In Vivo Alternatives

Ainhoa Letamendia Urraca and Carles Carrol Massot at Biobide
look at automated screening to detect risk of cardiotoxicity *in vivo*

The zebrafish is gaining relevance in biomedical research. However, limitations in the use of zebrafish in pre-clinical manual assays due to the increase in variability of the dispensed drug, and factors affecting the embryonic developmental stage have prevented pharmaceutical and biotechnological companies from incorporating it regularly. To approach the topic, a new, fully automated system, and the factors to be controlled are described, in order to provide standardised results for a validated cardiotoxicity assay.

The development process of a new drug is long and expensive (1). The main causes of drug withdrawal in advanced preclinical or clinical phases are toxicological effects, especially cardiotoxic and hepatotoxic (2). Cardiotoxicity is often described as the risk of causing a ventricular arrhythmia called Torsade de Pointes (TdP) through the alteration of the electrocardiogram (ECG) by prolonging the QT interval. Heart toxicological studies have proved the existence of a strong correlation between drugs known to induce a QT prolongation in humans and the appearance of an arrhythmia type 2:1 in zebrafish (3,4). The main protein involved in drug-induced QT prolongation is a potassium channel encoded by the ether-a-go-go-related gene (ERG), and its homologue in zebrafish can lead to arrhythmia type 2:1 (5). In addition, several other ion channel proteins and regulatory pathways are involved in the process of depolarisation and repolarisation, and they can compensate for the inhibition of ERG. Therefore, a wide spectrum of biological and environmental parameters must be controlled in order to develop an automated system. Nowadays, the predominant technique in early cardiotoxicological screenings is the 'patch clamp'. This technique measures the voltage current in the cell, revealing fundamental cell mechanisms, such as the probability of channels opening in response to depolarisation of the membrane. Unfortunately, this cellular based technique cannot reproduce the organism environment and has limitations when predicting the biological effect of drugs on heartbeat.

ZEBRAFISH MODEL

The zebrafish (*Danio rerio*) has become a popular model system to study human diseases and in the drug discovery research field. Since zebrafish embryos are small, transparent and undergo rapid development, *in vivo* analysis of different structures and dynamic processes, such as the heart, are feasible (6). At day two post-fertilisation (dpf) the zebrafish embryo already shows a functional circulatory system with cardiac tissues and structures analogous to those present in humans (7). Genetic studies have proven that they share common genes with a high degree of homology, involved in determining the heart morphology and its physiological activity (5). These characteristics have been exploited by researchers in developmental processes or disease studies and are powerful tools in understanding common molecular pathways in humans (8,9). Furthermore, the transparency and the ability to generate transgenics with fluorescent reporters have eliminated the need for invasive surgery and enabled an easier visualisation of drug effects in specific organs (10). Still, the development of zebrafish drug screenings has remained challenging in the last decade (11,12).

NEW APPROACH

An automated screening platform based on the zebrafish technology could potentially overcome the described limitations, allowing high-speed and cost-effective screenings, together with close translational results with other vertebrates. However, the automation of this application would first require the systematic control of the variables involved in the analysis, with the aim of avoiding misinterpretation of data and incorrect classification of drugs. So far, screenings on zebrafish have been developed based on previous cell-based assay technology and experience, but no

Figure 1: Drug screening platform flowchart. Zebrafish embryos are dispensed in 96 well plates. Compounds are then prepared and dispensed. After incubation, embryo heart rate is analysed.