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Review article

Zebrafish as potential model for developmental neurotoxicity testing: A mini review

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ABSTRACT

The zebrafish is a powerful toxicity model; biochemical assays can be combined with observations at a structural and functional level within one individual. This mini review summarises the potency of zebrafish as a model for developmental neurotoxicity screening, and its possibilities to investigate working mechanisms of toxicants. The use of zebrafish in toxicity research can ultimately lead to the refinement or reduction of animal use.

Contents

1. Introduction

Exposure to chemical pollutants poses a significant human health risk, especially when exposure takes place during fertilisation, pregnancy and/or early development of the individual (Andersen et al., 2000; Claudio et al., 2000; Goldman and Koduru, 2000; Mendola et al., 2002; Schettler, 2001; Slikker, 1994; Tilson et al., 1998). To obtain adequate and reliable safety information of an environmental toxicant an integrated approach, which includes exposure of an organism during all life stages, is necessary. By establishing the effect on

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different developing systems within one individual in time, specific information about the working mechanism of a toxicant may be obtained. Such an approach calls for a small, robust and easy to maintain organism which develops quickly and has a high fecundity. Moreover, the animal needs to be suitable for the analysis of all kinds of chemical stressors, even on site of an environmental disaster.

The zebrafish (*Danio rerio*) originates from Southeast Asia and was introduced as a genetic model organism by George Streisinger in the late 1960s (Barman, 1991; Bhat, 2003; Streisinger et al., 1981; Talwar and Jhingran, 1991). By now, it is one of the best described vertebrate model species in developmental biology; hundreds of mutations that disturb classic developmental processes have been defined (Driever et al., 1996; Haffter et al., 1996; Stainier, 2002; Thisse and Zon, 2002). Although zebrafish obviously lack some of the mammalian organs as lung, prostate, and mammary glands, their tissues and organs have been shown to be largely homologous to their mammalian counterparts at the anatomical, physiological and molecular level (Lewis and Eisen, 2003; Moens and Prince, 2002; Wilson et al., 2002). The zebrafish' genome is fully sequenced and zebrafish genes share a 60–80% homology with their human counterparts. More importantly, the amino acid sequences of functionally relevant protein domains has been proven to be even more evolutionary conserved (Reimers et al., 2004; Renier et al., 2007). By now, numerous genetic tools have been developed, such as *in situ* imaging tools in combination with transgenic variants expressing fluorescent proteins (Amsterdam and Hopkins, 2006; Chen and Ekker, 2004; Lekven et al., 2000; Patton and Zon, 2001). The transparency of the zebrafish larvae provides a huge advantage in research and mutant zebrafish lines that also express reduced pigmentation later in life have been developed as well (Lister et al., 1999; White et al., 2008). The purpose of this paper is to overview the potentialities of this model species in developmental neurotoxicology and whether this alternative model can be used as a replacement, refinement or reduction of the more laborious and more expensive toxicity studies in mammals.

2. Zebrafish in toxicity research

Traditionally, toxicity testing is performed with rodents according to the regulations from the Organisation for Economic Co-operation and Development (OECD) in Europe and the Environmental Protection Agency (EPA) in the United States. However, for aquatic toxicity testing, fish have already been established as a model organism by the OECD (OECD, 1992a, 1992b, 1998, 2000). In addition, the EPA includes fish in the Fish Acute Toxicity Test (OPPTS 850.1075) and the Fish Early-Life Stage Toxicity Test (OPPTS 850.1400) (EPA, 1996a, 1996b). In toxicity testing, a great advantage of using zebrafish is that exposure can take place by simple diffusion, while hydrophilic or larger molecules can be injected into the yolk sac, sinus venosus or circulation (common cardinal vein). From 72 hours post-fertilisation (hpf) onwards the larvae start to feed independently and compounds can also be administered orally. The small size of zebrafish larvae allows them to grow and develop in a single well of a microtiter plate. This makes zebrafish highly suitable for high-throughput screening while diminishing the need for large quantities of substances or chemicals at the same time (Barros et al., 2008). In addition, because of their small size, efficient low-cost pathological evaluation of all major organs can be carried out on a limited number of slides as well. Other advantages of the zebrafish have proven to be: its large numbers of offspring; its small size and translucency during embryonic and larval development. The later allows high resolution live imaging over time and for instance the use of fluorescent reporter molecules. In addition, zebrafish develop quickly, brain, heart, liver, pancreas, kidney, intestines, bones, muscles and sensory systems are fully functional at 5 days post fertilisation (dpf) (Kimmel et al., 1995). Up to date, direct visual assays in live animals can be carried out by using reporter fish lines which express fluorescent proteins as a read-out for active signalling pathways (Dorsky et al., 2002; Perz-Edwards et al., 2001). By now, many successful chemical screens using zebrafish have been reported, and after the necessary validation this could lead to the establishment of the zebrafish as a preferred animal for high-throughput toxicity screening which would ultimately lead to a reduction of costs and animal use (Anderson et al., 2007; Bowman and Zon, 2010; Burns et al., 2005; Cao et al., 2009; Chakraborty et al., 2009; Coffin et al., 2010; de Groh et al., 2010; Kokel et al., 2010; Molina et al., 2007, 2009; Murphey et al., 2006; North et al., 2007; Owens et al., 2008; Paik et al., 2010; Peterson et al., 2004; Rihel et al., 2010; Sachidanandan et al., 2008; Stern et al., 2005; Yeh et al., 2009; Yu et al., 2008). The above mentioned features make the zebrafish an excellent model for aquatic and high-throughput toxicity screening; however the zebrafish has some additional advantages for the use in other vertebrate toxicity testing methods.

3. Embryological screening techniques

Zebrafish are able to survive with malformations or loss of organ function well beyond the time one would expect, since zebrafish embryos can subsist on diffusion of nutrients from their yolk sac for the first few days and, more importantly, on diffused oxygen for the first week of life without a functional circulation (Berry et al., 2007; Burggren and Pinder, 1991; Chen et al., 1996; Incardona et al., 2004; Peterson et al., 1993; Sehnert et al., 2002; Zhang et al., 2004). Existing zebrafish embryonic assays take advantage of the transparency of the larvae and usually include skeletal (*eg* cranofacial), neuromuscular, physiological, morphological and behavioural parameters measured using simple light microscopy at distinct developmental time points (for details see the recent excellent review by McCollum et al., 2011) [cf also Fig. 1]. The developmental scoring parameters are generally used to determine the dose at which 50% of the embryos have died or show developmental malformations (LC50 or EC50 respectively). From these data the teratogenic index (TI; LC50/EC50) can be calculated as a general measure of teratogenicity (Bortagaray et al., 2010; Fraysse et al., 2006; Nagel, 2002; Ton et al., 2006). Some embryonic studies with zebrafish report a strong correlation with mammalian data. Atrazine, dichlorodiphenyltrichloroethane (DDT) and 2, 3, 7, 8-tetrachlorodibenzo-pdioxin (TCDD) have been shown to be primarily teratogenic, while 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, paraquat, dieldrin and nonylphenol were classified as specific neurotoxicants (Bretaud et al., 2004; Dong et al., 2002; Ton et al., 2006). However, other studies lack definite correlation because the zebrafish strains and screening protocols used still vary among laboratories. It is necessary to reach more definite consensus about the strains used, the main scoring time points, and the main endpoints used. Another relevant point before the zebrafish can be fully accepted as a vertebrate toxicity screening model is the translation to the human situation. The transparency of the embryonic and larval stages and the availability of fluorescent reporter molecules finally give the possibility to correlate the gross morphological effects of a toxicant and the affected molecular pathway responsible for this. Specific assays based on the development of different organ systems in zebrafish can be used to address questions about the working mechanism of a toxicant; an overview is presented in Fig. 1. Here, we will focus on the development of the central nervous system (CNS) in zebrafish to illustrate its use for advanced environmental toxicity testing.

4. Neurotoxicity in zebrafish

4.1. Development of the central nervous system

During gastrulation, which starts around 6 hpf, the body plan of the zebrafish is established and cells that will give rise to the nervous system move to distinct positions within the embryo (Kimmel et al., 1990). As with other vertebrates, the zebrafish central nervous system is formed out of the neural plate, a layer of ectodermal