BEHAVIOUR ALTERATION´S ASSAY

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

The zebrafish embryo is an emerging model due to its inherent properties (ease to manipulate, external fertilization and transparency) together with the need to apply the 3Rs. This model has a high genetic[1] homology with humans (over 85%) as well as important parallels in organogenesis and functional mechanisms.

Different studies have detected close similarities in neuronal signaling pathways and functional regions of the brain. Alterations in the mobility pattern of larvae induced by known drugs have been observed to strongly support the zebrafish as a predictive model of neuroactivity in humans. Behavioral profiling in zebrafish reveals relationships between drugs and their targets and demonstrates a conserved vertebrate neuropharmacology.

Biobide has set up an assay to evaluate general behavioral alterations, based on responses to dark-light changes.

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

METHOD DESCRIPTION

Experimental model

Zebrafish (Danio rerio) wild type embryos obtained from crossing adult zebrafish under strict environmental conditions of temperature and photoperiod.

Methodology

5 dpf embryos (Figure 1) are placed in 96 well-plate (one embryo per well) and are treated for 2 hours at 28.5ºC.
16 embryos are used at each condition and 5 concentrations per compounds are studied.

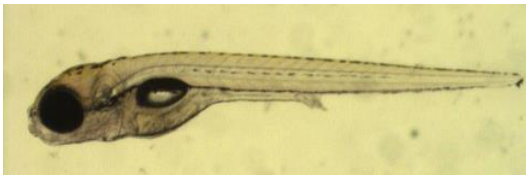

Figure 1. 5 days post fertilization wild type embryo

Zebrafish locomotor activity/photomotor response is tracked over 40 minutes alternating 10 minutes photoperiods using the DanioVision system powered by Ethovision (Noldus).

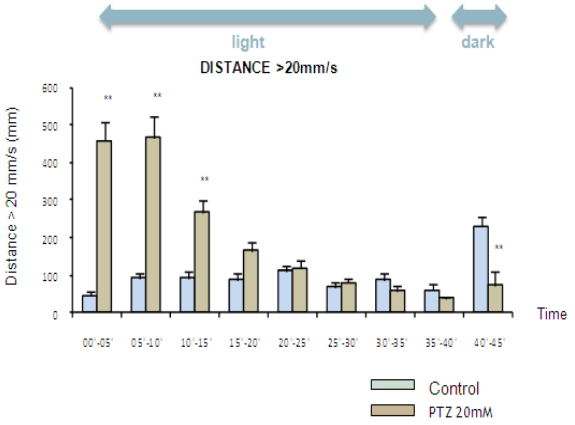

Figure 2. Behaviour of untreated zebrafish embryos exposed to different lighting conditions during the day

After tracking, embryos will be visualized under a stereoscope to assess the following items: embryo viability, severely affected, swim bladder formation, presence of oedema, body shape defects, and loss of equilibrium.

Over 10 parameters are measured based on distance, velocity, movement duration and frequency.

VALIDATION RESULTS

Drugs with different human therapeutic indications have been tested (Figure 3) at 1-100 µM for the validation study:

Figure 3. List of drugs with different human therapeutic indications that were obtained and tested.

COMPOUND	Therapeutic Classification	Adverse effects in human CNS	Results : TN, TP, FN, FP
5-Fluorouracil	Antineoplasic-cytotoxic	-	TN
Acetaminophen	Analgesic-Antipyretic	-	TN
Acetylcysteine	Mucolytic	-	TN
Artemisinin	Antimalaric	+	TP
Ascorbic Acid	Antioxidant	-	TN
Carbamazepine	GABA enhancing anxiolytic	+	FN
Chlorambucil	Alkylating antineoplastic	+	TP
Chloroquine	Antimalaric	+	TP
Dexamethasone	Anti-inflammatory	+	FN
Dieldrin	GABA receptors antagonist	+	TP
Disopyramide	Anti-arrhythmic	+	TP
Dopamine	Neurotransmitter	-	TN
Fluoxetine	SSRI antidepressant	+	TP
Foscarnet	Antiviral	+	TP
Halofantrine	Antimalaric	+	TP
Haloperidol	Antipsychotic	+	TP
Indirubin-3'-oxime	CDKs and GSK3β inhibitors	+	TP
Mefloquine	Antimalaric	+	TP
MPTP	Neurotoxin	+	TP
Norepinephrine	Hormone / Neurotransmitter	-	TN
PTZ	GABA antagonist	+	FN
Sotalol	Anti-arrhythmic	-	TN
Sucrose	Negative control	-	TN
Tacrine	Anticholinesterase	+	TP
Tetracycline	Antibiotic	+	TP
Thalidomide	Immunomodulatory	+	TP
Valproic Acid	Anticonvulsant	+	FN
Warfarin	Anticonvulsant	-	TN

TP: true positive

TN: true negative

FP: false positive

FN: false negative

SENSITIVITY	15/19	*75%*
SPECIFICITY	9/9	*100%*

- Behavioral alteration analysis is an alternative, rapid and non-invasive assay with good sensitivity (75%) and specificity (100%) that is suitable for use in early neuroactive drug screens.
- Zebrafish Behavioral assay fulfills 3R´s principles.

[1] Guo. 2009. Expert Opin Drug Discov. 4(7): 715-726
[2] Kokel & Peterson. 2008. Briefings in functional genomics and proteomics. 7 (6): 483-490
[3] Winter et al. 2008. Journal of Pharmacological and Toxicological Methods. 57: 176-187