Effects and Underlying Mechanisms of Refractory Period Pacing on Repolarization Dynamics in the Human Heart

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***Abstract*— Repolarization alternans is related to the initiation of life threatening cardiac arrhythmias. Experimental and computational studies suggest that the abolishment of alternans using dynamic pacing protocols may prevent abnormal heart rhythms. In a recent animal study, refractory period pacing (RPP) on every other beat has shown promising results in alternans reduction. However, the cellular mechanisms underlying this therapy and its efficiency in human patients remain unclear. In this study, *in vivo* unipolar electrograms acquired during RPP from 240 epicardial sites from one patient were analysed. Current clamp of 18 channels was performed *in silico* to elucidate the ionic mechanisms underlying action potential modulation by RPP. Its efficacy with positive and negative polarities was tested on a population of 87 calibrated human ventricular models exhibiting alternans. *In vivo* electrograms showed significant changes in T-wave alternans when applying RPP. *In silico*, results showed APD shortening for RPP with positive polarity and APD prolongation with RPP negative. Under current clamp protocols, voltage rectification of L-type Ca^{2+} (ICaL) and inward rectifier K+ (IK1) currents were identified as the key determinants for the observed changes. RPP pacing successfully reduced alternans on the *in silico* models using a negative polarity stimulus in the short beat.**

I. Introduction

T-wave alternans (TWA) are characterized as a beat-to beat oscillation on the morphology of the ST-segment or T-wave on electrocardiograms (ECG). Since the T-wave is a representation of ventricular repolarization, TWA are linked to repolarization alternans (RA) at the cellular level. RA can be measured from unipolar electrograms (UEGM) recorded on the surface of the myocardium. UEGM contains both local and far-field (remote) information. From the local component, it is possible to estimate local depolarization and repolarization times, and consequently the activation-recovery interval (ARI) as a surrogate of action potential duration (APD)[1]. The far-field component exhibits an ECG like waveform, with a ST-segment and T-wave. Therefore, UEGMs can be used to study both RA and TWA.

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RA are considered a factor of increased vulnerability to ventricular-tachycardia/ventricular-fibrillation (VT/VF) and sudden cardiac death (SCD)[2]. Increased repolarization dispersion across the heart has been demonstrated to be one possible mechanism of arrhythmogenisis in several experimental works, generating the substrate needed for 2:1 block, re-entry and life threatening cardiac arrhythmias[2][3]. Moreover, clinical studies have also reported increased TWA magnitude prior to the onset of spontaneous VT/VF[4][5]. This supports the hypothesis that, in certain cases, the heart goes through an intermediate state of increasing RA when transiting from normal to abnormal rhythms.

These findings suggest that an early detection and control of RA could potentially suppress the arrhythmogenic substrate and thus prevent VT/VF. Several experimental and computational studies have explored the possibility of applying dynamic pacing to control RA[6][7][8][9][10]. In a recent porcine study, a method that successfully reduced RA magnitude was proposed[11]. It consists in delivering early refractory period pacing (RPP) from the right ventricle every other beat with optimized amplitude, timing and polarity. This novel therapy showed positive results in the whole heart, while previous studies only showed limited spatial effectiveness using single-site pacing[9][10]. Therefore, further investigation on this procedure is needed. In particular, focus should be placed on the effect and feasibility in the human heart and on the underlying mechanisms.

In this study, we investigate the feasibility of APD modulation using RPP in one human heart. Additionally, we integrated the RRP protocol in the well-established OHara-Rudy computational model of the human ventricular myocyte[12] to investigate the underlying mechanisms and to test its efficacy on the reduction of alternans in a population of human calibrated models[13].

II. Methods

A. In vivo data acquisition and analysis

In vivo UEGMs were acquired from one patient prior to coronary artery bypass surgery[14]. The patient gave informed consent and was enrolled in the study. The protocol follows the ethical guidelines of the Declaration of Helsinki. Cardiopulmonary bypass was temporarily commenced to allow the surgeon to fit a multi electrode sock over the epicardium of both ventricles as in previous studies[14][15]. The heart was then refilled and bypass discontinued in order to allow the study to proceed with a normally beating heart. UEGMs from 240 electrodes sampled at 2 KHz were

recorded from the multi-electrode sock. The protocol consisted in 5 distinct parts with a duration of 40s each. Firstly, steady state ventricular pacing (S1) was delivered from the apex of the left ventricle (LV) at a cycle length (CL) of 500ms. During the second part, 10ms duration RPP stimulation was additionally delivered at the nearest electrode, 50ms after S1, every other beat, with positive polarity (RPP(+)). These two parts were then repeated using refractory stimuli with negative polarity (RPP(-)). The last part consisted in S1 steady state pacing only. Data were exported and analysed off-line. Presence of TWA was analysed using the spectral method as described previously[15]. It relies on spectral analyses to quantify the magnitude of alternation on the T-wave morphology at a frequency (f) of 0.5 cycles per beat:

$$K_{score} = \frac{P - P_N}{\sigma_N} \quad (1)$$

where P is the amplitude of the power spectrum at $f = 0.5$ and P_N and σ_N are the mean and standard deviation of the power spectrum at the noise band between $f = [0.44 : 0.49]$.

B. In silico current clamp experiment

In silico investigations were based on the O'Hara-Rudy (ORd) computation model of the human ventricular myocyte[12]. The ORd model is considered the state-of-the-art model of human ventricular electrophysiology, as it has been extensively formulated and validated based on human data. Simulations were performed at a CL of 500ms to mimic the protocol used in the human patient. Firstly, 1000 beats were generated to guarantee initial steady-state condition. Afterwards, 500 beats were generated for each of the RPP settings. RPP was delivered every other beat, 10ms after S1 with a duration of 10ms. It was tested with positive or negative polarities. Current clamp of 18 channels was performed to elucidate the relevant ionic currents responsible for RPP efficacy. The following channels were clamped individually: INa (Na^+ current), INaL (late Na^+ current), Ito (transient outward K^+ current), ICaL (Ca^{2+} current through the L-type Ca^{2+} channel), ICaNa (Na^+ current through the L-type Ca^{2+} channel), ICaK (K^+ current through the L-type Ca^{2+} channel), IKr (rapid delayed rectifier K^+ current), IKs (slow delayed rectifier K^+ current), IK1 (inward rectifier K^+ current), $INaCa_i$ (myoplasmic component of Na^+/Ca^{2+} exchange current), $INaCa_{ss}$ (subspace component of the Na^+/Ca^{2+} exchange current), INaK (Na^+ ATPase current), IpCa (sarcolemmal Ca^{2+} pump current), ICab (Ca^{2+} background current), IKb (K^+ background current), INab (Na^+ background current), Jrel (Ca^{2+} release, via ryanodine receptors, from jsr to myoplasm) and Jup (Ca^{2+} uptake, via SERCA pump, from myoplasm to nsr). All simulations were performed using MATLAB® software.

C. In silico RPP on alternating models

A population of 87 calibrated human ventricular models exhibiting alternans was used to access the efficacy of this therapy on the reduction of alternans [13]. Simulations were performed at CLs ranging from 600 to 350ms, in 50ms decrements. RPP was delivered every other beat with negative or positive polarity and in the short or long beats. The presence of APD alternans was defined as a difference superior to 5ms between APD_{80} as in previous studies[13].

III. Results

A. In vivo RPP analysis

Figure 1A shows an example of one epicardial electrogram during RPP with positive polarity. Beats at which RPP(+) stimulus was delivered showed a reduction in T-wave amplitude compared to the S1 beats. Analysis of TWA on 240 UGEMs was carried out using the spectral method. Figure 1C shows the K-scores results for the periods of baseline steady state pacing (No RPP), RPP with positive polarity (RPP +) and RPP with negative polarity (RPP -). A significant increase in the magnitude of TWA was observed (P<0.0001). Results from the computational model (Fig 1B/D) showed a decrease in APD due to a decrease in RT when positive RPP was delivered.

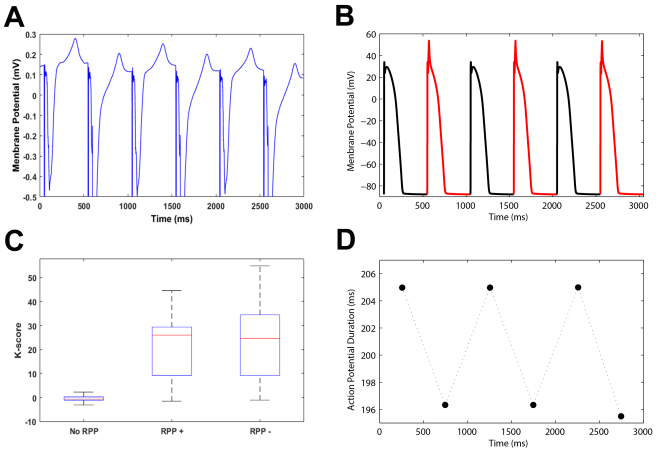

Fig. 1. Effects of refractory period pacing. A: electrograms from in vivo human ventricular epicardium. B: AP of the simulated ventricular myocyte with RPP. Black for normal beats and red for beats with positive RPP. C: K-score results for baseline steady state pacing (No RPP), RPP with positive polarity (RPP +) and RPP with negative polarity (RPP -). D: APDs for AP sequence in B.

B. In silico RPP effects on cellular physiology

The refractory pacing protocol was initially incorporated in the original ORd model to evaluate its effects on APD and channels behaviour on the normal myocyte. Results show that APD could be either increased or decreased depending on the stimulus polarity. A negatively charged pacing increased the APD by 5ms. On the other hand, a positively charged pacing decreased APD by almost 10ms. There was no significant difference in APD during steady state pacing. Figure 2A shows the action potential for a S1 beat (black), a beat with a positive stimulus during the RPP (red) and with a negative stimulus (blue). An increase in the membrane potential due to the positive stimulus can be clearly observed from 10ms after depolarization. ICaL was substantially reduced due to voltage gate inactivation

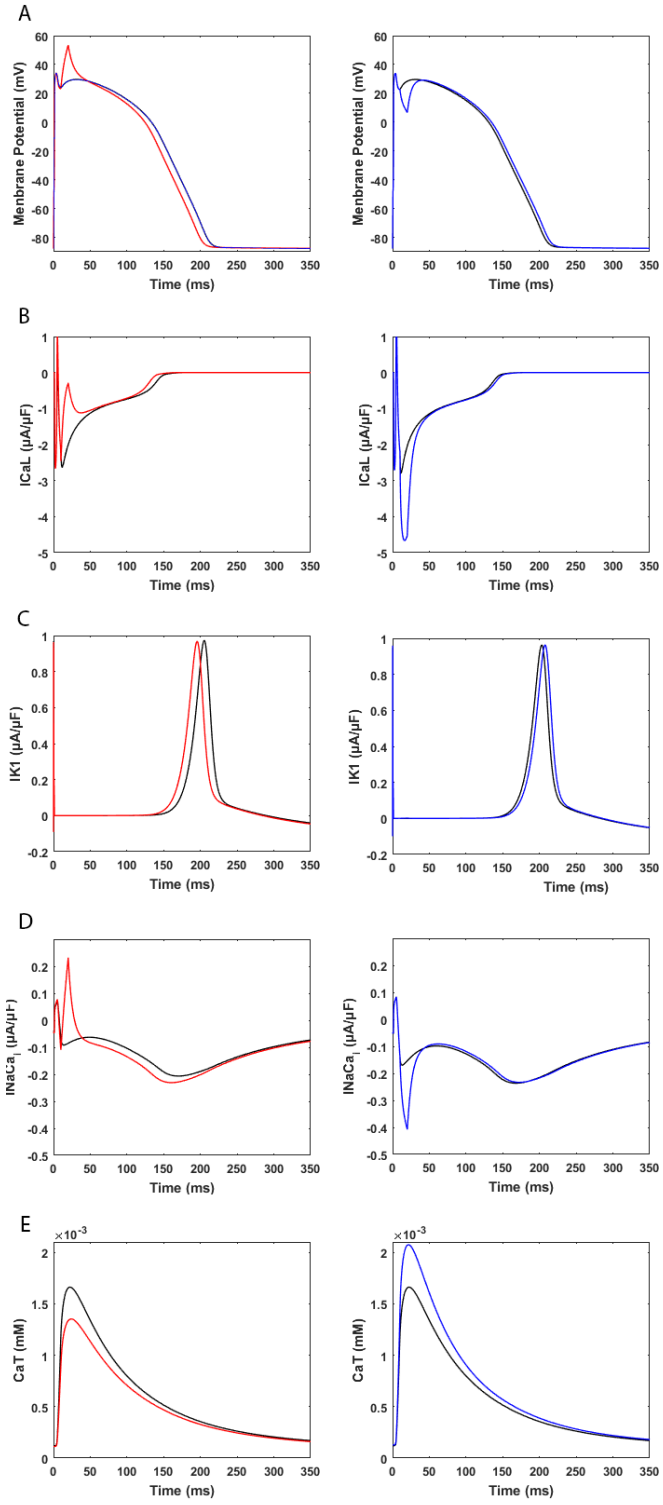

Fig. 2. Effects of RPP on channels behaviour. Black: last beat before RPP protocol. Red: last beat with RPP with positive polarity. Blue: last beat with RPP with negative polarity. A: membrane potential (mV). B: Ca^{2+} current through the L-type Ca^{2+} channel (ICaL). C: inward rectifier K^+ current (IK1). D: myoplasmic component of Na^+/Ca^{2+} exchange current ($INaCa_i$). E: intracellular Ca^{2+} transient (CaT)

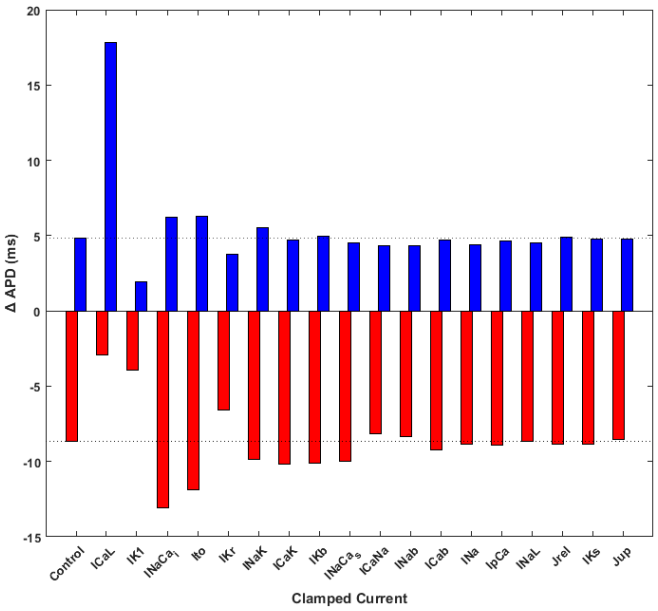

Fig. 3. Results from the current clamp computational study. The vertical axis shows the difference in APD_{90} between last beat with no RPP and with RPP. Red represents results for RPP with positive polarity and blue represent results for RPP with negative polarity. The first column (Control) represent the behaviour of the model when no currents are clamped.

at high membrane potentials (Fig 2B), whilst the reverse mode of the Na^+/Ca^{2+} exchanger is promoted due to the stimulation (Fig 2D). Despite no changes in magnitude, an earlier activation of IK1 was observed (Fig 2C). The reduced ICaL yielded a smaller Ca^{2+} release from the sarcoplasmic reticulum, resulting in a decreased amplitude of the calcium transient (CaT) (Fig 2E). The opposite occurs under negative polarity RPP stimulation (Fig 2, right column), where the reduction in membrane potential translates into the increase of ICaL peak, the forward $INaCa_i$ mode, increased amplitude of CaT and a later IK1 activation.

Current clamp experiments were performed to further dissect the role of each ionic current on RPP changes on APD. Results are shown in Figure 3. In the case of positive RPP (red), ICaL and IK1 are the main modulators of APD shortening, as they clamp to control severely abolishes the effect. $INaCa_i$ has the opposite effect to these two currents, due to the increase of its reverse mode. Interestingly, Ito clamp revealed that its effects are also opposed and significantly smaller to those of the two main ionic mechanisms. The same analysis holds for negative polarity RPP (Fig 3, blue). The remain currents showed negligible effects.

C. *In silico RPP on alternating models*

A population of 87 ORd models calibrated to *in vivo* human data and exhibiting RA[13] was used to assess the efficiency of this protocol to reduce or abolish RA. RPP was delivered in four different configurations: positive polarity on the short beat (RPP S+), negative polarity on the short beat (RPP S-), negative polarity on the long beat (RPP L-) and positive polarity on the long beat (RPP L+). Figure 4 shows

alternans magnitude for APD for all the simulated configurations. It can be observed that, as expected, this protocol can both increase or decrease APD alternans depending on the polarity of the stimulus and the beat in which it is delivered. Figure 4 shows a significant reduction in APD alternans magnitude for RPP S- ($P<0.0023$). The other configurations resulted on a significant increase ($P<0.0001$).

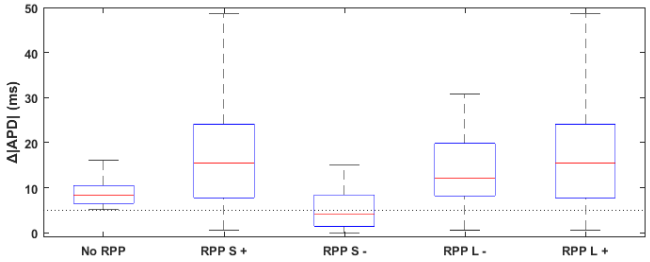

Fig. 4. Effects of RPP in our population of 87 alternating computational models. RPP was delivered in 4 different configurations: positive polarity on the short beat (RPP S+), negative polarity on the short beat (RPP S-), negative polarity on the long beat (RPP L-) and positive polarity on the long beat (RPP L+).

IV. Discussion

The aim of this work was to study the effects of RPP in the human heart motivated by the promising results from a porcine study[11]. We have delivered RPP to one human patient and used the ORd model to study the underlying mechanisms. To the extent of our knowledge this is the first time that this type of pacing protocol is assessed in the human. We also used a population of 87 models to test the efficacy on reduction of RA. *In vivo* results showed an increase in TWA during RPP. These indicates that electrical stimulation during the refractory period affects repolarization dynamics and can be potentially used to modulate repolarization time. *In silico* experiments showed either a decreased on APD with RPP+ or an increase on APD with a RPP- . Current clamp protocols were carried out to dissect the role of each ionic current and the physiologic mechanism underling the RPP efficacy. An increase in the calcium current through the L-type calcium channels increased the amount of CaT in the cell which results in a prolonged plateau phase. This means that the IK1 channels, which play a major role in the repolarization process, are triggered later and the APD is prolonged. In the case of a positive stimulus, the opposite effect was observed. ICaL is reduced and thus the membrane potential of phase 2 decays slightly faster. Therefore, IK1 channels open sooner and the APD is reduced. According to these results, it was expected that delivering positively charged stimuli in the long beat or negatively charged stimuli in the short beat would reduce alternans. In the population of alternans models this was confirmed for the negatively charged stimulus only. The fact that RPP L+ did not reduce alternans in the alternans model may due to the fact that these models have different electrophysiological properties with respect to the normal myocyte where RPP was originally tested. The results of this study show that RPP can modulate repolarization dynamics, and an appropriate pacing protocol should be established in order to reduce or abolish repolarization alternans rather than enhancing it.

V. Conclusion

In this study, we tested RPP in the human heart and demonstrate its potentially for TWA modulation. *In silico* experiments revealed that ICaL and IK1 are the main determinants for the response to therapy and that RPP successfully reduced APD alternans magnitude but the definition of appropriate stimulation protocols is needed.

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