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Research article

Shortening of the electromechanical window in the ketamine/xylazine-anesthetized guinea pig model to assess pro-arrhythmic risk in early drug development

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ABSTRACT

Background: A negative electromechanical window (EMw) was recently proposed as a better preclinical tool than QTc interval to predict clinical pro-arrhythmic potential. As such, we utilized the ketamine/xylazine anesthetized guinea pig to characterize the EMw and QTc interval for a diverse set of reference agents with known clinical pro-arrhythmic potential. Then we determined the clinical proarrhythmia predictive capacity of EMw shortening compared to hERG inhibition or QTc interval prolongation alone.

Methods: Changes in EMw and QTc interval by 26 reference agents were evaluated in the ketamine/xylazine-anesthetized guinea pig. Confusion matrix analysis using the hERG, QTc and EMw indexes (hERG IC_{50}, QTc EC_5 or the EMw EC_{-10} divided by their respective free therapeutic maximal plasma concentration) at various folds the therapeutic concentrations was conducted to assess the concordance of each index to predict clinical pro-arrhythmic risk.

Results: Shortening of the EMw concomitant to an increase in QTc interval was observed in the GP with known pro-arrhythmic drugs. Non-torsadogenic compounds did not cause EMw shortening, although some prolonged the QTc interval. The preclinical:clinical concordance of the EMw index (88%) was similar ($p > 0.05$) to using QTc interval prolongation alone (85%) but significantly greater ($p < 0.05$) than using hERG inhibition alone (69%). In addition, the specificity when using the EMw (87%) was largely greater ($p < 0.05$) than using QTc interval (73%) or hERG inhibition (60%) alone. When the components of the response (duration of left ventricular pressure (LVP) cycle (QLVPend) or QT interval) that caused EMw shortening were considered, the concordance is further improved (>95%).

Conclusion: EMw shortening improves QTc interval prolongation recording in early drug development and increases the translatability over existing preclinical tools in predicting clinical arrhythmias.

1. Introduction

Unintended drug-induced cardiac proarrhythmic risk and, in particular, Torsades de Pointes arrhythmias (TdPs: polymorphic ventricular tachycardia) have been a major reason of drug development attrition, causing about 21% of drug withdrawals between 1990 and 2010 (Heist & Ruskin, 2010). As such, the International Conference on Harmonization (ICH) S7B guidelines recommended in 2005 evaluating new drug candidates for effects on IKr or hERG in vitro and on the QT interval in vivo as primary preclinical studies to evaluate their potential for drug-induced QT interval prolongation and arrhythmogenesis. In addition, since 2005, the Food and Drug administration (FDA) has required almost all new drugs to be assessed in a "Thorough QT" (TQT) clinical study (ICH E14 guideline). The FDA has reviewed >250 TQT studies, of which approximately 20% have been positive for QT interval prolongation (Stockbridge, Morganroth, Shah, & Garnett, 2013). Though the ICH S7B and E14 guidelines were responses to a real public health concern, their reliance solely on QT interval prolongation may have limited the advancement of potentially safe and effective drugs with QT interval prolongation but with no or acceptable arrhythmogenic potential to market (Salvi, Karnad, Panicker, & Kothari, 2010).

Abbreviations: ANES GP, anesthetized guinea pig; AUC, area under the curve; CAVB, complete atrioventricular blockade; Cmax, maximal plasmatic concentration; ECG, electrocardiogram; EMw, electromechanical window; EMw-10, −10% shortening of the EMw; GP, guinea pig; FDA, Food and Drug Administration; hERG, human Ether-a-go-go Related Gene; HR, heart rate; ICH, International Conference on Harmonization; IKr, rapidly-activating delayed rectifier potassium current; LVP, left ventricular pressure; NCEs, new chemical entities; NBEs, new biological entities; QS_2, electromechanical systole; QTc, heart rate corrected QT interval; QTcb, Bazett's heart rate QT corrected interval; QTcVdW, Van de Water's heart rate QT-corrected interval; TdPs, Torsades de Pointes; ROC, receiver operating characteristic; TQT, thorough QT study.

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As such, several alternative methods for TdPs detection have been suggested to better evaluate the risk for drug-induced arrhythmia. The measurement of beat-to-beat instability (Champeroux et al., 2016; van der Linde et al., 2005; Wisialowski et al., 2006), QT interval restitution (Fossa, Zhou, Robinson, Purkayastha, & Martin, 2014), heterogeneity of repolarization (Antzelevitch, 2000; Antzelevitch & Fish, 2001) and changes in action potential shape ("triangulation") (Hondeghem, 2005, 2008) have all been proposed as more predictive markers of arrhythmia in vivo. More recently, the electro-mechanical window (EMw) has been suggested as an additional method to predict pro-arrhythmic risk in humans (Guns, Johnson, Van Op, Weltens, & Lissens, 2012). The EMw is defined as the delay between the electrical (QT interval) and the mechanical (QLVPend) systole (EMw = QLVPend − QT interval). An inversion (negative) of the EMw has been proposed as an indicator for the initiation of TdPs in several pre-clinical models: the pentobarbital-anesthetized guinea pig (ANES GP) (Guns, Johnson, Weltens, & Lissens, 2012); and a pharmacologic model of acquired-LQT1 syndrome in the fentanyl-etomidate anesthetized beagle dog (van der Linde et al., 2010). Clinically it was shown that in the healthy myocardium, the duration of electrical systole (QT) is shorter than that of the electromechanical systole (Second heart sound; QS_2 in the clinic), which it closely parallels throughout the range of resting heart rate (HR) (Boudoulas, Sohn, O'Neill, Brown, & Weissler, 1982). A negative EMw, formerly referred to in the clinic as inversed QT/QS2 ratio or "QT > QS2 Syndrome", occurs if either LV-contraction duration shortens or if the QT-interval prolongs (or a combination of both). Further, changes in autonomic tone (De Caprio et al., 1984), increases in intracellular Ca^{2+} or high circulating cathecolamines levels (Boudoulas, Geleris, Lewis, & Leier, 1981) were described to invert the normal QT/QS2 ratio. More recently, ter Bekke et al. (2015) observed that patients with genotype-positive long QT syndrome exhibit EMw inversion and it is most pronounced in patients with documented arrhythmic events.

The goals of our study were to: i) utilize the ketamine/xylazine ANES GP to characterize the EMw and QTc interval for a diverse set of reference pharmacological agents ($n = 26$) with known clinical pro-arrhythmic potential and ii) assess the clinical predictive capacity of using EMw shortening rather than the negative EMw as a biomarker of pro-arrhythmic risk as compared to QTc interval prolongation or hERG ion channel inhibition alone.

2. Methods

2.1. Anesthetized guinea pigs

All aspects of the animal use were in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 2011) and approved by the Merck Institutional Animal Care and Use Committee (IACUC).

2.1.1. Anesthesia

Male Hartley guinea pigs (BW: 350–500 g) were anesthetized with a bolus mixture of ketamine/xylazine (85/5 mg kg^{-1}, IM) then an intravenous (i.v.) infusion of ketamine/xylazine (40/0.5 mg kg^{-1} h^{-1}, to effect). Additional analgesics (buprenorphine: 0.05 mg kg^{-1}) were administered s.c. as needed prior to surgical instrumentation. The anesthetic influence of pentobarbital on the EMw was also assessed in male, Hartley guinea pigs (BW: 350–500 g) which were anesthetized with sodium pentobarbital (60 mg kg^{-1} IP) (Morissette et al., 2013). Animals were placed on an automated homeothermic blanket system for both ketamine/xylazine or pentobarbital ANED GPs to maintain body temperature between 37.5 and 38.0 °C for the entire duration of the experimentation.

2.1.2. Surgery

A longitudinal incision was made on the medial-ventral surface of the neck to access the trachea, jugular vein, and carotid arteries. An endotracheal tube was inserted and ANES GPs were allowed to breathe spontaneously. The jugular vein and left carotid arteries were cannulated with polyethylene catheters (PE50, I.D. ≈ 0.38 mm) for test article administration and arterial blood pressure (BP) monitoring/blood collection, respectively. A 2F solid-state pressure catheter (Millar Instruments, Houston, TX) was advanced into the left ventricule via the right carotid artery. The body surface ECG was measured via s.c. needle electrodes to record modified lead-II ECG. After surgery, ANES GPs were stabilized for a minimum of 20 min before beginning baseline data collection.

2.1.3. Test article administration

Solutions of varying test-article concentrations (Table 1) suitable for administering selected doses were prepared in appropriate vehicles. Vehicle alone or test-article solutions were administered i.v. with each dose level infused over a 20-min period using a programmable syringe infusion pump (Legato100, KD Scientific, Holliston, MA, USA). Each test article and vehicle were evaluated in $n = 6$ male ANES GP. Blood sampling for pharmacokinetic analysis was taken at 10 and 20 min of each dose level.

2.1.4. Pacing

Changes in HR were induced with atrial pacing. A 2F octapolar stimulating catheter (NuMed, Inc., Denton, TX, USA) was placed in the right jugular vein and advanced into the right atrium. Positioning was verified by examining electrogram tracings and a capture current of <100 μA. The paced frequency was controlled by a DS8000 digital stimulator (World Precision Instruments, Sarasota, FL, USA) connected to a constant current stimulus isolator, and the heart was paced at 2× excitation threshold with a pulse width of 1 ms.

Table 1
List of reference compounds, their mechanism of action, and dose range tested in the ketamine/xylazine anesthetized guinea pig model.

Drug	Mechanism of action	Doses (mg kg^{-1} 20 min^{-1})
Dobutamine	β1 receptor agonist	0.005, 0.01, 0.03
Milrinone	Phosphodiesterase 3 inhibitor	0.03, 0.07, 0.2
Ranolazine	Late inward sodium current inhibitor	3, 7, 20
Amlodipine	Dihydropyridine L-type calcium channel inhibitor	0.1, 0.2, 0.7
Verapamil	Phenylalkylamine L-type calcium channel inhibitor	0.03, 0.07, and 0.1
Diltiazem	Non-dihydropyridines L-type calcium channel inhibitor	0.03, 0.1, 0.3
Ivabradine	I_f Channel Inhibitor	0.1, 0.2, 0.7
Atenolol	Selective β1 adrenergic receptor inhibitor	1, 2, 7
Levosimendan	Calcium sensitiser	0.1, 0.2, 0.7
Mexiletine	Class Ib sodium channel inhibitor	3, 7, 20
Nicardipine	Dihydropyridine L-type calcium channel inhibitor	0.03, 0.07, 0.2
Ciprofloxacin	Antibiotic	20, 40, 80
Amiodarone	K-Channel antagonist (IKr)	1, 2, 7
Moxifloxacin	Antibiotic	3, 7, 20
Flecainide	Class Ic antiarrhythmic agent	0.3, 0.7, 2
Cisapride	Serotonin 5-HT4 receptor agonist	0.3, 0.7, 2
Dofetilide	K-Channel antagonist (I_{Kr})	0.003, 0.007, 0.02
Dl-Sotalol	Class III antiarrhythmic agent	0.5, 2.5, 5
Astemizole	Histamine H1-receptor antagonist	0.1, 0.2, 0.7
Haloperidol	Dopamine D2 receptor antagonist	0.3, 0.7, 2
Sparfloxacin	Antibiotic	1, 2, 7
Levofloxacin	Antibiotic	30, 40, 50
Ondansetron	Serotonin 5-HT_3 receptor antagonist	1, 2, 7
Pentamidine	Antimicrobial	3, 7, 20
Domperidone	Antagonist of the dopamine D2 and D3 receptors	0.3, 1, 3
Bepridil	Amine L-type calcium channel blocker	1, 2, 7