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Interactions of digitalis and class-III antiarrhythmic drugs: Amiodarone versus dronedarone

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ABSTRACT

Background: A post hoc analysis of the PALLAS trial suggested possible interactions of dronedarone and digitalis glycosides. The aim of the present study was to compare the effects dronedarone or amiodarone in combination with digitalis glycosides.

Methods and results: Eleven female rabbits underwent chronic oral treatment with amiodarone (50 mg/kg/d for 6 weeks). Ten rabbits were treated with dronedarone (50 mg/kg/d for 6 weeks). Ten rabbits were used as controls. Hearts were isolated and Langendorff-perfused. Monophasic action potentials and ECG showed a moderate prolongation of QT interval and action potential duration (APD). Both drugs also increased effective refractory period.

Additional application of ouabain (0.2 μM) resulted in a significant decrease of QT interval, APD, and ERP in all groups. Ventricular arrhythmias were induced by programmed ventricular stimulation and aggressive burst stimulation. Reproducible occurrence was defined as occurrence of at least 3 episodes. Under baseline conditions in control hearts, ventricular fibrillation (VF) was inducible in 1 of 10 hearts (7 episodes). After the application of 0.2 μM ouabain, 4 of 10 control hearts were inducible (24 episodes). One of 10 dronedarone-pretreated hearts (3 episodes) and 2 of 11 amiodarone-pretreated hearts (6 episodes) showed VF before ouabain infusion. After the application of 0.2 μM ouabain, 7 of 10 dronedarone-pretreated hearts were inducible (73 episodes). By contrast, only 4 of 11 amiodarone-pretreated hearts (13 episodes) showed VF.

Conclusion: In the present study, additional treatment with ouabain resulted in an increased ventricular vulnerability in al study groups. Of note, chronically dronedarone-pretreated hearts were significantly more vulnerable than amiodarone-pretreated hearts.

1. Introduction

Amiodarone and dronedarone are both frequently employed for rhythm control in atrial fibrillation (AF). Dronedarone was developed to be effective in suppression of AF without presenting the extra-cardiac side effects of amiodarone. Although amiodarone demonstrated superiority over dronedarone in suppression of AF in the clinical setting [1], dronedarone presented a more attractive safety profile as compared with amiodarone. This included a low risk of proarrhythmia [2]. In the ATHENA trial, dronedarone treatment resulted in a significant reduction of the combined primary end point consisting of hospitalization due to cardiovascular events and reduction of mortality [2]. In general, the electrophysiologic effects of dronedarone consist of a dose-dependent prolongation of repolarization [3]. However, dronedarone did not induce proarrhythmia in a highly sensitive experimental model of proarrhythmia where other antiarrhythmic drugs such as sotalol enhanced polymorphic ventricular tachycardia [4,5]. Of note, amiodarone and dronedarone presented a comparable electrophysiologic profile in isolated rabbit hearts [4].

The AMDROMEDA trial suggested negative effects of dronedarone treatment in patients with severe chronic heart failure [6]. In addition, an experimental study in dogs with chronic AV block revealed an increased incidence of proarrhythmia mediated by dronedarone [7]. In the PALLAS trial, the effects of dronedarone in patients with long-standing persistent or permanent AF and chronic heart failure were examined [8]. As a result of an increased mortality in the dronedarone group as compared with placebo the trial was terminated prematurely. Of note, a significant amount patients who died during this investigation received co-medication containing digitalis glycosides. The association of a combination therapy with dronedarone and digitalis glycosides has been confirmed in a retrospective analysis of the PALLAS trial [9] as well as in an experimental study in isolated rabbit hearts [10]. Of note, the combination of dronedarone and the digitalis

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glycoside ouabain resulted in a marked reduction of effective refractory period and post-repolarization refractoriness that was associated with an increased incidence of ventricular arrhythmias. Similar effects were observed both in healthy hearts as well as in an experimental model of non-ischemic chronic heart failure. As a result, the aim of the present study was, to compare the effects of chronic amiodarone therapy and chronic dronedarone therapy in combination with a digitalis glycoside to assess potential differences regarding the electrophysiologic profile.

2. Methods

All experimental protocols were approved by the local animal care committee and conformed with 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).

2.1. Oral drug treatment and preparation of hearts for perfusion

White female New Zealand rabbits (weighing 3.0–3.5 kg) received oral amiodarone ($n = 11$) or dronedarone ($n = 10$) treatment (50 mg/kg body weight p.o. per day) for a period of 6 weeks [4,11,12]. The drugs were mixed into the normal food. Ten female rabbits of comparable weight served as controls. Employment of this protocol previously demonstrated significant effects on ventricular repolarization [4,5].

The method of preparing the hearts has previously been described in detail [13]. In brief, all rabbits were anesthetized with sodium thiopental (200–300 mg i.v.). Hearts were removed and immediately placed in an ice-cold Krebs–Henseleit solution (composition in mM: $CaCl_2$ 1.80, KCl 4.70, KH_2PO_4 1.18, $MgSO_4$ 0.83, NaCl 118, $NaHCO_3$ 24.88, Na-pyruvate 2.0, and D-glucose 5.55). The spontaneously beating hearts were perfused at constant flow (52 ml/min) with warm (36.8 to 37.2 °C) Krebs–Henseleit solution. Perfusion pressure was continuously measured during the experiments and stayed stable at around 100 mmHg. The hearts were placed in a heated, solution-filled tissue bath. The perfusate was equilibrated with 95% O_2 and 5% CO_2 (pH 7.35; 37 °C). The cannulated and perfused hearts were attached to a vertical Langendorff apparatus (Hugo Sachs Elektronik, Medical Research Instrumentation, March-Hugstetten, Germany). A deflated latex balloon was inserted into the left ventricle and connected to a pressure transducer to control hemodynamic stability. The atrioventricular (AV) node was ablated to slow the intrinsic heart rate. This resulted in complete AV dissociation with a ventricular escape rate below 60 beats per minute. The slow heart rate remained stable throughout the whole protocol, independent of the used drug.

2.2. Electrocardiographic and electrophysiologic measurements

A volume-conducted ECG was recorded by complete immersion of the heart into a bath of Krebs–Henseleit solution. Signals from a simulated "Einthoven" configuration were amplified by a standard ECG amplifier (filter settings: 0.1–300 Hz). Monophasic action potential (MAP) recordings and stimulation were accomplished simultaneously using contact MAP pacing catheters (EP Technologies, Mountain View, CA, USA). The MAP electrograms were amplified and filtered (low pass 0.1 Hz, high pass 300 Hz). MAPs were analyzed using a specifically designed software, permitting precise definition of the amplitude (>5 mV) and duration of the digitized signals. The recordings were considered reproducible and, therefore, acceptable for analysis only if they had a stable baseline amplitude with a variation of less than 20% for at least 60 s during each cycle length and a stable duration with a variation of less than 10% in this time window measured at 90% repolarization (APD_{90}). Seven MAPs were evenly spread in a circular pattern around both ventricles; one MAP was recorded from the left endocardium. Pacing at twice diastolic threshold was performed for 1 min at each cycle length (CL) from 900 to 300 ms using a programmable stimulator (Universal Programmable Stimulator, UHS 20, Biotronik, Germany). All data were digitized at a rate of 1 kHz with 12-bit resolution and subsequently stored on a removable hard disk (BARD LabSystem; Bard Electrophysiology, Murray Hill, Massachusetts, USA).

2.3. Experimental protocol

Cycle length dependence was investigated under baseline conditions in all study groups by pacing the hearts at cycle lengths between 900 and 300 ms. Thereafter, hearts were perfused with ouabain (0.2 μM) over a period of 20 min. These sub-inotropic doses were previously used in Langendorff-perfused rabbit hearts [10,14]. Pacing, MAP recording, and measurement of ECG parameters were started 20 min after induction of AV block to ensure a stable data acquisition. Effective refractory periods (ERP) were determined after one minute of ventricular pacing using the extrastimulus technique [15].

Vulnerability to ventricular fibrillation (VF) was examined by VF induction through a standardized pacing protocol, including programmed ventricular stimulation with up to two extrastimuli (S2 and S3) and aggressive burst stimulation. Only sustained episodes requiring defibrillation were analyzed. Reproducible induction of at least 3 episodes per protocol part was required to exclude incidental episodes. The number of provocation maneuvers was exactly the same in all hearts. In case of sustained induction, VF was immediately terminated by defibrillation following a recovery period of at least one minute.

2.4. Data acquisition and statistical analysis

Data were entered into a computerized database (Microsoft Excel 2003) and statistical analysis was performed using the SPSS Software for Windows, release 22.0.0. (SPSS Inc., Chicago, USA). Before statistical testing, each continuous variable was analyzed for its normal distribution using the Kolmogorov–Smirnov test. Drug effects on cycle length dependence of PRR, QT interval, and APD were assessed using the repeated-measures ANOVA of SPSS 22.0: the general linear model (GLM) for repeated-measures test. After confirming significant group differences over CL, the differences between groups were calculated. Pairwise multiple comparisons with Bonferroni's procedure were applied to determine differences between groups. The chi-squared test and the Fisher test were used to compare the incidences of VF. The unpaired *t*-test was used for comparisons of differences between two independent groups, whereas the paired *t*-test between dependent groups. Differences are considered significant at $p < 0.05$. Categorical variables were expressed as frequency and percentage, whereas continuous variables are presented as mean ± SD.

3. Results

3.1. Effects of amiodarone, dronedarone, and ouabain on QT interval and action potential duration

Chronic amiodarone treatment resulted in a significant increase of QT interval (226 ± 27 ms vs. 253 ± 26 ms, $p < 0.05$). In addition, action potential duration was increased by trend (134 ± 23 ms vs. 149 ± 22 ms, $p = 0.14$). A comparable prolongation of myocardial repolarization was observed in hearts chronically treated with dronedarone (QT interval: 226 ± 27 ms vs. 244 ± 20 ms, $p = 0.09$, APD_{90}: 134 ± 23 ms vs. 155 ± 20 ms, $p < 0.05$).

Administration of ouabain (0.2 μM) led to a significant decrease in both APD_{90} and QT-interval. In control hearts, APD90 was reduced from 134 ± 23 ms to 89 ± 8 ms ($p < 0.001$). A similar decrease of QT interval was observed (226 ± 27 ms vs. 165 ± 23 ms, $p < 0.001$). In amiodarone-pretreated hearts, APD_{90} was decreased from 149 ± 22 ms to 111 ± 21 ms ($p < 0.001$), while QT interval was reduced from 253 ± 26 ms to 199 ± 32 ms ($p < 0.001$). Similar results were obtained in dronedarone-pretreated hearts (APD90: 155 ± 20 ms vs. 102 ± 17 ms; $p < 0.001$; QT interval: 244 ± 33 ms vs. 183 ± 29 ms; $p < 0.001$). Detailed cycle length-dependent effects in all study groups on APD_{90} are displayed in Fig. 1.

3.2. Drug effects on effective refractory period

Chronic treatment with amiodarone or dronedarone did not induce significant elevations of ventricular refractory period (control: 171 ± 23 ms; amiodarone: 185 ± 29 ms, $p = 0.24$; dronedarone: 182 ± 20 ms, $p = 0.27$). Administration of ouabain caused a significant decrease of effective refractory period in all groups. In control hearts, ERP was reduced from 171 ± 23 ms to 134 ± 33 ms ($p < 0.01$). A significant reduction of ERP was also observed in amiodarone-pretreated hearts (185 ± 29 ms vs. 148 ± 26 ms, $p < 0.01$) and in dronedarone-pretreated hearts (182 ± 20 ms vs. 121 ± 32 ms, $p < 0.001$). Of note, the decrease of ventricular ERP was significantly more marked in dronedarone-pretreated (121 ± 32 ms) hearts as compared with amiodarone-pretreated hearts (148 ± 26 ms, $p < 0.05$, Fig. 2).

3.3. Ventricular fibrillation

Regarding reproducible induction of VF, no significant differences between control hearts, amiodarone-pretreated hearts, and dronedarone-pretreated and control hearts were observed. In 1 of 10 control hearts, VF was inducible (7 episodes) as compared with 2 of 11 amiodarone-pretreated hearts (6 episodes) and 1 of 10 dronedarone-pretreated hearts (3 episodes, Fig. 3). Administration of ouabain led to an increased inducibility of ventricular fibrillation as compared with baseline in all study groups. Of note, after additional treatment with 0.2 μM ouabain more episodes of ventricular fibrillation were inducible in dronedarone-pretreated hearts (7 of 10 hearts, 73 episodes) as