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Digitalis Promotes Ventricular Arrhythmias in Flecainide- and Ranolazine-Pretreated Hearts

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Abstract
A post hoc analysis of the PALLAS trial suggested life-threatening interactions of digitalis and dronedarone. Thus, there is concern about an interplay between digitalis and other drugs that influence cardiac electrophysiology. We therefore investigated the interaction between digitalis and flecainide or ranolazine. Twenty-five rabbit hearts were Langendorff-perfused and treated with flecainide (2 µM, 12 hearts) or ranolazine (10 µM, 13 hearts). Infusion of flecainide prolonged mean action potential duration [APD_{90}, from 153 ms (interquartile range (IQR): 29.7 ms) to 159 ms (IQR: 24.9 ms, $p=0.04$)] and effective refractory period [ERP, 170 ms (IQR: 40 ms) vs. 200 ms (IQR: 32.5 ms, $p<0.01$)]. Administration of ranolazine prolonged APD_{90} [144 ms (IQR: 34.3 ms)) vs. 157 ms (IQR: 31.2 ms, $p<0.01$)] and ERP [180 ms (IQR: 40 ms) vs. 200 ms (IQR: 30 ms, $p<0.01$)]. Additional infusion of the digitalis glycoside ouabain (0.2 µM) abbreviated APD_{90} and ERP in both groups (flecainide: APD_{90}: to 128 ms (IQR: 19 ms), ERP: to 170 ms (IQR: 20 ms), $p<0.01$ each; ranolazine: APD_{90}: to 141 ms (IQR: 40 ms), ERP: to 160 ms (IQR: 30 ms), $p<0.01$ each). Ventricular vulnerability was assessed by a pacing protocol employing premature extra stimuli and burst stimulation. No proarrhythmic effect was observed with flecainide (1 vs. 3 episodes at baseline) or ranolazine (3 vs. 11 episodes at baseline). However, further infusion of ouabain had a proarrhythmic effect for both drugs (flecainide: 15 episodes, $p=0.04$; ranolazine: 21 episodes, $p=0.09$). Concomitant treatment of the sodium channel blockers flecainide or ranolazine with digitalis seems to be proarrhythmic. Abbreviation of repolarization and refractoriness that can facilitate re-entry was found as underlying mechanism.

Keywords Digitalis · Flecainide · Ranolazine · Ventricular arrhythmias · Sudden cardiac death

Introduction

Digitalis glycosides are recommended for acute and long-term heart rate control in patients with atrial fibrillation with preserved and reduced left ventricular ejection fraction [1]. Digitalis inhibits the sodium potassium ATPase pump in cardiac myocytes and thereby increases the intracellular sodium concentration. Mediated by an enhanced Na^+/Ca^+-exchanger activity, the intracellular calcium concentration is elevated which in turn leads to an increased calcium concentration inside the sarcoplasmatic reticulum [2, 3]. These molecular mechanisms alter the cardiac electrophysiology and have a positive inotropic effect. As a consequence, digitalis glycosides may be considered in the treatment of symptomatic heart failure to reduce the risk of hospitalization [4].

Subgroup analyses of the ROCKET AF and ENGAGE AF-TIMI 48 trials revealed a significant increase in all-cause mortality and sudden cardiac death in patients receiving digitalis glycosides and suffering of atrial fibrillation with and without heart failure [5, 6]. A meta-analysis demonstrated that this increased mortality can also be observed in heart failure patients without atrial fibrillation [7]. A recently published post hoc digoxin subgroup analysis of

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the ARISTOTLE trial indicated that the risk of death was associated with higher serum digoxin concentrations (≥1, 2 ng/ml) [8].

One of the underlying mechanisms explaining the increase in mortality is the capability of digitalis glycosides to provoke ventricular arrhythmias [7]. These arrhythmias are triggered by delayed afterdepolarizations due to a digitalis-mediated phosphorylation of the ryanodine receptor [2]. Furthermore, the digitalis-mediated calcium overload inside the sarcoplasmatic reticulum can provoke a calcium leak and subsequently trigger delayed afterdepolarizations [9].

It is recognized that further drug–drug interactions especially with other antiarrhythmic agents can amplify digitalis' proarrhythmic effects: A post hoc analysis of the PALLAS trial showed an increase in cardiovascular mortality and sudden arrhythmic death in patients receiving digitalis and the class III agent dronedarone [10]. Similar observations were made in experimental studies: in failing and non-failing rabbit hearts, concomitant treatment with the digitalis glycoside ouabain and dronedarone led to an increased ventricular vulnerability mediated by a shortening of ventricular repolarization and refractory periods [11, 12]. A concomitant therapy with ouabain and amiodarone had no significant proarrhythmic effect in this setting [12]. Of note, we recently demonstrated a potential benefit of ivabradine in digitalis-induced ventricular arrhythmias [13].

Flecainide is a class Ic antiarrhythmic agent that inhibits sodium currents. It is recommended for rhythm control in symptomatic patients with atrial fibrillation without structural heart disease [1]. Ranolazine inhibits the late sodium current $I_{Na,L}$ [14] and I_{Kr} and thereby leads to a minor prolongation of QTc interval [15]. It is recommended as a second-line therapy in chronic stable angina pectoris [16]. In several studies, ranolazine either alone or in combination with other antiarrhythmic drugs has been successfully employed for the treatment of atrial and ventricular arrhythmias [15, 17–19].

To our best knowledge, there are no whole-heart studies investigating the interplay of a concomitant use of the sodium channel inhibitors flecainide or ranolazine and digitalis glycosides. Therefore, aim of this study was to assess possible electrophysiologic effects of this combination therapy in a sensitive model of proarrhythmia.

Methods

All experimental protocols were approved by the local animal care committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 852-3, revised 1996).

The method of preparing and isolation of the hearts employing a Langendorff setup has been described in detail [20, 21].

In short, 25 hearts of healthy New Zealand white rabbits were excised and retrogradely perfused with a modified Krebs-Henseleit solution ($CaCl_2$: 1.80 mM, KCl 4.70 mM, KH_2PO_4 1.18 mM, $MgSO_4$ 0.83 mM, NaCl 118 mM, $NaHCO_3$ 24.88 mM, Na-pyruvate 2.0 mM and D-glucose 5.55 mM) at a constant flow (52 ml/h). Perfusion pressure remained stable at around 90 mmHg. The perfusate was equilibrated with 95% O_2 and 5% CO_2 (pH 7.35, 37 °C). The atrioventricular node was mechanically ablated by compressing the interatrial septum with surgical tweezers. A volume conducted pseudo 12 lead-ECG was recorded by immersion of the hearts in a heated, solution-filled tissue bath. Eight endo- and epicardially placed catheters were used to obtain monophasic action potentials. Hearts were paced at seven different cycle lengths (900–300 ms), thereby acquiring cycle-length dependent action potential duration at 90% of repolarization (APD_{90}) and QT interval.

APD_{90} was calculated between the fastest upstroke and 90% of repolarization employing a specifically developed software that analyzed 15 consecutive beats. QT interval was measured manually using software for electrophysiologic studies (Labsystem, Bard EP systems).

Thereafter, a predefined pacing protocol consisting of premature extra stimuli (S2 and S3) and burst stimulation was used to determine effective refractory periods (ERP) and to assess ventricular vulnerability. To be more precise, episodes of ventricular fibrillation that were induced by programmed ventricular stimulation were counted.

After generating baseline data, 13 hearts were treated with 2 µM flecainide and another 12 hearts were perfused with 10 µM ranolazine and the above-described protocol was repeated. Eventually, all hearts were additionally treated with 0.2 µM ouabain and the protocol was repeated again. Timing of the pacing protocol and drug administration is displayed in Fig. 1.

The employed drug doses of flecainide and ranolazine were derived from previous studies in the same model where similar doses were employed for the same purpose [11, 22, 23] and are within the range of peak plasma concentrations in humans. The sub-inotropic concentration of ouabain was previously used in isolated rabbit hearts in our and other groups [12, 24].

Statistics

Data analysis was performed with SPSS Statistics for Windows (version 24.0). Values are reported as mean ± SD (standard deviation) for normally distributed variables. Non-normally distributed variables are displayed as median and

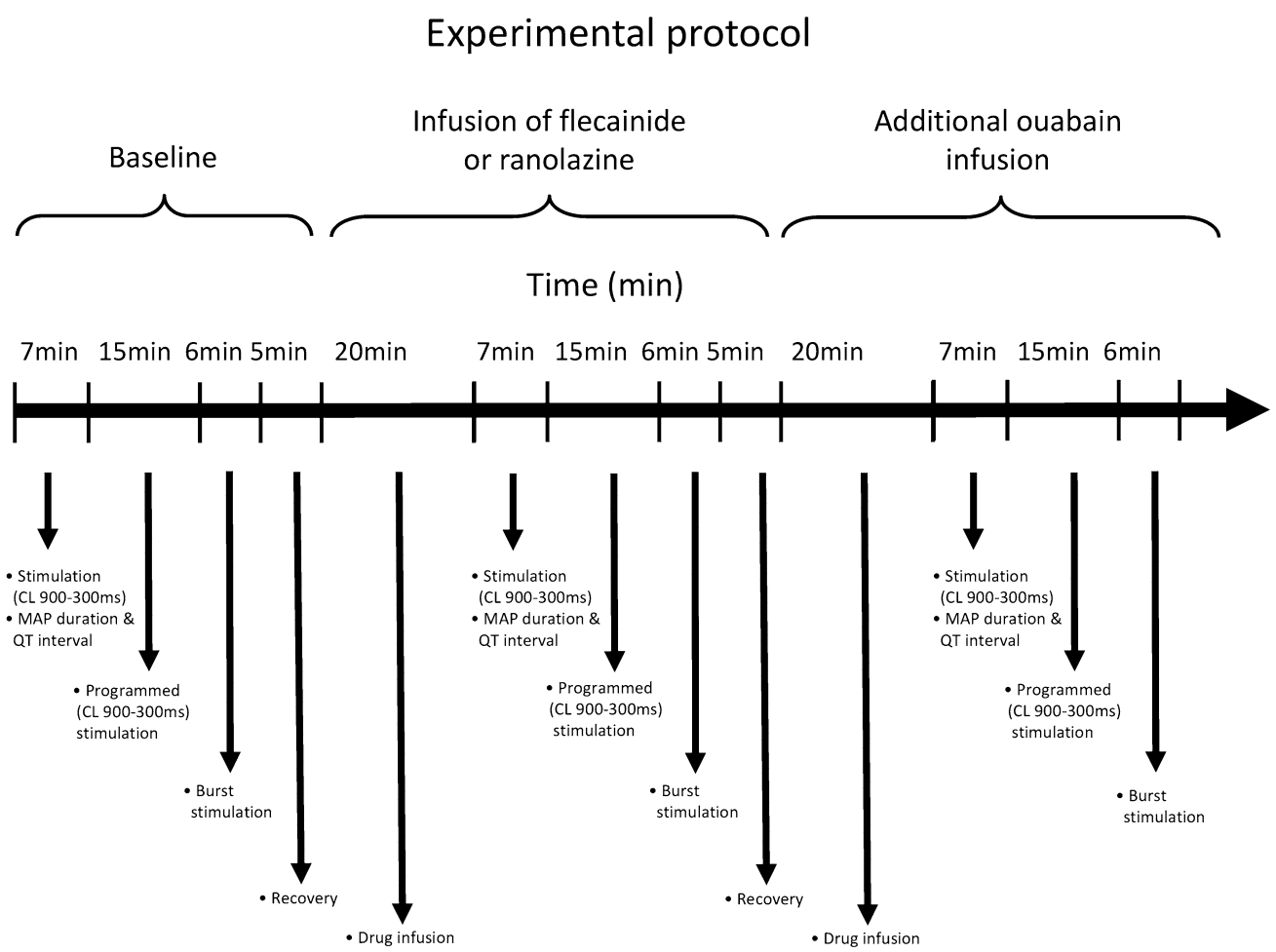

Fig. 1 Study protocol and timing of pacing protocols and drug administration

interquartile range (IQR). Normal distribution was analyzed using the Kolmogorov–Smirnov test. Different treatments were compared using the Wilcoxon signed rank test. Results with a p value < 0.05 were considered statistically significant. The sample size was determined by previous studies of our group with the same experimental model.

Results

Drug Effects on Action Potential Duration, QT Interval and Effective Refractory Period

Infusion of flecainide prolonged median QT interval from 250 ms (IQR: 44 ms) to 288 ms (IQR: 26 ms, $p < 0.01$) and slightly lengthened APD_{90} (153 ms (IQR: 29.7 ms) vs 159 ms (IQR: 24.9 ms), $p = 0.04$; Fig. 2). Treatment with ranolazine led to a prolongation of APD_{90} from 144 ms (IQR: 34.3 ms) to 157 ms (IQR: 31.2 ms, $p < 0.01$) and of QT interval from 258 ms (IQR: 50 ms) to 280 ms (IQR: 50 ms, $p < 0.01$). Flecainide- as well as ranolazine-treatment prolonged ERP significantly (flecainide: from 170 ms (IQR: 40 ms) to 200 ms (IQR: 32.5, $p < 0.01$); ranolazine: from 180 ms (IQR: 40 ms) to 200 ms (IQR: 30 ms, $p < 0.01$; Fig. 3)).

Additional perfusion with the digitalis glycoside ouabain (0.2 µM) abbreviated APD_{90} to 128 ms (IQR: 19 ms, $p < 0.01$) and QT interval to 252 ms (IQR: 45 ms $p < 0.01$) in flecainide-pretreated hearts. Similar results were obtained in ranolazine-pretreated hearts: Infusion of ouabain on top of ranolazine shortened APD_{90} to 141 ms (IQR: 40 ms, $p < 0.01$) and QT interval to 242 ms (IQR: 50 ms, $p < 0.01$). Concomitant perfusion with ouabain abbreviated ERP in both groups (flecainide group: to 170 ms (IQR: 20 ms, $p < 0.01$); ranolazine group: to 160 ms (IQR: 30 ms, $p < 0.01$)).

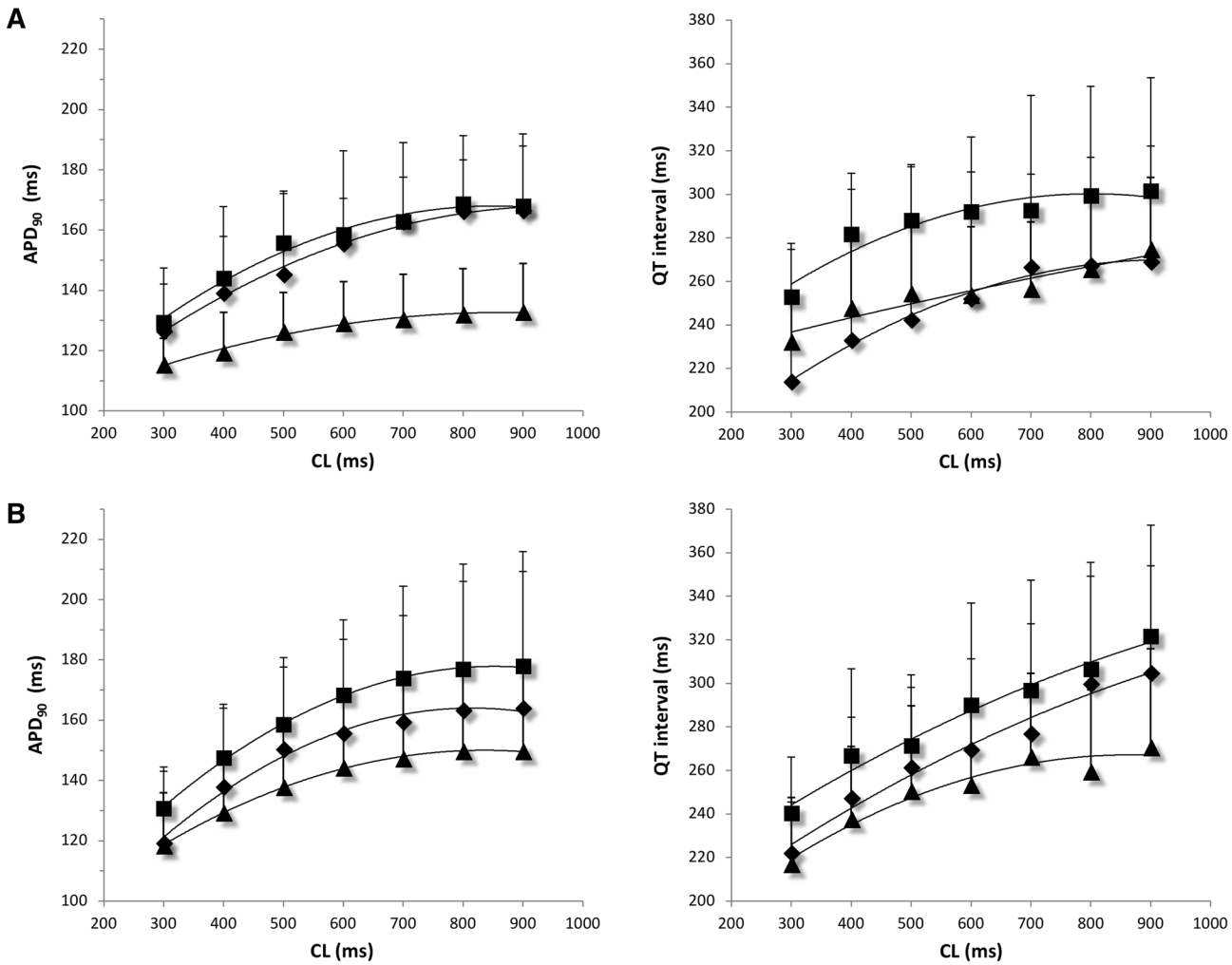

Fig. 2 Cycle length (CL)-dependent drug effects on APD_{90} (left) and QT interval (right) at baseline (filled diamond), after treatment with 2 µM flecainide (A, filled square) or 10 µM ranolazine (B, filled square) and after additional infusion of 0.2 µM ouabain (filled triangle)

Ventricular Arrhythmias

Infusion of flecainide had no proarrhythmic effects in isolated rabbit hearts (1 episode vs. 3 episodes under baseline conditions, $p = 0.41$; Fig. 4). Episodes of ventricular arrhythmias occurred less frequently under perfusion with ranolazine compared to baseline conditions (3 vs. 11 episodes, $p = 0.04$). However, in both groups, further treatment with ouabain led to a trend towards a proarrhythmic effect (flecainide group: 15 episodes, $p = 0.04$; ranolazine group: 21 episodes, $p = 0.09$; Fig. 5).

Discussion

The main finding of the present study is that a combination of sodium channel inhibitors and digitalis glycosides promotes ventricular arrhythmias in a sensitive experimental model of proarrhythmia. To our best knowledge, this is the first study demonstrating this effect in a whole-heart model.

Flecainide and ranolazine inhibit sodium currents and affect potassium channels as well. Thus, a slight prolongation of repolarization, in particular of APD_{90} and QT interval, was observed after treatment with flecainide and ranolazine. Furthermore, both drugs significantly augmented ventricular refractory periods. A prolongation of refractory periods is one of the main mechanisms of antiarrhythmic drugs to prevent cardiac arrhythmias by protecting the myocardium against premature beats. In an experimental model of acquired short QT syndrome, a prolongation of ventricular repolarization and refractory period after administration of ranolazine, vernakalant or antazoline was regarded as the main antiarrhythmic mechanism [20, 25].

In the present study, these effects were reversed by the application of the digitalis glycoside ouabain. It inhibits the sodium potassium ATPase pump and thereby abbreviates

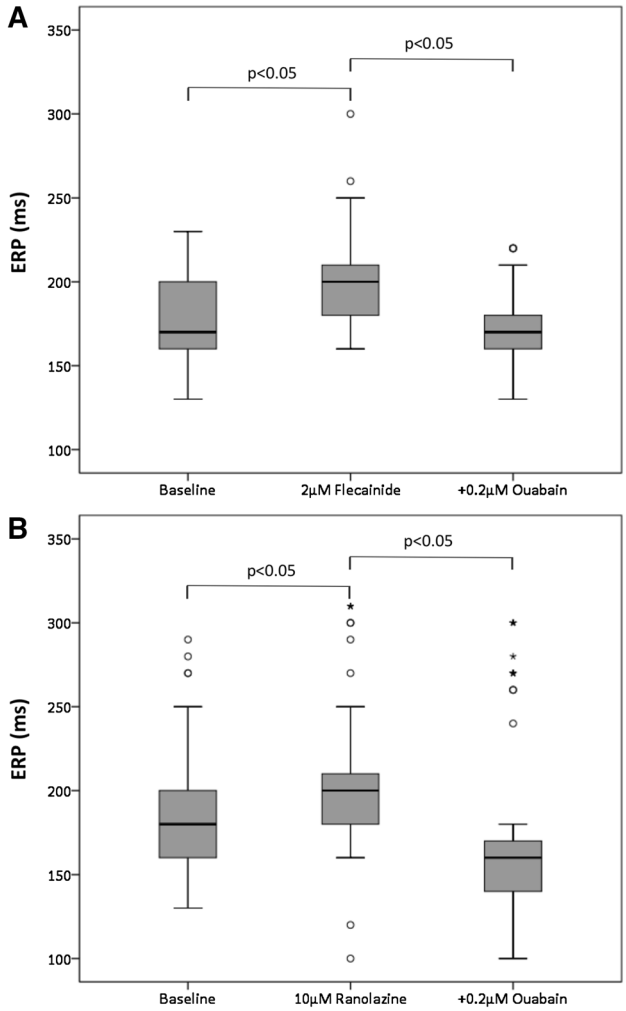

Fig. 3 Box plots of effective refractory periods (ERP) at baseline, after treatment with 2 μM flecainide (**a**) or 10 μM ranolazine (**b**) and after additional infusion of 0.2 μM ouabain

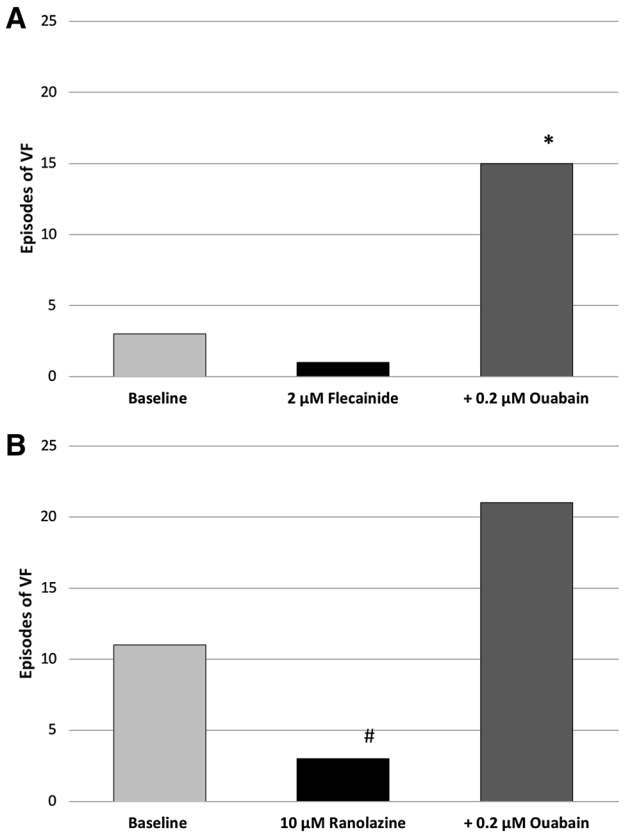

Fig. 4 Total number of induced episodes of ventricular fibrillation (VF) at baseline, after treatment with 2 μM flecainide (**a**) or 10 μM ranolazine (**b**) and after additional infusion of 0.2 μM ouabain (#$p < 0.05$ as compared with baseline; *$p < 0.05$ as compared with sole flecainide/ ranolazine infusion)

cardiac repolarization. Of note, effective refractory periods were significantly shortened. An abbreviation of ventricular refractory periods facilitates premature excitation and arrhythmia induction. In previous experimental studies in the same model, a reduction of effective refractory periods was related to an increased ventricular vulnerability [11, 12]. In accordance with these results, hearts were more prone to ventricular arrhythmias after ouabain treatment in the present study.

In a previous experimental investigation employing a canine ventricular septum preparation, less digitalis-mediated arrhythmias were observed after additional treatment with class I antiarrhythmic drugs [9]. This was explained by the capability of class I agents to inhibit the Na^+ influx and thereby to reduce the intracellular sodium concentration. Hereby, the digitalis-mediated increase of the intracellular concentration of sodium and subsequently of calcium can be attenuated. As a consequence, the calcium overload that leads to triggered activity is reduced [9]. In the present study, more ventricular arrhythmias were observed after ouabain infusion despite additional treatment with sodium channel inhibitors. This underlines the importance of other explanatory approaches for digitalis-mediated arrhythmias. More precisely, the phosphorylation of the ryanodine receptor might play a crucial role in triggering delayed afterdepolarizations and ventricular arrhythmias [2].

Several studies indicate a higher mortality in patients receiving digitalis glycosides either for rate control in atrial fibrillation or for symptomatic therapy of heart failure [5, 7]. In addition, the combination of antiarrhythmic drugs (especially dronedarone) and digoxin can lead to an increase of mortality and sudden cardiac death [10, 11].

In the light of the findings of the present study, a concomitant therapy of sodium channel inhibitors either as an antiarrhythmic or antianginal therapy and digitalis glycosides might be proarrhythmic. Even though this study is experimental and the results cannot be transferred directly to clinical practice, this combined therapy regimen should

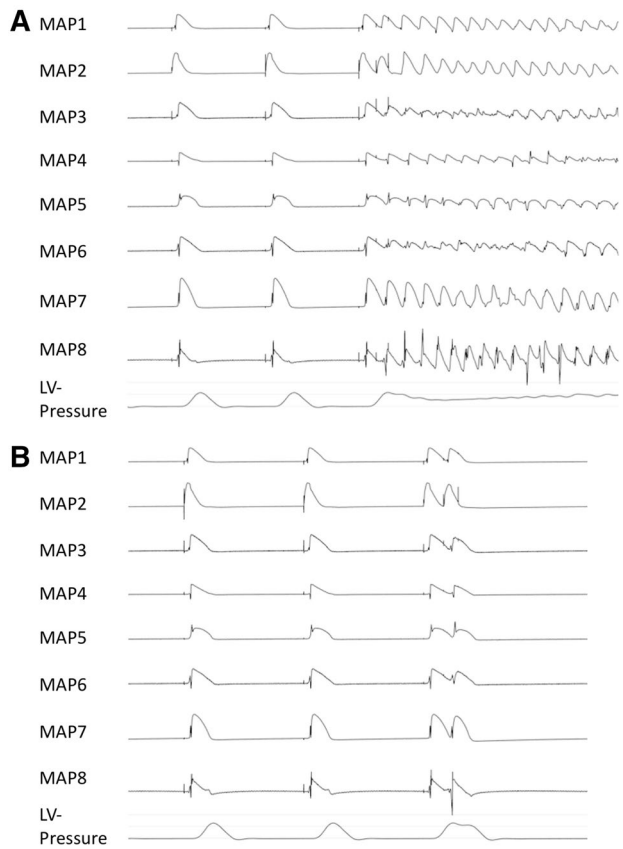

Fig. 5 **a** Representative example of ventricular fibrillation induced by a short-coupled extrastimulus (S3) in a ranolazine-pretreated heart under the influence of ouabain. **b** Representative example of programmed ventricular stimulation without induction of ventricular fibrillation in a ranolazine-treated heart *LV* left ventricular

be prescribed carefully. This is of particular interest since concomitant treatment with flecainide can elevate plasma digoxin levels [26] and higher serum digoxin levels are associated with an increased mortality [8].

Limitations of the Study

Our present study was performed in Langendorff-perfused rabbit hearts. Thus, results of this study cannot be directly transferred to the human heart. However, several studies indicated comparable electrophysiologic results in human and rabbit hearts. Due to the experimental setting of the isolated heart, humoral or nerval influencing factors are not considered. For instance, the autonomic reflex that is associated with heart failure patients cannot be simulated. Since a whole-heart model was used, no precise statements can be made on the impact of the administered drugs on ion currents.

Conclusion

The digitalis glycoside ouabain enhanced the ventricular vulnerability in flecainide- or ranolazine-pretreated hearts in this experimental whole-heart model by abbreviating the ventricular repolarization and ventricular refractory periods. Considering these findings, a concomitant therapy with the sodium channel inhibitors flecainide or ranolazine and digitalis glycosides should be carried out carefully.

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Compliance with Ethical Standards

Conflict of interest All other authors declare to have no conflict of interest.

Ethical Approval and Statement All applicable international, national and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

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