41.4.5 - Ion Channels, Electrophysiology

In vivo KCNQ1-suppression-replacement gene therapy in transgenic rabbits with type 1 long QT syndrome

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Background: Type 1 long QT syndrome (LQT1) is a genetic channelopathy characterized by both haploinsufficient and dominant-negative loss-of-function pathogenic variants in the KCNQ1-encoded Kv7.1 K+ channels conducting IKs. Patients with LQT1 may manifest QT prolongation and ventricular arrhythmias that can culminate in sudden cardiac death.
Purpose: With this project, we aim to investigate whether our mutation independent, KCNQ1-specific suppression-replacement (SupRep) gene therapy can restore the deficient K+-channel and thereby restore the healthy phenotype.
Methods: Our proprietary dual component SupRep gene therapy was created by combining a custom-designed KCNQ1 shRNA and a shRNA-immune (shIMM) KCNQ1 cDNA into AAV9. In vivo AAV9-mediated KCNQ1SupRep gene therapy was performed by targeted intra-aortic root construct injection (1E12 vg/kg body weight) during balloon occlusion using a Swan Ganz catheter. 2 weeks after in vivo gene therapy, 12-lead ECGs were assessed in adult transgenic LQT1 (KCNQ1-Y315S, loss-of IKs) and wild-type (WT) rabbits to determine the effect of SupRep therapy on the rabbit's QTc. Patch-clamp and calcium transient measurements were performed in isolated ventricular cardiomyocytes to evaluate the effect of SupRep on the cardiomyocyte's action potential duration (APD90) and Ca2+ transient duration (Ca2+90) both, in vitro – after plasmid transfection, and in vivo – after AAV9-mediated gene therapy. AAV9-KCNQ1-construct expression was verified with immunohistochemistry targeting the GFP-tag.
Results: After injection of 3 LQT1 rabbits so far, no significant changes were observed in the LQT1 rabbits' QTc before and after in vivo AAV9-mediated gene therapy. However, at the cellular level, LQT1 cardiomyocytes transfected in vitro with the SupRep-plasmid demonstrated a significant reduction of APD90 (ms, 1Hz stimulation at 37°) compared to LQT1 control cells (SupRep-in-vitro, 358±21 vs. control 447±22, $p<0.05$). No differences were observed between sham-plasmid transfected cardiomyocytes and LQT1 control cells after one day of culturing. Similarly, cardiomyocytes isolated from the 3 LQT1 rabbits that underwent in vivo SupRep gene therapy demonstrated pronounced shortening of both APD90 and Ca2+90 compared to LQT1 controls, leading to levels similar to WT controls (APD90, 1Hz, 37°: LQT1: 530±18, WT: 445±18, LQT1-SupRep: 375±25, $p<0.0001$ LQT1 vs. LQT1-SupRep, p=ns LQT1-SupRep vs. WT) (Ca2+90: LQT1: 487±23, WT: 346±16, LQT1-SupRep: 393±19, $p<0.0001$ LQT1 vs. LQT1-SupRep, p=ns LQT1-SupRep vs. WT).
Conclusion: In vivo KCNQ1-suppression-replacement gene therapy normalizes cellular action potential and calcium transient duration in transgenic LQT1 rabbits. Further in vivo experiments will be conducted to evaluate whether this therapeutic correction at the cardiomyocyte level will translate into normalization of the LQT1 rabbits' QTc both at rest and during stress testing.