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Original article

Assessment of ECG interval and restitution parameters in the canine model of short QT syndrome

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ABSTRACT

Introduction: The short QT syndrome (SQTS) is characterized by a short QT interval resulting from accelerated ventricular repolarization, and may be associated with ventricular fibrillation but not torsades de pointes. There are abundant data on the adverse effects of long QT, but knowledge of SQTS is sparse. The aim of this study was to examine whether analyses of several ECG biomarkers (QT, QTcB, QTcF, QTcV, QT_{btb}, and QT_{RR1000}) and dynamic restitution of the beat-to-beat QT–TQ relationship (TQ_{min}, %QT/TQ ratio>1, QT/TQ $ratio_{max}$) can be used to assess ECG changes in conscious dogs. **Methods:** Sling-trained dogs were infused with escalating concentration of levcromakalim (0, 1.0, 3.3, and 10.0 μg/kg/min), pinacidil (0, 3.3, 10.0, and 33.3 μg/kg/min), and nicorandil (0, 0.03, 0.1, and 0.3 mg/kg/min), drugs known to shorten QT. The RR, QT, QTcB, QTcF, QTcV, QT_{RR1000}, and TQ were measured before and after each concentration of the QT shortening test compounds. **Results:** Levcromakalim, pinacidil, and nicorandil but not vehicle significantly shortened RR, QT, QT_{btb}, QT_{RR1000}, and TQ but not QTc(B,F,V). The QT–RR cloud also shifted to the lower bounds of the normal QT–RR boundary by the test compounds. The percentage of beats with a QT/TQ ratio>1 was significantly increased in a dose response manner with levcromakalim and pinacidil and the lower TQ interval boundary (5th percentile) was decreased when compared to baseline or vehicle. **Discussion:** QT_{btb}, QT_{RR1000}, and dynamic beat-to-beat measurements of restitution constitute clinically applicable ECG biomarkers for assessment of changes associated with arrhythmogenic risk of ventricular fibrillation due to QT abbreviation.

1. Introduction

The short QT syndrome (SQTS) is a newly described disease characterized by an abbreviation of QT interval associated with episodes of syncope, paroxysmal atrial fibrillation (Gussak et al., 2000), or ventricular fibrillation, and may lead to sudden cardiac death in apparently healthy persons (Gaita et al., 2003; Brugada et al., 2004). Five forms of short QT syndrome have been described (Morita, Wu & Zipes, 2008). Each form is caused by a mutation of genes that encode for cardiac potassium and L-type calcium channels i.e., KCNH2 (I_{Kr}), KCNQ1 (I_{Ks}), KCNJ2 (I_{K1}), CACNA1C (α1 subunit of I_{CaL}), and CACNB2b (β2b subunit of I_{CaL}) (Brugada, Hong, Cordeiro & Dumaine, 2005; Morita et al., 2008). The functional mutation results in an abbreviation of the action potential duration (APD) and shortening of the QT interval on the body surface electrocardiogram (ECG). According to Schimpf et al. (2005), additional mutations in other ion channels (i.e. $I_{K\text{-}ATP}$, $I_{K\text{-}Ach}$) may produce shortening of APD.

Recently, extensive progress has been made in studying the congenital long QT syndromes (Antzelevitch & Francis, 2004) and in drug-induced long QT, particularly torsades de pointes (Fossa, 2008a; Fossa, 2008b; Hamlin & Kijtawornrat, 2008; Kijtawornrat, Nishijima, Roche, Keene & Hamlin, 2006). ECG interval biomarkers i.e., QT, QT corrected for heart rate (QTc), dynamic beat-to-beat QT–RR relationship (Fossa et al., 2005), short term variability of QT interval (STV; Hinterseer et al., 2007), transmural dispersion (duration from the peak of T to the end of T; Roche, Kijtawornrat, Hamlin & Hamlin, 2005) as well as restitution parameters i.e., QT/TQ ratio (Fossa et al., 2007) have been proven to beneficial in predicting drug-induced QT prolongation and consequently ventricular arrhythmias, especially torsades de pointes (Lu et al., 2008). While several ECG biomarkers and restitution parameters have been shown to predict arrhythmias in the model of long QT syndrome, these parameters have not been evaluated in the setting of short QT syndrome.

In this study, the abbreviation of QT interval was induced by infusion of several ATP sensitive "openers" of potassium channel ($I_{K\text{-}ATP}$) (pinacidil, levcromakalim, and nicorandil). The indications of potassium channel openers focused on the management of systemic and pulmonary hypertension, and stable angina pectoris (Pollesello &

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Mebazaa, 2004). These drugs stimulate $I_{K\text{-}ATP}$ channel activity by binding to the regulatory sulfonylurea receptor (SUR) subunit of $I_{K\text{-}ATP}$ channel in several tissues, including cardiac, smooth muscle, and skeleton muscle. Activation of $I_{K\text{-}ATP}$ channels in the cardiovascular system leads to shortening of the APD and relaxation of the vascular smooth muscle (Khan & Gowda, 2004).

The purpose of this study was to examine whether analyses of several ECG biomarkers (e.g., RR, QT, QTc, QT_{btb}, and QT_{RR1000}) and dynamic restitution (TQ_{min}, %QT/TQ ratio>1, and QT/TQ $ratio_{max}$) can be used to quantify ECG changes in conscious sling-trained dog infused with escalating concentration of levcromakalim, pinacidil, nicorandil and vehicle.

2. Methods

2.1. Approvals

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at QTest Labs, LLC in accordance with the *Guide for the Care and Use of Laboratory Animal* and USDA animal regulations.

2.2. Animal preparation and experimental procedures

A total of 5 beagle dogs were used in this study. All dogs (3 male and 2 female, weighing between 8.6 and 11.2 kg) were trained (at least 7 days) to lie comfortably in slings in a quiet room and monitored to ensure no cardiac arrhythmias or behavioral irregularities.

After clipping the hair from the ventral region of the thorax, dogs were placed in a ventrally recumbent position in a comfortable sling. A bipolar transthoracic electrocardiogram between points rV2 (right 4th intercostals space at the costochondral junction) and V2 (left, 5th intercostals space at the costochondral junction) was obtained on an IOX 1.7 (EMKA Technologies, Falls Church, VA). The signals were sampled at 1 kHz. All studies were performed at the same time of each day to minimize circadian rhythm effects.

On the day of each study, dogs were placed on a sling in a quiet room and a cephalic venous catheter was put in place for intravenous administration of vehicle or test compound via syringe infusion pump (KD scientific, Holliston, MA). Access to the lab during data collection was limited to a person who collected the data to avoid disturbing the dogs during data collection period.

2.2.1. Dosing protocol

The experiment was a crossover design in which five dogs received each of the following four treatments in random order: 1) vehicle (10% DMSO) 0, 0.01, 0.1and 0.3 ml/kg/min; 2) pinacidil 0, 3.3, 10.0, and 33.3 μg/kg/min; 3) levcromakalim 0, 1.0, 3.3, and 10.0 μg/kg/min; 4) nicorandil 0, 0.03, 0.1, and 0.3 mg/kg/min. Studies in individual animals were separated by at least 7 days to allow for washout for compounds. All doses were selected from the literature (Chinushi et al., 2002; Ghaleh, Dubois-Rande, Hittinger, Giudicelli & Berdeaux, 1995; Thomsen, Volders, Beekman, Matz & Vos, 2006; Uchida et al., 1994; van Opstal et al., 2002) because they were shown to cause significant shortening of the QT interval in the dog. Bipolar transthoracic electrocardiograms were obtained before dosing and at 10 min intervals during dosing (3 min of infusion and 7 min between each dose).

2.3. Beat-to-beat ECG interval parameters

ECG waveforms were analyzed on ECG auto 1.5 (EMKA Technologies, Falls Church, VA) and each cardiac cycle was reviewed for an accurate detection of the RR, PR, QRS, and QT interval measuring from the beginning of the Q wave to the end of T wave. QT intervals that could not be measured due to motion artifact or electrical noise on the ECGs were removed from the data set. Approximately 350 to 900 consecutive cardiac cycles (5 min of continuous data) at the time of steady state drug exposure, the last 5 min of each of the three dose levels, were analyzed. The baseline values, the measurement of the last 5 min of the 10 min predose period, were used as the reference for the analysis of significant QT shortening.

2.3.1. QT_{RR1000}

The QT was analyzed as a function of the previous RR interval for each cardiac cycle of a selected time period. This method has been previously described (Fossa, DePasquale, Raunig, Avery & Leishman, 2002). Briefly, an asymptotic decaying exponential growth curve fit was used to describe the relationship between QT and RR interval (Raunig, Depasquale, Huang, Winslow & Fossa, 2001).

$$QT = A - B \cdot \exp(-C \cdot RR / 1000)$$

The coefficients represent different aspects of the QT–RR relationship. The coefficient A represents the behavior of QT at very long RR intervals. The coefficient B represents the behavior of QT at short RR intervals. The coefficient C represents the relationship of the intermediate points and the steepness of the curve at low and high RR values. From the curve fit, the QT at an RR interval of 1000 ms (QT_{RR1000}) was calculated at baseline, and the changes in QT_{RR1000} from the baseline values were calculated for each dose level for all agents.

2.3.2. QTcF, QTcB, and QTcV

The QT corrected for heart rate, utilizing algorithms of Fridericia (QTcF; Fridericia, 1920), Bazett (QTcB; Bazett, 1920), and Van de Water (QTcV; Van de Water, verheyen, xhonneus & Reneman, 1989), was calculated from the same QT–RR data set that were used to calculate QT_{RR1000}.

2.3.3. QT_{btb}

The uncorrected QT cloud of data, using bootstrap technique, was analyzed from sequential sets of beat-to-beat ECG cycles. This method was discussed in previous work of Fossa et al. (2005).

2.4. Restitution parameters

2.4.1. TQ 5th percentile (TQ_{min})

The TQ interval of the ECG was calculated as the RR interval minus the QT interval of the previous beat. TQ 5th percentile is the measuring of the lower limit for 95% of the beats.

2.4.2. Percentage of beats with QT/TQ ratio greater than 1 (%QT/TQ ratio>1)

The percentage of beats with a QT/TQ ratio greater than 1 reflects the relative time spent on the restitution curve where stability is not as certain.

2.4.3. Upper 98th percentile of the QT/TQ ratio (QT/TQ $ratio_{max}$)

The upper 98th percentile of the QT/TQ ratio reflects the magnitude of the steepness of the restitution relationship.

2.5. Statistical analysis

All ECG parameters and restitution parameters were analyzed at baseline value and the changes from baseline values were calculated for each dose level and for all test compounds. The baseline values represent the least squares mean ± standard error of mean (SE). The differences from baseline were analyzed for significance using a *t* test for each dose level with no multiple comparison corrections (Fossa et al., 2002).