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Diverse perspectives in zebrafish seizure models: An exploration of chemical inducers

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Abstract

Numerous studies have investigated seizures in zebrafish, employing diverse methods to induce and observe epilepsy. Chemical inducers have become indispensable tools for precisely manipulating neural activity in controlled experiments. This research is dedicated to a meticulous comparison and analysis of various seizure-inducing chemicals in zebrafish. The selected chemical inducers include pentylenetetrazole (PTZ), kainic acid (KA), picrotoxin (PTX), pilocarpine, and other substances. The primary mechanism of these inducers involves altering neurotransmitter receptors, particularly GABA and glutamate, at inhibitory or excitatory synapses. This alteration creates an imbalance between inhibitory and excitatory signals in the brain, contributing to epileptogenesis. The resulting hyperactivity in the zebrafish brain disrupts metabolism, elevates reactive oxygen species (ROS), and causes neuronal damage. Chemical induction offers valuable insights into the neurological activity of zebrafish, enabling the observation of behaviors such as whole-body contractions, loss of posture, or freezing. Various parameters can be adjusted to examine the effects of chemical inducers on zebrafish development and to replicate various types of seizures, such as status epilepticus and temporal lobe epilepsy. Despite the array of options available, many chemical inducers remain underutilized in zebrafish studies. To ensure the successful induction of models, monitoring gene expression markers such as c-fos, which represents neuronal activity, becomes crucial. Additionally, a scoring system is employed to analyze seizure severity and identify treatment efficacy. The existing gaps in utilizing chemical inducers present great opportunities for future model development. This chemically-induced seizure model in zebrafish is pivotal for unraveling seizure mechanisms, contributing significantly to advancements in neurological research.

Keywords: Seizure Model; Chemical inducer; Epileptogenesis; Behavior; Zebrafish

1. Introduction

Epilepsy is a neurological disorder characterized by recurrent seizures caused by abnormal electrical activity of brain [1]. Zebrafish possess a well-preserved nervous system, making them ideal for studying neurological disorders, that potential for epilepsy treatment development [2,3]. Chemical inducers have become essential for manipulating neural activity in controlled experiments [4]. Therefore, most chemically induced models in aquatic vertebrates use compounds that disrupt the balance between inhibition and excitation in the animal's brain. Common chemical inducers include pentylenetetrazole (PTZ), kainic acid (KA), picrotoxin (PTX), pilocarpine, and others [5]. This exploration aims to carefully compare and analyze various chemicals used to induce seizures in zebrafish models, by assessing behavioral patterns and gene expression as biomarkers of seizure occurrence.

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2. Pentylenetetrazole

Pentylenetetrazole (PTZ) is a tetrazole derivative that acts as a GABA receptor antagonist [6]. PTZ is a pro-convulsant drug commonly used to study seizure activity [7]. The mechanism of action of PTZ involves antagonizing GABAergic neurotransmission, which is a fast inhibitory synaptic transmission mediated by the interaction of GABA with both ionotropic and metabotropic membrane receptors. The interaction between PTZ and GABAA receptors will block the chloride channel and reduce chloride ion influx so that subsequent membranes will be depolarized. This depolarization generates action potentials, increasing neuronal excitability and thus contributing to proconvulsant activity [8,9,10]. Furthermore, PTZ promotes the inactivation of voltage-gated K+ channels at slightly negative to positive membrane potentials. The inactivation decreases the outward K+ current and reduces the ability of neurons to repolarize. This modulation may affect neuronal excitability, alter their firing characteristics, and contribute to the generation and maintenance of seizure activity [11]. These changes may lead to an imbalance between excitation and inhibition in the brain, contributing to epileptogenesis and the development of a tendency to recurrent seizures [12,13].

The PTZ zebrafish model has proven to be a valuable tool for drug screening, the discovery of anti-seizure medications, and as a tool for identifying the adverse effects of seizure induction in drugs for other diseases [3,14]. In adult zebrafish, PTZ was injected intraperitoneally (170 mg/kg) or by dissolving PTZ solution (20 mM) in the tank water. The seizure behavior was characterized by the stage previously published, which was defined as a score from 0 - 6 (Table 2). The seizure behavior profile and PTZ brain level depend on PTZ concentration and exposure time in adult zebrafish [12, 15]. However, zebrafish models of chronic epilepsy that better resemble human epilepsy have already developed. Adult zebrafish were repeatedly exposed to sub-effective concentrations of PTZ until the onset of tonic-clonic seizures, which were considered to be kindled. Repeated exposure can increase seizure severity and induce kindling by altering brain neurotransmitter levels and gene expression [16]. This chemical kindling model is widely used in experimental epilepsy studies and has several advantages, including an easily repeatable protocol, no need for electrodes, and low mortality [17]. In a standard bathing medium, behavioral seizure activity can also be assessed in larval zebrafish by adding PTZ. The behavioral characteristics of larvae have been well described and have been used for various methods of study (Table 2) [18].

The concentration of PTZ can significantly influence the severity and characteristics of induced convulsions. The dose needs to be optimized to achieve the desired epileptic activity while avoiding excessive or lethal effects. For different study objectives, different concentrations may be appropriate [19,20]. Many studies of chemically induced seizures have shown that pharmacological seizure induction is an excellent model because researchers can control the timing, number, and severity of seizures in animals [21]. However, compared to any other model, the chemically induced model is not pharmacologically independent, as it may be involved in the interaction between the pro-convulsant and the compounds tested [22].

Recent investigations have explored numerous genes in the zebrafish model induced by PTZ. A pivotal gene in this context is c-fos, utilized to verify the successful induction of seizures. This is attributed to the rapid expression of c-fos following stimuli like PTZ, primarily localized in the zebrafish brain [23, 24]. Notably, c-fos is a proto-oncogene, and its expression can influence other gene expressions [25, 24]. Furthermore, PTZ induces the upregulation of the BDNF-TrkB signaling pathway, mediating inflammatory responses. Despite the controversial association between BDNF and epilepsy, its elevation may disrupt the balance between excitatory and inhibitory neurons [26,27]. Concurrently, the increased TrkB is believed to inhibit GABAergic neuron function by hindering Cl- efflux and reducing KCC2 expression [28]. The brain's inflammatory response induced by PTZ has been observed to enhance other inflammatory properties, including IL-1β, COX-2, NFκB, and leukocyte infiltration due to blood-brain barrier leakage [29, 30]. Additionally, the Adenosine A1 receptor plays a role in PTZ-induced seizure development, with seizure activity shown to decrease after treatment with adenosine deaminase inhibitors. This mechanism is associated with adenosine receptors that facilitate transmission [31].

3. Kainic Acid

Kainic acid (KA) is a naturally occurring glutamate receptor agonist and an analog of glutamate, an essential neurotransmitter in the brain. It can bind to and activate ionotropic glutamate receptors, leading to increased excitability of neurons and, at high concentrations, potentially causing neuronal damage or death. KA has been used to study the mechanisms of epilepsy, neurodegeneration, and other neurological disorders [32, 33].

KA exerts its neuroexcitatory property by binding to AMPA or kainate receptors, which are ionotropic glutamate receptors with presynaptic modulatory and postsynaptic excitatory actions. When KA binds to kainate receptors, it

causes activation of the receptor and leads to depolarization of the neuronal membrane, allowing the influx of Ca^{2+} into the cell. The influx of Ca^{2+} triggers a cascade of events that can lead to neuronal damage and death, including the activation of enzymes that break down cell membranes, the generation of reactive oxygen species (ROS), and the disruption of cellular metabolism [34, 35]. The production of ROS, the mediators of oxidative stress that play a critical role in excitotoxic cell damage, was increased by the entry of intracellular calcium and the overactivation of glutamate receptors after KA induction. Ca2+ overload also causes mitochondrial dysfunction, endoplasmic reticulum (ER) membrane fragmentation and ER stress, glial activation, and neuroinflammation, leading to neuronal death [35, 36].

KA can cause significant changes in cell proliferation and electrical activity, including the induction of interictal events and prolonged bursting discharges. However, the effects of KA exposure may vary depending on the developmental stage and duration of exposure. A study by Menezes et al. [37] suggests that exposure to KA early in development may affect brain development and alter susceptibility to seizures later in life. These findings highlight the importance of considering the developmental stage and duration of exposure when studying the effects of KA on zebrafish behavior and brain function [38]. KA also affects neurochemical changes related to astrocytes, resulting in lethargy in the zebrafish swimming pattern and a transient reduction in the level of GFAP cells and glutamate uptake. The reduction of GFAP-positive cells suggests that there is astrocyte damage within a short time after SE, but the reduction of S100B protein might, because GFAP-positive cells return to their control levels, present a neuroprotective effect. Both astrocyte stability and S100B expression affect transient impairment of glutamate uptake, particularly in the forebrain region of zebrafish, which is analogous to the limbic system in humans, a known feature of SE [39, 40].

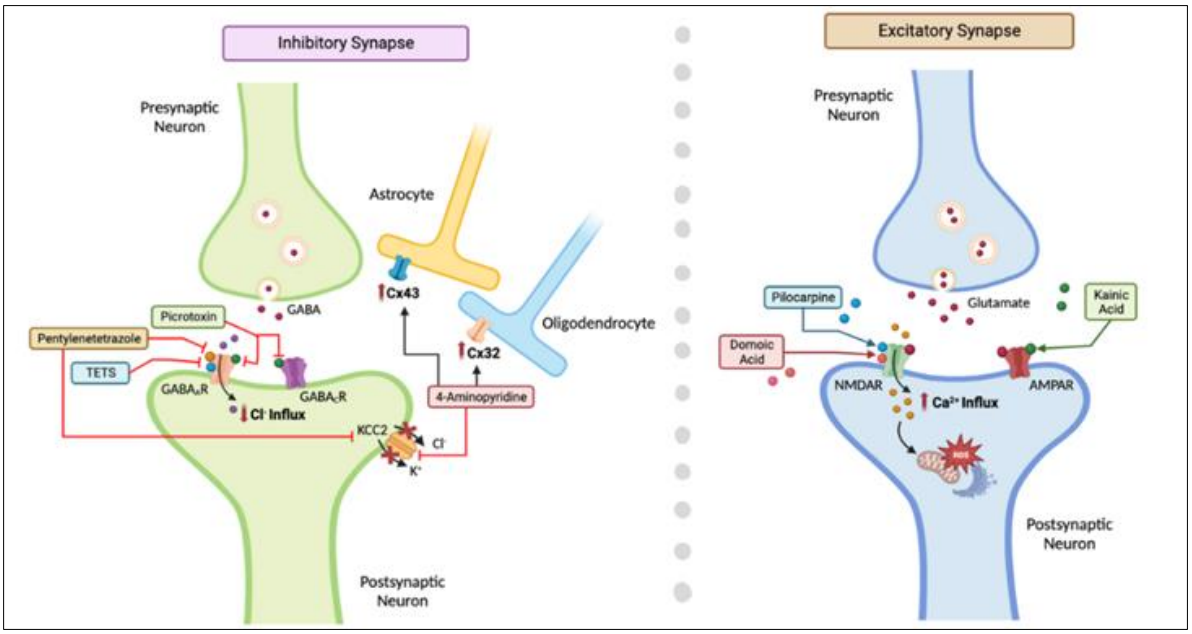

Figure 1 Chemically-induced seizures mechanism in zebrafish model. PTZ, PTX, and TETS interact with GABA-Receptor, which will reduce chloride ion influx. 4-AP increase the Cx32 and Cx43 expression resulting in hyperexcitability of neurons. In the excitatory synapse, KA, Pilocarpine and DA activate Glutamate-Receptor, which increases intracellular Ca2+ and leads to excitotoxicity of neurons (Figure was generated by https://www.biorender.com/) [84,8,46,75,78,38,59,95,12,9,32,66].

Immense brain damage and inflammation are reported to be induced by KA through direct pericardial administration in the larval zebrafish model, leading to seizure-like locomotor behavior. Similar to the previous study, KA-induced tissue opacity and upregulated pro-apoptotic markers indicate brain cell death. It was also found that the increased cell death is related to the significant elevation of pro-inflammatory cytokines such as IL-1B, IL-8, TNF-alpha, fas, and IRF1-Beta, signifying an acute inflammatory response, as seen in human temporal lobe epilepsy (TLE). Additionally, KA injection inflicts massive neuropathological changes characterized by the disarrangement of a neural circuit, indiscernible grey and white matter, and disrupted white matter with increased vacuolation. The KA-induced locomotor abnormalities gradually manifest as intermittent loss of posture, jerking movement, convulsion-like twitching such as tail or fin twitching, and even whole-body waving, persistently repeated for several days post-injection. Subsequently, the larvae become insensitive to tactile stimuli, experience posture loss, and show light-dark phases of hypoactivity [41].

4. Picrotoxin

Picrotoxin (PTX) is a non-competitive antagonist of the Gama-amino butyric acid A (GABA-A) receptor, which transports chloride ions across the cell membrane and has been used to induce epilepsy in animal models [42, 43]. PTX also antagonizes the GABA-C receptors, which are linked to the chloride channels, but these receptors mediate slow and prolonged responses [44]. The PTX mechanism leads to decreasing the inhibitory effect of GABA by decreasing the opening frequency and mean open time of the receptor [45]. PTX can be given in any route, as it dissolves quickly in saline. Seizures, therefore, develop more slowly than PTZ-induced seizures [46]. The study by L'Amoreaux et al. [47] showed that PTX is less potent when it is given in a substance that has the same binding site as taurine.

PTX causes various behavior alterations in zebrafish. Like PTZ, PTX can cause increases in locomotor activity in zebrafish in a dose-dependent manner [48, 49]. A previous study has shown that high-dose PTX treatment increases locomotion and thigmotaxis (an indicator of increased levels of anxiety) of zebrafish larvae in both light and dark environments. In comparison with PTZ, PTX gives more movement in light conditions and less movement in darkness [50]. Another study showed that acute exposure to PTX caused seizures without harm, and higher doses of acute antiepileptic drugs are needed to reduce locomotor seizure symptoms, suggesting that this may be beneficial in modeling treatment-resistant seizures [51]. Adult zebrafish showed increased hyperactivity and cortisol levels when exposed to PTX at a dose of 100 mg/L for 20 minutes. Additionally, there was an increase in seizure-like behavior characterized by spasms, increased time spent at the upper portion of the tank, corkscrew swimming, and circular swimming [52]. The results of the investigation utilizing a PTX-induced seizure mice model further confirmed these findings, demonstrating heightened anxiety and elevated levels of corticosterone [53]. The behavior of PTX in the zebrafish model was measured using scores that were identical to those used for PTZ or TETS, as previously reported [18, 50].

Not only was its effect on behavior evaluated but PTX was also explored with various methods. Similar to PTZ, the PTX also induced upregulation of fos transcripts after 30 minutes of administration in the zebrafish embryo model, caused by increased neuronal excitability as an indicator of seizure onset. Besides c-fos, there is also increased expression of gabra1 dan gabrg2 zebrafish brain induced by PTX [54]. However, many studies in PTX-induced seizure models were mainly found in the mammalian model. There was underutilization of this substance in zebrafish. A study by [55], using voltage-sensitive dye (VSD) in mouse hippocampal, found a prolonged depolarizing response and expansion of neuronal activity, confirming neurogenic response. PTX is also associated with PKA phosphorylation, which can reduce seizure activity in a rat's hippocampus. PKA plays a role in receptor trafficking, gating, and participation in neurotransmitter release [56]. In addition, exposing male mice to PTX caused increased expression of Camk1d, Platr26, Zfp599, Fyb, and Cdc7, a gene associated with brain development and represented ASD-like behavior [57].

5. Pilocarpine

Pilocarpine is a non-selective muscarinic ACh receptor agonist found primarily on the hippocampus, striatum, and cortex [58]. It is commonly used for seizure induction in an animal model, especially in the SE and TLE models. The mechanism of action of pilocarpine involves the activation of the muscarinic M1 receptor. Once a seizure is triggered, it is sustained by the activation of the NMDA receptor [59]. The seizures originate in the ventral forebrain, with the nucleus accumbens being the leading injury site due to its high concentration of muscarinic receptors [60]. EEG recordings have revealed a significant increase in spike activity in the hippocampus after the administration of pilocarpine. The brain damage occurred a few hours after the onset of the seizure and became more severe in specific regions, such as the hippocampal hilus and neocortical areas [61]. Terminating seizure activity in pilocarpine is challenging unless repetitive doses or a combination of AEDs are used. These conditions result in a high mortality rate in animal models [60, 62]. Several studies have addressed this issue by providing lithium prior to treatment in order to decrease the concentration of pilocarpine to minimize mortality [62]. However, research on using pilocarpine for seizures has been limited to rodents for years until recent studies explored its potential application in adult zebrafish [63, 64].

The mechanism of pilocarpine-inducing seizure is very diverse and related to its primary manifestation as SE. Administration of Lithium prior to pilocarpine activates T lymphocytes and mononuclear cells, increases IL-1β level, and damages BBB. Pilocarpine then will increase cholinergic response and trigger seizures. This pathogenic mechanism supports systemic inflammation and BBB as etiologic factors of SE in pilocarpine-induced [65]. Another mechanism that plays a crucial role is the poor neutralization of ROS. Pilocarpine-induced SE results in poor neutralization of ROS due to reduced activity of superoxide dismutase (SOD) and lowered levels of glutathione (GSH). Additionally, SE leads to increased activity of catalase (CAT), malondialdehyde (MDA) levels, and IL-1β levels [66]. Seizures will cause an excessive generation of ROS, but the antioxidant GSH is unable to regulate this production [67]. In addition, it is

exacerbated by the deficiency of SOD, an enzyme that catalyzes the conversion of superoxide radical O2 to H2O2 [68]. The CAT subsequently metabolizes the elevated production of H2O2 during the ongoing seizure to decrease the brain's vulnerability to seizures [69].

Pilocarpine seizure-like behavior has been observed in rodents to measure the stage or severity of seizures. The Racine scale is the pilocarpine-induced model's most widely used scoring system [70]. The scale can determine the transition phase towards SE, which consists of 5 stages [71]. A proposed method for assessing seizures in zebrafish induced by pilocarpine involves a scoring system with five stages representing the swimming behavior of epileptic zebrafish (Table 2) [63]. Scoring in larvae zebrafish was used in a study by Vermoesen et al., [72], which used a similar score to PTZ. A study by Szep et al., [73] used larva zebrafish to compare pilocarpine-induced epilepsy with PTZ. The study identified that pilocarpine-induced zebrafish larvae have more subtle epileptiform activity than PTZ-induced ones, making the zebrafish less sensitive to light. The mean total distance moved was significantly lower than in the PTZ one [73].

6. Other Chemical Agents

Tetramethylenedisulfotetramine (TETS) acts as a non-competitive GABAA receptor inhibitor, especially highly sensitive to $\alpha 2\beta 3\gamma 2$ subunit, which promote seizures and convulsions [74]. TETS is a highly lethal convulsive toxicant that is thought to cause seizures through the blockade of GABAA receptors in the central nervous system. This compound can immediately increase intracellular Ca2+, followed by a decrease in the frequency and an increase in the amplitude of Ca2+ oscillations [75]. This compound visibly changed Ca2+ dynamics in hippocampal neurons, which generate spontaneous network activity [76]. Like PTZ and PTX, these three GABAA Receptor antagonists increase seizure scores in correlation with a concentration-dependent manner. In displacing GABAAR ligands in rat brain membranes, TETS is significantly more potent than PTZ but not significantly more potent than PTX. TETS and picrotoxin have a similar potency in inhibiting the GABAAR-mediated uptake of GABA-activated chloride ions by membrane vesicles from the rat cerebral cortex [77,51].

4-Aminopyridine (4-AP) is an anticonvulsant that blocks potassium channels (KCC2) and prolongs neuronal action potentials, facilitating the unspecific release of neurotransmitters like glutamate in the hippocampus and striatum. This compound increases the neuron's excitability, which is key to causing seizure-like activity [78, 79]. Administration of 4-AP increases the protein expression of connexin (Cx) 32, expressed in oligodendrocytes, and plays a major role in buffering K+ released during neuronal activity. The expression of Cx43 in astrocytes also increased, resulting in constant hyperexcitability by activating the NMDA receptor and increasing glutamate release [78]. In zebrafish larvae treated with 4-AP, calcium activity was generated that increased the excitability of neurons in the cerebellum and hindbrain [80]. This compound also showed a widespread and significant increase in neuronal activity in the majority of brain regions [81].

Domoic acid (DA) is a neuroexcitatory amino acid naturally produced by certain marine diatom species of the genus Pseudo-nitzschia. Ingesting DA-contaminated seafood can lead to severe neurotoxicity, including seizures and neuronal damage due to the activation of ionotropic glutamate receptors. The toxin binds with higher affinity than glutamate to kainate and AMPA subclasses of ionotropic glutamate receptors by counteracting rapid desensitization of membrane channels at both synaptic terminals. Excess AMPA activity may be involved in progressive hippocampal damage in temporal lobe epilepsies and drug resistance [82, 83]. Excitotoxicity is initiated via the activation of these receptors and is mediated by the secondary activation NMDA receptors and subsequent Ca2+ influx. Besides neurobehavioral effects, acute exposure to DA causes neuronal cell death and obvious histopathological lesions in the brain, particularly in regions involved in memory processing, such as the CA1 and CA3 regions of the hippocampus [84, 83]. Chronic exposure to DA causes unilateral loss of hippocampal neurons, loss of hilar somatostatin-immunoreactive neurons and sprouting of mossy fibers in sea lions that have neuropathological similarities to humans with TLE [85]. In the zebrafish study, DA exposure leads to the loss of Mauthner neurons, which alters the excitability of neurons and their susceptibility to excitotoxicity [86]. DA also upregulates the mitochondrial calcium uniporter (MCU), which controls Ca2+ uptake after glutamate insult and drives neuronal death [87].

Table 1 Different Chemical Used in Zebrafish Seizure Model

Strain/Age	Dose/Route	Behavioral Pattern	Nonbehavioral Biomarker	References
Pentylenetetrazole (PTZ)				
Adult, strain wild-type/ 3-4 months-old	170 mg/kg / Intraperitoneal (i.p)	Reached seizure score 4 within 150-180 s ↓ Total distance traveled	↓ GABA ↑ Glutamate ↓ Acetylcholine (ACh) ↓ BDNF ↑ CREB1 ↓ NPY	[15]
Larvae, AB line/ 4-5 dpf	10, 20 and 40 mM / Immersed in water	10 mM PTZ increased locomotor activity and no clear signs of toxicity	-	[88]
Adult, strain wild-type, 4-5 months-old	1, 1.25, 1.5, and 2 mM / Immersed in water (Kindling model)	1.25 mM increased seizure severity score with repeated score 5 seizure	↑ Glutamate/ GABA ratio ↑ cfos ↑ crebbpa ↑ crebbpb	[19]
Kainic Acid (KA)				
Larvae, wild-type, 3 dpf	2.5, 5, 10 and 20 mg/kg / Microinjection into pericardium	5 mg/kg is an optimal dose that induce only mild malformations.	↑ early apoptosis marker: caspase-9 and bax ↑ il1b, irf1b, il8 ↑ c4, csf1ra	[41]
Adult, strain wild-type, 6 months-old	4,5, and 6 mg/kg / Intraperitoneal (i.p)	5 mg/kg increased seizure score with accepatable mortality ratio. ↓ Total distance travelled ↓ swimming speed ↑ Immobility	↑ GFAP ↓ Glutamate uptake in forebrain region	[39]
Larvae, 1. First exposure: 7,15 and 30 dpf 2. Second exposure: 2 mpf	First exposure: 100, 300, and 500 µM/ Immersion in water Second exposure: 6 mg/kg/ Intraperitoneal (i.p)	↓ locomotor activity in 7 and 15 dpf Pre-exposed group has less susceptible in second exposure to KA.	-	[37]
Picrotoxin (PTX)				
Embryo, 2 dpf	300 µM/ immersed in water	-	↑ c-fos	[54]
Larvae, wild-type, 5 dpf	1, 5, 25, 125, and 625 µM	125 µM increased distance moved 125 µM and 625 µM decrease thigmotaxis	-	[50]
Larvae, wild type, 5 dpf	0 - 4 mM/ Immersed in water	Concentration-dependent increase of seizure score ↑ distance moved in 0,4 mM	-	[51]

Pilocarpine				
Larvae, wild type 5 dpf	30 mM / immersed in water	↑ total distance moved in light/dark	-	[73]
Adult, wild-type-AB stock	400 mg/kg / intraperitoneal (i.p) repeated	↑ mean seizure score ↓ total distance travelled ↑ time in the upper half of the tank ↓ time in the lower half of the tank Abnormal and disruptive swimming pattern	↑ hmgb1 ↑ tlr4 ↓ nfkb ↑ tnfa ↑ il-1 ↑ bdnf ↑ creb-1 ↓ npy ↓ GABA ↑ glutamate ↓ Ach	[63]
Adult, 6 - 8 months	350 mg/kg, intraperitonal (i.p) single dose	↓ total distance travelled ↓ time spent in light zone ↑ aggressive events ↑ time spent in the proximal zone	↑ cleaved PARP1 ↑ cleaved Caspase-↑ BrdU cells number ↓ PSD95 ↓ SNAP25 ↓ GAD67 ↓ Gephyrin 93 ↑ gephyrin 75 ↑ GFAP ↓ Iba-1	[64]
Other Chemical Agent				
Larvae, wild-type, 5 dpf	TETS (0.1-100 μM)	There is no score 3 in any of the concentration ↑ distance moved in 4μM	-	[51]

Table 2 Scoring of Seizure Severity Based on the Behavior of a Variety of Chemical-Induced Models

Stage	**Description**		
	Adult		Larvae-
	PTZ, PTX, TETS	Pilocarpine	PTZ, PTX, Pilocarpine
0	Short swim	Normal Swimming	Normal swimming
1	increased swimming activity and high frequency of opercular movement	Jittery movement at the top of the tank	increased swimming activity
2	erratic movements	Ataxia/Hyperactivity	rapid "whirlpool-like" circling swimming behavior
3	circular movements,	Circular movement, Circling around the small area	series of brief clonus-like spasms leading to loss of posture

4	clonic seizure-like behavior	Erratic burst movement with loss of posture/Corkscrew swimming	
5	fall to the bottom of the tank and tonic seizure-like behavior		
6	Death		

7. Advantages and disadvantages of chemical induction of seizures in zebrafish

The zebrafish provides the opportunity to model various pharmacological and genetic epilepsy/seizures similar to those in rodents [89]. Using chemical induction seizure in zebrafish models has several advantages. Firstly, the time and duration of induced seizures depend on the chosen convulsant and its concentration, which allows for controlled experiments and reproducible results. Additionally, chemical induction enables the observation of behaviors such as whole-body contractions, loss of posture, or freezing, providing valuable insights into zebrafish's neurological activity. However, there are significant challenges associated with the use of chemicals for seizure induction. One challenge is the ambiguity in interpreting behavior, especially distinguishing between seizure-like behavior, hyperactivity, and abnormal behavior in zebrafish larvae. This challenge highlights the importance of careful behavioral analysis and consideration of potential confounding factors. Furthermore, only a few chemical seizure models have been investigated for non-behavioral seizure biomarkers, limiting the validation of observed effects. There are also limitations in the applicability of zebrafish as a model, primarily restricted to embryos and larvae, with the large size of adult zebrafish posing challenges for high-throughput studies [90]. In epilepsy research using animal models, including PTZ, KA, pilocarpine, and PTX, chemical induction offers advantages. These models simulate human seizure activity, deepen our understanding of seizure mechanisms, and serve as effective tools for potential treatment research [91,92,93]. However, there are limitations, as these models may not fully replicate the diverse mechanisms of seizures in humans [94,9.

8. Conclusion

The chemical inducer used for the zebrafish seizure model offers a range of possibilities, each with its distinct process. The commonly used drug primarily targets glutamate, GABA, and acetylcholine receptors. The effects of seizures can be investigated through several subjects, such as oxidative damage, systemic inflammation, or electrolyte imbalance. The methodology employed in the study can be adjusted based on the research objectives and inquiries, particularly regarding chemical administration, whether administered as a single dosage, repeated doses, immersion, or intraperitoneal injection. PTZ is the most often utilized chemical in seizure investigations due to its well-described nature, validation by electrophysiology methods, and availability in various concentrations. It is essential to acknowledge that the chemical-induced model has a drawback that requires careful consideration to address the potential confusion in understanding behavior. The vast subject matter that can be extensively examined, with numerous aspects yet relatively underutilized, presents additional research opportunities. These opportunities will facilitate a more profound comprehension of the mechanisms underlying seizures.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no competing interest.

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