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 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.

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.

RESULTS

Untreated embryos heart rate (HR)

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

Cardiotoxicity successfully detected

100% of NERG blockers were detected after 3 hours of exposure as arrhythmia type 2:1, ventricular 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.

Materials 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 5 µM as positive control. DSMO as vehicle at 0.5%.

Table 1: Compound Information and Classification

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Action</th>
<th>Characteristic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dofetilide</td>
<td>Ca2+/hERG blocker</td>
<td>A2:1 / VF</td>
</tr>
<tr>
<td>E4031</td>
<td>hERG blocker</td>
<td>1.49 A2:1 / VF</td>
</tr>
<tr>
<td>JN3030</td>
<td>Ca2+ blocker</td>
<td>2* 2.17* Bradycardia</td>
</tr>
<tr>
<td>Cisapride</td>
<td>Ca2+ antagonist</td>
<td>5.95 A2:1 / VF</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>Ca2+ antagonist</td>
<td>8.87 A2:1 / VF</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Failure/death</td>
<td>30 29.68 Failure/death</td>
</tr>
<tr>
<td>Salmeterol</td>
<td>100* 34.64* Failure/death</td>
<td></td>
</tr>
<tr>
<td>Flecainide</td>
<td>30 59.71 A2:1 / VF</td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>600 70.96 A2:1 / VF</td>
<td></td>
</tr>
<tr>
<td>BAYK8644</td>
<td>30 244.13 Bradycardia</td>
<td></td>
</tr>
<tr>
<td>Thioridazine</td>
<td>30 832.9 Arrhythmia</td>
<td></td>
</tr>
<tr>
<td>Torcetrapib</td>
<td>No block</td>
<td>- -</td>
</tr>
<tr>
<td>Atropine</td>
<td>No antagonist</td>
<td>- -</td>
</tr>
</tbody>
</table>

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).

Table: LOAEL in the well (µM) LOAEL in the embryo (µM) Characteristic effect

Dofetilide: 30 0.31 A2:1 / VF
E4031: 30 1.49 A2:1 / VF
JN3030: 2* 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 70.96 A2:1 / VF
BAYK8644: 30 244.13 Bradycardia
Thioridazine: 30 832.9 Arrhythmia
Torcetrapib: - - No block
Atropine: - - No antagonist

CONCLUSIONS

• A wide range of test concentrations should be used for compound screening.

• Compound uptake into the in vivo screening increases the number of compounds that can be evaluated, thus allowing the assay to detect low levels of pro-arrhythmic activity.

• 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

1Yurk et al., 2006. High-throughput assay for small molecules that modulate zebrafish embryonic heart rate. Nature Chemical Biology, 1 (5)
3Osu et al., 2014. Human cardiotoxic drugs delivered by soaking and microinjection induce cardiovascular toxicity in zebrafish. Journal of Applied Toxicology 34, 139-148

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