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Zebrafish: a predictive model for assessing drug-induced toxicity

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The zebrafish model organism is increasingly used for assessing drug toxicity and safety and numerous studies confirm that mammalian and zebrafish toxicity profiles are strikingly similar. This transparent vertebrate offers several compelling experimental advantages, including convenient drug delivery and low cost. Although full validation will require assessment of a large number of compounds from diverse classes, zebrafish can be used to eliminate potentially unsafe compounds rapidly in the early stages of drug development and to prioritize compounds for further preclinical and clinical studies. Adaptation of conventional instrumentation combined with new nanotechnology developments will continue to expand use of zebrafish for drug screening.

Zebrafish have several inherent advantages for drug screening: they are small, inexpensive to maintain and easily bred in large numbers – a single spawning produces 100–200 eggs. Adult zebrafish are 3-cm long. Larvae, which are only 1–4-mm long, can live for seven days in a single well of a standard 96- or 386-well microtiter plate supported by nutrients stored in the yolk sac. Administration of drugs is simple: zebrafish larvae absorb small molecules diluted in the surrounding water through their skin and gills. Drugs can be delivered orally for assays performed after this stage because zebrafish begin to swallow at 72 hours postfertilization (hpf). Highly hydrophobic compounds, large molecules and proteins can be injected into the yolk sac, the sinus venosus or the circulation. In adult zebrafish, drugs can also be delivered by oral intubation. Compared to testing in other animal models, statistically significant numbers of zebrafish can be used for each assay and small amounts (~mg) of drug are required. In addition, the transparency of zebrafish for several days postfertilization (dpf) enables *in vivo* observation of live or whole mount fixed specimens, including the visualization of vital dyes, fluorescent tracers, antibodies and riboprobes. By 120 hpf, zebrafish develop discrete organs and tissues, including brain, heart, liver, pancreas, kidney, intestines, bone, muscles, nerve systems and sensory organs (Fig. 1). These organs and tissues have been shown to be similar to their mammalian counterparts at the anatomical, physiological and molecular levels.

Although conventional *in vitro* assays using cultured cells can be used to evaluate potential drug toxicity effects, results are frequently not predictive of results *in vivo* which involve drug absorption, distribution, metabolism and excretion (ADME). To streamline the drug development time-line, prioritize drug candidates for animal testing and reduce unnecessary costs for mammalian studies, drug-screening assays using zebrafish are becoming increasingly popular [1–5]. This convenient, predictive animal model can serve as an intermediate step between cell-based evaluation and conventional animal testing. In this review, we describe a variety of assays for assessing cardiotoxicity, hepatotoxicity, neurotoxicity and developmental toxicity in zebrafish.

Cardiotoxicity assessment in zebrafish

Unforeseen cardiotoxicity is a major problem that can result in drug withdrawal. In 2004, rofecoxib (Vioxx), Merck's blockbuster antiarthritic drug was removed from the market because of increased risk of heart attack and stroke. Another example, propulsid (Cisapride), an oral gastrointestinal prokinetic agent, has been associated with ~400 adverse cardiac events (arrhythmias) and has resulted in more than 80 deaths in the USA [6,7]. These problems with cardiotoxicity led to the removal of propulsid from the US market along with terfenadine, an antihistamine and grepafloxacin, an antibacterial.

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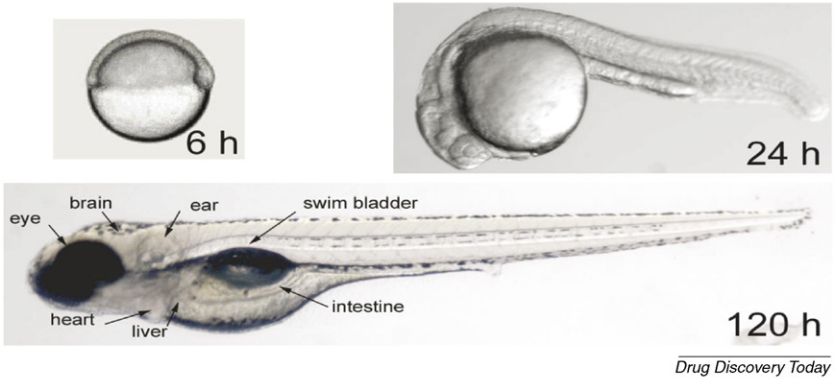

FIGURE 1

Zebrafish developmental stages. Zebrafish at 6, 24 and 120 hours postfertilization (hpf) are shown. By 120 hpf, zebrafish develop discrete organs and tissues, including brain, heart, liver, intestine, eye, ear and swim bladder.

In zebrafish, the heart is the first organ to develop and function and a beating heart forms by 22 hpf. By 48 hpf, the cardiovascular system is fully functional and exhibits a complex repertoire of ion channels and metabolic processes [8]. Zebrafish ERG (*ether-a-go-go-related gene*) is expressed in the early stages of zebrafish development and the amino acid sequence of the pore-forming domain of zebrafish ERG and human ERG are 99% conserved [9]. The zebrafish have been shown to be an excellent model for assessing drug-induced cardiotoxicity [1,3]. Although zebrafish and mammalian hearts differ in structure and zebrafish lack a pulmonary system, they exhibit similar functional characteristics, including (1) blood flows from a major vein atrium into an atrium; (2) blood moves through a muscular ventricle for delivery to the aorta; (3) valves direct blood flow; (4) a specialized endocardium musculature drives a high-pressure system; (5) an electrical system regulates rhythm; and (6) heart beat is associated with pacemaker activity [8,9]. Cardiac functions such as heart rate, contractility, rhythmicity and gross morphology can be visually assessed and some biomarkers can be evaluated using live and whole mount staining because zebrafish are transparent. Importantly, recent data have shown that zebrafish pharmacologic responses to well-characterized cardiotoxins are strikingly similar to responses in humans [1,3,9–12].

Zebrafish assays for cardiotoxicity testing

Drug effects on cardiac functions, including heart rate, rhythmicity, contractility and circulation are visually assessed in zebrafish at 48 hpf using a dissecting microscope. Mitoxantrone, terfenadine, clomipramine and thioridazine, shown to elicit cardiomyopathy, arrhythmia, negative inotropic effects or QT prolongation in humans, also caused bradycardia, abnormal atrial and ventricular (AV) ratio, decreased contractility and slow circulation in zebrafish.

Prolongation of the QT interval implies prolongation of the AV duration in a significant number of ventricular myocytes and is associated with an increased risk for Torsade de Pointes (TdP), a serious heart arrhythmia that often leads to death [13–15]. One hundred small molecules were recently tested and results showed that 22 of 23 drugs that cause QT prolongation in humans consistently caused bradycardia and blocked AV conduction in zebrafish [10]. QT prolonging 2:1 AV block was also observed in zebrafish that exhibited compromised HERG, the α-subunit of Ikr channels [12]. At sublethal concentrations, mitoxantrone, terfenadine, clomipramine and thioridazine caused hemorrhage and pericardiac edema. These visual assays are simple and well suited for predicting adverse cardiotoxic drug effects in humans.

Adult zebrafish electrocardiogram (ECG)

The FDA recently required preclinical testing for QT prolongation for all new drugs. The QT interval measures the time between the start of a Q-wave and the end of the T-wave in the heart's electrical cycle. Drug-induced QT prolongation is usually caused by drug binding to the ERG, which encodes for the inwardly rectifying potassium channel (Ikr) and is essential for maintaining normal cardiac functions [16]. Currently, new compounds are screened in assays that measure cardiac electrophysiological activity in cell culture or in mammalian models. These methods include a potassium channel binding assay, an ionic current assay, a repolarization assay and *in vivo* assessment frequently in dogs. Recent ECG studies show that heart functions in adult zebrafish are similar to heart functions in humans (Fig. 2), including the PR interval (activation of atrial action potential and conduction to the ventricle), the QRS complex (activation of the ventricle) and the QT interval (duration of ventricular action potential) [11–13]. Compared with the *ex vivo* patch clamp assay or *in vivo* mammalian ECG, advantages of performing ECG in adult zebrafish are (1) the amount of compound required is much lower than in mammals; (2) the cost of testing is lower than mammals ($1.00 per zebrafish versus $100 per rodent, and >$1000 per dog); (3) animal husbandry is comparatively cheap and easy; (4) zebrafish ECG can be combined with a broad repertoire of other zebrafish assays to rapidly profile potential toxicity during preclinical studies; and (5) use of zebrafish can reduce reliance on drug testing in higher vertebrates.

Hepatotoxicity assessment in zebrafish

Drug-induced liver injury has been recognized by the pharmaceutical industry as a major toxicological problem. Tests that are

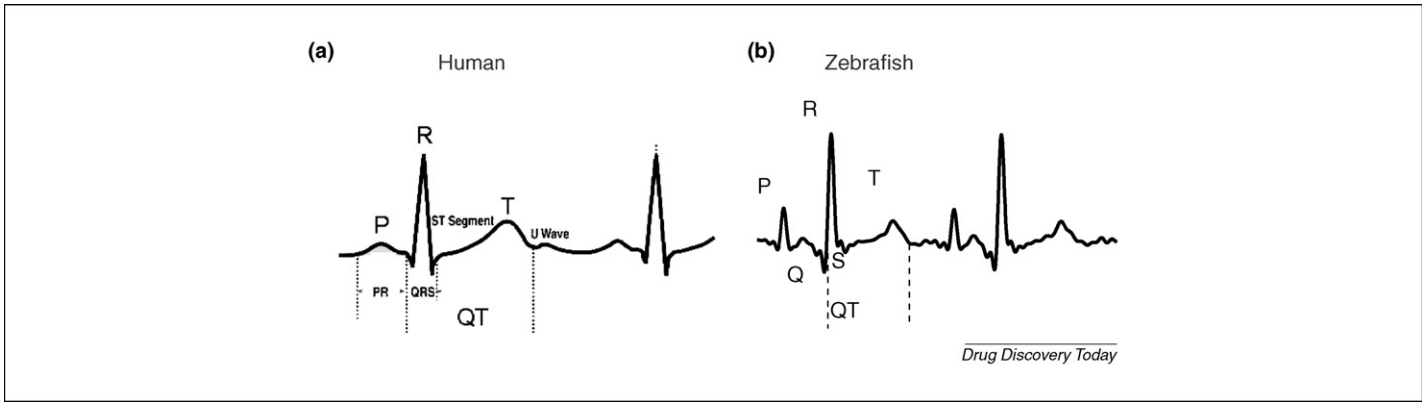

FIGURE 2

Comparison of human and zebrafish electrocardiograms (ECG). Humans (a) and adult zebrafish (b) exhibit similar ECG patterns. The P-wave, the first small peak, represents atrium excitation, followed by the QRS complex, which represents ventricle excitation. The T-wave, which follows the large R-wave, represents ventricle repolarization. The PR interval represents activation of atrial action potential and conduction to the ventricle; the RR interval measures one cardiac cycle, which can be converted to heart rate and QT interval.

useful for evaluating drug-induced hepatotoxicity in laboratory animals fall into four primary categories: (1) serum enzyme tests; (2) hepatic excretory tests; (3) assessment of alterations in the chemical constituents of the liver and (4) histological analysis. Tests for assessing repair and recovery of liver parenchyma and apoptosis are also used to study the effects of chemicals on the liver. Recent studies reveal that, as a general defense against xenobiotic chemicals, zebrafish exhibit mechanisms equivalent to mechanisms in mammals, including enzyme induction and oxidative stress [17,18]. Many zebrafish homologs of mammalian lipid metabolizing enzymes are present in the zebrafish liver, including 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (an enzyme which catalyzes the reaction during which acetyl-CoA condenses with HMG-CoA), HMG-CoA lyase (an enzyme that plays an essential role in breaking down dietary proteins and fats for energy) and members of the peroxisome proliferator-activated receptors (PPARs) [19]. PPARs are a group of nuclear receptors that exist across species and are intimately connected to cell metabolism and differentiation. Since zebrafish complete primary liver morphogenesis by 48 hpf and liver is fully formed and functioning by 72 hpf, zebrafish can be used as a convenient model for assessing hepatotoxicity.

Reporter enzyme assays

Native enzymes, including biotin and carboxylase, present in zebrafish liver and gut have been measured using an enzyme reporter assay, and results showed that after treatment with merbarone and carbamate, two mammalian liver toxicants, zebrafish exhibit organ-specific toxicity similar to effects in mice and humans [20].

Cytochrome P450 assays

Two cytochrome P450 (CYP) enzymes, CYP3A4 and CYP2D6, catalyze the majority of known drug-metabolizing reactions and most of these reactions are localized to the liver. Many clinically relevant drug–drug interactions are associated with inhibition and/or induction of these two CYP enzymes [21]. Although not all members of the mammalian CYP families and subfamilies have been identified in zebrafish, orthologs of CYP3A have been characterized [22] and a CYP3A ortholog, designated CYP3A65, has been shown to be homologous to the human CYP3A subfamily [23]. Using human CYP-specific substrates, CYP3A4 and CYP2D6 functional activity assays have been performed in zebrafish after drug treatment and CYP3A4 was upregulated in zebrafish treated with dexamethasone, similar to the CYP3A4 response in humans [24]. These results were consistent with a recent report showing that low doses of dexamethasone increased zebrafish CYP3A65 transcription, whereas high doses did not have this effect, assessed by *in situ* hybridization [23]. These results underscore the high degree of functional CYP conservation among species [25].

Visual assessment of liver necrosis

As an initial screen for drug-induced hepatotoxicity, drug effects on zebrafish liver tissue can be assessed visually without the need for complicated surgical procedures. As shown in Fig. 3, untreated zebrafish exhibited clear liver tissue, whereas after brefeldin A treatment, zebrafish liver was amorphous and gray, indicating necrosis [20].

Liver histopathology

Histopathology can be performed on zebrafish samples after drug treatment. Similar to effects in mammals, exposure with gamma-hexachlorocyclohexane induces fat deposits and fibrosis in zebrafish [26]. After treatment with valproic acid and simvastatin, liver phenotypes similar to those observed in mammalian fatty liver disease (FLD) were observed. Conventional Masson's trichrome staining combined with morphometric analysis can be used to quantify levels of collagen in liver tissues [27].

Hepatotoxicity assessment in adult zebrafish

Protocols for assessing hepatotoxicity in adult zebrafish have recently been optimized. Test compounds can be administered by injection or oral intubation, similar to drug delivery methods used in mammalian models. After drug dosing, liver histopathology can be performed and liver function enzymes, such as alanine transaminase (ALT), can be assessed in serum [28].

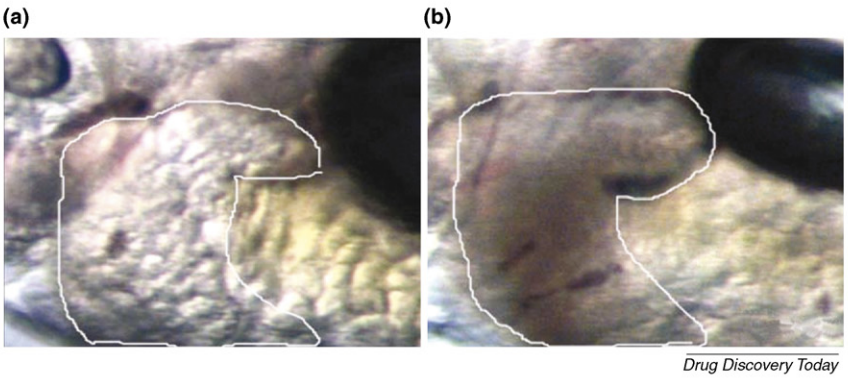

FIGURE 3

Visual assessment of liver necrosis in zebrafish. Untreated 144 hours postfertilization zebrafish exhibited clear liver tissue (a), whereas, after treatment with 5 μM brefeldin A, zebrafish liver was amorphous and gray (b), a color change linked to liver necrosis.

Neurotoxicity assessment in zebrafish

Neurotoxicity is another leading cause of drug withdrawal and examples of costly high-profile cases of neurotoxicity include diamthazole, vinyl chloride and clioquinol [29]. Neurotoxicity profiles of numerous approved drugs are incomplete and many cause neurotoxic side effects [29–31]. Increasing public concern about drug- and chemical-induced neurotoxicity and the inadequacy of current toxicological testing requirements resulted in initiation of a US Congressional review of Federal research and regulatory programs by the Office of Technology Assessment in 1988. Current approaches for assessing neurotoxicity in mammals rely on behavioral, morphological (neurohistopathology) and biochemical assays. However, compared with biochemical methods, behavioral testing and neurohistopathology are labor-intensive, require specialized training and are subjective. The application of biochemical markers for neurotoxicity testing is an area of ongoing research within Federal agencies and the research community. Although several biochemical markers, including changes in enzyme activity and protein phosphorylation, have been examined, these approaches have not proven useful for detecting toxicity in specific types of neurons. Compared with histopathology, biochemical assays that correlate brain functions with metabolism have not been rigorously tested [31]. For the reasons mentioned above, development of rapid assay methods and new animal models to predict neurotoxicity are urgently needed.

Zebrafish embryos and larvae are exceptionally well suited for neurotoxicity studies that combine cellular, molecular and genetic approaches. Because zebrafish are transparent for several dpf, specific neurons and axon tracts can be visualized *in vivo* using differential interference contrast microscopy (Nomarski microscopy) or by injecting live dyes. Specific types of neurons can be visualized in fixed intact zebrafish by immunohistochemistry or *in situ* hybridization. The function of individual neurons can be elucidated by inducing specific neural damage using behavioral- and bio-assays. In addition, the small size of early stage zebrafish permits performance of quantitative whole animal assays in a 96-well microplate format for neurotoxicity screening.

Neurotoxins previously tested in zebrafish

Zebrafish are susceptible to various toxins and have been used as an alternative model for assessing neurotoxic effects of drugs and industrial chemicals [32,33] as well as for identifying potential neuroprotectants [34]; many results in zebrafish have been strikingly similar to results in mammals. Neurotoxins tested in zebrafish include dopaminergic neurotoxins, non-NMDA type glutamate receptor (AMPA) agonists or antagonists, nicotinic acetylcholine receptors (nAChRs) antagonists or acetylcholinesterase (AChE) inhibitors and NMDA receptor antagonist (Table 1) [35–37]. The ability to examine the entire nervous system visually, including the brain, in live animals by staining a single slide makes zebrafish an exceptionally convenient model for assessing neurotoxicity.

Assessment of glial fibrillary acidic protein (GFAP) – a universal marker for neurotoxicity

Toxicity in the nervous system results in damage to, or death of, neurons and glial cells. Astrogliosis, which is activation of astrocytes (a glial cell type), has been linked to all forms of neuronal damage and this process has been shown to play a key role in neuronal repair [31,38]. The hallmarks of astrogliosis include (1) hypertrophy of astrocytic processes and (2) upregulation of astrocyte intermediate filament proteins, particularly GFAP, an astrocyte-specific marker. Increased GFAP levels in mammalian brain lysates have been observed (1) after neurotoxicity was induced by nitrile compounds and methamphetamine [39], (2) in ischemic brain injury [40] and (3) in Parkinson's disease [41]. Increased level of GFAP (astrogliosis) is an accepted indicator of neurotoxicity and measurement of GFAP level is now included in the neurotoxicity screening panel recommended by the US Environmental Protection Agency (EPA). Recently, the zebrafish GFAP gene was cloned and the corresponding protein was found to be highly conserved and to exhibit functions similar to those in mammals [42]. A microplate-based whole animal zebrafish GFAP ELISA assay has been used to assess neurotoxicity in zebrafish after the treatment with known neurotoxic compounds. GFAP level increased more than twofold in zebrafish treated with 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD) [43], a known mammalian neurotoxin [44],

TABLE 1

Neurotoxins tested in zebrafish [35–37]

Neurotoxin category	Representative neurotoxins tested
Dopaminergic neurotoxins	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) 6-Hydroxydopamine (6-OHDA) Rotenone Paraquat
Non-NMDA type glutamate receptor (AMPA) agonists or antagonists	Domoic acid (DA) Tetrodotoxins 6-Cyano-7-nitroquinoxaline-2,3-dione α-Latrotoxin Picrotoxin Strychnine
Nicotinic acetylcholine receptors (nAChRs) antagonists	Bungarotoxins Cobratoxins
NMDA receptor antagonist	DL-2-Amino-5-phosphonovalerate (AP-5)

confirming that a microplate GFAP assay can be used as a general neurotoxicity marker for drug screening.

Assessment of dopaminergic neuron-specific toxicity

In mammals, 6-hydroxydopamine (6-OHDA) has been shown to induce oxidative stress and dopaminergic neuron loss, which has been linked to pathogenesis of Parkinson's disease and to some types of neurotoxicity [45,46]. In zebrafish, drug effects on dopaminergic neurons can be assessed by whole mount immunostaining with an antityrosine hydroxylase antibody. α-Tyrosine hydroxylase (TH) catalyzes the first step in the catecholamine biosynthetic pathway and can be used as a biomarker for catecholaminergic neurons, including dopaminergic, noradrenergic and adrenergic neurons; all catecholaminergic neurons in zebrafish have been shown to be dopaminergic neurons [47,48]. By 96 hpf, there are several TH-positive cells in four clusters in the forebrain (anterior) and midbrain (posterior); the dopaminergic neurons are located primarily within the posterior clusters [3].

Assessment of neuronal apoptosis

Neuronal apoptosis has been observed in mammals after treatment with taxol (a chemotherapeutic drug), TCDD (a known teratogen and neurotoxin), neomycin (an antibiotic) and retinoic acid (an oxidized form of vitamin A). Apoptosis has been observed in several types of neuronal injuries and in various neurodegenerative diseases [49–51]. Similarly, in zebrafish, severe neuronal apoptosis has been observed in the brain and spinal cord after treatment with taxol using both TUNEL and acridine orange staining [2].

Assessment of motor neurons

In mammals, ethanol has been shown to affect neuronal proliferation and motor neuron survival [52]. Primary motor neurons in untreated zebrafish exhibit an organized, stereotypical vertical pattern and compound effects on motor neurons can be assessed by conventional immunostaining with axon-specific antibodies. Compared to untreated controls, after ethanol treatment, primary motor neuron loss was observed in the somite region of zebrafish (Fig. 4) [33].

Assessment of drug effects on motility

Complementary to other techniques for assessing drug-induced neurotoxicity, zebrafish locomotor activity can be analyzed visually or by continuous image acquisition using an infrared camera. The number of movements, duration and distance traveled in a given time period can be assessed. Pentylenetetrazole (PTZ), a convulsion-inducing agent, has been shown to cause seizures in zebrafish and the behavioral, electrophysiological

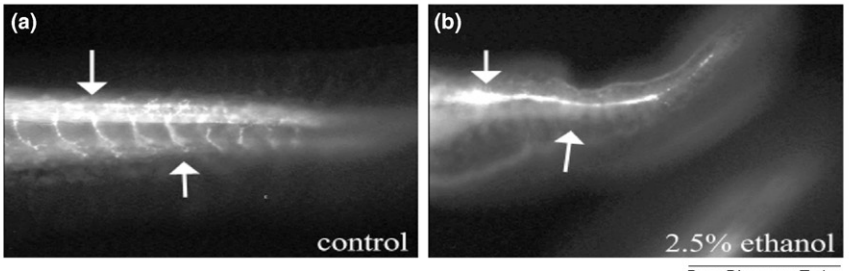

FIGURE 4

Zebrafish motor neuron damage after ethanol treatment. Forty-eight hours postfertilization zebrafish were untreated (a) or treated with 2.5% ethanol (b). Anti-Znp1 antibody was used to visualize motor neurons in the tail region (anterior: left; posterior: right) and ethanol treatment resulted in motor neuron loss.

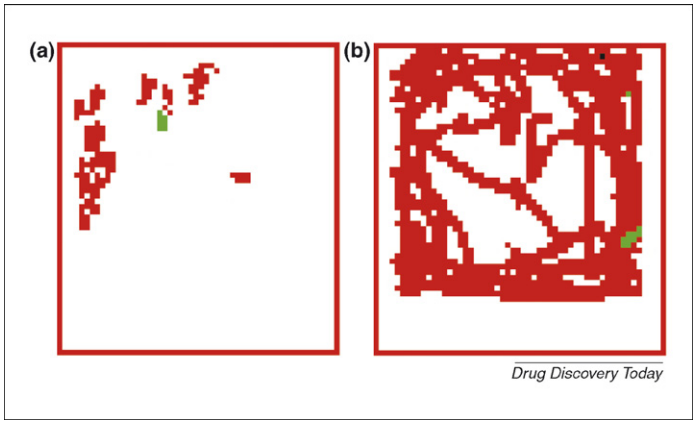

FIGURE 5

Graphical representation of movement of a single zebrafish. Graph (a) shows movement of an untreated 144 hours postfertilization zebrafish and graph (b) shows zebrafish treated with 7.5 mM pentylenetetrazole (PTZ). Each red bar represents a distinct movement during a 15-min period. The increasing amount of red color shown for PTZ-treated zebrafish indicates increased locomotor activity.

and molecular changes in PTZ-treated zebrafish are comparable to effects observed in a rodent seizure model [53]. A graphical representation of motility after incubation with PTZ is shown in Fig. 5.

Developmental toxicity assessment in zebrafish

After the teratogenic effects of thalidomide were recognized in 1966, the FDA established protocols to be used for assessing drug effects on reproduction and development before approval for human use. In addition, because of concerns about chemicals in human food, the EPA issued similar guidelines for pesticides in 1982 and for industrial chemicals in 1985. According to current international guidelines, developmental toxicity testing involves exposing pregnant animals, usually rats or rabbits, to compounds and subsequently assessing toxic effects on fetuses. Since the early 1980s, alternative methods for assessing developmental toxicity have been developed, including *in vitro* cell differentiation assays using either primary cell cultures or immortalized cell lines, the *in vitro* rodent whole embryo culture test and the *in vivo* frog embryo teratogenesis assay (FETAX) [54]. Unfortunately, *in vitro* tests have been of limited value in predicting the effect of drugs on human embryonic and fetal development [55]. Disadvantages of the *in vivo* FETAX assay include that xenopus do not have a characterized chemical metabolic pathway and they are insensitive to halogenated aromatic hydrocarbons [56], therefore, response to some toxicants in xenopus differs from the response in mammals. Although mammalian models remain the gold standard for assessing developmental toxicity, acceptance of zebrafish as a predictive model is increasing in the USA, mirroring its widespread use in Europe for environmental assessment [3,57].

There are strong rationale for performing developmental toxicity studies in zebrafish embryos including [58]; (1) zebrafish is a distinct species and has been shown to be sensitive to compounds which exhibit teratogenicity *in vivo* in mammals; (2) developmental processes in zebrafish are highly conserved; (3) in contrast to rodent embryo culture, which is limited to early organogenesis, zebrafish embryos can be cultured up until advanced organogenesis; and (4) the zebrafish genome is well characterized and dysmorphology phenotypes linked to genomic targets can potentially enable rapid evaluation of mechanisms of action for compound-induced teratogenicity. Recently, EPA established a five-year US$100M ToxCast Program (http://www.epa.gov/comptox/toxcast), a multifaceted initiative to prioritize chemicals requiring more rigorous toxicological testing [59]. EPA included zebrafish as the sole animal model for assessing environmental contaminants and it selected developmental toxicity as an initial screen. This ZETAX™ assay format assesses several morphological and functional endpoints which are similar to those used in conventional mammalian reproductive toxicity studies (segment II).

TABLE 2

Assessment of compound teratogenicity in zebrafish

Compound	Zebrafish					Zebrafish teratogen	Mammalian teratogen	Correct prediction
	LC_{50} (μM)	Body	Heart	Liver	GI			
Dimethylphthalate	ND	No	No	No	No	None	None	Yes
Retinol	1146	Yes	Yes	Yes	Yes	Weak	Weak	Yes
Valproic acid	110.9	Yes	Yes	No	No	Weak	Weak	Yes
BMS[a]-A (RAR alpha agonist-1)	4.7	Yes	Yes	Yes	Yes	Potent	Potent	Yes
BMS[a]-B (RAR alpha agonist-2)	1.3	Yes	Yes	Yes	Yes	Potent	Potent	Yes
BMS[a]-C (RAR beta agonist)	15	Yes	Yes	Yes	No	Potent	Potent	Yes
Penicillin G	ND	No	No	No	No	None	None	Yes
Isoniacide	ND	No	No	No	No	None	None	Yes
Ascorbic acid	ND	No	No	No	No	Cannot determine	None	Cannot determine
Diphenyl-hydantoin	ND	No	No	No	No	None	Weak	No
Cytosine arabinoside	1478	Yes	Yes	Yes	Yes	Potent	Potent	Yes
9-*cis*-Retinoic acid	258.4	Yes	Yes	Yes	Yes	Potent	Potent	Yes

None: TI (teratogenic index) ≤ 1; weak: TI > 1 but ≤ 15 and potent: TI > 15.

[a] Bristol-Myers Squibb.

In a recent study aimed at validating zebrafish as a predictive model for assessing developmental toxicity, 12 blinded reference compounds provided by Bristol-Myers Squibb Pharmaceutical Research Institute (New Brunswick, NJ, USA) were evaluated for developmental toxicity in zebrafish embryos using an LC_{50}/visual assessment protocol. In this study, zebrafish were treated with test compounds from 6 to 120 hpf and a range of developmental abnormalities in the body, heart, liver and gastrointestinal tract (GI) were observed [58]. The resulting data were subsequently compared to teratogenicity properties identified *in vivo* in mammals and assay predictivity and accuracy were assessed (Table 2). The assay was ranked good (>70% <80%) for specificity and excellent (>80%) for sensitivity. Furthermore, this assay format presented a 75% success rate in identifying nonteratogenic compounds and a 100% success rate in identifying teratogens [60]. This study demonstrated an approach for simple and direct assessment of developmental toxicity in zebrafish.

Summary

Zebrafish has been shown to be a predictive animal model for assessing drug toxicity and safety. Assessment of a large number of compounds from diverse drug classes is necessary to further validate the model. Although it is unclear how ADME after drug delivery in fish water compares to ADME after delivery by other routes of administration, performance of studies in adult zebrafish using conventional drug delivery methods such as injection and intubation promises to increase the use of this model for comparative animal studies. Reported results show that inter- and intra-laboratory standards vary widely, confounding interpretation of drug-induced toxicity and limiting wider acceptance of this model organism; thankfully, cooperation among academic and industry laboratories to develop standard operating procedures for performing compound assessment in zebrafish is increasing. Adaptation of conventional technology and instrumentation including microplate readers, automated sample handling devices, digital image systems, fluorescence activated cell sorters, electrophysiology sensors, ultrasound and biomicroscopy have dramatically improved the ability to perform quantitative whole animal analysis in zebrafish. Continued developments in the nanotechnology arena offer increasing promise for unlocking the full potential of this small animal model for drug screening and significantly improving zebrafish assay efficiency and reliability.

Conflict of interest

Both authors are employees of Phylonix. Ms McGrath is also an inventor of patents assigned to Phylonix.

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