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Objective: Nanoparticle-based imaging and nanocarriers therapies have emerged as essential tools for many fields of modern medicine, in order to track the fate of cells and optimize drug delivery. Up to now, however, there are only few reports on the effect of nanocarriers of different types on oxygen delivery, even though this would be of great interest for the design of high impact therapies in several cardiovascular diseases (CVDs). In particular, Cyclodextrin Nanosponges (C-NS) can be envisioned as innovative tools to improve the delivery of oxygen in a controlled manner in CVDs.

Methods: We tested oxygenated C-NS (OX-C-NS) at different concentrations (0.2, 2 and 20 μg/ml) for their capability to reduce cell mortality during hypoxia and reoxygenation (H/R) protocols. For comparative purpose, we also tested "blank materials" (C-NS filled with nitrogen gas without oxygen) and the effects of C-NS in Normoxia. To test the effectiveness of C-NS, we used H9c2, a cardiomyoblast cell line derived from rat heart, exposed to Normoxia (5% CO2 and 21% O2) or Hypoxia (5% CO2 and 95%N2) in a Hypoxic Chamber. The cellular mortality was measured with MTT assay.

Results: In Normoxia, regardless of OX-C-NS formulation, the H9c2 cells displayed a tendency to an increased proliferation, which seemed somewhat correlated to the concentration of OX-C-NS used.

The different concentration of OX-C-NS, applied before Hypoxia, induced a significant reduction of cell mortality compared to C-NS without oxygen. Also, the application of OX-C-NS at the beginning of reoxygenation induced a marked reduction of cell death.

Conclusions: OX-C-NS may induce H9c2 cell proliferation in Normoxia and may protect H9c2 from H/R injury *in vitro*. The administration of oxygen in a controlled manner during or after an ischemic event may be an innovative approach for reduction of Ischemia/Reperfusion injury, with consequent reduction of chronic CVDs. Our preliminary results, and in particular the observation of a remarkable efficacy in reoxygenation, suggest an interesting potentiality for medical application of C-NS during the treatment of myocardial infarction. Further studies are required to ascertain the protective potential of C-NS on cardiac I/R injury under *in vivo* conditions.

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Apelin-induced cardioprotection involves PTEN inhibition by Src kinase

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Objective: The cardioprotection against ischemia-reperfusion (I/R) injury consists in reduction of infarct size, limitation of myocardial contracture and improvement of post-ischemic mechanical recovery. Cardioprotection may be obtained with the administration in early reperfusion of the endogenous peptide apelin which acts with a mechanism triggered by the G-protein coupled receptor APJ and includes the PI3K-Akt-NO signaling pathway. PI3K-Akt activation is counteracted by the phosphatase and tensin homolog (PTEN), whose inhibition by Src has been suggested to be required for the effectiveness of the cardioprotective interventions. Since either oxidation or phosphorylation can inhibit PTEN, the present study aims to investigate whether apelin protective mechanism involves PTEN phosphorylation by Src.

Methods: The experiments were carried out on Langendorff-perfused rat hearts. In the control group the hearts underwent 30-min of global ischemia and 2-hours of reperfusion. In the apelin treated group, apelin-13 (0.5 μM) was infused during the first 20-min of reperfusion. In another group PP2, the specific inhibitor of Src kinase, was co-infused with apelin.

Left ventricular pressure was continuously recorded. After reperfusion infarct size was measured with nitro-blue tetrazolium technique. Western blot analysis was performed to test PTEN phosphorylation.

Results: Apelin significantly ($p < 0.001$) reduced infarct size from 60 ± 3 to 30 ± 3% of the left ventricle taken as the risk area. The effect of apelin on infarct size was suppressed by coinfusion of PP2. The increase in diastolic pressure, taken as an index of contracture, reached about 70 mmHg during the first 10 min of reperfusion and declined to about 45 mm Hg after 2 h of reperfusion in control group. This increase was significantly ($p < 0.001$) reduced by apelin so that it remained around 30 mm Hg for the entire period of reperfusion. Also in this case the effect of apelin was suppressed by PP2. In control group, left ventricle developed pressure (LVDevP) recovered to about 35% of pre-ischemic value at the end of reperfusion. If apelin was infused, this recovery reached about 70% of the pre-ischemic value at the end of apelin administration and remained almost unchanged for the entire period of reperfusion. Also in this case the effect of apelin was suppressed by PP2.

Western blot analysis revealed that apelin increased PTEN phosphorylation, an effect which was suppressed by inhibition of Src kinase with PP2.

Conclusion: myocardial protection by apelin against I/R injury includes the inhibition of PTEN by a phosphorylation induced by Src kinase.

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Nanoparticles at the neurovascular unit: In vitro and in vivo studies to assess the blood-brain barrier permeability and function

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Objective: The dilemma of the protection from noxious substances from the blood and the delivery of vital metabolites has always to be faced when dealing with the brain. Endothelial cells, forming the blood-brain barrier (BBB) with other cell types, regulate its trafficking. It is known that many common drugs cannot cross the BBB in appreciable concentration, decreasing the rate of success of possible available treatments for many central nervous system (CNS) diseases.

In the last decades, nanomedicine has played a pivotal role in developing strategies to deliver drugs to the CNS. In our previous studies we administered liposomes functionalized with phosphatidic acid and an ApoE-derived peptide (mApoE-PA-LIP) as a potential treatment for Alzheimer's disease (AD): their administration reduced brain beta-amyloid burden and ameliorated impaired memory in AD mice. We also evaluated the adaptability of warm microemulsion process for ligand surface modification of solid lipid nanoparticles (SLN) with ApoE to target the brain. Our *in vivo* biodistribution experiments, performed to study the influence of three different administration routes on SLN-mApoE bioavailability, showed that pulmonary administration increases the DiR-loaded SLN-mApoE bioavaibility to the brain in comparison to the intraperitoneal and intravenous ones, at the same concentrations and time points. In our ongoing experiments, we decided to further investigate the activities of NPs able to cross the BBB, independently from their administration routes. The aim of this study is to evaluate the interaction of mApoE-PA-LIP and SLN at the neurovascular unit. In light of our previous

results we here assess their interactions with human cerebral microvascular cells (hCMEC/D3) as in vitro BBB model. Our *in vitro* experiments by means of both the electrophysiological approach and the simultaneous calcium imaging will disclose if any active modulation on neuronal activities does occur after *ex vivo* and *in vivo* NPs administration. The obtained results will help us to better define the safety profile and active properties of NPs specifically developed to cross the BBB and to delivery their payload to the CNS.

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Catestatin induces glucose uptake and Glut4 trafficking in adult rat cardiomyocytes

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Obective: Catestatin is a cationic and hydrophobic peptide derived from the enzymatic cleavage of the prohormone Chromogranin-A. Initially identified as a potent endogenous nicotinic–cholinergic antagonist, Catestatin has recently been shown to act as a novel regulator of cardiac function and blood pressure and as a cardioprotective agent in both pre- and post-conditioning through Akt dependent mechanisms. The aim of this study was to investigate the potential role of Catestatin also on cardiac metabolism modulation, particularly on cardiomyocyte glucose uptake.

Methods: Experiments were performed on isolated adult rat cardiomyocytes. Glucose uptake was assessed by fluorescent glucose incubation and confocal microscope analysis. Glut4 plasma membrane translocation was studied by immunofluorescence experiments and evaluation of peripheral/internal Glut4 staining. Furthermore, we performed immunoblot experiments to investigate the involvement of the intracellular pathway Akt/AS160 in the Catestatin dependent Glut4 trafficking.

Results: Our results show that 10 nM Catestatin induces a significant increase in fluorescent glucose uptake, comparable to that exerted by 100 nM insulin which can be reverted by 100 nM Wortmannin (mean fluorescence intensity was 101.7 ± 13.8 in control, 346 ± 40.3 for Catestatin, 300.2 ± 42.7 for Ins, 137.12 ± 19.63 for Catestatin + Wm). Moreover, Catestatin stimulates Glut4 translocation to plasma membrane (peripheral/internal Glut4 staining was $0.86 \pm 0{,}04$ in Contr, $1.23 \pm 0{,}08$ for Cts, 1.04 ± 0.04 for Ins, 0.84 ± 0.02 for Cts + Wm) and phosphorylation of both Akt and AS160. All these effects were inhibited by Wortmannin.

Conclusions: On the whole, we show for the first time that Catestatin is able to modulate cardiac glucose metabolism, by inducing an increase in glucose uptake through Glut4 translocation to the plasma membrane, and that this mechanism is mediated by the Akt/AS160 intracellular pathway. Catestatin could therefore be an alternative agonist in respect to Insulin to increase glucose uptake in the heart, potentially relevant in diabetic cardiac malfunction.

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Chamazulene prevents ROS production in human dermal fibroblast and bovine aortic endothelial cells exposed to oxidative stress

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Objective: Cells are continuously exposed to oxidative stress due to production of reactive oxygen species (ROS) that may in some conditions induce cell damage. In this study we evaluated the capability of Chamazulene, an azulene compound from chamomile essential oil, to counteract ROS production in different cell models: Human Dermal Fibroblasts (HDF) and Bovine Aortic Endothelial Cells (BAEC) cultures.

Methods: Cells viability at different concentrations of Chamazulene was evaluated through the WST-1 Assay, while ROS production acutely induced by H2O2 (500 μM) or High Glucose (4.5 g/L) treatment was quantified with 2′-7′-Diclorofluorescein Diacetate probe and cytofluorimetric assay or confocal microscopy.

Results: Our results showed a reduction in ROS production induced by Chamazulene after cell treatment with H2O2 or High Glucose, thus suggesting an *in vitro* antioxidant activity of the compound. This preliminary study shows the possible role of Chamazulene as a scavenging molecule underlining its possible use to prevent ROS production and cell damage.

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HGF-mimic antibody administration to counteract doxorubicin cardiotoxicity

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Objective: Doxorubicin (Doxo) is a highly effective and widely used anti-cancer drug. Unfortunately, its use is limited by its cumulative dose-dependent cardiotoxicity (CTX). Various molecular mechanisms are involved in Doxo-mediated CTX, including DNA damage, oxidative stress, apoptosis and dysregulation of autophagy in cardiomyocytes. The addition of cardioprotectants to chemotherapy has been proposed as a preventing strategy to reduce the CTX risk. Thus, new agents targeting the detrimental activities of Doxo are attractive candidates as cardioprotective molecules. The Hepatocyte Growth Factor (HGF)/Met receptor couple has been shown to protect from cell death, oxidative stress and excessive autophagy in cardiac cells. In a previous work, we have demonstrated that agonist anti-Met antibodies, that mimic the biological effects of HGF, mitigate the cardiac damage derived from hypoxia. In this work, we exploited the potential cardioprotective function of an HGF-mimic antibody in the context of Doxo-CTX.

Methods: Adult male C57BL/6J mice were randomized to placebo (group 1), Doxo (group 2) and Doxo + the HGF-mimic antibody (group 3). Mice were treated with i.p. injections of PBS (group 1) or Doxo 7 mg/kg (group 2 and 3) for 3 weeks. Group 3 received the agonist antibody (5 mg/kg) the day before each cycle of chemotherapy. Body weight was measured weekly. The cardiac function was assessed by magnetic resonance imaging (MRI) at week 5 and 6 (2 and 3 weeks after cessation of chemotherapy). At sacrifice, the mice organs were weighted and the heart was examined through histological and molecular analysis.

Results: The treatment with the HGF-mimic antibody prevents the Doxo-induced cardiomyopathy in mice. In particular, MRI analysis showed that Met agonist antibody administration improves the heart systolic function through a thickening of contractile fibers, indicated by both MRI and heart weight measurement. In addition, Met receptor agonist antibody reduced the death rate and the loss of body weight and muscle volume produced by Doxo. From a molecular point of view, the presence of HGF-mimic antibody attenuated Doxo-mediated cell death mechanisms: apoptosis, excessive autophagy and mitochondrial dysfunction. In addition, the presence of antibody modulated DNA repair in response to DNA damage.