Characterization of PLN-R14Del mutation in human induced pluripotent stem cell-derived cardiomyocytes

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Background: Phospholamban (PLN) is the natural inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATP-ase (SERCA2a) in cardiac cells. Their interaction, and its regulation, is crucial for the excitation-contraction coupling (ECC) in cardiomyocytes. The R14Del mutation is characterized by a deletion of Arg-14 of PLN and it is associated with a severe phenotype of arrhythmogenic and dilated cardiomyopathy. The mutation has been already studied in several models, such as HEK293, mice, and human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Here we wanted to: 1) test how PLN-R14Del (MUT) hiPSC-CMs and their isogenic controls (WT) cultured in a standard monolayer (ML) format differ in terms of Ca^{2+} dynamics; 2) test in both groups a pure SERCA2a activator DRUG A, which prevents the interaction PLN/SERCA2a.

Methods: both WT and MUT hiPSC-CMs were thawed and plated in single glass petri dishes, to allow simultaneous electrophysiological recordings and Ca^{2+} measurements. Ca^{2+}-transient recordings were performed in field stimulation at 1 Hz (or increasing frequencies). All experiments were performed under Tyrode's (TYR) superfusion.

Results: V-induced Ca^{2+}-transients did not differ between WT and MUT MLs; the sarcoplasmic reticulum (SR) Ca^{2+}-content, was not significantly different between groups although it tended to be higher in MUT MLs. The half time of Ca^{2+}-transient decay ($t_{1/2}$) was significantly faster (small $t_{1/2}$) in MUT MLs than the WT ones ($p<0.05$). The pure SERCA2a stimulator DRUG A (1 µM) significantly accelerates the decay in both WT ($p<0.0001$) and MUT MLs ($p<0.01$). Data obtained from the staircase protocol (application of four increasing frequencies) for diastolic Ca^{2+}, showed a flat staircase in MUT MLs and a steeply positive one (implying accumulation of Ca^{2+} in the cytosol) in WT MLs ($p<0.05$ vs MUT). DRUG A 1 µM significantly reduced the accumulation of diastolic Ca^{2+} in WT ($p<0.01$), whereas it was similar to basal conditions in MUT MLs.

Conclusions: these results suggest that the PLN-R14Del mutation leads to a PLN loss-of-function for the following reasons: 1) the faster decay (small $t_{1/2}$) in MUT compared to WT MLs; 2) the lack of effect of DRUG A during the staircase protocol in MUT MLs. In particular, the latter indicates that in MUT MLs SERCA2a function is enhanced.