Zebrafish Embryo Developmental Toxicity Assay

Commonly used acronym: *ZEDTA*

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Organisation

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Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Regulatory use - Routine production, Translational - Applied Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Zebrafish
Type of cells/tissues/organs	Embryo

DESCRIPTION

Method keywords

development
toxicity
teratogen
screening
regulatory toxicology
malformations
drug development
chemicals
automated analysis
zebrafish
embryo

Scientific area keywords

toxicity testing
drug screening
drug development
preclinical
teratogenicity

Method description

In view of safety of pregnant women, a promising *in vitro* zebrafish embryo developmental toxicity assay has been developed to test pharmaceutical and chemical compounds for their teratogenic potential. The protocol deals with exposing zebrafish embryos to a range of compound concentrations at 28°C throughout organogenesis, i.e. from the gastrulation stage (5.25 hours post-fertilization [hpf]) up to 120 hpf. Morphological development is monitored at 5, 12, 24, 48, 72, 96 and 120 hpf. Larvae are evaluated for lethality in order to identify an LC25 (the compound concentration in which 25% lethality is observed) and morphological anomalies using a numerical scoring system to identify the NOAEL (no observed adverse effect level). These values are used to calculate the teratogenic index (LC25/NOAEL ratio) of each compound. If the teratogenic index is equal to or greater than 10 then the compound is classified as a teratogen, and if the ratio is less than 10 then the compound is classified as a non-teratogen. Currently the assay is optimized by including several skeletal endpoints after skeletal staining at 120 hpf and exogenous metabolic activation systems are developed to encompass the limited biotransformation capacity of the zebrafish embryos. Automation of the morphological scoring is also explored.

Lab equipment

Stereomicroscope ;
Aquaria ;
Incubator.

Method status

Still in development
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

Fast results ;
Medium-throughput ;
Cost effective ;
Limited compound requirements ;
Longitudinal follow-up.

Challenges

Compound uptake (internal concentrations) ;
Limited biotransformation ;
Less morphological endpoints compared to the mammalian *in vivo* tests.

Modifications

Skeletal staining methods and exogenous metabolic activation systems are currently developed to increase the sensitivity of the assay. The main focus is to reduce the number of false negative results.

Future & Other applications

The main goal is to optimize and use the assay for (regulatory) developmental toxicity testing, but the assay could potentially also be used for chronic toxicity testing in the future.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Ball, J.S., et al., Fishing for teratogens: a consortium effort for a harmonized zebrafish developmental toxicology assay. Toxicol Sci, 2014. 139(1): p. 210-9

Brannen, K.C., et al., Development of a zebrafish embryo teratogenicity assay and quantitative prediction model. Birth Defects Res B Dev Reprod Toxicol, 2010. 89(1): p. 66-77

Gustafson, A.L., et al., Inter-laboratory assessment of a harmonized zebrafish developmental toxicology assay - progress report on phase I. Reprod Toxicol, 2012. 33(2): p. 155-64

Pype, C., et al., Antioxidants reduce reactive oxygen species but not embryotoxicity in the metabolic Danio rerio test (mDarT). Reprod Toxicol, 2017. 72: p. 62-73

Pype, C., et al., Incubation at 32.5 degrees C and above causes malformations in the zebrafish embryo. Reprod Toxicol, 2015. 56: p. 56-63

Saad, M., et al., *In vitro* CYP-mediated drug metabolism in the zebrafish (embryo) using human reference compounds. Toxicol *In Vitro*, 2017. 42: p. 329-336

Verbueken, E., et al., *In Vitro* Biotransformation of Two Human CYP3A Probe Substrates and Their Inhibition during Early Zebrafish Development. Int J Mol Sci, 2017. 18(1)

Verbueken, E., et al., From mRNA Expression of Drug Disposition Genes to *In Vivo* Assessment of CYP-Mediated Biotransformation during Zebrafish Embryonic and Larval Development. Int J Mol Sci, 2018. 19(12)

Saad, M., et al., *In vitro* CYP1A activity in the zebrafish: temporal but low metabolite levels during organogenesis and lack of gender differences in the adult stage. Reprod Toxicol, 2016. 64: p. 50-6

Saad, M., et al., *In vitro* CYP-mediated drug metabolism in the zebrafish (embryo) using human reference compounds. Toxicol *In Vitro*, 2017. 42: p. 329-336

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