European Journal of Pharmacology 776 (2016) 185–190

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

Divergent electrophysiologic profile of fluconazole and voriconazole in an experimental whole-heart model of proarrhythmia

Gerrit Frommeyer [a,*], Christina Fischer [a], Philipp S. Lange [a], Patrick Leitz [a], Michael Fehr [b], Harilaos Bogossian [c], Peter Milberg [a], Lars Eckardt [a]

[a] Division of Electrophysiology, Department of Cardiovascular Medicine, University of Münster, Münster, Germany
[b] Clinic of Exotic Pets, Reptiles, Exotic and Feral Birds, University of Hanover, Hanover, Germany
[c] Märkische Kliniken GmbH, Department of Cardiology and Angiology, Klinikum Lüdenscheid, University of Witten-Herdecke, Germany

ARTICLE INFO

Article history:
Received 31 December 2015
Received in revised form
14 February 2016
Accepted 18 February 2016
Available online 19 February 2016

Keywords:
Fluconazole
Voriconazole
Dispersion of repolarization
Proarrhythmia
Sudden cardiac death

ABSTRACT

In several case reports a prolongation of the QT-interval and even proarrhythmic effects of fluconazole and voriconazole were reported. The aim of the present study was to investigate if application of fluconazole or voriconazole has the potential to provoke polymorphic ventricular tachycardia in a sensitive model of proarrhythmia. In female rabbits, fluconazole (10, 30 and 50 μM, n=6) and voriconazole (10, 30 and 50 μM, n=6) were infused after obtaining baseline data. Eight endocardial and epicardial monophasic action potentials and a simultaneously recorded 12-lead ECG showed a significant QT prolongation after application of fluconazole as compared with baseline (10 μM: +12 ms, 30 μM: +22 ms, 50 μM: +37 ms; $P<0.05$) accompanied by an increase of action potential duration (APD_{90}). Administration of voriconazole also induced QT prolongation (30 μM: +10 ms, 50 μM: +20 ms, $P<0.05$). Spatial dispersion of repolarization remained stable in voriconazole-treated hearts while fluconazole induced a significant increase (30 μM: +15 ms, 50 μM: +16 ms; $P<0.05$). Lowering of potassium concentration in bradycardic AV-blocked hearts did not provoke any early afterdepolarizations (EADs) or polymorphic ventricular tachycardia in voriconazole-treated hearts. Application of fluconazole led to the reproducible induction of EADs in 4 of 6 hearts and polymorphic ventricular tachycardia in 3 of 6 hearts (36 episodes). In the present study, voriconazole demonstrated a safe electrophysiologic profile despite significant QT prolongation. In contrast, fluconazole led to a more marked prolongation of myocardial repolarization combined with a more marked increase of dispersion of repolarization. These results imply that application of fluconazole might be torsadogenic and the QT-interval should be closely monitored.

1. Introduction

Fluconazole is regularly employed for antimycotic therapy. A significant prolongation of the QT-interval and occurrence of torsade de pointes has been described in several clinical case reports (Esch and Kantoch, 2008; Overbey et al., 2013; Pham et al., 2006) as well as larger cohort studies (Manosuthi et al., 2009; Poluzzi et al., 2010; Zeuli et al., 2013). An experimental study suggested a significant inhibition of K_v 11.1 potassium channels which may induce acquired long-QT-syndrome (Han et al., 2011). However, this study was conducted in isolated human kidney cells. In contrast, in a different study in neonatal rat ventricular myocytes only moderate effects of fluconazole on cardiac ion currents were described (Sung et al., 2012).

Application of voriconazole can also be associated with prolongation of the QT-interval. Several case reports describe this effect as well as the potential occurrence of torsade de pointes (Brown et al., 2014; Elbey et al., 2012).

The aim of the present study was to examine a possible proarrhythmic potential of fluconazole and voriconazole in a sensitive whole-heart model of proarrythmia (Frommeyer et al., 2011; Milberg et al., 2002).

2. 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

* Correspondence to: Abteilung für Rhythmologie, Department für Kardiologie und Angiologie, Universitätsklinikum Münster, Albert-Schweitzer Campus 1, D-48149 Münster, Germany.
E-mail address: gerrit.frommeyer@ukmuenster.de (G. Frommeyer).

http://dx.doi.org/10.1016/j.ejphar.2016.02.051
0014-2999/

Health (NIH Publication No. 852-3, revised 1996).

2.1. Preparation of hearts for perfusion

The method of preparing the hearts has previously been described in detail (Milberg et al., 2002). Adult New Zealand White rabbits were anaesthetized with sodium thiopental (200–300 mg i.v.). After midsternal incision and opening of the pericardium, the complete 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 aorta was cannulated, the pulmonary artery was incised, and the spontaneously beating hearts were perfused at constant flow (52 ml/min) with warm (36.8–37.2 °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 Electronic, Medical Research Instrumentation, March-Hugstetten, GER). The atrioventricular node was mechanically ablated using surgical tweezers resulting in a complete atrioventricular dissociation.

2.2. Electrocardiographic and electrophysiologic measurements

Signals from a simulated "Einthoven" configuration were amplified by a standard electrocardiogram (ECG) amplifier (filter settings: 0.1–300 Hz) and QT interval was measured. 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 specifically designed software. The recordings were considered reproducible and, therefore, acceptable for analysis only if they had stable baseline amplitude with a variation of less than 20% and a stable duration 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 ventricular endocardium. Pacing at twice diastolic threshold was performed for 1 min at each cycle length (CL) from 900 to 300 ms (Universal Programmable Stimulator, UHS 20, Biotronik, Berlin, GER). 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. Protocols for ex vivo experiments

Animals were randomly assigned to 2 groups for subsequent treatment with fluconazole (n=6) or voriconazole (n=6). Cycle length (CL)-dependence of MAP duration was investigated under baseline conditions by pacing the hearts at CLs between 900 and 300 ms. After this first part of the protocol, the extracellular K^+ concentration was lowered to 1.5 mM in order to facilitate the induction of EADs in spontaneously beating hearts at their slowest ventricular escape rate. Five minutes later, the [K^+] was returned to 5.8 mM. Then, fluconazole (10 μM) or voriconazole (10 μM) were administered over a period of 20 min and measurements were repeated. Thereafter, two increased concentrations (30 and 50 μM) were administered and measurements were repeated.

APD_{90} was measured as the interval between the fastest MAP upstroke and 90% repolarization. Spatial dispersion of APD_{90} was expressed as the difference between the maximum and the minimum of APD_{90}, simultaneously recorded from eight endocardial and epicardial catheters.

2.4. Data acquisition and statistical analysis

Electrograms, pressure, volume and MAPs were recorded on a multi-channel recorder. Data were digitized online at a rate of 1 kHz with 12-bit resolution and stored on a disk. The observed data were entered onto a computerized database (Excel 2003; Microsoft; Redmond, WA, USA) and statistical analysis was performed using the SPSS Software for Windows, release 22.0.0. (SPSS Inc., Chicago, IL, USA). Each continuous variable was analyzed for normal distribution using the Kolmogorov-Smirnov test. Drug effects on QT-interval, APD and dispersion of repolarization were assessed using General Linear Model (GLM) for repeated measures. 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 polymorphic ventricular tachycardia (VT). 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 means ± S.D.

3. Results

3.1. Drug effects on action potential duration and QT-interval

After a stabilization period of 10 min, MAP recordings and pacing thresholds (mean threshold 1.4 ± 0.2 mA) remained highly reproducible throughout the experimental protocol. After the period of stabilization, the MAP amplitude did not change by more than 20%. Drug influence on action potential duration is illustrated in Fig. 1. Application of fluconazole induced a concentration-dependent increase of QT-interval (baseline: 223 ± 15 ms; 10 μM: 235 ± 12 ms; 30 μM: 245 ± 22 ms; 50 μM: 260 ± 13 ms, $P < 0.01$, Fig. 1B). An increase in QT-interval was also observed in voriconazole-treated hearts (baseline: 224 ± 9 ms; 10 μM: 232 ± 8 ms; 30 μM: 241 ± 12 ms; 50 μM: 251 ± 12 ms, $P < 0.05$, Fig. 1D). The increase in QT-interval was accompanied by a significant increase in action potential duration (APD_{90}, Fig. 1A and C).

3.2. Dispersion of repolarization and action potential configuration

Application of fluconazole led to an increase of spatial dispersion of repolarization (baseline: 34 ± 8 ms, 10 μM: 36 ± 9 ms; 30 μM: 51 ± 17 ms, $P < 0.05$; 50 μM: 49 ± 16 ms, $P < 0.05$; Fig. 2A). This increase in total dispersion of repolarization resulted from an increase of interventricular (recorded between right epicardial and left epicardial catheters) and intraventricular (recorded between left endocardial and left epicardial catheters) dispersion. Voriconazole (baseline: 42 ± 11 ms; 10 μM: 42 ± 8 ms; 30 μM: 42 ± 12 ms; 50 μM: 43 ± 8 ms; P=ns, Fig. 2B) did not significantly increase spatial dispersion of repolarization.

In addition, application of fluconazole-induced action potential triangulation (Fig. 3A). The configuration of the action potential was assessed by calculation the APD_{90}/APD_{50} ratio. Application of fluconazole increased this ratio from baseline values of 1.36 to 1.62 with 50 μM Fluconazole (Fig. 3B). In contrast, the APD_{90}/APD_{50} ratio remained stable after application of voriconazole (baseline: 1.42, 50 μM: 1.47, Fig. 3C).

3.3. Occurrence of proarrhythmia

After lowering [K^+] concentration to 1.5 mM, no occurrence of