

Zebrafish Embryo as an Emerging Model Organism in Neurodevelopmental Toxicity Research

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ABSTRACT

Zebrafish is a model organism that has become increasingly popular in recent years due to some of the advantages it has when compared to traditional model organisms. Its genetic similarity with humans has contributed significantly to the elucidation of the molecular mechanisms underlying diseases. Moreover, external fertilization and rapid embryonic development of zebrafish embryos have made it attractive in many research areas. The genome of humans and zebrafish are found to be highly conserved having 76-82 % of the disease genes in humans that are also present in zebrafish. Zebrafish have been used in different studies in several concepts of neurogenesis. Unlike mammals, the external development of a zebrafish embryo makes it accessible for experimental manipulation in central nervous system research. It was observed that neurotoxic agents induced similar responses to other vertebral models in zebrafish embryos, whose brain development and blood-brain barrier were similar to those of other vertebrates. This review provides brief information about the availability of zebrafish embryos in neurodevelopmental toxicity research while giving brief information on embryogenesis and neurogenesis in zebrafish. Evaluation of neurotoxicity and the specific effects of various neurotoxins on motor and dopaminergic neurons, neuronal proliferation, mobility, and neurodevelopment are also explained.

Keywords: Zebrafish, zebrafish embryo, neurotoxicity, neurodevelopment

INTRODUCTION

The zebrafish (*Danio rerio*) is gaining popularity as a model organism in many human diseases as a small tropical freshwater fish. Since the zebrafish shares a high genetic similarity to humans, it is a powerful model for both identifying the actual mechanisms and improving therapeutic strategies in many diseases, including metabolic and neurological diseases (1-3). The genome of humans and zebrafish are found to be highly conserved having 76-82 % of the disease genes in humans that are also present in zebrafish. On the other hand, nearly 20-24 % of genes in zebrafish are duplicated (4). A high genetic similarity to humans enables discoveries of many genetic mutations leading to different hereditary neurological diseases in humans (5). To use the zebrafish

as a model organism, it is necessary to understand its physiology and behavioral characteristics well. Zebrafish belong to the class of teleosts, which constitutes 99% of the *Actinopterygii* species and emerged 350 million years ago. It inhabits vegetated freshwater streams and stagnant waters such as rice paddies in South and East Asia (Pakistan, Burma, India, and Nepal). Zebrafish live and lay eggs in shallow water at temperatures ranging from 24°C to 28°C. Appropriate conductivity and pH values are between 10 and 271 µS and pH 6.0-8.0, respectively (6). They feed on mosquito larvae and other insects. In the laboratory, embryos can hatch within 3 days after fertilization and mature within 2-3 months under good conditions (7). Zebrafish differ from previously used vertebrate models because of their external fertilization, transparency in development, small body



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size, short development time, production of a high number of eggs (200/female/week), and low maintenance costs (1).

Neurogenesis is defined as the progress of undifferentiated neural progenitor cells producing neurons that are mature and functional. The neural progenitor induction and the cell division phase are the first steps in neurogenesis that expands the progenitor pool. The specification of progenitors and differentiation into post-mitotic neurons follow the first step. All these steps are regulated to produce the various neuronal and glial cell types that will at the end form the mature central nervous system (CNS). Zebrafish have been used in many different studies on several concepts of neurogenesis. Unlike mammals, the external development of a zebrafish embryo makes zebrafish accessible for experimental manipulation in CNS research (8). Their small size and optical transparency allow observation of all processes *in vivo* throughout the early developmental stages, the early development of measurable behaviors, and the simplicity of the zebrafish nervous system compared to other model organisms provide advantages in nervous system studies (9,10). Thanks to major advances in genetic, embryological, and optical techniques in recent years, zebrafish have become a unique vertebrate model for studying neurogenesis (8).

To date, most of the studies have focused on neurogenesis in the embryonic stages of zebrafish (8-10). However, recent research has reported that the brain of mature zebrafish can be a very useful model for the study of neurogenesis in the adult (1). Furthermore, in the early 1960s, new neurons were suggested to arise in the adult mammalian brain, in the hippocampus and olfactory bulb through labeling newly synthesized DNA by *in-situ* [3H]-thymidine (11). The production of new neurons is observed mainly in the subventricular zone and the subgranular zone of the telencephalon in rodents and primates (12). Unlike mammals, teleosts such as zebrafish present a higher potential of reproduction because nearly 16 regions of proliferation were found in different areas of adult zebrafish brains (13,14). Approximately six thousand cells are produced in the adult zebrafish brain every 30 minutes which is the main reason for the popularity of zebrafish to study neurogenesis of the adult (15).

Embryogenesis in Zebrafish

In zebrafish, seven developmental stages are defined as the zygote, cleavage, blastula, gastrula, segmentation, pharyngeal, and hatching (exit from chorion) in the embryonic period (16). The time between the first cleavage (split) of the newly fertilized egg, approximately 40 minutes after fertilization, is called the zygote stage. The zygote is approximately 0.7-0.78 mm in diameter at fertilization. At the first cleavage 45 minutes after fertilization, two cells of equal size are formed. The cells or blastomeres then divide at intervals of about 15 minutes; 2-cell structure (0.75 h), 4-cell structure (1 h), 8-cell structure (1.25 h), 16-cell structure (1.5 h), 32-cell structure (1.75 h), and the 64-cell structure (2 h) is formed (16,17).

Blastula refers to the period from the 128-cell structure, in which

the blastodisc is ball-like, to the beginning of the gastrulation period. Structures formed during the blastula period are; 128-cell structure (2.25 h), 256-cell structure (2.5 h), 512-cell structure (2.75 h), 1 k-cell structure, high stage (3.3 h), oblong phase (3.7 h), sphere phase (4 h), dome phase (4.3 h), and 30% epiboly structures (4.7 h). Stages of the gastrula stage include the formations of 50% epiboly (5.3 h), germ ring (5.7 h), shield stage (6 h), 75% epiboly (8 h), 90% epiboly (9 h), and bud stage (10 h). The gastrula period is considered to be finished when the epiboly is complete and the tailbud is produced (16,18).

The identification as an embryo until the end of the third day continues as the larval stage after hatching or not. The pectoral fin, gills, and jaw continue to develop rapidly. Monitoring the development of endodermal structures such as the digestive tract is difficult at this stage (48 h) due to their deep location. The larval stage includes individuals that are no longer embryo but are not yet juvenile. The swim bladder is swollen, foraging and avoidance behaviors have started (72 h). The juvenile stage is the stage in which sexual maturity is not achieved but most of the adult characteristics are acquired. In the laboratory, this period starts after about 4 weeks and lasts up to 6-12 weeks, depending on the breeding and rearing conditions (up to 89 days, according to www.zfin.org). The adult stage in fish is defined as the production of healthy gametes and secondary sexual characteristics that reach reproductive capability (16-18). Zebrafish's developmental timeline is summarized in Figure 1 (19).

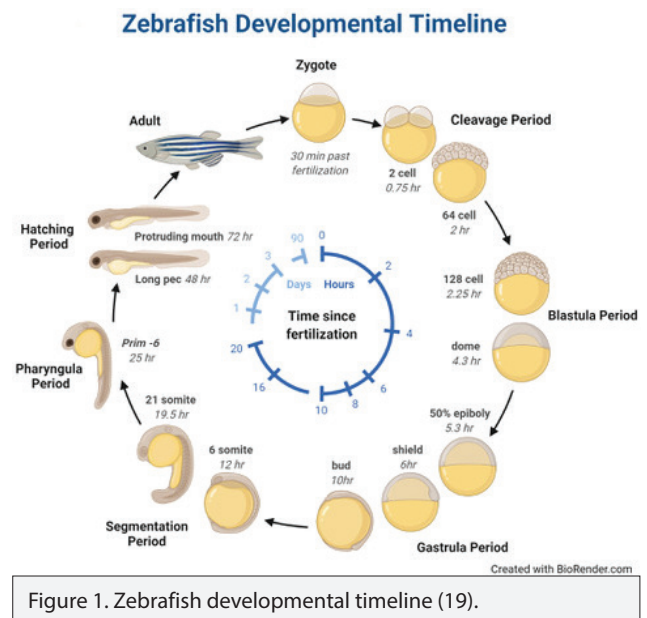


Figure 1. Zebrafish developmental timeline (19).

Neurogenesis in Zebrafish Embryos

Neuroectoderm is the first structure to be specified during the vertebrate nervous system development and this is named "neural induction" that occurs at the beginning of embryonic development (7,8). The mesodermal layer formed at the beginning of gastrulation folds in and interacts with the above ectoderm (20,21). This possible layer of mesodermal secretes some local factors that activate or inhibit neural activation in

the ectodermal layer. Cells in the ectodermal layer are thought to differentiate into neural tissue (22).

In recent years, vertebrate neural induction has been demonstrated by extrinsic signaling factors. The bone morphogenetic protein (BMP) is one of these factors to inhibit neural activity through epidermal regulation (23). Another factor is the wingless-integrated (Wnt) proteins that are among the earliest signals to regulate the formation of the neural plate primordium (24). The expression of *Wnt8* has been shown in non-axial mesendoderm required for the recovery of hindbrain fate as well as the expression of *gbx1* in the hindbrain (25). The anterior neural plate is organized through the complex interaction of the Wnt signals and the Wnt antagonists, including *TLC* (26).

Signaling pathways, as well as molecules that regulate the substantial processes during embryogenesis, are substantial for developmental biology. The Wnt pathway is often related to different diseases, especially with endocrine diseases as well as cancer, showing that these diseases are due to some impaired developmental processes (27). In our previous studies, we have shown that different endocrine-disrupting chemicals such as bisphenol A and DEHP activated the Wnt/ β -catenin pathway in zebrafish embryos as evidenced by the increased expressions of *c-myc* which is the target gene and *wnt3a* (28,29).

Another extrinsic factor is the fibroblast growth factor (Fgf). Fgf and the intrinsic transcription factors including the B1 (SoxB1) gene family are among the important cell proliferation modulators (30,31). Significant progress has been made showing that it is based on complex interactions between these molecules. These secreted proteins allow neural fate to occur in the dorsal ectoderm and permit the formation of the neural plate. In particular, it is important to note that neural ectoderm is determined by members of the SoxB1 family (27). To date, the members of the SoxB1 family have been shown in zebrafish as *sox1 (a/b)*, *sox2*, *sox3*, and *sox19 (a/b)* (28). *Sox2* may be observed in the neural progenitors of zebrafish embryos and also in the neural stem cells of adult zebrafish brain (23,32). Thus, *Sox2* is a very important factor needed for the maintenance of neural progenitor traits and their related functions for the vertebrates (33). In summary, SoxB1 members together with the inhibitory role of BMP signaling and induction of Fgf signaling are substantial determinants to assert the neural stem cell pool during the zebrafish embryo early gastrulation stages (8).

In the neural plate, the initiation of neurogenesis is maintained through the proneural genes in the zebrafish which is observed during the late gastrulation phase. The *neurog1 (neurogenin1)* is one of the first proneural genes to produce transcription factors together with *achaete-scute1 (asc1)*. These genes coding transcription factors are referred as the bHLH genes (33). On the other hand, *shh* has a key signal inducer role and is needed for the concentration-dependent activation of *neurog1* and *ascl1*, which causes the formation of thalamic nuclei in the vertebrates (34). In the midbrain-hindbrain boundary region of the neural tube of isthmus rhombencephali, regulation of the cellu-

lar fate takes place via Fgf8 signaling (35). Through the initiation of neurogenesis, progenitor pool cells induce the formation of the differentiated cells within the midbrain-hindbrain boundary region (36). The activity of the progenitor pool is evident through *her5* and *her11* expression together with the inhibition of E(Spl) factors. These determinants inhibit the expression of the proneural genes including *neurog1*, *ascl1a*, as well as cyclin-dependent kinase inhibitors (37,38).

The SoxB1 transcriptional targets are the E(Spl) family members which are the Her and Hes genes. They are named as the hairy genes, *her 3,5,6,9* and *11*. Ectopic Her and Hes gene expressions cause the downregulation of the expression of *neurog1* but loss-of-function trials reveal up-regulated *neurog1* expression (38). SoxB1 together with the hairy genes leads to neurogenesis inhibition which is an important mechanism increasing the cell pool (39). Therefore, there is a critical mechanism that modulates the neurogenetic gradients during the embryonic central nervous system and this process is maintained spatially through the inhibition of neurogenesis, regulated through the local inhibition of the Her and Hes proteins (8,40).

Zebrafish Embryo as a Model Organism in Neurodevelopmental Toxicity Studies

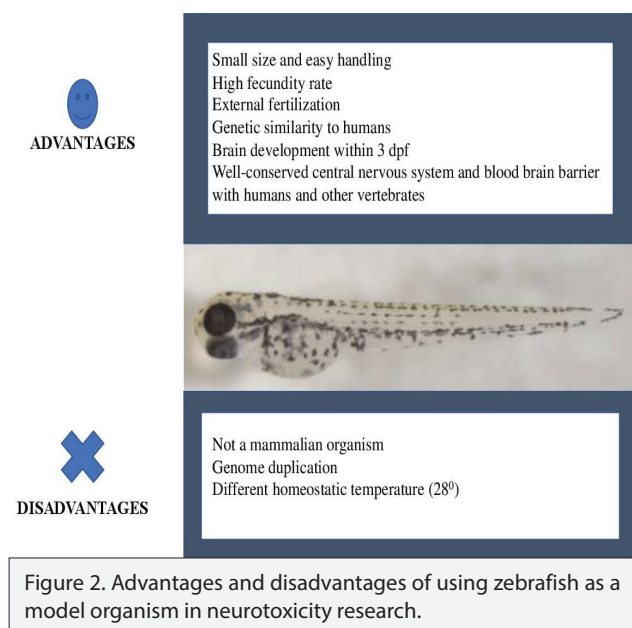
Neurotoxicity is characterized as a detrimental effect on the structure and/or function of the nervous system and may result from exposure to food additives and environmental toxicants, as well as drugs used during chemotherapy, radiation therapy, and organ transplantation. Neurotoxicity is the second leading cause of drug withdrawals after cardiovascular toxicity. Diamthazole and vinyl chloride aerosol can be given as examples of some drug withdrawals due to neurotoxicity (41). Although many approved drugs have been shown to cause neurotoxic side effects, they do not have well-defined neurotoxicity profiles. For instance, an antibiotic, chloramphenicol and antituberculosis drugs such as ethambutol and isoniazid lead to cause optic neuritis (42). Antiepileptic drugs and chemotherapy agents, as well as aminoglycosides may lead to some cerebellar syndromes including dysarthria (43). Other drug-related neurological complications have also been reported including cognitive impairment, headache, and neuromuscular disorders (41-43).

Different approaches are defined to determine the neurotoxicity of a molecule and they are generally divided into three groups as the behavioral, the morphological or histopathological and finally the biochemical approach that evaluates the cellular metabolism and functions that have been changed. Currently, the preclinical analysis of neurotoxicity is generally based on the evaluation of behavioral abnormalities and/or the observation of clear histopathological lesions in the nervous tissue (44).

The most efficient methods to analyze neurotoxicity are the animal behavioral studies including aggression, survival, feeding, motor function, reproduction and, maternal behavior. These parameters may be adversely affected due to exposure to vari-

ous neurotoxins. However, most effects on the central nervous system are mute and result in alterations in function, not easily identifiable in human studies, and are likely to cause minor changes in parameters including temperament, emotion, mood and, cognition that have not been sufficiently studied in animals (45). Histological methods are effective only when lesions in the nervous system are widespread and can be detected by immunohistochemical staining methods. Several specific biochemical markers (eg, alterations in the activity of enzymes and, protein phosphorylation) have been studied, but have only been shown to be beneficial for detecting specific types of neurotoxicity. There is a need to develop rapid assay methods and new animal models to predict neurotoxicity (46).

Zebrafish are very useful for neurotoxicity studies merging different approaches including genetic, cellular and, molecular methods. The main advantages of using zebrafish as a model organism in neurotoxicity research include its external development and genetic similarity to humans whereas, the main limitation may be suggested as its translational value (1). The main advantages and limitations of using zebrafish as a model organism in neurotoxicity research are summarized in Figure 2.



Because the zebrafish embryo is transparent and develops rapidly, the formation of specific neurons and axon pathways can be screened in living embryos through differential interference contrast microscopy or by injecting live dyes (47). Special neuron types can be screened in whole mounted and fixed embryos by using immunohistochemistry or in situ hybridization (48). Motor neuron activity can be screened in vivo by using calcium imaging and patch-clamp recording (49).

The blood-brain barrier is a special type of capillary endothelial system that serves to protect the brain from substances that may be harmful in the bloodstream. It also provides the nutrients that are needed for the brain to regulate the main physio-

logical functions. This barrier includes a complex system with special endothelial cells, macrophages, and astrocytes (50). The passage of macromolecules is prevented through tight junctions that are present between the endothelial cells (51). Lipid soluble, small molecules having molecular weight less than 400 daltons can pass the blood-brain barrier (50). The blood-brain barrier is also present in zebrafish and zebrafish is considered an excellent model organism to evaluate the molecular interactions as well as the permeability of drugs (52). It has been shown by Evans blue staining that blood-brain barrier functions are observed at 3 dpf zebrafish and the transparent feature of zebrafish embryo provides a major advantage to study the penetration of drugs during development (53).

Evaluation of Neurotoxicity in Zebrafish

Zebrafish have a population of early neurons that are named as "primary neurons". These neurons are part of a comparably basic nervous system that differentiate to regulate the movement of the larva (54). The distribution and projection properties of specific primary neurons have been explained in literature thoroughly (55). At 24 h after fertilization (24 hpf), these primary neurons of zebrafish differentiate, and in this timeline, substantially large neuronal cells may be identified by using Normarski optics *in vivo*. Brain ventricles of zebrafish are formed at 48 hpf (56). Zebrafish don't have a skull and, their body length may increase from 1 mm at 1 dpf (day post fertilization) to 5 mm at 6 dpf. Whole animal staining can be done to comprehensively evaluate the whole nervous system. Classical neurotoxins investigated in zebrafish include dopaminergic neurotoxins including rotenone, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), and paraquat. non-NMDA-type glutamate receptor agonists or antagonists including domoic acid, 6-cyano-7-nitroquinoxaline-2,3-dione, alpha-latrotoxin, and picrotoxin; nicotinic acetylcholine receptor antagonists such as bungarotoxins and cobratoxins or acetylcholinesterase inhibitors; and the NMDA receptor antagonist, DL-2-amino-5-phosphonovalerate (AP-5) (1).

Neurotoxicity can be evaluated in zebrafish after exposure to a neurotoxic compound in the optic nerves, motor, and dopaminergic neurons as well as the myelin sheath. Moreover, the molecular mechanisms including oxidant/antioxidant pathways, apoptosis and proliferation assays can be observed directly in transparent fish. Accordingly, zebrafish have been shown to be sensitive to different neurotoxins such as ethanol, acrylamide, retinoic acid, neomycin, and tetrachlorodibenzo-p-dioxin (57,58).

Effects of Neurotoxins on Motor Neurons and Neuronal Proliferation

In mammalian models, the effects of drug-induced neurotoxicity such as ethanol toxicity have been reported on motor neurons and also on neuronal proliferation. Ethanol has been reported to alter motor functions through the induction of motor neuron death and the inhibition of neuronal proliferation. Ethanol has also been found to affect brain and motor functions in humans (57).

Similarly, the compounds that lead to neurotoxicity in humans have been reported to induce complementary neurotoxicity in zebrafish. Ethanol caused defects in the optic nerves as well as in the motor neurons and altered neuronal proliferation. 6-OHDA led to the oxidation of neurons and loss of dopaminergic neurons and L-2-hydroxyglutaric acid caused neuronal apoptosis (58).

Effects of Neurotoxins on Dopaminergic Neurons

In the midbrain, dopaminergic neurons are the major source of dopamine (DA) in the mammalian CNS, and the loss of DA is related to Parkinson's disease which is the most important human neurological disorder (1). Dopaminergic neurons are found in the substantia nigra pars compacta, which is rich in DA and contains neuromelanin and high levels of iron. Dopaminergic neurons have a substantial role in controlling many different brain functions such as, voluntary movement and a wide range of behavioral parameters including mood, reward, addiction, and stress. To evaluate the compound effects on dopaminergic neurons, 6-OHDA was administered as a neurotoxin that destroys catecholaminergic terminals, and then immunostained using an anti-tyrosine hydroxylase antibody. Results showed that 6-OHDA led to dopaminergic neurotoxicity (1,59). Similarly, in mammals, 6-OHDA has been found to cause oxidative stress and result in neuronal death (59).

We have previously shown that as a neurotoxic pesticide that is capable of crossing the blood-brain barrier, rotenone exposure for 4 weeks led to decreased locomotor activity, dopamine, and serotonin levels and increased DOPAC and DOPAC/dopamine levels in zebrafish. Moreover, lipid peroxidation increased and antioxidant levels decreased suggesting the disrupted oxidant-antioxidant balance (60). We have also tested if exposure to rotenone disrupts the oxidant/antioxidant status in the intestine and brain of zebrafish and our results showed that lipid peroxidation increased whereas the activities of glutathione S-transferase and catalase decreased both in the intestinal tissues and brain of the rotenone exposed adult zebrafish (61).

Brain dopaminergic neurons of zebrafish embryos are sensitive to the MPTP. However, noradrenergic neurons in the medulla oblongata were not affected by MPTP exposure (62). Recently we have shown decreased locomotor activity in MPTP-exposed zebrafish embryos and morphine exerted neuroprotective effects against MPTP exposure by normalizing locomotor activity, acetylcholine esterase activity, Parkinson's Disease-related genes and oxidative stress (63). We have also shown the beneficial effects of 3-pyridineboronic in MPTP exposed zebrafish embryos by the amelioration of impaired locomotor activity and mitochondrial dysfunction (64). In zebrafish as the main dopamine-expressing neurons are located in the posterior tuberculum of the diencephalon decreased dopaminergic cells together with defects in swimming responses were also reported in zebrafish embryos (65).

Effects of Neurotoxins on Mobility

One of the biggest challenges in developing methods for assessing neurotoxicity is its association with behaviorally altered

neuromorphological, neurochemical, and neurophysiological changes that are often considered abnormal movement. In zebrafish larvae, locomotor or motility patterns are stage-specific and behavioral abnormalities are easily discernible (66). Embryonic motor behavior develops sequentially; The early period consists of transient spontaneous curling contractions, followed by the appearance of twitching in response to touch, and later the ability to swim (67). At 4 dpf, the embryos are free-swimming and spontaneously change direction with characteristic ranges of speed and distance. Zebrafish also exhibit basic behaviors that include memory, non-associative learning, conditioned responses, and social behaviors such as schooling (68).

Various studies have shown that zebrafish mobility can be monitored using a computer-assisted motion detector (69). It has been shown that this assay format is useful for assessing compound-induced neurotoxicity (including seizures). Similar to results in mammals, pentylentetrazole, a gamma-aminobutyric acid antagonist known to cause convulsions in humans, has been suggested to induce seizures in zebrafish. Moreover, electrophysiological, molecular changes, as well as the behavioral responses in zebrafish treated with pentylentetrazole are similar to the effects reported in the rodent seizure model.

Effects of Neurotoxins on Neurodevelopment

Developmental neurotoxicity includes changes in behavior, neurohistology, neurochemistry, neurophysiology, or overall dysmorphology due to exposure during development. The developing embryonic brain is more susceptible to confusion caused by toxins due to the differentiation of specific cell types and the disruption of delicate processes that occur only during development, such as proliferation and migration of newly formed neurons (70). In addition, the developing brain is more exposed to blood-borne toxins before blood-brain barrier formation. Therefore, critical assessment of developmental neurotoxicity requires the use of an embryonic model.

In one study, well-characterized compounds were selected to validate zebrafish as a model of developmental neurotoxicity. Embryos were exposed by quasi-static immersion at stage 6 hpf to 96 or 120 hpf; The induction of brain apoptosis or necrosis seen with the compounds was confirmed as an indicator of developmental neurotoxicity, and an effect on motor neurons in the caudal third of the embryo was shown to be associated with expected defects in motility. Different environmental pollutants were screened for their effects on brain apoptosis, axon tract and motor neuron formation, and catecholaminergic neurons in developing zebrafish, and compounds that act on several neurotoxicity parameters were identified. Some compounds even showed extensive toxicity at all neuroanatomical endpoints (71, 72). Overall, these results indicate a strong correlation with mammalian data, indicating that zebrafish is a suitable animal model for developmental neurotoxicity screening.

Harmful algal blooms (HABs) are formed when the growth of algae colonies derived from simple plants in sea and freshwater

Table 1. Neurodevelopmental genes in zebrafish and their functions.

Gene name	Gene symbol	Function
neurogenin 1	<i>neurog1</i>	Basal forebrain dopaminergic neuron determinant
myelin basic protein a	<i>mbp</i>	Structural constituent of myelin sheat
α 1-tubulin	<i>α1-tubulin</i>	Brain development
synapsin IIa	<i>syn2a</i>	Synaptogenesis
sonic hedgehog signaling molecule a	<i>shha</i>	Regulation of neurogenesis
ELAV like neuron-specific RNA binding protein 3	<i>elavl3</i>	Regulation of neurogenesis

are out of control. HABs have toxic effects on people and animals. Recently HABs have been also shown to cause potential neurotoxins. For instance, domoic acid (DomA) is a potent HAB neurotoxin that can accumulate in shellfish and finfish under certain environmental conditions. Exposure to the DomA resulted in long-lasting behavioral deficits in rodents and primate models (73). In humans and nonhuman primates, oral exposure to DomA induced gastrointestinal effects, neurological symptoms, seizures, memory impairment, and limbic system degeneration. Rodents are less sensitive than humans or nonhuman primates and induce behavioral abnormalities. DomA exhibits similar neurotoxic effects across species from sea lions to zebrafish (74).

The drinking water of many different geographic areas including the US, Latin America, Asia, Africa is co-contaminated by neurotoxic pollutants such as inorganic arsenic and fluoride. Inorganic arsenic and fluoride affect the neurodevelopment of children due to the exposure during the post and pre-natal periods. Although, co-contamination with inorganic arsenic and fluoride can create more risk than their exposure alone, there is a lack of information about the concurrent role in the deterioration of gut microbiota that acts as an organizer in neurodevelopment (75).

In zebrafish measuring the expression levels of different biomarkers is a sensitive and fast way to find out the alterations in the gene expression sequences in case of neurotoxin treatment. Different genes of the nervous system were suggested as potential neurotoxicity markers in zebrafish embryos that were treated with ethanol using RT-PCR. Neuronal stem cell transcripts were either decreased or increased during development, and the overexpression of an astrocytes marker was significant. It has been demonstrated that evaluation of the gene expression profile of the brain is useful in analyzing the neurotoxicity of many chemicals during development (76).

The microarray hybridization method has also been used to determine the effects of environmental toxins in zebrafish embryos and organ and cell-specific alterations in the gene expressions were detected by using in situ hybridization (77). When the genome profile and in situ studies were performed in methyl mercury exposed zebrafish embryos the gene expres-

sions were found to be altered significantly in brain different neuronal subregions (78).

To evaluate the developmental effects of neurotoxins in zebrafish embryos gene expression levels of some specific markers are determined. For instance, myelin basic proteins (MBP) was determined in propofol (anesthetic) exposed zebrafish and decreased MBP expression was evident in the central nervous system (79).

Some other neurodevelopmental genes include *syn2a* and *α 1-tubulin* that were found to be down-regulated in chlorpyrifos and 1,3-dichloro-2-propyl-phosphate exposed zebrafish embryos (80). On the other hand, *neurog1* is also an important neuro-developmental gene that is shown to be affected in ibuprofen and diclofenac exposed zebrafish embryos (79,81). Ans also, *elavl3*, *syn2a* and, *shha* have been shown to be down regulated in triphenyl phosphate exposed zebrafish embryos (82). Zebrafish genes having specific roles in neurodevelopment are listed in Table 1.

Autism spectrum disorder (ASD) is a crucial disorder of neurodevelopment (83). Valproic acid, a pharmaceutical agent used to treat seizures, is suggested to be an ASD inducer, and defective social behavior has been shown in valproic acid exposed zebrafish embryos (84). The preliminary findings of our ongoing study show that valproic acid affects the expressions of *shank3a*, *adsl* and *tsc1b* that are genes related to ASD.

The utilization of gene profiling patterns can be suggested as a useful endpoint for neurotoxicity research to reveal the developmental neurotoxicity of some different and potentially toxic compounds.

CONCLUSION

Although zebrafish embryo and adult regulatory pathways are mainly different they share some similarities during the production of functional neurons. Embryonal neurons originate from the neuroectodermal epithelium whereas, adult neurons are derived from glia cells. Accordingly, it is important to reveal the specific differences between the embryo adult neurogenesis in terms of neurotoxicity research. Understanding the specific

pathways of neurodevelopment will enable the improvement of therapeutic strategies. The translational value of zebrafish will also be improved through the incorporation of knowledge into the mammalian brain in terms of neuronal circuits.

Our review provides a brief overview of the emerging potential the zebrafish embryo has in neurotoxicity research during development. Although having some limitations, using the zebrafish as a model organism in neurotoxicology provides significant advantages. Many toxicity endpoints can be combined with different assays to evaluate the impact of large numbers of potential neurotoxic compounds.

We suggest the increasing employment of zebrafish in testing chemicals will speed up this process and facilitate the understanding of neurotoxicity mechanisms. Accordingly, we believe that the use of zebrafish and zebrafish embryos in research will become widespread, as *in vivo* imaging and analysis platforms, which are developing day by day, become more accessible.

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