

Original article

Relationship between prolongation of QTc and prolongation of the peak of T (Tp) to the end of T (Te)

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Abstract

Introduction: Lengthening of ventricular repolarization is known to be a risk factor for development of torsade de pointes, a form of ventricular tachycardia thought to be initiated by an early after depolarization and to be sustained by a novel reentrant mechanism precipitated, putatively, by heterogeneity of repolarization among ventricular myocytes. While prolongation of QT and QTc are good predictors of torsadogenic potential, the duration from the peak to the end of the T wave (Tp–Te) is thought to be a more accurate reflection of heterogeneity of ventricular repolarization. This study, conducted on Langendorff guinea pig hearts, was designed to compare lengthening of QTc with lengthening of Tp–Te for 16 test articles known to be and 7 known not to be torsadogenic. **Methods:** Bipolar, transventricular electrograms, recorded from 83 guinea pig hearts perfused according to methods of Langendorff, were exposed to escalating concentrations of test articles. RR, QT, QTc and Tp–Te were measured. QTc was calculated by the method of Fridericia. Data was analyzed using a mixed model ANOVA where least squared means (*t*-test) was used for comparing males vs. females and for concentration levels for each parameter studied. **Results:** QTc lengthened in 16 of 16 test articles known to be torsadogenic and did not lengthen in 7 of 7 test articles known not to be torsadogenic—sensitivity and specificity of 1.0. In 9 out of 16 torsadogenic test compounds, the Tp–Te interval increased parallel with QTc. In 7 test compounds known not to be torsadogenic, two test compounds increased Tp–Te. **Discussion:** It is clear that QTc prolongation is a more robust predictor of torsadogenicity than Tp–Te in the male guinea pig heart.

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Keywords: Guinea pigs; Heart; Heterogeneity; M fibers; Methods; QTc; Repolarization; Torsade de pointes; Tp–Te

1. Introduction

The Langendorff preparation has been proven useful for measuring effects of test articles on ventricular repolarization (i.e., QTc) (Hamlin, Cruze et al., 2004; Hamlin, Kijawornrat et al., 2004). Lengthening of ventricular repolarization is known to be a risk factor for development of torsade de pointes, a form of ventricular tachycardia thought to be initiated by an early after depolarization

(EAD) and to be sustained by a novel reentrant mechanism precipitated, putatively, by heterogeneity of repolarization among ventricular myocytes (Choi, Burton, & Salama, 2002). There is a significant difference between the electrical properties of myocytes isolated from different regions of the heart (Fig. 1). The QT interval of the electrocardiogram (ECG) represents the sum of both ventricular depolarization (QRS) and repolarization (ST–T). The peak of the T wave (Tp) is thought to correspond to the end of repolarization of the ventricular sub-epicardium, while the end of the T wave (Te) is thought to coincide with the end of repolarization of the mid-myocardium (M fibers) (Fig. 2) (Yan Antzelevitch, 1998). It is thought that the temporal difference between repolarization of mid-myocardium and sub-epicardium

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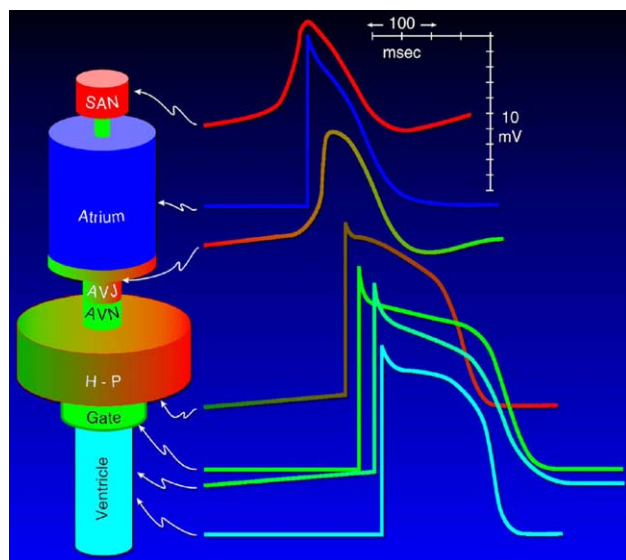


Fig. 1. Electrical heterogeneity of the heart. There is a significant difference between the electrical properties of myocytes isolated from different regions of the heart. This is due to the differences in ion channel composition, which determines the difference between myocytes from atria, ventricle and conduction system. SAN=sinoatrial node; AVJ=atrioventricular junction; AVN=atrioventricular node; H-P=His Purkinje conduction pathway; msec=millisecond; and mV=millivolt.

represents, in general, temporal heterogeneity of ventricular repolarization and that the greater the heterogeneity the greater anatomical/physiological substrate for reentrant arrhythmia (Shimizu Antzelevitch, 2000). While prolongation of QT and QTc are good predictors of torsadogenic potential (Kinter Valentin, 2002), the duration from the peak to the end of the T wave (Tp–Te) is thought to be a more accurate reflection of heterogeneity of ventricular repolarization and therefore a better predictor of torsadogenic substrate (Antzelevitch, 2001).

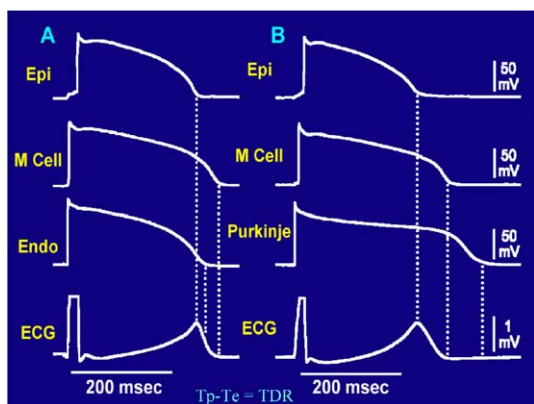


Fig. 2. Action potential durations of the different layers of the ventricular myocardium and the representative electrocardiogram (ECG) waveform, specifically peak to end of the T wave. TDR=transmural dispersion of repolarization (Shimizu, MaMahon, & Antzelevitch, 1999). *Courtesy of Charles Antzelevitch, PhD.

Table 1

Sixteen test articles known to be and 7 articles known not to be torsadogenic

Test articles known to be torsadogenic	Test articles known not to be torsadogenic
Arsenic trioxide	Aspirin
Astemizole	Captopril
Bretylium	Enalaprilat
Chloroquine	Mexilitine
Cisapride	Penicillin
Disopyramide	Propranolol
Erythromycin	Verapamil
Haloperidol	
Methadone	
Morphine	
Pimozide	
Quinidine	
Sotalol	
Sparfloxacin	
Tetracycline	
Thioridazine	

Review article, Redfern et al. (2003).

This study, conducted on Langendorff guinea pig hearts, was designed to compare lengthening of QTc with lengthening of Tp–Te for 16 test articles (Table 1) known to be and 7 articles (Table 1) known not to be torsadogenic.

2. Methods

These studies were conducted after approval of the Institutional Animal Care and Use Committee (IACUC) of QTest Labs and in compliance with USDA regulations. A total of 83, weighing 250–400 g, male guinea pigs were anesthetized with sodium pentobarbital (interperitoneal, 125 mg/kg). Hearts were removed quickly (1–2 min) after removal of palebral (blink) and pedal withdrawal reflex and were suspended on a Langendorff perfusion apparatus as described by previous authors (Hamlin, Cruze et al., 2004; Hamlin, Kijawornrat et al., 2004). Modified Krebs–Henseleit solution, gassed with 95% oxygen and 5% carbon dioxide, with pH between 7.35 and 7.4, temperature between 35.5 and 36 °C, and at a perfusion pressure of 64 mm Hg was used as perfusate. The Krebs–Henseleit solution contained (in mM) 118 NaCl, 4.7 KCl, 11.1 glucose, 25 NaHCO₃, 1.2 MgSO₄, 2.5 CaCl₂, and 1.2 KH₂PO₄. Bipolar transventricular electrograms (Ag:AgCl), with clear onset of QRS and end of T wave, were recorded from electrodes held gently on the epicardium on the right atrium and left ventricle. Recordings were made on a Biopac MP150 Data Acquisition Unit (Santa Barbara, CA USA). After control (Krebs) measurements were obtained (up to 15 min), hearts were perfused with 0.1% dimethylsulfoxide (DMSO) in Krebs (only if the test compound was formulated using DMSO) and then with escalating doses of test compounds (10⁻⁹–10⁻⁴ M), each dose perfused for 15 min, data of which were collected over the final 30 s of each 15 min epoch.

Table 2

Test articles known to prolong QTc (+=prolongation, 0=no change, -=shortening)

		Tp–Te		
		+	0	–
QTc	+	Arsenic trioxide	Astemizole	
		Bretylum	Haloperidol	
		Chloroquine	Methadone	
		Cisapride	Morphine	
		Disopyramide	Sotalol	
		Erythromycin	Sparfloxacin	
		Pimozide	Thioridazine	
		Quinidine		
		Tetracycline		
			0	
	–			

The RR, QT, and Tp–Te intervals were measured, at the targeted time points, from complexes originating from sinus rhythm. QTc was calculated by the cube root correction method of Fridericia (Fridericia, 1920). The data was analyzed using a mixed model analysis of variance setting where least squared means (*t*-test) was used for comparing males vs. females and for concentration levels for each parameter studied. Measurements were digitized on the Biopac MP150 Data Acquisition Unit (Santa Barbara, CA USA) at 1 kHz, which would allow for precision of measurement of less than 2 ms. The repeatability of the measurements for QT and Tp–Te had intracoefficients of variations of 0.5% and 6.0%, respectively. For accuracy of the measurement, when 10 complexes of known durations (generated by ECG 200 DNI Nevada, Inc. Carson City, NV, USA) were measured by the investigator, the average difference was 3%.

3. Results

QTc lengthened in 16 of 16 test compounds (Table 2) known to be torsadogenic and did not lengthen in 7 of 7 test compounds (Table 3) known not to be torsadogenic—sensitivity and specificity of 1.0. The average increase in QTc for the 16 test compounds known to prolong QTc was 44 ms, chloroquine produced the largest increase (82 ms) and sparfloxacin produced the smallest increase (18.3 ms).

Table 3

Test articles known not to prolong QTc (+=prolongation, 0=no change, -=shortening)

		Tp–Te		
		+	0	–
QTc	0	Enalaprilat	Aspirin	
		Propranolol	Captopril	
			Mexilitine	
			Penicillin	
	–			Verapamil

In 9 out of 16 torsadogenic test compounds, the Tp–Te interval increased parallel with QTc. The remaining seven test compounds that produced prolongation in QTc did not change the length of the Tp–Te interval. In 7 test compounds known not to be torsadogenic, two test compounds did not change QTc with an increase in Tp–Te, four test compounds did not change QTc or Tp–Te, and one compound (verapamil) shortened the QTc and Tp–Te intervals.

4. Discussion

It is clear that QTc prolongation is a more robust predictor of torsadogenicity than Tp–Te in the male guinea pig heart. These results are contrary to our initial belief; however, no doubt resides in the absence of heterogeneity in response to action potentials from endocardial to epicardial fibers (including M fibers), that is, all APD₉₀ must lengthen uniformly in all layers for compounds known to prolong QTc (Fig. 3).

Whether or not there were sufficient numbers of positive and negative test compounds studied, it is clear that uniformity in lengthening of Tp–Te is inadequate in the guinea pig, although M fibers have been established in the guinea pig (Sicouri, Quist, Antzelevitch, 1996). We are currently investigating the relationship between QTc and Tp–Te in other species—in particular rabbit. It is known that QTc lengthening or not lengthening in over 50 test articles produced sensitivities and specificities of 1. What is not known is if the Tp–Te interval lengthening

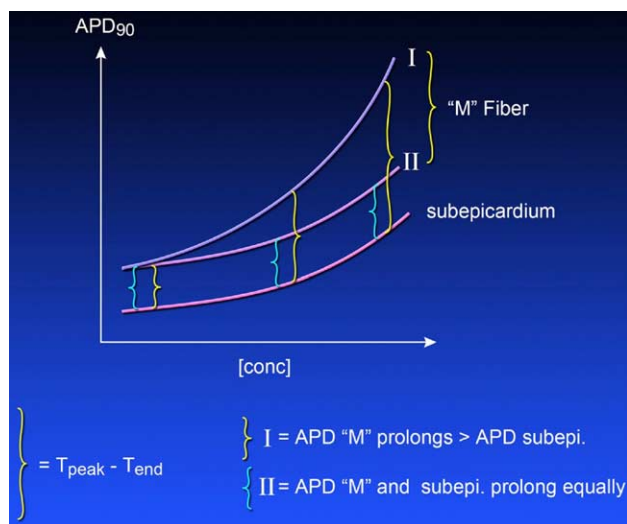


Fig. 3. Action potential durations of the sub-epicardium and mid myocardium (M fibers) of the ventricular myocardium with increasing concentrations of test compound. Example I shows a greater lengthening of the APD₉₀ for M fibers than for sub-epicardial fibers over the concentration range resulting in a lengthening of the Tp–Te interval. Example II shows equal lengthening of the APD₉₀ for M fibers and sub-epicardial fibers over the range of test compound concentrations resulting in no change in the Tp–Te interval. APD=action potential duration and conc.=concentration.

increases sensitivity and specificity, it appears not. Or does it detect a signal at a lower concentration of test compound that approximates more closely than QTc the in vivo effects? Another monumental issue is over the method of correcting QTc; we believe that the method (Fridericia) employed in this study is helpful but not perfect. That is, QTc is not independent of the RR interval when the RR interval is changed by test compounds thought not to alter repolarization (e.g., zatebradine and cilobradine). Would the percent change in QTc vs. the percent change in Tp–Te or other expressions of lengthening of these parameters produce different results? We have not yet explored this but intend to.

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