Molecular therapies for cardiac arrhythmias

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ENGLISH SUMMARY

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Despite the ongoing advances in pharmacology, devices and surgical approaches to treat heart rhythm disturbances, arrhythmias are still a significant cause of death and morbidity.\textsuperscript{1, 2} With the introduction of gene and cell therapy, new avenues have arrived for the local modulation of cardiac disease.\textsuperscript{3} This may prove to be an important advantage over pharmacological approaches that are often limited by their global (non-targeted) myocardial effects. This thesis reviews the current state of the field of gene and cell therapy focused on cardiac arrhythmia treatments, and reports a series of studies that are aimed at the advancement of this field.

Part I of this thesis introduces the various concepts of gene and cell therapy in the treatment of brady- and tachyarrhythmias. Chapter 1 discusses the basic concepts of cardiac electrophysiology and current challenges in the treatment of arrhythmias.\textsuperscript{3} In addition, this chapter reviews the complete arsenal of regenerative therapies in cardiac electrophysiology. With regards to bradyarrhythmias, gene and cell therapies are discussed in settings of pacemaker regeneration and repair of atrioventricular (AV) conduction. With regards to tachyarrhythmias, specific strategies are discussed in relation to reentry-based atrial fibrillation, myocardial infarction related ventricular tachycardy/fibrillation (VT/VF), and congestive heart failure. This chapter further elaborates on the duration of effect and potential deleterious effects of gene and cell therapy. Chapter 2 introduces the specific research questions addressed in this thesis. In brief, these questions include the following: 1) which gene targets generate pacemaker activity that best fits physiological demand, 2) which gene therapeutic or stem cell vehicle is most appropriate to deliver such pacemaker activity, and, 3) which gene therapy targets and delivery methods are best suitable to improve conduction in the epicardial borderzone (EBZ) and prevent post myocardial infarction VT/VF.

Part II describes some of the initial steps aimed at the optimization of gene-based biological pacemakers. Chapter 3 describes the consequences of lentiviral overexpression of HCN4 into neonatal rat ventricular myocytes (NRVMs).\textsuperscript{4} Perforated patch-clamp studies showed that single cardiac myocytes overexpressing HCN4 adopted hallmark features of cells isolated from the sinoatrial node (SAN), the dominant native pacemaker of the heart. These features included: a depolarized maximal diastolic potential (MDP), a slow action potential (AP) upstroke velocity, and slow phase 4 depolarization. Furthermore, robust modulation of the HCN4 current and spontaneous beating rates was shown in response to mediators of \(\beta\)-adrenergic stimulation. Because lentiviral vectors integrate their transgene into the host genome, this strategy has important potential for the fabrication of long-term physiologically controlled pacemaker function.

In Chapter 4 we sought for a novel \textit{in vitro} model to investigate cardiac gene and cell therapy. To this end, we studied organ explant cultures of neonatal rat ventricular preparations at day 6-8 after explantation, and compared these to freshly isolated tissue and monolayers of NRVMs.\textsuperscript{5} We found the cultured organ explant preparations to be electrophysiologically and structurally similar to freshly isolated tissue. Moreover, the cultured preparations could be easily modified via gene or
cell therapy. These findings validate the use of this model to study gene and cell therapies in vitro. In addition, we found the degree of spontaneous activity in organ explant cultures to be remarkably low as compared to the NRVM monolayers. This outcome is particularly relevant for studies that aim to characterize novel strategies in biological pacing.

In Chapter 5 we proceeded to use the organ explant culture system to study biological pacing based on cardiac myocyte progenitor cells (CMPCs). An advantage of these cells is that they are native to the heart. CMPCs are therefore expected to provide ideal cellular vehicles to generate long-term function in vivo. To characterize this approach, we first demonstrated that CMPCs are efficiently transduced by lentiviral vectors. Next, we showed efficient cell-to-cell coupling between CMPCs and NRVMs. Finally, we injected HCN4 overexpressing CMPCs into organ explant cultures and found significant pacemaker activity that was modulated by β-adrenergic stimulation.

As an alternative to the above discussed approaches to biological pacing, we also investigated the possibility of reprogramming cardiac myocytes towards pacemaker cells. To this end, the T-box transcription factor TBX3 has been reported to play a crucial role in the formation of the SAN and appeared capable of inducing the formation of novel pacemaker areas when expressed ectopically in embryonic atria. In Chapter 6 we therefore investigated TBX3-based reprogramming in terminally differentiated cardiac myocytes. In adult transgenic mice, we used tamoxifen to induce ectopic TBX3 expression which resulted in an efficient switch from the working myocardial expression profile to that of pacemaker myocardium. Important changes in gene expression included the suppression of the cardiac gap junction genes Cx40 and Cx43, the cardiac Na⁺ channel gene, SCN5A, and inwardly rectifying K⁺-channel genes (Kir genes). These changes resulted in significant conduction slowing and reductions in $I_{Na}$ and $I_{K1}$ which however, occurred without the induction of ectopic pacemaker activity. As an alternative to the tamoxifen system, we also explored the phenotypic consequences of lentiviral TBX3 expression in NRVMs. Here, we found TBX3-based reprogramming to induce a variety of phenotypes including depolarized and spontaneously active cardiac myocytes. However, in monolayers of NRVMs spontaneous activity was not enhanced. Based on the results in both systems, we concluded that TBX3-based reprogramming was not sufficient to generate de novo pacemaker function. However, the reductions in intercellular coupling and $I_{K1}$ are properties of the TBX3-based approach that reduce current-to-load mismatch, facilitate diastolic depolarization, and thus may be applied to enhance HCN-based biological pacing.

Part III focuses on the in vivo characterization of various gene therapy-based biological pacemaker strategies. Chapter 7 investigated the response of HCN2-based biological pacing to emotional arousal as induced by presenting food to dogs after an overnight fast. To this end, an HCN2 carrying adenovirus was injected into the left bundle branch (LBB) of AV-blocked dogs. An electronic pacemaker (set to VVI pacing at a lower rate of 35) was implanted for back-up pacing. During steady-state
gene expression, fasting followed by food presentation induced a significant increase in beating rates and heart rate variability as compared to saline injected controls. This work represents the first demonstration of a biological pacemaker responding to natural arousal stimuli, indicating a potential advantage over electronic pacing.

Because biological pacing based on HCN2 gene transfer operates at relatively slow idioventricular rates and incorporates only moderate autonomic responsiveness, we tested various alternatives in the following two chapters. In Chapter 8 we explored a novel approach to gene-based biological pacing via overexpression of the Ca\textsuperscript{2+}-stimulated adenylyl cyclase AC1.\textsuperscript{10} AC1 overexpression increases intracellular cAMP which impacts on a variety of pacemaker mechanisms including the pacemaker current $I_f$. This strategy was tested in vivo by implantation of HCN2, AC1, or HCN2/AC1 adenoviral constructs into the LBB of AV-blocked dogs that also received an electronic pacemaker (VVI 35) as a back-up and/or monitoring device. AC1 and HCN2/AC1 indeed generated highly efficient biological pacing with improved basal beating rates and greater sensitivity to autonomic modulation than HCN2.

In Chapter 9 we tested the hypothesis that HCN2-based biological pacing may be improved if the activation threshold is hyperpolarized via the additional overexpression of the Na\textsuperscript{+} channel SkM1.\textsuperscript{11} To test this hypothesis, we injected adenoviral constructs of HCN2, SkM1, or HCN2/SkM1 into the LBB of AV-blocked dogs that also received an electronic pacemaker (VVI 35). Indeed, we found the combination of HCN2/SkM1 to be superior to all other strategies tested. HCN2/SkM1 induced pacemaker activity with baseline batting rates in the 80 bpm range, brisk autonomic responsiveness, and a complete elimination of electronic back-up pacing with the latter being persistently required in previously explored strategies. In this study, we further showed function of HCN2/SkM1 to be critically dependent on a hyperpolarized activation threshold and injection into the LBB.

Part IV focuses on gene and cell therapies designed to normalize conduction and thereby prevent post-myocardial infarction tachyarrhythmias. Strategies tested within this part of the thesis evolved from the understanding that reentry is the predominant cause of ischemia-related arrhythmias.\textsuperscript{12} In the heart, ischemia generates an environment of extracellular hyperkalemia and acidification. An elevation in the extracellular potassium level depolarizes the membrane which inactivates cardiac Na\textsuperscript{+} channels, while a reduction in pH impacts directly on cardiac gap-junctions, causing them to close. In settings of ischemia, such as in the epicardial borderzone (EBZ) overlying myocardial infarcts, Na\textsuperscript{+} channel inactivation and closure of gap junctions importantly contribute to conduction abnormalities and the genesis of reentry. This type of arrhythmias may therefore be prevented by strategies that improve conduction in suppressed pathways, thereby removing unidirectional conduction block or causing the reentrant wave front to encounter its own refractory tail.

In the experiments described in chapter 10 we tested various viral constructs for their efficacy to improve conduction and prevent VT/VF, including: overexpression of GFP (control), overexpression of the depolarization resistant Na\textsuperscript{+} current SkM1,
overexpression of the acidification resistant liver-specific connexin Cx32, and overexpression of the combination SkM1/Cx32. Constructs were implanted in the canine EBZ immediately after ligation of the left anterior descending artery, and subsequent electrophysiological measurements and infarct sizing were performed 7 days later. Although all three interventions resulted in a significant normalization of conduction, as evidenced by shorter QRS durations, narrower local electrograms, and faster in vitro conduction velocities, only the SkM1 intervention significantly reduced the incidence of inducible VT/VF. In Cx32-treated animals, infarct size was increased which resulted in the predominant occurrence of monomorphic VT as compared to polymorphic VT/VF that predominated in the other groups. Thus, whereas SkM1 therapy reduces the incidence of inducible VT/VF, Cx32 gene transfer to improve gap junction conductance results in larger infarcts, a different VT morphology, and no antiarrhythmic efficacy.

In chapter 11, we explored the use of cellular delivery of Na⁺ channels in an effort to improve conduction. To this end, we compared the efficacy of SkM1 channels with SCN5A in their ability to restore fast impulse propagation in depolarized tissue. We used stably transfected HEK293 overexpressing either SkM1 or SCN5A and co-cultured them with cardiac myocytes. In HEK-cardiomyocyte cell pairs coupled through gap-junctions, patch-clamp experiments revealed an increase in AP upstroke velocity ($V_{\text{max}}$) when the HEK cells expressed SkM1. This effect of increased $V_{\text{max}}$ occurred especially at depolarized potentials and was not obtained with unloaded or SCN5A expressing HEK cells. Via Ca²⁺-based optical mapping techniques, we subsequently showed that SkM1 but not SCN5A expressing cells significantly accelerated the conduction velocity under both normal and depolarized conditions. When high frequency stimulation was used to induce reentry in vitro, expression of SkM1 also enhanced the angular velocity of the reentrant wavefront. These results suggest that cells carrying SkM1 can improve cardiac excitability and conduction in depolarized tissue.

In chapter 12, we continued our work with cellular delivery of SkM1, yet now after transfection into canine mesenchymal stem cells (cMSCs). Co-culture experiments showed that SkM1 loaded cMSCs, but not cMSC alone, maintain fast conduction at depolarized potentials, thus encouraging further experimentation in vivo. To this end, we studied dogs allocated to 3 different protocols: sham, cMSC and cMSC/SkM1, all applied immediately after ligation of the left anterior descending artery. Seven days later, we measured in vivo EBZ electrograms which were broad and fragmented in sham, narrower in cMSC, and narrow and unfragmented in cMSC/SkM1. Furthermore, during programmed electrical stimulation (PES) of EBZ, QRS duration in cMSC/SkM1 was shorter than in cMSC and sham. However, despite the potentially therapeutic actions of cMSC/SkM1, no protection against arrhythmias was obtained as the incidence of PES-induced VT/VF was equivalent in all groups. Comparing the outcomes of cellular and viral delivery of SkM1 thus highlights that the delivery platform is critical to obtain the antiarrhythmic effect.
Part V, chapters 13 and 14, discuss the findings of this thesis and puts them into perspective with other molecular therapies for cardiac disease and the ongoing advancements in pharmacology and devices.

REFERENCES


