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Istaroxime, a positive inotropic agent devoid of proarrhythmic properties in sensitive chronic atrioventricular block dogs

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ABSTRACT

Current inotropic agents in heart failure therapy associate with low benefit and significant adverse effects, including ventricular arrhythmias. Istaroxime, a novel Na^+/K^+-transporting ATPase inhibitor, also stimulates SERCA2a activity, which would confer improved inotropic and lusitropic properties with less proarrhythmic effects. We investigated hemodynamic, electrophysiological and potential proarrhythmic and antiarrhythmic effects of istaroxime in control and chronic atrioventricular block (CAVB) dogs sensitive to drug-induced Torsades de Pointes arrhythmias (TdP). In isolated normal canine ventricular cardiomyocytes, istaroxime (0.3–10 μM) evoked no afterdepolarizations and significantly shortened action potential duration (APD) at 3 and 10 μM. Istaroxime at 3 μg/kg/min significantly increased left ventricular (LV) contractility (dP/dt+) and relaxation (dP/dt−) respectively by 81 and 94% in anesthetized control dogs (n = 6) and by 61 and 49% in anesthetized CAVB dogs (n = 7) sensitive to dofetilide-induced TdP. While istaroxime induced no ventricular arrhythmias in control conditions, only single ectopic beats occurred in 2/7 CAVB dogs, which were preceded by increase of short-term variability of repolarization (STV) and T wave alternans in LV unipolar electrograms. Istaroxime pre-treatment (3 μg/kg/min for 60 min) did not alleviate dofetilide-induced increase in repolarization and STV, and mildly reduced incidence of TdP from 6/6 to 4/6 CAVB dogs. In six CAVB dogs with dofetilide-induced TdP, administration of istaroxime (90 μg/kg/5 min) suppressed arrhythmic episodes in two animals.

Taken together, inotropic and lusitropic properties of istaroxime in CAVB dogs were devoid of significant proarrhythmic effects in sensitive CAVB dogs, and istaroxime provides a moderate antiarrhythmic efficacy in prevention and suppression of dofetilide-induced TdP.

1. Introduction

The use of positive inotropic drugs, essential for the treatment of acute and chronic heart failure (HF), is strongly restricted due to inherent life-threatening adverse effects. Although prescribed to restore or maintain cardiac output, a number of clinical trials demonstrated increased risk to ventricular arrhythmias associated with dobutamine, milrinone or levosimendan in acute HF or digoxin in advanced stage of the disease [1]. In addition, clinical data showed that most of these inotropic agents would not improve overall clinical outcomes and even be deleterious due to marginal therapeutic benefits associated with significant adverse effects [1]. Therefore, development of an effective inotropic agent, devoid of proarrhythmic risk liability in vulnerable patients, is urgently needed [2].

Istaroxime is a Na^+/K^+-transporting adenosine triphosphatase (Na^+/K^+-ATPase) inhibitor, which exerts positive inotropic effects. However, in contrast to digoxin, which also inhibits the Na^+/K^+-ATPase, istaroxime stimulates the activity of the sarcoplasmic reticular Ca^{2+}-ATPase isoform 2a (SERCA2a), thereby enhancing Ca^{2+} sequestration by the sarcoplasmic reticulum (SR). This results in an increased Ca^{2+} release during systole (inotropy) and a reduced cytosolic Ca^{2+} levels during diastole, improving relaxation (lusitropy). This additional

Abbreviations: APD, action potential duration; ARI, activation recovery interval; AS, arrhythmia score; CAVB, chronic atrioventricular block; DAD, delayed afterdepolarization; dP/dt+, left ventricular maximal contraction pressure gradient; dP/dt−, left ventricular maximal relaxation pressure gradient; EAD, early afterdepolarization; ECG, electrocardiogram; EDP, end diastolic pressure; EGM, endocardial unipolar electrogram; ESP, end systolic pressure; HF, heart failure; LV, left ventricle; MAP, monophasic action potential; MAPD, monophasic action potential duration (measured at 80% repolarization); mEB, multiple ectopic beat; Na^+/K^+-ATPase, Na^+/K^+-transporting adenosine triphosphatase; NCX, Na^+/Ca^{2+} exchanger; RV, right ventricle; sEB, single ectopic beat; SERCA2a, sarcoplasmic reticular Ca^{2+}-ATPase isoform 2a; SR, sarcoplasmic reticulum; STV, short-term variability of repolarization; TdP, Torsade de Pointes arrhythmia

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pharmacological characteristic seems to confer istaroxime an improved safety profile compared to digoxin, known for its narrow therapeutic margin and enhanced proarrhythmic risk [1,3,4].

Although in HF patients the total mortality is the highest at the advanced (decompensated) stage due to cardiac pump dysfunction, sudden (arrhythmic) cardiac death mostly occurs at early and compensated stages of HF [5]. Therefore, in this patient group, additional proarrhythmic triggers should be minimized. To date, the proarrhythmic effects of istaroxime and its potency to improve cardiac systolic function in relation with its positive inotropy and molecular mechanisms have been studied exclusively in failing heart animal models [3,6–9] and HF patients [10–12]. Therefore, the proarrhythmic properties of istaroxime remain to be explored in a sensitive animal model representing the proarrhythmic vulnerability of this patient population (compensated/early stage of HF).

The chronic atrioventricular block (CAVB) dog is a model of compensated cardiac hypertrophy characterized by its enhanced sensitivity to afterdepolarizations-dependent ventricular arrhythmias upon administration of positive inotropes (ouabain) or I_{Kr} blockers (dofetilide or sertindole) [13–15]. This proarrhythmic vulnerability results from the alteration of Ca^{2+} handling and downregulation of repolarizing potassium currents [13], which together favour non-homogenous prolongation of repolarization at both temporal and spatial levels [16,17]. For this reason, this animal model has been widely used in proarrhythmic drug screening [16], but also to determine the antiarrhythmic efficacy of a number of interventions against drug-induced Torsades de Pointes arrhythmias (TdP) [18].

The goal of the present study was first to assess the hemodynamic, electrophysiological and proarrhythmic properties of istaroxime in control dogs in comparison to CAVB dogs sensitive to drug-induced ventricular arrhythmias. Secondly, potential antiarrhythmic effects of istaroxime were explored in prevention and suppression of dofetilide-induced TdP in CAVB dogs. Due to the enhanced diastolic Ca^{2+}reuptake, we hypothesized that the luso-inotropic properties of istaroxime would not be associated to significant proarrhythmic effects in the sensitive CAVB dog model. In addition, the improved Ca^{2+} cycling upon istaroxime administration may exert antiarrhythmic effects against TdP arrhythmias in this canine model of cardiac hypertrophy.

2. Materials and methods

2.1. Animal experiments

2.1.1. Animals

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication N0.85-23, revised 1985) and to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and to the Dutch Law on animal experimentation and was approved by the Committee for Experiments on Animals of Utrecht University.

Animal studies are reported in compliance with the ARRIVE guidelines [19]. The current study has no implications for replacement, refinement or reduction.

Dogs were housed in pairs in conventional dog kennels (approx. 8 m^2) containing wooden bedding material. Animals had access to water *ad lib*. and received dog food pellets twice a day. Cages were enriched with playing tools and animals were allowed to play in groups in an outdoor pen (50 m^2) once a day. Dogs were checked for comfort and health every day and body weight was established once a week.

2.1.2. Animal preparation

A total of 10 adult mongrel dogs (Marshall, New York, USA; 1 female, 26 ± 3 kg) were included in this study. Atropine (0.5 mg i.m., Pharmachemie BV, Haarlem, The Netherlands), methadone (10 mg i.m., COMFORTAN®, Eurovet Animal Health BV, Bladel, The Netherlands), meloxicam (2 mg/kg s.c., METACAM®, Boehringer-Ingelheim Vetmedica BV, Ingelheim am Rhein, Germany) and acepromazine (10 mg i.m., VETRANQUIL®, Alfasan BV, Woerden, The Netherlands) were given as premedication. In all experiments, general anesthesia was induced by pentobarbital (Nembutal, 25 mg/kg i.v.) and maintained by isoflurane (1.5%, Abbot Laboratories Ltd, Maidenhead, United Kingdom) via the ventilation system (O_2/N_2O mixture; 1:2 ratio). Perioperative care included antibiotic prophylaxis and analgesia using ampicillin (1000 mg, before and after the experiment, intravenously and intramuscularly, respectively; AMPI-DRY®, DopharmaBV, Raamsdonksveer, The Netherlands) and buprenorphin (Temgesic, 0.3 mg i.m. after the experiment; Indivior UK Ltd, Slough, United Kingdom).

After the sinus rhythm experiment was completed, a screw-in lead was brought to the right ventricular (RV) apex via the jugular vein and connected to an internal pacemaker (Medtronic, Maastricht, The Netherlands) implanted subcutaneously. Ablation of the His bundle was then performed by radiofrequency as previously described [13] in order to create complete and irreversible third degree AV block. A recovery interval of 2 weeks was given to the animals between 2 consecutive anesthetized experiments. At the end of the final *in vivo* experiment and while still under anesthesia, heparin (10 000 I.U., i.v.) was given and right side thoracotomy was performed after which the beating heart was excised from the thoracic cavity.

2.1.3. Data acquisition

All experiments comprised the continuous recording of surface electrocardiogram (ECG) along with endocardial electrical signals acquired from the left ventricle (LV) and RV using EPTracer software (Cardiotek, Maastricht, The Netherlands): a duo-decapolar catheter (St Jude Medical, St Paul, MN, USA) recording unipolar electrograms (EGM) from up to 10 different locations or a monophasic action potential (MAP) catheter (Hugo Sachs, Germany) in the LV (LV MAP), and a MAP in the RV (RV MAP). In addition, systemic arterial pressure (via femoral sheath) and LV pressure via a sensor catheter (CD Leycom Inc., Zoetermeer, the Netherlands) were also recorded.

2.1.4. Data analysis

Offline analysis of hemodynamic parameters (CD Leycom) included end systolic and end diastolic pressures from LV (ESP and EDP, respectively) and systolic and diastolic femoral artery pressures (SAP and DAP, respectively), along with the maximal rise and decay of LV pressure (dP/dt + and dP/dt −, respectively). All values were retrieved and averaged from 10 consecutive beats. Surface ECG intervals (RR, PQ, QRS and QTc using the van de Water correction for heart rate [20]) were measured offline with EPTracer. LV activation recovery interval (LV ARI, derived from EGM) or LV monophasic action potential duration (LV MAPD, measured at 80% repolarization and derived from MAP catheter), and RV MAPD (measured at 80% of repolarization) were determined with their associated short-term variability value (LV and RV STV calculated from Thomsen et al. [21]: $STV = \Sigma|D_{n-1}–D_n|/[30\times\sqrt{2}]$) using a custom-made software AutoMAPD written in Matlab (Mathworks, Natick, MN, USA).

Incidence of arrhythmic events, as being single and multiple EB (sEB and mEB, respectively) and TdP, was determined along with an arrhythmia score (AS) [22], calculated as the average of the 3 most severe arrhythmic events occurring within 10 min after the start of dofetilide infusion. Each arrhythmic event was scored as n + 1 points, n being the number of ectopic beats of the episode. Consecutive defibrillations required to restore normal heart rhythm were scored 50, 75 and 100 points for 1, 2 and ≥3 electrical cardioversions, respectively.

2.1.5. Sinus rhythm protocol

After a 10 min baseline period, istaroxime (APExBIO, Houston, TX, USA) was infused to 6 normally conducted sinus rhythm dogs at 3 μg/kg/min for 60 min followed by a 30 min washout period. This dose of