquaporin-1-dependent hyperosmolary changes decrease nitric oxide and induce VCAM-1 expression in endothelial cells exposed to high glucose
M. Zurro, R. Madonna, E. Montebello, G. Lazzerini, R. De Caterina

Reduction in endothelial progenitor cells anticipates endothelial dysfunction in normoglycaemic patients with family history for type 2 diabetes
M. Barsotti, R. Di Stefano, L. Pucci, D. Lucchesi, S. Sorbo, M. Iorio, L. Ghiadoni, G. Penno, S. Del Prato, A. Balbarini

Bindarit, an inhibitor of MCP-1, attenuates neointimal hyperplasia after balloon injury in rats
G. Grassia, M. Maddaluno, A. Guglielmotti, G. Mangano, C. Bartella, P. Maffia, A. Ialenti

12.20 - 14.30 **Colazione di Lavoro & Sessione Poster (Gruppo A)**

14.30 - 16.40 **Sessione III**
STRESS OSSIDATIVO
Moderatori: ***M. C. Cerra*** (Arcavacata di Rende - CS) - ***P. Pagliaro*** (Torino)

14.30 - 15.00 **Lettura**
Mitocondri e specie reattive dell'ossigeno
F. Di Lisa (Padova)

15.00 - 16.40 Comunicazioni libere

Positive effects of long-term L-triiodothyronine (LT3) treatment on cardiac remodelling related to T3-regulated mitochondrial transcription factors and respiration
S. Forini, V. Lionetti, F. Cecchetti, L. Sabotino, P. Nicolini, S. Balzan, M. Grana, M. Nannipieri, F.A. Recchia, G. Iervasi

Postconditioning and mitochondria
C. Penna, M.G. Perrelli, S. Raimondo, S. Geuna, P. Pagliaro

Expression of antioxidative enzymes in high glucose-exposed progenitor and mature endothelial cells
F. Felice, M.C. Barsotti, L. Pucci, D. Lucchesi, M.L. De Perna, G. Penno, S. Del Prato, R. Di Stefano, A. Balbarini

Oxidative stress, apoptosis and immunological activation in acute myocardial infarction
M. Fedele, P. Fabbi, M. Vercellino, M. Balbi, S. Garibaldi, M. Ghio, P. Contini, F. Indiveri, A. Barsotti

Trattamenti con dosi subapoptotiche sequenziali di epirubicina ed anticorpo anti ERBB2 inducono nei cardiomiociti apoptosi: ruolo protettivo del dexrazoxane
C. Aloi, P. Altieri, P. Spallarossa, M. Mura, S. Garibaldi, C. Barisione, G. Ghigliotti, U. Dorighi, A. Barsotti, C. Brunelli

Effect of a carbon monoxide-releasing molecule (CORM-3) on spontaneously hypertensive rat aortas
P. Failli, S. Franchi-Micheli, M. Cantore, A. Vannacci, M.C. Vinci, E. Masini

L'effetto cardiotropico dell'anione nitrossile (HNO) è mediato dall'interazione con tioli reattivi collocati in strutture dell'accoppiamento elettromeccanico del miocita
C. Tocchetti, N. Kaludercic, C. Vecoli, J.P. Froehlich, S. Betocchi, M. Chiariello, D.A. Kass, N. Paolocci

Human failing heart left ventricle is more protected than right ventricle versus oxidative stress damage
E. Borchi, V. Bargelli, C. Giordano, G. D'Amati, P.A. Nassi, E. Cerbai, C. Nediani

16.40 - 18.00 **Assemblea dei Soci**

18.00 - 19.00 **Sessione Poster (Gruppo B)**

SABATO 11 OTTOBRE

8.30 - 10.15 **Sessione IV**
TERAPIA CELLULARE NELLA RIGENERAZIONE MIOCARDICA
Moderatori: ***E. Musso*** (Parma) - ***P. Puddu*** (Bologna)

8.30 - 9.00 **Lettura**
Mesenchimal stem cells in cardiac cell therapy
A. Parini (Tolosa, Francia)

9.00 - 10.15 Comunicazioni libere

Expression of GATA-4 in bone marrow mesenchimal stem cells of the rat after cocolture with adult cardiomyocytes
R. Rastaldo, A. Folino, S. Cappello, G. Losano

Neovasculogenesis from resident adipose tissue precursors during adipose tissue development - Potential for cardiovascular tissue engineering
R. Madonna, R. De Caterina

Human fetal cardiac stem cells for heart repair
G. Forte, R. Fiaccavento, L. Sartiani, S. Pagliari, E. Cerbai, A. Mugelli, A.C. Vilela-Silva, F. Carotenuto, M. Minieri, P. Di Nardo

Amelioration of cardiac electrical performance following stem cell based regenerative therapies, in infarcted rat heart
M. Savi, C. Frati, L. Bocchi, G. Graiani, R. Berni, C. Lagrasta, F. Quaini, M. Vassalle, E. Musso

Isolation and characterisation of mesenchymal stem cells from dental pulp and their possible utilisation in experimental models of myocardial infarction
A. Sprio, S. Raimondo, P. Salamone, F. Di Scipio, A. Barberis, B. Mognetti, L. Marinos, S. Geuna, P. Pagliaro, G. Berta

HBR pre-treated term placenta human mesenchymal stem cells improve regional myocardial function in a swine model of transmural myocardial infarction
A. Simioniuc, C. Cavallini, M. Campan, G.D. Aquaro, M. Marinelli, C. Simi, F. Bernini, V. Lionetti, C. Ventura, F.A. Recchia

10.15 - 10.30 **Coffee break**

10.30 - 11.30 **Sessione Poster (Gruppo B)**

11.30 - 12.00 **Premiazione Young Investigator**
Moderatori: ***C.M. Caldarera*** (Bologna) - ***G. Di Sciascio*** (Roma)

12.00 Chiusura del Congresso

giovedì 9 ottobre 2008 ore 17.30 - 19.00
venerdì 10 ottobre 2008 ore 12.20 - 14.30

DISFUNZIONE ENDOTELIALE (1-11)

1 **Characterization of Ca2+ signals in endothelial progenitor cells from human peripheral blood and umbilical cord blood**
Y. Sanchez-Hernandez, J.E. Avelino-Cruz, E. Bonetti, V. Rosti, F. Moccia, F. Tanzi

2 **Effect of HDL quality on cellular cholesterol efflux pathways**
E. Favari, L. Calabresi, M.P. Adorni, F. Zimetti, G. Franceschini, W. Jessup, F. Bernini

3 **A new nitric oxide releasing anti-diabetic hybrid drug: from the design to the pharmacological characterization**
A. Martelli, A. Balsamo, M.C. Breschi, M. Digiacomo, P. Marchetti, S. Rapposelli, L. Testai, S. Torri, V. Calderone

4 **Insulin potentiates cytokine-induced VCAM-1 expression in human endothelial cells**
R. Madonna, M. Massaro, R. De Caterina

5 **The omega-3 fatty acid docosahexaenoate attenuates the insulin-induced pro-atherogenic phenotype in human umbilical endothelial cells**
F. Muscente, R. Madonna, R. De Caterina

6 **Ruolo dell'ApoE nel trasporto inverso del colesterolo (RCT) in vivo**
S. Costa, M. Pedrelli, F. Potì, G. Stomeo, I. Zanotti, F. Bernini

7 **Ruolo del complesso di adesione intercellulare caderina/beta-catenina nella modulazione del rimodellamento vascolare indotto da angiotensina II**
M. Mura, C. Barisione, S. Garibaldi, G. Ghigliotti, P. Fabbi, P. Altieri, C. Aloi, P. Spallarossa, A. Barsotti, C. Brunelli

8 **Endothelial micro-lesions induce nitric oxide production in rat aorta endothelium: preponderant role of gap junction hemichannels during Ca2+ influx**
J. Avelino-Cruz, Y. Sanchez-Hernandez, F. Moccia, F. Tanzi

9 **Acidi grassi polinsaturi nella regolazione della sintesi di colesterolo e trigliceridi: il ruolo di SREBP**
M. Di Nunzio, D. VanDeursen, A. Verhoeven, A. Bordoni

10 **Vasostatin-1 is a new physiological cell penetrating peptide acting by an heparan sulphate-endocytosis-PI3K-eNOS pathway**
R. Ramella, O. Boero, R. Levi, G. Alloatti, M.P. Gallo

11 **Urocortin II induces no production in porcine aortic endothelial cells through camp and Ca2+ related pathways leading to eNOS activation**
E. Grossini, C. Molinari, Dasg. Mary, Pp. Caimmi, F. Uberti, G. Vacca

ELETTROFISIOLOGIA CARDIACA (12-13)

12 **Deacetylase inhibitors reduce cardiac arrhythmogenesis in MDX mouse model of duchenne muscular dystrophy (DMD)**
R. Berni, C. Colussi, L. Bocchi, M. Savi, F. Delucchi, F. Quaini, D. Stilli, E. Musso, C. Gaetano

13 **Analisi dei cicli atriali durante la fibrillazione atriale persistente e chemioriflesso dopo ripristino del ritmo sinusale con cardioversione elettrica esterna**
G. Marchetti, R. Roncuzzi, A. Zaniboni, A. Barbieri, D. Vivoli, S. Urbinati

STRESS OSSIDATIVO (14-30)

14 **Pharmacological characterisation of the cardioprotective activity of a new spiro-cyclic benzopyran activator of mitochondrial KATP channels**
L. Testai, A. Balsamo, M.C. Breschi, M. Manganaro, A. Martelli, S. Rapposelli, V. Calderone

15 **Il trattamento dei cardiomiociti con creatina e ribosio previene l'arresto del ciclo cellulare indotto dall'ischemia via attivazione di AKT e delle cicline D1/E**
A. Caretti, P. Bianciardi, F. Lucchina, C. Terruzzi, A. Veicsteinas, M. Samaja

16 **Pro-survival effects of H2S donors against oxidative stress in H9C2 cells**
D. Mancardi, A. Merlino, M.G. Perrellli, F. Tullio, F. Moro, P. Pagliaro, C. Penna

17 **Role of superoxide dismutase and catalase in ischemic postconditioning**
F. Tullio, A. Merlino, F. Moro, M.G. Perrelli, D. Mancardi, C. Penna, P. Pagliaro

18 **Sildenafil reduces hypoxia-induced pulmonary hypertension and acute myocardial infarct in the chronically hypoxic rat**
G. Milano

19 **Myocardial protection in the rat heart and role of weak and strong no-release from a hybrid molecule containing an antioxidant substructure**
A. Folino, S. Cappello, R. Rastaldo, P. Pagliaro, A. Di Stilo, G. Losano

20 **The protective activity of apelin against reperfusion injuries in rat hearts**
S. Cappello, A. Folino, R. Rastaldo, G. Losano

21 **Advanced oxidation protein products (AOPP) as a factor affecting acute coronary syndromes**
P. Fabbi, M. Fedele, M. Vercellino, S. Garibaldi, P. Contini, G.P. Bezante, F. Indiveri, A. Barsotti

22 **Il training fisico controllato migliora la resistenza endoteliale allo stress ossidativo in pazienti claudicanti**
S. De Marchi, M. Prior, A. Rigoni, A. Cevese, E. Arosio

23 **L'inibizione della xantina ossidasi migliora la resistenza endoteliale allo stress ossidativo in pazienti arteriopatici**
S. De Marchi, A. Rigoni, M. Prior, P. Delva, A. Lechi, E. Arosio

24 **Rescue of oxidative stress by ergothioneine, a powerful natural antioxidant, in human endothelial cells**
R. Arici, M.C. Barsotti, R. Colognato, I. Laurenza, F. Franzoni, F. Galetta, L. Benzi, L. Migliore, A. Balbarini, R. Di Stefano

25 **Gli antiossidanti prevengono gli effetti protrombotici indotti dallo stress ossidativo**
V. Angri, P. Cirillo, S. De Rosa, L. Sasso, A. Paglia, V. Di Palma, P. Maietta, L.G. D'Ascoli, L. Brevetti, M. Chiariello

26 **Meccanismi chimici ed enzimatici di ossidazione della tetraidrobiopterina**
R. Biondi, I. Tritto, L. Formigli, M. Ricci, M. Bettini, G. Ambrosio

27 **Impatto della sindrome metabolica sulla presentazione ECG e sulla prognosi a breve e lungo termine dei pazienti con sindrome coronarica acuta**
I. Tritto, G. Spinucci, E. Carluccio, P. Biagioli, A. Cardona, S. Coiro, N. Viola, M. Bentivoglio, G. Ambrosio

28 **Levosimendan postconditioning reduces myocardial reperfusion injury through a redox-sensitive mechanism and mitochondrial ATP-sensitive potassium channel activation**
E. Tiravanti, G. Colantuono, N. Di Venosa, V. Lo Parco, A. Cazzato, A. Federici, T. Fiore

29 **Effetti del pre- e post-condizionamento ischemico sul reclutamento dei neutrofili nel microcircolo dopo ischemia-riperfusione: monitoraggio in vivo con videomicroscopia**
I. Tritto, I. Porchetta, C. Zuchi, M. Bettini, S. Coiro, A. Cardona, G. Ambrosio

30 **Losartan treatment restores the response to hypoxia of diabetic cardiomyocytes**
C. Alfarano, L. Raimondi, S. Suffredini, A. Mugelli, E. Cerbai

MISCELLANEA (31-40)

31 **A membrane G protein-coupled receptor (GPR30) mediates the cardiac effects of 17beta-estradiol in the male rat**
E. Filice, A.G. Recchia, D. Pellegrino, T. Angelone, M. Maggiolini, M.C. Cerra

32 **Inotropic and lusitropic effects of the antihypertensive catestatin: mechanisms of action in the rat heart**
A.M. Quintieri, T. Angelone, T. Pasqua, R. Mazza, A. Gattuso, B. Tota, S.K. Mahata, M.C. Cerra

33 **Cardiotropic effects of relaxin on myocardial infarction and adverse post-ischemic remodelling**
R. Mastroianni, S. Nistri, L. Formigli, E. Ragazzo, L. Cinci, D. Bani, E. Masini

34 **Regional myocardial hybernation as adaptation of severe mechanical dyssynchrony in non ischemic heart failure**
V. Lionetti, G.D. Aquaro, C. Di Cristofano, A. Simioniuc, M. Campan, S. Forini, F. Bernini, M. Lombardi, F.A. Recchia, A. Pingitore

35 **Reduced apoptosis in polyamine depleted rat cardiomyocytes treated with norepinephrine**
S. Cetrullo, B. Tantini, I. Stanic', F. Flamigni, C. Pignatti, C. Stefanelli, C. Guarnieri, C.M. Caldarera

36 **Control of insuline sensitivity an lipid metabolism by physical activity: plasma visfatin concentrations and metabolic parameters in physically active children**
J. Jürimäe, A. Cicchella, E. Lätt, K. Haljaste, P. Purge, T. Jürimäe, C. Pignatti, C.L. Passariello, C.M. Caldarera, C. Stefanelli

37 **La proteina chinasi attivata da AMP come modulatore di eventi precoci in un modello cellulare di ipertrofia cardiaca**
C.L. Passariello, D. Gottardi, M. Zini, S. Cetrullo, B. Tantini, C. Pignatti, F. Flamigni, C.M. Caldarera, C. Stefanelli

38 **Anti-inflammatory effects of a low molecular weight heparin-like derivative in a rat model of carrageenan-induced pleurisy**
E. Masini, S. Nistri, C. Uliva, M. Ceccarelli, C. Lanzi, D. Bani

39 **Nrg1 modulation of contractility and NO synthesis in isolated ventricular myocytes**
A. Brero, R. Ramella, O. Boero, B. Mautino, C. Dati, G. Alloatti, M.P. Gallo, R. Levi

40 **Effect of revascularizing viable myocardium on left ventricular diastolic function in patients with ischemic cardiomyopathy**
P. Pantano, E. Carluccio, P. Biagioli, V. Leonelli, G. Alunni, A. Murrone, G. Ambrosio

venerdì 10 ottobre 2008 ore 18.00 - 19.00
sabato 11 ottobre 2008 ore 10:30 - 11.30

MISCELLANEA (1-19 bis)

1 **Frequente riscontro di PFO in giovani pazienti affetti da episodi ricorrenti di fibrillazione atriale parossistica**
P. De Campora, G. Malferrari, S. Sanguigni, R. Sangiuolo

2 **Clinical results of bivalirudin in high risk patients undergoing percutaneous coronary intervention**
L. D'Antonio, S. Caroli, M. Macrì, R. Melfi, A. Nusca, M. Miglionico, A. D'Ambrosio, G. Patti, G. Di Sciascio

3 **Oxygen extraction in CHF patients after a period of resistance training**
C. Tarperi, A. Cevese, A. Baraldo

4 **Synergistic effects against post-ischemic cardiac dysfunction by sub-chronic nandrolone pretreatment and postconditioning: role of beta-2-adrenoreceptors**
C. Penna, G. Abbadessa, M. Mancardi, F. Tullio, F. Piccione, A. Spaccamiglio, S. Racca, P. Pagliaro

5 **Monitoring blood flow in the masseteric ramus of the facial artery, in conscious rabbit: a model for the investigation of metabolic and neural regulation of muscle blood**
M. Mohammed, S. Roatta, M. Passatore

6 **Determinazione dei potenziali elettrici rilevabili in un circuito sperimentale di CEC: dati preliminari su sangue eparinato**
C.M. Mazza, U. Carletti, D. Meglioli, T. Corazzari, R. Lodi, A.V. Mattioli

7 **Patients on angiotensin-converting enzyme inhibitors have improved outcome after PCI**
F. Mangiacapra, G. Patti, E. Ricottini, V. Vizzi, P. Gallo, A. Nusca, M. Miglionico, R. Melfi, G. Di Sciascio

8 **Is there a threshold value of platelet reactivity during clopidogrel treatment for identifying patients with higher risk of peri-procedural events during PCI?**
V. Vizzi, G. Patti, F. Mangiacapra, L. Gatto, A. D'Ambrosio, A. Nusca, S. Mega, G. Di Sciascio

9 **Measurement of clopidogrel responsiveness with verify-now predicts clinical outcome in patients undergoing PCI. Results of the Armyda-pro study**
V. Vizzi, G. Patti, F. Mangiacapra, L. Gatto, A. D'Ambrosio, A. Nusca, S. Mega, G. Di Sciascio

10 **Is additional clopidogrel loading before PCI necessary in patients on chronic therapy? Results of the Armyda-reload randomized trial**
F. Mangiacapra, G. Patti, L. Gatto, V. Pasceri, G. Colonna, A. D'Ambrosio, A. Montinaro, G. Sardella, F. Ciccirillo, G. Di Sciascio

11 **Follow-up evaluation of left ventricular function in patients with post-ischemic cardiomyopathy undergoing PCI: role of real time 3D echocardiography**
R. Contuzzi, L. Gatto, C. Goffredo, G. Patti, G. Di Sciascio

12 **In-lab clopidogrel loading versus pretreatment in patients undergoing PCI. Final results of the Armyda-5 randomized trial**
L. Gatto, G. Patti, F. Mangiacapra, V. Pasceri, G. Colonna, G. Sardella, A. Montinaro, A. Tondo, G. Di Sciascio

13 **Improved clinical outcome with eptifibatide in patients undergoing PCI: a meta-analysis of five randomized trials**
E. Ricottini, G. Patti, V. Pasceri, G. Di Sciascio

14 **Inhibition of class I histone deacetylases with an apicidin derivative prevents cardiac hypertrophy and failure: in vitro and in vivo data**
P. Gallo, P. Gallo, M. V. G. Latronico, S. Grimaldi, M. Todaro, P. Jones, K. Steikuhler, M. Todaro, G. Di Sciascio, G. Condorelli

15 **The protein synthesis and T-CAP translational control by AKT/MTOR/4E-BP1 signal prevent heart failure in pressure overload end dilated cardiomyopathy animal model**
P. Gallo, D. Catalucci, M.L. Bang, M. Ceci, G. Di Sciascio, G. Condorelli

16 **Head-up Tilt test in pazienti con sincope: follow-up a lungo termine**
G. Domenichini, I. Diemberger, C. Valzania, M. Bertini, D. Saporito, M. Ziacchi, A. Marziali, C. Martignani, M. Biffi, G. Boriani

17 **Effetti della resincronizzazione ventricolare sul flusso muscolare periferico alla luce del rimodellamento ventricolare inverso**
I. Diemberger, I. Corazza, J. Frisoni, C. Martignani, E. Cervi, A. Marziale, G. Domenichini, M. Biffi, R. Zannoli, G. Boriani

18 **Moderate-severe mitral regurgitation is independent predictor of clinical impairment in II NYHA class chronic heart failure pts with various etiology and physiopathology**
R. De Vecchis, C. Cioppa, A. Giasi, A. Pucciarelli, S. Cantatrione

19 **The furosemide single-dose test provides prognostic information about risk of cardiorenal sindrome in chronic congestive heart failure with marked hydrosaline retention**
R. De Vecchis, C. Cioppa, A. Giasi, A. Pucciarelli, S. Cantatrione

19 bis **Improvement of skeletal muscle functional performance in aged rats after amino-acid oral supplementation: increased rate of ATP production and protein availability**
R. Raddino, I. Bonadei, G. Caretta, M. Teli, D. Robba, G. Zanini, M.T. Scarabelli, O. Visioli

RIGENERAZIONE MIOCARDICA (20-41)

20 **Anti-apoptotic effects of cytokines on cultured mesenchymal stem cells**
M.G. Perrelli, C. Penna, S. Raimondo, A. Merlino, S. Geuna, P. Pagliaro

21 **Fibrin gel: a new scaffold for cardiovascular applications**
A. Magera, M.C. Barsotti, M. Lemmi, E. Simonetti, F. Chiellini, A. Minnocci, R. Solaro, G. Soldani, A. Balbarini, R. Di Stefano

22 **Development of a new technology for 3-D nanostructured scaffolds with potential cardiovascular applications**
M. Lemmi, E. Briganti, A. Magera, R. Arici, C. Ristori, E. Simonetti, F. Chiellini, G. Soldani, A. Balbarini, R. Di Stefano

23 **The abundance of adipose tissue-derived progenitor cells in the adipose tissue is affected by age and blood glucose levels**
R. Madonna, C. Cellini, F. Renna, L. Rinaldi, R. Ippedico, C. Palmieri, R. Cotellese, N. Picardi, R. De Caterina

24 **High-level transduction and gene expression of EGFP reporter gene in adipose tissue-derived stromal cells using a HIV type 1 -based lentiviral vector**
R. Madonna, R. De Caterina

25 **3-D Fibrin scaffold improves stemness of human peripheral blood endothelial progenitor cells**
C. Armani, A. Magera, M.C. Barsotti, M. Iorio, E. Simonetti, R. Arici, F. Chiellini, G. Soldani, R. Di Stefano, A. Balbarini

26 **Cardiomyocyte co-cultures to assess the cardiomyogenic potential of adult stem cell lines**
A. Vilela-Silva, S. Pagliari, G. Forte, O. Franzese, R. Fiaccavento, S. Pietronave, F. Carotenuto, F. Pagliari, M. Minieri, P. Di Nardo

27 **Effetto della severità dell'ischemia sul reclutamento delle cellule staminali nei tessuti postischemici: monitoraggio diretto in vivo mediante videomicroscopia**
I. Tritto, M. Di Ianni, F. Falzetti, C. Zuchi, I. Porchetta, S. Coiro, L. Moretti, A. Cardona, A. Tabilio, G. Ambrosio

28 **PGLA-based microspheres coated with adhesion molecules as carriers for mesenchymal (STEM) stromal cells**
F. Bonafé, L. Foroni, C. Orrico, E. Fiumana, S. Valente, G.P. Morselli, A. Stella, C. Guarnieri, C. Montero-Menei, C.M. Caldarera

29 **Telomerase protects SCA-1+ mesenchymal stem cells from apoptosis induced by high dose H2O2, while preserving stemness phenotype and multipotency**
S. Pagliari, G. Forte, O. Franzese, F. Pagliari, R. Fiaccavento, F. Carotenuto, E. Bonmassar, M. Prat, M. Minieri, P. Di Nardo

30 **Benefici funzionali e istopatologici sul cuore di ratto post-infartuato a seguito del trapianto di cellule mioblastiche producenti l'ormone cardiotropico relassina**
S. Nistri, M. Bonacchi, C. Nanni, S. Zecchi-Orlandini, E. Masini, D. Bani

31 **Stem cells on biodegradable beads to repair damaged myocardial tissue**
R. Fiaccavento, G. Forte, S. Pagliari, F. Pagliari, F. Carotenuto, M. Minieri, P. Di Nardo

32 **Adipose tissue-derived stromal cells for heart regeneration: objectives and preliminary results**
R. Madonna, F. Renna, G. Basta, G. Lazzerini, R. De Caterina

33 **Organ culture of arterial conduits from heart-beating donors**
S. Valente, L. Foroni, C. Orrico, F. Alviano, G.L. Faggioli, G. Pasquinelli

34 **Vascular wall MSCs could reside in virtually all human vascular segments**
A. Pacilli, F. Alviano, P.L. Tazzari, F. Ricci, M. Buzzi, M. Gargiulo, G. Pasquinelli

35 **A new user-friendly experimental protocol to analyze proliferation, survival and immunophenotype of cells cultured on electrospun nano scaffolds for tissue engineering**
L. Foroni, G. Dirani, P. Celio, M.L. Focarete, C. Gualandi, G. Pasquinelli

36 **Microsfere biomimetiche per il supporto di cellule progenitrici endoteliali umane**
C. Musilli, F. Ledda, A. Mugelli, A. Parenti

37 **Exogenous high-mobility group box 1 protein (HMGB1) ameliorates cardiac electrical performance in infarcted rat heart**
L. Bocchi, A. Rossini, M. Savi, R. Berni, S. Baruffi, G. Graiani, A. Germani, F. Quaini, M.C. Capogrossi, E. Musso

38 **Poly(L-lactic acid) electrospun nanofibrous scaffolds for cardiac tissue engineering**
C. Gamberini, M. Govoni, E. Giordano, C. Gualandi, M.L. Focarete, M. Scandola, L. Foroni, S. Valente, G. Pasquinelli, C. Guarnieri

39 **Studio in vitro ed in vivo della biocompatibilità di PAM (pharmacologically active micro-carriers) come substrato per la rigenerazione miocardica**
C. Frati, G. Graiani, L. Prezioso, B. Testa, L. Bocchi, M. Savi, C. Lagrasta, E. Musso, C.M. Caldarera, F. Quaini

40 **Physical factors can potentiate Sca-1pos CPC proliferation**
F. Carotenuto, G. Forte, S. Pagliari, F. Pagliari, R. Fiaccavento, S. Zava, A.M. Rizzo, M. Minieri, P. Di Nardo

41 **A biological self-assembling peptide suitable for cell delivery in ischemic tissues**
E. Simonetti, M.C. Barsotti, A. Magera, H. Hosseinkhani, S. Sorbo, D. Dinucci, F. Chiellini, R. Solaro, R. Di Stefano, A. Balbarini

ABSTRACT

MODULAZIONE DELLE CORRENTI IONICHE NEI CANALI DEL CALCIO DI TIPO-L (CAV1.2) NATIVI E CLONATI DI UN ANALOGO FUNZIONALE DEL DILTIAZEM

P. Ioan[1], R. Budriesi[1], M.P. Ugenti[1,3], I. Bodi[2], A. Schwartz[2], A. Chiarini[1]

[1]Dipartimento di Scienze Farmaceutiche, Università di Bologna, Bologna, Italy;
[2]Institute of Molecular Pharmacology and Biophysics, University of Cincinnati Medical Center, Cincinnati, USA;
[3]PhD Student, presso Institute of Molecular Pharmacology and Biophysics, University of Cincinnati Medical Center, Cincinnati, USA

Obiettivo: Il nostro gruppo di ricerca basandosi su un approccio di tipo multidisciplinare ha scoperto recentemente un nuovo calciomodulatore analogo funzionale del diltiazem con struttura fenilsulfonilpirrolidinica (M8). L'obiettivo di questo studio è la valutazione dell'attività di M8 e dei suoi enantiomeri sulle correnti ioniche, la cinetica dei canali Cav1.2 e sugli effetti del rilascio del Ca2+ intracellulare.

Materiali e Metodi: Le correnti del (ICa) o Ba2+ (IBa) nei canali Cav1.2 sono state registrate mediante patch-clamp e voltage-clamp rispettivamente in miociti di arteria caudale di ratto e cardiomiociti di topo, e in oociti di Xenopus che esprimono la variante cardiaca umana dei Cav1.2. L'attività vasorilassante, inotropa negativa e la modulazione del rilascio del Ca2+ intracellulare è stata determinata in vitro su tessuti isolati di cavia.

Risultati: Studi di binding precedenti avevano dimostrato che M8 spiazza [3H]-diltiazem dal proprio sito di legame in cardiomiociti di ratto. Studi di patch-clamp eseguiti su miociti di arteria caudale di ratto hanno evidenziato che M8 riduce la ICa e accelera la cinetica di inattivazione del canale. In cardiomiociti di topo M8 non ha effetti sulla intensità della ICa, allo stesso modo nel voltage-clamp su canali Cav1.2 cardiaci umani, M8 e diltiazem mostrano una trascurabile inibizione della IBa. In tutti gli esperimenti M8 accelera la cinetica di inattivazione del canale e perde il caratteristico use-dependence phenomenon del diltiazem. Su tessuti isolati di cavia i due enantiomeri hanno mostrato una diversa attività: solo uno di essi è responsabile dell'azione vasorilassante posseduta dal racemo mentre entrambi inibiscono la forza di contrazione cardiaca. Esperimenti condotti impiegando strips di aorta di cavia hanno mostrato che solo l'enantiomero dotato dell'attività vasorilassante inibisce il rilascio del Ca2+ dal reticolo sarcoplasmatico indotto da noradrenalina in assenza di Ca2+ extracellulare.

Conclusioni: Gli studi di patch e voltage-clamp hanno evidenziato che questo nuovo calciomodulatore blocca i canali Cav1.2 preferenzialmente nel loro stato aperto. La perdita dello use-dependence phenomenon potrebbe essere associata alla rapida dissociazione del composto dal sito di legame delle benzotiazepine. Infine uno dei suoi enantiomeri sembra inibire il rilascio di Ca2+ intracellulare con un meccanismo imputabile al blocco del recettore della ryanodina.

MOLECULAR AND CELLULAR REMODELING IN FAMILIAR HYPERTROPHIC CARDIOMYOPATHY: A ROLE FOR 5HT2 RECEPTORS

F. Stillitano[1], R. Coppini[1], L. Sartiani[1], S. Suffredini[1], J. Olivotto[2], F. Cecchi[2], A. Mugelli[1], E. Cerbai[1]

[1]Centro Interuniversitario di Medicina Molecolare e Biofisica Applicata - CIMMBA, Firenze; [2]Referral Ctr for Myocardial Diseases, Azienda Ospedaliero-Universitaria Careggi, Firenze.

Background: Familial Hypertrophic Cardiomyopathy (fHCM) is the most common of the genetic cardiovascular diseases, characterized by high rate mortality, especially in young people. Molecular mechanisms underlying ventricular hypertrophy and functional remodeling in fHCM, likely predisposing to fatal arrhythmias, remains largely unknown. Thus, we aimed to: (i) assess the role of serotonin 5-HT2B receptors, whose overexpression causes compensated hypertrophic cardiomyopathy in animal models and (ii) investigate the functional and molecular abnormalities of f-channel (HCN), taken as a marker of functional remodelling.

Methods: Biopsies were obtained from fHCM patients with severe obstructive disease undergoing septal myectomy; undiseased hearts not used for transplantation served as controls. Patch-clamp technique in isolated fHCM cells was used for electrophysiological recordings, and TaqMan Real Time PCR for relative quantification of HCN2/4 isoforms and 5-HT2B receptor genes.

Results: 5-HT2B mRNA was significant increased in fHCM with respect to the controls (5.83±1.6 vs 1.27±0.17, $p<0.05$). Functional coupling of 5-HT2 was confirmed by measuring the effect of the 5-HT2 selective agonist -metil-serotonin (α-Me5HT, 1μM, a) on action potential in single fHCM myocytes: α-Me5HT increased the action potential duration, an effect which was reverted by washout; no effect was detected in control cells. HCN2/HCN4 ratio was significantly increased in hypertrophied samples (6.82±4.6) relative to the controls (1.46±1.08, $p<0.01$). Consistently with a relative over-expression of HCN2 isoform, f-current activates at more negative potential (Vh ~-94 mV) in patch-clamped fHCM compared to control myocytes (-84 mV),

Conclusions: In fHCM, specific molecular mechanisms can be responsible for the induction of an altered functional phenotype in the human heart. Understanding the molecular mechanisms underlying ventricular and septal remodeling could identify functional arrhythmogenic mechanisms and, importantly, entirely novel targets for effective pharmacological interventions aimed to prevent and treat fHCM.

EFFETTI ACUTI DELL'ANGIOTENSINA II SULL'ELETTROFISIOLOGIA DEL MIOCARDIO VENTRICOLARE: EFFETTO ARITMOGENO ACUTO DELL'ATTIVAZIONE TISSUTALE DEL SISTEMA RENINA ANGIOTENSINA

P. Ferrero[1], R. Jabr[2], A. Kontogeorgis[1], M. Turner[1], C.H. Fry[2], N.S. Peters[1]

[1]Imperial College, London, United Kingdom;
[2]University of Surrey, Surrey, United Kingdom

Obiettivi: Vi sono evidenze circa l'effetto di modulazione del sistema renina angiotensina (RAS) sull'elettrofisiologia cardiaca nell'uomo. Allo scopo di verificare l'ipotesi di un possible effetto proaritmico auto-paracrino dell'angiotensina II, abbiamo caratterizzato l'effetto a breve termine della superfusione di tale peptide sul muscolo papillare di cavia.

Materiali e Metodi: Un muscolo papillare è stato prelevato dal ventricolo sinistro di cavie e disposto in un bagno di superfusione con soluzione di Tyrode e connesso con un trasduttore di tensione isometrica. Il muscolo è stato stimolato con un microelettrodo bipolare ad una estremità (ampiazza 1,5 volte la soglia; durata 0,02 ms). Il potenziale d'azione intracellulare propagato è stato registrato, ad una distanza non inferiore ad un millimetro dal punto di stimolazione, mediante un microelettrodo capillare riempito con una soluzione 3M di potassio cloruro. La durata del potenziale d'azione e la latenza tra l'artefatto di stimolazione e la massima ascesa della fase rapida del potenziale d'azione (dV/dt) sono stati misurati durante superfusione con soluzione di Tyrode (controllo) e Tyrode + Angiotensina II a diverse concentrazioni: (5/50/100 nM per 30 minuti). Le misurazioni sono state effettuate in condizioni basali (frequanza 1 Hz) e a frequenze crescenti (0,5-1-1,2-1,6- 2Hz) ottenendo le curve di restituzione del potenziale d'azione. La soglia di stimolazione è stata ripetutamente verificata durante l'esperimento.

Risultati: L'angiotensina II ha prodotto un accorciamento del potenziale d'azione (69% rispetto al controllo- p=0,03) accompagnato da un rallentamento della conduzione (11% in media p=0,04). L'effetto massimo è stato osservato dopo 10-20 min. di superfusione con 50 nM con una completa reversibilità dopo 30 min. La curva di restituzione non è risultata modificata. La fase passiva del potenziale d'azione (costante Tau) risulta ritardata suggerendo un effetto dell'angitensina sull'accoppiamento intercellulare.

Discussione: L'angiotensina II riduce la durata del potenziale d'azione e rallenta conduzione nel muscolo papillare di cavia. Tale effetto sembra essere indipendente dagli effetti sul potenziale d'azione e verosimilmente legato ad un effetto sulla resistività intercellulare mediata dalle gap junctions. Questa nuova osservazione suggerisce un effetto aritmogeno autocrino acuto da parte dell'angiotensina II rilasciata a livello tissutale.

WAVELET ANALYSIS OF INSTANTANEOUS HEART RATE IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION UNDERGOING PRIMARY CORONARY ANGIOPLASTY

C. Molinari[1], E. Grossini[1], C. Anchisi[1], A. Brunori[1], M. Invernizzi[1], A.S. Bongo[2], G. Vacca[1]

[1]Università del Piemonte Orientale, Novara;
[2]A.O.U. Maggiore della Carità, Novara.

Objectives: Depressed heart rate variability (HRV) has been associated with adverse outcome during and after acute myocardial infarction (AMI). Moreover the occurrence and timing of reperfusion after coronary angioplasty have crucial clinical implications, both for prognosis and for subsequent treatment. Myocardial ischemia induces strong autonomic reflexes. The perturbations of the sympathovagal balance induced by these reflexes can be observed by spectral analysis of heart rate variability. However, in earlier studies on HRV in patients with AMI undergoing primary coronary angioplasty, generally, inappropiate algorhythms of HRV analysis were used. We therefore decided to study the effect of coronary angioplasty and the subsequent reperfusion on autonomic cardiac rate control by applying a continuous time-dependent analysis to HR fluctuations focusing on few minutes before and after reperfusion.

Methods: A total of 19 patients with acute coronary syndrome (ACS) who received coronary angioplasty were recruited. The subjects were divided into two groups, ACS with STEMI (n=9) and ACS-NSTEMI (n=10). ECG recordings was obtained 200 s before and 300 s after coronary angioplasty. R waves were detected off-line and corrections were made for arrhythmias. The beat-to-beat spectral estimation analysis was based on the continuous wavelet transform (CWT) using Morlet basis wavelet.

Results: The most relevant findings of this study are: i) in ACS-STEMI group, coronary balloon inflation induces different alterations in high frequencies based on the location of the infarction. In the first minute of the angioplasty, HF changes detected from inferior wall (IW) AMI patients were significantly higher (normalized value: +2.1±0.8) in respect of those recorded from anterior wall (AW) AMI group (+1.4±0.6); ii) a significant increase of HF with a latency of 100 s was detected only from AW-AMI patients; iii) low frequencies, appear to be significantly affected by coronary angioplasty only in ACS-NSTEMI patients with AW-AMI (normalized value: +2.3±0.7) in respect of data obtained from ACS-NSTEMI with IW-MI group.

Conclusions: We found that time-dependent spectral analysis of HRV, using the CWT, enabled us to detect patterns of alteration in HRV, which were directly associated with infarction location. Therefore, the CWT provides a rich description of HRV evolution in a complex clinical setting.

MYOINTIMAL CELLS, RATHER THAN DENDRITIC CELLS OR MAST CELLS, ARE MAJOR SOURCES OF TNFALPHA AND NO IN ATHEROMATA

S. Bacci, L. Pieri, P. Romagnoli

Università di Firenze, Firenze.

Objective: Dendritic cells (DC) and mast cells (MC) can regulate, and intimal smooth muscle cells (myointimal cells) exert the injury response of arterial wall; TNFalpha and Nitric Oxide (NO) are candidate to play a role in the regulation of this process. Since both DC and MC can be source of these molecules, this study was aimed at investigating their expression by those cells and whether they were expressed also by myointimal cells in atheromata.

Methods: We have investigated by affinity cytochemistry the cell numbers and TNFalpha and iNOs expression in uncomplicated atheromata of the carotid arthery; the expression of TNFalpha was evaluated also by Western blot.

Results: DC as well as MC were significantly more numerous in atheromata than in controls. Appreciable fractions of DCs expressed TNFalpha and iNOs, and appreciable fractions of MC expressed TNFalpha, in both controls and atheromata. On the contrary, iNOs expression by MCs was significantly lower in atheromata. Phalloidin positive myointimal cells were more numerous in atheromata, and significantly more of these cells expressed iNOs and TNFalpha in atheromata than in controls. Higher amounts of iNOs and TNFalpha were demonstrated in atheromata, as compared with controls, by computerized image analysis; the finding for TNFalpha could be corroborated by Western blot.

Conclusions: We propose that TNFalpha and NO are generated by dendritic cells and, to a minor extent by MCs and activate injury responses during atherosclerosis and that myointimal cells - because of their high number - are the major sources of these molecules during progression of atheromata.

L'UROTENSINA II ESERCITA EFFETTI PRO-ATEROTROMBOTICI IN CELLULE ENDOTELIALI CORONARICHE UMANE IN COLTURA

G. Petrillo, P. Cirillo, M. Pacileo, L. Sasso, A. Paglia, V. Di Palma, P. Maietta, L.G. D'Ascoli, L. Brevetti, M. Chiariello

Cattedra di Cardiologia, Università Federico II di Napoli, Napoli.

Obiettivi: L'urotensina II (UII) è un peptide attivo sul tono della parete vascolare. I risultati di alcuni recenti studi hanno ipotizzato il coinvolgimento di tale peptide nella fisiopatologia dell'aterotrombosi, tuttavia i meccanismi responsabili di tale fenomeno restano sconosciuti. Pertanto, scopo del presente studio è stato di indagare, in cellule endoteliali coronariche umane (HCAECs), gli effetti dell'UII sull'espressione di molecole direttamente coinvolte nei meccanismi dell'aterosclerosi e della trombosi intravascolare: le molecole di adesione ICAM-1 e VCAM-1, responsabili del reclutamento dei monociti e coinvolte nella progressione della placca aterosclerotica, ed il Tissue Factor (TF), attivatore della cascata coagulativa, responsabile dello sviluppo delle sindromi coronariche acute.

Materiali e Metodi: HCAECs venivano incubate con dosi crescenti di UII, in un range di valori comprendente le concentrazioni riscontrabili nel plasma di pazienti con aumentato rischio cardiovascolare. Cellule stimolate con LPS (50 mcg/ml) servivano da controllo positivo. Veniva quindi valutata l'espressione del TF, di VCAM-1 e di ICAM-1 sulla superficie cellulare mediante analisi FACS. L'attività funzionale di tali molecole veniva verificata mediante saggio cromogenico del TF e saggio di adesione leucocitaria su un monostrato di HCAEC. Infine, in esperimenti aggiuntivi venivano indagati i possibili meccanismi molecolari coinvolti nella modulazione di tali fenomeni, attraverso la valutazione dell'attivazione dei noti pathways di signalling di NF-kB, RhoA e PKC.

Risultati: La stimolazione con UII induceva l'espressione del Tissue Factor e delle molecole di adesione ICAM-1 e VCAM-1 in HCAECs in coltura. In entrambi i casi, le molecole espresse risultavano essere funzionalmente attive. Tali fenomeni apparivano mediati dell'attivazione del fattore di trascrizione NF-kB, come dimostrato dall'EMSA (Electroforetic mobility shift assay) e dall'inibizione degli effetti dell'UII da parte dell'inibitore di NF-kB, pirrolidin-di-tio-carbammato (PDTC).

Conclusioni: L'urotensina II induce l'espressione del TF e delle molecole di adesione ICAM-1 e VCAM-1 in cellule endoteliali coronariche umane in coltura promuovendone la conversione ad un fenotipo pro-aterotrombotico. Questi risultati suggeriscono un potenziale nuovo ruolo per tale peptide vasoattivo nella fisiopatologia della malattia aterotrombotica.

HIGH INSULIN IMPAIRS PI3-KINASE/AKT/NITRIC OXIDE MEDIATED INSULIN SIGNALING IN HUMAN ENDOTHELIAL CELLS

R. Madonna, R. De Caterina

Cardiology Division, Center of Excellence on Aging, G. d'Annunzio University, Chieti.

Background and Aim: Insulin levels are a marker for cardiovascular events, but may also contribute to the pathogenesis of early atherosclerosis in hyperinsulinemic states. We studied the consequences of a prolonged insulin treatment of human umbilical vein endothelial cells (HUVEC) on the phosphatidylinositol 3'-kinase (PI-3 K)/AKT/nitric oxide (NO)-dependent insulin signaling pathway - mediating anti-atherogenic effects of insulin and promoting glucose transport - and on vascular cell adhesion molecule (VCAM)-1 expression - as a marker of a pro-atherogenic endothelial phenotype.

Methods: HUVEC were incubated with insulin (10^-10 to 10^-7 mol/L), during short- (30 min) and long-term (24-72 hours and 1 week) incubations. Constitutive and Ser473-phosphorylated AKT, as well constitutive and Ser1146-phosphorylated nitric oxide synthase (eNOS) were investigated by immunoblotting. Surface expression of VCAM-1 was analyzed by enzyme immunoassay. NO production was measured by the Griess assay.

Results: After short-term incubations, while constitutive AKT and eNOS did not vary at any insulin concentration, the expression of the functionally active phosphorylated forms of AKT and eNOS, as well as NO production, significantly increased in HUVEC treated with insulin in a concentration-dependent manner. After long-term incubations, increased VCAM-1 expression (average increase: 56 ± 8% and 25 ± 10% vs non-treated conditions after 24 and 72 hour incubations, respectively), as well as reduced constitutive and phosphorylated AKT, eNOS expression and NO production, were observed at 10^-8 and 10^-7 mol/L insulin concentrations.

Conclusions: Prolonged exposure of HUVEC to high insulin levels induces a downregulation of the PI-3 K/AKT/eNOS axis while increasing VCAM-1 expression. These alterations of the PI-3 K/AKT/eNOS axis might impair insulin signaling, thus contributing to detrimental effects of hyperinsulinemia on atherogenesis.

NA+/H+ EXCHANGER 1- AND AQUAPORIN-1-DEPENDENT HYPEROSMOLARY CHANGES DECREASE NITRIC OXIDE AND INDUCE VCAM-1 EXPRESSION IN ENDOTHELIAL CELLS EXPOSED TO HIGH GLUCOSE

M. Zurro, R. Madonna, E. Montebello, G. Lazzerini, R. De Caterina

Cardiology Division, Center of Excellence on Aging, G. d'Annunzio University, Chieti.

Background: High glucose induces endothelial dysfunction in terms of decreased nitric oxide (NO) availability and increased endothelial vascular cell adhesion molecule (VCAM)-1 expression. We investigated the contribution of hyperosmolarity and the involvement of membrane associated water channels aquaporin-1 (AQP1) and Na+/H+ exchanger 1 in these effects.

Methods and Results: Human aortic endothelial cells (HAEC) were incubated for short-term (1-3 days) or long-term (1-2 weeks) exposures to 5.5 mmol/L glucose (normoglycemia basal), high glucose (25 and 45 mmol/L, HG), or a hyperosmolar control (mannitol 25 and 45 mmol/L, HM), in the presence or absence of the Na+/H+ exchange 1 inhibitor cariporide (1 micromol/L, CA) or the AQP1 inihibitor dimethylsulfoxide (1% DMSO), the protein kinase C (PKC) inihibitor calphostin C or the PKCbeta isoform inhibitor LY379196 (LY).
Both short- and long-term exposures to HG and HM control decreased the expression of the active phosphorylated form of endothelial nitric oxide synthase (Ser1146-eNOS) and, in parallel, increased total VCAM-1 protein at immunoblotting. After 24 hour incubation with hyperosmolar stimuli, there was a significant and concentration-dependent enhancement of AQP1 expression. 1% DMSO and CA inhibited hyperosmolarity-induced APQ-1 and total VCAM-1 expressions, while increasing nitrite levels and Ser1146-eNOS expression. Calphostin C and LY blunted APQ-1- and Na+/H+ exchanger 1-induced VCAM-1 expression, while increasing the expression of Ser1146-eNOS and nitrite production (measured by the Griess assay).

Conclusions: High glucose decreases eNOS activation and induces total VCAM-1 expression in HAEC through a hyperosmolar mechanism. These effects are mediated by activation of membrane associated water channels aquaporin-1 (AQP1) and Na+/H+ exchanger 1, through a PKCbeta-mediated intracellular signaling pathway.

REDUCTION IN ENDOTHELIAL PROGENITOR CELLS ANTICIPATES ENDOTHELIAL DYSFUNCTION IN NORMOGLYCAEMIC PATIENTS WITH FAMILY HISTORY FOR TYPE 2 DIABETES

M. Barsotti[1], R. Di Stefano[1], L. Pucci[1], D. Lucchesi[1], S. Sorbo[1], M. Iorio[2], L. Ghiadoni[1], G. Penno[1], S. Del Prato[1], A. Balbarini[1]

[1]Università di Pisa, Pisa;
[2]Azienda Ospedaliera Universitaria Pisana, Pisa

Aims: Circulating endothelial progenitor cells (EPCs) contribute to integrity of endothelial monolayers. The concept of repair of damaged dysfunctional endothelium by EPCs has recently emerged. EPC reduction has been associated with vascular dysfunction.
Aim of our work was to assess if endothelial dysfunction is accompanied by EPC quantitative alterations in subjects with different cardiovascular risk.

Methods: The study population was composed of 34 subjects with impaired glucose regulation (IGR), 18 newly diagnosed type 2 diabetics (naiveT2D) and 26 normoglycaemic (NGT) with (n. 13, NGT/Fam+) and without (n. 13, NGT/Fam-) first-degree family history for type 2 diabetes. Endothelial function (flow-mediated dilation, FMD) of brachial artery was assessed by ultrasound; circulating EPCs (CD34/KDR double positive cells) were determined by flow cytometry.

Results: IGR and naiveT2D were older than NGT (55+/-5 and 58+/-8 vs 45+/-10 yrs, p=0.001), had higher systolic BP (p=0.001) and HbA1c values (6.0+/-0.4 and 65+/-0.6 vs 5.5+/-0.4%, p<0.0001). Endothelium independent dilation (glycerol trinitrate, GTN) was not different, while FMD was lower in naiveT2D (Delta % 4.4+/-2.3 M+/-SD) compared with IGR (Delta % 6.0+/-2.8) and in both naiveT2D and IGR compared to NGT (Delta % 7.9+/-3.6; Kruskal-Wallis p=0.0017). The same pattern was observed for the FMD/GTN ratio, an expression of the selective endothelial function impairment. Both patterns were confirmed between genders and in young (age <50 yrs, n. 27, p=0.07) and older (age >50 yrs, n. 51, p=0.04) subjects. EPCs were higher in NGT/Fam- (608+/-87 cells/ml, M+/-SE), similarly reduced in NGT/Fam+ (457+/-68 cells/ml) and in IGR (479+/-65 cells/ml), and even more reduced in naiveT2D (254+/-51 cells/ml, Kruskal-Wallis, p =0.01).

Conclusions: In conclusion, naiveT2D subjects showed EPC depletion as well as impaired FMD. In subjects with non-diabetic hyperglycemia, reduction in FMD was paralleled by an intermediate EPC depauperation. This impoverishment is already apparent in NGT/Fam+ suggesting that EPC reduction may precede impairment in endothelial function.

BINDARIT, AN INHIBITOR OF MCP-1, ATTENUATES NEOINTIMAL HYPERPLASIA AFTER BALLOON INJURY IN RATS

G. Grassia[1], M. Maddaluno[1], A. Guglielmotti[2], G. Mangano[2], C. Bartella[2], P. Maffia[3,1,4], A. Ialenti[1]

[1]Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy; [2]Department of Pharmacology, Angelini Research Center, S. Palomba-Pomezia, Rome, Italy; [3]Strathclyde Institute of Pharmacy & Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom;
[4]School of Biotechnological Sciences, University of Naples Federico II, Naples, Italy.

Introduction: Vascular smooth muscle cells (VSMCs) proliferation and migration is key to neointimal formation following balloon angioplasty or arterial stent. Several pro-inflammatory markers contribute to this process, and among them, monocyte chemoattractant protein 1 (MCP-1/CCL2) is receiving growing emphasis. In fact, beside its well known activity on monocyte/macrophage chemotaxis, MCP-1 induces VSMCs proliferation/migration.
Bindarit is an original indazolic derivative that has been shown to inhibit MCP-1 production both in vitro and in vivo. Therefore, the aim of the present study was to evaluate the effect of bindarit in a rat model of balloon injury-induced neointimal formation.

Methods and Results: Endothelial denudation of the left carotid artery was performed in rats by use of a balloon embolectomy catheter. Oral treatment with bindarit (200 mg/kg/die) significantly reduced the number of medial and neointimal proliferating cells at day 7 after injury (55% and 30% respectively, $P<0.001$) and inhibited neointimal formation at day 14 (40%; $P<0.01$). Interestingly, bindarit did not affect re-endothelialization. Activity on neointimal hyperplasia was correlated to a significantly reduction of MCP-1 levels both in sera and in injured carotid tissue.
In addition, in vitro data showed that bindarit (10-300 μM) reduces PDGF-BB-induced VSMCs proliferation and migration.

Conclusions: Obtained results show that bindarit is effective in reducing neointimal formation by inhibiting VSMCs proliferation and migration, and suggest that MCP-1 inhibition could represent an useful therapeutic strategy to reduce neointimal hyperplasia.

POSITIVE EFFECTS OF LONG-TERM L-TRIIODOTHYRONINE (LT3) TREATMENT ON CARDIAC REMODELLING RELATED TO T3-REGULATED MITOCHONDRIAL TRANSCRIPTION FACTORS AND RESPIRATION

S. Forini[1], V. Lionetti[2], F. Cecchetti[4], L. Sabotino[1], P. Nicolini[1], S. Balzan[1], M. Grana[3], M. Nannipieri[4], F.A. Recchia[2], G. Iervasi[1]

[1]Istituto di Fisiologia Clinica-CNR, Pisa;
[2]Settore di Medicina, Scuola Superiore Sant'Anna, Pisa;
[3]Servizio di Anestesia e Rianimazione, Chirurgia Generale, Vascolare e Trapianti, Ospedale di Cisanello, Pisa;
[4]Dipartimento di Medicina Interna, Università di Pisa, Pisa

Objectives: Heart failure (HF) is frequently associated to a low T3 syndrome. In our study we want to assess the effects of L-T3 infusion on myocardial repair and functional maintenance related to cardiomyocyte respiration and mitochondrial transcription factor levels in a rat model of ischemic HF.

Materials and Methods: Ischemic HF was produced by ligation of the left descending coronary artery of rats. 72h after ligation, the rats were treated for 4 weeks with a subcutaneous infusion of L-T3 (0.05μg/h/kg, T3+, n=10), or L-T3 vehicle (T3-, n=10) using implanted micro-osmotic pump. Healthy rats were used as control (Sham, n=10). Global and regional LV contractile function w a s assessed, respectively as ejection fraction (EF) and LV end-systolic wall thickening (LVESWT), by echocardiography. Myocardial mitochondrial transcription factor A (mTFA), thyroid hormone receptor beta 1 (TRβ1) expression, and cytochome c oxidase activity were measured in isolated mitochondria from frozen hearts. Results: L-T3 infusion determined a marked improvement of EF (70.7±5.53 vs 27.4±2.43 %, P<0.001) and LVESWT in the anterior wall (53.8±3.82 vs 2.66±0.99 %, P<0.001) at 1 month from myocardial infarction induction compared to infarcted T3- animals. When compared to T3- group, the T3+ rats showed a 3.9 fold increase of mTFA (p=0.002) and 3.0 fold increase of TR beta1 protein expression (p=0.01) in restored LV myocardium; no significant differences were found between T3+ and sham. Moreover, continuous T3 infusion elicited a significant compensatory increase of cytochrome c oxidase activity in the infarct border zone rather than remote zone of failing hearts.

Conclusions: Our results indicate that the long-term L-T3 infusion at physiological dose, started 72h after coronary artery ligation, increases mTFA levels and cytochrome c oxidase activity in recovered LV myocardium, and point to TRβ1 signalling as an essential component to induce cardiomyocyte adaptation to severe hypoxia.

POSTCONDITIONING AND MITOCHONDRIA

C. Penna[1], M.G. Perrelli[1,2], S. Raimondo[1], S. Geuna[1], P. Pagliaro[1,2]

[1]Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Orbassano;
[2]Istituto Nazionale di Ricerche Cardiovascolari, Bologna.

Background: Ischemia/reperfusion induces cell death via three interrelated processes: apoptosis, autophagy and necrosis.

Methods: Markers of apoptosis and mitochondrial-protection were studied with western-blotting analysis on cytosolic (CF) and/or mitochondrial (MF) fractions, in three groups of isolated rat hearts, which underwent a)Perfusion without ischemia (Sham), b)30-min ischemia plus 2-hours reperfusion, or c)Postconditioning (PostC) protocol (five cycles of 10-s ischemia/reperfusion immediately after the 30-min ischemia), respectively. Data are mean±SEM in arbitrary units. Results In CF Bcl-2 protein levels were higher in Sham and PostC, than the I/R group (26.35±0.13 and 23.95±0.25 vs 5.64±0.07). Also protective Pim-1 level was significantly reduced in I/R group with respect to Sham and PostC groups (20.02±0.20 vs 35.02±0.13 and 28.84±0.31). Cytochrome-C levels were higher in I/R than in Sham and PostC ((7.16±0.19 vs 2.96±0.12 and 3.25±0.12) groups. Also Caspase-3 levels resulted lower in Sham and PostC groups with respect to I/R group (5.24±0.08 and 5.27±0.10 vs 18.45±0.16). Heat shock protein 60 (HSP-60), Connexin-43 (Cx43) and GSK-3β were studied both in CF and MF. In the MF of Sham hearts, HSP was 33.8±0.58. HSP-60 level was reduced to 1.6±0.04 in hearts subjected to I/R and increased to 27.9±0.4 in PostC hearts. Yet, in the CF, HSP-60 level was higher in the I/R group than Sham and PostC groups (13.14±0.79, 1.25±0.09 and 1.27±0.09 , respectively). In CF, Cx43 level was high in Sham and PostC groups and lower in I/R group (24.00±0.03 and 28.33±0.19 vs 19.33±0.19). Moreover, in MF phospho-Cx43 level was higher in the I/R than Sham group (7.84±0.13 vs 0.71±0.19, $p< 0.001$). PostC treatment induced a reduction of phospho-Cx43 with respect to I/R group (5.55±0.21 vs 7.84±0.13). While in CF, total GSK-3β level was higher in I/R group than the other two groups, in MF phospho-GSK-3β was more expressed in PostC group with respect to Sham and I/R groups (5.98±0.10 vs 4.72±0.13 and 4.50±0.12). Electron microscopy confirmed that the I/R induced damages of cristae and of mitochondrial membranes were drastically reduced by PostC.

Conclusions: Data suggest that mitochondrial-protection and anti-apoptotic effects are central aspects of PostC protection. Pim-1 kinase plays a pivotal role in these protections.

EXPRESSION OF ANTIOXIDATIVE ENZYMES IN HIGH GLUCOSE-EXPOSED PROGENITOR AND MATURE ENDOTHELIAL CELLS

F. Felice, M.C. Barsotti, L. Pucci, D. Lucchesi, M.L. De Perna, G. Penno, S. Del Prato, R. Di Stefano, A. Balbarini

Università di Pisa, Pisa.

Purpose: Vascular diseases, including diabetes, are characterized by elevated levels of reactive oxygen species (ROS). Since recent studies demonstrated that endothelial progenitor cells (EPC) are more resistant to oxidative stress than human umbilical vein endothelial cells (HUVEC), we analysed the expression of intracellular antioxidative enzymes in human EPC and HUVEC exposed to high glucose, an in vitro setting simulating diabetes.

Methods: We studied the expression profile of catalase (CAT), glutathione peroxidase (GPX1), superoxide dismutase (SOD2) and of the transforming growth factor-beta 1 (TGF-beta1) by real-time PCR. Human EPC, obtained from peripheral blood mononuclear cells of healthy donors, and HUVEC, isolated from fresh umbilical cords, were exposed over a 96 hours period to the following experimental conditions: control (C) = constant exposure to 5 mM D-glucose; osmotic control (LG20) = constant exposure to 20 mM L-glucose + 5mM D-glucose; high glucose (HG25) = constant exposure to 25 mM D-glucose. The results are expressed as fold increase compared to control mRNA (means+/-SD).

Results: In EPC the transition from 5 (C) to 25 mM D-glucose (HG25) induced an increase of 1.87+/-0.39 folds in the expression of GPX1; 1.40+/-0.55 in SOD2 mRNA levels; the levels of catalase mRNA were not significantly affected (1.32+/-0.25). No increase in TGF-beta1 mRNA expression was observed (1.06+/-0.23). The exposure of HUVEC to 25 mM D-glucose or 20 mM L-glucose induced a weak increase in TGF-beta1 mRNA expression (1.32+/-0.88; 1.56+/-1.05 respectively). The exposure to 25 mM D-glucose induced a decrease of GPX1 mRNA (0.67+/-0.07) and of SOD2 mRNA (0.53+/-0.26) and of catalase mRNA (0.61+/-0.08) in HUVEC. Exposure to LG20 did not induce any differences from control (C) in enzymes expression.

Conclusions: The comparison of EPC and HUVEC antioxidative enzymes expression highlights that CAT, SOD2, GPX1 are more strongly expressed in EPC. For this reason, EPC seem to have a higher protection against oxidative stress.

OXIDATIVE STRESS, APOPTOSIS AND IMMUNOLOGICAL ACTIVATION IN ACUTE MYOCARDIAL INFARCTION

M. Fedele[1], P. Fabbi[1], M. Vercellino[1], M. Balbi[1], S. Garibaldi[1], M. Ghio[2], P. Contini[2], F. Indiveri[2], A. Barsotti[1]

[1]Cardiology Unit, DIMI-University of Genoa, Genoa;
[2]Immunology Unit, DIMI-University of Genoa, Genoa.

Purpose: To investigate the ratio between oxidative stress, apoptosis and immunological activation in a setting of acute coronary syndromes.

Methods: 15 patients (14 men) with acute myocardial infarction (AMI), and 18 normal volunteers (13 men) have been enrolled. At the time of admission into the hospital, peripheral blood samples were collected in order to determine oxidative stress markers (AOPP, now considered as a more reliable marker of oxidative stress), biomarkers of immunological response (sHLA-1, oxLDL and Hsp 60- specific CD8+ T-cells), cytokines (TGF-b, IL-10) and apoptosis markers (Fas-L).

Results: AOPP plasmatic levels were significantly higher in AMI group, respect to those observed in Controls ($p= 0,002$), showing a key role of oxidative stress on a setting of acute coronary syndromes. In AMI group, variations of plasmatic concentration of AOPP show a positive correlation with Fas-L, IL-10, TGF-b ($p< 0,05$), indicating an important role of oxidative stress in the activation of the apoptosis process and cytokines production. Levels of oxLDL and Hsp 60- specific CD8+ T-cells in AMI group were higher than Controls ($p< 0,05$). However, this variable was negatively correlated with sHLA-1, Fas-L and IL-10 plasma levels ($p< 0,05$). The behaviour of sHLA and Fas-L might be related with the high rate of apoptosis while the one of IL-10 might suggest an impaired homeostasis of the immune function.

Conclusions: Oxidative stress appears particulary activated during acute myocardial infarction, as shown by high levels of AOPP. Moreover the oxidative stress could activate the antigen specific (OxLDL-Hsp 60) CD 8+ cells that release sHLA, IL-10 and Fas-L, affect the homeostasis and trigger the apoptosis process. Since the apoptotic activity of Fas-L might involve the bystander smooth muscular cells as well as myocardiocytes it seems likely that such a mechanism may play an important role in the AMI pathogenesis.

TRATTAMENTI CON DOSI SUBAPOPTOTICHE SEQUENZIALI DI EPIRUBICINA ED ANTICORPO ANTIERBB2 INDUCONO NEI CARDIOMIOCITI APOPTOSI: RUOLO PROTETTIVO DEL DEXRAZOXANE

C. Aloi, P. Altieri, P. Spallarossa, M. Mura, S. Garibaldi, C. Barisione, G. Ghigliotti, U. Dorighi, A. Barsotti, C. Brunelli

Dipartimento di Medicina Interna (DiMI), Genova

Obbiettivi: Il Trastuzumab, anticorpo anti recettore erbB2, rappresenta il cardine del trattamento del carcinoma mammario che esprima tale recettore; ha un'azione cardiotossica responsabile di insufficienza cardiaca che diviene grave in caso di pretrattameto con antracicline e che limita il potenziale terapeutico dell'anticorpo. Abbiamo analizzato in H9c2: A) il tipo di danno indotto da dosi subapoptotiche di Epirubicina (EPI), di anticorpo B10 (che ha nel ratto attività simile al Trastuzumab nell'uomo) e dalla somministrazione sequenziale (come nell'uomo) di EPI e B10; B) l'effetto protettivo del Dexrazoxane (Dexra) nel trattamento sequenziale con EPI e B10.

Materiali e Metodi: Cellule pretrattate con Dexra [20µM], vengono esposte a basse dosi di EPI [0,3µM] e successivamente a B10 [1µM] per valutare: 1) senescenza; 2) apoptosi: analisi del potenziale mitocondriale e della positività per il Single-Stranded DNA (ssDNA); 3) analisi della F-actina; 4) permeabilità e danno di membrana con annessinaV-FITC/ioduro di propidio [AV/PI].

Risultati: Basse dosi di EPI e B10 somministrati singolarmente non generano apoptosi (ssDNA e potenziale mitocondriale simili al controllo [CT]), ma inducono: 1) senescenza marcata con EPI (32%) e lieve con B10 (5,8%) rispetto al CT 3,5%; 2) aumento area cellulare del 336% con EPI e del 247% con B10 (vs CT 100%) valutata con F-actina; 3) aumento della fluorescenza della F-actina misurata 45 Unità Arbitrarie [UA] con EPI, 34 UA con B10 vs 13 UA nel CT. Somministrazioni sequenziali di EPI e di B10 portano ad un danno precoce più grave con presenza di apoptosi: scomparsa di F-actina citoplasmatica (28% vs CT 100%); positività per ssDNA (74% vs CT 3%); alterato potenziale mitocondriale. Il pretrattamento con Dexra previene il danno del trattamento sequenziale con EPI e B10: riduce la positività per ssDNA (35% vs 74%) e la caduta del potenziale mitocondriale, previene la condensazione apoptotica [area maggiore rispetto ai controlli (231% vs 100%)].

Conclusioni: Basse dosi dell'anticorpo anti-erbB2 producono modesti danni ai cardiomiociti, ma sono notevolmente cardiotossiche se le cellule sono state esposte a basse dosi di EPI. Il pretrattamento con Dexra riduce il danno da EPI e previene il danno da anticorpo anti-erbB2. Questi dati sono il presupposto per pianificare studi clinici controllati.

EFFECT OF A CARBON MONOXIDE-RELEASING MOLECULE (CORM-3) ON SPONTANEOUSLY HYPERTENSIVE RAT AORTAS

P. Failli, S. Franchi-Micheli, M. Cantore, A. Vannacci, M.C. Vinci, E. Masini

Department of Preclinical and Clinical Pharmacology, Florence.

Carbon monoxide (CO) is generated in living organisms during degradation of heme by the enzyme heme oxygenase (HO), which exists in constitutive (HO-2 and HO-3) and inducible (HO-1) isoforms. Carbon monoxide gas is known to dilate blood vessels in a manner similar to nitric oxide and has been recently shown to possess anti-inflammatory and anti-apoptotic properties. We report that the effect of a carbon monoxide-releasing molecule (CORM-3) on aortas of normotensive Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats.
CORM-3 liberates dose- and time-dependently CO to elicit direct biological activities.

The isometric contraction of angiotensin II (AT-II) and endothelin-1 (ET-1) on endothelium-denuded aortic strips isolated from 15-week old SHR and age-matched normotensive WKY rats was recorded in control condition and in the presence of CORM-3 and of the NO-donor, S-nitroso-N-acetylpenicillamine (SNAP). Both agonists induced a concentration-dependent contraction, being the AT-II action similar in both strains, whereas ET-1 was more effective on aortic strips isolated from SHR rats. Both CORM-3 and SNAP relaxed in a concentration-dependent way the aortic preparations isolated from WKY and SHR rats. However, whereas the median inhibitory concentration (IC50) of SNAP was significantly lower in WKY than in SHR preparations, CORM-3 was similarly effective in both strains in reducing the effect of AT-II and ET-1. Inactivated CORM-3 (iCORM-3), the scaffold compound without the CO moiety, was totally uneffective. Pretreatments with charibdotoxin (CHTX), a blocker of K+ channels and with [1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one] (ODQ), a blocker of soluble guanylate cyclase (sGC), decrease CORM-3-induced relaxation in both rat strain.

In conclusion, Carbon Monoxide-Releasing Molecules could be new potential anti-hypertensive agents.

L'EFFETTO CARDIOTROPICO DELL'ANIONE NITROSSILE (HNO) È MEDIATO DALL'INTERAZIONE CON TIOLI REATTIVI COLLOCATI IN STRUTTURE DELL'ACCOPPIAMENTO ELETTROMECCANICO DEL MIOCITA

C. Tocchetti[1], N. Kaludercic[2,3], C. Vecoli[2,4], J.P. Froehlich[2], S. Betocchi[1], M. Chiariello[1], D.A. Kass[2], N. Paolocci[2,5]

[1]Università degli Studi di Napoli Federico II, Napoli, Italy;
[2]Johns Hopkins University, Baltimore-MD, USA;
[3]Università di Padova, Padova, Italy;
[4]Scuola Superiore Sant'Anna, Pisa, Italy;
[5]Università di Perugia, Perugia, Italy.

Introduzione: Molecole ossidanti come H2O2 sono anche delle molecole segnale. Donatori di anione nitrossile (HNO), specie ridotta dell'ossido nitrico (NO), aumentano la contrattilità cardiaca, accelerando il Ca2+ cycling cardiaco via aumentato rilascio di Ca2+ dai ryanodine receptors (RyR2) e accelerato Ca2+ re-uptake attraverso SERCA2a. L'azione di HNO sui RyR2 da parte di HNO è bloccata dall'agente riducente ditiotreitolo (DTT). Da ciò, si è ipotizzato che l'effetto cardiotropico di HNO derivi dalla sua interazione con tioli (-SH) altamente reattivi, strategicamente collocati in strutture dell'accoppiamento elettro-meccanico cardiaco.

Metodi: Cardiomiociti isolati da topi C57BL6 (età 2-6 mesi), sono stati risospesi in soluzione Tyrode (1mM Ca2+) e stimolati elettricamente (0.5 Hz, 22-25°C). L'accorciamento dei sarcomeri è stato valutato mediante real time imaging ed i transienti del calcio mediante la fluorescenza ad Indo-1.

Risultati: Inizialmente abbiamo testato gli effetti prodotti da concentrazioni crescenti dell'agente alchilante N-ethylmalemide (NEM). NEM aumentava la contrattilità dei miociti in maniera dose-dipendente: 13±10% (1µM, p=NS), 144±32%, 221±56% e 311±131% a 2.5, 5 e 50µM ($p<.05$ vs base), con un aumento parallelo dei transienti del Ca2+ (20±13%, 63±19%, 74±42% a 2.5, 5 e 50µM, sempre $p<.05$ vs base). Quindi agenti alchilanti hanno di per sè la capacità di alterare la contrattilità in cardiomiociti isolati. Tuttavia, diversamente da quella di HNO, l'azione di NEM (2.5µM) non veniva eliminata dal DTT (147±22%). In aggiunta, quando HNO, donato dal sale di Angeli (AS, 0.5mM), veniva infuso durante risposta stabile alla NEM, non si osservava alcun ulteriore incremento inotropo, suggerendo che i residui cisteinici bersaglio di NEM sono gli stessi di HNO. Risultati simili si osservavano quando i cardiomiociti venivano perfusi con la malemide fluorescente CPM (20µM), che blocca selettivamente gruppi sulfidrilici iper-reattivi, i.e. tiolati (-S.).

Conclusioni: HNO reagisce con residui cisteinici (verosimilmente con tioli iper-reattivi -S.) per iniziare e sostenere il suo effetto inotropo-lusitropo positivi in cardiomiociti isolati. Diversamente da alchilanti come NEM, le modificazioni indotte da AS/HNO sui siti bersaglio sono redox-sensibili e reversibili. Sebbene la produzione in vivo di HNO resti da validare, noi postuliamo che questo congenere di NO rappresenti un importante segnale endogeno ed un modulatore della funzione cardiovascolare.

HUMAN FAILING HEART LEFT VENTRICLE IS MORE PROTECTED THAN RIGHT VENTRICLE VERSUS OXIDATIVE STRESS DAMAGE

E. Borchi[1], V. Bargelli[1], C. Giordano[2], G. D'Amati[2], P.A. Nassi[1], E. Cerbai[1], C. Nediani[1]

[1]Università degli Studi di Firenze, Firenze;
[2]Università La Sapienza, Roma.

Increasing evidences suggest that oxidative stress plays a critical role in the development of human heart failure. During pathophysiological conditions, the balance between free radicals and antioxidants may shift towards a relative increase of free radicals resulting in oxidative stress. Few and conflicting data are available on antioxidant defences in human failing hearts and they are limited to left (LV) ventricle. The aim of this study was to investigate the source of free radicals and antioxidant enzyme activities in the right (RV) and LV ventricles of human failing hearts and their mutual relationship. We found a significant increase in NADPH oxidase activity in both ventricles of failing hearts. Protein expression and catalytic activity of catalase (CAT), glutathione peroxidase (GPx), manganese superoxide dismutase (Mn-SOD) were also evaluated. Despite unchanged protein expression of all enzymes, significant enhances in GPx and CAT activity were observed. A significant decrease in Mn-SOD activity was detected. Interestingly, a significant correlation was found between the values of GPx and catalase activity in LV and RV of the same heart. In addition, an increase in NADPH oxidase-dependent superoxide production positively correlated with the activation of both antioxidant enzymes, showing a more protection of the LV with respect RV. Consistent with this finding MDA levels, measured as an indirect index of oxidative stress, were significantly higher in the RV than LV. Our findings suggest that a similar adaptive response occurs in human failing LV and RV supporting the hypothesis of an antioxidant enzymes upregulation probably due to post-transductional modifications induced by an increased NADPH-oxidase superoxide generation. Moreover, human failing LV appeared more protected than RV versus oxidative stress damage.

Keywords: Heart failure; oxidative stress; glutathione peroxidase; catalase; SOD; NADPH oxidase

EXPRESSION OF GATA-4 IN BONE MARROW MESENCHIMAL STEM CELLS OF THE RAT AFTER COCOLTURE WITH ADULT CARDIOMYOCYTES

R. Rastaldo, A. Folino, S. Cappello, G. Losano

Università di Torino, Dipartimento di Neuroscienze, Sezione di Fisiologia, Torino.

Several reports state that bone marrow mesenchimal stem cells (MSC) can express myocardial phenotype if they are cocultured with cardiomyocytes. It has also been reported that the differentiation requires a contact between MSC and cardiomyocytes. These data are consistent with the results of an investigation of our group that after 3-7 days of coculture with adult rat cardiomyocytes, adult bone marrow MSC show the appearance of gap junctions and dihydropiridine calcium channels. While the expression of GATA-4 has been detected in a number of MSC cultured in the absence of cardiomyocytes, the present study aimed to see whether the cells expressing GATA-4 increase in number after coculture.

Method: MSC were obtained from the femur of adult male Green Fluorescent Protein positive (GFP+) rats and cultured in a MEM 10% FBS (Group I). At the third passage, GATA-4 expression was evidenced by immunostaining and the cells were observed with immunofluorescence microscopy. Another group of GFP+ MSC was cocultured with adult GFP- cardiomyocytes for 72 hours (Group II). Also the GFP+ MSC of this group were studied for the expression of GATA-4.

Results: In the absence of cardiomyocytes (Group I), a limited number of GFP+ MSC showed GATA-4 expression, while after coculture (Group II) the number of GFP+ cells expressing GATA-4 was increased. Surprisingly GATA-4 was present in a large number of apparently GFP- cells which were not identified with cardiomyocytes. The analysis of a culture of cardiomyocytes showed that similar non-cardiomyocyte GFP-/GATA-4+ cells were present 72 hours after isolation. Coculture induced a 3.5-fold increase of these cells. Moreover several GFP+/GATA-4+ cells had no contact with cardiomyocytes.

Conclusions: The differentiation of bone marrow MSC towards myocardial phenotype occurs with an increased expression of GATA-4. Since GFP+/GATA-4+ cells had no contact with cardiomyocytes, one may argue that the differentiation is not strictly dependent on contact between the two types of cells. It is also interesting that coculture induces an increase in number of non-cardiomyocyte GFP-/GATA-4+ cells. It is uncertain whether these cells belong to the side population of the heart or may be identified with fibroblast or cardiac stem cells capable to differentiate into cardiomyocytes.

NEOVASCULOGENESIS FROM RESIDENT ADIPOSE TISSUE PRECURSORS DURING ADIPOSE TISSUE DEVELOPMENT - POTENTIAL FOR CARDIOVASCULAR TISSUE ENGINEERING

R. Madonna, R. De Caterina

Cardiology Division, Center of Excellence on Aging, G. d'Annunzio University, Chieti.

Background and Objective: Adipose tissue development is associated with neovascularization, which might be exploited therapeutically. We investigated neovasculogenesis - and adipogenesis - specific gene expression profiles in an in vitro model of developing adipose tissue, to understand the potential of adipose tissue-derived stromal cells (ADSCs) to generate new vessels.

Methods and Results: Murine and human visceral adipose tissue were processed with collagenase I to obtain ADSCs from the stromal-vascular fraction, and immediately used for analyses, without further amplification in vitro or cell selection. By flow cytometry, we found that a considerable number of human or murine ADSCs co-express stem/progenitor markers CD34 and CD133, while being negative for the leuokocyte marker CD45. The expression of mesenchymal stem cell markers CD105 and CD44 was 5 ± 0.2% and 60 ± 4%, respectively. Compared with peripheral blood (PB), the percentage of CD45-/CD34+/CD133+ cells within the ADSCs was markedly higher than that in PB (6 ± 3% and 0.01 ± 0.01%, respectively). In methylcellulose medium, multi-locular cells with refringent cytoplasmic droplets, positive for oil red-O staining and expressing the adipogenesis marker perilipin, appeared after 6 days. After 10 days, clusters of ADSCs spontaneously formed branched tube-like structures, which were strongly positive for CD34, but little or totally negative for vWF, while loosing their ability to undergo adipocyte differentiation. In Matrigel, in the presence of endothelial growth factors, ADSCs formed a network of branched tube-like structures, quite similar to those formed by HUVEC.

Conclusions: These data demonstrate that adipogenesis and neovasculogenesis are closely related in the developing adipose tissue, based on the recruitment of local progenitors. Adipose tissue is therefore a viable autonomous source of cells for post-natal neovascularization.

HUMAN FETAL CARDIAC STEM CELLS FOR HEART REPAIR

G. Forte[1], R. Fiaccavento[1], L. Sartiani[2], S. Pagliari[1], E. Cerbai[2], A. Mugelli[2], A.C. Vilela-Silva[3], F. Carotenuto[1], M. Minieri[1], P. Di Nardo[1]

[1]Università di Tor Vergata, Roma, Italy;
[2]Universita' degli Studi di Firenze, Firenze, Italy;
[3]CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Rio de Janeiro, Brazil.

Aim: Aim of the present study was to identify human fetal c-kit+ cardiac stem cells (hfCSC) within the fetal heart and investigating their features in vitro. Finally, the feasibility of hfCSC-derived sheets using temperature-responsive technology was explored.

Materials and Methods: 9 week d.p.c. fetal hearts were obtained from abortive fetuses. C-kit, Nkx-2.5, GATA-4 and myosin antibodies were used to identify resident hfCSC within the myocardium. After digestion, the single cell suspension was subjected to immunomagnetic separation to purify c-kit+ cells. RT-PCR, immunofluorescence and western blot analysis were used to characterize the cells.

Results: We identified c-kit+ stem cells within 9 week d.p.c. fetal hearts and cultured them for more than 10 passages in standard conditions. In vivo, c-kit+ cells were organized in niches, mostly located in the presumptive areas of the apex and atria. Few c-kit+ cells were also detected among the mature cells of the myocardium, mostly in proximity of the primitive vasculature. To culture hfCSC, enriched c-kit+ cells were seeded and their phenotype monitored. FACS and immunofluorescence analysis clarified that more than 90% of the cells expressed c-kit and Oct3/4 in culture, while few cells spontaneously proceeded to cardiac differentiation within few passages, (GATA-4, Nkx-2.5). C-kit+ cells grew in culture while staining positive for proliferative markers. The cells also expressed a non-organized form of connexin 43, while were negative for markers of cardiac differentiated cells. To assess c-kit+ cells ability to repair cardiac tissue, a model of rat infarction by LAD ligation has been setup and the cells grown on temperature-responsive dishes. Such technique allows the generation of stem cell-derived cell sheets without scaffolds. The cells proliferated on the dishes and c-kit+ cell sheets prepared.

Conclusions: In conclusion, we confirmed the presence of c-kit+ cells within fetal human heart. We purified those cells and cultured them. The cells showed the spontaneous ability to differente towards the cardiac phenotype. Thus, using temperature-responsive technology, it was possible to prepare mono- and multilayered human stem cell sheets. In vivo experiments are required to assess the feasibility of hfCSC sheets transplants on animal hearts.

AMELIORATION OF CARDIAC ELECTRICAL PERFORMANCE FOLLOWING STEM CELL BASED REGENERATIVE THERAPIES, IN INFARCTED RAT HEART

M. Savi[1], C. Frati[1], L. Bocchi[1], G. Graiani[1], R. Berni[1], C. Lagrasta[1], F. Quaini[1], M. Vassalle[2], E. Musso[1]

[1]Interdept. Center for the Study of Biology and Clinical Application of Cardiac Stem Cells (CISTAC), University of Parma, Parma;
[2]Department of Physiology and Pharmacology, State University of New York, Downstate Medical Center, Brooklyn, NY, USA

Aim: Several cellular therapies used to promote myocardial regeneration have been shown to produce beneficial effects on cardiac contractile performance. However, our knowledge on the electrophysiological consequences of cell transplantation is still limited. We addressed this issue in a rat model of chronic myocardial infarction (MI). Regenerative treatment consisted of intra-myocardial injection of 500 x 103 syngeneic cardiac stem cells (CSCs), either alone or together with insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF).

Methods: Twenty-three male Wistar rats with 4-week old MI were studied. Eleven animals were subjected to injection of CSCs alone (MI-cells group) while in the remaining 12 rats IGF-1+HGF were added to the CSCs suspension (MI-cells+GF). To detect regenerative processes, CSCs were EGFP+ and Quantum Dots labeled. Ventricular arrhythmias (VAs) occurring during stress-induced autonomic stimulation (social stress: resident-intruder test) were telemetrically recorded in conscious animals, prior and two weeks after treatment. On the ECG tracings, the mean heart rate and heart rate based indices of the autonomic input to the heart (SDRR: standard deviation of the mean R-R interval: rMSSD: square root of the mean squared differences of successive R-R intervals) were also determined. Before sacrifice, invasive hemodynamic measurements were performed. The heart was then perfusion-fixed for morphometric and immunohistochemical studies.

Results: Myocardial infarction worsened cardiac mechanical performance and increased the proneness to stress-induced VAs. Hemodynamic parameters were ameliorated in both MI-cells and MI-cells+GF groups. Conversely, only the treatment with CSCs in combination with growth factors significantly reduced VAs (by four-fold in 100% of MI-cells+GF rats; $p<0.02$), whereas the injection of CSCs alone was ineffective. No differences among groups were found in heart rate, SDRR and rMSSD values. Clusters of cycling small myocytes expressing Cx43 and neovascularisation in the infarcted area were detected in all rats submitted to regenerative approaches.

Conclusions: Both stem cell based therapies resulted in newly formed mechanical competent cardiac tissue. Conversely, a marked recovery of the electrophysiological properties occurred only in MI-cells+GF group suggesting that cytokines increased the engraftment and differentiation of CSCs leading to an improvement of their natural role in heart repair, likely by changing an unfavorable microenvironment.

ISOLATION AND CHARACTERISATION OF MESENCHYMAL STEM CELLS FROM DENTAL PULP AND THEIR POSSIBLE UTILISATION IN EXPERIMENTAL MODELS OF MYOCARDIAL INFARCTION

A. Sprio, S. Raimondo, P. Salamone, F. Di Scipio, A. Barberis, B. Mognetti, L. Marinos, S. Geuna, P. Pagliaro, G. Berta

Department of Clinical and Biological Sciences, University of Turin, Torino, Italy.

Mesenchymal stem cells (MSCs) are considerably plastic, since they can differentiate into different cellular elements. They can easily be obtained by mammalian tissues, are autologous (so that do not cause rejection in homograft transplanted host) and do not raise ethical problems. MSCs are usually obtained from bone marrow, peripheral blood, cord blood, muscle, brain, skin or gut. Our research group recently pointed out an isolation and characterisation technique by which we obtain MSCs from dental pulp (MSCDPs); in our opinion it represents a good source of MSCs which are probably not differentiated because they are isolated from surrounding tissues very early (formation of the dental papillae).

Specifically, we isolated a cell line turned up to be very interesting because it is continuous and stable in normal culture conditions. Moreover, after adequate differentiating stimuli, it showed up to differentiate, in vitro, into many different cell types such as adipocytes, chondrocytes, osteocytes, nervous system cells and myocardiocytes too. This consideration moved us to evaluate MSCDPs behaviour in a model of infarcted heart. Specifically, we studied the homing of MSCDP implanted in normal hearts and in the border-zone of infarcted myocardium over the first 6 hours after transplantation. Interestingly, in normal hearts, MSCDP migrate very early through the interstitial milieu, while, in infarcted hearts MSCDP remain in the site of injection forming clusters of round-shaped cells in the border-zone of the infarcted area.

This preliminary observation could be represent a rational bases for a new potential source of cardiomiocytes to use in a hypothetical therapeutic approach.

HBR PRE-TREATED TERM PLACENTA HUMAN MESENCHYMAL STEM CELLS IMPROVE REGIONAL MYOCARDIAL FUNCTION IN A SWINE MODEL OF TRANSMURAL MYOCARDIAL INFARCTION

A. Simioniuc[1], C. Cavallini[2], M. Campan[1], G.D. Aquaro[3], M. Marinelli[1], C. Simi[1], F. Bernini[1], V. Lionetti[1], C. Ventura[2], F.A. Recchia[1]

[1]Sector of Medicine, Scuola Superiore Sant'Anna, Pisa;
[2]Laboratory of Molecular Biology and Stem Cell Engineering, Institute of Cardiology, University of Bologna, Bologna;
[3]Institute of Clinical Physiology, CNR, Pisa

Aims: To investigate whether hyaluronan mixed esters of butyric and retinoic acid (HBR) pre-treated term placenta human mesenchymal stem cells (FMhMSCs) implanted in the post-infarct myocardium improve regionally myocardial function.

Materials and Methods: A chronic transmural myocardial infarction (MI) was induced by direct ligation of the left anterior descendent coronary artery (LAD) under the third diagonal branch, during open chest surgery. 13 male farm pigs survived after MI and were randomly assigned to receive sterile saline solution (n=5, PBS, group A), FMhMSCs (n=4, group B) or HBR-FMhMSCs (n=4, group C). FMhMSCs, pre-treated with and without HBR, were transplanted into the infarcted area (IA) and border zone (BZ) 90 min after MI. Four weeks after transplantation, all the animals were evaluated by Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) in order to quantify the regional changes of myocardial contractility, perfusion and metabolism at the level of the IA and BZ.

Results: Area of MI, assessed by delayed-enhancement MRI, was reduced by 40% in group C, but not in group A and B. Myocardial perfusion and glucose uptake were significantly increased at the level of the BZ compared to IA in groups B and C, but not in group A. Although, end-systolic wall thickness was significantly increased in the BZ for the group C, compared to both groups A and B (group C: 7.02+/-1,6mm, group A: 5.44+/-0.2mm and group B: 4.84+/-0.4mm). End-systolic wall thickening, a well-known index of regional contractility, was also significantly improved in the BZ for the group C compared to the other 2 groups (group C: 36.79+/-2.5%, group A: 9.53+/-1.66%, group B: 9.28+/-8.81%).

Conclusions: Transplantation of HBR-FMhMSCs 90 min after coronary ligation reduces area of porcine MI and enhances myocardial contractility and adaptive remodelling in BZ more effectively than FMhMSCs or saline. Our results in large animals strongly support the potential clinical relevance of a cell therapy of MI based on HBR pre-treated mesenchymal stem cells.

CHARACTERIZATION OF Ca2+ SIGNALS IN ENDOTHELIAL PROGENITOR CELLS FROM HUMAN PERIPHERAL BLOOD AND UMBILICAL CORD BLOOD

Y. Sanchez-Hernandez[1], J.E. Avelino-Cruz[1], E. Bonetti[2], V. Rosti[2], F. Moccia[1], F. Tanzi[1]

[1]Università degli Studi di Pavia, Pavia;
[2]Medicina Interna ed Oncologia Medica (IRCCS), Policlinico San Matteo, Pavia.

Objective: Determine the Ca2+ sources responsible of [Ca2+]i variations in endothelial progenitor cells (EPCs) from peripheral blood (PB) and umbilical cord blood (UCB) of healthy subjects.

Material and methods: EPCs seeded on fibronectin-coated coverslips and, at the confluence stage, loaded with the Ca2+-sensitive dye Fura 2/AM and visualized under an epifluorescence microscope.

Results: We explored in the PB- and UCB-EPCs the response to several vasoactive molecules (ATP, acetylcholine, epinephrine and bradykinin). ATP caused a transient Ca2+ peak followed by a long decay phase due to Ca2+ influx, since in absence of extracellular Ca2+ the signal recovered immediately towards the baseline. To elucidate whether this Ca2+ influx occurred through SOCs, we applied a specific blocker, BTP-2, at the end of the decay phase. We found that in both PB-EPCs and UCB-EPCs the ATP response is shorter after BTP-2 application. Emptying the intracellular Ca2+ stores in absence of extracellular Ca2+ with CPA and then adding it back, we observed an initial transient in [Ca2+]i due to the CPA-induced blockade of Ca2+ ATPase, followed by a second intracellular Ca2+ transient upon Ca2+ addition to the bath. In according to ATP results, we found that BTP-2 also caused a decrease in [Ca2+]i almost to the basal level. In addition, preliminary results applying a high extracellular potassium concentration to induce a membrane depolarization did not provoke a detectable Ca2+ response. Subsequently, we applied a number of purinergic agonists and antagonists in order to elucidate the receptor isoforms involved in the Ca2+ response to ATP. PB-EPCs were activated by a preferential P2X agonist, α,β,-MeATP, while ATP-induced Ca2+ signal was reduced in the presence of 2-MeSAMP (a blocker of P2Y12,13 receptors), but not upon block of P2Y1 receptors with MRS 2179. Finally, we found that PB-EPCs did not response to acetylcholine or epinephrine, but shown a slight response to bradykinin.

Conclusions: As in mature endothelial cells, UCB- and PB-EPC shown a biphasic response to extracellular agonists characterized by an initial rapid increase in [Ca2+]i due to Ca2+ released from intracellular stores and a long decay phase due to Ca2+ entry though SOCs.

EFFECT OF HDL QUALITY ON CELLULAR CHOLESTEROL EFFLUX PATHWAYS

E. Favari[1], L. Calabresi[2], M.P. Adorni[1], F. Zimetti[1], G. Franceschini[2], W. Jessup[3], F Bernini[1]

[1]Dipartimento di Scienze Farmacologiche, Biologiche e Chimiche Applicate, Università degli Studi di Parma, Parma, Italy;
[2]Centro E. Grossi Paoletti Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Milano, Italy;
[3]Centre for Vascular Research, University of New South Wales, Australia.

HDL promote cholesterol efflux, their composition can modulate this process and a better understanding of this pathway may identify new targets to enhance the anti-atherogenic potential of these lipoproteins.

Objective: We compared different reconstituted HDL (rHDL) by size, apo-AI and phospholipids content to characterize their ability to promote cholesterol efflux through different pathways. Results showed that, in addition to lipid free apo-AI, a small rHDL particle (7.8nm) made by two apo-AI and with a phospholipids/protein (ph/pr) ratio of 40.6:1 (mol:mol) was a specific acceptor for the ABCA1 efflux. A 9.6nm rHDL, with the same apo-AI composition, but with a ph/pr ratio of 101.3:1 (mol:mol), lacked the ability to promote the ABCA1 efflux. This specificity was confirmed by probucol, since it was able to interfere with the efflux process mediated by the 7.8nm rHDL and had no effect on the efflux driven by the 9.6nm rHDL. The apo-AI and the 7.8nm rHDL did not promote the SR-BI efflux while the 9.6nm particle was very active as well as all the rHDL with size from 10.8nm to 17.0nm and ph/pr ratio from 80.7:1 to 178.0:1 (mol:mol). BLT-1 abolished the efflux to only the rHDL particles above 9.6nm. Interestingly all the particles, including the 7.8nm, were able to promote the ABCG1-mediated cholesterol efflux pathway.

Conclusions: We conclude that the higher cut-off of size and ph/pr ratio for driving ABCA1 efflux is represented by the 7.8nm while the 9.6nm particle represent the lower cut-off for SR-BI interaction. In addition the 7.8nm represented the lower cut-off for the ABCG1-mediated efflux, demonstrating that the same particle may be both an ABCA1 and ABCG1 acceptor.

A NEW NITRIC OXIDE RELEASING ANTI-DIABETIC HYBRID DRUG: FROM THE DESIGN TO THE PHARMACOLOGICAL CHARACTERIZATION

A. Martelli[1], A. Balsamo[2], M.C. Breschi[1], M. Digiacomo[2], P. Marchetti[3], S. Rapposelli[2], L. Testai[1], S. Torri[3], V. Calderone[1]

[1]Dip. di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Pisa;
[2]Dip. di Scienze Farmaceutiche, Pisa;
[3]Dip. di Endocrinologia e Metabolismo, Pisa.

Objective: The aim of this work is represented by the design, the synthesis and the pharmacological characterization of a new hybrid molecule (NO-Gli) possessing the hypoglycaemic action due to the native molecule, a hydroxylated active metabolite of glibenclamide (4-trans-hydroxyglibenclamide), and an additional nitric oxide (NO) releasing property conferred by the conjunction with an NO-donor moiety. This new molecule targets a multi-target pharmacotherapy of diabetes, in fact, diabetes is a multifactorial disease associated with a number of microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (myocardial ischaemia, cerebrovascular damage and peripheral vascular disease) complications. In fact, during the development of diabetes several biochemical (oxidative stress, modified low-density lipoprotein cholesterol, impaired NO production and chronic inflammation) and mechanical (low shear stress and hypertension) factors converge against the endothelium resulting in endothelial dysfunction and reduced nitric oxide biosynthesis, providing the pathophysiological basis for an increased cardiovascular risk.
On the basis of these remarks, a NO-releasing anti-diabetic hybrid drug has been projected, in order to confer the useful cardiovascular properties of exogenous NO to the hypoglycaemic activity of the insulin secretagogue agent.

Methods: The NO-Gli properties have been evaluated by several pharmacological tests both in vitro and in vivo: the release of NO by the amperometric method of the NO-detector on hepatic microsomial fractions, the anti-platelet activity by the Born's turbidimetric method on human platelet rich plasma, the vasorelaxing effect on rat aortic rings, the insulin secretagogue activity on human pancreatic insulae and the hypoglycaemic property on diabetic rats.

Results: NO-Gli shows a slow and modulated release of NO when analyzed by NO-detector, this NO release results able to evoke a significant anti-platelet effect and a vasorelaxing effect inhibited by ODQ an inhibitor of guanylyl cyclase, a key-enzyme in NO pathway. As concerns the anti-diabetic feature, NO-Gli shows an insulin secretagogue effect comparable to that of glibenclamide and its in vivo administration to diabetic rats evokes a reduction of glycaemia levels like glibenclamide.

Conclusions: NO-Gli represents a real pharmacodynamic hybrid possessing the features of a slow NO-donor and of a hypoglycaemic drug useful in the chronic pharmacotherapy of diabetes.

INSULIN POTENTIATES CYTOKINE-INDUCED VCAM-1 EXPRESSION IN HUMAN ENDOTHELIAL CELLS

R. Madonna, M. Massaro, R. De Caterina

Cardiology Division, Center of Excellence on Aging, G. d'Annunzio University, Chieti.

Background and Aim: Hyperinsulinemia is an independent risk factor for cardiovascular events and may contribute to cardiovascular disease. Low grade, chronic inflammation has been implicated in the pathogenesis of atherosclerosis. We aimed at determining the impact of pathophysiologically high insulin concentrations on cytokine-induced endothelial activation in human umbilical vein endothelial cells (HUVEC). Methods: HUVEC were incubated with insulin (0-24 h) ± tumor necrosis factor(TNF)-α or lipopolysaccharide (LPS). At pathophysiological/pharmacological concentrations (10^-9-10^-7 mol/L), insulin selectively induced VCAM-1 expression and potentiated the effects of TNF-á and LPS, effects reverted by the proteasome inhibitor lactacystin.

Results: Compared with TNF-α alone, insulin+TNF-α doubled U937 cell adhesion. Insulin markedly increased TNF-α-induced NF-êB activation and induced phosphorylated IêBα accumulation.

Conclusions: Hyperinsulinemia enhances cytokine-induced VCAM-1 expression in endothelial cells, thus potentially contributing to detrimental effects of other inflammatory stimuli on atherogenesis.

THE OMEGA-3 FATTY ACID DOCOSAHEXAENOATE ATTENUATES THE INSULIN-INDUCED PRO-ATHEROGENIC PHENOTYPE IN HUMAN UMBILICAL ENDOTHELIAL CELLS

F. Muscente, R. Madonna, R. De Caterina

Cardiology Division, Center of Excellence on Aging, G. d'Annunzio University, Chieti.

Background: Hyperinsulinemia predicts future cardiovascular events, but may also contribute to atherosclerosis. Free fatty acids (FFAs), which are increased in the metabolic syndrome, inhibit insulin signaling and may induce insulin resistance. We hypothesized that the type of FFA released in plasma from various sources may have differential effects on vascular consequences of high insulin. We therefore examined the effects of pathophysiologically high levels of insulin, in the presence or absence of saturated (palmitate, PA and arachidonic, AA acid) and unsaturated (linoleate, LA and docosahexaenoate, DHA) fatty acids, on the pro-atherogenic vascular cell adhesion molecule (VCAM-1) expression in human endothelial cells.

Methods and Results: Cultured human venous umbilical endothelial cells were incubated with 10^-9 to 10^-7 mol/L insulin (for 12 to 24 h), in the presence or absence of the phosphatidylinositol-3'-kinase inhibitor wortmannin (wt, 10^-7 mol/L). Parallel groups of cells were pretreated with DHA, or LA, or PA or AA (10^-7 to 5x10^-6 mol/L) overnight, then co-incubated with each single FFA (in serum-containing medium) and 10^-9 to 10^-7 mol/L insulin. Insulin markedly and concentration-dependently increased VCAM-1 surface expression (increment % after 10^-8 mol/L insulin: 147 ± 26, $p<0.01$ vs. untreated control, n=3) and mRNA (increment % after 10^-8 mol/L insulin: 190 ± 30, $p<0.01$ vs. untreated control, n=3), as assessed by enzyme immunoassay and Northern analysis. Wt significantly potentiated the effects of insulin (increment % after 10^-8 mol/L insulin + wt: 230 ± 68, $p<0.01$ vs. untreated control, n=3). A > 24 h exposure to DHA blunted both the constitutive (reduction % after 2.5x10^-5 mol/L DHA: 30 ± 6% $p<0.05$ vs. untreated control, n=3) and insulin+wt-induced VCAM-1 surface expression (reduction % after 2.5x10^-5 mol/L DHA: 50 ± 8 %, $p<0.01$ vs. insulin+wt-treated control, n=3). Neither PA, nor LA, PA or AA altered insulin-induced VCAM-1 expression. PA concentration-dependently increased VCAM-1 surface expression (increment % after 2.5x10^-5 mol/L PA: 32 ± 6, $p<0.05$ vs. untreated control, n=3).

Conclusions: DHA selectively attenuates insulin-induced VCAM-1 expression, and therefore may quench insulin-induced pro-inflammatory and pro-atherogenic effects in type 2 diabetes and the metabolic syndrome.

RUOLO DELL'APOE NEL TRASPORTO INVERSO DEL COLESTEROLO (RCT) IN VIVO

S. Costa, M. Pedrelli, F. Potì, G. Stomeo, I. Zanotti, F. Bernini

Dipartimento di Scienze Farmacologiche, Biologiche e Chimiche Applicate, Università degli Studi di Parma, Parma.

Il topo knock out per l'apolipoproteina E (ApoE -/-) è ampiamente accettato come modello sperimentale per lo studio della patologia aterosclerotica, presentando elevati livelli plasmatici di colesterolo totale e trigliceridi, ridotti livelli di colesterolo HDL e uno spontaneo e precoce sviluppo di lesioni ateromasiche.L'ApoE potrebbe svolgere un ruolo importante nel trasporto inverso del colesterolo (RCT), un processo fisiologico, antiaterosclerotico mediante il quale le HDL, trasferiscono il colesterolo dalle cellule dei tessuti extraepatici, quali i macrofagi presenti nella parete lesa del vaso, al fegato, per la successiva escrezione nella bile.

Obiettivo: Nel nostro lavoro abbiamo valutato l'RCT in topi ApoE -/-, in confronto con i topi controllo. Metodi: l'RCT è stato quantificato mediante una metodica che consente di monitorare ogni step di tale processo, dall'efflusso di colesterolo dai macrofagi, arricchiti in colesterolo radiomarcato e iniettati nel peritoneo degli animali, sino alla sua escrezione nelle feci.

Risultati: Rispetto ai topi controllo si evidenziano significative riduzioni nell'RCT nei topi ApoE -/-, i quali mostrano un minor contenuto di colesterolo radiomarcato nel fegato (2,3% vs 3,8%; p=0,0069), nelle feci escrete nell'arco delle 0-24 e 24-48 ore dall'iniezione dei macrofagi (0,20% vs 0,34% e 0,29% vs 0,52% rispettivamente; p=0,0001). La percentuale di radioattività nel plasma dei topi Apo-E -/- risulta più alta rispetto ai controlli, ma non si raggiunge la significatività statistica. Il plasma dei topi ApoE -/- mostra una maggior capacità di rimuovere il colesterolo dai macrofagi promuovendone l'efflusso nel mezzo di coltura. L'efflusso di colesterolo mediato dalla diffusione passiva e dal trasportatore SR-BI era 14.2±1.9% vs. 9.0±2,9%, ($p<0,05$), e 9,9±2,0% vs. 5,5±1,4% ($p<0,01$), rispettivamente. Gli stessi risultati sono stati ottenuti iniettando i macrofagi dei topi controllo in topi ApoE -/- e viceversa, escludendo in questo modo un coinvolgimento dell'apo-E periferica nell'RCT. Tale conclusione è supportata inoltre dall'assenza dell'apolipoproteina apoE nel plasma di topi ApoE -/- iniettati con le cellule dei topi controllo, come osservato nell'analisi di western blot.

Conclusioni: Possiamo concludere che la mancanza di apoE nei topi induce una significativa riduzione nell'RCT dai macrofagi, suggerendo un possibile meccanismo che può spiegare la maggior suscettibilità a sviluppare aterosclerosi di questi animali.

RUOLO DEL COMPLESSO DI ADESIONE INTERCELLULARE CADERINA/BETA-CATENINA NELLA MODULAZIONE DEL RIMODELLAMENTO VASCOLARE INDOTTO DA ANGIOTENSINA II

M. Mura, C. Barisione, S. Garibaldi, G. Ghigliotti, P. Fabbi, P. Altieri, C. Aloi, P. Spallarossa, A. Barsotti, C. Brunelli

Dip. Medicina Interna e Specialità Mediche (DiMI) Università di Genova, Genova

Obiettivi: L'interazione cellula-cellula preserva la sopravvivenza delle cellule muscolari lisce vascolari (VSMCs) attraverso la via delle caderine, proteine transmembrana che promuovono l'adesione omofila calcio-dipendente. Tale legame avvia l'assemblaggio della caderina con b-catenina (b-cat), una proteina adattatoria che può anche partecipare al signalling proliferativo Wnt. Durante l'aterosclerosi, caratterizzata da uno stato infiammatorio di cui Angiotensina (Ang) II è uno dei responsabili, si verifica danno alle suddette interazioni, con proliferazione e de-differenziazione cellulare nelle lesioni di tipo stenotico, o deplezione delle VSMCs nella progressione dell'aneurisma. Abbiamo definito il ruolo del complesso caderina-catenina nei riguardi di vitalità, proliferazione e differenziazione di VSMCs di aorta di ratto (A7R5), e nella modulazione della risposta alla somministrazione di AngII in termini di proliferazione, ipertrofia e produzione di radicali liberi.

Materiali e Metodi: A7R5 confluenti sono state mantenute come monostrato aderente o come aggregati cellulari in sospensione su una matrice di agarosio per inibire il contatto cellula-matrice. Nelle due condizioni di coltura sono stati valutati gli effetti di AngII (100nM) su apoptosi, ciclo cellulare, stress ossidativo e ipertrofia, sull'espressione di IGF-1R, b-cat, IGFBP-3, Caveolina-1 (Cav-1), Angiotensin-type 1 receptor (AT1r). La localizzazione di b-cat, AT1r e Cav-1, elementi necessari alla trasduzione del segnale di AngII, è stata ottenuta in microscopia confocale.

Risultati: L'incubazione in agarosio induce: 1) quiescenza cellulare con blocco della fase di sintesi, ridotta espressione di IGF-1R e IGFBP-3, aumento dei raft lipidici di membrana e dell'espressione di Cav-1 2) ridotta risposta ad AngII in termini di produzione di radicali liberi, ipertrofia, espressione proteica di IGF-1R, Cav-1 e b-cat, mentre l'espressione totale di AT1r non subisce variazioni nelle diverse condizioni di coltura e trattamento. B-cat, Cav-1 e AT1r, che in cellule aderenti sono distribuite sia a livello della membrana che in sede nucleare o perinucleare (Cav-1), in funzione del trattamento con AngII, negli aggregati cellulari in sospensione rimangono localizzate a livello corticale lungo la membrana a prescindere dal trattamento.

Conclusioni: La formazione del complesso di adesione intercellulare caderina-catenina determina quiescenza cellulare e contrasta l'insorgenza di fenomeni legati al rimodellamento vascolare indotti da AngII mediante stabilizzazione a livello plasmalemmale di b-cat, Cav-1 e At1r.

ENDOTHELIAL MICRO-LESIONS INDUCE NITRIC OXIDE PRODUCTION IN RAT AORTA ENDOTHELIUM: PREPONDERANT ROLE OF GAP JUNCTION HEMICHANNELS DURING CA2+ INFLUX

J. Avelino-Cruz, Y. Sanchez-Hernandez, F. Moccia, F. Tanzi

Università degli Studi di Pavia, Pavia.

Objectives: After a vascular trauma, repairing mechanisms are activated and a sustained increase in [Ca2+]i in endothelial cells is one of the earlier responses to injury. Since nitric oxide production (NOP) can be dependent on the [Ca2+]i, the aim of this work was to determine if injury induces the nitric oxide production in endothelial cells and the sources of Ca2+ involved in the response.

Material and Methods: Wistar rats were anesthetized and then the aorta was dissected out, cut in segments of ~4 mm that were then opened and fixed in a Petri dish treated with sylgard. After loading aorta strips with either 16μM Fura-2AM or 10μM DAF-FM DA, endothelial cells were visualized under an epifluorescence microscope and the response to a provoked and controlled injury (2 to 3 rows of cells were scraped) evaluated.

Results: Injury induced a clearly increase in the DAF-fluorescence which is prevented by L-NAME, an inhibitor of eNOS activity, and by the absence of extracellular Ca2+. These results suggest that raise in fluorescence is due to injury induced- NOP and that it depends on extracellular Ca2+. Accordingly, previous emptying of intracellular Ca2+ pools did not alter injury-induced NOP. Surprisingly, blockade of capacitative-Ca2+ entry (BTP-2; 20μM), the main Ca2+ entry pathway in non excitable cells, did no alter the response to injury. Previous work from our lab has shown that sustained Ca2+ entry after injury probably occurs through unopposed gap junctions hemichannels. In the present study, we sought to extend such evidence at molecular level by using gap junction-mimetic peptides, which specifically inhibit connexins 37 and 43. We found that acute application of these blockers decreased the plateau phase of Ca2+ response to injury by about 60%. Similarly, injury induced-NOP decreased by 75%. Additionally, we assessed the effect of several classical gap junction blockers (palmitoleic acid, heptanol, octanol and oleamide) on the production of nitric oxide finding in all cases a strongly inhibited response.

Conclusions: During injury response gap junctions hemichannels are playing a central role in the Ca2+ influx responsible of the sustained increase in the [Ca2+]i and of the following NO production.

ACIDI GRASSI POLINSATURI NELLA REGOLAZIONE DELLA SINTESI DI COLESTEROLO E TRIGLICERIDI: IL RUOLO DI SREBP

M. Di Nunzio[1], D. VanDeursen[2], A. Verhoeven[2], A. Bordoni[1]

[1]Centro Ricerche sulla Nutrizione e Vitaminologia, Dip. Biochimica G. Moruzzi, Università di Bologna, Italy;
[2]Dip. Biochimica, Erasmus MC, Università di Rotterdam., Rotterdam, The Netherlands.

L'effetto protettivo verso le malattie cardiovascolari da parte degli acidi grassi polinsaturi (PUFA) della serie n-6 ed n-3 è in parte mediato dalla loro azione ipotrigliceridemizzante ed ipocolesterolemizzante, che coinvolge una famiglia di fattori trascrizionali denominati Sterol Regulatory Element Binding Protein (SREBP). La forma attiva di SREBP (nSREBP) si lega allo sterol responsive element (SRE) nelle regioni promotrici di geni chiave del metabolismo lipidico, modulandone l'espressione. L'entità di formazione di nSREBP, regolata dalla concentrazione intracellulare di colesterolo, è influenzata anche dai PUFA, in maniera diretta ed indiretta.

In questo studio è stato valutato l'effetto della supplementazione con differenti concentrazioni di PUFA n-3 ed n-6 sull'attività di SREBP in una linea cellulare di epatomi umani (HepG2) transfettati con SRE-luciferase (SRE-luc). In queste cellule è stata inoltre valutata la composizione acilica per via cromatografica e la distribuzione intracellulare di colesterolo libero mediante colorazione con filipina in microscopia a fluorescenza, in assenza/presenza di U18666A, un inibitore della traslocazione intracellulare di colesterolo.
In contrasto all'acido oleico, tutti i PUFA supplementati sono stati incorporati nei lipidi cellulari, e hanno determinato una significativa riduzione dell'attività di SRE-luc. I PUFA più attivi in questo senso si sono dimostrati l'acido linoleico (LA, 18:2 n-6), l'arachidonico (ARA, 20:4 n-6) e l'eicosapentaenoico (EPA, 20:5 n-3). La colorazione del colesterolo libero ha mostrato che esso è distribuito lungo le membrane plasmatiche ed in compartimenti intracellulari, sia nelle cellule controllo che in quelle supplementate. L'aggiunta di U18666A nelle cellule controllo ha determinato un confinamento del colesterolo negli organuli intracellulari, mentre nelle cellule supplementate esso ha continuato ad apparire distribuito anche lungo le membrane plasmatiche.

I PUFA supplementati appaiono quindi in grado di regolare l'attività di SREBP, probabilmente anche grazie a modifiche a livello della mobilizzazione del colesterolo intracellulare. Questi dati confermano l'efficacia dei PUFA nel controllo della sintesi di colesterolo e trigliceridi, chiarendo ulteriormente il loro meccanismo di azione. Ciò appare utile per sviluppare nuove strategie terapeutiche per una migliore gestione del metabolismo lipidico e delle malattie cardiovascolari.

VASOSTATIN-1 IS A NEW PHYSIOLOGICAL CELL PENETRATING PEPTIDE ACTING BY AN HEPARAN SULPHATE-ENDOCYTOSIS-PI3K-ENOS PATHWAY

R. Ramella, O. Boero, R. Levi, G. Alloatti, M.P. Gallo

Department of Animal and Human Biology, University of Turin, Turin, Italy.

Objectives: Vasostatins (VSs) are vasoactive peptides derived from Chromogranin A, a protein contained in secretory granules of chromaffin and other cell types. The negative inotropic effect and the reduction of isoproterenol-dependent inotropism induced by VSs in the heart, suggest that they play an anti-adrenergic function. Previous studies in our laboratory showed that the Chromogranin A - derived peptide Vasostatin-1 (VS-1) induces a PI3K-dependent-NO release by endothelial cells. However, in the absence of a specific VS-1 membrane receptor, the molecular mechanisms and the cellular processes upstream the eNOS activation by this peptide are still unknown. In this report we investigated the hypothesis that VS-1 acts as a cationic cell penetrating peptide, binding to heparane sulfate receptors and activating eNOS phosphorylation (Ser1179) through a PI3K dependent-endocytosis-coupled mechanism. Materials and methods: Bovine Aortic Endothelial (BAE-1) cells (European Collection of Cell Cultures, Salisbury, Wiltshire, UK) were maintained in Dulbecco's Modified Eagle's medium (DMEM, Sigma, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, at 37°C in a humidified atmosphere of 5% CO2 in air. Cells were maintained in 1% FCS 24h before the experiments. Endocytotic vescicles traffiking was quantified by confocal microscopy with the water-soluble styryl pyridinium dye N-(3-triethylaminopropyl)-4-(p-dibutylaminostyryl) pyridinium dibromide (FM 1-43) to label plasmalemma-derived vesicles. VS-1 dependent eNOS phosphorylation was studied by immunofluorescence and western blot experiments. Confocal fluorimetric measurements were performed using an Olympus Fluoview 200 laser scanning confocal system mounted on an inverted IX70 Olympus microscope, equipped with a 60X oil-immersion objective (NA 1.25). Results: In endocytosis experiments, BAE-1 cells stimulated with VS-1 showed a marked increase in the endocytotic processes, which was blocked by both heparinase III and Wortmannin. To understand the molecular mechanism responsible for the eNOS activity, we investigated on the increase of P(Ser1179) eNOS, by immunofluorescence and western blot experiments. Our results showed that VS-1 (100 nM) induces a significant increase in the level of phosphorylation of eNOS (Ser1179), blocked by Wortmannin.

Conclusions: Our results suggest that VS-1 binds to heparane sulphate receptors and stimulates endocytotic vesicles formation associated with the PI3K dependent eNOS phosphorylation.

UROCORTIN II INDUCES NO PRODUCTION IN PORCINE AORTIC ENDOTHELIAL CELLS THROUGH CAMP AND CA2+ RELATED PATHWAYS LEADING TO ENOS ACTIVATION

E. Grossini, C. Molinari, D.A.S.G. Mary, P.P. Caimmi, F. Uberti, G. Vacca

Dipartimento di Fisiologia, Facoltà di Medicina e Chirurgia A. Avogadro, Novara.

Aim: urocortin II was shown in anesthetized pigs to increase coronary blood flow through a nitric oxide synthase (eNOS) related pathway and the involvement of the subtype 2 of CRF receptors (CRFR2). However information regarding the intracellular signalling induced by urocortin II involving CRFR2 and nitric oxide (NO) is scarce. The present study was planned to better characterize the mechanism of action of urocortin II leading to NO production.

Methods: in porcine aortic endothelial cells the effects of urocortin II on NO production and ERK, Akt, p38 and eNOS phosphorylation were examined in the absence or presence of various agents such as the adenylyl cyclase agonist and antagonist (forskolin and 2'5' dideoxyadenosine), the Ca2+ ionophore A23187, the Ca2+-calmodulin-kinase inhibitor KN93, the CRFR2 blocker astressin 2B and of the specific inhibitors of intracellular kinases (UO126, wortmannin, SB203580). In some samples the experiments were performed in the presence of EDTA.

Results: urocortin II caused an increase of NO production of about 36.4% ($p<0.05$), which was amplified by forskolin (155.8%; $p<0.05$) and A23187 (107.6%; $p<0.05$). All effects of urocortin II were prevented by l-NAME, 2'5' dideoxyadenosine, KN93, EDTA and astressin 2B ($p>0.05$). Similarly, pre-treatment of cells with UO126, wortmannin and SB203580 abolished all effects of urocortin II on NO production. Western Blot analysis confirmed the involvement of ERK, Akt and p38 in the eNOS activation.

Conclusions: in porcine aortic endothelial cells urocortin II interaction with CRFR2 causes a cAMP-dependent and Ca2+-related phoshorylation of ERK, Akt and p38 leading to eNOS activation.

DEACETYLASE INHIBITORS REDUCE CARDIAC ARRHYTHMOGENESIS IN MDX MOUSE MODEL OF DUCHENNE MUSCULAR DYSTROPHY (DMD)

R. Berni[1], C. Colussi[2], L. Bocchi[1], M. Savi[1], F. Delucchi[1], F. Quaini[1], D. Stilli[1], E. Musso[1], C. Gaetano[3]

[1]Interdept. Center for the Study of Biology and Clinical Application of Cardiac Stem Cells (CISTAC), University of Parma, Parma;
[2]Laboratory of Vascular Biology and Gene Therapy, Istituto Cardiologico Monzino, Milano;
[3]Laboratory of Vascular Pathology, Istituto Dermopatico dell'Immacolata, Roma.

Aim: To acquire more insight into the mechanisms underlying arrhythmogenesis in DMD cardiomyopathy, we analyzed the effects of histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) on cardiac electrical performance and myocardial protein expression, in MDX mice.

Methods: The following three groups of 5-month old mice were studied: untreated MDX animals (MDX, n=16), MDX mice treated with deacetylase inhibitor (5 mg/kg, for 90 days; SAHA group, n=15), and wild type mice (WT, n=14). Electrocardiograms were telemetrically recorded in conscious freely moving animals, during both baseline conditions and stress-induced autonomic activation (restraint test), in order to evaluate: (i) heart rate, (ii) heart-rate-based indices of cardiac autonomic control (SDRR: standard deviation of the mean R-R interval; rMSSD: square root of the mean squared differences of successive R-R intervals), and (iii) proneness to ventricular arrhythmias (VAs). At sacrifice hearts were either frozen or perfusion-fixed for respectively determining: (i) expression levels of Connexin 43 (Cx43), Connexin 40 (Cx40), Connexin 32 (Cx32), and Nav 1.5 Sodium channel (immunoblotting), and (ii) spatial distribution and intercellular organization of connexin proteins (immunohistochemistry and confocal microscopy).

Results: Heart rate, SDRR and rMSSD had similar values in all animals at rest as well as during restraint. MDX mice exhibited a significantly higher vulnerability to stress-induced VAs as compared with WT animals (range of VAs: 1-36 in MDX vs. 0-2 in WT, $p<0.01$; percentage of animals showing ventricular arrhythmic events: 100% vs. 56%; $p<0.05$). In SAHA treated mice, the incidence of VAs was reduced to WT values. The expression level of Cx43 was similar in all groups, whereas in SAHA mice an up-regulation of Cx32 associated with a down-regulation of Cx40 was observed. In addition, SAHA abolished the reduced Nav 1.5 expression occurring in MDX mice.

Conclusions: The restoring of a normal Nav 1.5 expression and the remodeling of connexin proteins induced by SAHA was associated with a decreased risk of arrhythmias in treated MDX mice suggesting that changes in these molecules contribute to ventricular electrical instability in DMD cardiomyopathy.

ANALISI DEI CICLI ATRIALI DURANTE LA FIBRILLAZIONE ATRIALE PERSISTENTE E CHEMIORIFLESSO DOPO RIPRISTINO DEL RITMO SINUSALE CON CARDIOVERSIONE ELETTRICA ESTERNA

G. Marchetti[1], R. Roncuzzi[1], A. Zaniboni[1], A. Barbieri[2], D. Vivoli[2], S. Urbinati[1]

[1]Unità Opearyiva di Cardiologia, Bologna;
[2]Policlinico di Modena, Modena.

Il periodo refrattario atriale in corso di fibrillazione atriale persistente subisce progressivamente un processo di accorciamento .Lo studio del ciclo atriale e della sua frequenza per valutare in modo non invasivo il fenomeno del rimodellamento elettrico atriale può esser eseguito mediante analisi amplificata con campionamento delle frequenza a 500Hz dall'elettrogramma atriale.
Il sistema nervoso autonomo può essere un importante trigger delle aritmie e della F.A. e influenza il periodo refrattario atriale . Il chemioriflesso studia questo aspetto.

Metodi: 42 pazienti (30 maschi e 12 donne) di età media di 69 anni, con F.A. persistente da 2 a 42 mesi (media di 11 mesi) sono stati sottoposti a cardioversione elettrica esterna.
Il 33% dei pazienti aveva una ipertensione arteriosa, il 36% ipertrofia ventricolare sinistra , il 16% una sottostante valvulopatia, il 14% una cardiopatia ischemica, il 13% una cardiomiopatia dilatativa.
Il diametro atriale era compreso tra 43 mm e 52 mm, e la frazione d'eiezione del ventricolo sinistro del 45% (dal 25 al 55%).
Il pre-trattamento farmacologico prevedeva amiodarone + inibitore del recettore 1 della angiotensina II, 3 settimane prima della procedura, oltre all'anticoagulante orale.
Sono stati analizzati i cicli atriali della F.A., prima della cardioversione elettrica (CVE). In prima giornata dopo cardioversione è stata studiata la chemioreflessisensitività: sulla campionatura di 10 successivi R-R somministrando al paziente 5 litri di ossigeno/min x 5 minuti in posizione clinostatica e calcolando la differenza tra basale e dopo ossigeno degli intervalli R-R.

Risultati: Nelle 4 recidive di F.A. precoce dopo cardioversione elettrica esterna i cicli atriali erano a frequenza di 400 min. versus una media di 340 min. dei pazienti che non presentavano recidive precoci. La analisi del ciclo atriale dimostrava elevata predittività del mantenimento del ritmo sinusale anche al follow up a 1 anno: il 62% dei pazienti rimaneva in ritmo sinusale a 12 mesi (ciclo atriale pre CVE con frequenze tra 320 e 360 min) ; il ciclo atriale dei pz con recidive era di 380 min.
Un ridotto chemioriflesso era presente nelle 4 recidive precoci di F.A., ma la sua predittività del mantenimento del ritmo sinusale a distanza risultava non elevata.

PHARMACOLOGICAL CHARACTERISATION OF THE CARDIOPROTECTIVE ACTIVITY OF A NEW SPIRO-CYCLIC BENZOPYRAN ACTIVATOR OF MITOCHONDRIAL KATP CHANNELS

L. Testai[1], A. Balsamo[2], M.C. Breschi[1], M. Manganaro[2], A. Martelli[1], S. Rapposelli[2], V. Calderone[1]

[1]Dip. Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Pisa;
[2]Dip. Scienze Farmaceutiche, Pisa.

Objectives: Ischemic preconditioning (IPC) is an endogenous phenomenon whereby brief periods of ischemia improve the ability of the heart to tolerate a subsequent prolonged ischemia, reducing the injury in the damaged region.
A pivotal role in IPC is played by ATP-sensitive potassium channels expressed on the inner mitochondrial membrane (mitoKATP), whose activation reduces a calcium overload into mitochondrial matrix, a key step leading to cell death. Therefore the possibility to use pharmacological tools triggering the IPC process represents a rational basis for the development of new interesting antiischemic drugs. Indeed, well-known KATP-openers, such as the benzopyranic derivative cromakalim, showed cardioprotective effects, but they cannot be used in therapy because of wide side-effects, due to unsatisfactory selectivity vs the other type of KATP channel. In a recent paper, we reported some spiro-substituted benzopyrans exhibiting good anti-ischemic properties on Langendorff perfused rat hearts subjected to ischemia/reperfusion cycles and modest hypotensive activities. This work is aimed to a more detailed pharmacological characterisation of the cardioprotective effects of a selected benzopyran derivative, 4'-(N-(4-acetamidobenzyl))-2,2-dimethyl-2,3-dihydro-5'H-spiro[chromene-4,2'-[1,4]oxazinan]-5'-one.

Methods: The anti-ischemic properties of the molecule and of reference compounds have been evaluated in in vivo model of myocardial acute infarct on rats, obtained through reversible ligature of left descending coronary artery and in cultured cardiomyoblasts (H9c2 cells) subjected to anoxia/reperfusion cycles. Furthermore, the involvement of the mitoKATP channel has been investigated through the selective blocker 5-hydroxydecanoate. Finally, the benzopyran derivative and reference compounds have been tested on calcium-loaded cardiac mitochondria, in order to evaluate their influence on calcium movements across the mitochondrial membranes, recorded through a potentiometric assay.

Results: In rats subjected to acute infarct, the selected molecule reduced dose-dependently the ischemic area of left ventricle and increased the cell viability in injured cardiomyoblasts with a mechanism involving the activation of mitoKATP. Finally the benzopyran derivative induced calcium-release from calcium pre-loaded mitochondria.

Conclusions: In this work we demonstrate that this spiro-cyclic benzopyran compound has an interesting cardioprotective profile due to mitoKATP activation and probably linked to a reduced calcium uptake into mitochondria during ischaemia.

IL TRATTAMENTO DEI CARDIOMIOCITI CON CREATINA E RIBOSIO PREVIENE L'ARRESTO DEL CICLO CELLULARE INDOTTO DALL'ISCHEMIA VIA ATTIVAZIONE DI AKT E DELLE CICLINE D1/E

A. Caretti[1], P. Bianciardi[1], F. Lucchina[2], C. Terruzzi[2], A. Veicsteinas[3], M. Samaja[1]

[1]Dipartimento di Medicina, Chirurgia e Odontoiatria, Università di Milano, Ospedale San Paolo, Milano;
[2]Giellepi Chemicals, Milano;
[3]Istituto di Esercizio Fisico, Salute e Sport, Università di Milano, Fondazione Don Gnocchi, Milano.

Obiettivi: La funzione cardiaca, che dipende criticamente dai processi che generano energia e dal continuo rifornimento di sangue, è severamente compromessa nell'ischemia. Per alleviare alcuni sintomi associati all'ischemia, la somministrazione di Creatina (Cr) può favorire il metabolismo energetico muscolare, mentre quella di D-ribosio (Rib) rifornisce la cellula dei precursori necessari per la sintesi di ATP. Ma l'efficienza della somministrazione combinata di entrambe le sostanze non è mai stata provata. Pertanto, in questo studio si è testata l'ipotesi che la somministrazione combinata di Cr and Rib (Cr+Rib) ai cardiomiociti ischemici fornisce vantaggi aggiuntivi rispetto alle singole somministrazioni, e soprattutto che tale effetto non è dovuto semplicemente alla combinazione di Cr+Rib sulla bioenergetica cardiaca, ma influenza specifiche vie di segnalazione cellulare.

Metodi: Abbiamo utilizzato cardiomiociti H9c2 esposti per 24 h ad ischemia simulata (1% O2 con deprivazione del glucosio dal mezzo di cultura) e analizzato le cellule per la loro sopravvivenza e progressione del ciclo cellulare.

Risultati: La somministrazione combinata Cr+Rib migliora la sopravvivenza e allevia l'arresto del ciclo cellulare nella fase S, mentre le somministrazioni separate di Cr e Rib non hanno effetti rilevanti. Inoltre, la somministrazione combinata Cr+Rib correla con l'aumento della fosforilazione di Akt, il recupero del livello di espressione della ciclina D1 e l'ulteriore inibizione dell'inibitore del ciclo p21, mentre non si sono verificati effetti sulla ciclina E e sull'inibitore p27.

Conclusioni: Il trattamento dei cardiomiociti ischemici H9c2 con Cr+Rib conferisce vantaggi aggiuntivi rispetto ai trattamenti con Cr e Rib mediante effetti diretti sulla segnalazione cellulare mediata da Akt, ciclina D1 e p21. Pertanto la combinazione Cr+Rib può essere considerata per la sua potenziale efficacia terapeutica in alcune categorie di pazienti.

PRO-SURVIVAL EFFECTS OF H2S DONORS AGAINST OXIDATIVE STRESS IN H9C2 CELLS

D. Mancardi[1], A. Merlino[1], M.G. Perrellli[1,2], F. Tullio[1], F. Moro[1], P. Pagliaro[1,2], C. Penna[1]

[1]Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Orbassano;
[2]Istituto Nazionale di Ricerche Cardiovascolari, Bologna.

Background: Known as a toxic and poisoning gas H2S has emerging properties in cell-signalling. It has been proven to be endogenously produced at micromolar concentration in mammalian cells by the activity of cystathionine-β-synthase and cystathionine-γlyase. In recent studies H2S has been reported to confer myocardial protection against ischemia/reperfusion injuries in rats through the opening on mitochondrial K-ATP sensitive channels. Therefore, in the present study we investigated the role of mitochondria on the H2S-induced tolerance to oxidative stress in an immortalized myocytes cell line.

Methods: The myoblast cell line H9c2 was obtained from the European Type Culture Collection. The cells were maintained at 37 °C in a 5% CO2 humidified atmosphere, in Dulbecco's modified Eagle's medium nutrient mixture F-12 HAM supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 Units/mL of penicillin and 100 µg/mL streptomycin). Cells were routinely grown to 80% confluence in 175 cm2 flasks prior to passage and seeding for experiments.
The H9c2 cells were seeded in 96-well plates at a cellular density of 0.5x104 and treated over-night with different concentrations of NaSH or modified-donors were tested for the same final concentrations of H2S (0,1-1-10-100 _M). After the overnight treatment cells were either exposed to 150 µM H2O2 (1 hour) or 1 µM Doxorubucin (18 hours) in order to mimic oxidative stress. To assess cell death multiwells were processed with MTT assay (5 mg/ml) and absorbance was measured at 540 nm in a microplate reader. Cells undergoing immune blot analysis were plated on 100mm Petri-dishes.

Results: H2S resulted in a marked reduction of oxidative stress-induced cell death. The optimal response in terms of cell-survival has been obtained for concentrations ranging from 50 through 100 µM either for NaSH and for modified-donors. In fact, while 150 mM H2O2 induced a 65% cell death, pre-treatment with H2S (100 mM) reduced cell death to 25%. Western-blot analysis for mitochondrial enzymes showed a marked increase in anti-apoptotic kinases (phospho-GSK-3β, AkT and Bcl-2).

Conclusions: data show that H2S exerts protective effects against oxidative stress. H2S-protection converges on mitochondria via activation of mitochondrial pro-survival kinases and anti-apoptotic factors.

ROLE OF SUPEROXIDE DISMUTASE AND CATALASE IN ISCHEMIC POSTCONDITIONING

F. Tullio[1], A. Merlino[1], F. Moro[1], M.G. Perrelli[1,2], D. Mancardi[1], C. Penna[1], P. Pagliaro[1,2]

[1]Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Orbassano;
[2]Istituto Nazionale di Ricerche Cardiovascolari, Bologna.

Background: The contribute of reactive oxygen species (ROS) in inducing Preconditioning have been widely confirmed. Recently, the involvement of ROS in Postconditioning (PostC) triggering have been suggested in vivo and in vitro models. However, the nature of ROS involved in this protection is unknown. H2O2 administered during reperfusion induces cardioprotective effects. It has been suggested that PostC is triggered by peroxynitrite (ONOO-) produced by nitric oxide (NO·) and superoxide anion (O2-) reaction. The aim of this preliminary study was to investigate the effect of Catalase (CAT) and Superoxide Dismutase (SOD) in the cardioprotection induced by PostC.

Methods: Isolated rat hearts were perfused with oxygenated Krebs-Henseleit buffer at constant flow (9 ml/min/g) and paced at 280 bpm. After stabilization, all hearts were subjected to 30-min of global ischemia followed by 120-min of reperfusion (I/R). The hearts of the Control group (Ctrl, Group 1; n=10), after stabilization, were subject to I/R only. In Group 2 (n=10) immediately after ischemia the hearts were exposed to a protocol of PostC (i.e. 5 cycles of 10-sec ischemia/reperfusion). Group 3 (n=5) and Group 4 (n=5) hearts underwent I/R and PostC, respectively, in the presence of CAT (100 U/ml); Group 5 (n=5) and Group 6 (n=5) hearts underwent I/R and PostC, respectively, in the presence of SOD (10 U/ml). Myocardial necrosis was evaluated with nitroblue-tetrazolium staining.

Results: Infarct size was 61±4% of risk area in Ctrl hearts. PostC reduced infarct size to 33±5% ($p<0,05$ vs Ctrl). In the presence of CAT or SOD, in hearts subjected to I/R only, infarct size was similar to control hearts (P=NS vs Ctrl for both). While SOD infusion during PostC maneuvers abolished PostC protection (75±2%, NS vs Ctrl; $p<0,05$ vs PostC), CAT infusion did not alter the cardioprotection induced by PostC (38±5%, NS vs PostC; $P< 0,05$ vs Ctrl).

Conclusions: Our preliminary results suggest that H2O2 may not be a trigger of PostC In fact, CAT preserves PostC protection while reducing H2O2. Yet, SOD leads to the formation of H2O2 through the reduction of O2- and abolishes PostC effects. Data support the hypothesis that ONOO- may be responsible for PostC cardioprotection.

SILDENAFIL REDUCES HYPOXIA-INDUCED PULMONARY HYPERTENSION AND ACUTE MYOCARDIAL INFARCT IN THE CHRONICALLY HYPOXIC RAT

G. Milano[1,2,3]

[1]Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland;
[2]University Hospital Geneva, Geneve, Switzerland;
[3]University of Milan, Milan, Italy.

Background: Chronic hypoxia (CH) causes pulmonary artery hypertension (PAH), which leads to right ventricle hypertrophy (RVH). Furthermore, CH has been associated with divergent effects on tolerance to myocardial ischemia (MI). While earlier studies suggested protective effects of CH to subsequent hypoxia/ischemia, we observed that CH actually depresses MI tolerance, whereas short episodes of normoxia during CH, or aeration, can reverse this effect.

Methods and Results: This study compared the efficacy of sildenafil, an inhibitor of phosphodiesterase-5, and aeration during CH in preventing CH-induced impairment in PAH, CHV and MI tolerance. Adult male Sprague-Dawley rats were exposed to CH (2 weeks at 10% O2) with no treatment, sildenafil (1.4 mg/kg/day, i.p.), intermittent aeration episodes (1 h normoxia/day) and sildenafil plus aeration. Hearts were either subjected to 30-min regional ischemia followed by 3 h-reperfusion, or right/left ventricle pressure measurement, or freeze-clamp for biochemical analyses. Both sildenafil and aeration alleviated PAH, which was normalized by the combination of both intervention (but not by either one alone). Sildenafil, but not aeration, prevented RVH. CH increased infarct size with respect to normoxia. Sildenafil suppressed this detrimental effect of CH, but aeration showed an effect greater than sildenafil. Both sildenafil and intermittent aeration reversed the abnormalities in myocardial cGMP, activated caspase-3 and phosphorylated eNOS levels.

Conclusions: Either inhibition of phosphodiesterase-5 by sildenafil during CH, or aeration provided an efficient strategy for the treatment of PAH and cardiac hypertrophy, and improved tolerance to MI. These outcomes appear to depend on improved NO metabolism.

MYOCARDIAL PROTECTION IN THE RAT HEART AND ROLE OF WEAK AND STRONG NO-RELEASE FROM A HYBRID MOLECULE CONTAINING AN ANTIOXIDANT SUBSTRUCtURE

A. Folino[1], S. Cappello[2], R. Rastaldo[2], P. Pagliaro[3], A. Di Stilo[4], G. Losano[2]

[1]Università di Torino, Diartimento di Neuroscienze, Sezione di Fisiologia, Torino;
[2]Istituto Nazionale per le Ricerche Cardiovascolari - Consorzio Interuniversitario, Bologna;
[3]Università di Torino, Dipartimento di Scienze Cliniche e Biologiche, Orbassano;
[4]Università di Torino, Dipartimento di Scienza e Tecnologia del Farmaco, Torino.

In the rat it was found that an effective protection against ischemia-reperfusion (I/R) injuries is achieved if an hybrid molecule containing NO-donor furoxan moiety and antioxidant phenol substructures is given at the concentration of 1 but not 10 mM. The investigation aims to assess the role of NO-donor moiety. For this reasons we used 2 different hybrids, with the same phenol substructure but different (weak and strong) NO release at 1 mM concentration.

Method: Experiments were performed on 55 Langendorff perfused rat hearts. A latex balloon in the left ventricle was connected to a pressure transducer to detect left ventricular pressure. I/R consisted of 30 min of global ischemia and 2 hours of reperfusion.
Seven groups were considered. Group I (n = 8; control) underwent I/R only. Group II (n=8) received the anitoxidant compound only. Group III (n= 6) received strong NO-donor. Group IV (n=6) received the weak NO-donor. Group V (n=8) received a mixture of strong NO-donor and antioxidant. The mixture given to Group VI (n=12) contained the weak NO-donor. Group VII (n=6) was treated with hybrid containing the strong and Group VIII (n=9) the weak NO-donor. All compounds were given at 1 mM concentration during the first 20 min of reperfusion. Infarct size was determined with the nitro-blu tetrazolium technique.

Results: Infarct size is reported as percent of left ventricle (mean±SE). Group I (control): 55±3%; Group II: 47±8%; Group III: 49±14%; Group IV: 46±10%; Group V: 52±10%; Group VI: 46±9%; Group VII: 64±3%; Group VIII: 37±6%. With respect to Group I, only Group VIII, treated with hybrid containing the weak NO-donor moiety, showed a significant ($p<0.01$) reduction of the infarct size. As observed from left ventricular pressure, pulsatility reappeared in almost all experiments of each group.

Conclusions: The study confirms that protection occurs to a better extent if hybrid releases only a limited quantity of NO. It is likely that low concentrations of NO can induce mitochondria to produce a small amount of ROS which activates PKC without damaging myocardial fibers. The results also show that hybrid cannot be replaced by the mixture of NO-donor and antioxidant.

THE PROTECTIVE ACTIVITY OF APELIN AGAINST REPERFUSION INJURIES IN RAT HEARTS

S. Cappello[1], A. Folino[2], R. Rastaldo[1], G. Losano[1]

[1]Istituto Nazionale di Ricerche Cardiovascolari - Consorzio Interuniversitario, Bologna;
[2]Università di Torino, Dipartimento di Neuroscienze, Sez. di Fisiologia, Torino.

Apelin is an endogenous peptide found in the gastrointestinal tract, fat, brain, lung, kidney, liver, skeletal muscle and cardiovascular system . On the cardiovascular system apelin exerts potent vasodilator and positive inotropic activities. Since apelin activates PLC and, consequently, PKC, the possibility that it can induce myocardial protection against the extension of infarct size has been considered.

Method: The experiments were performed on 21 rat hearts perfused with oxygenated Krebs-Henseleit solution. After stabilizazion the hearts underwent 30 min of global ischemia followed by 2 hours of reperfusion. A latex balloon placed in left ventricle and filled with saline was connected via a catheter to an electric pressure transducer to detect left ventricular pressure. The hearts were divided in three groups. In Group I (n=8) only ischemia and reperfusion were performed. In Group II (n=6), apelin was infused during the first 20 min of reperfusion at 500 nM concentration . In Group III (n=7), apelin was infused at the same concentration for 20 min before ischemia. Infarct size was assessed with nitro-blue tetrazolium technique and calculated as percent of the left ventricle. Samples of coronary effluent were taken in the control and during reperfusion to test lactic dehydrogenase (LDH) concentration.

Results: With respect to the control Group I, where it was 55.±3% of the left venricle, infart size was significantly ($p<0.0001$) reduced in Group II (27±3%) , but was not significantly different in Group III (45±4%). Total LDH, taken during reperfusion and expressed in U./g of dry heart weight, was reduced in both Group II (207±60; $p<0.01$) and III. (363±61; $p<0.03$) with respect to the control (810±152). After ischemia, left ventricular developed pressure (LVDP) showed a significant ($p<0.05$) recovery in Group II. In ongoing additional experiments the protection is removed if the NOS-inhibitor L-NNA is infused with apelin.

Conclusions: At the concentration of 500 nM, apelin protects rat heart from reperfusion injuries only if given during reperfusion, while it is poorly effective if given before ischemia. The absence of protection when L-NNA accompanies apelin infusion suggests that nitric oxide rather than PLC is involved in the limitation of the injuries via PKC.

ADVANCED OXIDATION PROTEIN PRODUCTS (AOPP) AS A FACTOR AFFECTING ACUTE CORONARY SYNDROMES

P. Fabbi[1], M. Fedele[1], M. Vercellino[1], S. Garibaldi[1], P. Contini[2], G.P Bezante[1], F. Indiveri[2], A. Barsotti[1]

[1]Cardiologia Di.MI, Università degli Studi, Genova;
[2]Immunologia Di.MI, Università degli Studi, Genova.

Objective: We investigated the course of oxidative stress and inflammatory activity in acute coronary syndromes.

Methods: We divided 50 subjects into 3 groups: 16 subjects with unstable angina (UA), 18 subjects with acute myocardial infarction with ST elevation (STEMI), 16 control subjects (CTR). Oxidative stress markers (OxLDL, AOPP, Thiols), and biomarkers of inflammation (hs-CRP, IL-6, IL-1β) were determined in plasma samples, at the time of the inclusion in the study (T0), at 30 (T1) and 180 days (T2).

Results: Plasma levels of OxLDL levels were significantly higher in CTR group than patients, at admission and follow-up. AOPP were significantly higher in STEMI and UA compared to CTR at each time point, particulary at T0 ($p< 0,001$). At follow-up, a significant decrease of AOPP was observed in STEMI, at T2 in UA a increase of AOPP was found. CTR groups showed elevated levels of Thiols compared to STEMI at each time point, and respect UA only at T0 and T2.
At T0, levels of hs-CRP and IL-6 in STEMI and UA were significantly higher compared to the CTR; T1-T2 follow-up in STEMI and UA showed a significant decrease in hs-CRP and IL-6 levels. STEMI and UA showed elevated levels of IL-1β compared to CTR group at each time point. Moreover, a positive relation between IL-1β vs AOPP ($p= 0,002$) and hs-CRP vs IL-6 ($p= 0,005$) was observed in STEMI at T0.

Conclusions: in this study STEMI and UA patients use of hypolipemic drugs, before the admission in the study indeed, OxLDL levels were lower than CTR group. In STEMI and UA the high levels of AOPP, associated with a large consume of Thiols on acute setting, show a key role of oxidative stress in plaque destabilization. Inflammation markers were significantly higher in STEMI group,in particular, hs-CRP and IL-6 was strictly associated with the acute phase of the disease, while IL-1β remained high till 180 days.
These observations suggest that AOPP could represent the major factor involved in plaque destabilization, and they could be used as a prognostic factor for severe forms of cardiovascular disease.

IL TRAINING FISICO CONTROLLATO MIGLIORA LA RESISTENZA ENDOTELIALE ALLO STRESS OSSIDATIVO IN PAZIENTI CLAUDICANTI

S. De Marchi[1], M. Priori[1], A. Rigoni[1], A. Cevese[2], E. Arosio[1]

[1]U.O. di Riabilitazione Vascolare, Università di Verona;
[2]Scienze Motorie, Università di Verona.

Obiettivi: L'effetto positivo dell'esercizio fisico sul processo aterosclerotico è documentato tramite l'analisi della vasodilatazione endotelio dipendente (EDD). Lo scopo del nostro studio è stato quello di valutare come il training controllato possa prevenire la disfunzione endoteliale acuta indotta da esercizio massimale in pazienti affetti da arteriopatia obliterante periferica al II stadio di Fontaine.

Materiali e Metodi: 12 arteriopatici (69-78 aa) sono stati sottoposti a misura della EDD all'arteria omerale e studio della reattività microcircolatoria tramite laserDoppler (test post ischemico e vasodilatazione endotelio mediata tramite acetilcolina) prima e dopo esercizio su treadmill condotto sino all'induzione del massimo dolore sopportabile. La medesima sequenza è stata ripetuta dopo ciclo di training controllato della durata di 20 giorni, su treadmill, al di sotto della soglia del dolore.

Risultati: Il training ha consentito un miglioramento dell'intervallo di marcia libero (131±12 vs 66,6±21 m; $p<0,05$) e dell'intervallo di marcia assoluto (275±15 vs 125,8±40 m; $p<0,05$). L'EDD è migliorata dopo il ciclo (14,8±0,7 vs 10,3±0,5; $p<0,05$). L'esercizio fisico massimale ha causato una riduzione dell' EDD che si è significativamente attenuata dopo il ciclo di training (delta decremento 21,6±4,2% vs 54,3±3,6%; $p<0,05$) . L'iperemia postischemica al laser-Doppler è incrementata (85,3±3,9% vs 66,2±1,5%; $p<0.05$). La vasodilatazione endotelio dipendente con acetilcolina è aumentata nei tre livelli di stimolazione dopo il ciclo riabilitativo (I livello: 147±38 vs 35±9 % incremento - $p<0.005$; II livello: 182±22 vs 70±15 % incremento - $p<0.005$; III livello: 470±54 vs 120±15 % incremento - $p<0.005$). Non abbiamo registrato alcuna modifica con lo stimolo al nitroprussiato.

Conclusioni: Nel nostro studio si documenta il significativo miglioramento della EDD e, una minor caduta della funzione endoteliale, dopo uno stress di tipo ossidativo e metabolico. In conclusione si dimostra come un ciclo di esercizio fisico controllato determini il miglioramento della vasodilatazione endotelio-dipendente sia omerale che microcircolatoria e incrementi la riserva microcircolatoria all'arto inferiore in pazienti anziani affetti da severa arteriopatia aterosclerotica degli arti inferiori.

L'INIBIZIONE DELLA XANTINA OSSIDASI MIGLIORA LA RESISTENZA ENDOTELIALE ALLO STRESS OSSIDATIVO IN PAZIENTI ARTERIOPATICI

S. De Marchi[1], A. Rigoni[1], M. Prior[1], P. Delva[2], A. Lechi2, E. Arosio[1]

[1]U.O. di Riabilitazione Vascolare, Università di Verona;
[2]Medicina Interna C1, Università di Verona.

Obiettivi: La xantina ossidasi è uno dei sistemi enzimatici responsabili della produzione di radicali dell'ossigeno, in particolare durante il fenomeno dell'ischemia-riperfusione. Lo scopo è stato quello di valutare come l'inibizione di questo enzima possa prevenire la disfunzione endoteliale indotta da esercizio massimale, in pazienti con arteriopatia obliterante periferica (AOAI).

Materiali e Metodi: 14 arteriopatici con uricemia normale, sono stati sottoposti a misura della vasodilatazione endotelio dipendente (EDD) omerale e del flusso all'arteria femorale, prima e dopo esercizio su treadmill condotto sino all'induzione del dolore massimo. La medesima sequenza di valutazioni veniva ripetuta dopo 24 ore. Si somministravano 600 mg di allopurinolo subito dopo i test basali il primo giorno, quindi altri 600 mg il secondo giorno, 1 ora prima dei test strumentali. Si misurava l'intervallo di marcia e veniva dosata l'uricemia è stato inoltre dosato il lattato capillare.

Risultati: Il test al treadmill massimale si è dimostrato in grado di ridurre acutamente la EDD (0,406±0,06 vs 0,442±0,06 cm; $p<0,005$). La somministrazione di allopurinolo ha migliorato la EDD a riposo (12,1±2,3 vs 7,3±1,2 %; $p<0,05$) e ha evidenziato una minor riduzione della EDD dopo stress ossidativo indotto da test massimale (-0,92±0,14 vs -2,5±0,23%; $p<0,05$). Il flusso all'arteria femorale è incrementato dopo esercizio fisico (0,702±0,012 vs 0,675±0,102 l/min; $p<0,05$); è incrementato anche dopo esercizio fisico in corso di trattamento con allopurinolo (0,714±0,011 vs 0,702±0,012 l/min; $p<0,05$). L'intervallo di marcia assoluto è incrementato dopo trattamento (342±101 vs 228±98 m; $p<0,05$). L'uricemia è risultata nelle norma, ridotta dopo trattamento (3,55±1,22 vs 5,34±0,66 mg/dl; $p<0,005$). Il lattato è incrementato dopo test massimale analogamente prima e dopo trattamento con allopurinolo (base: 3,5±0,8 vs 1,3±0,3 mmol/l; $p<0,05$ - allopurinolo: 3,4±1,1 vs 1,5±,5 mmol/l; $p<0,05$).

Conclusioni: Documentiamo come il test al treadmill massimale in AOAI, induce disfunzione endoteliale e che allopurinolo la prevenga bloccando la sintesi di radicali liberi (incrementando il flusso femorale dopo sforzo e l'autonomia di marcia). Lo stress ossidativo aumenta per il meccanismo di ischemia-riperfusione che si realizza nell'arto durante il treadmill. La xantina ossidasi è responsabile dell'aumentato stress ossidativo durante ischemia-riperfusione all'arto affetto in pazienti con AOAI e la sua inibizione migliora l'emodinamica periferica.

RESCUE OF OXIDATIVE STRESS BY ERGOTHIONEINE, A POWERFUL NATURAL ANTIOXIDANT, IN HUMAN ENDOTHELIAL CELLS

R. Arici[1], M.C. Barsotti[1], R. Colognato[2], I. Laurenza[1], F. Franzoni[1], F. Galetta[1], L. Benzi[1], L. Migliore[1], A. Balbarini[1], R. Di Stefano[1]

[1]Università di Pisa, Pisa;
[2]J.R.C. - Unità Ecvam, Ispra.

Purpose: To evaluate the potential effect of a novel natural antioxidant, ergothioneine (EGT), in modulating the oxidative stress induced in vitro on human umbilical vein endothelial cells (HUVEC).

Methods: The total antioxidant activity EGT was assessed by its ability to antagonize the oxidation of ƒÑ-keto-g-methiolbutyric acid by hydroxyl, peroxyl and peroxynitrite radicals. The results are expressed as Total Oxyradical Scavenging Capacity (TOSC) units.
Different concentrations of H2O2 and incubation times were tested on HUVEC isolated from umbilical veins. 1 hour pre-treatment with 500ƒYM or 1mM EGT, followed by 1 hour of incubation with 100 ƒYM, 500ƒYM, 1 mM or 2 mM H2O2 was used for the experimental setting.
Ability of EGT to prevent H2O2¡Vdependent cell death was tested by MTT assay. Western blot analysis on mitogen-activated protein kinase (MAPK) protein cascade genes (ERK 1/2 and p38) was also performed.

Results: The scavenging capacity towards hydroxyl radicals for EGT was 5.53¡Ó1.27 units, a value 26% higher than the value obtained with the reference antioxidant Trolox, a vitamin E analog (4.40¡Ó0.60 units, $p<0.01$). When the antioxidant capacity of EGT was evaluated towards peroxyl and peroxynitrite radicals, the values obtained were 0.34¡Ó0.09 and 5.20¡Ó1.00, with an increase of 60% and 10% compared to glutathione (0.21¡Ó0.04 units, $p<0.001$), and uric acid (4.7¡Ó0.9 units, $p<0.05$), respectively.
EGT exerted antioxidant capacity against induced-oxidative stress as observed by MTT assay. 500ƒ_M EGT was able to rescue cell death induced by 1 mM H2O2 and 2 mM H2O2 of 16 % ($p<0.005$) and 26 % ($p<0.05$), respectively. 1mM EGT was able to rescue cell death induced by 2mM H2O2 of 24 % ($p<0.05$). Western blot analysis of the activation of MAPKs revealed that EGT acts as a p38 inhibitor since treatment of cells with 1mM of EGT, followed by 500mM of H2O2, was able to induce a dephosphorilation of p38 of 32%. The same treatment upregulated ERK1/2 phosphorilation of 37%, with a protective role in rescuing oxidative stress.

Conclusions: Because of its protective role in rescuing oxidative stress, a dietary supplementation with EGT could be useful for preventing ROS accumulation in oxidative stress-induced diseases.

GLI ANTIOSSIDANTI PREVENGONO GLI EFFETTI PROTROMBOTICI INDOTTI DALLO STRESS OSSIDATIVO

V. Angri, P. Cirillo, S. De Rosa, L. Sasso, A. Paglia, V. Di Palma, P. Maietta, L.G. D'Ascoli, L. Brevetti, M. Chiariello

Università degli Studi di Napoli Federico II, Napoli.

Obiettivi: Numerose evidenze sperimentali indicano come i radicali liberi dell ossigeno (ROS) siano coinvolti nella patogenesi delle malattie cardiovascolari. É stato dimostrato che alcuni sistemi enzimatici cellulari o la disfunzione dei processi di respirazione cellulare determinano la produzione di ROS. I ROS agiscono sulle principali vie di trasduzione del segnale, coinvolte nei meccanismi dell infiammazione e della trombosi vascolare. Il Tissue Factor (TF) una glicoproteina di membrana, direttamente coinvolta nell attivazione della trombosi intravascolare. Numerosi studi clinici hanno valutato gli effetti degli antiossidanti nella prevenzione delle malattie cardiovascolari. I meccanismi eventualmente coinvolti nella modulazione di tali potenziali effetti protettivi non sono ancora completamente noti. Nel presente studio abbiamo valutato se il pretrattamento con l acido transretinoico (atRA, vitamina A), l acido ascorbico (vitamina C) e l αtocoferolo (vitamina E), vitamine con note proprieta antiossidanti, o con la quercetina (Q) e l acido carnosico (AC), sostanze antiossidanti presenti in alimenti comuni della dieta mediterranea, potesse modulare l espressione del TF in cellule endoteliali coronariche umane (HCAECs) in coltura.

Materiali e Metodi: HCAECs venivano coltivate in mezzo arricchito alternativamente con concentrazioni crescenti di Vit.A, Vit. C, Vit. E, Q o AC. Le cellule venivano quindi sottoposte a stress ossidativo con attivazione della NADPH Ossidasi (NADPH 100μM; PMA 150nM) ed infine processate per valutare: a) i livelli di trascrizione del TFmRNA mediante RTPCR; b) l espressione del TF valutandone l attivita funzionale sulla superficie cellulare mediante un saggio cromogenico.

Risultati: Lo stress ossidativo induceva la trascrizione del TFmRNA ed il conseguente incremento dell espressione di TF attivo sulla superficie cellulare. Tale effetto veniva significativamente ridotto dal pretrattamento con vitamina E, vitamina C ed in misura piu evidente dall atRA. Analogamente, la Q e l AC riducevano significativamente gli effetti dello stress ossidativo sul TF.

Conclusioni: I risultati del presente studio, sebbene condotto in vitro, evidenziano il potenziale ruolo protettivo svolto dagli antiossidanti nel ridurre gli effetti protrombotici dello stress ossidativo su cellule endoteliali coronariche umane.

MECCANISMI CHIMICI ED ENZIMATICI DI OSSIDAZIONE DELLA TETRAIDRO-BIOPTERINA

R. Biondi[2], I. Tritto[1], L. Formigli[2], M. Ricci[2], M. Bettini[1], G. Ambrosio[1]

[1]Cardiologia, Università di Perugia, Perugia;
[2]Patologia Clinica, Università di Perugia, Perugia.

Obiettivi: La funzione della ossido nitrico sintetasi endoteliale (eNOS) è compromessa nel cuore ischemico. L'attività della eNOS richiede diversi cofattori, tra i quali la tetraidrobiopterina (BH4) riveste un ruolo primario. Recenti studi nel cuore isolato e perfuso hanno evidenziato che l'ischemia causa trasformazione della BH4 cardiaca, con deplezione dei suoi livelli tessutali e formazione di un metabolita, la diidroxantopterina (XP-H2); questo effetto si accompagnava a ridotta sintesi di NO. Per comprendere i meccanismi molecolari coinvolti nel catabolismo della BH4 in corso di ischemia, la BH4 è stata sottoposta in vitro a trattamenti ossidativi chimici ed enzimatici.

Materiali e Metodi: Specie ossidanti, quali perossido di idrogeno (H2O2) e radicale idrossidrilico (OH.) erano utilizzati quali ossidanti chimici, mentre la xantina ossidasi (XO) era utilizzata come ossidante enzimatico. I prodotti derivati dalle reazioni di trasformazione della BH4 erano separati e misurati mediante HPLC corredata con due rivelatori disposti in serie, uno colorimetrico e l'altro fluorimetrico.

Risultati: Incubando la BH4 con H2O2 (15 min a 25°C), la reazione ossidativa iniziava alla concentrazione di 1 mM; dava come prodotto intermedio la formazione di BH2 e come metabolita finale la XP-H2; la concentrazione di XP-H2 aumentava con l'incremento della concentrazione di H2O2 raggiungendo il valore di 20.65±1.12% a una concentrazione di H2O2 di 16.0 mM. Anche OH. causava ossidazione della BH4 con prevalente formazione di XP-H2 (54,72±1.55 %), ma anche con la comparsa di pterina (Pt) (36.50±2.54% dopo 60 min di incubazione a 25°C). Anche questo tipo di ossidazione aveva come metabolita intermedio la BH2. Caratteristica risultava invece essere l'ossidazione enzimatica della BH4 da parte di XO. Infatti, il metabolita finale era solamente la XP-H2, senza composti intermedi. La reattività della XO compariva alla concentrazione di 0.01 U/ml di reazione quando l'incubazione era condotta a 37°C per 15 min.

Conclusioni: La conversione della BH4 in vitro da parte di ossidanti chimici ed enzimatici è sovrapponibile a quanto riscontrato nel cuore ischemico dove la BH4 è metabolizzata a XP-H2, il prodotto ossidato irreversibile. E' verosimile ipotizzare un ruolo dell'ossidazione della BH4 come meccanismo regolante l'attività della eNOS in vivo, e di conseguenza la funzione endoteliale a livello della circolazione cardiaca.

IMPATTO DELLA SINDROME METABOLICA SULLA PRESENTAZIONE ECG E SULLA PROGNOSI A BREVE E LUNGO TERMINE DEI PAZIENTI CON SINDROME CORONARICA ACUTA

I. Tritto, G. Spinucci, E. Carluccio, P. Biagioli, A. Cardona, S. Coiro, N. Viola, M. Bentivoglio, G. Ambrosio

Cattedra di Cardiologia, Università di Perugia, Perugia.

Obiettivi: La sindrome metabolica (SM) aumenta il rischio di sindromi coronariche acute (SCA). Tuttavia dati recenti mostravano che, fra i pazienti con SCA, l'incidenza di morte e reinfarto è inferiore nei pazienti obesi, mettendo così in discussione il valore prognostico dell'obesità e della SM in questo contesto. Poiché si ritiene che la SM aumenti il rischio soprattutto a lungo termine, abbiamo valutato, in pazienti ricoverati per SCA, se la presenza di SM ne influenzi la prognosi, a breve e lungo termine.

Metodi: Dal 1995 al 2000 nel nostro reparto sono stati ricoverati 1430 pazienti consecutivi con diagnosi di SCA; 602 (42,2%) erano affetti da SM (definizione 2005 NCEP-ATP III). I pazienti erano suddivisi in base alla presentazione clinica: STEMI oppure NSTEMI/UA, e quindi seguiti nel tempo.

Risultati: I pazienti con SM mostravano un aumento significativo della presentazione NSTEMI/UA (56,6% vs 48,6%; $p<0,01$), e parallellamente una ridotta incidenza di STEMI (43,4% vs 51,4%; $p<0,01$). All'analisi multivariata, corretta per i fattori confondenti, la SM restava un predittore indipendente di NSTEMI/UA (OR 1,30; 95% IC 1,0181-1,792; $p<0,05$). Durante il ricovero, i pazienti con SM mostravano minore incidenza di complicanze (32,4% vs 39,5%; $p<0,01$) e minore mortalità (2,3% vs 4,7%; $p<0,05$). Tuttavia, nonostante una presentazione clinica e un decorso intraospedaliero in apparenza più benigni, a un follow up medio di 59 ± 42 mesi i pazienti con SM mostravano una prognosi significativamente peggiore, con maggiore incidenza dell'endpoint composito di reinfarto, angina e insufficienza cardiaca ($p<0,05$). Quando i pazienti erano suddivisi in base alla presentazione clinica al momento dell'evento indice, l'impatto prognostico negativo a lungo termine della SM era confermato nel gruppo NSTEMI/UA, mentre nei pazienti con STEMI l'incidenza degli eventi non mostrava differenze significative fra i gruppi.

Conclusioni: Nei pazienti con SCA, la presenza di sindrome metabolica sembra quindi favorire la presentazione NSTEMI/UA, in accordo con la ridotta incidenza di complicanze e mortalità intraospedaliere, e potrebbe contribuire a spiegare le controversie sul valore prognostico a breve termine di obesità e sindrome metabolica in questo contesto. Tuttavia, nel lungo periodo la SM esercita un effetto prognostico negativo nei pazienti che si presentavano con NSTEMI/UA come evento indice.

LEVOSIMENDAN POSTCONDITIONING REDUCES MYOCARDIAL REPERFUSION INJURY THROUGH A REDOX-SENSITIVE MECHANISM AND MITOCHONDRIAL ATP-SENSITIVE POTASSIUM CHANNEL ACTIVATION

E. Tiravanti[1], G. Colantuono[1], N. Di Venosa[1], V. Lo Parco[1], A. Cazzato[1], A. Federici2, T. Fiore[1]

[1]Università degli Studi di Bari, DETO, sez Anestesia e Rianimazione, Bari;
[2]Università degli Studi di Bari, Dipartimento di Farmacologia e Fisiologia Umana, Bari.

Purpose: Levosimendan is a novel inotropic and vasodilator agent that has been shown to protect against myocardial ischemia/reperfusion injury. The aim of this study was to investigate whether the rat heart could be postconditioned using levosimendan (LevoPost-C) and to evaluate the possible role of oxygen free radicals (ROS) and mitochondrial KATP (mKATP) channels in the mechanism of cardioprotection.

Methods: Isolated rat hearts (n=4 each group) underwent 30 min global ischemia (I) and 120 min reperfusion (R) with or without LevoPost-C (6 cycles of 15 s Levosimendan (0.1 microM)/vehicle immediately after 30 min of ischemia). 4 groups received the infusion of either the mKATP channel blocker, 5-hydroxydecanoate (5-HD, 100 microM) or the ROS scavenger 2-mercaptopropionyl glycine (MPG, 300 microM) during the LevoPost-C maneuvers (3 min only). In other 4 groups the infusion of 5-HD and MPG was started after the first 3 min of reperfusion and continued until the end of the experiment. Infarct size after 2 hours of reperfusion was assessed by triphenyltetrazolium chloride staining and expressed as a % of the risk area.

Results: LevoPost-C attenuated myocardial infarct size (31 ± 3% vs. 57 ± 5% in control; $p < 0.01$). This cardioprotective effect was abolished by 5-HD (55 ± 7% and 60± 5% respectively) given during either the first 3 min or just after the third min of reperfusion as well as by MPG (52 ± 6%) given during the initial 3 min of reperfusion. It is relevant that MPG given just after the third min of reperfusion did not modify the protective effect of Post-C (infarct size 34 ± 5%; $p < 0.01$ vs. control, NS vs.LevoPost-C). 5-HD or MPG given in the absence of LevoPost-C did not alter the effects of I/R.

Conclusions: Our data show that intermittent infusion of levosimendan at reperfusion may induce cardioprotection in rat heart. The protective effect seems to be related to an early redox-sensitive mechanism as well as to a persistent activation of mKATP, suggesting that the mKATP/ROS pathway is involved in Levosimendan-induced postconditioning.

EFFETTI DEL PRE- E POST-CONDIZIONAMENTO ISCHEMICO SUL RECLUTAMENTO DEI NEUTROFILI NEL MICROCIRCOLO DOPO ISCHEMIA-RIPERFUSIONE: MONITORAGGIO IN VIVO CON VIDEOMICROSCOPIA

I. Tritto, I. Porchetta, C. Zuchi, M. Bettini, S. Coiro, A. Cardona, G. Ambrosio

Cardiologia, Università di Perugia, Perugia.

Obiettivi: Il precondizionamento ischemico (preC) è efficace nel ridurre l'infiltrazione dei neutrofili nei tessuti postischemici. Tuttavia, poiché il verificarsi di un attacco ischemico è imprevedibile, la sua applicazione clinica è stata finora molto limitata. Alcuni studi hanno suggerito indirettamente che anche il postcondizionamento ischemico (PostC) possa ridurre il reclutamento dei neutrofili nei tessuti. Abbiamo quindi valutato direttamente se il postC può inibire il reclutamento dei neutrofili nel microcircolo durante riperfusione postischemica, e paragonato i suoi effetti con quelli del preC.

Metodi: In ratti anestetizzati, il cremastere era sottoposto 90 min di ischemia seguiti da 90 min di riperfusione. I controlli non ricevevano ulteriori interventi; il postC era indotto all'inizio della riperfusione con 5 cicli di 10 sec di riperfusione e 5 sec di ischemia. Il preC era indotto prima dell'ischemia con 5 min di ischemia e 10 min di riperfusione. I leucociti erano marcati con rosso di acridina, e la loro interazione con l'endotelio vasale era monitorata nelle venule postcapillari mediante videomicroscopia intravitale, che permette di misurare l'interazione dei leucociti con la parete vasale, con misurazioni ripetute nel tempo.

Risultati: Nei controlli, durante riperfusione postichemica si osservava un marcato aumento dell'interazione leucociti-endotelio, con aumento del numero dei leucociti che mostravano rolling e di adesione ferma alla parete vasale. Sia il preC che il postC erano in grado di ridurre l'interazione leucociti-parete vasale (rolling al picco: 22.5±6.5, 12.2±3.7 e 14.9±8, rispettivamente; adesione al picco: 2.3±1, 1.3±.6 e 1.1±.8, rispettivamente; $p<0.05$).

Conclusioni: Anche il postcondizionamento ischemico è in grado di ridurre il reclutamento dei neutrofili nei tessuti. Questo è un punto potenzialmente molto importante, poichè potrebbe risultare nella riduzione del fenomeno del no-reflow, con notevoli implicazioni cliniche.

LOSARTAN TREATMENT RESTORES THE RESPONSE TO HYPOXIA OF DIABETIC CARDIOMYOCYTES

C. Alfarano[1], L. Raimondi[1], S. Suffredini[2], A. Mugelli[1], E. Cerbai[2]

[1]Dipartimento di Farmacologia Preclinica e Clinica, Università di Firenze, Firenze;
[2]Centro Interuniversitario di Medicina Molecolare e Biofisica Applicata (C.I.M.M.B.A.), Firenze.

Background: Metabolic derangement occurring in the diabetic heart reduces cardiomyocyte tolerance to ischemia, likely impairing protective mechanism against hypoxic injury, e.g. the activation of KATP channels. We aimed to (i) characterize the cellular response to simulated hypoxia and (ii) assess the protective effect of in-vivo treatment with losartan, an angiotensin II type 1 receptor antagonist, on cardiomyocytes isolated from diabetic and normoglycemic rats.

Methods: Cardiomyocytes were isolated from not-treated normoglycemic (C) or streptozotocin-injected (STZ-55 mg/Kg) (D) rats and from the same groups treated with losartan (20 mg/Kg/day in drinking water) for 3 weeks (CL and DL respectively). Simulated hypoxia was induced by superfusion with a modified glucose-free Tyrode's solution containing NaCN (2 mmol/l). Time to rigor and time to KATP current activation were measured as index of cellular response to simulated hypoxia. Glucose-6-phosphate (G6PDH) and cytoplasmic/mitochondrial glycerol-3-phosphate dehydrogenase (G3PDH) activities were assayed as an index of the cell metabolic state.

Results: Cardiomyocytes from D rats showed higher G6PDH and cytoplasmic G3PDH than C or CL cells. Rigor occurred significantly later in D than in C cells (20.5±2.0 min, n=16 and 10.0±2.0 min; n=15 respectively; $p<0.001$). In parallel, KATP current activation occurred faster in C and CL cells (7.5±1.1 and 7.1±0.9 min) than in D cells (25.7±7.1 min) ($p<0.05$). Blood glucose was equally enhanced in D and DL rats. However, in cells from DL rats, the time to rigor and KATP current activation returned to values (respectively: 9.9±1.3 min, n=16 and 5.8±1.8 min, n=16) similar to those measured in C and CL cells and significantly shorter than in D cells ($p<0.05$). Also, the G6PDH and the cytoplasmic G3PDH activities decrease reaching values of activity similar to those found in C and CL cells.

Conclusions: A delayed activation of protective mechanisms against hypoxic injury occurs in the diabetic heart. Losartan treatment modifies the response of diabetic cardiomyocytes to simulated hypoxia which returns similar to that of normoglycemic cells. This effect is associated with the amelioration of anaerobic metabolism.

A MEMBRANE G PROTEIN-COUPLED RECEPTOR (GPR30) MEDIATES THE CARDIAC EFFECTS OF 17BETA-ESTRADIOL IN THE MALE RAT

E. Filice[1], A.G. Recchia[1], D. Pellegrino[1], T. Angelone[2], M. Maggiolini[1], M.C. Cerra[1]

[1]Dip. Farmaco-Biologico, Università della Calabria, Cosenza;
[2]Dip. Biologia Cellulare, Università della Calabria, Cosenza.

Aim: Even though multidisciplinary data have revealed E2-induced cardiovascular actions, the direct influence of 17β-estradiol (E2) on the male heart has received little attention. Both genomic and nongenomic effects of E2 require activation of the estrogen receptor (ER) α and ERβ.
Recently, a novel membrane G protein-coupled receptor, named GPR30, has been implicated in rapid E2 signaling. We aimed to investigate the influence of E2 on the performance of the mammalian male heart and the molecular mechanisms involved.

Methods: The expression of ERα, ERβ and GPR30 at both mRNA and protein levels were assessed through reverse-transcription PCR and western blotting. cGMP concentration was evaluated by EIA assay.

Results: We observed that E2 negatively affected cardiac performance through a dose-dependent reduction of contractility. This action, was mimicked by selective agonists for ERα and ERβ, and blocked by the ER inhibitor ICI 182,780. The putative role of GPR30 was demonstrated by using the selective ligand G-1. Moreover, E2-induced cardiac responses involved ERK, PI3K, PKA, and eNOS transduction pathways.

Conclusions: Taken together these data suggest that the cardiotropic effect of E2 in the male rat heart is mediated by ERα and ERβ, whereas the involvement of GPR30 signaling warrants further evaluation.

INOTROPIC AND LUSITROPIC EFFECTS OF THE ANTIHYPERTENSIVE CATESTATIN: MECHANISMS OF ACTION IN THE RAT HEART

A.M. Quintieri[1], T. Angelone[2], T. Pasqua[1], R. Mazza[2], A. Gattuso[2], B. Tota[2], S.K. Mahata[3], M.C. Cerra[1]

[1]Dip. Farmaco-Biologico, Università della Calabria, Cosenza, Italy;
[2]Dip. Biologia Cellulare, Università della Calabria, Cosenza, Italy;
[3]Dept. Medicine, University of California & VA San Diego Healthcare System, San Diego, USA.

Aim: Circulating levels of catestatin (Cts: human chromogranin A352-372) decrease in patients with essential hypertension. Genetic ablation of the chromogranin A (Chga) gene in mice increases blood pressure and pre-treatment of Chga-null mice with Cts prevents blood pressure elevation, indicating a role of Cts in preventing hypertension. Using the Langendorff-perfused rat heart it was found that WT-Cts dose-dependently induces negative inotropic and lusitropic effects, while its natural variants G364S-Cts and P370L-Cts did not affect basal cardiac performance.

Methods: The Langendorff-perfused rat heart was used to test the involvement of β2-adrenergic receptors (ARs)-Gi/o proteins and the endothelial nitric oxide synthase (eNOS)-NO-cGMP-PKG pathway in the WT-Cts-dependent cardiomodulation. SDS-PAGE immunoblot analysis was used to analyse phosphorylated-protein kinase B (Akt)-Ser473 (P-Akt), total-Akt (T-Akt), phosphorylated GSK-3 (P-GSK-3-Ser9) and actin.

Results: We found that WT-Cts inhibited phospholamban phosphorylation. Moreover, WT-Cts-dependent inotropic and lusitropic effects were abolished by chemical inhibition of β2 ARs, Gi/o protein, eNOS, or cGMP.

Conclusions: Our data indicate that the inhibitory influence exerted on the basal mechanical performance and the counter-regulatory action agains β-adrenergic and ET-1 stimulations exerted by Cts in the rat heart require the involvement of β2-ARs-Gi/o protein-eNOS-cGMP signaling mechanisms.

CARDIOTROPIC EFFECTS OF RELAXIN ON MYOCARDIAL INFARCTION AND ADVERSE POST-ISCHEMIC REMODELLING

R. Mastroianni[1], S. Nistri[2], L. Formigli[2], E. Ragazzo[1], L. Cinci[2], D. Bani[2], E. Masini[1]

[1]Department of Preclinical and Clinical Pharmacology, Florence;
[2]Department of Anatomy, Histology and Forensic Medicine, Florence.

The hormone relaxin (RLX) has been shown to cause coronary vasodilatation and to prevent ischemia/reperfusion-induced cardiac injury in rodents.

This study provides evidence that RLX, used as an adjunctive drug to coronary reperfusion, reduces the functional, biochemical and histopathological signs of myocardial injury in an in vivo swine model of heart ischemia/reperfusion, currently used to test cardiotropic drugs for myocardial infarction.
Human recombinant RLX, given at reperfusion at doses of 1.25, 2.5 and 5 µg/kg b.wt., upon a 30-min ischemia, caused a dose-related reduction of key markers of inflammation, myocardial damage and cell apoptosis, by a mechanism involving reduction of oxygen free radical-induced tissue injury. Moreover, RLX reduces the inflammatory activation of resident cardiac mast cells, the main source of cardiac histamine and prostanoids, thereby preventing the onset of severe arrhythmias, cardiodepressive effects and coronary spasm. Overall, RLX reduced the extension of necrotic myocardium and leads to a substantial improvement of heart contractility.
Then we explored whether RLX, which is able to promote extracellular matrix turn-over and neoangiogenesis, could beneficially influence post-infarction heart remodelling and dysfunction using a swine model of stem cell therapy with C2C12 myoblasts genetically engineered to express RLX. One month after cell engraftment, histological analysis of the tissue showed that C2C12/RLX myoblasts selectively settled in the ischemic scar, where they induce extracellular matrix remodeling by the secretion of matrix metalloproteases (MMP) and increase microvessel density through the expression of VEGF. By echocardiography, C2C12/RLX-engrafted swine showed improved heart contractility compared with the ungrafted controls.

In conclusion, our studies offer additional evidence for the potential therapeutic effects of relaxin in acute myocardial infarction and in preventing deleterious cardiac remodeling in the post-infarcted heart.

REGIONAL MYOCARDIAL HYBERNATION AS ADAPTATION OF SEVERE MECHANICAL DYSSYNCHRONY IN NON ISCHEMIC HEART FAILURE

V. Lionetti[1], G.D. Aquaro[2], C. Di Cristofano[3], A. Simioniuc[1], M. Campan[1], S. Forini[2], F. Bernini[1], M. Lombardi[2], F.A. Recchia[1], A. Pingitore[1]

[1]Settore di Medicina, Scuola Superiore Sant'Anna, Pisa;
[2]Istituto di Fisiologia Clinica, CNR, Pisa, 3ICOT, Università La Sapienza, Roma.

Objectives: Regions of the left ventricle (LV) exposed to sustained mechanical dyssynchrony can develop chronic dysfunction leading to failure even in absence of coronary stenosis. We hypothesized that LV mechanical dyssynchrony causes regional myocardial hibernation.

Materials and Methods: Seven adult male minipigs (35-40 kg) were chronically instrumented with an unipolar pacemaker connected to the epicardial surface of the LV free wall to induce sustained LV dyssynchronous contraction. The heart was paced at 180 beats/min for 21 days and regional LV contractile and perfusion reserve were assessed, respectively, as end-systolic wall thickening (LVESWT) and myocardial perfusion reserve index (MPRI) by low-dose dobutamine stress -MRI. Dobutamine was infused at 10 µg/kg/min i.v. for 10 min with the pacemaker turned off. Pacing caused LV mechanical dyssyncrhony, assessed as the delay between the time to end-systolic peak in the pacing site (anterior and lateral-anterior regions) and in opposite regions (inferior and septal-inferior regions).

Results: After 21 days of pacing, dobutamine caused a marked increase in LVESWT (27±2.98 vs 7.45±3 %, $P<0.05$) and MPRI (2.1±0.5 vs 1.3±0.3, $P<0.05$) in the pacing site, but not in the opposite site, in absence of gadolinium delayed contrast-enhancement. Conversely, the end-systolic LV wall stress in failing hearts was uniformly reduced by 37 % during dobutamine stress. Histological analysis showed a marked and not homogenous increase of glycogen deposits in cardiomyocytes of pacing site, further suggesting hibernation development, but not in the opposite site.

Conclusions: Our results indicate that prolonged LV mechanical dyssynchrony causes myocardial hibernation in the region that is activated first.

REDUCED APOPTOSIS IN POLYAMINE DEPLETED RAT CARDIOMYOCYTES TREATED WITH NOREPINEPHRINE

S. Cetrullo[1], B. Tantini[1], I. Stanic'[1], F. Flamigni[1], C. Pignatti[1], C. Stefanelli[1], C. Guarnieri[1], C.M. Caldarera[2]

[1]Department of Biochemistry G.Moruzzi, University of Bologna, Bologna;
[2]Institute of Cardiovascular Research, Imola.

Introduction: Chronic heart failure is associated with neurohormonal activation and alterations in autonomic control. Although these mechanisms provide valuable support for the heart in physiological circumstances, they also have a fundamental role in the development and subsequent progression of chronic heart failure. Increased sympathetic nerve activity in the myocardium is a central feature of patients with heart failure. Moreover norepinephrine (NE), the primary transmitter of the sympathetic nervous system, is able to induce apoptosis of cardiomyocytes in many studies. Accumulating evidence shows that apoptosis of cardiomyocytes plays an important role in causing heart failure.
Polyamines are biogenic amines involved in many cellular processes, including apoptosis. Actually it appears that these molecules can act as promoting, modulating or protective agents in apoptosis depending on apoptotic stimulus and cellular model.
In the present work we have studied the involvement of polyamines in apoptosis of rat neonatal cardiomyocytes treated with NE.

Methods: Cardiomyocyte cultures were prepared from 1-3 days old neonatal Wistar rat hearts. After 20 h of serum starvation, confluent cells were treated with NE. Polyamine depletion was obtained by culturing the cells in the presence of α-difluoromethylornithine (DFMO). Caspase activity was measured by the cleavage of a fluorogenic peptide substrate. Ornithine decarboxylase (ODC) activity was measured by estimation of the release of 14C-CO2 from 14C-ornithine. Signal transduction pathway activation was investigated by western blotting.

Results: The results indicate that NE causes an early induction of the activity of ODC, the first enzyme in polyamine biosynthesis, followed by a later increase of caspase activity, an apoptosis marker. Polyamine depletion obtained with DFMO cell pretreatment reduces apoptosis. Moreover, preliminary experiments indicated an involvement of AMP activated protein kinase (AMPK) and AKT, key signalling proteins correlated with cell death as well as with cell proliferation, in the response of cardiomyocytes to norepinephrine. The activation of these signal transduction pathways is altered in polyamine depleted cells.

Conclusions: These data suggest that polyamines are involved in the execution of the death program activated by norepinephrine in neonatal rat cardiomyocytes.
This work was supported by a Grant from the Compagnia di San Paolo, Torino, and University of Bologna (R.F.O.).

CONTROL OF INSULINE SENSITIVITY AN LIPID METABOLISM BY PHYSICAL ACTIVITY: PLASMA VISFATIN CONCENTRATIONS AND METABOLIC PARAMETERS IN PHYSICALLY ACTIVE CHILDREN

J. Jürimäe[1], A. Cicchella[2], E. Lätt[1], K. Haljaste[1], P. Purge[1], T. Jürimäe[1], C. Pignatti[3], C.L. Passariello[3], C.M. Caldarera[4], C. Stefanelli[2,3,4]

[1]Institute of Sport Pedagogy and Coaching Sciences, Tartu, Estonia;
[2]Faculty of Exercise and Sport Science, University of Bologna, Bologna, Italy;
[3]Department of Biochemistry, University of Bologna, Bologna, Italy;
[4]INRC, National Institute for Cardiovascular Research, Bologna, Italy.

Visfatin is a newly identified adipocytokine with insulin-like metabolic effects that may improve insulin sensitivity. Plasma visfatin concentrations are elevated in patients with type 2 diabetes mellitus and associated with abdominal obesity and fasting insulin in adults and Visfatin has been suggested to be one of the links between intra-abdominal obesity and type 2 diabetes. In this context, it is important to look at the relationships between plasma visfatin concentrations and insulin sensitivity in regularly physically active people.

The purpose of the present study was to investigate the association of fasting plasma visfatin concentration with insulin resistance and body fat parameters according to sex in physically active children. Thirty four healthy children (17 boys and 17 girls) aged between 13 and 16 years participated in this study. All children were swimmers recruited from local training groups and trained for at least eight hours per week during at least last two years. The distribution of age and body mass index (BMI) was not different between boys and girls. Plasma visfatin concentrations were not different between sexes (boys: 1.3±0.9 ng/ml; girls: 1.2±1.0 ng/ml). No differences were also observed for insulin, glucose and fasting insulin resistance index (FIRI) values between studied groups, while girls presented significantly higher values for leptin compared to boys. Plasma visfatin concentrations were related to the markers of overall obesity (BMI, body fat%, fat mass) and insulin sensitivity (insulin, FIRI) only in boys, while no relationship between these parameters was observed in girls.

In conclusion, the results of this study suggested that plasma visfatin concentrations are not different between regularly physically active boys and girls. In addition, our findings indicated that the associations of fasting plasma visafatin concentrations with metabolic and body composition parameters were sex-dependent in children.

This study was supported by Estonian Science Foundation Grant No 6220 and Grant No 2695 of the Estonian Ministry of Education and Science, by italian MIUR (PRIN 2004) and by INRC.

LA PROTEINA CHINASI ATTIVATA DA AMP COME MODULATORE DI EVENTI PRECOCI IN UN MODELLO CELLULARE DI IPERTROFIA CARDIACA

C.L. Passariello[1], D. Gottardi[1], M. Zini[1], S. Cetrullo[1], B. Tantini[1], C. Pignatti[1], F. Flamigni[1], C.M. Caldarera[2], C. Stefanelli[1,2]

[1]Dipartimento di Biochimica, Università di Bologna, Bologna;
[2]INRC, Bologna.

La proteina chinasi attivata da AMP (AMPK) rappresenta un legame tra crescita cellulare e disponibilità di energia. A causa del suo ruolo omeostatico, la AMPK potrebbe contrastare la crescita sia iperplastica che ipertrofica. Per valutare il ruolo della AMPK nell'ipertrofia cardiaca, abbiamo studiato la risposta della AMPK in un modello cellulare di induzione di ipertrofia rappresentato da cardiomioblasti H9c2.

Nelle cellule esposte all'agonista β-adrenergico isoproterenolo si assiste ad un rapido aumento della fosforilazione della AMPK, che viene attivata anche da altri effettori adrenergici, come fenilefrina e noradrenalina. Questo effetto degli agonisti adrenergici, che scatenano una risposta ipertrofica nei cardiomiociti, apparentemente sembra in contrasto con il ruolo omeostatico attribuito alla AMPK. Abbiamo quindi studiato alcuni processi molecolari correlati all'induzione dell'pertrofia. Uno degli eventi precoci in seguito all'esposizione a stimoli ipertrofici, sia in vivo che in vitro, è rappresentato dall'induzione dell'Ornitina decarbossilasi (ODC). Nel nostro modello cellulare, come nei cardiomiociti di ratto, il trattamento con isoproterenolo induce uno rapido aumento dell'attività ODC, che è già visibile dopo 30 minuti ed è correlato alla attivazione della proteina chinasi Akt. Per valutare il ruolo della AMPK nell'induzione dell'attività ODC, abbiamo inibito l'espressione della AMPK attraverso silenziamento mediante RNA interference, transfettando le cellule con siRNA con target le due isoforme $\alpha 1$ e $\alpha 2$ della subunità catalitica della AMPK. Il knock down della AMPK causa un aumento significativo dell'induzione ODC, indicando un ruolo di modulatore negativo della chinasi. Un effetto paragonabile è stato ottenuto anche in cardiomiociti di ratto neonato trattati con noradrenalina.

Queste ricerche hanno mostrato che il trattamento con isoproterenolo attiva rapidamente in cellule cardiache due vie apparentemente contrastanti tra loro, una legata alla crescita e una seconda in grado di modulare negativamente la prima. Si può prospettare un modello secondo cui la AMPK svolge un ruolo regolatore nella transizione verso un fenotipo ipertrofico. La rapida attivazione della AMPK in seguito a stimoli adrenergici ha probabilmente lo scopo di garantire alla cellula i substrati energetici necessari rispondere ad una accresciuta richiesta di ATP. La mancanza di condizioni trofiche ottimali (substrati energetici) attraverso una prolungata attivazione della AMPK potrebbe invece contrastare l'instaurarsi dell'ipertrofia.

ANTI-INFLAMMATORY EFFECTS OF A LOW MOLECULAR WEIGHT HEPARIN-LIKE DERIVATIVE IN A RAT MODEL OF CARRAGEENAN-INDUCED PLEURISY

E. Masini[1], S. Nistri[2], C. Uliva[1], M. Ceccarelli[1], C. Lanzi[1], D. Bani[2]

[1]Department of Pharmacology Preclinical and Clinical, Florence;
[2]Department of Anatomy, Histology and Forensic Medicine, Florence.

Low molecular weight heparin derivates are characterized by low anticoagulant activity and marked anti-inflammatory effects that allow for these molecules to be viewed as a new class of non steroidal anti-inflammatory drugs (NSAIDs).

We report that the K5NOSepiLMW, an O-sulphated heparin-like semi-synthetic polymer of D-glucuronic acid-N-acetyleparosan disaccharide unit with low molecular weight, has marked anti-inflammatory effects in a rat model of acute inflammation, the carrageenan-induced pleurisy, commonly used to test NSAID efficacy. A 30 min pre-treatment with K5NOSepiLMW (1 mg/Kg b.wt., given intra-pleurally) attenuated the recruitment of inflammatory leukocytes in the lung tissue and the pleural exudate, inhibited the induction of inducible nitric oxide synthase and cyclooxygenase-2, thereby abating the generation of harmful nitric oxide and pro-inflammatory prostglandins, such as PGE2 and PGF1α, reduced the inflammation-induced oxidative/nitrosative lung tissue injury, as shown by tissue malondialdehyde and nitrotyrosine, and blunted the local generation of cytokines such as IL-1β and TNF-α. All these parameters were markedly increased by intra-pleural carrageenan in the absence of any pre-treatment. The biologically inactive polysaccharide B4/110 had no therapeutic effect when given in the place of K5NOSepiLMW, this suggesting that its anti-inflammatory action is specific. Morover, K5NOSepiLMW showed a similar potency as celecoxib, a cyclooxygenase-2 inhibitor and well known NSAID.

This study provides further insight into the mechanisms underlying the beneficial effects of heparin derivates in inflammation and identifies K5NOSepiLMW as novel promising anti-inflammatory drugs.

NRG1 MODULATION OF CONTRACTILITY AND NO SYNTHESIS IN ISOLATED VENTRICULAR MYOCYTES

A. Brero, R. Ramella, O. Boero, B. Mautino, C. Dati, G. Alloatti, M.P. Gallo, R. Levi

Department of Animal and Human Biology, University of Turin, Turin.

Objectives: Neuregulins (Nrg) are growth factors that mediate cellular interactions in different systems through the activation of tyrosine kinase receptors of the ErbB family. The ErbB-Nrg pathway is a mediator of cardiac development and different long-term effects on postnatal and adult cardiomyocytes have been demonstrated. As it is not clear whether Nrg1 has any rapid effects on cardiac activity, we investigated acute effects in isolated adult rat ventricular myocytes (ARVCMs).

Methods: The effects induced by recombinant binding domain of Nrg1beta1 on nitric oxide (NO) production were studied in unstimulated ARVCMs using simultaneous Ca2+ and NO fluorimetric confocal imaging. Nrg1 effect on Ca2+ handling was investigated by Ca2+ transients and Ca2+ current (ICa2+) measurements. eNOS and phospholamban (PLN) phosphorylation state were evaluated by western blot analysis and immunofluorescence staining.

Results: In isolated cardiomyocytes Nrg1 induced a Ca2+ independent increase in NO production, blocked by the PI3K inhibitor Wortmannin (Wm). Western blot analysis revealed an increase in eNOS phosphorylation in Nrg1 treated myocytes respect to control and phosphorylation was attenuated by Wm. Nrg1 treatment induced a significant increase in Ca2+ transients amplitude under basal condition while it was ineffective upon beta-adrenergic stimulation. Moreover Nrg1 treatment accelerated the recovery of cytosolic Ca2+ as indicated by the decrease of the constant of cytosolic Ca2+ decline, suggesting a role of Nrg1 in Ca2+ release and/or reuptake from SR. The increase in Ca2+ transients amplitude exerted by Nrg1 didn't involve the regulation of L-Type Ca2+ channels. The role of PI3K and PKG were also investigated as potential signaling steps involved in Nrg1 mediated Ca2+ handling: the presence of Wm or PKG inhibitor DT2 abolished the increase in transient Ca2+ amplitude and the acceleration of Ca2+ recovery induced by Nrg1 treatment. Immunofluorescence analysis revealed that Nrg1 treatment increased PLN phosphorylation, blocked by inhibitors of PI3K, eNOS and PKG.

Conclusions: In ARVCMs Nrg1 increases eNOS phosphorylation and NO production through PI3K-Akt pathway, without affecting basal Ca2+ and ICa2+. The stimulation of NO synthesis activates the cGMP-PKG dependent pathway, with consequent increase in PLN phosphorylation, acceleration of cytosolic Ca2+ recovery in the SR and increase in Ca2+ transients amplitude.

EFFECT OF REVASCULARIZING VIABLE MYOCARDIUM ON LEFT VENTRICULAR DIASTOLIC FUNCTION IN PATIENTS WITH ISCHEMIC CARDIOMYOPATHY

P. Pantano, E. Carluccio, P. Biagioli, V. Leonelli, G. Alunni, A. Murrone, G. Ambrosio

Cardiologia e Fisiopatologia Cardiovascolare, Università e Azienda Ospedaliera di Perugia, Perugia.

Background: In patients with ischemic left ventricular (LV) dysfunction and viable myocardium, revascularization improves systolic function. Diastolic dysfunction is also present in such patients; however, whether revascularization beneficially influences also diastolic function is largely unknown.

Methods: Twenty-six patients with chronic ischemic LV dysfunction (EF 32±6%, WMSI 2.45±0.33) and viable myocardium (low-dose dobutamine echocardiography), underwent echocardiography examination at baseline and >4 months after revascularization. By pulsed-wave Doppler-derived transmitral filling indices patients were categorized in restrictive (RF) and non-restrictive (non-RF) filling groups. Diastolic function and LV filling pressures were evaluated by load-independent pulsed-wave Tissue-Doppler (TDI).

Results: At baseline, 16 (62%) patients showed a non-RF, and 10 (38%) patients a RF pattern. Degree of diastolic dysfunction was correlated to the number of viable segments ($p<0.05$). After revascularization, along with improvement in systolic function (EF 43±10%, WMSI 1.78±0.47, $p<0.0001$ for both), diastolic filling significantly improved in most patients, with only 3 patients still exhibiting a RF pattern ($p<0.05$). By TDI analysis, revascularization was associated with increased E' velocity (20±27%, $p<0.05$) and decreased E/E' (-18±37%, $p<0.05$). Filling pressure also decreased from 17.5±6.8 mmHg to 13.1±6.5 mmHg ($p<0.05$). Improvement of diastolic function by TDI was largely related to the extent of viability and LV reverse remodeling after revascularization ($p<0.01$).

Conclusions: In patients with ischemic cardiomyopathy, LV diastolic filling may largely improve after revascularization. Improvement of diastolic dysfunction is related to the amount of viable myocardium and it may represent an additional advantage of revascularizing dyssinergic but viable myocardium.

FREQUENTE RISCONTRO DI PFO IN GIOVANI PAZIENTI AFFETTI DA EPISODI RICORRENTI DI FIBRILLAZIONE ATRIALE PAROSSISTICA

P. De Campora[1], G. Malferrari[2], S. Sanguigni[3], R. Sangiuolo[1]

[1]Ospedale Fatebenefratelli, Napoli;
[2]Ospedale S.Maria Nuova, Reggio Emilia;
[3]Ospedale Madonna del Soccorso, S. Benedetto del Tronto.

Il riscontro ecocardiografico della Pervieta' del Forame Ovale (PFO) è piuttosto frequente nella popolazione occidentale e sembra associarsi ad Emicrania con "Aura" (MA+) ed Ictus giovanile criptogenetico.
Scopo del nostro lavoro studiare, con analisi retrospettiva, le caratteristiche ecocardiografiche di giovani pazienti (età < 50 anni) giunti alla nostra osservazione per episodi di Fibrillazione Atriale Parossistica (FAP).

Metodi: Abbiamo sottoposto 35 pazienti (19 uomini di età 32,65+/- 5 anni: 16 donne 29,93 +/- 5 anni) affetti da FAP e 30 controlli © matchati ad Ecocardiogramma Trans Toracico ColorDoppler (TTE), Ecografia Trans-Cranica Color-Doppler (TCCD), ed Ecocardio Trans-Esofageo (TEE). I 2 gruppi sono stati sottoposti a TTE per valutare funzione sistolica, diastolica, volumetria delle camere cardiache ed analisi della regione della Fossa Ovale. L'esame TCCD eseguito con test Dinamico (10 cc di NaCl e.v.) per descrivere le caratteristiche dello shunt (grado; rapporto con M. di Valsalva); il TEE per investigare l'associazione con aneurismi della Fossa Ovale. Esclusi dallo studio soggetti con cardiopatia nota, diabetici oppure con finestra ecografica inadatta.

Results: 11 patienti del gruppo (FAP) hanno mostrato la presenza di PFO (11/36, pari al 31.4 % totale. 6 donne (37 %); 5 uomini (26.3 %). Nel gruppo controllo © abbiamo riscontato la PFO in 5 soggetti (14.2 %). Degno di nota che, nel gruppo FAP, la PFO piu' frequentemente (4/11: 36 % vs 1/5, 20 % gruppo C) si associava ad Aneurisma della Fossa Ovale - osservato al TEE - e gli shunts presentavano una maggiore rilevanza ossia, un maggior numero di microbolle al TCCD.
Da un punto di vista statistico, i Pazienti FAP hanno rispetto ai controlli un ODD RATIO di 2,2 per il riscontro di PFO.

Conclusioni: Nei pazienti affetti in epoca giovanile da episodi ricorrenti di fibrillazione atriale parossistica sembra sussistere una maggiore prevalenza di Pervietà della Fossa Ovale rispetto ai coetanei non affetti. La PFO, inoltre, in questi pazienti si associa maggiormente alla presenza di aneurismi della Fossa Ovale e di shunts significativi da un punto di vista emodinamico che potrebbero, in certe circostanze,fungere da triggers innescando l'aritmia.
Si tratta di un'ipotesi diagnostica che richiede, tuttavia, ulteriori conferme sperimentali.

CLINICAL RESULTS OF BIVALIRUDIN IN HIGH RISK PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION

L. D'Antonio, S. Caroli, M. Macrì, R. Melfi, A. Nusca, M. Miglionico, A. D'Ambrosio, G. Patti, G. Di Sciascio

Campus Bio-Medico, Roma.

Background: Randomized trials demonstrated that use of bivalirudin, a direct thrombin inhibitor, is associated with similar efficacy and decreased bleeding risk as compared with unfractioned heparin (UFH) in patients at low to moderate risk undergoing percutaneous coronary intervention (PCI). Aim of this study was to evaluate the efficacy and safety of bivalirudin in patients at high risk of periprocedural ischemic and bleeding complications treated with PCI.

Methods: A total of 88 patients with age >75 years, chronic renal failure or diabetes mellitus scheduled to undergo PCI were randomized to receive bivalirudin (bolus … followed by infusion during the procedure; N=44) or unfractioned heparin (70 UI/kg; N=44). Creatine kinase MB (CK-MB), Troponin-I (TnI) and haemoglobin levels were measured at baseline and at 8 and 24 hours after intervention. Evaluation of entry-site complications was performed in all patients. Primary efficacy end-point was 30 day incidence of major adverse cardiac events (MACE): death, myocardial infarction (MI), target vessel revascularization (TVR) or ischemic stroke. Primary safety end-point was occurrence of bleeding complications after PCI.

Results: Primary efficacy end-point occurred in 13% of patients randomized to bivalirudin vs 6% of those in the heparin group (P= 0.50). No patients had catheter thrombosis. Patients with post-PCI elevation of cardiac markers above the upper normal limit were also similar in the 2 arms (CK-MB: 25% vs. 29%; P=0.8; TnI: 52% vs. 61%; P=0.5). No patient developed major bleeding. Patients in the bivalirudin group showed a trend towards reduction in minor bleedings (5% vs. 18%; P=0.09) and a significantly lower incidence of entry-site >10 cm haematomas (0% vs. 13%; P=0.034).

Conclusions: Use of bivalirudin compared with unfractioned heparin is associated with lower rates of entry-site bleeding complications in high risk patients undergoing PCI; efficacy of bivalirudin in this setting of patients needs to be evaluated after completion of the enrollment.

OXYGEN EXTRACTION IN CHF PATIENTS AFTER A PERIOD OF RESISTANCE TRAINING

C. Tarperi, A. Cevese, A. Baraldo

Università di Verona, Facoltà di Scienze Motorie, Verona.

Chronic heart failure (CHF) patients are generally discouraged from performing resistance training. As a consequence skeletal muscle atrophy which parallels the progressive impairment of heart function, worsens. We put CHF patients through a program of resistance training, and performed cardiovascular and skeletal muscle force tests before and after the training. Among other variables, we studied the maximal oxygen extraction, which reflects the muscle ability to utilise blood born oxygen.

Subjects: patients with an implanted automatic defibrillator were selected by the Division of Cardiology of the Civil Hospital in Verona.

Training: 12 subjects performed resistance training and 8 classical aerobic training. Initial loads were calculated as 80% 1RM, or 100% WVT (aerobic threshold), respectively. The loads were increased every fortnight by re-calculation of 1RM and by 5% of WVT. The physical activity had a cadence of 3 sessions (1h 30') per week and lasted 16 weeks. TESTING: Pre and post training evaluation included estimate of tissue oxygenation by near-infrared spectroscopy (NIRS - OxiplexTS, ISS, USA) during an incremental cycle ergometer test (10 W/min) to exhaustion. We considered the percent deoxy-hemoglobin concentration at peak work, relative to maximal deoxygenation after a 3 min total ischemia.

Results indicate an overall increase in force by 50% in resistance trained subjects. They also showed an increase in peak haemoglobin deoxygenation, reflecting an improved ability to extract oxygen from circulating blood. This was not seen in the aerobic training group.

We conclude that resistance training in strictly controlled conditions is feasible in CHF patients and leads to a remarkable increase in muscle strength. The NIRS results, although the mechanism involved may not be resolved by the method, indicate that a consistent peripheral adaptation takes place, that can result from augmented capillarity density, increased availability of mitochondrial enzymes, or both. Since this is not a generally recognised effect of resistance training, we may speculate that the isometric component also had a positive effect on the oxygen transport system (cardio-respiratory), thus increasing the availability of oxygen to exercising muscles, in aerobic condition.

SYNERGISTIC EFFECTS AGAINST POST-ISCHEMIC CARDIAC DYSFUNCTION BY SUB-CHRONIC NANDROLONE PRETREATMENT AND POSTCONDITIONING: ROLE OF BETA-2-ADRENORECEPTORS

C. Penna[1], G. Abbadessa, M. Mancardi, F. Tullio, F. Piccione, A. Spaccamiglio, S. Racca, P. Pagliaro

[1]Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Orbassano;
[2]Istituto Nazionale di Ricerche Cardiovascolari, Bologna.

Background: β-2-adrenoreceptor overexpression is beneficial against ischemia/reperfusion (I/R) injury. Whether β-adrenoreceptors are involved in postconditioning (PostC) is unknown. We have shown that sub-chronic nandrolone pretreatment induces cardiac β-2-adrenoreceptor overexpression without hypertrophy. However, other groups showed that nandrolone pretreatment induces cardiac hypertrophy and less resistance to I/R injury. We investigate whether nandrolone-decanoate (ND)-pretreatment can modulate (1) β-adrenoreceptor expression and (2) post-ischemic cardiac function in response to I/R and PostC.

Methods: Isolated rat hearts from ND-pretreated (15 mg/kg/day i.m., for 14 days) and untreated-animals underwent 30-min ischemia and 120-min reperfusion. In subgroups, at the beginning of reperfusion a PostC protocol (five cycles of 10-s reperfusion and 10-s ischemia) was applied. Left ventricular pressure (LVP) was measured with an electromanometer, and infarct-size was evaluated using nitro-blue-tetrazolium staining.

Results: ND-pretreatment increased β-2-adrenoreceptor expression, but did not alter cardiac-weight, LVP and maximum rate of increase of LVP (dP/dtmax). After I/R, infarct-size resulted smaller in ND-pretreatment than in untreated-animals. Infarct-size was also reduced by PostC, both in untreated and ND-pretreated animals. End-diastolic-LVP revealed that contracture was less marked in ND-pretreated animals. PostC reduced contracture in both ND-pretreated and untreated hearts. Moreover PostC improved post-ischemic recovery of developed LVP and dP/dtmax much more in hearts of ND-pretreated than untreated-animals.

Conclusions: These preliminary data show that two-weeks ND-pretreatment induces 1) an overexpression of β2-ARs without cardiac hypertrophy and 2) improves the post-ischemic diastolic and systolic cardiac function. Intriguingly, ND-pretreatment potentiates the improvement of systolic function induced by postconditioning.

MONITORING BLOOD FLOW IN THE MASSETERIC RAMUS OF THE FACIAL ARTERY, IN CONSCIOUS RABBIT: A MODEL FOR THE INVESTIGATION OF METABOLIC AND NEURAL REGULATION OF MUSCLE BLOOD

M. Mohammed, S. Roatta, M. Passatore

University of Torino, Department of Neuroscience, Physiology Section, Torino.

Background: The regulation of muscle blood flow and the competition between neural vasoconstriction and metabolic dilatation at rest and during exercise is still a matter of debate. In fact variable/conflicting results are obtained depending on different techniques adopted to measure or infer muscle blood flow, to induce or control muscle activity, and to exclude neural or metabolic regulatory pathways.

Aim: To present an experimental model allowing to measure muscle blood flow; 1) with high reliability and time resolution, 2) selectively from a pure muscle artery, 3) in a district allowing for easy denervation of the local sympathetic supply, 4) in a conscious animal.

Method: Chronic perivascular flow probes (0.7PSB Transonic, USA) are implanted bilaterally on the masseteric ramus of facial artery in rabbits weighing 2.4-2.7 Kg. The animals are also equipped with a chronic probe for telemetric arterial blood pressure measurement (PhysioTel PA-D70, DSI, USA) and with intramuscular electrodes for detection of electromyografic activity from the masseter muscle. The cervical sympathetic nerve could be unilaterally sectioned.

Results: Stable blood flow measurements could be obtained starting 4-5 days after surgery and some preliminary observations could be gathered. Average blood flow in resting conditions ranged between 0.2 and 0.4 ml/min,. Alerting stimuli/stressors, like airjet and box oscillations, decreased blood flow up to 50%; smaller effects were observed following unexpected noise and pinprick. Whenever transient episodes of EMG activity appeared during the trials, rapid vasodilatory responses (+ 200-400%, latency < 1s) were correspondently observed. Huge blood flow increases appeared during mastication that reached 4000 % of control when the rabbit chewed hard food.

Conclusions: The present model appears to be very reliable and potentially useful to clarify the complex interaction among neural and metabolic mechanisms controlling muscle blood perfusion in jaw muscles under physiological conditions. It allows to correlate local perfusion with systemic autonomic variables (blood pressure and heart rate) and to test the local efficacy of pharmacological interventions.

Supported by Regione Piemonte 2004, fellowship by San Paolo Bank for MM and by SIRC.

DETERMINAZIONE DEI POTENZIALI ELETTRICI RILEVABILI IN UN CIRCUITO SPERIMENTALE DI CEC: DATI PRELIMINARI SU SANGUE EPARINATO

C.M. Mazza[2], U. Carletti[1], D. Meglioli[2], T. Corazzari[2], R. Lodi[3], A.V. Mattioli[1]

[1]Università di Modena e R.E. Dip di Scienze Biomediche, Modena;
[2]Università di Modena e R.E. Fisica Medica, Modena;
[3]Università di Modena e R.E. Dottorato in Cardiochirurgia, Modena.

Obiettivi: In condizioni fisiologiche, lo scorrimento della componente corpuscolata del sangue all'interno dei vasi è assiale per effetto della interazione elettrostatica della carica elettrica negativa delle cellule e dell'endotelio, che contrasta l'adesione cellulare alla parete. Lo scorrimento assiale riduce la viscosità, soprattutto nel microcircolo. Questo studio preliminare ha valutato i parametri legati alla presenza di carica elettrica del sangue in un circuito di circolazione extracorporea (CEC).

Materiali e Metodi: Per rilevare la presenza di cariche elettriche nel circuito è stato progettato un dispositivo elettromagnetico, che riprende i principi dei sensori e dei trasduttori, ed opera nelle condizioni del trasporto collettivo di cariche in regime pulsato. Al moto collettivo stazionario delle cariche elettriche è teoricamente associata una differenza di potenziale, tra gli elettrodi applicati al condotto CEC, registrata con oscilloscopio a memoria. Sono stati testati diversi liquidi, acqua deionizzata (A) e sangue bovino con anticoagulante (B) e registrati i seguenti parametri: periodo di ripetizione δT degli impulsi (msec), ampiezza degli impulsi di differenza di potenziale (Volt) tra il liquido in moto e il potenziale di riferimento.

Risultati: Test effettuati a volumi crescenti di liquido hanno dimostrato che fino a 5 l/min la differenza di potenziale δ sovrapponibile nel gruppo A vs gruppo B (a 2 l/m 1.85 vs 1.30, a 3 l/m 2.01 vs 2.27 a 4 l/m 2.57 vs 3.06), oltre i 5 l/min il sangue presenta un segnale di ampiezza significativamente maggiore. Questo indica un aumento delle cariche elettriche nel fluido e non sulla superficie di contatto (a 5l/m 2.65 vs 3.19 a 6 l/m 3.44 vs 5.04 e a 7 l/m 3.59 vs 7.08 $p<0.001$). Analogo comportamento si osserva per il periodo di ripetizione δT che a 5 l/min è significativamente maggiore nel sangue rispetto all' acqua deionizzata.

Conclusioni: Per flussi superiori a 5 l/min, la parte corpuscolata del sangue subisce modificazioni conseguenti all'aumento delle cariche elettriche di superficie cellulare che possono influenzare lo scorrimento assiale del flusso e quindi favorire la trombogenesi. Diventa importante identificare tali alterazioni così da poter sviluppare materiali biocompatibili.

PATIENTS ON ANGIOTENSIN-CONVERTING ENZYME INHIBITORS HAVE IMPROVED OUTCOME AFTER PCI

F. Mangiacapra, G. Patti, E. Ricottini, V. Vizzi, P. Gallo, A. Nusca, M. Miglionico, R. Melfi, G. Di Sciascio

Department of Cardiovascular Sciences, Campus Bio-Medico University, Roma.

Background: In experimental models, angiotensin-converting enzyme inhibitors (ACE-I) have been demonstrated to protect myocardium from ischemia and to reduce microvascular injury during balloon angioplasty. In this observational study, we have investigated whether a chronic treatment with ACE-I may influence peri-procedural outcome in patients undergoing percutaneous coronary intervention (PCI).

Methods: A total of 423 consecutive patients undergoing PCI were analyzed. Creatine-kinase MB and Troponin I levels were measured at baseline and at 8 and 24 hours after intervention.

Results: Post-procedural peak levels of Troponin I were significantly lower in patients on ACE-I therapy (0.6±2.5 ng/ml vs. 1.7±7.8 ng/ml in those without ACE-I; P=0.016), whereas no difference was found in post-PCI creatine-kinase MB values (5.2±16.3 ng/ml vs. 4.6±9.2 ng/ml; P=0.720). Patients receiving different kinds of ACE-I had similar post-procedural release of myocardial markers (P=0.945).

Conclusions: In patients undergoing PCI, chronic treatment with ACE-I is associated with reduced minor peri-procedural myocardial damage, as assessed by Troponin elevation. This protective effect seems to be a class effect.

IS THERE A THRESHOLD VALUE OF PLATELET REACTIVITY DURING CLOPIDOGREL TREATMENT FOR IDENTIFYING PATIENTS WITH HIGHER RISK OF PERI-PROCEDURAL EVENTS DURING PCI?

V. Vizzi, G. Patti, F. Mangiacapra, L. Gatto, A. D'Ambrosio, A. Nusca, S. Mega, G. Di Sciascio

Department of Cardiovascular Sciences, Campus Bio-Medico University, Rome.

Background: Degree of clopidogrel responsiveness is relevant in influencing clinical outcome in patients undergoing percutaneous coronary intervention (PCI). Aim of this study was to identify a threshold value of residual platelet reactivity during clopidogrel treatment able to predict occurrence of peri-procedural myocardial infarction (MI).

Methods: A total of 223 patients receiving clopidogrel and undergoing PCI were prospectively enrolled. Platelet reactivity was measured before intervention as P2Y12 reaction units (PRU) by the VerifyNowTM P2Y12 assay. Creatine kinase-MB and Troponin-I levels were measured at baseline and at 8 and 24 hours after the procedure.

Results: Receiver-operating characteristic (ROC) curve analysis showed that PRU values significantly discriminate between patients with and without peri-procedural MI (area under the curve 0.69, 95% CI 0.56-0.81; P=0.016). A PRU value > 240 was identified as the optimal cut-off point to predict peri-procedural MI, with a specificity of 78% and a negative predictive value of 97%.

Conclusions: This study indicates that a pre-intervention PRU value > 240, as measured by a point-of-care assay, significantly predicts occurrence of peri-procedural MI in patients receiving clopidogrel and undergoing PCI. This threshold value may help identifying patients at higher risk in whom individualized antithrombotic strategies may be indicated to improve peri-procedural outcome in coronary intervention.

MEASUREMENT OF CLOPIDOGREL RESPONSIVENESS WITH VERIFY-NOW PREDICTS CLINICAL OUTCOME IN PATIENTS UNDERGOING PCI. RESULTS OF THE ARMYDA-PRO STUDY

V. Vizzi, G. Patti, F. Mangiacapra, L. Gatto, A. D'Ambrosio, A. Nusca, S. Mega, G. Di Sciascio

Department of Cardiovascular Sciences, Campus Bio-Medico University, Roma.

Background: Individual variability of clopidogrel response may influence results of percutaneous coronary intervention (PCI). Aim of this study was to prospectively evaluate the correlation of point-of-care measurement of platelet inhibition with clinical outcome in patients undergoing PCI.

Methods: A total of 160 patients receiving clopidogel pre-PCI were enrolled. Platelet reactivity was measured by the VerifyNowTM P2Y12 assay. Primary endpoint was 30-day occurrence of major adverse cardiac events (MACE) according to quartile distribution of P2Y12 reaction units (PRU).

Results: Primary end-point was significantly more frequent in patients with pre-procedural PRU levels in the 4th quartile vs those in the lowest quartile (20% vs 3%; P=0.034), and it was entirely due to peri-procedural myocardial infarction (MI). Mean PRU absolute values were more elevated in patients developing peri-procedural MI (258±53 vs 219±69 in patients without infarction; P=0.030) Multivariable analysis revealed that pre-PCI PRU levels in the 4th quartile were associated with a 6-fold increased risk of 30-day MACE (OR 6.1, 95% CI 1.1-18.3; P=0.033).

Conclusions: This prospective study indicates that high pre-PCI platelet reactivity may predict 30-day events. Use of a rapid, point-of-care assay for monitoring residual platelet reactivity after clopidogrel administration may help identifying patients in whom individualized antiplatelet strategies may be indicated with coronary intervention.

IS ADDITIONAL CLOPIDOGREL LOADING BEFORE PCI NECESSARY IN PATIENTS ON CHRONIC THERAPY? RESULTS OF THE ARMYDA-RELOAD RANDOMIZED TRIAL

F. Mangiacapra[1], G. Patti[1], L. Gatto[1], V. Pasceri[2], G. Colonna[3], A. D'Ambrosio1, A. Montinaro[3], G. Sardella[4], F. Ciccirillo[3], G. Di Sciascio[1]

[1]Department of Cardiovascular Sciences, Campus Bio-Medico University, Roma;
[2]Interventional Cardiology Unit, San Filippo Neri Hospital, Roma;
[3]Department of Cardiovascular and Respiratory Sciences, Interventional Cardiology Unit, Vito Fazzi Hospital, Lecce;
[4]Department of Cardiovascular and Respiratory Sciences, La Sapienza University, Roma.

Background: Laboratory evidence suggests that additional 600 mg clopidogrel loading further inhibits platelet function in patients already on chronic treatment; however, there are no clinical data on safety and efficacy of this strategy in patients undergoing percuteous coronary intervention (PCI).

Methods: A total of 436 patients on chronic (>10 days, 75 mg/day) clopidogrel therapy (38% with non ST-segment elevation ACS) were randomized to receive a further 600 mg loading dose of clopidogrel 4-8 hours before PCI (N=219) or placebo (N=217). Main end-point was 30-day incidence of major adverse cardiac events (MACE - death, myocardial infarction and target vessel revascularization) evaluated both in the overall population and in prespecified subgroups of clinical presentation.

Results: Overall, primary end-point occurred in 7% of patients in the reload vs 9% in the placebo arm (P=0.70) and was mainly due to peri-procedural myocardial infarction in either arm. In patients with stable angina, 1-month occurrence of MACE was not significantly different in the 2 arms (8% vs 4%; P=0.23); conversely, patients with ACS randomized to reloading had significant clinical benefit (MACE incidence: 7% vs 18%; Odds Ratio 0.36 at multivariable analysis, 95% CI 0.29-0.92; P=0.035). There was no excess bleeding in the reload arm (5% in both groups). Point-of-care aggregometry with the VerifyNowTM assay showed lower platelet reactivity during intervention in the reload vs the placebo arm (205±55 vs 227±85 P2Y12 Reaction Units; P=0.046) in patients with ACS.

Conclusions: The ARMYDA-RELOAD trial indicates that patients with stable angina on chronic clopidogrel therapy can safely undergo PCI without need of further reload; however, in patients with ACS, a 600 mg reload strategy can significantly improve outcome without increasing bleeding risk.

FOLLOW-UP EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH POST-ISCHEMIC CARDIOMYOPATHY UNDERGOING PCI: ROLE OF REAL TIME 3D ECHOCARDIOGRAPHY

R. Contuzzi, L. Gatto, C. Goffredo, G. Patti, G. Di Sciascio

Department of Cardiovascular Sciences, Campus Bio-Medico University, Roma.

Objectives: Usefulness of transthoracic 3D echocardiography in evaluating follow-up variations of left ventricular (LV) volumes and ejection fraction (LVEF) in patients with post-ischemic cardiomyopathy undergoing percutaneous coronary intervention (PCI) was assessed.

Methods: A total of 30 patients with significant coronary artery disease and depressed cardiac function (LVEF <45%) were prospectively enrolled to undergo 3D echocardiography at baseline (T0), and at 1 (T1) and 4 (T4) months after intervention. LV end-diastolic volume (EDV), end-systolic volume (ESV) and LVEF were measured.

Results: No adverse cardiac event occurred during follow-up in the study population. LVEF significantly increased at 1 and 4 months after the procedure compared with baseline ($P<0.001$), with a concomitant decrease of EDV and ESV ($P < 0.01$). A significant improvement of echocardiographic parameters was demonstrated both in myocardial wall segments related to the treated coronary artery ($P < 0.04$) and in those remote ($P < 0.017$) from the treated artery. In the subgroup of patients (N=7) with baseline LVEF <30%, LVEF improvement was early (+11.2% and +15.7% from baseline at 1 and 4 months, respectively; $P < 0.02$), whereas LV volumes reduction occurred later (EDV: -11.6 ml from baseline at 1 month, P=0.47, and -28 ml at 4 months, P=0.021; ESV: -20.4 ml from baseline at 1 month, P=0.06, and -35.8 ml at 4 months, P=0.001).

Conclusions: In patients with post-ischemic cardiomyopathy, PCI is associated with diffuse improvement of LV geometry and function during follow-up, as measured by a high sensitive ultrasound diagnostic technique.

IN-LAB CLOPIDOGREL LOADING VERSUS PRETREATMENT IN PATIENTS UNDERGOING PCI. FINAL RESULTS OF THE ARMYDA-5 RANDOMIZED TRIAL

L. Gatto[1], G. Patti[1], F. Mangiacapra[1], V. Pasceri[2], G. Colonna[3], G. Sardella[4], A. Montinaro[3], A. Tondo[3], G. Di Sciascio[1]

[1]Department of Cardiovascular Sciences, Campus Bio-Medico University, Roma;
[2]Interventional Cardiology Unit, San Filippo Neri Hospital, Roma;
[3]Department of Cardiovascular and Respiratory Sciences, Interventional Cardiology Unit, Vito Fazzi Hospital, Lecce;
[4]Department of Cardiovascular and Respiratory Sciences, La Sapienza University, Rome.

Background: Clopidogrel therapy improves procedural outcome in patients undergoing percutaneous coronary intervention (PCI); however, a strategy of clopidogrel pretreatment prior to coronary angiography may increase bleeding risk in those patients requiring urgent surgical revascularization. A high clopidogrel loading dose given in the cath-lab at the time of PCI (after coronary anatomy is known) may help avoid these risks, but it is unknown whether this strategy will maintain the same benefit of clopidogrel pretreatment several hours before the procedure.

Methods: Four-hundred-nine patients were randomized to receive a 600 mg clopidogrel loading dose 4-8 hours before PCI (preload group, N=204) or a 600 mg loading dose given in the cath-lab after coronary angiography, but prior to PCI (in-lab group, N=205). In patients with acute coronary syndromes (ACS) PCI was performed <8 hours from admission. Primary end-point was 30-day incidence of major adverse cardiac events (death, myocardial infarction or unplanned revascularization).

Results: Clopidogrel in-lab load was not inferior to a preload strategy with regard incidence of the primary end-point (8.8% vs 10.3%, P=0.017 for non-inferiority), which was mainly due to peri-procedural myocardial infarction in either arm (8.8% vs 9.3%, P=0.020 for non-inferiority). In the clopidogrel preload arm, no increased risk of bleeding/vascular complications was observed. Using VerifyNowTM assay, patients in the in-lab group showed higher platelet reactivity during PCI and at 2 hours after intervention vs those in the preload arm (P2Y12-Reaction-Units: 276±63 vs 250±75, P=0.043 and 245±72 vs 212±72, P=0.01, respectively)

Conclusions: ARMYDA-5 results indicate that a strategy of 600 mg in-lab clopidogrel load pre-PCI has no unfavourable influence on outcome vs. a 4-8 hours preload in patients with stable angina or ACS undergoing early intervention. When needed, in-lab clopidogrel administration can be a safe alternative to pretreatment given before knowing patients coronary anatomy.

IMPROVED CLINICAL OUTCOME WITH EPTIFIBATIDE IN PATIENTS UNDERGOING PCI: A META-ANALYSIS OF FIVE RANDOMIZED TRIALS

E. Ricottini[1], G. Patti[1], V. Pasceri[2], G. Di Sciascio[1]

[1]Department of Cardiovascular Sciences, Campus Bio-Medico University, Roma;
[2]Interventional Cardiology Division, San Filippo Neri Hospital, Roma.

Background: Randomized studies have demonstrated an overall improvement in clinical outcome in patients receiving eptifibatide in the setting of percutaneous coronary intervention (PCI); however, these studies were underpowered to detect differences in individual efficacy end-points, whereas discordant results have been reported regarding the possible increase in the bleeding risk.

Methods: Randomized trials comparing outcome of patients treated with eptifibatide vs placebo during PCI were included. The following 30-day end-points were analyzed: a) composite end-point of death or myocardial infarction; b) individual components of the composite end-point (death, myocardial infarction); c) repeat revascularization; d) major bleedings; e) minor bleedings. Data were extracted by 2 independent reviewers.

Results: A total of 5 trials, including 7524 patients, were found. Composite end-point occurred in 7% of patients receiving eptifibatide vs 10% of those receiving placebo (pooled OR 0.70, 0.59-0.83; P<0.0001). In the eptifibatide group, there was a non significant mortality reduction (0.7% vs 1% in the placebo group, OR 0.78, 0.47-1.29; P=0.33), and a significantly lower incidence of myocardial infarction (27% risk reduction; OR 0.73, 0.61-0.87; P=0.0004) and repeat revascularization (27% risk reduction, OR 0.73, 0.56-0.97; P=0.03). Occurrence of major bleedings was not different in the 2 arms (4% eptifibatide vs 3% placebo, OR 1.17, 0.87-1.56; P=0.29), whereas the eptifibatide group had a higher risk of minor bleedings (OR 1.55, 1.36-1.77; P<0.0001).

Conclusions: An extensive peri-procedural use of eptifibatide significantly reduces early major coronary events in patients undergoing PCI, without increased risk of major bleeding.

INHIBITION OF CLASS I HISTONE DEACETYLASES WITH AN APICIDIN DERIVATIVE PREVENTS CARDIAC HYPERTROPHY AND FAILURE: IN VITRO AND IN VIVO DATA

P. Gallo[1], P. Gallo[2], M.V.G. Latronico[2], S. Grimaldi[2,3], M. Todaro[4], P. Jones[4], K. Steikuhler[4], M. Todaro[4], G. Di Sciascio[1], G. Condorelli[2,3]

[1]Department of Cardiovascular Science, Campus Bio Medico University, Rome;
[2]Laboratory of Genetic and Molecular Cardiology, Scientific and Technology Pole, IRCCS MultiMedica, Milan;
[3]Laboratory of Molecular Cardiology, San Raffaele Science Park Foundation, Rome;
[4]Istituto di Ricerche di Biologia Molecolare P. Angeletti, IRBM-Merck Research Laboratories, Rome.

Aims: Recent studies have demonstrated the importance of chromatin remodeling via histone acetylation/deacetylation for the control of cardiac gene expression. Specific histone deacetylases (HDACs) can, in fact, play a positive or negative role in determining cardiac myocyte (CM) size. Here, we report on the effect on hypertrophy development of three inhibitors (HDACi) of class I HDACs.

Materials and Methods: we used three HDAC inhibitors, MS27-275, PXD-101 and API-D, were chosen based on suitable pharmacokinetic properties and bioavailability for in vivo studies. For the in vitro studies hypertrophy was induced by the addition of 50ìM phenylephrine (PE) and treated with HDACi. Samples were analyzed by Western Blotting RNA Extraction, Real-Time PCR and lactate dehydrogenase (LDH) release and terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) analysis. Echocardiographic and invasive hemodynamic assessment of cardiac function were performed in vivo after thoracic aortic constriction (TAC) and intra peritoneal administration of the drug in nine week old mice.

Results: The compounds were first analyzed in vitro by scoring hypertrophy, expression of fetal genes, and apoptosis of neonatal rat CMs stimulated with phenylephrine (PE), an á1-adrenergic agonist. This initial screening indicated that a truncated derivative of apicidin with class I HDAC specificity, denominated API-D, had the highest efficacy to toxicity ratio, and was thus selected for further analysis in vivo. Administration of this drug significantly decreased myocardial hypertrophy and fetal gene expression after 1 week of pressure overload induced by TAC in mice. After 9 weeks of TAC, when manifest heart failure is encountered, mice treated with APID presented with significantly improved echocardiographic and hemodynamic parameters of cardiac function when compared to untreated TAC-operated mice

Conclusions: The apicidin derivative, API-D, is capable of reducing hypertrophy and, consequently, the transition to heart failure in mice subjected to TAC. Treatment with this substance, therefore, holds promise as an important therapeutic option for heart failure.

THE PROTEIN SYNTHESIS AND T-CAP TRANSLATIONAL CONTROL BY AKT/MTOR/4E-BP1 SIGNAL PREVENT HEART FAILURE IN PRESSURE OVERLOAD END DILATED CARDIOMYOPATHY ANIMAL MODEL

P. Gallo[1], D. Catalucci[3], M.L. Bang[3], M. Ceci[2], G. Di Sciascio[1], G. Condorelli[2,3]

[1]Department of Cardiovascular Science, Campus Bio-Medico University, Rome;
[2]San Raffaele Biomedical Science Park, Rome;
[3]I.R.C.C.S. Multimedica, Milan.

Aims: Idiopathic dilated cardiomyopathies (DCM) are caused by single gene mutations, often in a component of the mechanotransduction machinery. Muscle LIM protein (MLP) is a component of the Z disk, the cytoskeletal structure of the cardiomyocyte (CMC) which defines the lateral boundaries of the sarcomere. MLP gene mutation induces DCM in mice and in humans. Recent studies suggested the role of the IGF-1/PI-3K/Akt pathway in myocardial adaptation to physical stress.

Materials and Methods: we used MLP-/- mice as model for DCM and E40K-AKT transgenic mice, overexpressing an active form of AKT. Cardiac function was evaluated by echocardiography and hemodynamic assessment. Immunofluorescence, electrophoresis and immunoblotting were used for assessing the T-CAP protein, Akt and mTOR activities.

Results: Fractional shortening (FS) and inotropic function was dramatically increased in E40K/MLP-/- mice as compared to MLPKO mice, which show severe contractile impairment. Other parameters, such as interventicular septum, LV posterior wall thickness and LV mass index were increased in Akt E40K/MLP-/- mice compared to WT. In Akt E40K/MLP-/- mice basal LV dP/dt max and cardiac function after increasing doses of dobutamine were greatly improved (FS% 42,04 in Akt E40K/MLP-/-, vs 22,85 in MLPKO, $p<0.0001$; Max dP/dT at rest: 8353 Akt E40K/MLP-/- vs 2800 mmHg in MLPKO, $p<0.05$). HW/BW ratio was increased in Akt E40K/MLP-/- mice compared to WT and MLP KO mice. In E40K/MLP-/- mice, the expression levels of three embryonic cardiac genes (βMyosin Heavy Chain, skeletal actin and Atrial Natriuretic Factor) were dramatically decreased as compared to MLPKO mice. Myocardial tissue from E40K/MLP-/- mice showed a decreased accumulation of collagen, increased cellularity and increased cell size; electron microscopy analysis of myocardial tissue showed the normalization of the Z disk structure, remarkably altered in MLP-/-myocytes

Discussion: In MLP-/-/E40K-AKT mice, the mTOR signal activated by E40K-AKT induced the increase T-CAP protein, which is profoundly decreased in MLP-/- mice. Moreover, the cardiac hypertrophy, determined by an increase in protein synthesis, reduced the diastolic wall, maintaining T-CAP protein in the myofilament fraction and associated it with Titin. This molecular mechanism favoured the Z disks reorganization and prevented the onset of the dilated cardiomyopathy of the MLP KO mice.

HEAD-UP TILT TEST IN PAZIENTI CON SINCOPE: FOLLOW-UP A LUNGO TERMINE

G. Domenichini, I. Diemberger, C. Valzania, M. Bertini, D. Saporito, M. Ziacchi, A. Marziali, C. Martignani, M. Biffi, G. Boriani

Istituto Di Cardiologia, Bologna.

Questo studio ha valutato la predittività del risultato dell'head-up tilt test (HUTT) in termini di recidive sincopali, analizzando pazienti (pz) sottoposti a HUTT per sincope inspiegata o sospetta neuromediata, e ha cercato di identificare parametri clinici predittivi di recidiva, sia nel gruppo di pz con HUTT positivo che nell'intera popolazione sottoposta a HUTT.

Sono stati inclusi 107 pz (56 uomini, 51 donne; mediana dell'età 51.4 ± 20.2 anni). L'HUTT prevedeva una fase passiva (angolo di 70° per 25 minuti) seguita, se negativa, da una fase attiva con somministrazione di isoproterenolo o di isosorbide dinitrato. La mediana del follow-up (f-up) è stata di 3390 giorni (range 216-4838).
76 pz hanno presentato una risposta positiva (vasodepressiva in 58, mista in 13 e cardioinibitoria in 5). Il 31.8% di tutti i pz ha avuto recidive di sincope al termine del f-up. A distanza di 1 anno dall' HUTT l'80.8% di tutti i pz è risultato libero da recidiva; a distanza di 10 anni il 67.3%. La mediana del tempo libero da recidiva è stata di 360 giorni (range 5-4099). All'analisi di Kaplan-Meier non sono emerse differenze statisticamente significative, in termini di recidive, quando sono state valutate le risposte all'HUTT (positiva/negativa), i vari tipi di risposta positiva ed il timing della risposta positiva (fase passiva/attiva). All'analisi multivariata un numero di sincopi > 4 nei 12 mesi antecedenti l'HUTT è risultato un fattore di rischio per recidiva sia nei pz con HUTT positivo ($p = 0.028$; HR: 1.83; CI (95%) = 1.07-3.17) che in tutti i pz sottoposti a HUTT ($p = 0.023$; HR: 1.7; CI (95%) = 1.07-2.69).

In questo studio è stato possibile evidenziare la scarsa predittività dei risultati dell'HUTT in termini di recidive sincopali, che appaiono essere associate alla storia stessa della sincope. Infatti un numero di sincopi > 4 nei 12 mesi antecedenti l' HUTT risulta un importante fattore di rischio di recidiva sia nei pz con HUTT positivo, che in tutti i pz sottoposti a HUTT. L'HUTT nella pratica clinica appare quindi indicato a scopi diagnostici (conferma della genesi neuromediata), ma non sembra rivestire un particolare ruolo in termini prognostici.

EFFETTI DELLA RESINCRONIZZAZIONE VENTRICOLARE SUL FLUSSO MUSCOLARE PERIFERICO ALLA LUCE DEL RIMODELLAMENTO VENTRICOLARE INVERSO

I. Diemberger, I. Corazza, J. Frisoni, C. Martignani, E. Cervi, A. Marziale, G. Domenichini, M. Biffi, R. Zannoli, G. Boriani

Istituto di Cardiologia prof. A. Branzi; Università degli Studi di Bologna, Bologna.

Obiettivi: Valutare l'effetto della stimolazione biventricolare (sBIV) sul flusso muscolare a riposo (FMR) e relazioni con il rimodellamento ventricolare inverso.

Materiali e Metodi: In 33 candidati ad impianto di defibrillatore con sBIV si è proceduto, all'impianto e a 3-mesi, a valutazione del FMR (mL/100mL/min) mediante pletismografia di occlusione (prima/dopo attivazione della sBIV) e a valutazione dei volumi ventricolari sinistri e dei parametri emodinamici. Sono stati definiti responders (per positivo rimodellamento ventricolare) i pazienti con riduzione del volume telesistolico del ventricolo sinistro >/=15%. Non presentando tutte le variabili una distribuzione normale, si è provveduto ad esprimerle con mediana e range-interquartile (riq) e ad analizzarle con test non-parametrici.

Risultati: i pazienti presentavano di base un significativo incremento del FMR durante sBIV rispetto alla valutazione a funzione inattiva (4,7 riq 3,0-5,9 vs. 3,9 riq 2,8-5,0; p<0,001). Dopo 3-mesi dall'impianto si confermava l'incremento del FMR durante sBIV (5,7 riq 4,1-7,5 vs. 4,0 riq 2,5-5,5; p<0,001), significativamente superiore rispetto ai valori di base (p=0,01). Non vi erano differenze significative nelle valutazioni con sBIV inibita (p=0,224). Si è osservato un positivo rimodellamento a 3-mesi, con riduzione dei volumi (telediastolico: 196 ml riq 138-265 vs. 220 ml riq 193-270, p=0.001; telesistolico: 140 ml riq 84-206 vs. 170 ml riq 138-214, p=0.001) e ad un aumento della frazione d'eiezione (30% riq 24-37 vs. 23% riq 20-25; p<0.001), in assenza di un miglioramento dell'indice cardiaco (2,2 l/min/mq riq 1,8-2,5 vs. 2,1 l/min/mq riq 1,7-2,5; p=0,76). È interessante notare come a fronte di un significativo incremento percentuale dell'indice cardiaco (18% riq -10-38% vs. -5% riq -22%-5%; p=0,04) presentato nei responders a 3-mesi (n=19) non si sia verificato un significativo miglioramento del FMR sia in termini assoluti a 3-mesi (indipendentemente dall'attivazione della sBIV), che di incremento percentuale (vs. non-responders).

Conclusioni: il pacing biventricolare si associa ad un incremento del FMR sia in acuto che a medio termine. Tale effetto si accresce nel tempo durante sBIV ma non in condizioni di base. Il miglioramento del FMR appare indipendente dalla presenza di rimodellamento ventricolare inverso e al miglioramento dell'emodinamica.

MODERATE-SEVERE MITRAL REGURGITATION IS INDEPENDENT PREDICTOR OF CLINICAL IMPAIRMENT IN II NYHA CLASS CHRONIC HEART FAILURE PTS WITH VARIOUS ETIOLOGY AND PHYSIOPATHOLOGY

R. De Vecchis[1], C. Cioppa[1], A. Giasi[1], A. Pucciarelli[2], S. Cantatrione[1]

[1]Cardiology Elena d'Aosta Intermediate Infirmary, Napoli;
[2]Department of Cardiology, University of Naples Federico II Medical School, Napoli.

Background: The passage from II to III New York Heart Association (NYHA) class is indicative of cardiopulmonary impairment and unfavourable prognosis.Among chronic heart failure (CHF) II NYHA class pts, the topic has been debated what criteria have be assumed for identifying the pts prone to accelerated progression towards III NYHA class.

Methods: 58 pts,70±12a, whose 28 females, were enrolled, and subjected to clinical follow-up with regular echographic assessment over two years; all were treated with pharmacologic therapy, according to their respective clinical features and typology of basal heart disease. The pts were subdivided in 3 categories, as follows:1) diastolic CHF, i.e. heart failure with normal ejection fraction(HFNEF)-20pts-2) systolic CHF without mitral regurgitation(MR) or with only mild MR(CHF MR-neg)-19 pts- 3)systolic CHF with moderate or severe MR(CHF MR-pos)-19 pts-.The sensitivity,specificity,positive predictive value(PPV),negative predictive value(NPV)for the composite endpoint death and hospitalization due to worsening CHF were investigated,concerning each of the following ultrasonographic criteria: I) EF<40% II)moderate or severe MR III) restrictive mitralic pattern IV)pulmonary systolic arterial pressure (PAP)>40mmHg. Moreover, the hazard ratio(HR)was calculated,by Cox multivariate model,to achieve information about risk of death and/or worsening CHF, descending from each of three types of CHF and four echographic patterns included in our case record, as well as the respective profiles of risk,assessed by relative risk(RR)and absolute(ARR) and relative(RRR)reduction of risk.

Results: 15 (26%) pts showed clinical deterioration,requiring one hospitalization at least over two years,due to breathlessness (9 pts) and/or generalized edema(6 pts). Noteworty, the moderate or severe MR only seemed to play the role of independent predictor for worsening CHF (sensitivity: 66%; specificity: 70%; PPV: 43,5%; NPV: 85,7%; RR:3,1; OR:4,6; ARR:-29,3%; RRR:-67,3%); likewise,CHF MR-pos was identified as the class at highest risk of adverse clinical outcome (HR:3,3 95%IC:1,7-6,5).On the contrary, a poor predictive value was exhibited by each of the other six variables analyzed by Cox model.

Conclusions: The chances of successful pharmacological prevention of clinical impairment in CHF MR-pos pts, II NYHA class, seem to be slight.Likewise, the sensitivity and PPV, shown by moderate or severe MR, for prediction of worsening CHF, exceed far and away the values of sensitivity and PPV associated to each of the other echographic variables of our study, as depressed (<40%) EF or restrictive mitralic pattern or increased(>40 mm Hg) PAP.

THE FUROSEMIDE SINGLE-DOSE TEST PROVIDES PROGNOSTIC INFORMATION ABOUT RISK OF CARDIORENAL SINDROME IN CHRONIC CONGESTIVE HEART FAILURE WITH MARKED HYDROSALINE RETENTION

R. De Vecchis[1], C. Cioppa[1], A. Giasi[1], A. Pucciarelli[2], S. Cantatrione[1]

[1]Cardiology Elena d'Aosta Intermediate Infirmary, Napoli;
[2]Department of Cardiology, University of Naples Federico II Medical School, Napoli.

Background: The intensive iv diuretic therapy can be associated in chronic heart failure (CHF) pts to acute renal dysfunction (ARD)- especially in the presence of the maximal ACE-inhibition -and increase in mortality, usually arrhythmic.Moreover, the standard deviation of RR normal intervals (SDNN) has been recognized as independent predictor of mortality from all causes in CHF pts with ejection fraction of left ventricle (LVEF)<45%.Therefore, we have investigated the relationship between LVEF and reaction of SDNN and serum creatinine(cr) to single dose of 250 mg iv of furosemide (fur) for better selection of CHF pts eligible for iv intensive diuretic therapy.

Methods: 57 pts, III NYHA class, with marked signs of hydrosaline retention (anasarca), were subdivided in 3 subsets: 1) LVEF>50%-10 pts- 2) subnormal LVEF(50-40 %) -27 pts- and 3) depressed LVEF(40-30%)-20 pts-.All pts were treated with iv fur at dose of 250 mg (0,25 mg / min).SDNN was measured in all pts with two 12 h Holter registrations, before and after fur. A control (C)group of 13 pts, whose the clinical and echografic pattern was similar to former, received a drip of saline, aimed to investigate spontaneous changes in SDNN or cr. The remaining therapy (ACE-inhibitor,inotropic iv support with dobutamine and dopamine at low doses, started 12 h before drip of fur or saline, and prolonged up to end of their infusion) was identical . ARD was defined as acute decrease ->25% in cr, found after saline or fur.

Results: A significant reduction in SDNN values, fur-related, was found; moreover, a significant positive correlation ($r = 0, 84$; $p < 0,001$) was identified by comparing LVEF with decrease in SDNN after drip of fur. 12 pts (21 %) exhibited ARD versus 0 cases in C. The risk of ARD, fur-related, resulted associated with normal systolic left ventricular function (HFNEF)-Odds Ratio (OR):10,27 95% CI : 2,23-47,18 -,while the location in the most severely (39-30%) depressed ejection fraction's class seemed to denote a trend towards the role as protective factor -OR:0,124 95% CI: 0,015-1,05 .

Conclusions: HFNEF appears at higher risk of ARD, and associated with more pronounced fall in SDNN, fur-related, compared to CHF with reduced LVEF.

IMPROVEMENT OF SKELETAL MUSCLE FUNCTIONAL PERFORMANCE IN AGED RATS AFTER AMINO - ACID ORAL SUPPLEMENTATION: INCREASED RATE OF ATP PRODUCTION AND PROTEIN AVAILABILITY

R. Raddino[1], I. Bonadei[1], G. Caretta[1], M. Teli[1], D. Robba[1], G. Zanini[1], M.T. Scarabelli[2], O. Visioli[3]

[1]Sezione di Malattie Cardiovascolari, Dip.to Medicina Sper.le Applicata, Brescia, Italy;
[2]Center for Heart and Vesse Preclinical Studies, St. John Hosp. Med.Center Wayne State Univ. School, Detroit, Michigan, USA;
[3]Istituto Nazionale di Ricerche Cardiovascolari (I.N.R.C.), Bologna, Italy.

Sarcopenia is an inevitable age-related degenerative process chiefly characterized by decreased synthesis of muscle proteins and impaired mitochondrial function, leading to progressive loss of muscle mass.

Here, we sought to probe whether long-term administration of oral amino acids (AAs) can increase protein and adenosine triphosphate (ATP) content in the gastrocnemius muscle of aged rats, enhancing functional performance. To this end, 6- and 24-month-old male Fisher 344 rats were divided into 3 groups: group A (6-month-old rats) and group B (24-month-old rats) were used as adult and senescent control group, respectively, while group C (24-month-old rats) was used as senescent treated group and underwent 1-month oral treatment with a mixture of mainly essential AAs. Untreated senescent animals exhibited a 30% reduction in total and fractional protein content, as well as a 50% reduction in ATP content and production, compared with adult control rats ($p < 0.001$). Long-term supplementation with mixed AAs significantly improved protein and high-energy phosphate content, as well as the rate of mitochondrial ATP production, conforming their values to those of adult control animals ($p < 0.001$). The improved availability of protein and high-energy substrates in the gastrocnemius muscle of treated aged rats paralleled a significant enhancement in functional performance assessed by swim test, with dramatic elongation of maximal exertion times compared with untreated senescent rats ($p < 0.001$). In line with these findings, we observed that, after 6 hours of rest following exhaustive swimming, the recovery in mitochondrial ATP content was approximately 70% in adult control rats, approximately 60% in senescent control rats, and normalized in treated rats as compared with animals of the same age unexposed to maximal exertion ($p < 0.001$).

In conclusion, nutritional supplementation with oral AAs improved protein and energy profiles in the gastrocnemius of treated rats, enhancing functional performance and accelerating high-energy phosphate recovery after exhaustive exertion.

ANTI-APOPTOTIC EFFECTS OF CYTOKINES ON CULTURED MESENCHYMAL STEM CELLS

M.G. Perrelli[1,2], C. Penna[1], S. Raimondo[1], A. Merlino[1], S. Geuna[1], P. Pagliaro[1,2]

[1]Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Orbassano;
[2]Istituto Nazionale di Ricerche Cardiovascolari, Bologna.

Background: Cytokines, a group of low-molecular-weight polypeptides, play a crucial role in inflammatory reactions during myocardial ischemia/reperfusion injury. Elevated interleukin-6 (IL-6) and Tumor Necrosis Factor á (TNFα) levels have been observed in patients with acute myocardial infarction (AMI). However, low doses of IL-6 and TNFα activate signaling pathways involved in preconditioning. In fact, IL-6 and TNFá induce a PI3-kinase and NO-dependent protection of cardiomyocytes, which is associated with improvement in mitochondrial Ca2+ handling. Injection of stem cells (SCs) has been proposed as new therapy against AMI. However, in the infarcted area many injected SCs are apoptotic. In this preliminary study we investigated the action of IL-6 and TNFá in the apoptotic death of mesenchymal SC (MSCs).

Methods: MSCs were isolated from the bone marrow of femurs and were cultured in complete αMEM (20% FBS) at 37°C and 5% CO2. MSCs were then exposed to IL-6 (1-10ng/ml) and TNFα (0.5-1ng/ml) for 9 days. We performed western blotting analysis for Bcl-2, cytochrome c and total GSK-3β. The effect of IL-6 and TNFα on the cellular vitality was tested with the growth curve. Parameters were studied at 0, 3, 6 and 9 days.

Results: While treatments with IL-6 and TNFá induced a time-dependent increase of Bcl-2, the other studied proteins were not changed during the observation time. Treatments at both concentrations of either IL-6 or TNFα were able to induced a rise in growth of MSCs both at 6th day and 9th day of observation.

Conclusions: These preliminary results suggest a relationship between these cytokines and Bcl-2. Bcl-2 is one of the most important factors involved in anti-apoptotic pathways in different cellular model. Our data underline an involvement of IL-6 and TNFα in the activation of Bcl-2 and MSCs survival. Injection in the infarcted myocardium of MSC pre-treated with low doses of IL-6 or TNFα could be considered a useful approach to minimize apoptotic death of these cells.

FIBRIN GEL: A NEW SCAFFOLD FOR CARDIOVASCULAR APPLICATIONS

A. Magera[1], M.C. Barsotti[1], M. Lemmi[1], E. Simonetti[1], F. Chiellini[2], A. Minnocci[3], R. Solaro[2], G. Soldani[4], A. Balbarini[1], R. Di Stefano[1]

[1]Dip. Cardio-Toracico Vascolare, Università di Pisa, Pisa;
[2]Dip.di Chimica e Chimica Industriale, Università di Pisa, Pisa;
[3]BioLabs, Scuola Superiore S. Anna, Pisa;
[4]Laboratorio di Biomateriali, Istituto di Fisiologia Clinica CNR, Massa.

Aims: Peripheral blood endothelial progenitor cells (EPC) are promising therapies for irreversible myocardial damage, heart failure and peripheral ischemia disease. Natural biopolymers as fibrin are appealing in tissue engineering, because fibrin is biocompatible and bioresorbable. In vitro studies indicate that fibrin can support the growth and proliferation of several cells types. No studies are available with fibrin as scaffold for EPC.
The goal of this study was to investigate if fibrin is a suitable matrix for EPC culture as compared with fibronectin and if different concentrations of fibrinogen (Fb) and thrombin (Th) can influence fibrin structure and EPC behaviour.

Methods: Fibrin was prepared mixing Fb (final 4.5-9-18-36 mg/ml) and Th (final 6-12.5-25-50 U/ml).Scaffolds were maintained for 1 hour at 37°C, 5% CO2 before cell seeding. The ultrastructure of fibrin was investigated by scanning electron microscopy (SEM), cryogenic SEM (CRYO-SEM) and atomic force microscopy (AFM). EPC were obtained from peripheral blood of healthy donors and cultured for 1 week on fibrin. EPC seeded on fibronectin were used as control. Metabolic cell activity on the different scaffolds was assessed after 7 and 14 days by WST1 while cell viability by confocal microscopy (Calcein AM incorporation).

Results: Fibrin polymerization rate ranged between 17 and 68 seconds and increased at higher Fb or Th concentrations. Both AFM and SEM analysis revealed a nanometric fibrous structure, with a decrease in fiber diameter with higher fibrinogen concentrations (4.5 mg/ml: 166±4 nm. vs. 36 mg/ml: 119±3 nm, $p<0.005$, $n=5$). Different concentrations of Th didn't affect fibre diameter and density. CRYO-SEM suggested a reticulate structure with mesh-size up to 10µm. WST1 assay showed that EPC metabolic activity was better with lower fibrinogen concentrations (4.5 mg/ml: 0.890±0.134 a.u. vs. 36 mg/ml 0.234±0.046 a.u., $p<0.05$, $n=5$), while Th had no significant effect. Calcein staining demonstrated that EPC were viable at 14 days and even organised in cluster.

Conclusions: Fibrin combines important properties of an ideal biological scaffold, like the nanometric structure, important for the growth and migration of cells. Fibrin is also an ideal scaffold for EPC but the ratio between fb and th is important for cell viability.

DEVELOPMENT OF A NEW TECHNOLOGY FOR 3-D NANOSTRUCTURED SCAFFOLDS WITH POTENTIAL CARDIOVASCULAR APPLICATIONS

M. Lemmi[1], E. Briganti[2], A. Magera[1], R. Arici[1], C. Ristori[1], E. Simonetti[1], F. Chiellini[3], G. Soldani[2], A. Balbarini[1], R. Di Stefano[1]

[1]Dip.to Cardiotoracico e Vascolare Università di Pisa, Pisa;
[2]Laboratorio di Biomateriali, Istituto di Fisiologia Clinica CNR, Massa;
[3]Dipartimento di Chimica e Chimica Industriale Università di Pisa, Pisa.

Aims: The in situ release and maintaining of cells to promote revascularization is a new goal of cardiovascular therapy. Endothelial progenitor cells (EPC) may contribute to the process of vascular repair. Medical devices realized according to tissue engineering are composed by a cellular component and by an artificial component, made of a biocompatible polymer. Scaffolds may be coated with bio-polymers like fibrin to enhance cell adhesion and growth.
Aim of this study was to realize nanocomposite 3D scaffolds composed by a synthetic polymer coated with fibrin to support EPC growth and to promote in vivo angiogenesis.

Methods: PEtU-PDMS scaffolds were studied in vitro for their biocompatibility (viability and proliferation tests; citokine release). In vivo biocompatibility was studied by intramuscular implant in a rabbit model. The scaffolds were fabricated by spray-phase inversion technique. 25U/mL thrombin was sprayed during the fabrication process. The scaffold morphology was analysed by stereomicroscopy and by scanning electron microscopy (SEM).
EPC obtained from peripheral blood were cultured on 3D-scaffolds. Fibronectin coating was used as control. Cell viability was assessed by confocal laser microscopy (Calcein-AM incorporation).
To test in vivo angiogenesis, EPC-seeded scaffolds were subcutaneously implanted into the back of rats for 14 days. After harvesting, the scaffolds were examined histologically and immunohistochemically.

Results: In vitro and in vivo biocompatibility data demonstrated absence of any citotoxic effect, immunocompatibility and a slight inflammatory reaction without any sign of encapsulation and implant rejection. Morphological analyses showed an homogeneus fibrin coating of the scaffolds, tightly bound and interconnected to the PEtU-PDMS surface. SEM showed the presence of a well organized layer of fibres in a nm scale (mean diameter ~140nm). Cell viability and phenotype were not affected when EPC were seeded on 3D-scaffolds. The histological observation of explanted scaffolds revealed a slightly inflammatory response and a significant increased numbers of neovessels in tissues surrounding the EPC-seeded scaffold as compared to the scaffold without cells.

Conclusions: Our data suggest that 3D-scaffold obtained with a new spray manufacturing technology can support in vitro EPC growth and promote in vivo neovascularisation. Further studies are currently under way in an ischemic hindlimb rat model.

THE ABUNDANCE OF ADIPOSE TISSUE-DERIVED PROGENITOR CELLS IN THE ADIPOSE TISSUE IS AFFECTED BY AGE AND BLOOD GLUCOSE LEVELS

R. Madonna[1], C. Cellini[2], F. Renna[1], L. Rinaldi[1], R. Ippedico[1], C. Palmieri[2], R. Cotellese[2], N. Picardi[2], R. De Caterina[1]

[1]Cardiology Division, Center of Excellence on Aging, G. d'Annunzio University, Chieti;
[2]Department of Surgical Science, G. d'Annunzio University, Chieti.

Adipose tissue-derived stromal cells (ADSCs) are being recognized as a source of stem cells potentially useful for cardiovascular repair. The influence of aging on ADSC levels and their viability in elderly patients are currently unknown. We analyzed the abundance and angiogenic activity of adipose tissue-derived progenitor cells in patients most likely to benefit from this novel source of stem cells, i.e., elderly patients in a general adult population.

Methods: 40 subjects (age 67.9 ± 11.7 years) with variable degrees of cardiovascular risk, underwent abdominal surgery for intercurrent diseases. Visceral adipose tissue (3±1 g visceral fat/patient) was processed by collagenase type I to obtain ADSCs from the stromal-vascular fraction. The number of total ADSCs in primary cultures after digestion was automatically quantified by a Multisizer Coulter Counter. Progenitor cells (PCs) within ADSCs were quantified by flow cytometry as %CD45-/CD34+/CD133+ cells of total ADSCs. Matrigel angiogenesis assay was used to analyze the ability of ADSC to form tubes or networks.
Increasing age ($r = -0.48$, $p<0.05$) and blood glucose levels ($r = -0.35$, $p<0.05$) significantly and inversely correlated with the abundance of adipose tissue-derived PC. We found however no correlations between number of PC or total ADSCs and other quantitative parameters such as total cholesterol ($r = 0.12$; $p=0.45$), LDL cholesterol ($r = 0.15$; $p=0.6$), HDL cholesterol ($r = -0.17$; $p=0.27$), waist circumference ($r = -0.77$; $p=0.63$), body mass index ($r = -0.12$; $p=0.44$), systolic ($r = 0.48$, $p= 0.77$) and diastolic ($r = 0.144$; $p = 0.375$) arterial pressure. No associations were found between number of PC or total ADSCs and physical exercise, gender and family history for coronary heart disease. In Matrigel angiogenesis assays, increasing age ($r = -0.29$, $p = 0.05$) and body mass index ($r = -0.31$, $p=0.05$) exhibited some reduction of ADSC-derived tubulization.

Conclusions: Aging and impaired glucose regulation may alter the availability of adipose tissue-derived progenitor cells and their angiogenic functional capacity. Such changes may impair the use of adipose tissue as source of autologous progenitor cells in elderly patients.

HIGH-LEVEL TRANSDUCTION AND GENE EXPRESSION OF EGFP REPORTER GENE IN ADIPOSE TISSUE-DERIVED STROMAL CELLS USING A HIV TYPE 1 -BASED LENTIVIRAL VECTOR

R. Madonna, R. De Caterina

Cardiology Division, Center of Excellence on Aging, G. d'Annunzio University, Chieti.

Background and Objective: Heart transplantation of human adipose tissue-derived stromal cells (ADSCs) is under evaluation for cardiac repair. To test the in vivo regenerative potential of ADSCs, their fate needs to be traced after delivery in in vivo models.
In this study, we have tested a vesicular stomatitis virus G envelope protein (VSV-G)-pseudotyped human immunodeficiency virus type 1 (HIV-1) lentiviral-based vector system expressing the Emerald Green Fluorescence Protein (EGFP) reporter gene to label ADSCs.

Methods and Results: Human visceral adipose tissue was processed with collagenase I to obtain ADSCs from the stromal-vascular fraction. To enhance the level of EGFP gene expression in ADSCs, we used a lentiviral vector incorporating the spleen focus-forming virus (SFFV) long terminal repeat (LTR) sequences, and the Woodchuck hepatitis virus posttranscriptional regulatory element (WPRE). To increase transduction efficiency we compared three different protocols of infection, namely transduction with non-concentrated lentiviral supernatants or separated supernatant or pellet fractions after ultracentrifugation. Transduction efficiency and transgene expression levels in ADSCs were analyzed by quantitative flow cytometry at 5 and 28 days after transduction.
Of the three protocols tested, transduction of ADSCs with pellet after ultracentrifugation provided the highest rate of transduction (flow cytometry estimated titer: 6.5 ± 0.3 x 105 transduction units (TU)/mL and 20-0 ± 1.2 x 106 TU/mL at day 5 after transduction with non-concentrated lentiviral supernatant and pellet, respectively). In contrast, the titer in the supernatant after ultracentrifugation was undetectable. Reporter gene expression was detectable for up to 4 weeks in vitro and had no adverse effects on cell viability, proliferation, or differentiation to endothelial cells and adipocytes. Indeed, in methylcellulose medium, multi-locular cells with refringent cytoplasmic droplets, positive for oil red-O staining, appeared after 6 days in wild type as well as EGFP-labelled ADSCs. In Matrigel, in the presence of endothelial growth factors, wild type and EGFP-labelled ADSCs formed a network of branched tube-like structures, quite similar to those formed by human umbilical vein endothelial cells (HUVEC).

Conclusions: Lentiviral vectors are highly effective for gene labeling of human ADSCs with concentrated lentivirus-based protocols providing the highest titer of transduction for primary cultures of ADSCs.

3-D FIBRIN SCAFFOLD IMPROVES STEMNESS OF HUMAN PERIPHERAL BLOOD ENDOTHELIAL PROGENITOR CELLS

C. Armani[1], A. Magera[1], M.C. Barsotti[1], M. Iorio[2], E. Simonetti[1], R. Arici[1], F. Chiellini[1], G. Soldani[3], R. Di Stefano[1], A. Balbarini[1]

[1]Università di Pisa, Pisa;
[2]Azienda Ospedaliera Universitaria Pisana, Pisa;
[3]C.N.R., Massa.

Aims Fibrin is a natural biopolymer appealing for cell-based regenerative therapies, because it can support cell growth, migration and differentiation. Endothelial progenitor cells (EPC) represent a very interesting cell source: they can be easily isolated from the peripheral blood, making them ideal in applications in the field of regenerative medicine. We have demonstrated that fibrin can support EPC viability and growth.

Aim of this study was to evaluate if fibrin can affect EPC differentiation and stem cell markers expression.

Methods: Fibrin was prepared mixing commercially available (Kedrion S.p.A. Lucca, Italy) fibrinogen (9 mg/ml) and thrombin (25 U/ml). Clot ultrastructure was investigated by scanning electron microscopy (SEM) and cryogenic SEM (CRYO-SEM) to measure fibre diameter and density. Clot elasticity was evaluated by atomic force microscopy (AFM). EPC were obtained from peripheral blood and cultured on fibrin. Fibronectin coating was used as a control. Metabolic activity was assessed after 7 and 14 days by WST1 assay and viability by confocal microscopy (calcein incorporation). The expression of both endothelial (CD31, KDR, vWF, Ve-Cadherin) and stem cell markers (nanog, oct-4) was assessed by flow cytometry, confocal microscopy and Real Time RT-PCR. Results SEM analysis revealed a nanometric fibrous structure, with mean fiber diameter of 165±4 nm and mean density of 95.9±0.2 %. CRYO-SEM suggested a reticulate structure with mesh-size up to 10 μm. Fibrin clot elasticity was 1.78 MPa, as in literature. WST1 assay showed that fibrin increased EPC metabolic activity as compared to fibronectin (fibrin: 0.606±0.056 a.u. vs. fibronectin: 0.311±0.067). Calcein staining demonstrated that EPC were still viable at 14 days. Flow cytometry showed the expression of endothelial markers (CD31=41.8±8.4%; vWF=32.3±3.0%; KDR=89.3±3.7%; VE-Cadherin=41.2±3.8%), confirmed also by confocal microscopy and Real Time RT-PCR. Interestingly, nanog and oct-4 (embryonic stem cell markers) expression was significantly greater on fibrin ($p<0.001$) as compared to fibronectin.

Conclusions: These findings suggest that fibrin it is not only a suitable scaffold for EPC growth and viability but also induces EPC differentiation. The observation that Nanog, known as the most important marker of stemness, is maintained longer than on fibronectin, may offer a surplus value to stem cell-based therapies.

CARDIOMYOCYTE CO-CULTURES TO ASSESS THE CARDIOMYOGENIC POTENTIAL OF ADULT STEM CELL LINES

A. Vilela-Silva[1], S. Pagliari[2], G. Forte[2], O. Franzese[2], R. Fiaccavento[2], S. Pietronave[3], F. Carotenuto[2], F. Pagliari[2], M. Minieri[2], P. Di Nardo[2]

[1]Conselho Nacional de Desenvolvimento Científico e Tecnológico, Rio de Janeiro, Brazil;
[2]Università di Roma Tor Vergata, Roma, Italy, 3Università del Piemonte Orientale, Novara, Italy.

Aim: Aim of the present study was to assess the cardiomyogenic potential of murine immortalized stem cell lines derived from Sca-1+ Mesenchymal and Cardiac Progenitor Cells (mTERT-MSC and mTERT-CPC, respectively), as compared to their native counterparts.

Materials and Methods: Different preparations of cardiac myocytes purified from adult (CMs) and neonatal (nCMs) mouse hearts as well as the HL1 cardiac cell line, were co-cultured in a transwell system or in direct contact with mTERT-MSC, mTERT-CPC, MSC and CPC for 1 week. In direct co-cultures, GFP-positive stem cells were used, and immunofluorescence and confocal microscopy techniques adopted. RT-PCR and Western blot analysis were employed to study stem cell commitment after transwell co-culture. Conditioned medium incubation was used as internal control.

Results: Preliminary results showed that mTERT-MSC established functional heterotypic gap junctions with nCMs, as shown by the appearance of connexin 43-positive spots between the different cell types. Moreover, nCMs, which showed continuous beating and cell proliferation all over the week in co-culture, were able to induce stem cell cardiac commitment through direct contact, as witnessed by GFP-positive cells expressing cardiac markers as GATA-4 and Nkx-2.5. Neonatal CMs were able to survive and beat for at least 1 week in culture even when grown alone. Concerning adult cardiomyocyte (CMs and HL1) effects on stem cells, no significant differences in stem cell phenotype was reported after co-culture. Finally, no stem cell commitment could be ascribed to soluble factors acting in the transwell system, nor present in the conditioned media.

Conclusions: In conclusion, the co-culture systems here described can be considered valuable tools to investigate the molecular processes leading to cardiac differentiation in multipotent stem cells. At the same time, these systems could be utilized to validate the cardiomyogenic potential of ex vivo-derived stem cells and immortalized stem cell lines. In particular, only neonatal CMs seemed to be effective in inducing cardiac commitment of adult stem cells, as compared to adult cardiomyocytes. Finally, the preliminary results obtained suggest that cell-to-cell contact is necessary to induce adult stem cell commitment to cardiac phenotype.

EFFETTO DELLA SEVERITÀ DELL'ISCHEMIA SUL RECLUTAMENTO DELLE CELLULE STAMINALI NEI TESSUTI POSTISCHEMICI: MONITORAGGIO DIRETTO IN VIVO MEDIANTE VIDEOMICROSCOPIA

I. Tritto[1], M. Di Ianni[2], F. Falzetti[2], C. Zuchi[1], I. Porchetta[1], S. Coiro[1], L. Moretti[2], A. Cardona[1], A. Tabilio[2], G. Ambrosio[1]

[1]Cattedra di Cardiologia, Università di Perugia, Perugia;
[2]Cattedra di Ematologia, Università di Perugia, Perugia.

Obiettivi: Molto interesse ha suscitato la possibilità che le cellule staminali ematopoietiche (HSC) possano riparare tessuti danneggiati dall'ischemia. Si conosce molto poco sui meccanismi di reclutamento nei tessuti bersaglio. 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 anche ai leucociti, e mostrano un incremento della 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. Scopo dello studio era valutare se il reclutamento delle HSC nei tessuti postischemici segua il paradigma delle tre fasi, e se venga influenzato dalla severità dell'ischemia.

Metodi: In ratti anestetizzati, il cremastere era sottoposto a tempi variabili di ischemia (5, 30 o 180 min), seguiti da 90 min di riperfusione (R). I gruppi sham erano sottoposti alla procedura chirurgica ma non ad ischemia. A 90 minuti di R, 20 milioni di HSC marcate con rosso di acridina erano infuse ev, ed il loro comportamento nel microcircolo era monitorato mediante videomicroscopia in vivo per i successivi 45 minuti. Alla fine dell'esperimento, le HSC aderenti erano contate nel muscolo cremastere, e la migrazione nei tessuti era valutata mediante immunoistochimica per CD-34 e real-time PCR per CD-34 mRNA.

Risultati: Le HSC marcate erano ben visualizzate nelle venule post-capillari, dove apparivano come punti luminosi. Nel muscolo normale, l'interazione fra HSC ed endotelio era modesta, mentre dopo ischemia protratta (180 min) le HSC mostravano un significativo aumento del rolling e dell'adesione (circa tre volte rispetto agli altri gruppi); anche il numero di HSC aderenti al termine della riperfusione era significativamente aumentato (32± 6.1, 37.3±3.7, e 53±5.8 cellule, rispettivamente; $p<0.05$). L'immunoistochimica confermava l'infiltrazione delle HSC nei tessuti; la real-time PCR nei muscoli sottoposti a 180 di ischemia mostrava aumentata espressione del CD-34 (9,9 volte rispetto al muscolo controlaterale non ischemico; range 2,3-20,6).

Conclusioni: L'interazione cellule staminali ematopoietiche/parete vasale è molto bassa in condizioni normali; durante riperfusione postischemica, il reclutamento delle cellule staminali ematopoietiche incrementa, e sembra proporzionale alla severità del danno ischemico.

PGLA-BASED MICROSPHERES COATED WITH ADHESION MOLECULES AS CARRIERS FOR MESENCHYMAL (STEM) STROMAL CELLS

F. Bonafè[1], L. Foroni[2], C. Orrico[2], E. Fiumana[1], S. Valente[2], G.P. Morselli[3], A. Stella[2], C. Guarnieri[1], C. Montero-Menei[4], C.M. Caldarera[1]

[1]INRC Research Unit of Biochemistry, University of Bologna, Bologna;
[2]INRC Research Unit of Vascular Surgery, University of Bologna, Bologna;
[3]U.O. Plastic Surgary, Policlinico S. Orsola Malpighi, University of Bologna, Bologna;
[4]INSERM U646, Ingenierie de la Vectorisation Particulaire, Universitè d'Angers, Angers, France.

Aim of this study was to evaluate the ability of mesenchymal stromal cells (MSCs) to adhere on pharmacologically active microcarriers (PAM) effective for cell therapy/tissue engineering applications. Biodegradable particles were made with poly (D, L-lactic-co-glycolic acid) (PLGA) and coated with adhesion molecules as a support for cell culture or cell carriers. The mean diameter of PAM was 60 µm as measured by a cell Coulter counter. hMSCs were isolated from human lipoaspirates (stroma-vascular fraction) and femoral artery (mesenchymal stromal cells). Both cell populations expressed markers of mesenchymal stem cells (CD90, CD44, STRO-1, CD166), while haematopoietic markers (CD34 and CD45) were absent. Cells were used at different densities (1x10000-2x100000/mg PAM) and analyzed by light and electron microscopy (SEM, TEM). Cells adhered to PAM quite completely just after 4 h from seeding. The optimal cell density ranged between 0.5 to 1 x100000 cells/mg PAM, each single PAM carrying not more than three cells. Large aggregates of PAM and hMSCs were also observed; this finding was particularly frequent with higher cell seeding density values and prolonging the time of cell incubation over 24 h. SEM revealed that PAM surface was quite rough, facilitating cells to adhere tightly. TEM of day 3 cultures showed that hMSCs were viable with euchromatic nuclei and prominent nucleoli; also hMSCs retained their basic mesenchymal features, i.e., loosely dispersed intermediate filaments of vimentin-type and well-developed rough endoplasmic cisternae; a few cells showed intracytoplasmic phagocyted PAM fragments without any sign of cell degeneration; this finding confirms that PAM are inert and atoxic.

These results show that: a) hMSCs derived from adipose tissue and femoral artery tightly cover PGLA-based microspheres coated with adhesion molecules; b) short times of cell incubation with PAM are preferred to reduce the formation of large hMSCs-PAM aggregates.

This work was supported by a grant from Compagnia di San Paolo (Turin, Italy).

TELOMERASE PROTECTS SCA-1+ MESENCHYMAL STEM CELLS FROM APOPTOSIS INDUCED BY HIGH DOSE H2O2, WHILE PRESERVING STEMNESS PHENOTYPE AND MULTIPOTENCY

S. Pagliari[1], G. Forte[1], O. Franzese[1], F. Pagliari[1], R. Fiaccavento[1], F. Carotenuto[1], E. Bonmassar[1], M. Prat[2], M. Minieri[1], P. Di Nardo[1]

[1]Università di Tor Vergata, Roma;
[2]Università del Piemonte Orientale, Novara.

Aim: Ex vivo-derived progenitor cells display high donor variability and limited lifespan in culture, proceeding to senescence within few passages. To overcome these drawbacks, a new stable murine Sca-1posLinneg MSC stem cell line (Sca-1 mTERT-MSC) was generated. Thus, the present study was aimed at investigating the effects of Telomerase ectopic expression on survival and multipotency of immortalized Sca-1pos Mesenchymal Stem Cells (MSC).

Materials and Methods: Senescent MSC from murine bone marrow were transfected with pCINeo vector encoding for the catalytic subunit of telomerase enzyme. RT-PCR, Immunofluorescence and FACS analysis were used to investigate the phenotypical features of the cell line. Standard differentiative protocols were employed to assess mTERT-MSC multipotency.

Results: Murine Sca-1+ mesenchymal stem cells were cultured until they proceeded to senescence, as shown by flattened morphology, high beta-galactosidase activity and loss of proliferative ability. Senescent cells (p10) were therefore immortalized. The over-expression of mTERT counteracted senescence, while restoring MSC spindle-shaped morphology, undifferentiated phenotype (Sca-1+, c-kit+, Nanog+, Islet-1+, Nucleostemin+, Nestin+) and indefinite in vitro lifespan. Moreover, telomerase ectopic expression did not affect the functional features of MSC. In fact, mTERT-MSC preserved their multipotency as witnessed by the appearance of adipocyte, bone and muscle markers after stimulation with appropriate factors. Moreover, TERT-transduced MSC showed enhanced resistance to H2O2-induced apoptosis, in a fashion that appeared similar to that shown by early passaged cells. Such effects appeared to be mediated by telomerase-induced rearrangement of cell cycle-related proteins and by a direct effect of the enzyme on DNA repair.

Conclusions: These data suggest that the ectopic expression of TERT, while leading to cell immortalization, results in the protection of Sca-1+ mesenchymal stem cells from apoptosis induced by high dose H2O2, while preserving stemness phenotype and multipotency.
This cell line represent an invaluable model of multipotent stem cells preserving stable features and function.

BENEFICI FUNZIONALI E ISTOPATOLOGICI SUL CUORE DI RATTO POST-INFARTUATO A SEGUITO DEL TRAPIANTO DI CELLULE MIOBLASTICHE PRODUCENTI L'ORMONE CARDIOTROPICO RELASSINA

S. Nistri[1], M. Bonacchi[2], C. Nanni[3], S. Zecchi-Orlandini[1], E. Masini[4], D. Bani[1]

[1]Dipartimento di Anatomia, Istologia e Medicina Legale, Firenze;
[2]Dipartimento dell'Area Critica Medico-Chirurgica (Sez. Cardiochirurgia), Firenze;
[3]U.O. Medicina Nucleare, Ospedale S.Orsola-Malpighi, Bologna;
[4]Dipartimento di Farmacologia Preclinica e Clinica, Firenze.

Sebbene il miocardio contenga elementi staminali in potenza suscettibili di rigenerare il tessuto a seguito di un danno ischemico, il loro effettivo ruolo nel processo di guarigione cardiaco post-infartuale è di fatto trascurabile. Per tale ragione, il trapianto di cellule staminali extra-cardiache è attualmente visto come un promettente approccio terapeutico per la disfunzione cardiaca conseguente all'infarto del miocardio. Modernamente, più che non un ruolo diretto nella neo-genesi di tessuto contrattile, le cellule staminali vengono intese come una sorgente locale di fattori cardiotropici capaci di influenzare positivamente il rimodellamento del cuore post-infartuato.

Il presente studio mira a valutare se il trapianto di mioblasti scheletrici C2C12, geneticamente modificati per esprimere la green fluorescent protein (C2C12/GFP) o la GFP e l'ormone cardiotropico relassina (C2C12/RLX), sia in grado di migliorare i parametri cardiaci in un modello di infarto miocardico cronico nel ratto. Ratti maschi del peso di circa 200-250 g. sono stati sottoposti ad induzione chirurgica dell' infarto miocardico e, un mese dopo, sottoposti a trattamento con solo terreno di coltura (controlli), con C2C12/GFP, con C2C12/RLX più relassina esogena (1 µg/die x 25 giorni, somministrata mediante pompe osmotiche) o con sola relassina esogena (ibid.), i cui effetti sono stati valutati nel corso dei 2 mesi successivi. Sia il trapianto cellulare che la relassina esogena miglioravano i principali parametri ecocardiografici di funzionalità cardiaca, incrementavano la vitalità miocardica, valutata mediante micro-PET, decrementavano la sclerosi cardiaca e l'apoptosi cardiomiocitaria e aumentavano la densità micro-vascolare nella cicatrice post-infartuale. Tali effetti erano massimamente evidenti nei ratti trattati con C2C12/RLX più relassina esogena.

Nell'insieme, i dati funzionali ed istopatologici qui riportati offrono ulteriore supporto al concetto che il trapianto di cellule staminali mioblastiche migliora le prestazioni contrattili e la sopravvivenza del miocardio durante il rimodellamento post-infartuale. Essi forniscono inoltre elementi probativi che indicano come le cellule staminali trapiantate, opportunamente modificate geneticamente, possono essere ottimi veicoli per il rilascio locale di sostanze cardiotropiche, quali appunto la relassina, aventi valenza terapeutica nel rimodellamento cardiaco post-infartuale.

STEM CELLS ON BIODEGRADABLE BEADS TO REPAIR DAMAGED MYOCARDIAL TISSUE

R. Fiaccavento, G. Forte, S. Pagliari, F. Pagliari, F. Carotenuto, M. Minieri, P. Di Nardo

Università Tor Vergata, Roma.

In the last years, a major effort has been made by several laboratories in an attempt to acquire fundamental knowledge on the growth and differentiation of cells for the regeneration of acutely and chronically diseased hearts. Among various stem cell delivery systems, recent studies were performed by biodegradable and biocompatible polymeric beads.

Objective: The aim of this study was to evaluate the cardiac differentiation potential of two populations of stem cells from mouse bone marrow (mTERT-MSC) and heart (mTERT-CSC) cultured on PLA and PLGA beads and to compare bead size effects on the differentiation processes. These cells were Sca-1+, and displayed stem cells markers c-kit, islet-1 and Nanog.

Methods: MSC and CSC cells were cultured on PLA and PLGA beads. Beads were: A) PLA beads of 30 µm; B) PLA beads of 100 µm; C) PLGA beads of 60 µm coated with poly-D-lisin and fibronectin-like sequences, molecules that stimulate cell adhesion. Cultures were maintained for 4, 8, 24 hours in a humidified culture incubator. Cells/beads ratio was changed and cells were seeded on Falcon 8 well slides. After 8 and 24 hours, cells clustered independently of the material or coating used. Cell growth differences were mainly observed depending on beads size. Cells clusters were observed with light microscopy with standard staining and were also sectioned (7 µm) to observe cell growth on beads surface. Both cell populations displayed a fibroblast-like shape, indicating a clear adhesion process.

Conclusions: Since mTERT-MSC and mTERT-CSC were both GFP+, the stem cells/beads clusters obtained could be useful to follow the fate of cells in cardiac tissue damage repair.

ADIPOSE TISSUE-DERIVED STROMAL CELLS FOR HEART REGENERATION: OBJECTIVES AND PRELIMINARY RESULTS

R. Madonna, F. Renna, G. Basta, G. Lazzerini, R. De Caterina

Chair of Cardiology and Center of Excellence on Aging G. d Annunzio University, CNR Institute of Clinical Physiology, Pisa.

Recent studies have documented that a population of cells derived from collagenase-digested human adipose tissue could be induced to differentiate into multiple cell lineages including chondrocytes, adipocytes, osteoblasts, myocytes, neurons and endothelial cells. The adipose tissue, like the bone marrow, is derived from the embryonic mesoderm and contains a heterogeneous stromal cell population, including mesenchymal stem cells and endothelial cell progenitors.

We aimed at determining the potential of adipose tissue-derived stem cells (ADSC), in comparison with bone marrow derived-stem cells (BMSC), to promote neovascularization and improve perfusion and myocardial contractility in an animal model of myocardial infarction. In this ongoing project we expect to demonstrate the feasibility and safety of ADSC transplantation as a potential new resource for cardiac repair. Secondary outcome of the study, at 4 weeks after cell transplantation, will be the change from baseline in global left ventricular ejection fraction, and histological evidence of increased neovascularisation in the ischemic region. Such studies are preliminary to in vivo application in humans. We have developed several protocols for harvesting and digesting the subcutaneous and visceral adipose tissue from humans, pigs and mice, with the subsequent obtainment of primary cultures of stromal cells and mesenchymal stem cells. We have determined in vitro the capability of ADSC to differentiate into contractile cardiomyocytes and vessels, and identified important biomarkers such as myocardin A and telomerase, involved in the regulation of myogenesis from the adipose tissue. Furthermore, we have demonstrated the feasibility and safety of ADSC intracoronary delivery in isolated-perfused mouse hearts. Finally, we have developed a technology of labeling ADSC with the Emerald Green Fluorescence Protein (EGFP) reporter gene using a lentiviral vector. This system provided effective transduction of ADSC and high transgene expression levels in vitro, without adverse effects on cell viability, proliferation, or differentiation to endothelial cells and adipocytes.

This research line is thus generating important experimental data, which will help in identifying suitable and alternative sources of stem cells for cardiac repair and dissecting new mechanisms that underlie age-dependent deterioration of cardiac function. Ultimately, this will contribute to the design of new therapies for heart failure.

ORGAN CULTURE OF ARTERIAL CONDUITS FROM HEART-BEATING DONORS

S. Valente[1], L. Foroni[1], C. Orrico[1], F. Alviano[2], G.L. Faggioli[1], G. Pasquinelli[3,4]

[1]Chair of Vascular Surgery, Department of Anestesiological and Surgical Sciences, University of Bologna, Bologna;
[2]Dept of Histology and Cardiovascular Tissue Banking Facility, University of Bologna, Bologna;
[3]Clinical Pathology, Clinical Department of Radiological and Histocytopathological Sciences, University of Bologna, Bologna,
[4]INRC, National Institute for Cardiovascular Research, Bologna.

The organ culture is an in vitro method for studying the vascular wall biology. The value of organ culture is twofold; it retains the in vivo structural tissue architecture while preserving the interaction between vascular cell subtypes and extracellular matrix components.

This study is aimed at establishing whether putative resident progenitors i) remain niched within human vascular segments after collection in tissues banking facilities and ii) are able to develop an intimal lining in long-term cultures without VEGF support.
Human arterial vascular conduits were collected from heart-beating donors (n. 3); before culturing, samples were processed for light microscopy (LM) and transmission electron microscopy (TEM); TUNEL assay and ultrastructural investigation were used to establish the degree of cell preservation; the remaining tissue was cut in 3 x 3 mm slices and cultured for up to 70 days in medium containing 5% of FBS. The samples were processed for LM and TEM weekly.
Before culturing, TUNEL assay revealed that the vast majority of the vascular wall cells had significant nuclear damage; this feature was confirmed by TEM. At 4 day of culture, TEM showed the presence of a few structurally preserved cells in correspondence with the adventitia layer; after 42 days, several undifferentiated cells with a high N/C ratio and prominent nucleoli were seen at this same location. LM revealed that spindle-shaped and epithelioid cells migrated near the inner and outer surfaces of the arterial segments; an almost continuous endothelial-like lining became evident starting after 56 days of culture. Immunostaining for CD34 surface molecule was negative in the adventitial lining and focally positive in the intimal side.

This study demonstrates that human vascular conduits, after collection in tissues banking facilities, still contain a resident cell population which is particularly resistant to physical and chemical stresses; under plain tissue culture conditions these cells are able to expand and migrate toward the arterial surfaces and give origin to a pseudo-intimal lining. Noteworthy is the fact that we can exclude any contribute of hematopoietic cells to this phenomenon that, in this case, is dependent only on putative resident progenitors.

VASCULAR WALL MSCS COULD RESIDE IN VIRTUALLY ALL HUMAN VASCULAR SEGMENTS

A. Pacilli[1], F. Alviano[2,3], P.L. Tazzari[3], F. Ricci[3], M. Buzzi[3], M. Gargiulo[1], G. Pasquinelli[4,5]

[1]Chair of Vascular Surgery, Department of Aenestesiological and Surgical Sciences, Bologna;
[2]Department of Histology,Embriology and Applied Biology, Bologna;
[3]Cardiovascular Tissue Banking Facility, Policlinico S. Orsola, Bologna;
[4]Clinical Pathology, Clinical Department of Radiological and Histocytopathological Sciences, Bologna;
[5]INRC, National Institute for Cardiovascular Research; University of Bologna, Bologna.

Aim: To isolate a different source of Mesenchymal Stem Cells (MSCs) endowed with angiogenic ability from different human vascular segments collected in tissue banking facilities from healthy and young heart beating and non-heart beating donors (HBD and NHBD respectively) for allogenic use in clinical practice.

Materials and Methods: Twelve fresh vascular segments (6 thoracic aortas, 3 femoral arteries, 3 saphena veins), after decontamination, were mechanically minced and enzimatically digested with liberase 2 (0,3 mg/ml) 4 hours at 37°C in a fluttering support. The omogenate was filtered and seeded in biocoated multiwell plate (1x105cells/cm2). The growing cells were expanded and their immunophenotype analyzed by flow-cytometry.

Results: The immonophenotypical profile of VW-MSCs was consistent with that reported in the literature for BM-hMSCs, i.e., CD105+, CD90+, CD44+, STRO1+, OCT4+, CD34low, CD31-, CD133-, CD45-. We also proved that the same mesenchymal population could be isolated from different vascular segments anatomically distinct.

Conclusions: Angiogenic properties and multipotency of MSCs make them the best candidate for cell-based therapies, regenerative medicine and tissue-engineering. Human VW-MSCs are endowed with angiogenic ability (probably due to contribute of the niche in establishing the phenotype of stem cells it interacts with), and could be very useful in clinical practice in patients affected with vascular injury without any surgical options. Since vascular segments are an alternative source of MSCs, the presence of tissue banking facilities in local health authorities is very important to collect them not only from HBD, but also from NHBD whose vessels cannot be cryopreserved for autologous and allogenic grafts.

A NEW USER-FRIENDLY EXPERIMENTAL PROTOCOL TO ANALYZE PROLIFERATION, SURVIVAL AND IMMUNOPHENOTYPE OF CELLS CULTURED ON ELECTROSPUN NANO SCAFFOLDS FOR TISSUE ENGINEERING

L. Foroni[1], G. Dirani[2], P. Celio[2], M.L. Focarete[3], C. Gualandi[3], G. Pasquinelli[2,4]

[1]Dept of Specialistic Surgical and Anaestesiological Sciences, Bologna University, Bologna;
[2]Dept of Radiological and Histocytomorphological Sciences, Bologna University, Bologna;
[3]G. Ciamician, Chemistry Dept., Bologna University, Bologna;
[4]INRC, Bologna University, Bologna.

Tissue engineering is a fascinating area of regenerative medicine that encompasses the use of cells seeded on biocompatible and bioresorbable scaffolds in order to replace diseased or damaged tissues. Nowadays, different strategies are available to fabricate polymeric porous scaffolds with controlled architecture to be used as support for in vitro cell culturing. Among various technologies, electrospinning allows the production of polymeric scaffolds with a 3D nanofibrous structure that mimics the morphological nano-features of native extracellular matrix.
In literature immunofluorescence and cryosections are the most common biochemical techniques used to characterized cells seeded on electrospun scaffolds, even if these methods show several practical limits.
In this work poly(L-lactic acid) (PLLA) electrospun nanofiber scaffolds were seeded with cells and a preliminary study was performed with the aim of developing a new user-friendly scaffold embedding protocol that is based on an experimental procedure commonly used for native tissues.

The results suggest that the optimized protocol can be applied to the present scaffolds with the advantage to obtain not only en face sections but also cross sections that are easy to handle and to process. It was therefore possible to perform different informative stainings on histological sections; these include assays for evaluating cell proliferation, apoptosis and cell differentiation abilities.

MICROSFERE BIOMIMETICHE PER IL SUPPORTO DI CELLULE PROGENITRICI ENDOTELIALI UMANE

C. Musilli[1], F. Ledda[1], A. Mugelli[2], A. Parenti[1]

[1]Dipartimento di Farmacologia, Università degli Studi di Firenze, Firenze;
[2]C.I.M.M.B.A., Università delgi Studi di Firenze, Firenze.

Le cellule progenitrici endoteliali (EPC) rivestono un ruolo primario nella rigenerazione vascolare cellulo-mediata per la riparazione del miocardio infartuato. Le molteplici problematiche connesse all'inoculazione delle EPC, ha portato alla creazione di strumenti di supporto e veicolo per le cellule staminali autologhe. Tra queste, microparticelle polimeriche (PAM) di 60 μm di diametro mediamente, biodegradabili e biocompatibili rivestite con sequenze di fibronectina e con poli-D-lisina (Tatard et al., Biomaterials, 2005).

Obiettivo della presente ricerca è stata la valutazione dell'adesione delle EPC umane alle PAM. EPC sono state ottenute, in vitro, per differenziamento della popolazione mononucleata (PBMC) di donatori sani. Il fenotipo pre-endoteliale è stato caratterizzato mediante l'analisi dell'espressione di antigeni (VEGFR-2, VE-caderina, vWF) con citometria a flusso e immunocitochimica. È stato messo a punto inizialmente un protocollo per la semina e coltura delle EPC sulle microsfere, cercando di ottimizzare il numero di cellule/mg di PAM. Successivamente, la concentrazione ottimale di 125000 cellule/0.5 mg PAM, è stata lasciata in incubatore a 37°C per 4 giorni. A vari tempi le colture sono state esaminate al microscopio ottico per valutare l'adesione delle EPC alle microsfere. Le osservazioni sono state effettuate dopo 6, 12, 24, 48 ore e 4 giorni. Dopo poche ore dalla semina, è possibile osservare già un certo numero di EPC adese alle PAM. Il numero di EPC che aderisce alle PAM cresce dopo 24-48 ore dalla semina e rimane costante fino ai 4 giorni di coltura. Se le PAM venivano ulteriormente rivestite di fibronectina, si osservava un numero maggiore di EPC adese alle microsfere e maggiori connessioni tra cellule e microsfere, quasi a formare delle "corde".

Questi risultati dimostrano che le EPC aderiscono alle PAM e che tale adesione può migliorare se le PAM sono ulteriormente rivestite di fibronectina.

EXOGENOUS HIGH-MOBILITY GROUP BOX 1 PROTEIN (HMGB1) AMELIORATES CARDIAC ELECTRICAL PERFORMANCE IN INFARCTED RAT HEART

L. Bocchi[1], A. Rossini[2], M. Savi[1], R. Berni[1], S. Baruffi[1], G. Graiani[1], A. Germani[3], F. Quaini[1], M.C. Capogrossi[4], E. Musso[1]

[1]Centro per lo Studio della Biologia e delle Applicazioni Cliniche delle Cellule Staminali Cardiache, Università-Parma, Parma;
[2]Laboratorio di Biologia Vascolare e Terapia Genica Centro Cardiologico Fondazione Monzino, Milano;
[3]Fondazione Livio Patrizi, Laboratori di Ricerca Gruppo Bios, Roma;
[4]Laboratorio di Patologia Vascolare, Istituto Dermopatico dell'Immacolata, I.R.C.C.S., Roma.

Aim: HMGB1 is a cytokine released into the extracellular space by necrotic cells and activated macrophages in response to injury. Previous findings demonstrated that intra-myocardial injection of exogenous HMGB1 promotes muscle regeneration and recovery of mechanical function in the infarcted mouse heart. The effects of the regenerative manoeuvre on cardiac electrical remodelling and propensity to arrhythmias are not known. In the present study, we analyzed the electrophysiological consequences of HMGB1 treatment in a rat model of acute myocardial infarction (MI).

Methods: In 22 male adult Wistar rats, MI was induced by left coronary artery ligature and four hours later, 200 ng of purified HMGB1 (HMGB1 group, n=10) or inactivated protein (Untreated MI group, n=12) were injected in the peri-infarction area. Three additional rats, assigned to the sham-operated group (SO) were treated similarly, except that the ligature around the coronary artery was not tied and saline solution was intra-myocardially injected. After two weeks, HMGB1-dependent cardiac electrical remodelling was assessed in each rat at the organ and tissue levels by analyzing: (i) the proneness to arrhythmias during baseline and stress-induced-autonomic-stimulation in conscious animals, by means of telemetry ECG recordings and (ii) excitability, refractoriness and conduction velocity at multiple sites of the heart surface, by means of epicardial potential mapping. Eventually, the heart was perfusion-fixed for morphometrical analysis.

Results: In comparison with SO animals, untreated MI rats were characterized by (i) high level of cardiac electrical instability as documented by the increased incidence of baseline and stress-induced ventricular arrhythmias, (ii) reduced excitability of ventricular myocardium, and (iii) worsening of left ventricular geometry including chamber dilation and wall thinning. MI-related morpho-functional changes were markedly attenuated in HMGB1-treated rats which exhibited (i) significant reduction in baseline and stress-induced ventricular arrhythmogenesis, (ii) partial recovery of tissue excitability, and (iii) better ventricular structural remodelling with an increase in the wall thickness-to-chamber radius ratio.

Conclusions: We conclude that, in acute MI, intra-myocardial injection of HMGB1, in addition to promoting a partial recovery of cardiac mechanical function, may also represent an effective approach to achieve a substantial improvement of the electrical competence of the regenerated heart.

POLY (L-LACTIC ACID) ELECTROSPUN NANOFIBROUS SCAFFOLDS FOR CARDIAC TISSUE ENGINEERING

C. Gamberini[1], M. Govoni[1], E. Giordano[1], C. Gualandi[2], M.L. Focarete[2], M. Scandola[2], L. Foroni[3], S. Valente[3], G. Pasquinelli[4], C. Guarnieri[1]

[1]Department of Biochemistry Moruzzi, University of Bologna, Bologna;
[2]Department of Chemistry Ciamician, University of Bologna, Bologna;
[3]Dept. of Specialistic Surgical and Anaestesiological Sciences, Policlinico S. Orsola, University of Bologna, Bologna;
[4]Dept. of Experimental Pathology, Clinical Pathology Section, Policlinico S. Orsola, University of Bologna, Bologna.

Introduction: The use of three-dimensional (3-D) porous scaffolds as a guide for cellular growth in culture drives remarkable hopes in the field of regenerative medicine, due to the possibility to modulate cellular distribution in a construct intended for the implant in a patient to promote the regeneration of irreversibly damaged tissues, such as the infarcted myocardium. Nanostructured polymeric fibrous scaffolds with morphology and fiber diameter comparable to that of the extracellular matrix in natural tissues can be obtained by means of electrospinning, a very versatile technology to produce polymeric nanofibers. For this reason electrospun scaffolds are believed to provide a better biomimetic environment than other types of porous scaffolds and to offer an ideal support for cell attachment, migration, proliferation and differentiation. In addition, a suitable design of the electrospinning apparatus, and the careful tuning of the processing parameters, may produce nanofibres with the desired alignment.

Methods: Bioresorbable polymeric [poly(L-lactic acid) (PLLA)] nanofibrous scaffolds were fabricated by electrospinning technology integrated with a flexible mechatronic equipment, allowing controlled and reproducible 3-D architectures. Cardiac H9c2 myoblasts were seeded onto scaffolds to evaluate their survival, adhesion and proliferation. Morphology and spatial cellular distribution were evaluated via scanning electron microscopy (SEM) and cryomicroscopy of H&E stained sections. Cell attachment and proliferation were measured with the continual fluorescence AlamarBlueTM assay.

Results: Good cell adhesion and proliferation was obtained. SEM observations revealed abundant cellular interactions with the scaffold nanofibers and the presence of cells into the 3-D mesh after two weeks of culture.

Conclusions: These results show an evidence that electrospun nanostructured PLLA 3-D scaffolds support survival, adhesion and proliferation of cardiac-derived cells, suggesting their potential use to support the regeneration of a damaged heart.

The University of Bologna (Progetto strategico di Ateneo - SCAFELMED), the Fondazione Cassa di Risparmio di Bologna (2007.0058) and the INRC, are gratefully acknowledged for their financial support.

STUDIO IN VITRO ED IN VIVO DELLA BIOCOMPATIBILITA' DI PAM (PHARMACOLOGICALLY ACTIVE MICROCARRIERS) COME SUBSTRATO PER LA RIGENERAZIONE MIOCARDICA

C. Frati[1], G. Graiani[1], L. Prezioso[1], B. Testa[1], L. Bocchi[1], M. Savi[1], C. Lagrasta[1], E. Musso[1], C.M. Caldarera[2], F. Quaini[1]

[1]Università degli Studi, Parma;
[2]Università degli Studi, Bologna.

Obiettivi: l'utilizzo di biomateriali in terapia cellulare è stato introdotto allo scopo di migliorare la sopravvivenza, la differenziazione e l'integrazione cellulare nel tessuto ospite. In questo contesto PAM, particelle sferiche biodegradabili composte da poly D,L-lactic-co-glycolic acid (PLGA) e rivestite con molecole di adesione, potrebbero servire come supporto per la costruzione in vitro di patch cellulari e/o impiegate come "carriers" ingegnerizzati per il rilascio continuo di peptidi attivi e fattori di crescita.

Materiali e Metodi: Studio in vitro: sono state allestite colture di cellule simil-mesenchimali miocardiche e midollari umane con PAM. È stata valutata la capacità di crescita e proliferazione ad 1 e 2 settimane dalla semina e dopo re-plating a seguito di tripsinizzazione.
Studio in vivo: 0.5 mg di PAM venivano iniettati in cuori di ratti con infarto cronico (MI) e ratti Sham Operated (SHAM) sacrificati ad 1-2 settimane dall'iniezione.
All'interno di ciascuno dei 2 gruppi veniva utilizzato come controllo un gruppo di animali cui veniva iniettato il solo veicolo d'iniezione.

Risultati: Studio in vitro: la modalità di crescita delle cellule non sembra essere influenzata dalla presenza di PAM. Tuttavia l'adesione cellulare delle microparticelle è associata per l'80% ai clusters caratteristici delle cellule mesenchimali in coltura e più raramente a singola cellula. Dopo tripsinizzazione la capacità di crescita cellulare viene mantenuta e PAM sono preferenzialmente associate a singole cellule ed solo il 50% a clusters cellulari.
Studio in vivo: il riconoscimento di PAM all'interno del tessuto murino risulta difficoltoso in assenza di uno specifico colorante, tuttavia sono stati ritrovati piccoli aggregati di particelle sferiche riconducibili alle PAM. Risultati preliminari mostrano che nel miocardio normale di ratti SHAM, PAM inducono negli animali sacrificati ad una settimana una modesta risposta infiammatoria che si attenua ulteriormente a due settimane dall'iniezione. Nei miocardio infartuato dei ratti MI è stata individuata una maggior densità di PAM principalmente localizzata in zona infartuale e periinfartuale.

Conclusioni: risultati preliminari sulla biocompatibilità in vitro che in vivo suggeriscono che PAM possano essere impiegate negli approcci sperimentali di rigenerazione miocardica, in quanto efficaci supporti per cellule umane e veicoli potenzialmente impiegabili nella ricostituzione funzionale del cuore danneggiato.

PHYSICAL FACTORS CAN POTENTIATE SCA-1POS CPC PROLIFERATION

F. Carotenuto[1], G. Forte[1], S. Pagliari[1], F. Pagliari[1], R. Fiaccavento[1], S. Zava[2], A.M. Rizzo[2], M. Minieri[1], P. Di Nardo[1]

[1]Lab di Cardiol. Mol. e Cell. Universita Tor Vergata Roma, Roma;
[2]Istituto di Fisiol. Gen. e Chim. Biologica, Università Milano, Milano.

Cardiac progenitors cells (CPC) are a fundamental myocardial component supporting cardiac tissue regeneration through the replacement of damaged cells. At present, a variety of soluble growth factors that modulate stem cell migration, differentiation, proliferation and death, have been identified. Recent studies have testified for a pivotal role of physical factors in guiding stem cell fate. By contrast, no information is presently available on the possible effects that gravity, the major physical factor to which cells are exposed, can exert on cardiac stem cells.

Aim: The present investigation has been carried out to elucidate whether microgravity (*f*Yg) could affect CPC biological behaviour and if it could be dissimilar in two different CPC populations.

Materials and Methods: Therefore, CPC Linneg and Sca-1pos CPC Linneg subpopulation were exposed to *f*Yg, as emulated by the 3D-clinostat, in order to elucidate respective responses.

Results: Data show that 24h-*f*Yg provokes alterations in the cytoskeletal architecture of CPC Linneg by altering microtubule and actin fibers disposition. Moreover, the number of nuclei/field was significantly reduced in the same conditions although the number of CPC Linneg mitotic nuclei increased twice vs. controls. After exposure to long-term *f*Yg (72h) CPC Linneg microfilaments were again well-organized, microtubules and actin networks regained their ordinary disposition, and cell number appeared even higher than controls at 1xg. After 24h-clinorotation, Sca-1pos CPC Linneg did not show significant cytoskeletal alterations. Moreover, no decrease in nuclei/field ratio was observed and mitotic nuclei showed 4-fold increase vs. controls. The exposure to long-term *f*Yg (72h) considerably increased cell number (2-fold) and mitotic nuclei (1.5-fold) vs. controls.

Conclusions: Data showed that the CPC survivability in a hostile environment was mainly contributed by the Sca-1pos CPC Linneg subpopulation that demonstrated to be substantially insensitive to gravity modifications preserving the phenotypical, structural and functional characteristics of the control cells independently of the severity of the gravitational stress. The unexpected capability of Sca-1pos CPC Linneg to withstand and adapt to extreme environmental conditions, even through an amplified proliferative ability, could represent a strategy to expand a specific CPC subpopulation suitable to regenerate damaged myocardium.

A BIOLOGICAL SELF-ASSEMBLING PEPTIDE SUITABLE FOR CELL DELIVERY IN ISCHEMIC TISSUES

E. Simonetti[1], M.C. Barsotti[1], A. Magera[1], H. Hosseinkhani[2], S. Sorbo[1], D. Dinucci[1], F. Chiellini[1], R. Solaro[1], R. Di Stefano[1], A. Balbarini[1]

[1]Università di Pisa, Pisa;
[2]National Institute for Materials Science, Tsukuba, Japan.

Aims: Autologous transplantation of endothelial progenitor cells (EPC) is a promising approach for revascularization of ischemic tissues, however strategies for in situ prolonged stay of the cells are needed. Scaffolds mimicking the nanometric structure and the biological function of the extracellular matrix (ECM) should be ideal. Self-assembling peptide-amphiphiles (PA) are biocompatible molecules, forming nanofibers when mixed with opposite charged solutions. PA may include sequences such as arginine-glycine-aspartic acid (RGD), also found in ECM and involved in cell attachment. When injected with growth factors or stem cells in experimental myocardial infarction in rats, PA resulted in a significant neovascularization and a reduction of necrotic area.
Purpose of our work was to evaluate PA containing RGD as potential scaffolds for EPC growth and to compare 2-D and 3-D EPC growth in PA gels.

Methods: PA gel scaffolds were formed mixing an aqueous PA solution (0.1%-1%-2%) with endothelial medium with 5% FBS and growth factors containing different CaCl2 concentrations (10-20mM). The morphology of PA was analysed by scanning electron microscopy (SEM) and by atomic force microscopy (AFM). Mononuclear cells obtained from peripheral blood of healthy donors were seeded either on the surface (2-D) or inside the gel (3-D) and cultured for 1 week to obtain EPC. EPC obtained on fibronectin were used as a control. EPC viability was assessed by confocal microscopy after calcein-AM staining and by WST-1 assay.

Results: PA had a native pH of 4.0 but gained solubility in water when pH=7.4 was reached. A transparent gel-like solid was obtained when PA concentration was ¡Y1%, irrespectively from CaCl2 concentration. Both SEM and AFM analysis showed the presence of 3-D networks of nanofibers. The assessment of EPC viability either with confocal microscopy and WST-1 showed no effect of CaCl2 concentration. However, the higher viability was observed when PA concentration was =1% in the 3-D seeding model (0.660¡À0.140 a.u. vs. fibronectin: 0.311¡À0.067, p=0.05).

Conclusions: The 3-D gel formed by the self assembling PA containing RGD can be a suitable scaffold for EPC growth and transplantation. This scaffold has the potential to be used as injectable matrix to support EPC cardiovascular tissue engineering applications.