

clinical application of β -blocker agents requires patient and disease oriented approaches.

doi:10.1016/j.vph.2015.11.029

Is NLRP3 inflammasome a new pharmacological target in myocardial ischemia/reperfusion injury?

S. Femminò^a, F. Chiazza^b, R. Mastrocola^a, F. Tullio^a, C. Penna^a, D. Nigro^a, G. Alloattì^c, M. Cocco^b, D. Garella^b, M. Bertinaria^b, R. Fantozzi^b, M. Aragno^a, M. Collino^b, P. Pagliaro^a

^aDepartment of Clinical and Biological Sciences, University of Turin, Italy

^bDepartment of Drug Science and Technology, University of Turin, Italy

^cDepartment of Life Sciences and Systems Biology, University of Turin, Italy

Objectives: Recently it has been documented that the NLRP3 inflammasome plays a pivotal role in the inflammatory response to tissue injury. The NLRP3 inflammasome is a large multimeric danger-sensing platform that induces activation of the caspase-1 and mediates the cleavage of inactive pro-IL-1 β , among other proteins, into its active form. We have shown that the activation of the NLRP3 inflammasome exacerbates myocardial ischemia/reperfusion (I/R) injury in diabetic mice. Moreover, our group previously demonstrated that INF-4E inhibits NLRP3, ATPase and caspase-1 activities in THP-1 cells. Here we test in an ex-vivo model whether INF-4E inhibiting NLRP3 may positively affect I/R injury, post-ischemic cardiac function and molecular response in the rat hearts.

Materials and methods: Isolated hearts from male Wistar rats (body weight 450,550 g; n = 7) underwent perfusion without ischemia (Sham) or I/R (30-min ischemia plus 20-min or 60 min reperfusion) with and without INF-4E treatment (50 M for 20-min before ischemia). Coronary perfusion pressure and left ventricular pressure (LVP) were monitored, and dP/dt_{max} assessed during the entire period of perfusion. Biopsies obtained at the end of reperfusion were used for Western blotting evaluations of NLRP3 and caspase-1 (apoptosis index) levels/activities and for assessment of RISK pathway involvement. At the end of 60 min reperfusion infarct size was measured with nitro-blue-tetrazolium technique by an independent observer.

Results: In this preliminary study, the 20 min INF-4E pre-ischemic administration induced a significant reduction of infarct size and an improvement in post-ischemic LVP recovery. Western blot analysis demonstrated NLRP3 and caspase-1 activation by I/R procedure, which were strongly attenuated after INF-4E pre-treatment. Moreover, an important modulation of RISK kinase phosphorylation was observed, though a clear-cut correlation between the reduction in infarct size and phosphorylation of RISK kinases was not observed.

Conclusions: These preliminary results demonstrate that the INF-4E inhibits the formation of the NLRP3 inflammasome in the rat heart and ameliorates the response to myocardial I/R injury, confirming the ability of this drug to affect NLRP3 inflammasome complex activation.

doi:10.1016/j.vph.2015.11.030

Spread bio oil diet and cardiac response to ischemia/reperfusion: A preliminary study in mice

F. Tullio^a, S. Femminò^a, G. Alloattì^b, T. Angelone^c, M.C.C. Cerra^b, B. De Cindio^d, F. Lupi^d, A.M. Quintieri^b, P. Pagliaro^a, C. Penna^a

^aDepartment of Clinical and Biological Sciences, Italy

^bDepartment of Life Sciences Systems Biology, University of Turin, Turin, Italy

^cDepartment of Biology, Ecology and Earth Science, Italy

^dDepartment of Information, Modeling, Electronics and System Engineering (D.I.M.E.S.), University of Calabria, Arcavacata di Rende (CS), Italy

Objectives: The role of diet is of paramount importance in the prevention and development of cardiovascular disease (CVD). A diet high in saturated fat increases the risk of heart disease and stroke, in particular it causes about 31% of coronary heart disease and 11% of stroke worldwide. The Mediterranean diet is the symbol of the Italian style and is considered able to reduce the burden of CVD, but this protective alimentary modality is frequently neglected for incorrect life style. Recently it has been suggested that “spread bio oil” (SBO), an innovative food product, at hard fat phase, mainly based on olive oil, may be beneficial, thus in the present study we aimed to compare three different diets, standard, high fat and SBO diet, in terms of cardiac response to the ischemia/reperfusion (I/R).

Materials and methods: Male mice were fed with standard diet (SD), high fat (HF) or spread SBO diet for 4 weeks. Before, during and at the end of these diets blood samples were collected and the anthropometric parameters, such as body mass index (BMI), weight, height and abdominal circumference, were evaluated. The hearts of each group were randomly assigned to two different protocols: only Tyrode perfusion (Sham) or I/R (30 min ischemia and 60 min reperfusion), at the end of experiments the infarct size was evaluated with nitro-blue tetrazolium techniques.

Results: While HF diet did not induce significant variations of anthropometric parameters (BMI, weight, and abdominal circumference), left ventricular weight resulted significantly higher in comparison to SD animals. Also, the infarct size resulted higher in HF than in SD hearts (infarct size 65 \pm 5% and 43 \pm 5%, respectively). Although, the animals fed with SBO diet displayed a significant increase of abdominal circumference with respect to SD only, surprisingly enough, their hearts had a smaller infarct size (26 \pm 6%) with respect to both SD and HF animals.

Conclusions: These preliminary data suggest that the SBO increases the resistance of heart to I/R challenge, despite partial modifications of anthropometric parameters. To explain these preliminary results further investigations and analyses of cardioprotective pathways are necessary.

doi:10.1016/j.vph.2015.11.031

Obestatin exerts post-conditioning-like cardioprotective effects via nitrosative/oxidative signaling

C. Penna^a, G. Alloattì^b, T. Angelone^d, M.C. Cerra^d, S. Femminò^a, M.P. Gallo^b, E. Ghigo^c, P. Pagliaro^a, C. Rocca^d, F. Tullio^a, L. Trovato^c, R. Granata^c

^aDepartment of Clinical and Biological Sciences, Italy

^bDepartment of Life Sciences and Systems Biology, Italy

^cDepartment of Medical Sciences, University of Turin, Turin, Italy

^dDepartment of Biology, Ecology and Earth Science, University of Calabria, Arcavacata di Rende (CS), Italy

Objectives: Obestatin (Obe), a 23-amino acid peptide derived from preproghrelin, affects several neurometabolic and cardiac physiological functions. A preconditioning-like effect of obestatin in limiting apoptosis and ischemia/reperfusion (I/R) injury in both isolated rat heart and cultured adult cardiomyocytes has been recently demonstrated. We hypothesized an obestatin-postconditioning-like cardioprotective effect via a nitrosative/oxidative mitochondrial signaling (NO/ROS/PKC ϵ /mitoKATP channel).

Materials and methods: Isolated hearts underwent the following protocols: control group (I/R, 30 min global ischemia followed by 120 min reperfusion); Obe group (obestatin 75 nM, for 20 min immediately after ischemia and 100 min reperfusion). Other hearts received obestatin plus different antagonist of NO/ROS/PKC ϵ /mitoKATP channel pathway. Each inhibitor was given during the last 5 min of stabilization and the initial 20 min reperfusion. Developed left ventricular pressure

(dLVP) was monitored and infarct size was measured with nitro-blue tetrazolium technique.

Results: The I/R damage was reduced by the administration of Obe in early reperfusion. In particular, postconditioning with Obe reduced infarct size from $61 \pm 4\%$ (control group) to $32 \pm 6\%$ (Obe group) of LV mass. Post-ischemic cardiac dysfunction was avoided by Obe-postconditioning (dLVP variation with respect to pre-ischemic value: -41 ± 7 mm Hg in control group vs $+2 \pm 8$ mm Hg in Obe group, $p < 0.05$). These protective effects were abolished by antagonists of NO/ROS/PKC ϵ /mitoKATP channel pathway. In fact the co-infusion of Obe with PKC ϵ antagonist (ϵ V1-2, 0.1 μ M) or nitric oxide synthase inhibitor (L-NIO, 1 μ M) abrogated the cardiac protection (infarct size $66 \pm 7\%$ and $65 \pm 11\%$ of LV mass, and dLVP variation: -44 ± 10 mm Hg and -55 ± 7 mm Hg, respectively). The ROS scavenger N-acetylcysteine (NAC, 10 μ M) and the mitoKATP channel blocker 5-hydroxydecanoate (5-HD, 10 μ M), demonstrated that Obe protection was dependent on the opening of mitoKATP channels and ROS signaling. In fact infarct size was $59 \pm 4\%$ of LV in Obe + NAC and $73 \pm 25\%$ of LV in Obe + 5-HD ($p = \text{NS}$ vs I/R control group). Also dLVP recovery was blunted by NAC or 5-HD (dLVP variation: -19 ± 8 mm Hg and -11 ± 9 mm Hg, respectively).

Conclusions: These results suggest a novel cardioprotective role for Obe, which appears mainly due to a direct reduction of post-ischemic myocardial damage and dysfunction. Intriguingly, obestatin-postconditioning includes a redox/NO dependent signaling. These data suggest a potential role for obestatin as innovative pharmacological agent to counteract the development of reperfusion injury.

doi:10.1016/j.vph.2015.11.032

Modulation of SERCA2 function by direct lysine acetylation

M.C. Florio^{a,f}, L. Bocchi^b, C. Piubelli^{a,f}, M. Savi^b, C. Weichenberger^{a,f}, C. Corti^{a,f}, R. Sacchetto^c, S. Suffredini^{a,f}, C. Gaetano^d, G. Pompilio^e, P.P. Pramstaller^{a,f}, F. Domingues^{a,f}, D. Stilli^b, A. Rossini^{a,f}

^aCenter for Biomedicine, European Academy Bozen/Bolzano (EURAC), Bolzano/Bozen, Italy

^bDepartment of Life Sciences, University of Parma, Parma, Italy

^cDepartment of Comparative Biomedicine and Food Science Agripolis, Legnaro (PD), Italy

^dDivision of Cardiovascular Epigenetics, Department of Cardiology, Goethe University, Frankfurt am Main, Germany

^eLaboratory of Vascular Biology and Regenerative Medicine, Centro Cardiologico Monzino, IRCCS, Milan, Italy

^fAffiliated Institute of the University Lübeck, Germany

Objectives: Acetylation and deacetylation at specific lysine (K) residues is mediated by histone acetylases (HATs) and deacetylases (HDACs), respectively. HATs and HDACs act on both histone and non-histone proteins, regulating various processes, including cardiac impulse propagation. Aim of the present work was to establish whether the function of the Ca²⁺ ATPase SERCA2, one of the major players in Ca²⁺ reuptake during excitation-contraction coupling in cardiac myocytes (CMs), could be modulated by direct K acetylation.

Materials and methods: HL-1 atrial mouse cells (donated by Prof. Claycomb), zebrafish and Streptozotocin-induced diabetic rat CMs were treated with the pan-inhibitor of class I and II HDACs suberanilohydroxamic acid (SAHA) for 1.5 h. Evaluation of SERCA2 acetylation was analyzed by co-immunoprecipitation (Co-IP). SERCA2 activity was measured on microsomes by pyruvate/NADH coupled reaction assay. SERCA2 mutants were obtained after cloning wild-type and mutated sequences into the pCDNA3 vector and transfected into HEK cells. Ca²⁺ transients in CMs (loading with Fluo3-AM, field

stimulation, 0.5 Hz) and in transfected HEK cells (loading with FLUO-4, caffeine pulse) were recorded.

Results: Co-IP experiments performed on HL-1 cells demonstrated a significant increase in the acetylation of SERCA2 after SAHA-treatment (2.5 μ M, $n = 3$). This was associated with an increase in SERCA2 activity in microsomes obtained from HL-1 cells, after SAHA exposure ($n = 5$). Accordingly, SAHA-treatment significantly shortened the Ca²⁺ reuptake time of adult zebrafish CMs. Further, SAHA 2.5 nM restored to control values the recovery time of Ca²⁺ transients decay in diabetic rat CMs. HDAC inhibition also improved contraction parameters, such as fraction of shortening, and increased pump activity in microsomes isolated from diabetic CMs ($n = 2$). Notably, the K464, identified by bioinformatic tools as the most probable acetylation site on human SERCA2a, was mutated into glutamine (Q) or arginine (R) mimicking acetylation and deacetylation respectively. Measurements of Ca²⁺ transients in HEK cells revealed that the substitution of K464 with R significantly delayed the transient recovery time, thus indicating that deacetylation has a negative impact on SERCA2 function.

Conclusions: Our results indicate that SERCA2 function can be improved by pro-acetylation interventions and that this mechanism of regulation is conserved among species. Therefore, the present work provides the basis to open the search for novel pharmacological tools able to specifically improve SERCA2 activity in diseases where its expression and/or function is impaired, such as diabetic cardiomyopathy.

doi:10.1016/j.vph.2015.11.033

Apelin protects the heart against ischemia-reperfusion injury via epidermal growth factor receptor (EGFR) transactivation

A. Folino^a, A. Rolfo^b, P.G. Montarolo^a, G. Losano^a, R. Rastaldo^c

^aDepartment of Neuroscience, University of Turin, Turin, Italy

^bDepartment of Surgical Sciences, University of Turin, Turin, Italy

^cDepartment of Clinical and Biological Sciences, University of Turin, Turin, Italy

Objectives: Apelin peptide is a ligand of the G protein coupled receptor (GPCR) APJ. Apelin-13 (Ape) is the most active isoform on the cardiovascular system, where it exerts positive inotropy and vasodilation. It also mimics ischemic postconditioning (IPost) against ischemia-reperfusion (I/R) injury. Protection is caused by NO after phosphatidylinositol 3-kinase (PI3K)-Akt activation. Various GPCRs activate PI3K-Akt via epidermal growth factor receptor (EGFR) transactivation by ligand-dependent and -independent pathways, the former due to the shedding of epidermal growth factor (EGF)-like ligands by matrix metalloproteinase (MMP), the latter to the non-receptor tyrosine kinase Src. We investigated the main steps of the pathway of Ape-induced cardioprotection focusing the attention on a possible role of EGFR transactivation.

Materials and methods: Rat hearts underwent 30 min of global ischemia and 2 h of reperfusion. Left ventricular pressure (LVP) was measured from a balloon in the left ventricle. After reperfusion infarct size (IS) was measured. EGFR phosphorylation was assessed on hearts undergone 10 min of reperfusion with western blot. These inhibitors were used: GM6001 to prevent EGF-like shedding by MMP, AG1478 to inhibit EGFR, PP2 to inhibit Src, LY294002, ODQ and 5HD to inhibit PI3K, guanylyl cyclase (GC) and mitochondrial ATP-dependent K⁺ channels (K⁺mito) respectively.

Results: Global ischemia caused an increase in left ventricular diastolic pressure (LVDP) to about 50 mm Hg. Ape limited ($p < 0.001$) this increase to about 30 mm Hg. All inhibitors prevented this effect. I/R reduced the recovery of developed LVP to about 35% only of the baseline. The recovery was improved by Ape (75% of baseline