

Pharmacokinetics in Zebrafish Embryos (ZFE) Following Immersion and Intrayolk Administration: A Fluorescence-Based Analysis

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SCOPE OF THE METHOD

The Method relates to	Other: Pharmacokinetics in zebrafish embryos after different routes of administration
The Method is situated in	Translational - Applied Research
Type of method	In vivo
Used species	Zebrafish
Targeted organ system or type of research	General pharmacokinetics

DESCRIPTION

Method keywords

exposure routes
fluorescence-based analysis

Scientific area keywords

zebrafish embryo
pharmacokinetics

Method description

Immersion treatment: zebrafish embryos at the two-cell stage were immersed in Danieau's medium containing the compound. A compound concentration of 10 μ M and DMSO concentration of 0.1% (v/v) in a volume of 5 mL was used per well (6-well-plates). At 72 h, the zebrafish medium containing the compound was removed, and the animals rinsed 3 x with Danieau's medium. Next, the embryos were kept for another 48 h in Danieau's medium supplemented with DMSO (0.1%, v/v) in the absence of compound. In the case of control experiments, the embryos were exposed to Danieau's medium

supplemented with DMSO (0.1%, v/v). Intrayolk microinjection (IY): zebrafish embryos at the two-cell stage were positioned in a Petri dish at room temperature. IY microinjection was performed using glass needles fitted to a micromanipulator connected to a gas pressure microinjector. Needles were filled with compounds dissolved in the vehicle (DMSO/saline (1:1)), placed under the microscope, and using forceps, the tip was cut off in a manner to allow for a consistent volume to be injected. Afterwards, the embryos were transferred to 6-well plates. Control embryos were exposed to vehicle only. Previous to fluorescence imaging, the embryos were dechorionated (up to 72 hpf) and immobilized by hypothermia, rinsed three times with Danieau's medium, and positioned latero-lateral (right lateral recumbency) on a single cavity glass slide and covered by a drop of agarose (0.1%). Then, the fluorescence in the selected area was quantified as integrated fluorescence intensity (RFU). The RFU values of the non-yolk compartment (i.e., RoB: rest of body) was assessed by subtracting the yolk results from the corresponding integrated fluorescence intensities found in the WB.

Lab equipment

- Microinjector,
- Fluorescent stereomicroscope.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

By using a fluorescence-based approach in this study, it was shown that a 72 h-long immersion of embryos starting at a two-cell stage results in an intrabody exposure which is similar or higher than that seen after a 2 mg/kg intrayolk microinjection, at least in the case of a lipophilic compound (log D: 1.73). In contrast, zero to low intrabody exposure was reached after immersion of the embryos with less lipophilic compounds, possibly resulting in a false-negative outcome in screening programs. In the latter case IY microinjection, a technical procedure that can be easily automated, is highly recommended.

Challenges

Higher immersion concentrations than the one used in this study could possibly be deployed in order to increase intrabody exposure to compounds. Future studies should consider examining the relationship between immersion concentrations and the relative uptake in ZFE, essential information that is presently missing in literature.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

Associated documents

[Pharmacokinetics in Zebrafish Embryos \(ZFE\) Following Immersion and Intrayolk Administration-A Fluorescence-Based Analysis.pdf](#)

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