International Journal of Cardiology 228 (2017) 583–587

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Experimental evidence for a severe proarrhythmic potential of levosimendan☆

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ARTICLE INFO

Article history:
Received 15 August 2016
Received in revised form 7 November 2016
Accepted 10 November 2016
Available online 14 November 2016

Keywords:
Levosimendan
Therapeutic safety
Ventricular refractory period
Ventricular fibrillation

ABSTRACT

Background: The calcium sensitizer levosimendan is established for therapy of acutely decompensated congestive heart failure. Clinical experience suggests a possible proarrhythmic potential. The aim of the present study was to assess possible proarrhythmic effects and underlying electrophysiological mechanisms.
Methods and results: Ten rabbit hearts were isolated and Langendorff-perfused. Thereafter, levosimendan was infused in 3 concentrations (0.5, 1, and 2 μM). Eight *endo*- and epicardial monophasic action potentials and a 12-lead ECG showed a dose-dependent reduction of QT interval (0.5 μM: −27 ms, 1 μM:-33 ms, 2 μM: −77 ms; $p < 0.05$) and action potential duration at 90% of repolarization (APD_{90}; 0.5 μM: −12 ms, 1 μM: −12 ms, 2 μM: −20 ms). There was no significant increase in dispersion of repolarization. The described abbreviation of myocardial repolarization was accompanied by a significant decrease of effective refractory period (ERP; 0.5 μM: −16 ms, 1 μM: −20 ms, 2 μM: −27 ms; $p < 0.05$).
Under baseline conditions, ventricular fibrillation was inducible by programmed stimulation and aggressive burst stimulation in 3 of 10 hearts (4 episodes). After application of 1 μM levosimendan, 8 of 10 control hearts were inducible (27 episodes). Of note, in 8 of 10 hearts after infusion of up to 2 μM levosimendan, incessant ventricular fibrillation that could not be terminated by multiple external defibrillations occurred.
Conclusion: In the present study, acute infusion of levosimendan resulted in an abbreviation of ventricular repolarization and a reduction of ERP. This led to a significantly elevated inducibility of ventricular fibrillation. In 8 of 10 hearts, incessant ventricular fibrillation occurred. These results suggest a proarrhythmic effect of levosimendan and might explain an increased mortality that coincided levosimendan treatment in a few small clinical studies.

1. Introduction

Levosimendan is a calcium-sensitizer that exerts positive inotropic effects without increasing intracellular calcium concentration [1]. It is regularly employed for symptomatic therapy of acutely decompensated heart failure [2].

In several clinical studies, a remarkable symptomatic relief by levosimendan therapy has been described. In two sequential clinical studies in patients with acutely decompensated heart failure, levosimendan led to clinical improvement at different predefined time points as compared with placebo-treated patients [3]. In the same study, improvements in patient self-assessment and a reduction of B-type natriuretic peptide plasma levels were also observed in the levosimendan group. Further clinical data also suggest a beneficial effect of levosimendan in patients undergoing cardiac surgery [4] as well as in patients after coronary revasculization [5].

However, the cited clinical studies also unmasked relevant side effects of levosimendan treatment. Apart from gastrointestinal side effects and hypotension a potential association with ventricular arrhythmias and deaths have also been observed in large clinical study [3]. In contrast, no increase of arrhythmic events by levosimendan was observed in the randomized, placebo-controlled LEAF-trial [6]. The aim of the present study was, therefore, to examine the electrophysiologic effects of levosimendan treatment in an experimental whole heart model.

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).

☆ All authors declare: No conflict of interest.

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http://dx.doi.org/10.1016/j.ijcard.2016.11.251
0167-5273/

2.1. Preparation of hearts for perfusion

The method of preparing the hearts has previously been described in detail [7]. In brief, 10 rabbits were anaesthetized 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 (36.8–37.2 °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 <20% for at least 60 s during each cycle length and a stable duration with a variation of <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 levosimendan in increasing concentrations (0.5, 1, and 2 μM) over a period of 20 min. The employed doses resemble those employed in previous experimental studies in similar models [8] as well as reported doses in clinical investigations [9]. 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 1 min of ventricular pacing using the extrastimulus technique [10].

Dispersion of MAP_{90} was expressed as the difference between the minimum and the maximum of MAP_{90}. 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 an aggressive burst stimulation. Three burst stimulations were performed per protocol part. Only sustained episodes requiring defibrillation were analyzed. The number of provocation manoeuvres was exactly the same in all hearts. In case of sustained induction, VF was attempted to be immediately terminated by defibrillation following a recovery period of at least 1 min.

2.4. Data acquisition and statistical analysis

Data were entered into a computerized database (Microsoft Excel 2010) and statistical analysis was performed using the SPSS Software for Windows, release 23.0.0. (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 refractory period, QT-interval and APD were assessed using the repeated-measures ANOVA of SPSS 23.0: the General Linear Model (GLM) for repeated measures test. 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 levosimendan on QT interval, action potential duration and dispersion of repolarization and effective refractory period

During electrophysiologic examination, all electrocardiographic parameters reached equilibrium within 10 min. After this stabilization period, MAP recordings and pacing thresholds (mean threshold 1.3 ± 0.4 mA) remained highly reproducible throughout the experimental protocol.

Levosimendan treatment resulted in a dose-dependent decrease of APD_{90} (baseline: 126 ± 18 ms; 0.5 μM 114 ± 23 ms, $p = 0.21$; 1 μM: 114 ± 14 ms, $p = 0.10$; 2 μM 106 ± 16 ms, $p < 0.02$; Fig. 1). This decrease of APD_{90} was paralleled by a comparable decrease in QT-interval (baseline: 253 ± 71 ms; 0.5 μM 226 ± 66 ms, $p = 0.39$; 1 μM: 220 ± 67 ms, $p = 0.30$; 2 μM 176 ± 60 ms, $p < 0.02$; Fig. 1).

Levosimendan did not induce significant alterations of spatial dispersion of repolarization (baseline: 57 ± 20 ms; 0.5 μM 60 ± 27 ms, $p = 0.78$; 1 μM: 60 ± 23 ms, $p = 0.76$; 2 μM 53 ± 11 ms, $p = 0.58$).

In addition, administration of levosimendan caused a significant decrease of effective refractory period (baseline: 158 ± 29 ms; 0.5 μM 142 ± 27 ms, $p = 0.22$; 1 μM: 138 ± 19 ms, $p = 0.08$; 2 μM 131 ± 14 ms, $p < 0.02$; Fig. 2).

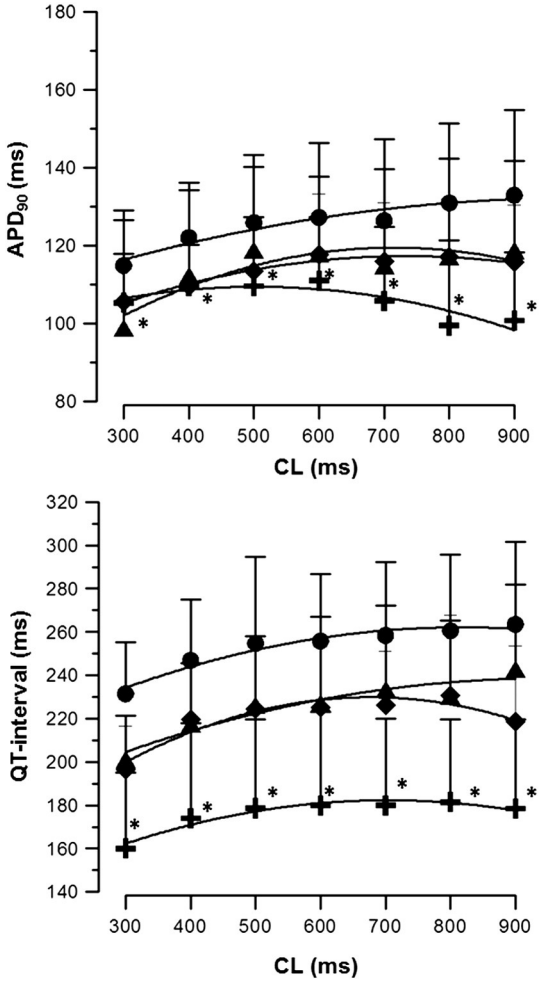

Fig. 1. Cycle length-dependent effects on APD_{90} (top) and QT-interval (bottom) at baseline (●), after administration of 0.5 μM levosimendan (▲), 1 μM levosimendan (♦), and 2 μM levosimendan (+) (*$p < 0.05$ as compared with baseline).