ZEBRAFISH: A PREDICTIVE MODEL FOR ASSESSING DRUG-INDUCED CARDIOTOXICITY

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Abstract

The goals of this study were to assess the effects of short term exposure of zebrafish to 10 cardiotoxic drugs. Heart rate, rhythmicity, circulation and morphology were assessed. For heart rate and rhythmicity, 48 hour post fertilization (hpf) zebrafish were exposed to varying concentrations of compounds for 4 hours. For morphology assessment, zebrafish were exposed continuously to compounds for 24 hours. Compounds were identified that had significant effects on each of the cardiac-specific endpoints measured. Results from our study were in good agreement with results observed by published data in zebrafish, other vertebrate models, and humans. Together the results reported here demonstrate that: 1) zebrafish exhibit a clear dose-response to known cardiotoxic compounds, and 2) effects of known cardiotoxic drugs in zebrafish are similar to effects observed in primates and other pre-clinical animal models.

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 10 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.

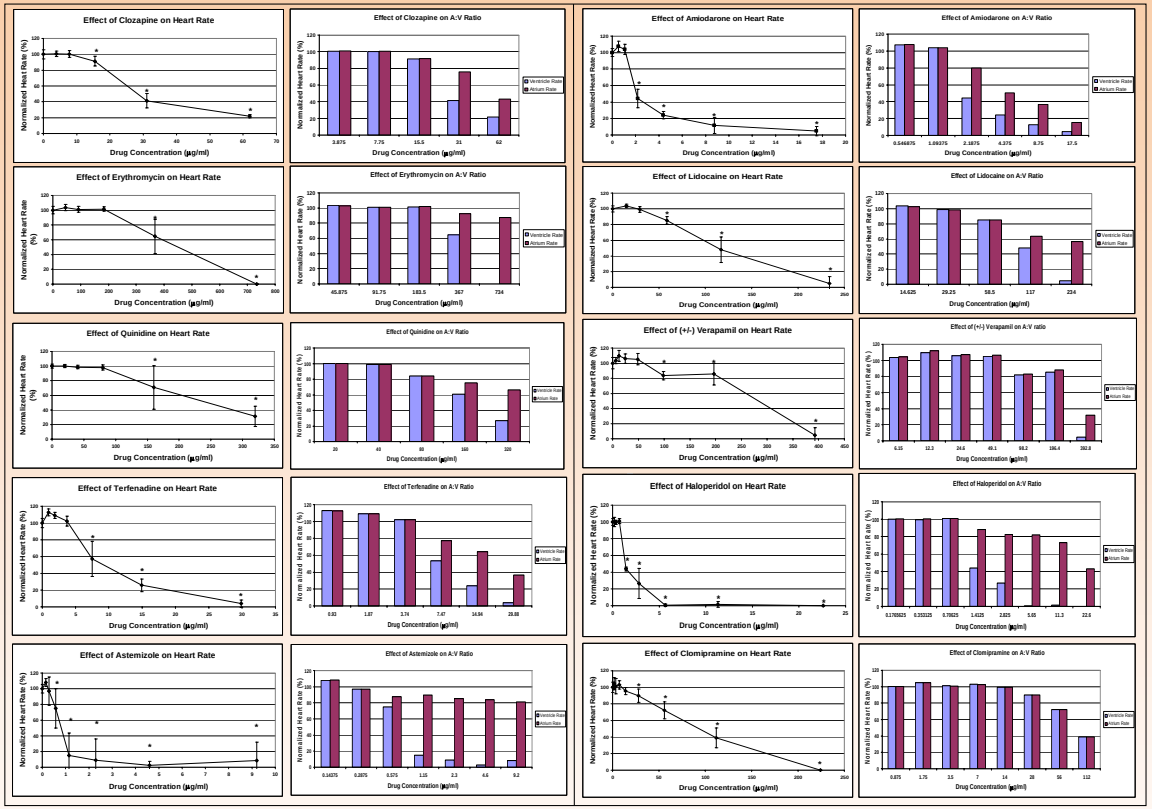
Methods

Heart Rate Assessment: 48hpf 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.

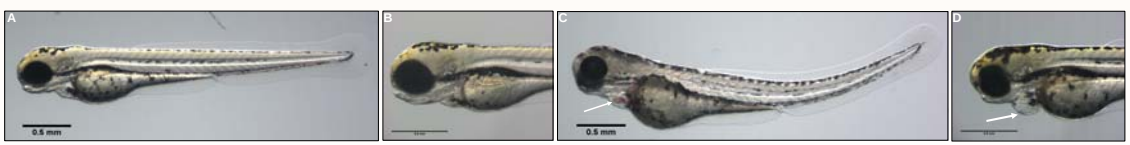
Cardiac Morphology and Circulation: 2dpf zebrafish were incubated with a single concentration of drug for 24h at 28°C. Following treatment, zebrafish (N=10) were observed for circulation defects, gross morphological defects, edema, and thrombosis. The % incidence for each class of defect was calculated.

Results



Effect of selected drugs on zebrafish heart rate and Atrial-Ventricular ratio. Zebrafish (2dpf) were incubated at 28°C with selected drugs at various concentrations for 4h. To facilitate comparison, the results are normalized to the recorded heart rates of zebrafish treated with 1% DMSO (carrier control). The bar graph for each drug shows associated drug induced changes in atrial contraction rates. The ventricle is more sensitive to the presence of cardiotoxic compounds and usually showed a more significant decrease in activity than the atrium. Normal A:V contraction ratios of 1:1 would increase to 10:1 or higher with increasing drug concentration. A notable exception was Clomipramine, which maintained a 1:1 A:V ratio with increasing drug concentration despite a decrease in heart rate. ANOVA with a *post-hoc* Dunnett's test (based on raw data) was used to identify concentrations that significantly altered heart rate, indicated on each graph with an *****. P<0.05 was considered significant, and error bars are equal to ± 1 SD.

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 3pdf 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 a curvature of the body. **D)** A milder drug-induced toxicity example at 8.0x magnifcation. The white arrow points to the pericardium, which is clearly enlarged. Otherwise the fish exhibits no evidence of cardiotoxicity.

Summary of Morphology and Circulation Effects Observed in Zebrafish - % Incidence

Drug Name	Concentration (µg/ml)	Gross Morphology	Circulation	Pericardial Edema	Thrombosis
DMSO (control)	1%	0%	0%	0%	0%
Clozapine	31	10% atrium and ventricle swollen	90% slow	40%	0%
Erythromycin	734	100% atrium and ventricle swollen	60% slow 30% absent	100%	20% yolk 10% pericardium
Quinidine	200	80% atrium and ventricle swollen	100% absent	30%	100% yolk 40% ventricle
Terfenadine	20	50% atrial swelling caused compression of ventricle	100% absent	60%	80% yolk 10% tail
Astemizole	4.6	40% atrium and ventricle swollen	80% absent 20% slow	100%	100% yolk
Amiodarone	8.75	20% atrium and ventricle swollen	100% absent	50%	0%
Lidocaine	117	20% atrium and ventricle swollen	20% slow	10%	0%
Verapamil	98.2	0%	0%	30%	0%
Haloperidol	5.65	80% atrium and ventricle swollen with asynchrony btw. chambers	80% slow 20% absent	60%	50% yolk
Clomipramine	56	70% swollen ventricle	80% absent 20% slow	70%	10% yolk

Summary of Observed Zebrafish Cardiotoxicity. The most common gross morphological defect induced by all drugs was an associated swelling of the heart chambers. If the ventricle stopped beating, blood often pooled and clotted in the chamber. Circulation was often reduced or absent entirely. Note that this does not immediately kill the fish, as there is sufficient oxygenation acquired through passive diffusion. Rates of pericardial edema are noted, as well as the presence of thrombi and their location. Most thrombi occur at the yolk just posterior to the entrance to the atrium.

Summary of Cardiotoxicity in Humans and Zebrafish

Drug Name	Known cardiotoxic adverse side effects in humans?	Cardiotoxicity observed in zebrafish?	Abnormal A:V ratio in zebrafish?	Correlation of cardiotoxicity between humans and zebrafish?
Clozapine	Yes	Yes	Yes	Yes
Erythromycin	Yes	Yes	Yes	Yes
Quinidine	Yes	Yes	Yes	Yes
Terfenadine	Yes	Yes	Yes	Yes
Astemizole	Yes	Yes	Yes	Yes
Amiodarone	Yes	Yes	Yes	Yes
Lidocaine	Yes	Yes	Yes	Yes
Verapamil	Yes	Yes	Yes	Yes
Haloperidol	Yes	Yes	Yes	Yes
Clomipramine	Yes	Yes	No	Yes

Summary of similarities in zebrafish and human cardiotoxicity. Drugs known to have adverse cardiotoxic side effects in humans also demonstrate cardiotoxicity in zebrafish. Note that Clomipramine did not cause an abnormal A:V ratio even at the highest tested concentration.

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.

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