CARDIOVASCULAR DEPARTMENT

Project title

"Therapeutic impact of autophagy activation on cardiac remodeling and cardiovascular damage after acute myocardial ischemia"

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Abstract

Autophagy is emerging as a new potential therapeutic target for preventing and treating cardiovascular diseases. Experimental studies demonstrated that activation of autophagy reduces cardiovascular damage in response to acute myocardial ischemia, pressure overload, and chronic ischemic remodeling.

Trehalose is a natural activator of autophagy, which proved effective at reducing chronic cardiac remodeling and atherosclerosis in recent experimental studies. Our translational research proposal is aimed at clarifying whether: 1) autophagy activation reduces endothelial dysfunction; 2) trehalose reduces cardiac remodeling through transcription factor EB-dependent activation of mitochondrial autophagy; 3) autophagy dysregulation is associated with the development of cardiovascular diseases in human patients; and 4) Trehalose administration reduces cardiac remodeling and endothelial dysfunction in patients with coronary artery disease. If successful, our work may provide new evidence supporting the potential therapeutic role of autophagy activation in the treatment of cardiovascular diseases.

Main technical approaches

- Previous experimental work demonstrated that autophagy defects in immune mononuclear cells contribute to the development of atherosclerosis. Peripheral blood mononuclear cells will be obtained from 2 groups of patients: 1) patients affected by CAD (group 1, N=63); 2) patients affected by essential hypertension without overt cardiovascular diseases (group 2, N=63). Inclusion criteria will be: 1) age between 18 and 85 years; 2) ejection fraction >50%. Exclusion criteria will be: 1) acute myocardial infarction in the last 6 months; 2) acute and chronic inflammatory diseases; 3) immunological diseases; 4) cancer; 5) infection; 6) previous organ transplantation; 7) intake of pharmacological therapy modulating autophagy (e.g. mTOR inhibitors). Diagnosis of CAD in group 1 will be based on angiographic evidence of one or more coronary artery plaques with diameter stenosis >50%. On the other hand, the presence of CAD in group 2 will be excluded based on the absence of positive family history, signs and symptoms, and lack of stress-induced myocardial ischemia. Autophagy will be assessed in the two groups, by evaluating the levels of autophagy markers LC3 and p62 in circulating mononuclear cells. In the group 1, autophagy levels in circulating mononuclear cells will be correlated with the

extension of CAD and with the number of endothelial progenitor cells (EPCs). EPCs obtained from a subset of patients from groups 1 ad 2 will be cultured and transdifferentiated into endothelial cells. EPC-derived endothelial cells from the two groups will be treated or not with trehalose (50 mM) for 24 hours. After treatment, angiogenesis and cell survival will be assessed.

- Circulating Mononuclear Cells will be isolated from peripheral blood. Briefly, after withdrawal in Vacutainer® heparin tubes, whole blood will be centrifuged by Ficoll's gradient. After the last centrifugation at 200 g to remove platelet, mononuclear cells will be used for biochemical analyses or plated to induce endothelial transdifferentiation. The percentage of EPCs among circulating mononuclear cells will be assessed by FACS analysis. Survival and angiogenesis of EPC-derived endothelial cells will be assessed by propidium iodide/annexin V assay and Matrigel assay, respectively.
- We will conduct a clinical study open-label, multicenter, assessors-blinded randomized trial. A total of 162 patients with acute STEMI with a culprit lesion located in the left anterior descending artery undergoing primary PCI will be included. Exclusion criteria will be: 1) three-vessel disease; 2) prior myocardial infarction; 3) diabetes mellitus; 4) acute and chronic inflammatory diseases; 5) immunological diseases; 6) cancer; 7) infection; 8) previous organ transplantation; 9) intake of pharmacological therapy modulating autophagy (e.g. mTOR inhibitors); 10) contraindications to undergo cardiac magnetic resonance imaging (MRI) study. Patients will be 1:1:1 randomly allocated to receive trehalose 30 g daily, trehalose 60 g daily or no trehalose for 6 months starting the day of primary PCI on top of standard therapy (randomization will be performed using a web-based system). The 30 g lower dose of trehalose was chosen based on the calculated daily intake of the molecule, which was proved to be effective in the reduction of chronic ischemic remodeling in mice, according to the results of our recent study (2% in the drinking water) [17]. We made a conversion of trehalose dosage from mice to humans according to validated FDA conversion charts. We will also test the effects of a doubled 60 g dose to check whether the properties of trehalose are dose-dependent. The two doses of trehalose will be combined in the analysis of the primary and secondary endpoints vs. control patients (no trehalose) and patients taking 30 or 60 g of trehalose will be considered as part of the same group (see power assumption and statistical considerations in the following text). Exploratory analyses for the comparison of trehalose doses and the comparison of each doses vs. placebo will be performed. The primary endpoint of the study will be the change in left ventricular end-diastolic volume index (LVEDVi) as assessed by cardiac MRI between baseline and 6-month.
- Trehalose will be produced by Nyl Laboratories (Monterotondo, RM, Italy) in packages of 30 g. Compliance of patients to trehalose treatment will be measured by counting the number of consumed packages. Primary and secondary endpoints on cardiac function will be assessed by cardiac MRI performed at baseline (within 10 days after inclusion in the study and after primary PCI) and after 6 months (360 days

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± 15 days). All cardiac MRI scans will be performed using a 1.5 Tesla system. Steady state free precession cine images will be performed for the assessment of left ventricular ejection fraction, mass, and left and right ventricular volumes. Further sequences for myocardial structural characterization and evaluation of fibrosis will be acquired by using paramagnetic contrast media. All examinations will be conducted according to acquisition and post-processing protocols standardized by the Society for Cardiovascular Magnetic Resonance. The analysis of cardiac MRI will be performed by a central core-laboratory and performed by assessors blinded to treatment allocation.

Scientific references

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