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Review

Zebrafish (*Danio rerio*): A valuable tool for predicting the metabolism of xenobiotics in humans?

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ABSTRACT

Zebrafish has become a popular model organism in several lines of biological research sharing physiological, morphological and histological similarities with mammals. In fact, many human cytochrome P450 (CYP) enzymes have direct orthologs in zebrafish, suggesting that zebrafish xenobiotic metabolic profiles may be similar to those in mammals. The focus of the review is to analyse the studies that have evaluated the metabolite production in zebrafish over the years, either of the drugs themselves or xenobiotics in general (environmental pollutants, natural products, *etc.*), bringing a vision of how these works were performed and comparing, where possible, with human metabolism. Early studies that observed metabolic production by zebrafish focused on environmental toxicology, and in recent years the main focus has been on toxicity screening of pharmaceuticals and drug candidates. Nevertheless, there is still a lack of standardization of the model and the knowledge of the extent of similarity with human metabolism. Zebrafish screenings are performed at different life stages, typically being carried out in adult fish through *in vivo* assays, followed by early larval stages and embryos. Studies comparing metabolism at the different zebrafish life stages are also common. As with any non-human model, the zebrafish presents similarities and differences in relation to the profile of generated metabolites compared to that observed in humans. Although more studies are still needed to assess the degree to which zebrafish metabolism can be compared to human metabolism, the facts presented indicate that the zebrafish is an excellent potential model for assessing xenobiotic metabolism.

1. Introduction

Fish are the most numerous and phylogenetically diverse group of vertebrates and are useful in the study of fundamental processes in vertebrate evolution, development, toxicology and disease processes (Garcia et al., 2016; Langheinrich, 2003; Spitsbergen and Kent, 2003). In particular, zebrafish (*Danio rerio*), which is a tropical fish of the Cyprinidae family, has been a prominent genetic model since 1981 (Streisinger et al., 1981), when its genetic amenability allowed the first genetic screening for mutations that affect organ development in a vertebrate (Kamel and Ninov, 2017). Since then, zebrafish has become a popular model organism in several lines of biological research beyond genetics, including developmental biology (Carten et al., 2011; Ho et al., 2003), toxicology (Thompson et al., 2010; Weigt et al., 2011; Zhang et al., 2016), drug discovery (Fleming et al., 2005; Kithcart and MacRae, 2017), disease models (Brugman, 2016; Capiotti et al., 2014) and neurobiology (Pinho et al., 2016; Shams et al., 2017). Therefore, zebrafish represents a viable alternative to the classical mammalian models currently used in biological research because it has some unique properties: it is an intact organism with similar development, anatomy and physiology to higher vertebrates (Otte et al., 2017); it is easier and less expensive than popular rodent models; it is a genetically tractable vertebrate model that can be easily injected with modified genes and can absorb chemical mutagens through water; embryos are transparent and develop externally, allowing the use of noninvasive imaging techniques; embryos develop very rapidly compared to mammalian models,

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potentially reducing the experimentation time (Garcia et al., 2016); and it has a high reproduction capacity, where a pair of zebrafish can generate hundreds of fertilized eggs, which develop rapidly into larvae with functional metabolic organs (Kamel and Ninov, 2017).

Although there are some major differences resulting from adaptation to aquatic life, most zebrafish organs perform the same functions as their human counterparts and exhibit well-conserved physiology (MacRae and Peterson, 2015). In fact, zebrafish share physiological, morphological and histological similarities with mammals (Diekmann and Hill, 2013) and its genome has been sequenced, revealing that ~70% of human genes have a zebrafish orthologue and approximately 82% of potential human disease-related genes have at least one obvious zebrafish orthologue (Howe et al., 2013). Not surprisingly, in terms of publication, zebrafish are one of the fastest growing model organisms (Garcia et al., 2016). Furthermore, chemical-genetic screens in zebrafish have already rendered the drug ProHema, which is undergoing phase II clinical trials for improving hematopoietic stem cell engraftment and expansion (Kamel and Ninov, 2017; North et al., 2007). Assessing the toxicity of a compound is a critical step in the discovery of new drugs. In forensic toxicology, it is also very important to assess the toxicity of a compound, since the knowledge of its metabolism will be fundamental for determining the analytical targets to be monitored as the emergence of ever newer designer drugs is an ongoing challenge for analytical toxicologists in forensic as well as clinical toxicology (Peters and Martinez-Ramirez, 2010). To understand or predict the toxic potential of a compound, knowledge of the absorption, distribution, metabolism, and elimination of the compound is required (Garcia et al., 2016).

Notably, extensive metabolism of a xenobiotic, an external and foreign compound to the organism, could lead to reduced activity, whereas creation of new active metabolites could lead to increased toxicity (Diekmann and Hill, 2013). Zebrafish embryos represent an attractive model for studies of developmental toxicity of xenobiotics both for human and environmental risk assessment (Carlsson et al., 2013). The reason for the preference in the use of embryos instead of adult fish is because in Europe, zebrafish embryos are not considered laboratory animals until the independent feeding stage (European Commission, 2016), which makes them ideal candidates for several chemical screenings. On the other hand, in the United States of America fish are not protected for research use, while in Brazil, China and India, all animals are protected for use in research (Sneddon et al., 2017). In fact, the zebrafish embryo has already been accepted as a validated alternative for the acute fish toxicity test (OECD TG236) (OECD, 2013). In addition, toxicology may be the most prevalent use of zebrafish in the industry, with the majority of large pharmaceutical companies reporting some use of zebrafish for toxicology (MacRae and Peterson, 2015). The metabolism of the substance as a critical step in the toxicological evaluation. Therefore, the purpose of this review is to analyse publications involving xenobiotic metabolism studies at various stages in the zebrafish's evolution over the years. Considerations will also be made on the development of zebrafish, enzymes involved in the metabolism of xenobiotics, and analytical methods for the detection of metabolites, highlighting advantages and limitations of zebrafish as a model for the evaluation of xenobiotic metabolism.

2. Development

The genetic signals and responses that drive early embryonic development are fundamentally similar between zebrafish and mammalian embryogenesis (Sipes et al., 2011). The formation of different organ systems can be followed under the microscope during the developmental period, offering many opportunities for the study of organogenesis (Dawid, 2004). The zebrafish has a rapid development, progressing from zygote to larvae in 72 h. During the early embryonic stages, zebrafish are covered by a chorionic membrane (egg shell) which will be lost between 3 or 4 dpf (days post-fertilization) (Kimmel et al., 1995). The chorion is an acellular envelope containing pores that are approximately 0.5 μm in diameter with 2 μm spacing (Lee et al., 2009). A limitation to using the zebrafish embryo is its need to take up substances through the chorion and other membranes, because the gills of the adult fish are not yet developed (Vallverdú-Queralt et al., 2015). Therefore, the chorion can significantly confound the early life zebrafish toxicity assay by leading to false positives because of size-dependent limitations in the uptake of large compounds (Langheinrich et al., 2003; Mandrell et al., 2012). The chorion may be removed but not without potentially affecting embryo integrity and behavior (Mandrell et al., 2012; Sipes et al., 2011).

The morphogenesis of the liver, which is an organ that plays a key role in the biotransformation of xenobiotics, starts at approximately 24 hpf (hours post-fertilization), and liver-specific markers occur even earlier (16 hpf) (Tao and Peng, 2009). By 5 dpf, the heart, liver, brain, pancreas, and other organs are developed (Garcia et al., 2016; Kimmel et al., 1995). The zebrafish larvae begin independent feeding at approximately 6 dpf, growing rapidly and developing into juveniles at approximately 30 dpf, and subsequently into adults at approximately 90 dpf (Kamel and Ninov, 2017). Similar to the mammalian liver, the zebrafish liver is an essential organ in the body and performs several vital activities, including metabolism, detoxification and homeostasis (Menke et al., 2011; Tao and Peng, 2009). However, zebrafish have a unique hepatic anatomy and cellular architecture, despite the high conservation of cell types within the liver, compared with mammals (Goessling and Sadler, 2015; Tao and Peng, 2009).

3. Enzymes involved in the metabolism of xenobiotics

Drug-metabolizing enzymes play central roles in the metabolism, elimination and/or detoxification of xenobiotics introduced into the organism (Xu et al., 2005). Zebrafish have the ability to perform both phase I (oxidation, *N*-demethylation, *O*-demethylation and N-dealkylation) and phase II (sulfation and glucuronidation) metabolism reactions, and the metabolic enzymes responsible for these reactions are highly conserved in relation to mammals (Brox et al., 2016; Chng et al., 2012; Diekmann and Hill, 2013). Phase I enzymes consist primarily of the cytochrome P450 (CYP) superfamily of microsomal enzymes, which are found abundantly in the liver, gastrointestinal tract, lung and kidney, catalyzing the oxidative and reductive metabolism of many chemicals and endogenous compounds (Tseng et al., 2005; Xu et al., 2005). In humans, it is believed that five CYP gene families, namely, CYP1, CYP2, CYP3, CYP4 and CYP7, play crucial roles in hepatic as well as extra-hepatic metabolism and elimination of xenobiotics and drugs, as CYP1A2, CYP2C9, CYP2D6 and CYP3A4/5 are responsible for the oxidative biotransformation of approximately 70% of most clinically used drugs (Saad et al., 2017; Xu et al., 2005). Therefore, CYP families 1-3 are the main metabolizing enzymes responsible for xenobiotic metabolism (Guengerich, 2008; Saad et al., 2016).

Many human CYP enzymes have direct orthologs in zebrafish (Goldstone et al., 2010), suggesting that zebrafish metabolic profiles may be similar to those of mammals. In addition, there are other CYPs described in fish that lack orthologs in humans, such as CYP1C, CYP2AE and CYP2X (Table 1) (Goldstone et al., 2010). Nevertheless, as CYP activity is not necessarily correlated with its gene expression or even protein levels, more focus is needed on CYP activity (Saad et al., 2017). Like the human CYP3A genes, CYP3A65 transcription in the foregut region was enhanced by treatment of the zebrafish larvae with dexamethasone and rifampicin (Tseng et al., 2005). On the other hand, 7-benzyloxy-4-(trifluoromethyl)coumarin, a vertebrate CYP3A substrate not usually associated with CYP1 activity, was metabolized more efficiently by CYP1A than CYP3A65 in zebrafish (Scornaienchi et al., 2010). That is, a zebrafish CYP that has an ortholog in human will not necessarily have the same behavior on a certain substrate. Another interesting case is the metabolism of dextromethorphan in zebrafish; although no ortholog of human CYP2D6 has been found in the fish