Zebrafish as an alternative method for toxicological studies

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Abstract

According to the Directive 2010/63/EU fish embryos do not fall into regulatory frameworks dealing with animal experimentation. Therefore, in compliance with the 3Rs principle, zebrafish embryos are considered as replacement or refinement methods. Since more and more industrial chemicals are recognized causes of skin sensitization, it is needed a thorough understanding of the mechanisms to make predictions of the toxic potential of novel compounds. Thus, the FET test was performed and up to four apical observations were recorded as indicators of lethality: coagulation of fertilized eggs, lack of somite formation, no detachment of the tail bud from the yolk sac and lack of heartbeat. Then, in order to assess whether the skin sensitization due to chemical incubation was really measurable, the Fish Interleukin 8 (IL8) ELISA Kit was carried out. The preliminary results obtained so far seem encouraging. However, they need to be confirmed through further ELISA tests and compared with other in vitro methods.

Materials and Methods

Zebrafish mating and eggs production

Fish selected for breeding were transferred to breeding tanks in the afternoon at a 2:1 M:F ratio. Zebrafish eggs were obtained from natural spawning of wild type animals in the early morning of the day after, following standard husbandry practices. After collecting embryos, these ones were placed in incubator at 28°C.

Dechorionation

To remove the chorion, barrier that would hinder the passage of substances, of which a possible indicator of lethality, while malformations such as a pronounced yolk sac oedema, a pericardial oedema or also a spinal curvature (scoliosis) were considered development toxicity endpoints. At the end of the exposure period, acute toxicity was determined based on any positive outcome in one of the four apical observations, then the LC50 was calculated.

Tissue homogenization

For this assay, 100 zebrafish larvae were collected in a 1.5mL tube and homogenated: inside the Eppendorf tube was added a small bead and the instrument was setted for shaking at 30Hz for 5 minutes. After that, the sample was centrifuged again for 10 minutes at 5000g, then the supernatant was removed and centrifuged for 30 minutes at 20000g.

ELISA analysis

Through the ELISA kit (performed according to the manufacturer’s directions), the IL8 was evaluated.
Results

From the LC50 values of each chemical agent, the substance that turned out to be the most toxic is the DNCB: 0.228 mg/L are indeed sufficient to determine the death of 50% of treated embryos. On the other hand, LA has proved to be the least toxic among those tested with an LC50 value of 1.172 mg/mL. As regards Sodium lauryl sulphate, its LC50 value observed was 0.041 mg/mL.

Once the range of concentrations in which there is 100% survival was identified, IL8 production was measured. The results relating to the production of IL8 do not support, at the moment, a response in zebrafish larvae specifically correlated with the exposure to a sensitizing or non-sensitizing chemical. The quantitative evaluation of IL8 production was carried out by exposing the embryos for a period of 96 hours at the highest sublethal concentration of each compound (DNCB, LA and SLS) as shown in Figure 1.

Conclusions

From the preliminary results obtained, the FET test proved to be an excellent tool to evaluate the toxicity of selected sensitizing substances and to analyze phenotypes that can be correlated with them. For this purpose, the test with chemicals already in use will be implemented and the range of chemical compounds to be evaluated will also be expanded. Moreover, this experimental model is extremely advantageous in the toxicological field thanks to the high fertility of the females: it was possible, indeed, to have a very large number of experimental units and to treat and analyze many embryos simultaneously. Further investigations are needed to clarify whether the mechanism of production of IL8 in zebrafish is correlated with the allergenic potency of a substance and also to obtain a complete framework to possibly widely apply this model in larger screening.

References

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