Supplementary information

Effects of antiarrhythmic drugs on hERG gating in human-induced pluripotent stem cell-derived cardiomyocytes from a patient with short QT syndrome type 1

Running title: Drug effects on hERG gating

Mengying Huang1, Zhenxing Liao1,2*, Xin Li1,3, Zhen Yang1,2, Xuehui Fan1,6, Yingrui Li1, Zhihan Zhao1, Siegfried Lang1,4, Lukas Cyganek4,5, Xiaobo Zhou1,4,6, Ibrahim Akin1,4, Martin Borggrefe1,4, Ibrahim El-Battrawy1,4

1First Department of Medicine, Faculty of Medicine, University Medical Centre Mannheim (UMM), University of Heidelberg, Mannheim, Germany; 2North Sichuan Medical College; 3College of Medical Technology, Chengdu University of Traditional Chinese Medicine; 4DZHK (German Center for Cardiovascular Research), Partner Sites, Heidelberg-Mannheim and Göttingen, Germany; 5Stem Cell Unit, Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Germany; 6Key Laboratory of Medical Electrophysiology of Ministry of Education and Medical Electrophysiological Key Laboratory of Sichuan Province, Institute of Cardiovascular Research, Southwest Medical University, Luzhou, China.

*equally contributed.

Address for correspondence:

Xiaobo Zhou, MD

First Department of Medicine, Faculty of Medicine, University Medical Centre Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany. E-mail: Xiaobo.zhou@medma.uni-heidelberg.de

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Material and Methods

Generation of human iPS cells (hiPSC)

Somatic cell isolation and primary culture

Human fibroblast cultures were derived from skin punch biopsies of a healthy donor and patient with SQTS type 1 carrying p.N588K in hERG channel. Skin punch biopsy (3-4 mm) was taken aseptically by a clinical doctor, placed in DMEM (Thermo Fisher Scientific, #11960044) containing 200 U/ml penicillin and 200 µg/ml streptomycin (Thermo Fisher Scientific, #15140122) and transferred to the lab within 48 h. Biopsies were mechanically cut in pieces of 0.5-1 mm, were placed epidermis upside in the cell culture dish and cultured in human fibroblast medium (HFBM) composed of DMEM (Thermo Fisher Scientific, #11960044) supplemented with 10% Fetal Bovine Serum (Thermo Fisher Scientific, #10270106), 1x MEM Non-Essential Amino Acids Solution (Thermo Fisher Scientific, #11140035), 2 mM L-Glutamine (Thermo Fisher Scientific, #25030024), 50 µM β-mercaptoethanol (Serva Electrophoresis, #28625), 10 ng/ml bFGF (Peprotech, #100-18B), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, #15140122) at 37°C with 5% CO2 atmosphere. Medium was changed every other day. Fibroblasts before passage 3 (p3) was used for generation of iPS cells.

Generation of hiPSCs

HiPSC lines isSTQSa1.7 (GOEi091-A.7, here abbreviated as SQTS1), isSQTSa1.8 (GOEi091-A.8) and isSQTSa1.15 (GOEi091-A.15) were generated from fibroblasts in feeder free culture conditions using the integration-free CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, #A16517) with the reprogramming factors OCT4, KLF4, SOX2, c-MYC according to manufacturer’s instructions with modifications. In brief, 1.5x10⁴ early passage fibroblasts were plated in two wells of a Matrigel-coated 24-well plate in HFBM two days before transduction. Cells were transduced at 40-50% confluence with Sendai virus cocktail (hKOS: hc-Myc: hKlf4) at a MOI of 10:10:6 according to the counted cell number of extra well (typically 2.5x10⁴ cells/well) in HFBM. Virus was removed after 24 h and HFBM was changed every other day.
HiPSC lines ipWT1.1 (GOEi014-B.1), ipWT1.3 (GOEi014-B.3, here abbreviated as donor) and ipWT1.6 (GOEi014-B.6) were generated from fibroblasts in feeder free culture conditions using the integration-free episomal 4-in-1 CoMiP reprogramming plasmid (Addgene, #63726) with the reprogramming factors OCT4, KLF4, SOX2, c-MYC and short hairpin RNA against p53, as described previously with modifications (1). In brief, 5x10^5 early passage fibroblasts were used for electroporation with the Nucleofector 2b Device (Lonza) with program P22 or U23 by using the NHDF Nucleofector Kit (Lonza, #VPD-1001) and 2 µg of the reprogramming plasmid. Transfected cells were plated in one well of a Matrigel-coated 6-well plate in HFBM supplemented with 500 µM sodium butyrate (Sigma-Aldrich, #B5887), 2 µM Thiazovivin (Millipore, #420220), 100 U/ml penicillin and 100 µg/ml streptomycin. Medium was changed every other day with HFBM supplemented with 500 µM sodium butyrate.

At day 7 post transduction/transfection, cells were replated in various dilutions in Matrigel-coated 6-well plates in HFBM supplemented with 500 µM sodium butyrate and 2 µM Thiazovivin. From day 8, medium was changed to E8 medium (Thermo Fisher Scientific, #A1517001) supplemented with 500 µM sodium butyrate (day 8-day 11) with daily medium change. Cells were monitored for morphology change and appearance of colonies (typically after 2-3 weeks). Individual colonies with iPSC-like morphology were picked mechanically in Matrigel-coated 12-well plates in E8 medium supplemented with 2 µM Thiazovivin. Newly established iPSC lines were passaged with Versene solution (Thermo Fisher Scientific, #15040066) and cultured in E8 medium supplemented with 2 µM Thiazovivin on the first day after passaging in Matrigel-coated plates for at least ten passages before being used for pluripotency characterization and differentiation experiments.

**Characterization of hiPSCs**

To examine pluripotent characteristics of generated hiPSCs, cell morphology, alkaline phosphatase activity, expression of endogenous pluripotency markers, Spontaneous differentiation potential were assessed. The hiPSC lines from the donor and STQS1 patient displayed a typical morphology for human pluripotent stem cells and were positive for alkaline phosphatase. In comparison to fibroblasts, generated hiPSC lines showed expression of endogenous pluripotency markers SOX2, OCT4, NANOG, LIN28, FOXD3 and GDF3 at mRNA level proven by RT-PCR. Human
embryonic stem cells (hESCs) were used as positive control, mouse embryonic fibroblasts (MEFs) were used as negative control. The expression pluripotency markers OCT4, SOX2, NANOG, LIN28, SSEA4 and TRA-1-60 was detected in generated iPSC lines. Spontaneous differentiation potential of generated iPSC lines was analysed by embryoid body (EB) formation. Germ layer-specific genes like α-fetoprotein (AFP) and albumin (ALB) (endoderm), cTNT and α-MHC (mesoderm), and tyrosine hydroxylase (TH) and MAP2 (ectoderm) were detected in a developmentally controlled manner during differentiation of EBs (days 0, 8, or 8+25), whereas endogenous OCT4 expression was decreased during spontaneous differentiation. Immunocytochemical staining of spontaneously differentiated hiPSC lines showed expression of endodermal marker AFP, mesodermal-specific α-SMA and ectodermal βIII-tubulin. All data together confirmed the pluripotency and differentiation potential of generated hiPSCs.

**Spontaneous in vitro differentiation of hiPSCs**

For embryoid body (EB) formation, 5x10^4 hiPSCs together with 2.5x10^4 mouse embryonic fibroblasts were plated in each well of a 96-well U-bottom plate in hES medium composed of DMEM-F12 (Thermo Fisher Scientific, #31331028), 15% Knockout Serum Replacement (Thermo Fisher Scientific, #10828028), 1x MEM Non-Essential Amino Acids Solution (Thermo Fisher Scientific, #11140035), 50 µM β-mercaptoethanol (Serva Electrophoresis, #28625) and 2 µM Thiazovivin, the plate was centrifuged at 250 g for 5 min and co-cultures were cultivated in suspension to form multicellular EB aggregates. At day 2, medium was changed to differentiation medium composed of IMDM Glutamax (Thermo Fisher Scientific, #31980022), 20% Fetal Bovine Serum (Thermo Fisher Scientific, #10270106), 1x MEM Non-Essential Amino Acids Solution and 450 µM 1-Thioglycerol (Sigma-Aldrich, #M6145) for further 6 days with medium change every other day. At day 8, EBs were plated onto 0.1% gelatin-coated 6-well plates and cultured for up to one month in differentiation medium with medium change every other day.

**Generation of hiPSC-CMs**

Frozen aliquots of hiPSCs were thawed and cultured without feeder cells and differentiated into hiPSC-CMs as described with some modifications (2).
Thawing and culture of hiPSCs
To thaw iPSs, the following steps were performed.

One day before thawing cells, a T-25 flask coated with Matrigel was put in the incubator. Mix medium (13 ml E8 medium + 6.5 ul from 10 mM stock solution of Rock inhibitor) was prepared. 2.5ml mix medium was added in the T-25 flask and put into incubator. Frozen hiPS cells were taken out from liquid N2 tank and thawed in hand until only small lumps of ice exist. Then, the cell suspension was transferred to 50 ml Falcon prepared with 5 ml of mixed medium. The cell suspension was centrifuged at 250 x g (1200 rpm), 4min, 20 ° C. The supernatant was discarded and 2.5ml Mix medium was added. After 4x pipetting up and down, the cell suspension was transferred into the T-25 with 2.5 ml mix medium. The T-25 stayed into incubator until the next day.

Differentiation
On the next day, the medium in T-25 flask was changed to E8 medium without ROCK inhibitor. Then the E8 medium was changed every two days. 2 to 4 days later, when cells reached 85-95% confluence, the differentiation was started.

First, cells were treated with EDTA and dissolved in E8 Medium. Then, the cells were counted and plated on Matrigel / Geltrex coated plates (optimal cell density for plating is cell line dependent; 15.000 cell/cm2 - 26.000 cells/cm2 = 150.000-260.000 cells per 6-well). Lastly, E8 medium was added to final volume (3ml per 6-well with 5µM ROCK inhibitor on the first day. The plate was shaken and incubated at 37°C with 5% CO2.

Day -x to -1: Daily medium change with E8 Medium was performed.
Day 0: When cell density reached 85-95% confluence, the medium was changed to cardiac medium (consisting of RPMI, 1% sodium pyruvate, 1% Pen / Strep, 2% B27 and 200 uM ASC) freshly enforced with 1 uM CHIR ,.5 ng / ml BMP4 ,.9 ng / ml activin A ,.5 ng / ml FGF.
Day 2: After around 48h, the medium changed to cardiac medium freshly enforced with 5 uM IWP4.
Day 4 and later: the medium was changed every 2-3 days to cardiac medium only. Normally, on day 8 of differentiation some cells start to beat.
Day 13: the cardiac medium was changed to selection medium (consisting of RPMI
without glucose and glutamine, 1% Pen / Strep) freshly added with 440 mM Sodium Lactate Stock and 50 mM 2-mercaptoethanol. The selection medium was changed every day for 4-7 days.

Day 18: the selection medium was changed to cardiac medium (consisting of RPMI, 1% sodium pyruvate, 1% Pen / Strep, 2% B27 and 200 uM ASC) every 2-3 days.

From Day 40: Cells were ready for experiments.
Supplementary Table 1: Summary of drug effects on cardiac ion channel currents

| Drug | I_{Na} | I_{Ca-L} | I_{NCX} | I_{to} | I_{Kr} | I_{Ks} | I_{K1} | I_{KATP} |
|------|--------|----------|---------|--------|--------|--------|--------|----------|
| Qui  | I\(^3\) | I\(^4\)  | N\(^6\), I\(^33\) | I\(^5\), I\(^9\) | I\(^7\) | I\(^8\)  | N\(^9\), I\(^80\) | I\(^11\) |
| Ajm  | I\(^{12}\) | I\(^{13}\) | NA | I\(^{13}\) | I\(^{14}\) | NA | I\(^{14}\) | I\(^{14}\) |
| Ami  | I\(^{15}\) | I\(^{5,17}\) | I\(^{16}\) | N\(^{17}\), I\(^{17}\) | I\(^{17}\) | I\(^{17}\) | N\(^{17}\), I\(^{36}\) | I\(^{3}\) |
| Iva  | I\(^{18}\) | N\(^{18}\) | NA | NA | I\(^{18}\) | N\(^{18}\) | N\(^{19}\) | NA |
| Fle  | I\(^{20}\) | N\(^{20}\), I\(^{15}\) | A\(^{17}\) | I\(^{20}\) | I\(^{21,22}\) | N\(^{22}\) | A\(^{23}\) | I\(^{24}\) |
| Mex  | I\(^{25}\) | I\(^{25,27}\) | I\(^{32}\) | I\(^{26}\) | N\(^{22}\), I\(^{27}\) | N\(^{22}\), I\(^{35}\) | NA | A\(^{30,31}\) |
| Ran  | I\(^{28}\) | I\(^{28}\) | I\(^{29}\) | N\(^{28}\), I\(^{34}\) | I\(^{28}\) | I\(^{28}\) | N\(^{28}\) | NA |

I\(_{Na}\): peak sodium channel current. I\(_{Ca-L}\): L-type calcium channel current. I\(_{NCX}\): Na/Ca exchanger current. I\(_{to}\): transient outward potassium channel current. I\(_{Kr}\): rapidly activating delayed rectifier potassium channel current. I\(_{Ks}\): slowly activating delayed rectifier potassium channel current. I\(_{K1}\): inward rectifier potassium channel current. I\(_{KATP}\): ATP-sensitive potassium channel current.

I: inhibition. A: activation. N: no effect. NA: no analysis.

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