

ECG ASSESSMENT OF DRUG-INDUCED PROARRHYTHMIA IN ADULT ZEBRAFISH

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Abstract

In this study, we examined effects of drug treatment on cardiac proarrhythmia using a conventional ECG technique adapted for adult zebrafish. Proarrhythmia is defined as drug induced disturbance of cardiac rhythm. Clinical manifestations include: bradycardia, tachycardia, AV block and QT prolongation. To assess proarrhythmia predictability in the zebrafish model, we tested 7 drugs known to cause QT prolongation in humans: terfenadine, astemizole, thioridazine, haloperidol, quinine, procainamide and sotalol, and 3 negative QT drugs, verapamil, penicillin and propranolol. ECG was obtained from anesthetized zebrafish perfused orally with tricine, a well known fish anesthetic. ECG control baseline was established for 10 min before drug treatment and served as an internal control for each compound followed by 20 min drug treatment. Averaged ECG were obtained at 15 sec or 1 min intervals to generate a time course of drug effect. RR, PR, QRS and QT intervals were measured from averaged ECG and QT was corrected using the Fredericia method. Our results showed that zebrafish ECG was highly reproducible. Baseline ECG parameters were similar to previously published results. Astemizole, thioridazine, haloperidol, and quinine, which caused QT prolongation in humans also resulted in QT prolongation in zebrafish. We have shown that ECG can be reliably obtained from adult zebrafish and that this model system is predictive of human cardiac proarrhythmia.

Results



Figure 2. Comparison of human and zebrafish ECG. ECG pattern in human (A) and adult zebrafish (B). The P wave, the first small peak, represents atrium excitation, followed by the QRS complex, which represents ventricle excitation. The T wave, which follows the large R wave, represents ventricle repolarization. The PR interval represents activation of atrial action potential and conduction to the ventricle; the RR interval measures one cardiac cycle which can be converted to heart rate and QT interval, a key component in LQTS.

	n	RR	PR	QRS	QT	BPM
CTX (+)	5	348 +/- 43	52 +/- 19	26 +/- 3	225 +/- 11	
CTX (+)	58-110	328 +/- 77	65 +/- 14	34 +/- 11	242 +/- 34	151 +/- 38
Tricine	151-175	432 +/- 95	54 +/- 14	22 +/- 6	208 +/- 45	147 +/- 41

Table 1. Baseline electrocardiogram signals in Tricine and CTX treated zebrafish: Electrocardiogram signals from adult zebrafish with (+) and without (-) m-conotoxin GIIIB (CTX), and with tricine alone (Ethy 3-aminobenzoate methanesulfonate) were compared. Results from tricine and conotoxin-treated zebrafish were similar. Values are means +/- standard deviation in ms except in BPM which is beats/min, n = number of adult male zebrafish. ** (n), not reported.

*Modified from Milan et al (2006).

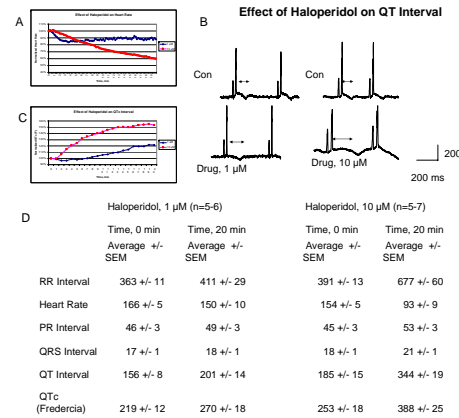


Figure 3. Effect of Haloperidol on ECG. A. Haloperidol, 1 µM, decreased maximally averaged heart rate approximately 5 min following drug application, but there was a slow increase in heart rate during the remaining drug treatment time. Statistical analysis indicated that decrease in heart rate was significant 4 to 7 min following drug application; Heart rate decreased ~15% which was statistically significant. Haloperidol, 10 µM, also decreased heart rate but with different kinetics. Statistical analysis indicated that decrease in heart rate was significant 10 min following drug application. There was ~41% decrease in heart rate after 20 min of drug treatment. Heart rate was averaged at 15 sec intervals. B. Representative ECG showing increase in QT interval (line with arrows) 20 min after exposure to Haloperidol. C. QT interval was averaged at 1 min intervals. QT interval was corrected using Fredericia method. Corrected QT was normalized and plotted against time. After treatment with 1 µM haloperidol, QTc increased by 23% (P<0.0159) or an average of 51 ms and with 100 µM QTc increased by 57% (P<0.0001) or an average of 135 ms after 20 min of drug treatment. D. Effect of haloperidol on ECG parameters measured before and after 20 min of drug treatment. After 1 µM drug treatment, no significant change in PR and QRS interval was observed. After treatment with 100 µM, PR interval increased by 18% and QRS by 17%. All values were average +/- standard error mean.

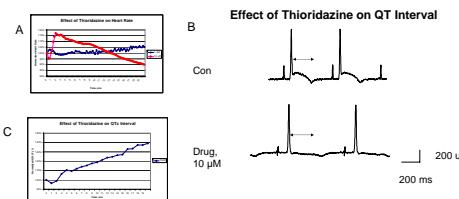


Figure 4. Effect of Thioridazine on ECG. A. Thioridazine, 10 µM, initially increased heart rate for 1 min then heart rate decreased. Statistical analysis indicated that decrease in heart rate was significant after 20 min of drug treatment (P<0.0001). B. Representative ECG showing increase in QT interval (line with arrows) 20 min after exposure to thioridazine. C. Corrected QT was normalized and plotted against time. After treatment with 10 µM thioridazine, QTc increased by 39% (P<0.0001) or an average of 88 ms after 20 min of drug treatment. D. Effect of thioridazine on ECG parameters measured before and after 20 min of drug treatment.

Introduction

Cardiotoxicity is a major problem with hundreds pharmaceutical agents, industrial chemicals and naturally occurring products. Due to its genetic and physiological similarity to humans (Chen and Fishman, 1996; Granaio and Nusslein-Volhard, 1996), zebrafish has been shown to be a reliable and efficient model for drug screening (Semino et al., 2001; Pang et al., 2001). Use of zebrafish as an animal model for drug screening can accelerate the screening process, decrease development costs, and provide more accurate results compared to cell-based screening assays.

Zebrafish heart: The zebrafish (*Danio rerio*) is a major new animal model system for studying cardiovascular development, genetics, and cardiotoxicity. Similar to other fish and larval amphibians, zebrafish use gills for respiration and have a single-loop for a circulatory system. The heart consists of two chambers: an atrium that receives blood and a ventricle that pumps blood to the body.

Evolutionary conservation: Although the overall structures in zebrafish and mammalian hearts are different, both develop specialized chambers, outflow tracts to an intricate vasculature, valves to ensure directionality, specialized endothelial cells (endocardium) musculation to drive a high-pressure system, and an electrical system to regulate rhythm. In addition, both exhibit similar characteristics such as: there is inflow of blood from a major vein to an atrium, the blood moves to a muscular ventricle for delivery to the aorta, valves are present to direct blood flow, and the heartbeats is associated with pacemaker activity (Thisse & Zon, 2002). The underlying development, patterning, genes, functions, as well as disease characteristics are similar to humans, making zebrafish a valuable animal model for studying cardiotoxicity.

Methods

Adult AB strain zebrafish (2-4 cm body length; mass: 0.4-0.6 g) were used in all experiments. Zebrafish were anesthetized with 0.64 mM tricaine for 1 min. Sedated zebrafish were placed in a recording chamber and a 1 mm perfusion needle was inserted into the oral cavity. Zebrafish were then orally perfused with 10 mM HEPES (pH 7.5) in E3 solution (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl₂, 0.33 mM MgSO₄, 0.64 mM tricaine) at a rate of 15 ml/min. Following placement of the perfusion needle, a 29 gauge needle electrode (AD Instruments, Colorado Springs, CO) was inserted into the ventral midline directly between the pectoral fins at a depth of 1 mm. A second electrode was introduced into the ventral midline 2/3 of the total body length from the head and positioned at a depth of 1 mm. ECGs were recorded for 10 min for control baseline recording, and 20 min for recording drug response. Drugs were diluted from DMSO stocks to obtain final concentrations in E3 saline solution. ECG signal was amplified (Octal Bio Amp, AD Instruments, Colorado Springs, CO), filtered at 0.3-500 Hz, digitized (PowerLab 16/30, AD Instruments), and then recorded continuously at an acquisition rate of 2000 Hz with digital filtering at 1 Hz (ChartPro, AD Instruments). Offline processing was performed with ChartPro (AD Instruments) and IgorPro (WaveMetrics). Each drug was perfused until stable RR and QT intervals were attained (~20 min).

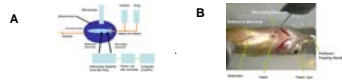


Figure 1: ECG recording setup. A. An overview of ECG recording setup. A microscope was used to visualize zebrafish. Control and drug solutions were gravity fed to zebrafish at 15 ml/min. Solutions were switched with a solenoid switch valve system. Solution waste was removed by a vacuum pump. Recording electrodes were connected to a differential amplifier (Octal Bio Amp) and the analog signals were digitized (PowerLab) and stored in a computer. ECGs were acquired and analyzed with ChartPro software. B. The ventral surface of an adult zebrafish under a microscope. The perfusion needle was placed in the zebrafish mouth and the recording electrodes were placed in the pericardial area and abdomen.

D	Quinidine, 500 µM (n=4-7)		Time, 20 min
	Time, 0 min	Average +/- SEM	
RR Interval	474 +/- 26		501 +/- 19
Heart Rate	128 +/- 7		121 +/- 4
PR Interval	45 +/- 3		52 +/- 5
QRS Interval	23 +/- 1		28 +/- 3
QT Interval	176 +/- 12		265 +/- 22
QTc (Fredericia)	224 +/- 11		330 +/- 23

Figure 5. Effect of Quinidine on ECG. Pronounced ECG changes were observed after treatment with 500 µM DMSO concentration was increased to 0.2% to improve drug solubility. A. After treatment with 500 µM quinidine, there was ~15% increase in heart rate which peaked around 5 min of drug treatment, which was statistically significant (P<0.0001). This increase was followed by a slow decrease in heart rate. Decrease in heart rate was greater at low drug concentrations, however, there was an overall decrease for all drug concentrations tested. B. Representative ECG showing increase in QT interval (line with arrows) 20 min after exposure to 500 µM quinidine. C. Corrected QT was normalized and plotted against time. After treatment with 500 µM astemizole, QTc increased by 47% (p=0.0009) or an average of 106 ms after 20 min of drug treatment. D. Effect of quinidine on ECG parameters measured before and after 20 min of drug treatment. For 1, 10, 100 and 250 µM drug concentrations, there were not any significant changes in ECG parameters including QT interval. QT interval was not analyzed for 250 µM drug concentration.

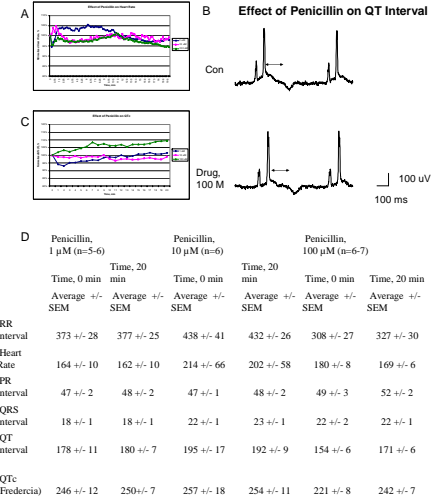


Figure 5. Effect of Penicillin on ECG. A. No significant change in heart rate was observed after treatment with penicillin. B. Representative ECG shows increase in QT intervals (line with arrows) 20 min after exposure to penicillin, 100 µM. C. Corrected QT was normalized and plotted against time. After treatment with 100 µM penicillin, QTc increased by 9%, an average of 21 ms after 20 min of drug treatment. This increase was not statistically significant (p=0.9815). D. Effect of penicillin on ECG parameters measured before and after 20 min of drug treatment.

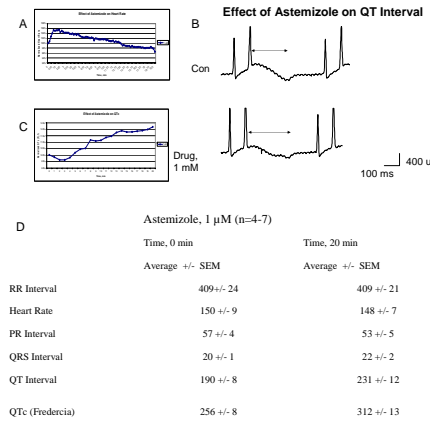


Figure 5. Effect of Astemizole on ECG. A. Astemizole, 1 µM, has no effect on heart rate. B. Representative ECG showing increase in QT intervals (line with arrows) 20 min after exposure to astemizole. C. Corrected QT was normalized and plotted against time. After treatment with 1 µM astemizole, QTc increased by 22% (P<0.0001) or an average of 41 ms after 20 min of drug treatment. D. Effect of astemizole on ECG parameters measured before and after 20 min of drug treatment.

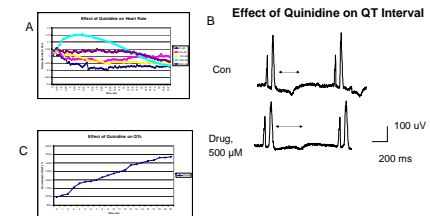


Figure 5. Effect of Quinidine on QT Interval. A. Quinidine, 500 µM, increased heart rate for 5 min after treatment, which was statistically significant (P<0.0001). B. Representative ECG showing increase in QT interval (line with arrows) 20 min after exposure to quinidine. C. Corrected QT was normalized and plotted against time. After treatment with 500 µM quinidine, QTc increased by 47% (p=0.0009) or an average of 106 ms after 20 min of drug treatment.

	Human ¹	Zebrafish ²	Monkey ³	Dog ⁴
Terfenadine	↑	↑	↑	↑
Astemizole	↑	↑	-	↑
Thioridazine	-	-		
Penicillin				

Table 2. Comparison of drug effect on QTc interval.

1. Yap and Camm (2006); 2. Milan et al (2006); 3. Ando et al (2005); 4. Toyoshima (2005)

Summary

*Zebrafish exhibits comparable responses to compounds as responses in mammals
 *Zebrafish assays are rapid, reproducible and quantitative
 *Statistically significant numbers of animals can be used per drug concentration