

Assessment of Drug-Induced Cardiotoxicity in Zebrafish

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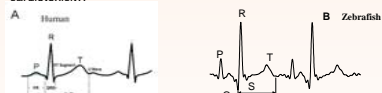
Abstract

The goal of this study was to validate use of zebrafish for assessing drug-induced cardiotoxicity. We treated 2-day zebrafish with varying concentrations (0.01 μ M-1000 μ M) of 24 validation drugs, including: doxorubicin, 5-fluorouracil, cyclophosphamide, mitoxantrone, terfenadine, clomipramine, thioridazine, gentamicin, tetracycline, amantadine, disopyramide, lidocaine, metoprolol, mexiletine, phenytoin, procainamide, propafenone, clozapine, erythromycin, quinidine, astemizole, amiodarone, verapamil, haloperidol, and clomipramine. Heart rate, rhythmicity, circulation, and morphology were easily assessed in the transparent animals. Drugs that elicited cardiomyopathy, arrhythmia, negative inotropic effects, or QT prolongation in humans including: doxorubicin, 5-fluorouracil, terfenadine, and lidocaine also caused bradycardia, AV block, and slow circulation in zebrafish. Severe impairment of cardiac function after treatment with terfenadine and clomipramine resulted in the formation of pericardial edema, hemorrhage, bradycardia, as well as death at higher concentrations. QT-prolonging drugs such as terfenadine, quinidine, and thioridazine induced acute atrioventricular block (AV block); A:V ratios ranging from 2/1 to 8/1 were observed. Gentamicin, amantadine and tetracycline, which rarely induce cardiotoxicity in normal clinical use, did not cause significant cardiotoxicity in zebrafish. Results for drug effects on zebrafish heart rate show 100% concordance with results in mammals, which according to ECVAM standards, is excellent. This data supports use of zebrafish as a predictive animal model for assessing drug-induced cardiotoxicity.

Introduction

Cardiotoxicity: Cardiotoxicity is a major problem with hundreds of pharmaceutical agents, industrial chemicals and naturally occurring products. In the pharmaceutical sector, several compounds have been shown to lengthen cardiac repolarization, leading to arrhythmia and its clinical manifestation, Torsades de pointes. Previous research has shown that compounds capable of inducing repolarization abnormalities cause bradycardia in zebrafish. In current research, we look at the effect of 24 drugs on cardiotoxicity in zebrafish.

The Zebrafish Heart: The zebrafish (*Danio rerio*) is a useful animal model system for studying cardiovascular development, genetics, and cardiotoxicity. Zebrafish use gills for respiration and have a single-loop circulatory system. The heart consists of two chambers: an atrium that receives blood and a ventricle that pumps blood to the body. Both the mammalian and zebrafish heart share the development of specialized chambers, outflow tracts to an intricate vasculature, valves to ensure directionality, specialized endothelial cells (endocardium) to drive a high-pressure system, and an electrical system to regulate rhythm. 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 heartbeat is associated with pacemaker activity. The underlying development, patterning, genes, functions, as well as disease characteristics are similar to humans, making zebrafish a valuable animal model for studying cardiotoxicity.



Comparison of human and zebrafish ECG. ECG pattern in human (A) and adult zebrafish (B). The zebrafish and human heart share strikingly similar patterns of electrical activity, including distinct P, QRS, and T waves.

Methods

Heart Rate Assessment: 2 days post fertilization (dpf) zebrafish were incubated with drugs for 4 hours at 28°C. After incubation, zebrafish (N=10) were visualized on a Zeiss Stemi-1000 dissecting scope, and the number of ventricular contractions in a 30 second period was counted manually. The number of contractions was multiplied by 2 to calculate the heart rate, reported in beats per minute (BPM).

Atrial-Ventricular Ratio: Following ventricular measurement, atrial contractions were counted using the same methods described above.

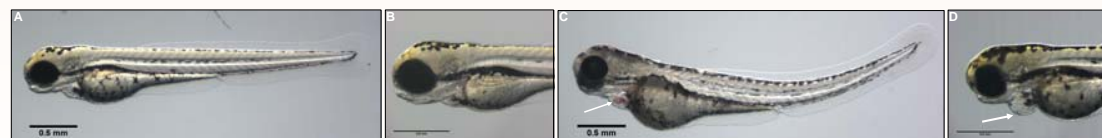
Cardiac Morphology and Circulation: 2dpf zebrafish were incubated with a single concentration of drug for 24h at 28°C. Following treatment, zebrafish (N=10) were observed for circulation defects, gross morphological defects, edema, and thrombosis.

Results

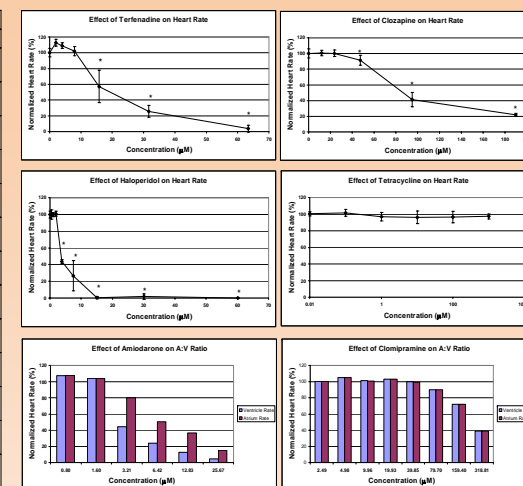
Drug	Cardiotoxic manifestations in humans	Cardiotoxicity in Zebrafish			
		Heart rate	Rhythmicity	Morphology	Circulation
Doxorubicin Chemotherapeutic	Cardiomyopathy, decreased heart rate, contractility and heart failure	Decreased	Normal	Hemorrhage	Slower
5-Fluorouracil Chemotherapeutic	Proarrhythmic	Decreased	Normal	No defects observed	Slower
Cyclophosphamide Chemotherapeutic	Cardiac myocyte death, decreased heart rate, contractility and heart failure	Decreased	Normal	No defects observed	Slower
Mitoxantrone Chemotherapeutic	Acute heart failure, decreased left ventricular ejection fraction (LVEF), decreased heart rate.	Decreased	AV block	Pericardial edema; Hemorrhage	Slower
Terfenadine Antihistamine	QT prolongation, proarrhythmic	Decreased	AV block	Pericardial edema; Hemorrhage	Slower
Clomipramine Anticonvulsant	QT prolongation	Decreased	AV block	Pericardial edema; Hemorrhage	Slower
Thioridazine Antidepressant	Negative inotropic effect, QT prolongation, PR interval prolongation	Decreased	AV block	Hemorrhage	Slower
Gentamicin Aminoglycoside	Rare clinical finding: Negative inotropic effect	Normal	Normal	No defects observed	Normal
Tetracycline Antibiotic	Rare clinical finding: Negative inotropic effect	Normal	Normal	No defects observed	Normal
Amantadine Antiviral	Rare clinical finding: cardiomyopathy	Normal	Normal	No defects observed	Normal
Disopyramide Antiarrhythmic	Decreased myocardial contractility	Decreased	Normal	No defects observed	Slower
Lidocaine Anesthetic	Arrhythmias; cardiac arrest	Decreased	AV block; Arrhythmia	Pericardial edema	Slower
Metoprolol Beta-blocker	Bradycardia	Decreased	Arrhythmia	Pericardial edema	Slower
Mexiletine Antiarrhythmic	Bradycardia	Decreased	Arrhythmia	Pericardial edema	Slower
Phenytoin Anticonvulsant	Bradycardia	Decreased	Normal	Pericardial edema	Slower
Procainamide Antiarrhythmic	Arrhythmias	Decreased	Arrhythmia	Pericardial edema	Slower
Propafenone Antiarrhythmic	Arrhythmias	Decreased	Arrhythmia	Pericardial edema	Slower
Clozapine Antipsychotic	Myocarditis; arrhythmias	Decreased	AV block	Pericardial edema	Slower
Erythromycin Antibiotic	Arrhythmia; torsades de pointes	Decreased	AV block	Pericardial edema; swollen heart chambers	Slower
Quinidine Antiarrhythmic	Arrhythmia; bradycardia; abnormal prolongation of the QT interval	Decreased	AV block	Pericardial edema; swollen heart chambers	Absent
Astemizole Antihistamine	Arrhythmia; Torsades de pointes	Decreased	AV block	Pericardial edema	Absent
Amiodarone Antiarrhythmic	Arrhythmia; bradycardia; SA node dysfunction	Decreased	AV block	Pericardial edema	Absent
Verapamil Antiarrhythmic	AV block; bradycardia; pulmonary edema	Decreased	AV block	No defects observed	Normal
Haloperidol Antipsychotic	QT prolongation; ventricular arrhythmias; tachycardia	Decreased	AV block	Pericardial edema; swollen heart chambers	Slower

Summary of drug-induced cardiotoxicity in zebrafish. 24 compounds were tested for cardiotoxicity in 2dpf zebrafish embryos. The most common form(s) of cardiotoxicity observed in humans are listed for each drug, and the corresponding effects observed in zebrafish are noted. Gentamicin, tetracycline, and amantadine were predicted to show no signs of cardiotoxicity in zebrafish based on findings in humans. Several compounds known to have adverse cardiotoxic effects in humans caused a decrease in heart rate in zebrafish. Atrioventricular block was observed at higher concentrations of a number of compounds. Defects in cardiovascular morphology were primarily noted by an increase in pericardial edema, though instances of hemorrhage, as well as swelling of the atrium and ventricle were also noted. Some compounds caused a corresponding decrease in circulation, particularly at higher concentrations.

Circulation and Morphology Assessment



Examples of Malformations Observed in Cardiotoxicity Assessment. A & B) Images of 3dpf zebrafish treated for 24h with 1% DMSO (2.5x and 8x, respectively). No abnormalities are observed. **C)** 2.5x image of a drug-treated 3dpf zebrafish. The white arrow points to the heart, where blood can clearly be seen in the chambers. Ventricular contractions have stopped, allowing blood to accumulate in both chambers, and pool just posterior to the atrium (surrounding the yolk). Secondary toxicity has caused necrosis of the musculature along the tail, and caused curvature of the body. **D)** A milder drug-induced toxicity example at 8.0x magnification. The white arrow points to the pericardium, which is clearly enlarged. Otherwise the zebrafish exhibits no evidence of cardiotoxicity.



Examples of dose-response and A:V ratio analysis. Results are normalized to the recorded heart rates of zebrafish treated with 1% DMSO (carrier control). A decrease in ventricular rate is observed when zebrafish are treated with terfenadine, clozapine, and haloperidol, while tetracycline has no significant effect on heart rate (note log scale). Data are reported as mean \pm SD, and P<0.05 was used as the significance level. One-way ANOVA with a post hoc Dunnett's test was used to identify concentrations that significantly altered heart rate, indicated with an ***. The bar graphs demonstrate a dose dependent increase in A:V block. Amiodarone treatment resulted in a \sim 2:1 A:V ratio at 3.21 and 6.42 μ M, and increased at higher concentrations. Conversely, Clomipramine, despite causing a dose-dependent decrease in heart rate, does not generate an AV block.

Drug Name	Known cardiotoxicity in humans?	Cardiotoxicity observed in zebrafish?	Correlation of cardiotoxicity between humans and zebrafish?
Doxorubicin	Yes	Yes	Yes
5-fluorouracil	Yes	Yes	Yes
Cyclophosphamide	Yes	Yes	Yes
Mitoxantrone	Yes	Yes	Yes
Terfenadine	Yes	Yes	Yes
Clomipramine	Yes	Yes	Yes
Thioridazine	Yes	No	Yes
Gentamicin	No	No	Yes
Tetracycline	No	No	Yes
Amantadine	No	No	Yes
Disopyramide	Yes	Yes	Yes
Lidocaine	Yes	Yes	Yes
Metoprolol	Yes	Yes	Yes
Mexiletine	Yes	Yes	Yes
Phenytoin	Yes	Yes	Yes
Procainamide	Yes	Yes	Yes
Propafenone	Yes	Yes	Yes
Clozapine	Yes	Yes	Yes
Erythromycin	Yes	Yes	Yes
Quinidine	Yes	Yes	Yes
Astemizole	Yes	Yes	Yes
Amiodarone	Yes	Yes	Yes
Verapamil	Yes	Yes	Yes
Haloperidol	Yes	Yes	Yes

Correlation of human and zebrafish cardiotoxicity. The correspondence between human and zebrafish cardiotoxicity demonstrates the utility of the zebrafish model as a tool for assessing drug-induced cardiotoxicity.

Conclusions

Drugs known to cause cardiotoxic adverse side effects in humans show similar effects in zebrafish.

Zebrafish assays are rapid, quantitative, and reproducible. Only small amounts of drug are needed.

Zebrafish *in vivo* cardiotoxicity assays provide useful information that supplements conventional hERG assays.