UNVEILING HUMAN CARDIAC STEM CELLS REGENERATIVE POTENTIAL IN ISCHEMIA/REPERFUSION INJURY

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After an Acute Myocardial Infarction (AMI), Ischemia-Reperfusion (I/R) injury is responsible for a critical decrease in the number of viable cardiomyocytes (hCMs). Human myocardium harbors a population of endogenous cardiac stem cells (hCSCs) that is activated upon I/R injury, contributing to myocardial repair through the establishment of an auto/paracrine molecular crosstalk between hCSCs and hCMs in stress. Clinical trials involving transplantation of hCSCs into the infarcted myocardium have demonstrated the potential of these cells. Although some improvements have been reported regarding increase in viable myocardium and improved tissue contractility, extensive data indicates that transplanted cells do not survive in the myocardium and this has led to the postulation of a paracrine mechanism for the observed beneficial effects.

Using the same cells currently employed in the allogeneic hCSCs transplantation clinical trial CARE-MI (EUDRA 2013-001358-81), our work aims at setting up the first in vitro human I/R injury model in order to better decipher the mechanisms of action of hCSCs upon AMI using proteomic tools.

Mono-cultures of donor derived hCSCs, hCMs and co-cultures with the two cell types were established using human donor derived CSCs and cardiomyocytes derived from human induced pluripotent stem cells at different maturation stages (hiPSC-CMs). Ischemia was mimicked by substituting growth media by Ischemia Mimetic Solution and placing the cells at 0% O₂ for 5 hours. In the reperfusion step, cells were placed back in their physiological culture conditions (3% O₂). The effect of I/R injury in hCSCs was accessed by total proteome analysis (using LC-MS) at different time points. Growth factor secretion, cells’ viability, as well as hCSCs proliferation was also monitored in both mono- and co-culture systems.

More than 2000 proteins were identified in hCSCs exposed to injury including proteins associated with mitochondrial dysfunction and oxidative stress. Important features of I/R injury were successfully captured, namely hCSCs proliferation activation upon insult, increase in key growth factors secretion, and the protective effect of hCSCs on hiPSC-CMs. This system will allow further understanding on the molecular landscape of the myocardium during AMI, namely regarding hCSCs regenerative response and hCMs survival. The knowledge generated in this work will hopefully potentiate the development of novel molecular and cell-based therapies for myocardium regeneration.

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