

Zebrafish in toxicology research: advantages and limitations



The abuse of psychoactive substances is a serious public health problem and understanding the brain disorder induced by psychoactive substances is an important challenge in biomedical research that requires extensive clinical and preclinical investigation (Neelkantan et al. 2013; Stewart et al. 2011). Recreating the complex network of neurochemical interactions in organisms *in vitro* is not possible, especially for psychotropic drugs (Riehl et al. 2010). In this way, experimental animal models are an important tool for investigation of the toxicology and chemistry of the drugs of abuse.

Animal models in toxicological and pharmacological research using mammals present high cost, consume large amounts of chemical compounds and are laborious to perform the tests and maintenance of animals. In this context, animal models such as the zebrafish, which outweigh these disadvantages, are an interesting and promising alternative in research.

Since the 1970s, the zebrafish (*Danio rerio*) is used in research in neuroscience and developmental biology (Serra et al. 1999). In the 1990's, zebrafish were used for the first vertebrate large scale mutagenesis screen. In last few years, the use of zebrafish (*Danio rerio*) in scientific research has increased rapidly in other science fields, including Pharmacology and Toxicology (Chakhaborty et al. 2009).

The zebrafish represents an interesting model for integration of research of genetic, neural and behavioral aspects (Miklósi and Andrew, 2006). The coupling between behavioral assays and analytical and molecular techniques permits the elucidation of mechanisms of toxicity, the test of new drugs for

therapeutic treatments and the study of new drugs (Tierney et al. 2011). The aim of this paper is to outline recent developments and futures perspectives in drugs of abuse research with zebrafish, in Experimental and Analytical Toxicology.

Zebrafish as a new animal model in Toxicology: advantages and limitations

The zebrafish is a small tropical fish native of northern India and adjacent countries. Many factors and animal characteristics make zebrafish an attractive and efficient model to analyze the mechanisms of action and effects of drugs in general (Chakhaborty et al. 2009). Zebrafish and humans share about 75% of their genome and have physiological similarity (Chakhaborty et al. 2009, Zhu et al. 2014). Genome, transcriptome and proteome of zebrafish have been widely studied and described (Maximino et al. 2010). The similarity level between zebrafish and humans is also observed in the nervous system (Mathur and Guo, 2010).

Zebrafish are small (at adulthood, 2.5 - 4.0 cm) and maintenance costs of this fish in laboratory are considerably low, permitting logistical and economical advantages over rodent models (Key and Devine, 2003). The fertility rate and the number of embryos generated are higher those in mammalian models (Chakhaborty et al. 2009). In general, an adult female can produce 200-300 eggs and reproduce 2 or 3 times per week (Blaser and Gerlai, 2006; Gerlai 2003; Patton and Zon, 2001; Zon and Peterson, 2005).

The zebrafish cycle of life and development period is well-characterized and short (3 to 4 months) (Cadet, 2009; Hill et al. 2005). The body is formed in the first 24 hours post fertilization (hpf) and the internal organs are fully

developed at 96 hpf (Chakhaborty et al. 2009; De Esch et al. 2012b; Ninkovic and Bally-Cuif, 2006b; Parng et al. 2002; Patton and Zon, 2001). The development outside of the uterus and the optical transparency of eggs and tissues during embryogenesis allows the visualization of tissues and organs *in vivo*, making possible the visual analyses in real time of early developmental processes, organ morphology and dysfunctions caused by drugs of abuse and quantification of cell proliferation and cell death in specific tissues (Chakhaborty et al. 2009; Friedrich et al. 2010; Hill et al. 2002; Ingham 2009; Miklósi and Andrew, 2006;; Mathur and Guo, 2010; Ninkovic et al. 2006b; Parng et al. 2002; Patton and Zon, 2001; Peterson and MacRae, 2012; Xu et al. 2011). In addition, blastomeres of zebrafish are large and stable for biophysical and electrophysiological assays (Zhu et al. 2014).

The zebrafish has become a widely utilized model organism in pharmacological and toxicological research, particularly due to evidence that they may share with humans and other mammals some key receptors targeted by drugs of abuse (Miller et al. 2014). In addition, zebrafish are highly social animals which enables them to display robust behavioral responses, such as shoaling, aggression and social preference, and emerge as a sensitive alternative model to investigation of drugs of abuse-evoked states (Cachat et al. 2013; Pham et al. 2012). Another advantage is that the size of zebrafish provides a rapid absorption of drugs via the gills and leads to bioaccumulation in Central Nervous System (CNS) and other tissues (Echevarria et al. 2008).

Zebrafish is amenable to molecular and genetic analysis (Bailey et al. 2013; Chakhaborty et al. 2009; Miklósi and Andrew, 2006; Parng et al. 2002). Since genetic mutations can interfere in brain function, the use of molecular and biochemical techniques in zebrafish allows the identification of molecular substrates for drugs in brain and the analysis of function and regulation of the genes, the production of transgenic strains and the induction of specific mutations, inducing overexpressing of the genes or decreasing genes expression (Cadet 2009; Goldsmith 2004; Key and Devine, 2003). Use of zebrafish mutant strains has allowed the understanding of mechanisms and pathways and neural expression of specific genes. Due to the increasing progress in this area, studies of mutant zebrafish have investigated specific behaviors, diseases, deformities and functional processes (Spitsbergen and Kent, 2003). The performance of point mutations in zebrafish, generating mutant lines, enables the molecular investigation of the mechanism of action of drugs of abuse, determining specific receptors and target molecules.

Although the model advantages are numerous, some limitations should be considered in the use of zebrafish in toxicological, pharmacological and neuroscience research. The normal or defective functioning of the human brain is complex to be fully modeled in zebrafish brain. Some areas do not have the same degree of development of the mammalian brain, making it difficult to map for mammalian counterparts (Kalueff et al. 2014; Rinkwitz et al. 2011; Stewart et al. 2014). Moreover, the social behavior is not clearly defined in fish larvae (Kalueff et al. 2014; Stewart et al. 2014).

Zebrafish has a genome 30-40% tetraploid due to an genome duplication with some of their genes having two copies instead of one - as in mammals - <https://assignbuster.com/zebrafish-in-toxicology-research-advantages-and-limitations/>

and one of these two genes are not represented in the human genome (Aleström et al. 2006; Klee et al. 2011; Stewart et al. 2014).

One disadvantage associated with the zebrafish model is the fact that some drugs are not soluble in water, making it difficult to administration by immersion. This potential problem can be solved by using organic solvents or other routes of administration (Stewart et al. 2014). In other hand, exposition to drug by immersion reduces the stress-induced by injection (Stewart et al. 2011b). Finally, although the barrier between brain and blood presents development similar to humans, the distribution of some drugs in organism may be different in different species (Stewart et al. 2014).

Although the pattern has some limitations, the advantages over conventional models mammals make zebrafish a very interesting alternative in research. In this sense, the use of zebrafish in research involving drugs of abuse, in order to characterize drugs of abuse effects and to quantify these compounds in a biological fluid from the animal is increasing and represents a great target for research.

Zebrafish research to study the drugs of abuse

The conduct of toxicology research in laboratory animals has become a well-established and essential practice and chemical and toxicological information on chemical compounds and drugs are obtained from the results of these studies (Gad 2007; Olson et al. 2000). The use of animals in research has several advantages: low cost, easily use and functional homology with humans (Gerlai et al. 2010). The animal experiments to

predict the action, metabolism and effects of drugs of abuse in humans are extremely important in Toxicology.

Introduced as a model for neural development by George Streisinger in the 1960's, zebrafish has become a promising aquatic model for study of drugs. The zebrafish is an efficient alternative model of drug delivery via the gills, by direct application of drugs in water and posterior submersion of the animal. Between 12 and 14 days after fertilization (dpf), molecular oxygen is mainly absorbed by the skin, suggesting that this is the main route of uptake for small molecules, and after 72 h the embryos begin to swallow indicating the availability of the oral route for absorption of chemical compounds (Goldsmith 2004). Exogenous compounds, such as ethanol, have been shown to rapidly enter in systemic circulation of the fish, demonstrating the high sensitivity of the zebrafish to various psychotropic agents (Dlugos and Rabin, 2003; Echevarria et al 2006; Kyzar et al. 2012). In the larval and embryonic stage, the performance of rapid and high-throughput analysis of multiple behaviors and screening of chemical compounds is possible and suitable (De Esch et al. 2012b; Guo 2009; Richendrfer et al. 2012).

The first response to the action of a psychoactive substance is the behavior. In this sense, the zebrafish has been widely used in the development and validation of behavioral assays to different drugs of abuse, due to its sensitivity to drugs and their robust responses. Locomotor activity, learning, sleep, aggression, social and antipredator behavior are some of the observed and well-characterized behavioral parameters in zebrafish (Bailey et al. 2013; Cachat et al. 2013; Guo 2004; Pham et al. 2012; Spitsbergen and Kent, 2003).

<https://assignbuster.com/zebrafish-in-toxicology-research-advantages-and-limitations/>

Endocrine responses to stress in zebrafish are also an important tool for toxicological effects induced by drugs of abuse. In zebrafish, the hypothalamus-pituitary-interrenal axis (HPI) is homologous to the hypothalamic-pituitary-adrenal axis (HPA), found in mammals and humans, and cortisol is involved in physiological responses to stress situations (Stewart et al. 2010).

Another interesting pattern of physiological change for toxicological investigation of drug in zebrafish is the color of the skin. As mammals, fish have cells containing pigments in the collagen layer of the dermis and these cells have hormonal and nervous regulation (Nguyen et al. 2013). In this sense, effects on skin color may contribute in part of the elucidation of the mechanism of action of many drugs of abuse.

The toxicity induced by abuse of drugs in the digestive and cardiovascular systems of zebrafish can also be determined. These systems are developed and become fully functional in the first days after fertilization (Patton and Zon, 2001). The characterization and monitoring of blood vessels in embryos is easily achievable (Rubinstein 2003). The heart rate is an indicator of cardiac toxicity widely used. Morphological examination of the heart and digestive organs can be performed to identify organ-specific toxicity of drugs of abuse (Rubinstein 2006). For example, the effects of environmental toxin tetrachlorodibenzo- *para* -dioxin (TCDD) on heart rate (Henry et al. 1997), the functional lipid metabolism (Farber et al. 2001), the effects of fungicide riphenyltin acetate (APTT) in hepatocytes (Strmac and Braunbeck, 1999) were investigated using histological and immunochemical analysis.

The development of analytical techniques to determine compounds of interest in biological fluids zebrafish also plays an important role in the elucidation of mechanisms of action and toxicity of drugs of abuse.

Moreover, it is extremely important to develop methods of extraction and concentration of analytes and biomarkers that make possible dosages required to understanding toxicological aspects of these drugs of abuse. The main analytical methods developed to study the drugs of abuse in zebrafish are listed in Table 1.

The new psychoactive substances (NPS) that have emerged in recent years have action and effect mechanisms partially or totally unknown. According to UNODC, the number of NPS significantly increased between 2009 and 2013 (UNODC 2014). In many poisoning deaths caused by these unknown substances, the lack of information makes it difficult to identify the cause of death. Therefore, the use of zebrafish in research represents an important tool to scan and evaluate the chemical and toxicological properties of both known and unknown drugs of abuse.