ASSESSING CARDIOTOXICITY IN THE ZEBRAFISH EMBRYO

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INTRODUCTION

Zebrafish models are gaining recognition in their applications within several fields, such as developmental biology and toxicology.

The **advantages of the zebrafish** are mainly their low cost and ease of maintenance and breeding. Moreover, the zebrafish is ideal for research purposes due to its small size, ease of handling and transparency. In fact, integrating the use of fluorescent reporter genes into this model allows the visualization of specific tissues, such as the heart. Despite clear anatomic differences between the zebrafish two-chambered (one atrium and one ventricle) heart and four-chambered mammalian heart, several studies have highlighted similarities in the genes and regulatory networks driving cell fate[1,2,3].

Importantly, the use of zebrafish larvae is in accordance with the **3R principle**.

OBJECTIVE

To evaluate a zebrafish embryo model for screening compounds with potential to cause cardiotoxicity

MATERIAL AND METHODS

Material: zebrafish embryos from BBD-T-010 strain (Figure 1).

Compounds: 13 reference compounds sent by Roche were analysed blinded (Table 1).

Treatment: 48-52 hpf (hours post fertilization) dechorionated embryos placed in 96-well plates, one embryo/well, 20 embryo/condition, 5 concentrations per compound. Embryos treated with Terfenadine at 5µM as positive control. DMSO as vehicle at 0.5%.

Table 1: **Compound information and classification**

COMPOUNDS	ACTION	
Dofetilide	hERG blockers	
E4031		
Cisapride		
Terfenadine		
Quinidine		
Thioridizine		
Verapamil	Ca^{2+}/hERG blocker	Other ion channels
Flecainide	NaV 1.5 blocker	
BAYK8644	Ca^{2+} antagonist	
JNJ303	Iks blocker	
Salmeterol	β_2 agonist	Other
Torcetrapib	CETP inhibitor	
Atropine	muscarinic antagonist	

Assessment: After 3 and 24 hours of exposure.

Imaging: A 15 second video of the beating heart was recorded and analysed using non-commercial Cardio v3.0.0.5 software to obtain the heart rate, the presence of arrhythmia and the absence of heartbeat (death / fibrillation). Embryos were not anesthesized to avoid potential beating alterations.

Bioanalysis: after the second video recording embryos were euthanized, washed, dried and frozen at -80ºC until the bioanalysis by liquid chromatography and mass spectrometry (LC/MS/MS).

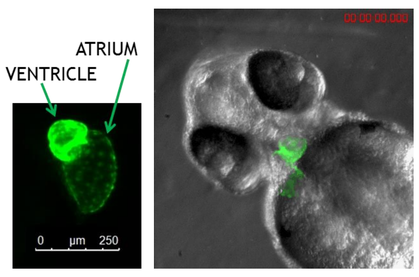

Figure 1: Zebrafish BBD-T-010 strain (GFP expressed in the heart).

RESULTS

Untreated embryos heart rate (HR)

HR increased from 3h to 24h of exposure ($p<0.0001$) as the embryo is developing (Figure 2).

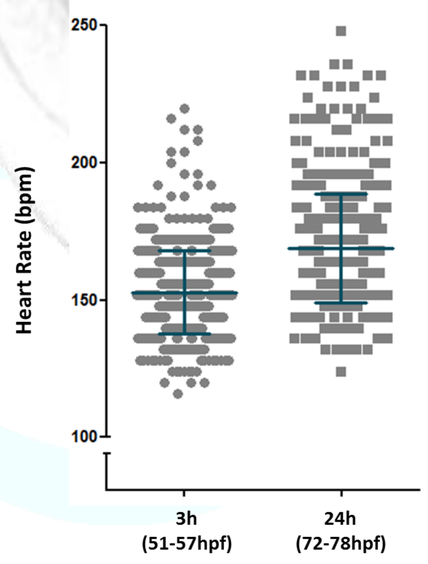

Figure 2: untreated embryos heart rate after 3h and 24h of incubation. n (3h)= 638; n(24h) =491

Cardiotoxicity succesfully detected

100% of hERG blockers were detected after 3 hours of exposure as arrhythmia type 2:1, vertricular failure and death in a dose dependent manner (Table 2).

100% of compounds affecting other ion channels were detected as several cardiotoxicity types (Table 2).

2/3 compounds with other mechanism of action (Atropine and Torcetrapib) were not detected.

Compounds	LOAEL in the well (µM)		LOAEL in the embryo (µM)	Characteristic effect
	3h	24h	24h	
Dofetilide	30		0.31	A2:1 / VF
E4031	30		1.49	A2:1 / VF
JNJ303	20*		2.17*	Bradycardia
Cisapride	3		5.95	A2:1 / VF
Terfenadine	3	1	8.87	A2:1 / VF
Verapamil	30		29.68	Failure/death
Salmeterol	-	100*	34.64*	Failure/death
Flecainide	100		35.71	A2:1 / VF
Quinidine	600	300	70.96	A2:1 / VF
BAYK8644	30		244.13	Bradycardia
Thioridazine	30		832.9	Arrhythmia
Torcetrapib	-		-	-
Atropine	-		-	-

*precipitation

Table 2: Lowest Observable Adverse Effect Level (LOAEL) in the well (corresponding to treatment concentration) and in the embryo (measured by bioanalysis after 24 hours of exposure).

3h *vs.* 24h of exposure

More severe effects may be detected after 24 hours of exposure. However, all hERG blockers, as well as compounds affecting other ion channels were detected within the 3 hours incubation period.

Bioavailability

Measuring embryo exposure to the compounds confirmed that both Torcetrapib and Atropine concentrations were above 10µM (negative compounds).

Bioaccumulation, of varying degrees, was shown by Cisapride, Terfenadine, BAYK8644 and Thioridazine (blue line shift towards the right in Figure 3); others, such as Dofetilide and E4031 showed reduced penetration in the embryo (blue line shift towards the left in Figure 3).

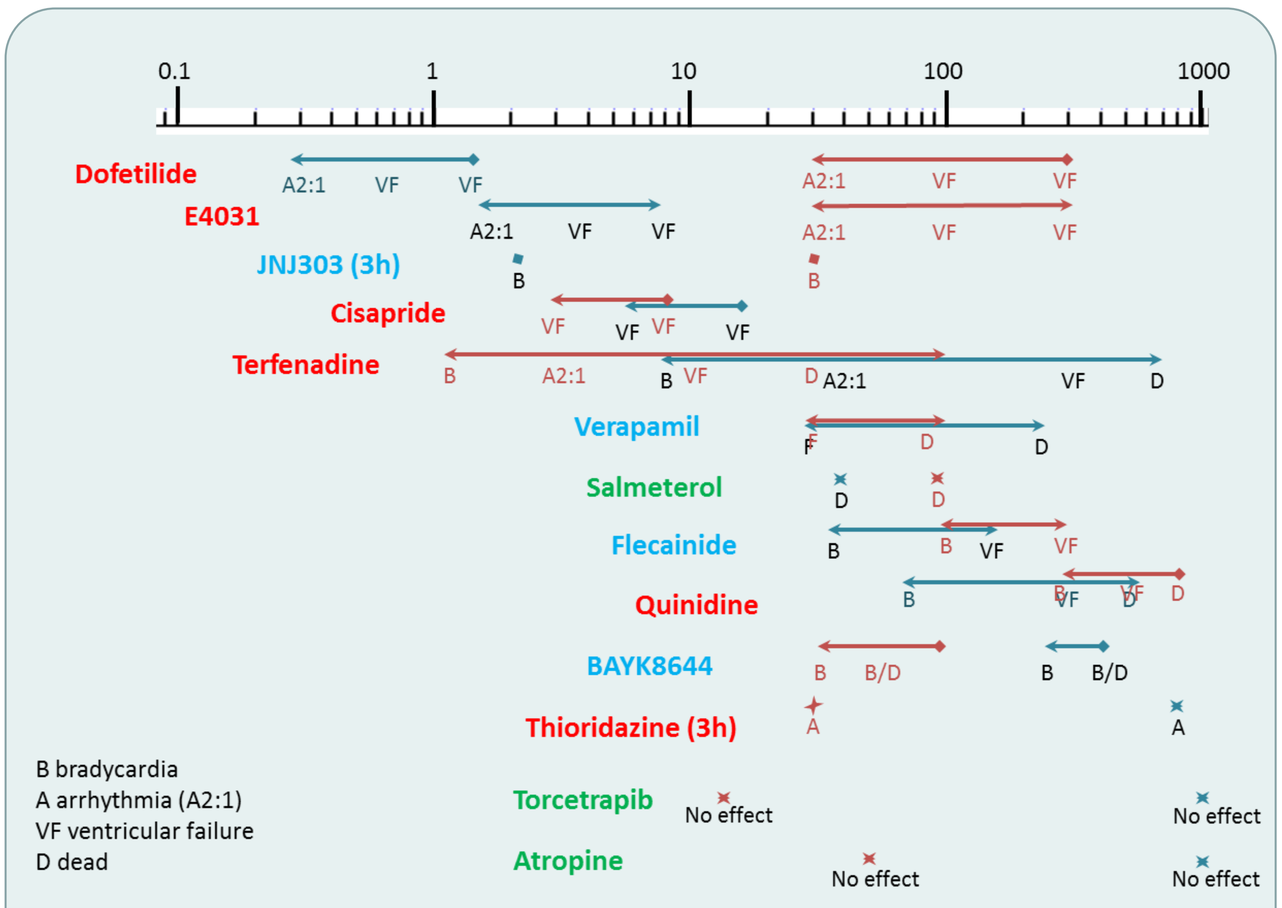

Figure 3: Cardiotoxic effects after 24 hours of exposure related to **EMBRYO** and **WELL** concentrations (µM) for each compound. Bradycardia (B); Arrhythmia (A), type 2:1 arrhythmia A2:1; failure (F), ventricular failure (VF); Dead (D). If drug precipitated the end of the arrow is a diamond instead of an arrowhead. The compounds are sorted by EMBRYO LOAEL values.

CONCLUSIONS

- A wide range of test concentrations should be used for compound screening.
- Compound uptake into the embryo can vary, some compounds accumulate in the embryo whilst others have reduced penetration.
- Although translation into the *in vivo* setting needs more investigation, the assay successfully detects clinically established pro-arrythmic agents.
- Adequate exposure should be determined by bioanalysis to confirm a true inactive compound.
- Zebrafish embryos can be used in drug discovery to provide early screening assays which comply with 3R principles.

References

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