Epilepsy Research 157 (2019) 106207

Contents lists available at ScienceDirect

Epilepsy Research

journal homepage: www.elsevier.com/locate/epilepsyres

Anticonvulsant action of a selective phosphatidylinositol-3-kinase inhibitor LY294002 in pentylenetetrazole-mediated convulsions in zebrafish

Arindam Ghosh Mazumder[a,b], Savita Kumari[a,b], Damanpreet Singh[a,b,*]

[a] Pharmacology and Toxicology Laboratory, CSIR-Institute of Himalayan Bioresource Technology, Palampur, 176061, Himachal Pradesh, India
[b] Academy of Scientific and Innovative Research (AcSIR), CSIR-Institute of Himalayan Bioresource Technology, Palampur, 176061, Himachal Pradesh, India

ARTICLE INFO

Keywords:
Epilepsy
Zebrafish
LY294002
PI3K/AKT/mTOR
Seizure
Phosphatidylinositol-3-kinase

ABSTRACT

The role of PI3K/AKT/mTOR signalling pathway has been widely deciphered in pathogenesis of epilepsy. LY294002 is a selective inhibitor of phosphatidylinositol-3-kinase (PI3K). The present study was designed to explore the anticonvulsant potential of LY294002 in a zebrafish model of pentylenetetrazole (PTZ)-mediated convulsions. Zebrafish larvae at 7*dpf* (days post fertilization) were pre-incubated with varying concentrations of LY294002, prior to PTZ exposure. The adult zebrafish were also exposed to PTZ after intraperitoneal injection with different concentrations of LY294002, followed by gene expression studies in the brain. The hyperactive responses indicated by total distance travelled and the mean speed of larva was drastically decreased, whereas latency to first clonic-like seizure was increased following LY294002 pre-treatment. Additionally, a marked decrease in *c-fos* expression was also observed in the larvae group exposed to LY294002 in comparison to control. Furthermore, PTZ evoked seizure severity was considerably decreased, while latency to clonic-like seizure was increased in adult zebrafish group treated with 100 nM of LY294002. Furthermore, the occurrence of tonic-like seizures was also reduced in the adult zebrafish treated with LY294002. The mRNA levels of *PIK3CA, PIK3R1, AKT1, mTOR*, *Rps6* and *Rps6kb1* in adult zebrafish brain was significantly reduced as compared to vehicle control group. Our results provided conclusive support for the anticonvulsant potential of LY294002.

1. Introduction

Epilepsy is a chronic neurological condition that is characterized by periodic seizures, occurring as a result of an electrical imbalance between the excitatory and inhibitory neurotransmission in the brain (Fisher et al., 2014). Apart from basic excito-inhibitory mechanism, there are other factors that are responsible for generation of epileptic seizures like, non-synaptic electrical transmission through the gap junctions, participation of non-neuronal glial cells *viz*. astrocytes and microglia in precipitating seizures and oxidative stress leading to metabolic deregulation (Dudek et al., 1998; Devinsky et al., 2013; Pearson-Smith and Patel, 2017). Data from the Global Health Metrics denotes that epilepsy ranks 5th globally in disease adjusted life years among all the neurological disorders in the world (Feigin et al., 2017). The conventional antiepileptic drugs (AEDs) act on the synaptic neuronal signal transmission, either by prolonging the blocking of excitation or by facilitating the inhibitory neurotransmission. Of all the research done so far on epilepsy, its exact epileptogenic mechanism precipitating seizures remain poorly understood. Moreover, the available AEDs only provide a symptomatic relief, rather than providing an insight into factors responsible for abrogation of epileptic seizures (Zeng et al., 2009a, 2009b). In some cases, a patient remains resistant to the conventional AEDs therapy with accumulating incidence of seizure along with associated conditions like memory impairment, depression, anxiety-like behaviour, *etc*., thus decreasing the quality of life of the patients. In recent times, certain biomarkers (genetic, structural, functional, electrophysiological and neuroinflammatory) of epilepsy have been identified as potential targets for better outcome of the disease condition, but they have come up with their own set of challenges which need to be addressed very rationally (Pitkänen et al., 2016). In this regard, constant efforts are being made to understand the exact molecular pathways entailing the epileptogenic processes to develop newer effective agents for better therapeutic management of epilepsy (Kobow et al., 2012; Laxer et al., 2014).

In the last decade, phosphatidylinositol-3-kinase (PI3K) pathway has gained a lot of importance as a molecular target in the pathogenesis of various disorders. PI3K is an enzyme of the family of lipid kinases responsible for various cellular functions like development,

* Corresponding author at: Pharmacology and Toxicology Laboratory, CSIR-Institute of Himalayan Bioresource Technology, Palampur, 176061, Himachal Pradesh, India.
E-mail address: damanpreet@ihbt.res.in (D. Singh).

https://doi.org/10.1016/j.eplepsyres.2019.106207
Received 18 April 2019; Received in revised form 18 August 2019; Accepted 14 September 2019
Available online 17 September 2019
0920-1211/ © 2019 Elsevier B.V. All rights reserved.

proliferation, metabolism and distinctive cellular functions (Engelman et al., 2006). It gets stimulated when growth factors (epidermal growth factor, insulin-like growth factor, human epidermal growth factor, *etc*.) or receptor tyrosine kinases get attached to it, thus activating the novel phosphatidylinositol pathway (Kapeller and Cantley, 1994). Upon receptor activation, the PI3K gets employed to the membrane to generate a group of secondary messenger, PIP2 (phosphatidylinositol-4,5-bisphosphate), which gets converted to PIP3 (phosphatidylinositol-3,4,5-triphosphate) in the presence of PI3K. This conversion takes place when PI3K transfers the terminal phosphate of the ATP to D-3 position of PIP2 (Carpenter and Cantley, 1990; Gharbi et al., 2007; Cantrell, 2001). It has been reported that PI3K phosphorylates the AKT to trigger mTOR pathway and its downstream genes for progression of epilepsy (Berdichevsky et al., 2013). Accumulating evidence has shown that inhibition of mTOR hyperactivation using specific inhibitors resulted in suppression of acquired epilepsy (Huang et al., 2010). Hence, inhibition of mTOR pathway acts as a good therapeutic intervention for the management of epilepsy (Citraro et al., 2016). Moreover, it has been well reported that the phosphorylation of PI3K is essential for the induction of its activity and subsequent cellular processes to occur (Cohen et al., 1990). Among all the eight classes of PI3K, the role of Class I PI3Ks (consisting of a catalytic subunit, p110 and a regulatory adaptor unit, p85) has been widely elucidated in different diseased conditions that alter the normal physiological functions (Vanhaesebroeck et al., 2016).

LY294002 [2-(4-morpholinyl)-8-phenyl-4H-l-benzopyran-4-one or 2-(4-morpholinyl)-8-phenylchromo], is a low molecular weight, cell permeable and a selective inhibitor of PI3K activity (Vanhaesebroeck and Waterfield, 1999). It acts as a selective competitive inhibitor of the ATP binding site of class I PI3K, thus preventing the conversion of PIP2 to PIP3 (Vlahos et al., 1994; Xing et al., 2008). The role of LY294002 has been implicated in a number of studies and has shown to act through the PI3K/AKT/mTOR signaling pathway (Alvarez et al., 2009; Mazumder et al., 2016). The experiments conducted *in vitro* on cultured hippocampal neurons treated with LY294002, blocked the PI3K/AKT interceding the mTOR signalling pathway, thus reducing the inflammatory and pathophysiological processes associated with epilepsy (Xiao et al., 2015). Interestingly, previous research has also shown direct inhibition of mTOR auto kinase property by LY294002 (Brunn et al., 1996). Certain studies conducted on cortical cultures showed that LY294002 possesses neuroprotective effects in oxidative stress instigated degeneration of neurons (Levinthal and DeFranco, 2004). In addition to this, LY294002 has also been proposed to mitigate neuroprotective effect of estrogen against glutamate provoked neurotoxicity (Honda et al., 2000). It is a fact that the PI3K/AKT pathway is essential for brain development and propagates neuroprotection, but surprisingly, experimental reports have claimed that treatment with LY294002 during embryogenesis in zebrafish led to neuronal death and it also initiated apoptosis in primary cultures (Chen et al., 2017; Dai et al., 2012). Therefore, it is evident from the available literature that LY294002 plays a dual role, *i.e.* acting as a neuroprotectant and inducing apoptosis. However, a similar characteristic profile was depicted by rapamycin, a potent mTOR inhibitor that has antiepileptic property, but possesses neuroprotective and pro- apoptotic activity (Ding et al., 2015; Saqcena et al., 2015), thus justifying the use of LY294002 in our study.

The popularity of using zebrafish (*Danio rerio*) model in neuroscience research has increased considerably because its brain structural organization shows homology with that of human especially with the presence of diencephalon, telencephalon, and cerebellum. It also possesses a basic vertebrate neural plan that comprises of the fore, mid and hindbrain and shows complex behaviour like memory, conditioned responses, and training. (Xi et al., 2011). It has emerged out to be a useful model for AEDs screening. Apart from adult zebrafish, 7 *dpf* (days post fertilization) larva model is widely used in epilepsy research as it is a small, full vertebrate that has all its organ systems functional (Berghmans et al., 2007). For induction of epileptic seizures, chemoconvulsants are either directly added to the water or injected into the intraperitoneal cavity (Winter et al., 2008; Alfaro et al., 2011). Therefore, taking into account all the previous research done, the present study was designed to explore the anticonvulsant potential of LY294002 in a zebrafish model of pentylenetetrazole (PTZ)-mediated convulsions.

2. Materials and methods

2.1. Drugs and chemicals

PTZ, Trizol reagent and SYBR Green Jump start Taq Ready Mix was purchased from Sigma Aldrich, USA. The salts for system water that includes sodium bicarbonate and sea salt were procured from Central Drug House, New Delhi and Aquarium Systems, Germany, respectively. LY294002 was obtained from Cell Signalling Technologies, USA. Dimethyl sulphoxide (DMSO) was procured from SD Fine-Chem Limited, Mumbai. RNase-free DNase kit was acquired from Promega, Madison, USA, while, High capacity cDNA-RT kit was purchased from Applied Biosystems, USA.

2.2. Zebrafish maintenance and egg collection

4-5 months old adult wild-type zebrafish were maintained in a ZebTEC Stand-Alone system (Tecniplast, Buguggiate, Varese, Italy) with conductivity from 400 to 600 µS, temperature of 26–28 °C and pH 7.0–7.5. The light: dark cycle of the room was kept at 14:10 h respectively, whereas the live freshly hatched *Artemia* (Inve Aquaculture, Inc., Salt Lake City, USA) were fed to the fish, twice daily with a dropper. Natural spawning was induced to obtain the healthy eggs from the adult zebrafish in separate breeding tanks. Precisely, zebrafish were relocated in a breeding tank setup (Tecniplast, Buguggiate, Varese, Italy) consisting of an internal grid base tank placed in an external transparent chamber covered with a clear transparent lid. The breeding tank setup was prefilled with system water at 28.5 °C. The female to male ratio was 2:1 (each tank contained 4 females and 2 males) and were estranged by a movable transparent divider at the start of the dark cycle, a day preceding egg collection. The divider was removed the next day, 30 min after the start of the light cycle to induce spawning. Clean pipettes were used to collect the healthy fertilized eggs in sterile petri dishes, cleaned with system water and maintained at 28.5 °C in a BOD incubator (Relitech, Ambala, India) till 7*dpf*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of CSIR-IHBT.

2.3. PTZ-mediated clonic-like seizures in larva

At 7 *dpf*, the larvae were incubated with varying concentrations of LY294002 (1–50 µM) for 1 h at 28.5 °C. DMSO was used to make the stock solution of LY294002, and all the working solutions were made using system water (DMSO concentration remained 0.01% in final solution). Subsequently after incubation, individually each larva was transferred to a transparent, top opened circular chamber (3.5 × 2 cm; D × H), filled with 8 mM PTZ solution and behaviour was recorded with an upper cut-off time of 15 min. The observations of the convulsive behaviour as a consequence of PTZ were made on the basis of a three-point scale as, Stage 1: enhanced swimming activity or hyperactivity; Stage 2: circular whirlpool-like movements and; Stage 3: clonic-like seizures accompanied by loss of posture (plummeting on either side, bent body position and remaining motionless for around 1–3 s) and falling (sinking to the bottom primarily due to rigid extensions of the body). The locomotor activity of each larva was recorded using a video tracking software (SMART V3.0., Panlab, Barcelona) connected to a camera (c922 Pro Stream, Logitech Asia Pacific Ltd, Hong Kong) attached at the centre of the chamber. The latency to first clonic-like

seizure *i.e.* stage 3 was also recorded (Baraban et al., 2005; Kumari et al., 2019). Three concentrations of LY294002 *i.e.* 10 μM (*LY-10-L* group), 20 μM (*LY-20-L* group) and 30 μM (*LY-30-L* group) were selected based on the results of our initial studies, and 8 larvae/group were used. Two different groups of larvae ($n = 8$) maintained in system water (containing 0.01% DMSO) for 1 h, served as *Vehicle-L* group (received PTZ exposure) and *Naïve-L* group (without PTZ exposure).

2.4. PTZ mediated seizures in adult zebrafish

Adult male zebrafish (3 months old) were intraperitoneally injected with different concentrations of LY294002 (10–400 nM). The procedure for intraperitoneal injection was performed as described previously (Kinkel et al., 2010). Briefly, each fish was fasted to empty the intestinal bulb 24 h prior to cryoanaesthesia. Thereafter, the fish was placed into a system water soaked sponge trough (10–15 mm deep) with the abdomen facing upwards, providing constant aeration to the gills. An injection volume of 20 μL was administered between the pelvic fins into the abdominal cavity, posterior to the pelvic girdle, using a Hamilton syringe (Model: P/N 80408, SYR 25 μl, 702 N) under a stereozoom microscope (SMZ800 N, Nikon Trinocular Stereo Zoom Microscope, Tokyo, Japan) and quickly transferred to a separate chamber filled with system water at 28.5°C for recovery. After an incubation period of 1 h, each fish was transferred to a transparent glass chamber, containing PTZ (6 mM) solution at 28.5°C and seizure behaviour was recorded for 30 min. The behavioural endpoints for seizure scoring was made on a modified 7-point scale as described earlier with slight changes (Mussulini et al., 2013; Stewart et al., 2012). Briefly, the stages were compiled as, Stage 1: increased swimming activity; Stage 2: erratic movements with frequent left and right motions; Stage 3: rapid whirlpool like swimming; Stage 4: series of brief clonic-like seizures, wild jumping, whole body rhythmic contractions; Stage 5: tonic-like seizure behaviour with loss of posture (occurring due to inability of coordination and dorso-ventral body position of the fish, reflecting major seizure associated neurological deficits) and falling (sinking of the fish to the bottom of the tank due to rigid extension of the body); Stage 6: freezing behaviour or immobility for > 2 s except for continuous respiratory and ocular movements and; Stage 7: Death. Based on the initial results, three concentrations of LY294002 *i.e.*50 nM (*LY-50-A* group), 100 nM (*LY-100-A* group) and 200 nM (*LY-200-A* group) were selected and recording was performed ($n = 6$). The vehicle control (*Vehicle-A*) and naïve (*Naïve-A*) group ($n = 6$) were intraperitoneally injected with vehicle (0.01% DMSO solution) and after 1 h, the former group was exposed 6 mM PTZ solution to record seizure behaviour, whereas the latter group was not exposed to PTZ. The seizure severity score, latency to clonic-like seizures and occurrence of stage 5 *i.e.* tonic-like seizure behaviour with loss of posture and falling was recorded in each fish exposed to PTZ.

2.5. Real time quantitative PCR studies

The gene expression studies were conducted by quantitative real time polymerase chain reaction (qRT-PCR) analysis on Step One Plus Real Time PCR system (Applied Biosystems, USA) using SYBR Green Jump start Taq Ready Mix. The effect of LY294002 on *c-fos* mRNA level of 7 *dpf* zebrafish larvae was studied. Precisely, pre-incubated 7 *dpf* zebrafish larvae (3 sets of larvae/group, $n = 20$/set) with 20 μM concentration of LY294002 for 1 h were exposed to 8 mM PTZ for 15 min. Additionally, two separate groups (3 sets of larvae/group, $n = 20$/set) served as the vehicle control (exposed to PTZ) and naïve control group (not exposed to PTZ). Trizol reagent was used to extort total RNA from whole larva following 1 h of PTZ exposure. The homogenate obtained thereafter was incubated at room temperature for 5 min, treated with chloroform and centrifuged at 12,000 *g* for 15 min, 4 °C. The aqueous layer formed was separated and treated with isopropanol and centrifuged at 12,000 *g* for 10 min, 4 °C. The RNA pellet obtained was repeatedly washed with ethanol (75%), centrifuged (7500 *g* for 5 min, 4 °C), dissolved in nuclease free water and quantified using Nanodrop ND-1000 (Thermo Scientific, USA). Additionally, the DNA traces were removed with RNase-free DNase kit (Promega, Madison, USA) and cDNA synthesis was done using high capacity cDNA-RT kit (Applied Biosystems, USA), as per manufacturer's instructions. Elongation factor-1-α (*elf1α*) of zebrafish was considered as a reference standard for normalization (Tanwar et al., 2019). The mean of each sample was considered after performing the reaction in triplicates, to minimize the sampling error.

Table 1
Primer sequence of target genes.

Genes	Forward primer (5' 3')	Reverse primer (5' 3')
c-fos	AACTGTCACGGCGATCTCTT	GCAGGCATGTATGGTTCAGA
PIK3CA	CGCAATGAGAGGATGAGCGA	ACGCTGTCACGATGGAACAA
PIK3R1	ACATGGCTCTGCAAGATGCT	GGAGGCATCTCGGACCAAAA
AKT1	TCGGCAGGTGTCTTCTCAAT	ACCCATTGCCATACCACGAG
mTOR	AGATCATCAACCGAGTGCGG	AGGGCACCATCCAATGTAGC
Rps6	TCACTCTTGTTACCGTCCTC	TGACAATGACCAAGTTGAGA
Rps6kb1	AAAACTCCCAAAGACTCTCC	CTAGTGGCGCACTTTTACTT
elf1α	GATGCACCACGAGTCTCTGA	TGATGACCTGAGCGTTGAAG

The gene expression studies in adult zebrafish brain were carried out following 30 min of PTZ exposure. All the adult zebrafish in vehicle control group and the group pretreated with 100 nM concentration of LY294002 were sacrificed, their brains were isolated and total RNA was harvested. Following cDNA synthesis, qRT-PCR was performed as discussed above. The naïve group was also used to calculate the basal level. The primers (Table 1) were designed using Primer Express Software 3.0 (Applied Biosystems, USA). Each gene had an annealing temperature of 55 °C and all the other qRT-PCR conditions remained same as described previously in our study (Mazumder et al., 2018). The gene expression was denoted as fold change using comparative 2ˆddCT method and evaluated as performed earlier (Livak and Schmittgen, 2001).

2.6. Statistical analysis

All the results were expressed as mean ± standard error, unless otherwise specified. The statistical significant difference in latency to clonic seizures, distance travelled, speed and gene expression among different groups was examined by one-way analysis of variance followed by Tukey's *post hoc* test. The results of seizure severity score in adult zebrafish were analysed using a non-parametric Kruskal-Wallis test, and statistical differences was studied using Mann-Whitney *U* test whereas, the statistical significance of difference in occurrence of Stage 5 in adult zebrafish subjected to PTZ was analysed by Chi-square test. The results were regarded as significant at $P < 0.05$.

3. Results

3.1. Effect of LY294002 on PTZ-mediated hyperactive responses in zebrafish larva

PTZ exposure at 8 mM to 7*dpf* zebrafish larva resulted in induction of hyperactive responses in *Vehicle-L* group indicated by increased swimming activity. *Vehicle-L* group showed significant ($P < 0.001$) increase in total distance travelled as compared to normal *Naïve-L* group of larvae [$F_{(4,35)} = 15.756$, $P < 0.001$]. The total distance travelled was significantly ($P < 0.001$) decreased in the larvae group pre-exposed to LY294002 at all the three concentrations followed by PTZ (Fig. 1A). The mean swimming speed was also found to be significantly ($P < 0.001$) increased in the *Vehicle-L* larvae group as compared to *Naïve-L* group [$F_{(4,35)} = 15.80$, $P < 0.001$]. Similarly, LY294002 pre-exposure at 10, 20 and 30 μM concentrations resulted in a marked

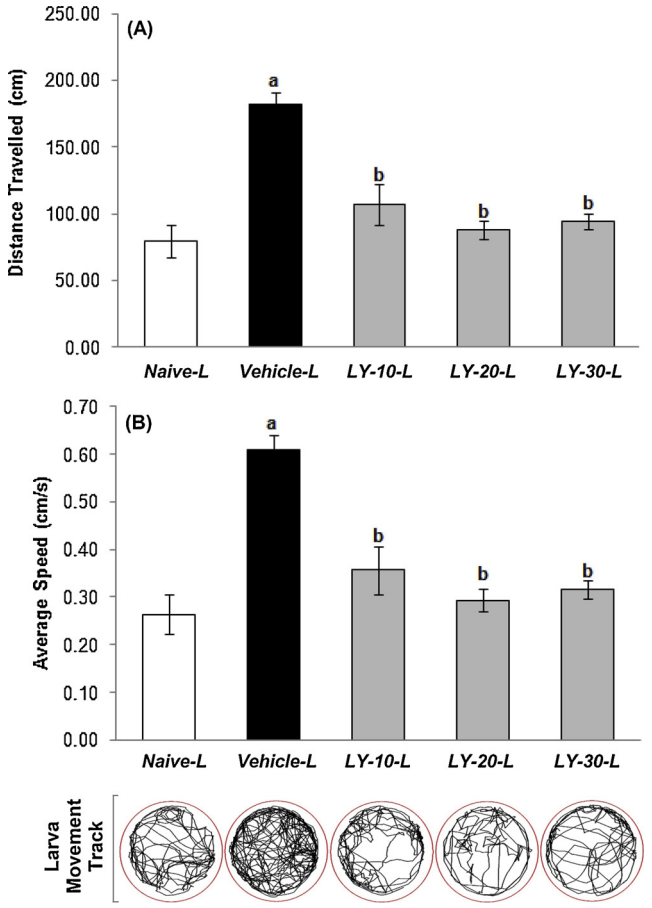

Fig. 1. Effect of LY294002 on PTZ-mediated hyperactive responses in 7 *dpf* zebrafish larvae as (A) total distance travelled and (B) mean swimming speed. [a]P < 0.05 as compared to naive group; [b]P < 0.05 as compared to vehicle control group. ***LY-10-L***: 10 μM of LY294002 treated group exposed to PTZ; ***LY-20-L***: 20 μM of LY294002 treated group exposed to PTZ; ***LY-30-L***: 30 μM of LY294002 treated group exposed to PTZ; ***Naïve-L***: Vehicle treated group not exposed to PTZ and; ***Vehicle-L***: Vehicle treated group exposed to PTZ.

(P < 0.001) decrease in the mean swimming speed as that of *Vehicle-L* group (Fig. 1B).

3.2. Effect of LY294002 on latency to PTZ-mediated convulsions in zebrafish larva

There was induction of clonic-like seizures in larvae at 3.73 ± 0.16 min following 8 mM PTZ exposure in *Vehicle-L* group (Fig. 2). The latency to first clonic-like seizure was increased in the groups of larvae pre-treated with different concentrations of LY294002, however significantly (P < 0.001) change was only observed at 20 μM concentration (*LY-20-L* group) as compared to *Vehicle-L* [$F_{(3,28)}$ = 46.434, P < 0.001].

3.3. Effect of LY294002 on larva c-fos expression

The level of *c-fos* mRNA was significantly (P < 0.001) increased following PTZ exposure in larvae of *Vehicle-L* group as compared to *Naïve-L* group [$F_{(2,6)}$ = 48.431, P < 0.001]. The increased *c-fos* mRNA level was found to be significantly (P < 0.001) decreased in 20 nM LY294002 pre-incubated group (*LY-20-L*) as compared to *Vehicle-L* group (Fig. 3).

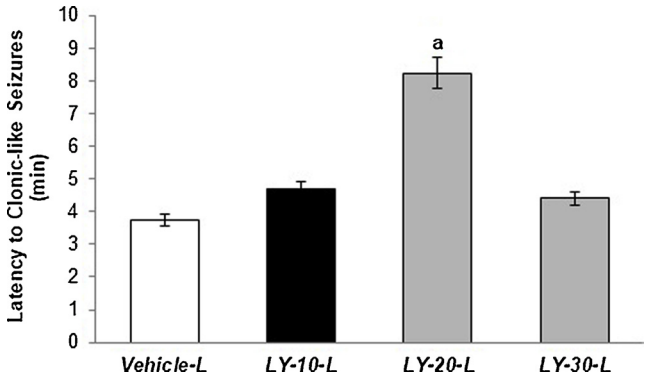

Fig. 2. Effect of LY294002 on latency to PTZ-mediated clonic-like seizures in 7 *dpf* zebrafish larvae. [a]P < 0.05 as compared to vehicle control group.***LY-10-L***: 10 μM of LY294002 treated group exposed to PTZ; ***LY-20-L***: 20 μM of LY294002 treated group exposed to PTZ; ***LY-30-L***: 30 μM of LY294002 treated group exposed to PTZ and; ***Vehicle-L***: Vehicle treated group exposed to PTZ.

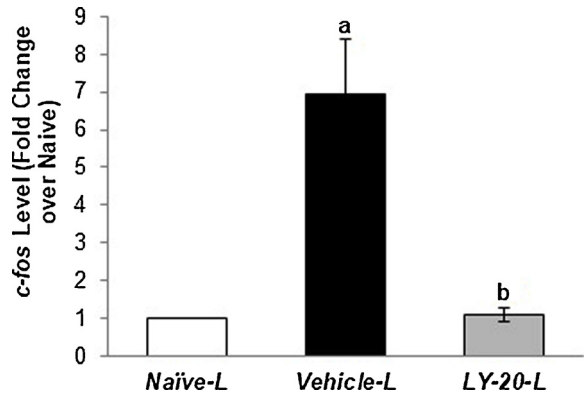

Fig. 3. Effect of LY294002 on *c-fos* mRNA expression in 7 *dpf*zebrafish larvae. The results expressed as mean ± S.D. [a]P < 0.05 as compared to naive group; [b]P < 0.05 as compared to vehicle control group. ***LY-20-L***: 20 μM of LY294002 treated group exposed to PTZ; ***LY-30-L***: 30 μM of LY294002 treated group exposed to PTZ and; ***Vehicle-L***: Vehicle treated group exposed to PTZ.

3.4. Effect of LY294002 on PTZ-mediated seizures in adult zebrafish

The seizure severity score was significantly (P = 0.001) decreased in the fish treated with 100 nM concentration of LY294002 as compared to *Vehicle-A* group. However, insignificant (P = 0.138) change was observed at 50 and 200 nM concentrations of LY294002 as that of *Vehicle-A* group (Fig. 4A). The latency to first clonic-like seizure was found to be 3.8 ± 0.2 min in *Vehicle-A* group (Fig. 4B). A marked (P < 0.001) increase in the latency was observed in *LY-100-A* group treated with LY294002 at 100 nM concentration as compared to *Vehicle-A* group [$F_{(3,20)}$ = 42.009, P < 0.001]. *LY-50-A* and *LY-200-A* groups treated with 50 and 200 nM LY294002 concentrations, respectively showed insignificant increase in the latency to clonic-like seizure as that of *Vehicle-A* group. Tonic-like seizures *i.e.* stage 5 appeared in all the tested adult zebrafish of *Vehicle-A* group. However, the incidence of tonic-like seizures significantly (P < 0.001) reduced to 66.66% in groups treated with 50 and 200 nM concentrations of LY294002, as compared to *Vehicle-A* group. Interestingly, tonic-like seizures were completely abolished in *LY-100-A* group treated with 100 nM concentration of LY294002 (Fig. 4C). Since naïve group (*Naïve-A*) was not administered with PTZ, hence not shown in Fig. 4).

3.5. Effect of LY294002 on mRNA levels of adult zebrafish brain

The effect of 100 nM concentration of LY294002 on the brain mRNA level following PTZ treatment is shown in Fig. 5. The expression of

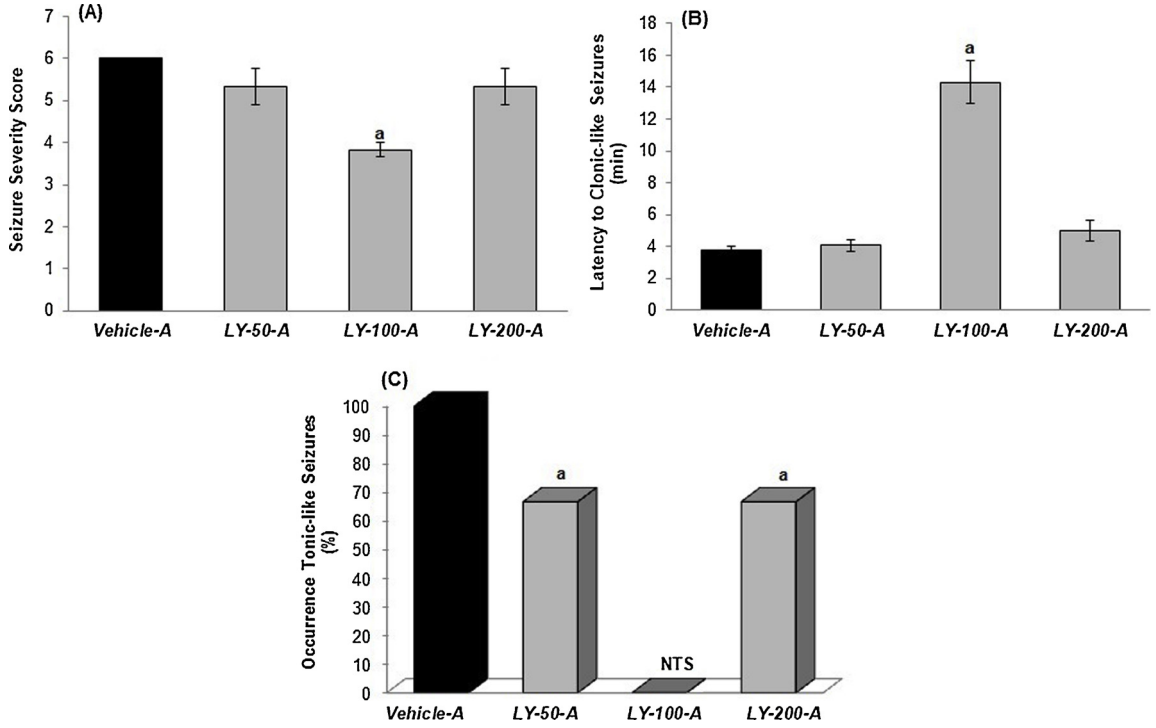

Fig. 4. Effect of LY294002 on PTZ-induced seizures in adult zebrafish as (A) seizure severity score, (B) latency to first clonic-like seizures and (C) occurrence of tonic-like seizures (stage 5). [a]P < 0.05 as compared to vehicle control group. **NTS**: No tonic-like seizures; ***LY-50-A***: 50 nM of LY294002 treated group exposed to PTZ; ***LY-100-A***: 100 nM of LY294002 treated group exposed to PTZ; ***LY-200-A***: 200 nM of LY294002 treated group exposed to PTZ and; ***Vehicle-A***: Vehicle treated group exposed to PTZ.

PIK3CA [$F_{(2,15)}$ = 19.292, P < 0.001] and *AKT1* [$F_{(2,15)}$ = 15.321, P < 0.001] was drastically (P < 0.001) increased in *Vehicle-A* group as that of *Naïve-A* group. Treatment with 100 nM concentration of LY294002 in *LY-100-A*group showed a marked (P < 0.001) reduction in *PIK3CA* and *AKT1* mRNA levels as that of *Vehicle-A* group. PTZ exposure to adult zebrafish resulted in a significant (P = 0.008) increase in *PIK3R1* level in *Vehicle-A* group in contrast to *Naïve-A* group. Further the LY294002 (100 nM) treatment reduced (P = 0.006) *PIK3R1* level as compared to *Vehicle-A* group [$F_{(2,15)}$ = 8.671, P = 0.003]. The level of *mTOR* was also found to be significantly (P = 0.012) reduced following treatment with LY294002 (100 nM) as compared to *Vehicle-A* [$F_{(2,15)}$ = 7.709, P = 0.005]. The brain *mTOR* level was significantly (P = 0.009) increased as a consequence of PTZ in *Vehicle-A* control as that of *Naïve-A* group. As indicated in Fig. 5, the brain *Rps6* [$F_{(2,15)}$ = 208.951, P < 0.001] and *Rps6kb1* [$F_{(2,15)}$ = 22.423, P < 0.001] was significantly (P < 0.001) increased following PTZ exposure in *Vehicle-A* group as compared to normal *Naïve-A* group. The level of *Rps6* and *Rps6kb1*was found to be significantly (P < 0.001) reduced following treatment with LY294002 at 100 nM concentration in *LY-100-A* group as compared to *Vehicle-A* group.

4. Discussion

The present study is the first attempt to explore the anticonvulsant potential of LY294002 in an experimental model. The study showed that there was a significant decrease in the locomotor activity (depicted

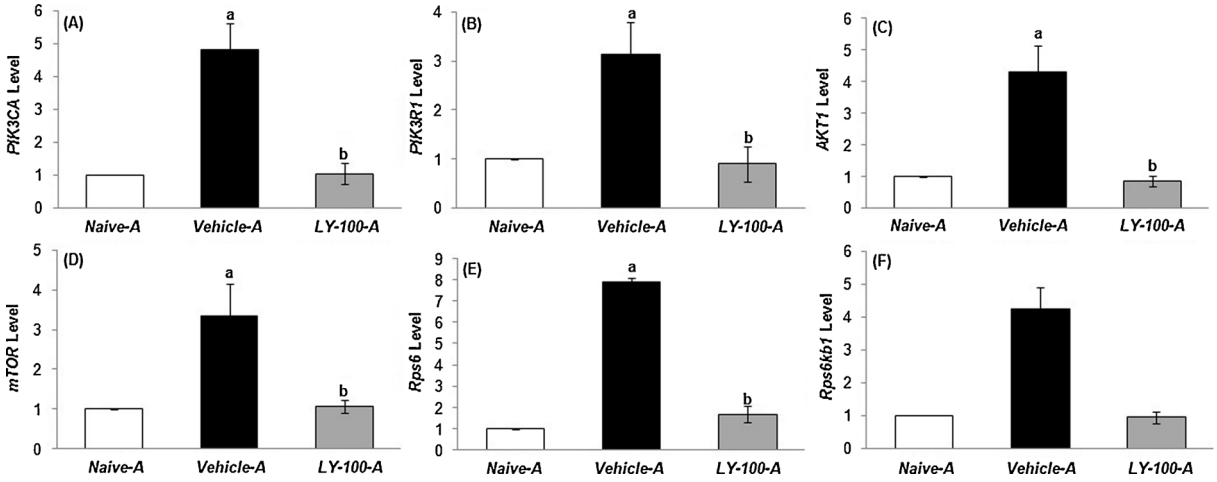

Fig. 5. Effect of LY294002 on mRNA levels of (A) *PIK3CA,* (B) *PIK3R1,* (C) *AKT1,* (D) *mTOR,* (E)*Rps6* and(F)*Rps6kb1*in adult zebrafish brain. The data represented as fold change over naive group. [a]P < 0.05 as compared to naive group; [b]P < 0.05 as compared to vehicle control group. ***LY-100-A***: 100 nM of LY294002 treated group exposed to PTZ; ***Naïve-A***: Vehicle treated group not exposed to PTZ and; ***Vehicle-A***: Vehicle treated group exposed to PTZ.

by total distance travelled and mean swimming speed) in the larvae group pre-incubated with LY294002 at all the three concentrations when compared to the vehicle control group. Moreover, the latency to attain first clonic-like seizure (Stage 3) in case of larva considerably increased following pre-treatment with 20μM of LY294002 when compared to the diseased group. Our results also depicted that the severity of seizures in adult zebrafish treated with 100 nM concentration of LY294002 decreased significantly, whereas the seizure latency to achieve clonic-like seizures increased following treatment. Furthermore, tonic-like seizures were completely abolished in *LY-100-A* group treated with 100 nM concentration of LY294002.

The use of zebrafish in epilepsy research has gained importance since Baraban and his group developed the PTZ-induced seizure model in 7*dpf* larva, which simulate stages of convulsion in rodents. PTZ, a common chemoconvulsant, when used in the medium of zebrafish larva induce seizures and burst of hyperlocomotor activity. The criteria for the induction of epileptic seizures includes hyperlocomotion, behavioural seizures, electrophysiological parameters (electrical events occurring in the brain following PTZ exposure) and altered *c-fos* expression (Baraban et al., 2005). Studies have shown that conventional antiepileptic drugs reduce locomotion in PTZ model of zebrafish larva and decrease seizure severity (Afrikanova et al., 2013; Liu et al., 2016). Similarly, our study showed that, 7 *dpf* zebrafish larva when pre-treated with 20 μM of LY294002 depicted an increase in latency to clonic-like seizures as compared to the vehicle control group. The increased locomotor activity as a consequence of PTZ was gradually reduced following LY294002 treatment indicated by decrease in total distance travelled and speed. These results of the present study supported the anticonvulsant effect of LY294002.

It is observed that neuronal excitation during seizure results in alteration in different gene expression. Hence, to establish the fact that occurrence of seizure caused alteration in expression of immediate early genes in the brain (Stewart et al., 2012), *c-fos* expression at the most active concentration of LY294002 *i.e.* 20 μM was evaluated in the larvae. Experimental studies conducted earlier have shown that repetitive seizures result in long lasting impact on altered gene transcription in the brain. Moreover, seizure-linked *c-fos* activation served as a common link between the stimulus for the emergence of seizures and the development of epileptic condition (Simler et al., 1999). Several studies conducted on rodent models of epilepsy showed increased expression of *c-fos* in the brain following seizures through electrical stimulation or induction through subconvulsive dose of PTZ (Dragunow and Robertson, 1987; Szyndler et al., 2009). Similarly, an analogous *c-fos* upregulation was also observed in zebrafish larva submitted to PTZ exposure, thus establishing the fact that induction of seizure results in increased expression of *c-fos* (Baraban et al., 2005). In line with these findings, our study revealed increase in *c-fos* expression in vehicle control group. Earlier studies suggested that compounds with anticonvulsant potential downregulates the expression of *c-fos* in zebrafish larva (Baxendale et al., 2012). Hence, reduced levels of *c-fos* in PTZ exposed larvae pre-treated with LY294002 further supported its anticonvulsant potential.

Based on the results of our larval studies, the anticonvulsant effect of LY294002 was further studied in adult zebrafish model of PTZ-induced convulsions. The seizure severity score was decreased and latency to first clonic-like seizure increased considerably at 100 nM of LY294002, whereas occurrence of Stage 5 (tonic-like seizures) was reduced at all the tested concentrations in contrast to the vehicle control group. Since earlier experiments indicated that conventional antiepileptic drugs reduce PTZ-evoked seizure severity in adult zebrafish with increase in onset to first clonic seizures (Kundap et al., 2017), hence our findings further supported the anticonvulsant potential of LY294002.

The central signaling pathway of PI3K/AKT/mTOR plays a major role in various neural disorders and its role in epilepsy has been widely explored (Crino, 2015; Mazumder et al., 2017). Studies have also suggested that hyperactivation of this pathway leads to further seizure generation and propagation (Pene et al., 2002; Dai et al., 2012; Xiao et al., 2016). However, certain studies also support the neuroprotective role of PI3K/AKT signaling cascade in embryonic development of zebrafish (Chen et al., 2017). The available scientific literature has revealed that PI3K when activated by growth factors and receptor tyrosine kinases is responsible for the phosphorylation of AKT to activate its downstream gene, mTOR, the master regulator of various cellular processes (Laplante and Sabatini, 2012; Mazumder et al., 2016). Evidence from literature states the importance of the mTOR pathway in the propagation of epilepsy, thus citing mTOR as an important target to control the generation of epileptic seizures (Galanopoulou et al., 2012). The role of mTOR inhibitors has been widely reported in various therapeutic interventions of epilepsy, particularly in case of acquired epilepsy. However, the use of these inhibitors particularly rapamycin, is associated with a number of untoward side effects (Zeng et al., 2009a, 2009b; Ostendorf and Wong, 2015; Mazumder et al., 2016). Hence, continuous research is in progress to identify safe and effective, directly or indirectly acting mTOR inhibitor for management of epilepsy. In the present study, an increased expression of *PIK3CA, PIK3R1, AKT1, mTOR, Rps6kb1*and *Rps6* genes in the brain of vehicle control group of adult zebrafish associated with PI3K/AKT/mTOR pathway was observed as a result of acute exposure to PTZ. These results were supported by a study in a rat model of acute seizures induced by PTZ that indicated acute activation of mTOR pathway (Zhang and Wong, 2012). Hence it can be correlated that seizure activity due to acute exposure of PTZ caused overexpression of *PI3K* which further activated its downstream genes *AKT* and *mTOR*. The expression of studied genes in the zebrafish brain gradually diminished upon treatment with 100 nM of LY294002 that might be due to inhibition of *PI3K*.

Interestingly, LY294002 treatment groups in both larva and adult zebrafish showed U-shaped response while assessing the parameters of behavioural endpoints. These responses might be attributed due to the fact that PI3K/AKT/mTOR, being the central signal transduction pathway occurring in all cells that leads to activation of a large number of its downstream genes thus modulating various cellular processes. Moreover, previous reports depicted that the central pathway of PI3K/AKT/mTOR, co-occur with an amalgamation of a large number of smaller pathways which directly or indirectly modulate the central pathway (Mazumder et al., 2016). LY294002 being a selective inhibitor of class I group of PI3K, might be responsible for alteration or modulation of other molecular targets in the pathway, thus altering the central signalling pathway, thereby reorganising the normal cellular physiology. However, more studies are required in this regard to consolidate these results. Earlier findings have linked the complex mechanism of PI3K/AKT/mTOR hyperactivation to its altered gene expression in epileptic rodent models (Meng et al., 2013; Mazumder et al., 2018). Thus, our study showed that the inhibition of PI3K using its specific inhibitor LY294002, led to decreased overexpression of AKT and mTOR which were responsible for the generation and progression of seizures.

5. Conclusion

The results of our study concluded that LY294002, a specific inhibitor of PI3K, reduced the PTZ mediated seizures in larva and adult zebrafish. The observed anticonvulsant effect of LY294002 was due the inhibition of PI3K/AKT/mTOR pathway. The results of our study provided a convincing platform and outlined the fundamental role of LY294002 as a better therapeutic intervention for epilepsy. However, more studies need to be conducted for determining its safety and efficacy in other models of epilepsy.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to the Director of CSIR-IHBT, Palampur (HP), India for providing the necessary facilities for conducting the experimental procedures. The financial assistance for the work was provided by the CSIR, New Delhi, India through a project MLP-0204. Arindam Ghosh Mazumder is indebted to the Department of Science and Technology, New Delhi, India for providing DST-INSPIRE fellowship vide letter no. DST/INSPIRE Fellowship/2013/820. The authors are also thankful to the Director and Dr. Sridhar Sivasubbu of CSIR-Institute of Genomics and Integrative Biology, New Delhi for providing initial stock of adult wild-type strains of zebrafish (ASWT). The institute communication number of the manuscript is 4377.

References

Afrikanova, T., Serruys, A.S.K., Buenafe, O.E., Clinckers, R., Smolders, I., de Witte, P.A., Crawford, A.D., Esguerra, C.V., 2013. Validation of the zebrafish pentylenetetrazol seizure model: locomotor versus electrographic responses to antiepileptic drugs. PLoS One 8 (1), 54166.

Alfaro, J.M., Ripoll-Gómez, J., Burgos, J.S., 2011. Kainate administered to adult zebrafish causes seizures similar to those in rodent models. Eur. J. Neurosci. 33 (7), 1252–1255.

Alvarez, Y., Astudillo, O., Jensen, L., Reynolds, A.L., Waghorne, N., Brazil, D.P., Cao, Y., O'Connor, J.J., Kennedy, B.N., 2009. Selective inhibition of retinal angiogenesis by targeting PI3 kinase. PLoS One 4 (11), 7867.

Baraban, S.C., Taylor, M.R., Castro, P.A., Baier, H., 2005. Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. Neuroscience 131 (3), 759–768.

Baxendale, S., Holdsworth, C.J., Santoscoy, P.L.M., Harrison, M.R., Fox, J., Parkin, C.A., Ingham, P.W., Cunliffe, V.T., 2012. Identification of compounds with anti-convulsant properties in a zebrafish model of epileptic seizures. Dis. Models Mech. 5 (6), 773–784.

Berdichevsky, Y., Dryer, A.M., Saponjian, Y., Mahoney, M.M., Pimentel, C.A., Lucini, C.A., Usenovic, M., Staley, K.J., 2013. PI3K-Akt signaling activates mTOR-mediated epileptogenesis in organotypic hippocampal culture model of post-traumatic epilepsy. J. Neurosci. 33 (21), 9056–9067.

Berghmans, S., Hunt, J., Roach, A., Goldsmith, P., 2007. Zebrafish offer the potential for a primary screen to identify a wide variety of potential anticonvulsants. Epilepsy Res. 75 (1), 18–28.

Brunn, G.J., Williams, J., Sabers, C., Wiederrecht, G., Lawrence, J.C., Abraham, R.T., 1996. Direct inhibition of the signaling functions of the mammalian target of rapamycin by the phosphoinositide 3-kinase inhibitors, wortmannin and LY294002. EMBO J. 15 (19), 5256–5267.

Cantrell, D.A., 2001. Phosphoinositide 3-kinase signalling pathways. J. Cell. Sci. 114 (8), 1439–1445.

Carpenter, C.L., Cantley, L.C., 1990. Phosphoinositide kinases. Biochemistry 29 (51), 11147–11156.

Chen, S., Liu, Y., Rong, X., Li, Y., Zhou, J., Lu, L., 2017. Neuroprotective role of the Pi3 kinase/akt signaling pathway in zebrafish. Front Endocrinol. 8, 21.

Citraro, R., Leo, A., Constanti, A., Russo, E., De Sarro, G., 2016. mTOR pathway inhibition as a new therapeutic strategy in epilepsy and epileptogenesis. Pharmacol. Res. 107, 333–343.

Cohen, B., Liu, Y.X., Druker, B., Roberts, T.M., Schaffhausen, B.S., 1990. Characterization of pp85, a target of oncogenes and growth factor receptors. Mol. Cell. Biol. 10 (6), 2909–2915.

Crino, P.B., 2015. mTOR signaling in epilepsy: insights from malformations of cortical development. Cold Spring Harb. Perspect. Med. 5 (4), 022442.

Dai, R., Xia, Y., Mao, L., Mei, Y., Xue, Y., Hu, B., 2012. Involvement of PI3K/Akt pathway in the neuroprotective effect of Sonic hedgehog on cortical neurons under oxidative stress. J. Huazhong Univ. Sci. Technol. Med. Sci. 32 (6), 856–860.

Devinsky, O., Vezzani, A., Najjar, S., De Lanerolle, N.C., Rogawski, M.A., 2013. Glia and epilepsy: excitability and inflammation. Trends Neurosci. 36 (3), 174–184.

Ding, K., Wang, H., Wu, Y., Zhang, L., Xu, J., Li, T., Ding, Y., Zhu, L., He, J., 2015. Rapamycin protects against apoptotic neuronal death and improves neurologic function after traumatic brain injury in mice via modulation of the mTOR-p53-Bax axis. J. Surg. Res. 194 (1), 239–247.

Dragunow, M., Robertson, H.A., 1987. Kindling stimulation induces c-fos protein (s) in granule cells of the rat dentate gyrus. Nature 329 (6138), 441.

Dudek, F.E., Yasumura, T., Rash, J.E., 1998. 'Non-synaptic'mechanisms in seizures and epileptogenesis. Cell Biol. Int. 22 (11–12), 793–805.

Engelman, J.A., Luo, J., Cantley, L.C., 2006. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat. Rev. Genet. 7 (8), 606.

Feigin, V.L., Abajobir, A.A., Abate, K.H., Abd-Allah, F., Abdulle, A.M., Abera, S.F., Abyu, G.Y., Ahmed, M.B., Aichour, A.N., Aichour, I., Aichour, M.T.E., 2017. Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol. 16 (11), 877–897.

Fisher, R.S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J.H., Elger, C.E., Engel Jr., J., Forsgren, L., French, J.A., Glynn, M., Hesdorffer, D.C., 2014. ILAE official report: a practical clinical definition of epilepsy. Epilepsia 55 (4), 475–482.

Galanopoulou, A.S., Gorter, J.A., Cepeda, C., 2012. Finding a better drug for epilepsy: the mTOR pathway as an antiepileptogenic target. Epilepsia 53 (7), 1119–1130.

Gharbi, S.I., Zvelebil, M.J., Shuttleworth, S.J., Hancox, T., Saghir, N., Timms, J.F., Waterfield, M.D., 2007. Exploring the specificity of the PI3K family inhibitor LY294002. Biochem. J. 404 (1), 15–21.

Honda, K., Sawada, H., Kihara, T., Urushitani, M., Nakamizo, T., Akaike, A., Shimohama, S., 2000. Phosphatidylinositol 3-kinase mediates neuroprotection by estrogen in cultured cortical neurons. J. Neurosci. Res. 60 (3), 321–327.

Huang, X., Zhang, H., Yang, J., Wu, J., McMahon, J., Lin, Y., Cao, Z., Gruenthal, M., Huang, Y., 2010. Pharmacological inhibition of the mammalian target of rapamycin pathway suppresses acquired epilepsy. Neurobiol. Dis. 40 (1), 193–199.

Kapeller, R., Cantley, L.C., 1994. Phosphatidylinositol 3-kinase. Bioessays 16 (8), 565–576.

Kinkel, M.D., Eames, S.C., Philipson, L.H., Prince, V.E., 2010. Intraperitoneal injection into adult zebrafish. J. Vis. Exp. 42, 2126.

Kobow, K., Auvin, S., Jensen, F., Löscher, W., Mody, I., Potschka, H., Prince, D., Sierra, A., Simonato, M., Pitkänen, A., Nehlig, A., 2012. Finding a better drug for epilepsy: antiepileptogenesis targets. Epilepsia 53 (11), 1868–1876.

Kumari, S., Mazumder, A.G., Bhardwaj, A., Singh, D., 2019. Early α-linolenic acid exposure to embryo reduces pentylenetetrazol-induced seizures in zebrafish larva. Prostaglandins Leukot. Essent. Fatty Acids 143, 15–20.

Kundap, U.P., Kumari, Y., Othman, I., Shaikh, M., 2017. Zebrafish as a model for epilepsy-induced cognitive dysfunction: a pharmacological, biochemical and behavioral approach. Front. Pharmacol. 8, 515.

Laplante, M., Sabatini, D.M., 2012. mTOR signaling in growth control and disease. Cell 149 (2), 274–293.

Laxer, K.D., Trinka, E., Hirsch, L.J., Cendes, F., Langfitt, J., Delanty, N., Resnick, T., Benbadis, S.R., 2014. The consequences of refractory epilepsy and its treatment. Epilepsy Behav. 37, 59–70.

Levinthal, D.J., DeFranco, D.B., 2004. Transient phosphatidylinositol 3-kinase inhibition protects immature primary cortical neurons from oxidative toxicity via suppression of extracellular signal-regulated kinase activation. J. Biol. Chem. 279 (12), 11206–11213.

Liu, C., Zhou, J., Zhang, Y., Zhu, T., Wang, R., Li, C., 2016. Validation of the zebrafish pentylenetetrazol seizure model: behavior assay for assessing anti-epileptic drug efficacy. Biochem. Anal. Biochem. 5, 270. https://doi.org/10.4172/2161-1009.1000270.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods 25 (4), 402–408.

Mazumder, A.G., Patial, V., Singh, D., 2018. Mycophenolate mofetil contributes to downregulation of the hippocampal interleukin type 2 and 1β mediated PI3K/AKT/mTOR pathway hyperactivation and attenuates neurobehavioral comorbidities in a rat model of temporal lobe epilepsy. Brain Behav. Immun. 75, 84–93.

Mazumder, A.G., Shantaram Padwad, Y., Singh, D., 2016. Anticancer mammalian target of rapamycin (mTOR) signaling pathway inhibitors: current status, challenges and future prospects in management of epilepsy. CNS Neurol. Disord. Drug Targets 15 (8), 945–955.

Mazumder, A.G., Sharma, P., Patial, V., Singh, D., 2017. *Ginkgo biloba* L. attenuates spontaneous recurrent seizures and associated neurological conditions in lithium-pilocarpine rat model of temporal lobe epilepsy through inhibition of mammalian target of rapamycin pathway hyperactivation. J. Ethnopharmacol. 204, 8–17.

Meng, X.F., Yu, J.T., Song, J.H., Chi, S., Tan, L., 2013. Role of the mTOR signaling pathway in epilepsy. J. Neurol. Sci. 332 (1–2), 4–15.

Mussulini, B.H.M., Leite, C.E., Zenki, K.C., Moro, L., Baggio, S., Rico, E.P., Rosemberg, D.B., Dias, R.D., Souza, T.M., Calcagnotto, M.E., Campos, M.M., 2013. Seizures induced by pentylenetetrazole in the adult zebrafish: a detailed behavioral characterization. PLoS One 8 (1), 54515.

Ostendorf, A.P., Wong, M., 2015. mTOR inhibition in epilepsy: rationale and clinical perspectives. CNS Drugs 29 (2), 91–99.

Pearson-Smith, J., Patel, M., 2017. Metabolic dysfunction and oxidative stress in epilepsy. Int. J. Mol. Sci. 18 (11), 2365.

Pene, F., Claessens, Y.E., Muller, O., Viguie, F., Mayeux, P., Dreyfus, F., Lacombe, C., Bouscary, D., 2002. Role of the phosphatidylinositol 3-kinase/Akt and mTOR/P70S6-kinase pathways in the proliferation and apoptosis in multiple myeloma. Oncogene 21 (43), 6587.

Pitkänen, A., Löscher, W., Vezzani, A., Becker, A.J., Simonato, M., Lukasiuk, K., Grohn, D., Bankstahl, J.P., Friedman, A., Aronica, E., Gorter, J.A., 2016. Advances in the development of biomarkers for epilepsy. Lancet Neurol. 15 (8), 843–856.

Saqcena, M., Patel, D., Menon, D., Mukhopadhyay, S., Foster, D.A., 2015. Apoptotic effects of high-dose rapamycin occur in S-phase of the cell cycle. Cell Cycle 14 (14), 2285–2292.

Simler, S., Vergnes, M., Marescaux, C., 1999. Spatial and temporal relationships between C-Fos expression and kindling of audiogenic seizures in Wistar rats. Exp. Neurol. 157 (1), 106–119.

Stewart, A.M., Desmond, D., Kyzar, E., Gaikwad, S., Roth, A., Riehl, R., Collins, C., Monnig, L., Green, J., Kalueff, A.V., 2012. Perspectives of zebrafish models of epilepsy: what, how and where next? Brain Res. Bull. 87 (2–3), 135–143.

Szyndler, J., Maciejak, P., Turzyńska, D., Sobolewska, A., Taracha, E., Skórzewska, A., Lehner, M., Bidziński, A., Hamed, A., Wisłowska-Stanek, A., Krząścik, P., 2009. Mapping of c-Fos expression in the rat brain during the evolution of pentylenetetrazol-kindled seizures. Epilepsy Behav. 16 (2), 216–224.

Tanwar, G., Mazumder, A.G., Bhardwaj, V., Kumari, S., Bharti, R., Yamini, Singh, D., Das, P., Purohit, R., 2019. Target identification, screening and in vivo evaluation of pyrrolone-fused benzosuberene compounds against human epilepsy using Zebrafish model of pentylenetetrazol-induced seizures. Sci. Rep. 7904. https://doi.org/10.1038/s41598-019-44264-6.

Vanhaesebroeck, B., Waterfield, M.D., 1999. Signaling by distinct classes of phosphoinositide 3-kinases. Exp. Cell Res. 253 (1), 239–254.
Vanhaesebroeck, B., Whitehead, M.A., Piñeiro, R., 2016. Molecules in medicine mini-review: isoforms of PI3K in biology and disease. J. Mol. Med. 94 (1), 5–11.
Vlahos, C.J., Matter, W.F., Hui, K.Y., Brown, R.F., 1994. A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). J. Biol. Chem. 269 (7), 5241–5248.
Winter, M.J., Redfern, W.S., Hayfield, A.J., Owen, S.F., Valentin, J.P., Hutchinson, T.H., 2008. Validation of a larval zebrafish locomotor assay for assessing the seizure liability of early-stage development drugs. J. Pharmacol. Toxicol. Methods 57 (3), 176–187.
Xi, Y., Noble, S., Ekker, M., 2011. Modeling neurodegeneration in zebrafish. Curr. Neurol. Neurosci. Rep. 11 (3), 274–282.
Xiao, Z., Peng, J., Yang, L., Kong, H., Yin, F., 2015. Interleukin-1β plays a role in the pathogenesis of mesial temporal lobe epilepsy through the PI3K/Akt/mTOR signaling pathway in hippocampal neurons. J. Neuroimmunol. 282, 110–117.
Xiao, Z., Peng, J., Gan, N., Arafat, A., Yin, F., 2016. Interleukin-1β plays a pivotal role via the PI3K/Akt/mTOR signaling pathway in the chronicity of mesial temporal lobe epilepsy. Neuroimmunomodulation 23 (5–6), 332–344.
Xing, C., Zhu, B., Liu, H., Yao, H., Zhang, L., 2008. Class I phosphatidylinositol 3-kinase inhibitor LY294002 activates autophagy and induces apoptosis through p53 pathway in gastric cancer cell line SGC7901. Acta Biochim. Biophys. Sin. 40 (3), 194–201.
Zeng, L.H., Rensing, N.R., Wong, M., 2009a. Developing antiepileptogenic drugs for acquired epilepsy: targeting the mammalian target of rapamycin (mTOR) pathway. Mol. Cell. Pharmacol. 1 (3), 124.
Zeng, L.H., Rensing, N.R., Wong, M., 2009b. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. J. Neurosci. 29 (21), 6964–6972.
Zhang, B., Wong, M., 2012. Pentylenetetrazole-induced seizures cause acute, but not chronic, mTOR pathway activation in rat. Epilepsia 53 (3), 506–511.