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The combined effects of ranolazine and dronedarone on human atrial and ventricular electrophysiology

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ABSTRACT

Introduction: Pharmacological rhythm control of atrial fibrillation (AF) in patients with structural heart disease is limited. Ranolazine in combination with low dose dronedarone remarkably reduced AF-burden in the phase II HARMONY trial. We thus aimed to investigate the possible mechanisms underlying these results.
Methods and results: Patch clamp experiments revealed that ranolazine (5 μM), low-dose dronedarone (0.3 μM), and the combination significantly prolonged action potential duration (APD_{90}) in atrial myocytes from patients in sinus rhythm (prolongation by 23.5 ± 0.1%, 31.7 ± 0.1% and 25.6 ± 0.1% respectively). Most importantly, in atrial myocytes from patients with AF ranolazine alone, but more the combination with dronedarone, also prolonged the typically abbreviated APD_{90} (prolongation by 21.6 ± 0.1% and 31.9 ± 0.1% respectively). It was clearly observed that neither ranolazine, dronedarone nor the combination significantly changed the APD or contractility and twitch force in ventricular myocytes or trabeculae from patients with heart failure (HF). Interestingly ranolazine, and more so the combination, but not dronedarone alone, caused hyperpolarization of the resting membrane potential in cardiomyocytes from AF. As measured by confocal microscopy (Fluo-3), ranolazine, dronedarone and the combination significantly suppressed diastolic sarcoplasmic reticulum (SR) Ca^{2+} leak in myocytes from sinus rhythm (reduction by ranolazine: 89.0 ± 30.7%, dronedarone: 75.6 ± 27.4% and combination: 78.0 ± 27.2%), in myocytes from AF (reduction by ranolazine: 67.6 ± 33.7%, dronedarone: 86.5 ± 31.7% and combination: 81.0 ± 33.3%), as well as in myocytes from HF (reduction by ranolazine: 64.8 ± 26.5% and dronedarone: 65.9 ± 29.3%).
Conclusions: Electrophysiological measurements during exposure to ranolazine alone or in combination with low-dose dronedarone showed APD prolongation, cellular hyperpolarization and reduced SR Ca^{2+} leak in human atrial myocytes. The combined inhibitory effects on various currents, in particular Na^+ and K^+ currents, may explain the anti-AF effects observed in the HARMONY trial. Therefore, the combination of ranolazine and dronedarone, but also ranolazine alone, may be promising new treatment options for AF, especially in patients with HF, and merit further clinical investigation.

1. Introduction

Atrial fibrillation (AF) is the most common clinically relevant arrhythmia. It is a major risk factor for embolic stroke and aggravation of heart failure (HF) and is therefore associated with increased morbidity and mortality [1]. Anti-arrhythmic drugs and/or ablation of left atrial tissue/pulmonary vein are first line therapies for rhythm control in patients with AF [2]. Pharmacological rhythm control of AF is particularly

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limited in patients with structural heart disease. Currently available drugs for these patients exhibit limited efficacy, poor tolerability and pronounced adverse side effects, notably life-threatening ventricular arrhythmias [3]. For most patients with severe structural heart disease, particularly HF, clinical guidelines recommend treatment with amiodarone. Although relatively effective against arrhythmias, chronic treatment with amiodarone may cause severe extra-cardiac side effects and organ toxicity. There is a need for new and safer innovative compounds for the treatment of AF, particularly in patients with structural heart disease.

Results of preclinical and some clinical studies have already provided evidence that the antianginal compound ranolazine may exert anti-AF effects [3–8]. Ranolazine is known to inhibit a number of ion currents, preferentially the late sodium current (late I_{Na}), a persistent current which occurs after the peak sodium current (peak I_{Na}) during the action potential (AP) [8–12]. In ventricular myocytes the most sensitive ion currents that are inhibited by ranolazine are the late I_{Na} and, to a lesser extent, the rapidly activating delayed rectifier potassium current (I_{Kr}). In atrial myocytes ranolazine also inhibits peak I_{Na} [4,8]. Dronedarone, a multichannel blocker, is already an approved anti-arrhythmic agent for the treatment of non-permanent AF. Recently a phase II trial of the combination of ranolazine and low dose dronedarone (HARMONY) showed a synergistic effect of these two drugs to reduce AF burden in patients with paroxysmal AF [3].

Our aim, therefore, was to investigate the underlying electrophysiological mechanisms of these drugs alone and in combination (as in the HARMONY trial) in isolated atrial myocytes from patients with AF and ventricular myocytes from patients with end-stage HF.

2. Materials and methods (for details see Supplement)

2.1. Human myocardial tissue

All procedures performed conform to the declaration of Helsinki and were approved by the local ethics committee. Right atrial appendages were obtained from patients in sinus rhythm. Left atrial myocardium was obtained from patients with AF (Table 1). Human left ventricular myocardial tissue was taken from explanted failing hearts (Table 1). Isolation of myocytes was performed as previously described [13,14].

2.2. Patch clamp experiments

For action potential recordings the whole-cell patch-clamp technique was used. Briefly, action potentials were continuously elicited by square current pulses of 1–2 nA amplitude and 1–5 ms duration at increasing stimulation frequency (0.5–3 Hz). When appropriate, superfusion with ranolazine, dronedarone or the combination of both was commenced. Experiments were conducted at 37 °C.

2.3. Confocal microscopy (measurement of SR Ca^{2+} leak)

Ca^{2+} imaging was performed as previously described [15]. Briefly, myocytes were incubated with Fluo-3 AM, followed by de-esterification. Field stimulation (1 Hz) was applied before measurement at rest, to ensure appropriate SR Ca^{2+} loading. Ca^{2+} spark measurements were performed on a laser scanning confocal microscope.

2.4. Human myocardial muscle strip preparation and experiments

Myocardial muscle strips were isolated from the right ventricle and isometric twitch experiments were performed in an organ bath as previously described [14,16].

2.5. Statistics

All data are presented as mean ± SEM. For APD measurements, statistical testing was performed with 2-way RM ANOVA and Bonferroni post-test. For analysis of resting membrane potential (RMP) measurements, paired Student's *t*-test was used. For confocal measurements Student's t-test was performed and Chi Square test used for analysis of sparking and pro-arrhythmic myocyte numbers. Results with $P < 0.05$ were considered as statistically significant.

Table 1
Characteristics of patients included in the different studied groups. AF: atrial fibrillation; HF: end stage heart failure; DCM, dilated cardiomyopathy; ICM: ischaemic cardiomyopathy, EF: ejection fraction; LA: left atrial, LAVI: left atrial volume index; LVEDD: left ventricular end diastolic diameter; ACE: angiotensin-converting enzyme; CABG: coronary artery bypass graft surgery; AVR: aortic valve replacement; MVR: mitral valve replacement, HTX: heart transplantation. Values are n, mean ± SEM or n (%). *$P < 0.05$ sinus rhythm vs. AF.

	Sinus rhythm ($n = 27$)	AF ($n = 24$)	HF ($n = 20$; 9 DCM, 11 ICM)
Male sex (%)	74.1	62.5	65.0
Age (mean ± SEM, y)	68.04 ± 2.1	68.25 ± 2.1	55.5 ± 3.16
EF (mean ± SEM, %)	52.36 ± 2.64	47.68 ± 3.18	26.95 ± 3.44
LA diameter (mean ± SEM, mm)	38.08 ± 1.88 *	51.53 ± 2.32	45.31 ± 1.88
LAVI (mean ± SEM, ml/m^2)	40.75 ± 4.72 *	70.57 ± 9.78	
LVEDD (mean ± SEM, mm)	43.8 ± 2.98	50.29 ± 2.1	64.2 ± 3.58
HR (mean ± SEM, /min)	71.22 ± 2.36 *	90.46 ± 3.53	77.88 ± 4.52
Ischemic heart disease (%)	85.2	83.3	42.1
Diabetes (%)	18.5	41.7	10.5
ACE inhibitors (%)	44.4	25.0	40.0
β-Blockers (%)	63 *	87.5	87.5
Diuretics (%)	55.6	79.2	87.5
Digoxin (%)	3.7 *	33.3	12.5
Catecholamines (%)	0.0	0.0	12.5
Amiodaron (%)	0.0	8.3	31.3
AT1 receptor antagonists (%)	33.3	54.2	37.5
Aldosterone antagonists (%)	11.1	16.7	50.0
PDE inhibitors (%)	3.7	8.3	31.3
Ca^{2+}-channel blockers (%)	29.6	41.7	6.3
Statin (%)	55.6	47.8	25.0
CABG (%)	66.7	29.2	
AVR (%)	7.4	29.2	
MVR (%)	3.7	12.5	
CABG + AVR (%)	11.1	12.5	
CABG + MVR (%)	0.0	8.3	
HTX (%)	3.7	8.3	100
AVR + MVR (%)	7.4	0.0	