biobide

Your Zebrafish Partner

CARDIOTOX ASSAY

Biobide is a biotechnological company offering drug discovery services to Pharma, Biotech, Chemical, Cosmetic and Nutraceutical companies. Our service is based on the **zebrafish model** and the capacity to offer highly efficient tailor made assays.

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.

The assay to evaluate cardiotoxicity assay is performed under Good Laboratory Practice (GLP) environment.

METHOD DESCRIPTION

Experimental model

Zebrafish (*Danio rerio*) strain expressing Green Fluorescent Protein obtained from crossing adult zebrafish under strict environmental conditions of temperature and photoperiod.

Methodology

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

Assessment: After 3 and 24 hours of exposure the embryos are analyzed.

Imaging: A 15 second video of the beating heart is recorded and analyzed using non-commercial Cardio v3.0.0.5 software (Biobide property) to obtain the heart rate, the presence of arrhythmia and the absence of heartbeat (death / fibrillation). Embryos are not anesthetized to avoid potential beating alterations.

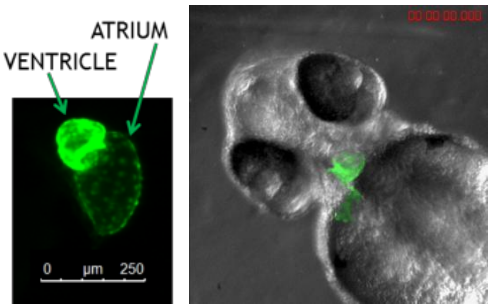

Figure 1.Zebrafish strain expressin Cop-GFP

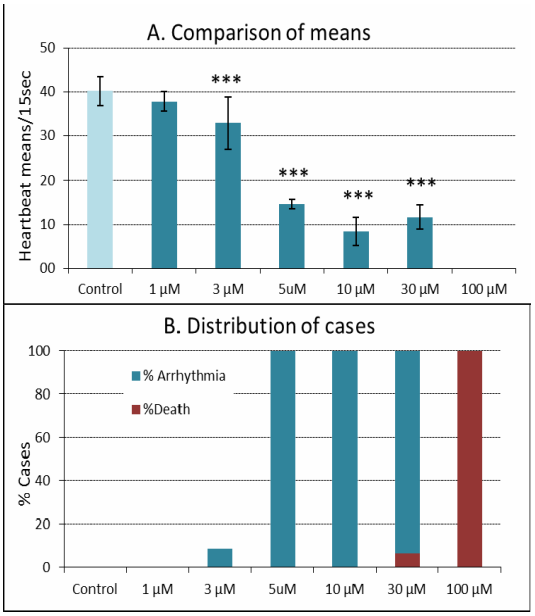

Figure 2. Example of graphs showing the results of embryos treated for 3 hours with terfenadine. Bar graphs representing mean and standard deviation of the number of heartbeats counted in 15 seconds (A); Percentage of embryos displaying arrhythmia and dead embryos (B); p>0.05; ** p>0.01; *** p>0.001.

VALIDATION RESULTS

Figure 1. List of drugs with different human therapeutic indications that were tested and results obtained.

TP: true positive

TN: true negative

FP: false positive

FN: false negative

High sensitivity: 100%
High specificity: 93.3%

Classification of products	Reference Compounds	Cardiac effect in humans	Cardiotox assay	Classification
Antiarrhythmics	*Lidocaine*	Bradycardia	Bradycardia	TP
	Flecainide	QT prolong.	Arrhythmia 2:1	TP
	Propafenone	QT prolong.	Arrhythmia 2:1	TP
	Amiodarone	QT prolong.	Arrhythmia 2:1	TP
Beta-blockers	*Propanolol*	Bradycardia	Bradycardia	TP
	Timolol	Bradycardia	Bradycardia	TP
Antipsychotics	*Thioridazine*	QT prolong.	Arrhythmia 2:1	TP
	Haloperidol	QT prolong.	Arrhythmia 2:1	TP
	Ziprasidone	QT prolong.	Bradycardia	FN
	Pimozide	QT prolong.	Arrhythmia 2:1	TP
	Sertindol	QT prolong.	Arrhythmia 2:1	TP
	Risperidone	QT prolong.	Arrhythmia 2:1	TP
Antidepressants	*Fluoxetine*	Arrhytmia	Arrhythmia	TP
Antitumorals	*Tamoxifen*	Arrythmia	Arrhythmia	TP
	Lapatinib	No effect	No effect	TN
Calcium blockers	*Verapamil*	Bradycardia	Bradycardia	TP
	Diltiazem	Bradycardia	Bradycardia	TP
	Nitrendipine	Bardycardia/ Cardiac arrest	Bardycardia/ Cardiac arrest	TP
	Terodiline	QT prolong.	Arrhythmia 2:1	TP
Colinergic agonist	*Pilocarpine*	No effect	No effect	TN
	Nicotine	Bradycardia	Bradycardia	TP
Antihistaminics	*Astemizol*	QT prolong.	Arrhythmia 2:1	TP
	Terfenadine	QT prolong.	Arrhythmia 2:1	TP
	Fexofenadine	No effect	No effect	TN
Anti-infective	*Halofantrine*	QT prolong.	Arrhythmia 2:1	TP
	Foscarnet	Bradycardia	Bradycardia	TP
Gastrointestinal agents	*Cisapride*	QT prolong.	Arrhythmia 2:1	TP
Hormones	*Estradiol*	Bradycardia	Bradycardia	TP
	Testosterone	Bradycardia	Bradycardia	TP
Iks blockers	*L-768763*	QT prolong.	Bradycardia	FN
Ina Openers	*SDZ-201106*	QT prolong.	Arrhythmia 2:1	TP
Others	*Digitoxin*	Bradycardia	Cardiac arrest	TP
	Ketanserin	Bradycardia	Bradycardia	TP

FURTHER ANALYSIS CAN BE PERFORMED:

- BIOAVAILABILITY BY HPLC MS/MS TO INVESTIGATE FURTHER FALSE NEGATIVE RESULTS
- HISTOPATHOLOGY
- GEN EXPRESION

- Zebrafish cardiotoxicity study is an alternative, rapid and non-invasive assay with good sensitivity (100%) and specificity (93.3%) that is suitable to be used to early evaluate cardiotoxicity.

- Zebrafish embryos can be used in drug discovery to provide early screening assays which comply with 3R principle.

[1] Burns et al., 2005. High-throughput assay for small molecules that modulate zebrafish embryonic heart rate. Nature Chemical Biology, 1 (5)
[2] Letamendia et al., 2012. Development and Validation of an Automated High-Throughput System for Zebrafish In Vivo Screenings. *PlosOne* 7(5): e36690
[3] Zhu et al., 2014. Human cardiotoxic drugs delivered by soaking and microinjection induce cardiovascular toxicity in zebrafish. *Journal of Applied Toxicology* 34: 139–148