UNIVERSITÄTSKLINIKUM
Schleswig-Holstein

axol
your discovery stems from here

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

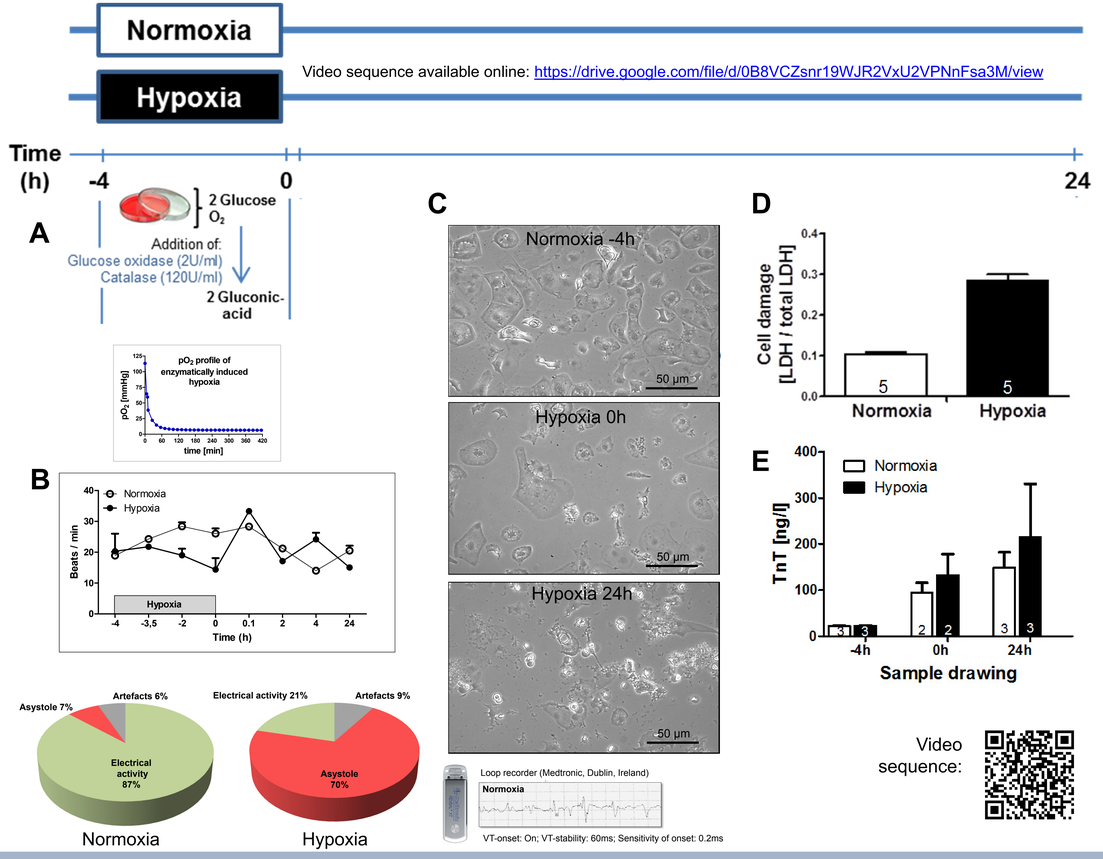
Background: Hypoxia/ischemia is a central elicitor of ischemia/reperfusion (I/R) injury in the human heart. However, there is an apparent lack of suitable in vitro models for the investigation of I/R-induced cellular mechanisms in human cardiac cells.

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 (TnT; 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 pO_2 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 7.65 to 6.98. iPSC-derived cardiomyocytes showed spontaneous synchronized contractions after 10 days in culture with a beating frequency of 24.41±4.02 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 asynchronized 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 reveal 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 (TnT) 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.



Contact:
Prof. Dr. Martin Albrecht
Department of Anaesthesiology and Intensive Care Medicine,
University Hospital Schleswig-Holstein, Kiel, Germany
Email: Martin.Albrecht@uksh.de