β3-ARs selective agonist BRL37344 elicited a concentration-dependent increase of contractility abolished by SR_{59230A}, a specific β3-AR antagonist, but not by α/β1/β2-ARs inhibitors (phentolamine, nadolol, and ICI118,551). Under acute hypoxia, BRL_{37344} did not affect the goldfish heart performance. However, SR_{59230A}, but not phentolamine, nadolol, and ICI118,551, abolished the time-dependent enhancement of contractility which characterizes the hypoxic goldfish heart. Under both normoxia and hypoxia, adenylate cyclase and cAMP were found to be involved in the β3-ARs-dependent downstream transduction pathway. Moreover, unlike normoxia, under hypoxia the mechanism of action activated by β3-ARs involves the Nitric Oxide Synthase (NOS)/Nitric Oxide (NO) system. Our results are the first to propose β3-ARs as components of the complex molecular machinery that allows hypoxia tolerance in cyprinid fish.

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The involvement of GRK2 in stress response to radiation during cardiovascular invasive procedure

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Background

G protein coupled receptor kinase type 2 (GRK2) has a pivotal role in the development and progression of cardiovascular diseases. Moreover, this kinase is a stress protein which is able to move quickly within the cell in response to acute stimuli such as ionizing radiation exposure (IR). Indeed, we have previously shown that in HEK-293 cells the acute exposure to IR promotes GRK2 translocation in different cellular compartment. The cardiovascular invasive procedures such as coronary angiography or percutaneous coronary interventions (PCI) are diagnostic examination, that use a tolerated dose of X-ray. In PBMCs, GRK2 levels increase during acute myocardial infarction and are associated with worse cardiacfunction.

Purpose

To evaluate the effects of IR exposure on GRK2 levels in PBMCs isolated from patients undergoing cardiovascular interventions and in Human Endothelial Progenitor cells (EPC) after IR exposure.

Methods

GRK2 levels are evaluated by western blot analysis in PBMCs isolated from peripheral blood by Ficoll gradient before, immediately after, at 24 hours from the procedure. GRK2 levels are evaluated also in EPC cells by western blot analysis 24 hours after IR exposure.

Results

In PBMCs from patients undergoing cardiovascular invasive procedure, changes in GRK2 levels are recorded in response to IR exposure occurring during procedure. In particular GRK2 levels are inversely proportional with dose-area product (DAP) of patients undergoing PCI both immediately after and 24 hours from the procedure. To evaluate the direct effects of IR on cells and to confirm the trend observed in patients undergoing PCI, GRK2 levels are measured in EPC cells whose source of origin is represented by PBMCs. In detail, in these cells the GRK2 levels are assessed after 24 hour from IR, by using radiation doses (0,3 Gy and 1 Gy) comparable with IR exposure of cardiovascular invasive procedure. Similarly to PCI, also in EPC cells the GRK2 levels are inversely proportional with radiation dose after 24 hours from IR exposure. These in vitro results confirm the changes of GRK2 levels in response to IR, reflecting the trend observed in PBMCs of patients undergoing PCI.

Conclusion

These preliminary data suggest that *GRK2 could be involved in stress response associated to IR exposure during cardiovascular interventions.* The physiopathological relevance of this damage is to be established.

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Silica nanoparticle internalization by human mesenchymal stem cells enhances their adhesion properties in dynamic conditions

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Introduction

Human mesenchymal stem cell (hMSC)-based therapies have shown to partially improve cardiac functionality upon myocardial infarction. However, their therapeutic effect is limited by their engraftment capacity. Indeed, most of the MSCs die during the delivery and fail to adhere and therefore to survive upon intramyocardial injection. In previous studies, we demonstrated that fluorescent-labelled silica nanoparticles (SiO_2NPs) are well-tolerated by hMSCs and efficiently track cells in vivo. Interestingly, SiO_2NP internalization increased the area and the maturation level of hMSC focal adhesion complexes, and enhanced the expression of Connexin-43 (Cx43), the main isoform that form the intracellular GAP junction, during *in vitro* static culture. Based on these findings, we hypothesized that internalization of SiO_2NPs could enhance MSC adhesion also in dynamic conditions, resembling interstitial shear stresses physiologically present in the contractile myocardium.

Methods

hMSCs were isolated from three different bone marrow donors and incubated with 50 μg/ml SiO_2NPs overnight for internalization. hMSCs without SiO_2NPs were used as control. Custom- made shear stress chambers were used to mimic the heart physiological shear stress of 0,43 dyne/cm^2. Adhesion capability of hMSCs (pre-loaded with FITC-labeled Calcein) was evaluated on either a fibronectin-coated glass or a rat-origin cardiac cell monolayer (80% cardiomyocytes and 20% fibroblasts) following two hours. Gap junction formation between the hMSCs and the cardiac monolayer was evaluated by assessing Cx43 expression in immunofluorescent images, and their functionality by quantification of Calcein (gap-junction permeant dye) transfer in flow cytometry experiments.

Results

On fibronectin substrates the adhesion area of SiO_2NP-hMSC focal adhesion complexes was higher than in control cells (5122 ± 701 and 1469 ± 236 cm^2, respectively, $p<0.01$) Adhesion on the cardiac monolayer was also superior in SiO_2NP-treated hMSCs. SiO_2NP-hMSC were also characterized by the highest number of polarized

adhesion focal complexes per cell (between 7-13 *vs* 3-6 in control condition). Cx43 expression was higher in SiO_2NP-hMSCs compared to control. In addition, cells from the cardiac monolayer showed a higher positivity for FITC-labeled Calcein in the presence of SiO_2NP-hMSCs compared to the control (Calcein transfer fold change value of 2.58 $\pm$ 0.278 for SiO_2NP-hMSCs condition).

Conclusions

SiO_2NP incorporation clearly increases the hMSC adhesion capacity and the formation of functional GAP junction with the cardiac cells, showing a high potential to improve their engraftment and consequently their therapeutic effect *in vivo*.

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Cardiac mitochondrial alterations in a mouse model of fabry disease

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Background

Fabry disease (FD) is a genetic disorder caused by deficiency of α-Gal A activity with intralysosomal accumulation of globotriaosylceramide (GB3). Fabry patients show cardiac involvement developing left ventricular hypertrophy and dysfunction (LVHD), whose pathogenesis remains unclear. Beside GB3 accumulation, impairment of energetic metabolism is hypothesized as putative mechanism for LVHD, and mitochondrial dysfunction is suggested by reduced activity of mitochondrial respiratory chain observed in fibroblasts from FD patients.

Purpose

To explore the pathogenetic mechanism of cardiac dysfunction in FD evaluating the role of mitochondria.

Methods

Transgenic mouse that lacks endogenous GLA but expresses a human R301Q GLA transgene [Tg]/mα-Gal A knockout (KO)] was used as model of FD. On cardiac tissue, we evaluated cell area through WGA staining. Living cardiomyocytes (CM) were isolated by Langendorff system and cell contractility analysis was performed by MUSCLEMOTION software. The levels of BNP, SerCa, PGC-1α, Tfam and NRF1 were evaluated by Real-time PCR while, the levels of Mitofusin 2 (MFN2) and Citochrome C (Cit C) by western blot on mitochondrial and cytosolic extract, respectively.

Results

In cardiac tissue from FD mice (FD-M) we observed an increase of cardiac cell area compared to wild- type tissue. The size of the CM isolated from FD-M was increased, confirming cardiac cell hypertrophy in our model. Accordingly, transcription levels of BNP and SerCa were higher in the hearts of FD mice respect to wild type. To evaluate cardiac cell performance, single cell contractility under field stimulation was recorded. In basal condition, FD-M CM showed higher contractility than wild type cells. However, an impaired contractile response to adrenergic stimulation was recorded for FD-M CM. To verify whether the observed phenotype was linked to mitochondrial dysfunction we explored mitochondrial health. Mitochondrial biogenesis was impaired in FD-M hearts concurrently with altered expression of the master regulators PGC- 1α, Tfam and NRF1. Moreover, increased levels of Mfn2, DRP1 and LC3II on mitochondria from FD-M hearts suggest that also mitochondrial fusion-fixion equilibrium as well as mitophagy were affected. In the extracts from FD-M hearts, we observed an increase cytosolic levels of Cit C, marker of a mitochondrial damage and permeabilization.

Conclusions

Cardiac cell hypertrophy was observed in FD-M hearts together with alterations in mitochondrial biology, suggesting that the LVHD could be a compensatory response to the reduced contractile force due to inadequate energy supply from dysfunctional mitochondria.

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Human left atrial dilation is associated to increased expression of type-1 hyperpolarization cyclic-nucleotide gated channel and MinK-related peptide 1 subunit: Implications for atrial electrophysiological remodeling

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Atrial electrical remodeling is a fundamental event to set the basis for the initiation and the maintenance of atrial fibrillation (AF). Although it may be a consequence of AF, atrial electrical remodeling also precedes its onset, thus conferring great interest to predictive clinical markers associated with its development. Enlargement of atrial size and deformation are two echocardiographic parameters often detected in patients prone to develop AF, including those affected by mitral valve disease. Following on our previous studies demonstrating the occurrence of functional modifications of Hyperpolarization Cyclic-Nucleotide gated (HCN) channels in AF patients, in this study we aimed to investigate whether changes in HCN channels, the regulatory subunit MinK-related peptide 1 (MiRP1), and the upstream transcriptional regulator 5' AMP- activated protein kinase (AMPK), occurred in the left atrial samples of patients in sinus rhythm undergoing mitral valve surgery. Additionally, we investigated whether echocardiographic parameters are linearly related to these modifications.

Material and methods

Left atrial specimens were obtained from patients affected by mitral valve disease undergoing cardiac surgery. Before surgery all patients were analyzed by Speckle Tracking Echography allowing the evaluation of parameters related to atrial deformation, strain and degree of atrial and ventricular dilation. Atrial samples were then processed to perform Western blot analysis of the following target