Effects of transient hypoxia/ischemia on induced human pluripotent stem cell (iPSC)-derived cardiomyocytes

Martin Albrecht PhD 1, Kerstin Parczany 1, Matthias Gruenewald MD 1, Ole Broch MD 1, Lars Hummitzsch MD 1, Rouven Berndt MD 2, Jochen Cremer MD 2, Markus Steinfath MD 1, Karina Zitta PhD 1

1 Department of Anesthesiology and Intensive Care Medicine, University Hospital Schleswig-Holstein, Kiel, Germany; 2 Department of Cardiovascular Surgery, University Hospital Schleswig-Holstein, Kiel, Germany

Background: Hypoxia/ischemia is a central elicit of ischemia/reperfusion (I/R) injury in the human heart. However, there is an apparent lack of suitable in vitro models for the investigation of I/R-induced cellular mechanisms in human cardiac cells.

Aim: To investigate whether induced human pluripotent stem cell (iPSC)-derived cardiomyocytes are susceptible to physiologically defined transient hypoxic/ischemic conditions.

Materials and Methods: Spontaneously contracting human iPSC-derived cardiomyocytes (ax2505; kindly provided by Axol Bioscience Ltd., Cambridge, UK) were subjected to enzymatically induced hypoxic/ischemic conditions (Zitta et al. Eur J Pharmacol 2010, Zitta et al. Mol Med 2012, Zitta et al. Exp Cell Res 2012, Hummitzsch et al. Exp Cell Res 2014) by using glucose oxidase (GO, 2U/ml) and catalase (CAT, 120U/ml). Morphological assessment as well as measurements of LDH activity released from damaged cells (Cytotoxicity Detection Kit; Roche, Mannheim, Germany) and Troponin T (TrT; Department of Clinical Chemistry, Kiel) were used for evaluating and quantifying hypoxia/ischemia induced cytotoxicity. Electrophysiological parameters were assessed using a loop recorder (Medtronic, Dublin, Ireland) which was attached to the bottom of the culture dish.

Results: Hypoxic/ischemic conditions were rapidly established after the addition of GO/CAT and resulted in pO2 levels <10mmHg after 60 minutes (lasting for at least 6 hours; A), a gradual decrease of glucose concentration (from 2g/l to <1g/l after 4 hours) and a decline of pH from 7.05 to 6.98. iPSC-derived cardiomyocytes showed spontaneous synchronized contractions after 10 days in culture with a beating frequency of 24.41±0.02 bpm under normoxia and 18.87±3.21 bpm under hypoxic/ischemic conditions (online video). Directly after replacing the hypoxic/ischemic medium by normoxic culture medium, frequency of contractions increased by 1.8-fold in the hypoxia/ischemia group (B). The electrophysiological signal of the cardiomyocytes corresponded to a standard ventricle polarisation with an amplitude of 0.73±0.26mV and a QRS-interval of 0.11±0.02s. During hypoxia a 10-fold increase in numbers of episodes of asystole were detected (normoxia: 7%; hypoxia/ischemia: 70%; B). 24 hours after the 4 hour hypoxia period, iPSC-derived cardiomyocytes showed clear morphological signs of cell damage such as cell rounding, swelling and detachment from the growth surface (C) which was associated with irregular and asynchronous beating of single cells (online video). In cultures that were subjected to 4 hours of hypoxia, LDH release as a marker of cell damage was increased 3-fold while iPSC-derived cardiomyocytes that were cultured under normoxic conditions did not show morphological changes or increased LDH release after 24 hours (normoxia: 0.10±0.00au; hypoxia/ischemia: 0.29±0.02au; P<0.05; D). In addition, concentrations of Troponin T (TrT) were evaluated in cell culture supernatants and showed increased levels in the hypoxia group (E).

Conclusion: Human induced pluripotent stem cell-derived cardiomyocytes are susceptible to transient hypoxia/ischemia in vitro. The described culture system closely resembles hypoxic/ischemic conditions in vivo and may help to elucidate cellular/molecular mechanisms of ischemia/reperfusion injury as well as facilitate the search for cardioprotective strategies.