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Zebrafish as a Versatile Model System for Investigating Neurodegenerative Diseases: Focus on Alzheimer's Disease Pathogenesis and Drug Discovery

Logeshwari B[1†], Srikanth Jeyabalan[1†*], Chetan Ashok[1], Gayathri Veeraraghavan[2], Krishnaraj K[3],

[1]Department of Pharmacology, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai 600116, Tamil Nadu, India

Email id: logeshwarib@sriramachandra.edu.in (L.B.); srikanth.j@sriramachandra.edu.in (S.J.); chetana@sriramachandra.edu.in (C.A.)

[2]Centre For Toxicology & Developmental Research, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai 600116, Tamil Nadu, India Email id: gayathriv@sriramachandra.edu.in. (G.V.)

[3]Formulation and development (R&D), Himalaya Wellness Company, Bengaluru, Karnataka, India Email id: krishnrajsk@gmail.com. (K.K.)

***Corresponding Author:** Dr. Srikanth Jeyabalan

Associate Professor, Department of Pharmacology, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Chennai 600 116, Tamilnadu, Indiasrikanth.j@sriramachandra.edu.in (S.J.), Tel: +919094020093

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Abstract

The zebrafish (Danio rerio), a prominent model organism since the 1970s, has proven influential in neurodegenerative disease research, notably Alzheimer's disease (AD). This review examines the zebrafish's distinct advantages in unraveling AD pathogenesis. Genetic benefits, coupled with transparent embryos enabling real-time disease visualization, have facilitated effective AD models. The zebrafish shares significant genetic similarity with humans, particularly in neurodegenerative disease-related genes, enhancing its role in drug discovery. The article focuses on zebrafish models for AD, emphasizing the amyloid cascade hypothesis and tau hyperphosphorylation. Aβ accumulation in zebrafish is pivotal for cerebrovascular function. Applications include studying genetic and environmental factors, investigating therapeutic compounds, and understanding molecular mechanisms. High-throughput drug screening with zebrafish expedites potential drug candidate identification. The zebrafish's rapid development cycle and prolific offspring minimize research time and costs, ensuring a consistent sample pool. Neurotransmitter systems, neuroanatomy, and genome similarities with humans further enhance its value in AD research. Limitations, such as unpredictable chemical absorption and the zebrafish's regenerative capacity, are acknowledged as challenges in AD modeling.

Despite controversies surrounding Aβ functionality in zebrafish, the model remains intriguing for neurodegeneration studies, providing opportunities for comprehensive AD insights. The future of AD research with zebrafish involves refining models, exploring new domains, and establishing a transgenic model expressing both Aβ and Tau pathologies, solidifying the zebrafish's status as a promising neurodegenerative disease research model.

Introduction:

The zebrafish (*Danio rerio*) is a tropical freshwater teleost that belongs to the Cyprinidae family and was introduced to biological research by George Streisinger in the 1970s. Zebrafish were originally used to monitor water pollution, teratogenesis and toxic substances. In 1981, Streisinger et al. presented the zebrafish as an effective animal model similar to the mammalian system (Mullins et al., 1994). The use of zebrafish as a model organism for studying neurodegenerative diseases has provided valuable insights and advancements in drug discovery. The unique genetic and experimental advantages of zebrafish, coupled with the ability to easily manipulate their genomes, have allowed researchers to create zebrafish models of various neurodegenerative diseases, such as Alzheimer's and Parkinson's disease (Wang et al., 2023). These models have helped researchers understand the underlying mechanisms of these diseases and identify potential therapeutic targets. Furthermore, the optical transparency of zebrafish embryos allows for real-time visualization of disease progression and drug response, making them an ideal model for high throughput screening of compounds for drug discovery (Saleem et al., 2018). Researchers are able to conduct high resolution live in vivo imaging of the entire central nervous system (CNS) without relying on small invasive optical windows typically utilized in traditional mammalian research models (Cong et al., 2017). The straightforward utilization of transgenes through the binary UAS/Gal4 system enables the labelling of cells and structures, as well as the expression of human genes in all, specific, or individual neurons (Kawakami et al., 2016). Additionally, the zebrafish's fast development cycle and ability to produce a large number of offspring

significantly decrease both time and expenses involved in research. This abundance of samples also helps to minimize variations between individuals (Lieschke et al, 2007). Moreover, the transparent nature of zebrafish embryos and larvae makes them ideal for optical manipulation and imaging of neural activity. Lastly, the high similarity in genome between zebrafish and humans positions them as a valuable model for studying human diseases in fields such as pharmacology and developmental genetics (Randlett et al., 2015). Due to their sensitivity to various pharmacological agents, zebrafish serve as a perfect model in the realm of pharmaceutical science.

Zebrafish development progresses at a remarkably rapid pace. By the time they reach 12 weeks of age, zebrafish become sexually mature and have the ability to generate numerous embryos on a weekly basis (Lawrence et al., 2012). These embryos develop outside the mother's body, enabling researchers to easily manipulate them for experimental purposes and observe their growth from the very beginning. Remarkably, all vital organs and the central nervous system (CNS) are fully operational within 72 hours after fertilization (Schmidt et al., 2013). The zebrafish brain exhibits both structural and functional resemblances to the mammalian brain, along with similar neural circuitry that encompasses the majority of crucial neurochemical signal transduction pathways (Blader et al., 2000). The neuroendocrine function in the hypothalamus of zebrafish closely resembles that of mammals, while the layer structure of the zebrafish cerebellum is also comparable to that found in humans (Pitchai et al., 2019). The neurotransmitter system remains conserved in zebrafish from the early stages of development, displaying distinct similarities to mammalian systems, including clusters of dopaminergic cells in the olfactory bulb and hypothalamus. Additionally, zebrafish produce neurotransmitters such as dopamine (DA), serotonin (5-HT), acetylcholine (ACh), histamine (HA), glutamate, and GABA (Kaslin et al., 2001).

The zebrafish exhibits a significant degree of genetic similarity to humans. Through an extensive sequencing study, it has been revealed that zebrafish possess at least one ortholog

for over 70% of all human genes. This includes numerous risk genes associated with various neurodegenerative diseases in humans. Notably, these genes play a role in the development of Parkinson's disease (SNCA, PINK1, LRRK2, and Parkin), familial Alzheimer's disease (PSEN1 and PSEN2), amyotrophic lateral sclerosis (SOD1, TARDBP, C9orf72, and FUS), and Huntington's disease (Huntingtin) (Howe et al., 2013). Zebrafish have been proven to be a viable research tool in the study of neurodegenerative diseases (NDD), particularly Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). Recent advancements in imaging techniques, behavioural tests, and high-throughput drug screening methods in zebrafish have led to the discovery of new drug targets for the treatment of NDD. Utilizing zebrafish as a platform for drug discovery in NDD offers a time- and cost-efficient approach (Wasel et al., 2020).

In spite of the numerous benefits, embryonic fish and their larval stages do not always serve as an optimal model for studying human neurodegenerative diseases that typically manifest in adulthood or old age. Similar to mouse models, zebrafish may not exhibit the same pathological consequences resulting from gene deletion or mutation as observed in humans. In this review, we outline the contributions of the zebrafish model in advancing our understanding of the pathogenesis of four neurodegenerative diseases in humans (Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease). Furthermore, we discuss how recent advancements in gene editing and manipulation of neuronal activity have enhanced the potential of zebrafish as a robust model for investigating human neurodegenerative diseases.

Alzheimer’s disease

Alzheimer’s disease (AD), the most prevalent type of senile dementia, is represented by the extracellular repository of amyloid-beta plaques and intracellular accretion of neurofibrillary tangles in the brain, causing memory, intelligence, personality and other disorders that eventually lead to death from traumatic brain injury (Pakdeepak et al., 2005). The illness

results in progressive shrinkage of the hippocampus and parietal region of the brain (Frisoni, et al., 2010). GWAS have discovered multiple genes in high-risk areas that are responsible for controlling immune responses, indicating a possible involvement of microglia in the development of Alzheimer's disease (McQuade et al., 2019). It is worth noting that Aβ plaques can be observed in the brain prior to the manifestation of cognitive decline. Conversely, NFTs have been linked to neurodegeneration, cell demise, and impairment in cognitive abilities (DeTure et al., 2019). Scientists have put forth a number of compelling hypotheses, based on previous research that could potentially trigger the onset of Alzheimer's disease (AD). These hypotheses include the amyloid cascade hypothesis, tau hyperphosphorylation, the cholinergic hypothesis, neuroinflammation, and the role of metal ions. To delve deeper into the understanding of AD pathogenesis, researchers have created various pharmacological and transgenic (Tg) AD models using zebrafish.

Potential applications of zebrafish in Alzheimer disease research

Zebrafish have shown great potential in Alzheimer's disease research, with various applications that can enhance our understanding of the disease and aid in the development of therapeutics. These applications include: -

- Studying the impact of genetic and environmental factors on Alzheimer's disease development: Zebrafish models allow researchers to manipulate genes and expose the fish to environmental factors to study their impact on the development of Alzheimer's disease. This provides valuable insights into the complex interactions between genetic predisposition and environmental influences in the onset and progression of the disease.
- Investigating the effects of potential therapeutic compounds: Zebrafish models offer a unique opportunity to visualize and assess the effects of candidate drugs in real time, providing crucial information on their efficacy and potential side effects. This

contributes to the identification of promising drug candidates for further preclinical and clinical studies.

- Understanding the cellular and molecular mechanisms underlying Alzheimer's disease: Zebrafish models allow for the visualization and manipulation of specific cell types and molecular processes involved in the pathology of Alzheimer's disease. This provides a comprehensive understanding of disease mechanisms and potential targets for therapeutic intervention.
- Screening for novel therapeutic targets: The amenability of zebrafish to high-throughput screening makes it an excellent platform for identifying new molecular targets that may play a critical role in Alzheimer's disease pathogenesis. This can lead to the discovery of novel pathways for therapeutic intervention.

Zebrafish models of Alzheimer's disease

The theory known as the 'amyloid hypothesis' was initially introduced in the 1980s. It suggests that the accumulation and formation of Aβ aggregates, which are neurotoxic, play a significant role in the development of Alzheimer's disease (Fagan et al., 2009). Nevertheless, recent studies have shown that the accumulation of Aβ in the brain is not influenced by the age at which Alzheimer's disease (AD) begins or its severity (Luna et al., 2013). However, Aβ still plays a significant role in the pathology of AD. Interestingly, in zebrafish, Aβ serves a vital function in maintaining the proper functioning of the cerebrovascular system, as higher levels of Aβ have been linked to abnormal branching of blood vessels in the developing hindbrain (Bhattarai, et al., 2017). Aβ peptides are derived from the amyloid precursor protein (APP), which is a glycoprotein embedded in the cell membrane. The cleavage of APPβ involves the participation of β- and γ-secretases, resulting in the formation of Aβ peptides. On the other hand, the production of P3 peptide from APPα involves the action of α- and γ-secretases. These processes give rise to various isoforms of Aβ with different lengths, with Aβ42 and Aβ40 being particularly toxic. The accumulation of Aβ leads to the

formation of oligomers, protofibrils, and fibrils, ultimately resulting in the development of Aβ plaques. When Aβ42 is injected into the zebrafish brain through cerebroventricular microinjection, it triggers the accumulation of Aβ protein, apoptotic cell death, activation of microglia, and degeneration of synapses (Bhattarai et al., 2016). Human Aβ was successfully recapitulated in the hindbrain ventricular injections, resulting in the manifestation of all characteristic features of AD. These included the rapid formation of β-sheet aggregations, heightened neuronal toxicity, cell death, and impaired motor functioning (Vaquer-Alicea et al., 2019). It is intriguing to note that while progenitor proliferation remained intact, neurogenesis showed a decline as the individual aged (Bhattarai et al., 2020). A recent study conducted by the same research team further revealed, through single-cell transcriptomic data, that IL-4 plays a role in regulating serotonin production and subsequently influences the downstream regulation of brain-derived neurotrophic factor (BDNF) expression. This ultimately leads to enhanced plasticity and proliferation of neural stem cells in the adult zebrafish brain (Tomasiewicz et al., 2002).

The initial zebrafish tau model exhibited a temporary expression of human tau protein in neurons, resulting in the hyperphosphorylation and build-up of tau in neuronal cell bodies. This led to a disturbance in the cytoskeletal structure, closely resembling the neurofibrillary tangles observed in human diseases (Paquet et al., 2009). The validation of these initial tau transgenic models was confirmed through in vivo imaging, which showcased the swift hyperphosphorylation and aggregation of tau, alongside the occurrence of neuronal cell death (Cosacak et al., 2017). The expression and excessive phosphorylation of the mutant human P301L-tau in mature fish neuronal cells did not lead to any neurodegenerative effects, nor did it cause the formation of neurofibrillary tangles (NFTs) in the brain (Sonawane et al., 2018). A well-established zebrafish model with stable Tg, carrying mutations on the MAPT gene encoding microtubule-associated protein tau, such as Tau-P301L and Tau-A152T, has been

developed to accurately replicate the key pathological characteristics of tauopathies in Alzheimer's disease (AD) (Kim et al., 2010).

Zebrafish Models of Alzheimer's disease and Neurotoxicity

Investigating the etiology of Alzheimer's disease (AD) can be effectively done by utilizing environmental neurotoxins that trigger AD-like progression. AD patients often exhibit cholinergic dysfunction and cholinergic neuronal loss in their brains. This suggests that a possible cause of AD is a decrease in the synthesis of the neurotransmitter acetylcholine. To establish a neurotoxin-induced AD model in zebrafish, scopolamine, an antagonist of the acetylcholine muscarinic receptor, has been employed. Scopolamine has been found to induce learning deficits in zebrafish, but these deficits can be reversed by using acetylcholinesterase inhibitors like physostigmine (Greenamyre et al., 1989).

The mechanisms underlying the association between AD and the glutamatergic and GABAergic systems are still not fully understood (Louzada et al., 2004). However, studies have shown that the loss of glutamatergic neurons in the medial temporal lobe and hippocampal network is linked to excitotoxicity, which plays a role in the development of AD. Additionally, the positive effects of GABAergic compounds in improving cognitive impairment caused by Aβ further highlight the involvement of the GABAergic system in the pathogenesis of AD (Nada et al., 2016). The presence of Okadaic acid (OKA) in the AD zebrafish model leads to the inhibition of protein phosphatase 2A (PP2A), an enzyme responsible for dephosphorylating tau protein. This inhibition is associated with cognitive decline. Additionally, the zebrafish brain in this model shows the presence of Aβ protein aggregation, senile plaque, increased levels of phosphorylated tau (p-tau), and the activation of glycogen synthase 3β (GSK-3β) kinase (Wang et al., 2020).

Aluminum, a metal ion, is believed to have neurotoxic properties and is associated with the development of Alzheimer's disease (AD) (Senger et al., 2011). In experiments conducted on zebrafish exposed to aluminum trichloride (AlCl3), it was observed that concentrations ranging from 50 to 250 µM led to increased activity of the enzyme acetylcholinesterase (AChE) and impaired locomotor activity under acidic conditions (pH 5.8). These findings suggest that the increased AChE activity induced by aluminum is responsible for the development of AD (Lee et al., 2020). Another study investigated the neurotoxic effects of lead (Pb), a heavy metal, on zebrafish fibroblast cells. The results indicated that exposure to Pb caused alterations in the DNA copy number in a dose-dependent manner. Although most of the genes affected by these copy number alterations were associated with the amyloid precursor protein (APP) molecular pathway, further research is needed to determine the involvement of Pb in neurological diseases, particularly AD (Banote et al., 2020).

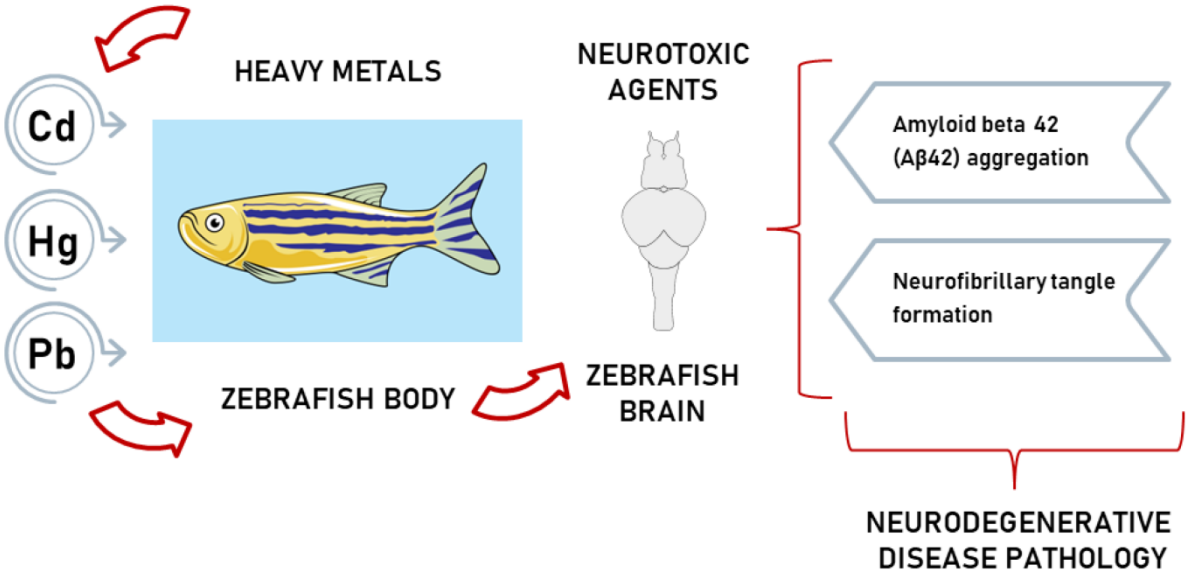


Figure 1. Zebrafish as a model for investigating the effect of heavy metals and other pollutants (Paduraru et al., 2023)

Study	**Method(s)**	**Aβ aggregation**	**Neuronal cell death**	**NSPC proliferation and neurogenesis**	**Other phenotype**
Nery et al., 2014	Aβ 1–42 (ventricular injections)	Not reported	Yes	Not reported	Increased tau phosphorylation. Impaired avoidance of aversive stimulus.
Bhattarai et al., 2017	Human TR-Aβ 42 (ventricular injections)	Yes	Yes	Yes	Increased microglia activation. Increased synaptic degeneration
Newman et al., 2010	Human Aβ 42 (expression in melanophores under mitfa promoter)	Not reported	Not reported	Not reported	Abnormal pattern and loss of skin pigmentation
Bhattarai et al., 2016	Human Aβ 42 (ventricular injections)	Yes	Yes	Yes	Formation of intracellular Aβ-sheets. Impaired conditioning and reduced learned anxiety response

Table 1 Zebrafish models of Aβ toxicity

Table 2 Zebrafish models of tauopathy

Study	**Method(s)**	**Target of tau phosphorylation**	**NFT formation**	**Other phenotype**
Bai et al., 2007	MAP-Tau4R mutation	Enolase-2 promoter – Neurons	Yes	
Lopez et al., 2017	Tau A152T mutation	PanN:Gal4VP15 driver – Pan-neuronal	Yes	Increased neuronal cell death
Tomasiewicz et al., 2002	FTDP-17 mutation	GATA-2 promoter – Neurons	Yes	Disruption of cytoskeletal filaments in cell axon
Cosacak et al., 2017	Tau P301L mutation	her4.1 promoter – NPCs (with radial glial identity) and neurons	No – Investigated in adult zebrafish	

Zebrafish Models of Alzheimer's disease with Genetic modifications

The expression of the disease-causing protein in the brain, heart, eyes, and vasculature of zebrafish with a mutated APP gene under the control of the appb promoter has been successfully achieved (Pu et al., 2017). This zebrafish model demonstrates behavioural symptoms similar to Alzheimer's disease and the presence of cerebral β-amyloidosis. Additionally, it shows neuronal loss and an enlarged perivascular space. By thoroughly understanding the characteristics of zebrafish gene duplication, we can potentially uncover the mechanisms underlying key disease-related genes that may be lethal in mammals (Wong et al., 2020). A significant area of interest in zebrafish AD research centers on the Presenilin (PSEN) genes, which have been linked to the hereditary types of AD (Veugelen et al., 2016). PSEN is a component of the γ-secretase complex, which is responsible for controlling cellular proliferation. Mutations in PSEN have been proposed to contribute to the production of Aβ peptides that form plaques (Kabir et al., 2020). In the context of disease pathology, PSEN mutations associated with AD have been demonstrated to expedite the catalytic breakdown of APP by γ-secretase, leading to heightened generation of longer Aβ peptides that are amyloidogenic (Sundvik et al., 2013). The zebrafish counterparts of PSEN1 and PSEN2 are psen1 and psen2, respectively. The elimination of psen1 in zebrafish leads to the loss of histamine neurons, and it is proposed that the histaminergic system plays a role in cognitive functions in Alzheimer's disease (AD) (Hin et al., 2020). Additionally, the zebrafish's genetic makeup has been modified to replicate the heterozygous K115fs mutation found in the human PSEN2 gene. As a result, this zebrafish model demonstrates an expedited aging process in the brain and immune responses associated with microglia, all while lacking any apparent histopathological abnormalities. However, in order to utilize this transgenic model for drug discovery purposes, it is imperative to obtain substantial evidence regarding the functionality of both PSEN1 and PSEN2 in zebrafish (Nery et al., 2014).

Exploration of Drug discovery using Zebrafish models of Alzheimer's disease

GSK3 is recognized for phosphorylating tau on various sites, and its isoform GSK-3β plays a vital role in the abnormal hyperphosphorylation of tau. In zebrafish, GSK3β functions similarly to its human counterpart. The inhibition of GSK-3β using lithium chloride has the ability to reverse cognitive impairments and the hyperphosphorylation of tau in an in vivo model of Alzheimer's disease. This model was created by injecting Aβ42 into the hindbrain ventricle of zebrafish embryos (Paquet et al., 2019). The Tau-P301L mutation zebrafish model demonstrated that the synthesized GSK3 inhibitor AR-534 effectively reduces tau hyperphosphorylation (Lo Monte et al., 2013). In their study, Monte et al. utilized zebrafish embryos to assess the safety and permeability of AR-A014418, compounds 9e, and 26 days as potential GSK inhibitors. The findings support the use of GSKβ inhibitors for Alzheimer's disease treatment. Nevertheless, there remains insufficient evidence to determine the most effective GSKβ inhibitor (Boiangiu et al., 2021).

The regulation of the cholinergic system plays a crucial role in the treatment of Alzheimer's disease (AD) by suppressing cholinesterase activity. Numerous drugs have been studied and identified for their potential in modulating this system. For instance, cotinine and 6-hydroxy-L-nicotine have been found to alleviate anxiety-like behaviour and memory impairment in zebrafish models induced by scopolamine. These drugs also demonstrated a reduction in oxidative stress and acetylcholinesterase (AChE) activity in the zebrafish brain (Capatina, et al., 2020). Similarly, Thymus vulgaris L. essential oil (TEO) showed promising results in improving zebrafish amnesia and anxiety, as well as reducing AChE activity and enhancing brain antioxidant capacity in the same zebrafish model. Gas chromatography analysis revealed that the main compounds present in TEO are thymol and p-cymene (Pan et al., 2019). Additionally, linarin, an active component of the flavonoid glycoside, exhibited protective effects against AlCl3-induced zebrafish dyskinesia by inhibiting AChE activity. The binding of linarin with AChE active sites was demonstrated through molecular docking

simulation (Hu et al., 2019). Behavioural symptoms were alleviated and AChE and BuChE activity was blocked by compounds derived from 3-(4-aminophenyl)-coumarin, particularly compound 4m. Compound 4m shows promise as a potential lead for drug design in the treatment of Alzheimer's disease (Reinhardt et al., 2019).

The prevention of tau hyperphosphorylation enables the identification of new drugs that can combat tauopathies associated with Alzheimer's disease (AD). LDC8, a cyclin-dependent kinase 5 (CDK5) inhibitor, has shown the ability to protect against neurodegeneration, neuroinflammation, and synapse density loss in a zebrafish model of AD induced by Aβ42 (Naini et al., 2018). This protection is achieved by modulating the phosphorylation of microtubule-associated proteins. Heparan sulfate, a highly sulfated polysaccharide, is closely linked to tau aggregates in AD (Koehler et al., 2019). The use of surfen or its derivative oxalyl surfen, both of which possess heparan sulfate antagonist properties, can reduce tau hyperphosphorylation and rescue neuronal and behavioral impairments in zebrafish models carrying the Tg mutation. Furthermore, certain GSK-3β inhibitors have demonstrated a decrease in tau hyperphosphorylation, suggesting that inhibiting p-tau may be a common approach to alleviate AD (Basnet et al., 2019).

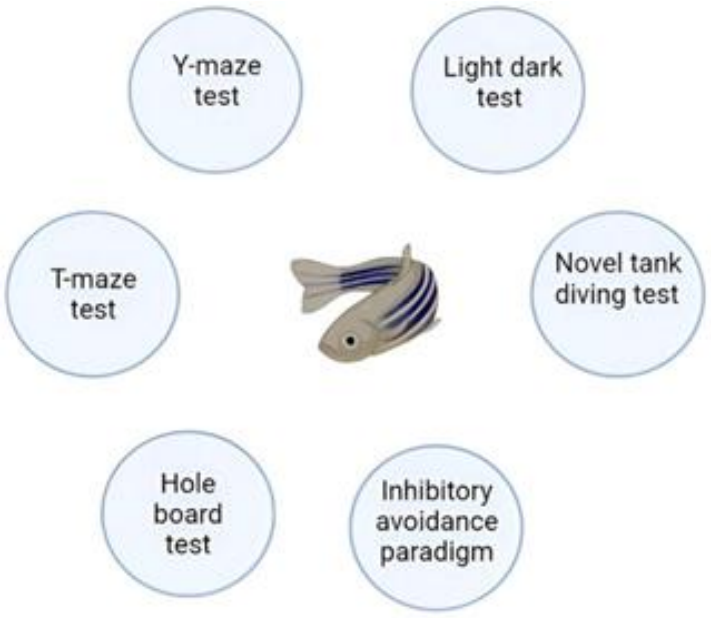
Neurobehavioral assessment of Zebrafish in Alzheimer's disease

Visible behaviors exhibited by zebrafish play a crucial role in determining the success of disease models or verifying the efficacy of drugs at the organism level. Zebrafish embryos undergo rapid development and transform into larvae within 72 hours after fertilization (hpf). By 48 hpf, the entire body plan is established. Metamorphosis occurs, and zebrafish reach the juvenile stage at 30 days post fertilization (dpf), with adulthood commencing at 90 dpf. The development of the brain-blood barrier (BBB) begins at 72 hpf. Additionally, zebrafish are diurnal creatures and possess advantageous visual, auditory, and olfactory functions. Zebrafish and their larvae offer distinct advantages and applications in research. Larvae have been extensively utilized for large-scale, high-throughput behavioral analysis, whereas adult

zebrafish exhibit a more sophisticated range of behaviors (Vaz et al., 2018). Administering drugs to zebrafish is convenient in their aqueous environment, allowing for the evaluation of drug efficacy, bioavailability, and toxicity at various stages. However, it is important to note that this model has limitations, as the route of drug administration differs from that in humans and mammals, who typically take drugs orally (Zanandrea et al., 2018). The possibility of drug absorption through the skin or other organs cannot be disregarded, potentially leading to discrepancies in drug efficacy assessments. The diverse array of behaviors observed in zebrafish larvae and adult fish underscores their potential as a promising model for neurobiological investigations.

Figure 2 Zebrafish as model for analysing diverse array of behaviours (Shenoy et al., 2022)

Novel Tank Diving Test



In order to evaluate the cognitive abilities of zebrafish, their locomotor and exploratory activities are observed through the novel tank test. Each zebrafish is placed individually in the novel tank, and their swimming path is recorded. The tank is divided into three horizontal sections: the bottom, middle, and top areas. The zebrafish's vertical exploration can be assessed by measuring the number of entries and the time spent in the bottom or top area.

Additionally, anxiety-related behaviors can be identified by observing the number and duration of freezing bouts or erratic movement during the test. To facilitate the measurement of a large sample, cuvettes can be utilized as novel apparatus arranged in parallel in front of the camera (Wang et al., 2017).

Locomotor activity

In order to assess the spontaneous swimming behaviour of zebrafish, individuals are positioned on a light and optical stage equipped with an infrared light source. Digital videos are then recorded to monitor the horizontal or vertical movement of zebrafish in water over a specific time period. The swimming pattern of zebrafish can be observed through an automatically generated trajectory using specialized software systems. Various representative parameters of swimming behavior, such as mean velocity, total moving distance, and turning angle, are analyzed simultaneously. Another method to measure zebrafish movement is by counting the total number of lines they cross. Vertical lines are drawn at equal intervals to divide the vessel into multiple zones. This particular method is more suitable for adult zebrafish as their behavior tends to be less random compared to larvae (Williams et al., 2002).

T/Y-Maze Test

Spatial discrimination tasks, such as the T/Y-maze, have been utilized to assess learning and memory in zebrafish, similar to how they are used in rodents. In the T/Y-maze test, a positive stimulus is employed as a reward to motivate zebrafish when they exhibit the correct response in locating an objective. For instance, the T-maze consists of a starting zone, a long arm, two short arms, and two chambers. Food is used as the positive stimulus and is delivered in one of the chambers for zebrafish training. The fish are deprived of food for a period of one or two days and then placed in the start zone to perform the test. The behavior of the fish is recorded using a camera, and the time spent in finding the objective is measured. A

simplified apparatus and procedure have been developed based on the T-maze. The tank is divided by a central white divider, and there is sufficient space at the bottom for the zebrafish to freely swim between the two sections. A red card attached to one end enables the fish to differentiate between the two halves of the tank. The correct response is considered when the zebrafish present on the side with food within 5 seconds after a light tap at the center of the tank (Rubinstein et al., 2006).

Disadvantages of zebrafish as an AD model

There are numerous benefits to using zebrafish as a model system for studying Alzheimer's disease (AD), but there are also a few limitations when using them in translational neuroscience research. One advantage is that it is easy to make pharmacological modifications in the fish by adding desired chemicals to the water. However, the quantification of chemical compounds entering the fish is unpredictable because substances can be absorbed randomly through the gills and skin due to the fish's exposure to the entire aquatic environment (Xi et al., 2011). Additionally, the lack of knowledge about the zebrafish-specific Aβ peptide is a drawback. Further research is needed to determine if the post-translational processing of APP in humans is also present in zebrafish (Bhushan et al., 2016).

Zebrafish have a unique ability to regenerate neurons along their brain axis throughout their lives, unlike mammals. In an interesting finding, researchers discovered that zebrafish injected with Aβ1–42 peptide showed regeneration of neurons, specifically neural cell/progenitor cell proliferation and neurogenesis. They examined the regenerative capacity in both old and young fish to understand the impact of aging and Aβ deposition on neuroregeneration. The study revealed that in Aβ-induced neurodegeneration, microglia become activated to prevent synaptic degeneration and promote neurogenesis (Murphy et al., 2010). This establishes a potential connection between neurodegeneration, neuroinflammation, and neurogenesis. While this may pose a challenge to the feasibility of

the AD model, it also presents exciting opportunities for exploring the molecular mechanisms of signaling pathways that play a crucial role in neuron regeneration. This research will undoubtedly contribute to our understanding of the molecular programs necessary for regenerating the mammalian central nervous system.

Debate around the use of zebrafish for research on AD

Aβ plaques are widely considered to be crucial in the development of AD. They result in synaptic dysfunction, disruption of neuronal connectivity, and neuron death. Interestingly, studies using the zebrafish model of AD suggest that Aβ may also play a role in maintaining cerebrovascular functions (Mueller et al., 2004). These studies demonstrate that a deficiency in Aβ leads to a reduction in cerebrovascular branching and vessel length in the developing hind brain of zebrafish embryos. Additionally, other researchers have reported that Aβ is involved in regulating angiogenesis in the human umbilical cord vein and zebrafish hind brain. This contradicts its functionality in humans, where it causes cerebrovascular dysfunction and cognitive defects. One possible reason for this discrepancy is that Aβ in teleosts, including zebrafish, is different from Aβ in other vertebrates, including humans. The researchers further explain that drugs targeting Aβ production failed in recent clinical trials because the potential role of Aβ in regulating angiogenesis may interfere with the mechanism of action of the drug. Further research on the molecular mechanism of this functionality will help us better understand this discrepancy. This also highlights the existence of other non-amyloid hypotheses (such as the cholinergic hypothesis, tangles hypothesis, calcium hypothesis, and mitochondrial hypothesis) in AD (Smith et al., 2009). Several zebrafish AD models have already been established, focusing on Tauopathy and the cholinergic hypothesis, but there is still a need for better models that exhibit a combination of several pathologies (Shenoy et al., 2022) . Elaborate studies involving real-time imaging in the zebrafish model may uncover the potential roles of these hypotheses in AD pathogenesis. With these controversies, the zebrafish model emerges as an even more intriguing system for studying

neurodegeneration, which requires further research. Furthermore, it possesses potential as a comprehensive framework for comprehending Alzheimer's disease (AD) and serving as a foundation for investigating unexplored domains that could potentially yield advantages in the realm of pharmaceutical advancements.

Future of Alzheimer's disease models of zebrafish

In this review, our objective is to demonstrate the significant progress that zebrafish offers in comprehending the pathological mechanisms of AD. The zebrafish model has become an intriguing tool for strategically studying AD. Research utilizing this model system can effectively bridge the gap between drug discovery based on cellular models and preclinical assays. The zebrafish is highly suitable for conducting high-throughput pharmacological screening of drugs before validating them in rodent models. Numerous studies have already explored the potential of zebrafish as a model for understanding AD. However, there is still a need to further elucidate areas such as the behavior, physiology, neuroanatomical circuitry of the fish, and the connection between neurodevelopment and neurodegeneration. A desirable AD transgenic model that expresses both Aβ and Tau pathologies is currently necessary. This model will aid in completing the puzzle of understanding AD, as there are still missing pieces. In conclusion, the zebrafish has made significant strides as a potential model in the field of neurodegeneration and continues to emerge as an attractive model system for future AD research. It undeniably holds immense potential for developing therapeutic interventions for AD.

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