Cellular models of cardiac channelopathies

Chapter 6

Impact

This doctoral thesis addresses the cellular basis of inherited and drug-induced cardiac arrhythmias owing to ion-channel dysfunction, using *in-vitro* and *in-silico* models with emerging complexity.

Societal impact

Cardiovascular diseases are the leading cause of death worldwide. Their impact on healthcare systems is phenomenal, estimated to cost around 169 billion euros per year in Europe alone[1]. Due to their heavy burden, cardiovascular-diseases-related research projects receive high priority in competitive open-funding schemes: at least 876 million euros were invested by the European Union between 2010 and 2012[2].

Sudden cardiac death (SCD) accounts for approximately half of the deaths linked to cardiovascular diseases, and for 15-20% of total deaths in the Western society[3]. Despite an increased understanding of the mechanisms causing SCD, mortality remains high and also affects young, otherwise healthy, individuals. These fatal events, which are often the first symptom of an arrhythmogenic condition, have tremendous psychosocial impact. Improved knowledge of the mechanisms and management of cardiac arrhythmias proved crucial in the primary and secondary prevention of SCD in the last decade[3]. Translational research using cellular and computational models combined with clinical investigations has a crucial role in further reducing the incidence of SCD, as exemplified by the results of this doctoral thesis. Here, I focus particularly on the mechanisms of genetically- and drug-induced cardiac arrhythmias caused by ion-channel disease or pharmacological modulation.

Scientific impact and dissemination

In my doctoral studies, I have explored the value of different cellular models to advance our understanding of inherited and drug-induced arrhythmias. For each chapter, I used different cellular systems, combined with *in-silico* models and the results of clinical studies.

Chapter 2 offers a multiscale characterization of a sodium-channel mutation found in a Dutch-German familial cohort, called the "Worm population"[4]. In this population, many cases of SCD were reported, also at young age, and different ECG phenotypes indicating proarrhythmia were identified. Sodium-current characteristics were assessed by expressing the human isoform of the channel (wild-type versus mutation) in a heterologous cell system and by computational studies using a model of the human ventricular myocyte. Cellular simulation of the conditions identified in patients carrying the

mutation correlated with the true occurrence of arrhythmia in these subjects. Integrating the expertise of *in-vitro*, *in-silico* and clinical scientists was key to the success of this project. It was awarded with a travel grant from the Working Group on Cardiac Cellular Electrophysiology of the European Society of Cardiology (EWGCCE) in 2019 and with two poster awards (at the 16th Dutch-German Joint Meeting of the Molecular Cardiology Working Groups (2018) and at the 1st Translational Cardiovascular Research Meeting of the Netherlands Heart Institute (2017)). Moreover, this work was presented at the Annual Meeting of the Biophysical Society in San Francisco (USA), in 2018.

Chapter 3 explores the regulation of the sodium channel by intracellular calcium, using two different heterologous cellular systems. This is a contentious topic in the field, with several reports showing conflicting results. Our preliminary data suggested that increasing the intracellular calcium concentration induces a potentiation of sodium current. However, subtle differences were detected between the two cellular models indicating that the genetic/proteomic background influences the experimental outcomes. As such, this work highlights the importance of choosing the right cellular models when investigating ion-channel function in disease. Further work will clarify how calcium modulates sodium currents in different genetic backgrounds, and may help solving the current dispute in the field.

In **Chapter 4**, a characterization of the main ionic components of a commercially-available human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) line is presented. These cells shared many similarities with other hiPSC-CM lines, while showing some differences with adult isolated human CMs. Repolarization instability and arrhythmia induction revealed interesting perspectives for the use of beat-to-beat variability of repolarization as a marker of drug-induced arrhythmias. Of particular relevance was the application of beat-to-beat variability of repolarization as proarrhythmic parameter, for the first time in hiPSC-CMs, at the single-cell and 2D-monolayer level. This offers great opportunities for the medium-throughput analysis of medicinal compounds in cardiac-safety studies, e.g., as part of the *Comprehensive in vitro Proarrhythmia Assay* (CiPA) initiated by the US Food and Drug Administration (http://cipaproject.org), and with global impact.

Chapter 4 has been published in *Europace*, the official journal of the European Heart Rhythm Association of the European Society of Cardiology[5]. The manuscript therefore has an exposure to diverse audiences, from basic scientists to clinical electrophysiologists. The paper is published as open-

access, and freely available. A poster describing the main results of this project was awarded as "Outstanding Scientific Work" at the 42nd EWGCCE Meeting in Essen, Germany (2018).

Target groups

Patients are the main stakeholder of the work detailed in this doctorate thesis. Nowadays, the management of cardiac arrhythmias is still challenging and makes use of pharmacological interventions and device implantation (implantable cardioverter defibrillators and pacemakers). The main advantage of cellular models is that they enable patient-/mutation-specific mechanistic and therapeutic approaches (personalized medicine). Cellular models are also used to predict the safety and efficacy of drugs, based on the genetic background of patients (pharmacogenetics) and gender-related variability, especially considering the progress in the generation of hiPSC-CM. As detailed in Chapter 2, mutation carriers of the Worm population displayed a diverse spectrum of proarrhythmic characteristics. Based on the novel cellular and computational results, my work clarified how Worm-mutation carriers are more predisposed to develop (often-lethal) arrhythmias, and it explained the mechanistic importance of arrhythmia triggers that were identified in patients, such as a low potassium concentration in the blood or sympathetic arousal. In the future, similar approaches could be used to study the basic phenotypes of other mutations and their modulation by drugs or external stimuli, towards personalized and mutation-specific pharmacological or lifestyle interventions. In the example of the Worm population, it is estimated that thousands of potential carriers with the founder mutation are actually living in the Maastricht-Aachen Euregio; they will benefit from the cellular results described in this doctorate thesis once these are translated into preventive and/or therapeutic measures. Regional dissemination of information regarding the Worm project, and the engagement of patients and other stakeholders, occurs via Maastricht University Medical Center+ (https://hartenvaatcentrum.mumc.nl/wetenschappelijke-studies/cardiogenetica-studies/de-worm-studie) and the Health Foundation Limburg (https://www.hartenvaatonderzoekfondslimburg.nl/plotse-hartstilstand).

A second target group is the scientific and medical community. This thesis offers a combination of results from various cellular methodologies, complemented by *in-silico* and clinical findings. For example, in Chapter 4, we

introduced the dynamic-clamp technique to overcome resting membrane-potential instability due to the intrinsic immaturity of hiPSC-CMs. This enabled to investigate beat-to-beat variability of repolarization as proarrhythmic marker, which may be relevant for many research groups around the globe.

In addition, the development of more accurate and predictive *in-vitro* models may lead to a reduction of *in-vivo* studies using laboratory animals, with the latter often raising ethical concerns. Although, at this moment, animal-based research is indispensable for the mechanistic research of cardiac electrophysiology and arrhythmias, a reduction in the number of animal studies is desirable and the use of experimental animals should be limited to conditions where no adequate *in-vitro* models exist. Substitutions with cellular models can only be reached if the latter "*are simple enough to be testable and complex enough to be informative*" (Figure 5.1. *Features of an Ideal Model*; this doctorate thesis) for the elements of study for which they are employed. This includes that cellular models should adequately represent disease mechanisms and predict the efficacy and safety of medicinal compounds, in the context of personalized medicine[6].

Finally, pharmaceutical companies can benefit from our translational approaches, especially in drug discovery and safety pharmacology. By applying them in medium-throughput assays, multiple compounds can be efficiently screened, leaving only the best drug candidates for upscale testing in animal models. This will have a large impact on costs and on ethical concerns by contributing to the implementation of the 3Rs (reduction, replacement and refinement of animals in research).

References

[1] Leal J, Luengo-Fernández R, Gray A, Petersen S, Rayner M. Economic burden of cardiovascular diseases in the enlarged european union. *Eur Heart J* 2006; **27**: 1610-9.

[2] Pries AR, Naoum A, Habazettl H, Dunkel M, Preissner R, Coats CJ *et al.* Cardioscape mapping the cardiovascular funding landscape in europe. *Eur Heart J* 2018; **39**: 2423-30.

[3] Wong CX, Brown A, Lau DH, Chugh SS, Albert CM, Kalman JM *et al.* Epidemiology of sudden cardiac death: Global and regional perspectives. *Heart, lung & circulation* 2019; **28**: 6-14.

[4] ter Bekke RMA, Isaacs A, Barysenka A, Hoos MB, Jongbloed JDH, Hoorntje JCA *et al.* Heritability in a scn5a -mutation founder population with increased female susceptibility to non-nocturnal ventricular tachyarrhythmia and sudden cardiac death. *Heart Rhythm* 2017;

[5] Altrocchi C, de Korte T, Bernardi J, Spätjens R, Braam SR, Heijman J *et al.* Repolarization instability and arrhythmia by ikr block in single human-induced pluripotent stem cell-derived cardiomyocytes and 2d monolayers. *Europace* 2020; **22**: 1431-41.

[6] Kirchhof P, Sipido KR, Cowie MR, Eschenhagen T, Fox KA, Katus H *et al.* The continuum of personalized cardiovascular medicine: A position paper of the european society of cardiology. *Eur Heart J* 2014; **35**: 3250-7.