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Taurine Protects from Pentylenetetrazole-Induced Behavioral and Neurochemical Changes in Zebrafish

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Abstract

Epilepsy is a common neurological disorder characterized by recurrent unprovoked seizures, which culminate in various neurobehavioral and neurochemical changes. Taurine (TAU) is an amino sulfonic acid which acts an endogenous inhibitory neuromodulator. Moreover, TAU displays intrinsic antioxidant activity, contributing to its beneficial actions in the CNS. Here, we evaluated whether TAU pretreatment protects from pentylenetetrazole (PTZ)-induced behavioral alterations and oxidative stress-related parameters in zebrafish brain tissue. Fish were pretreated with 42, 150, and 400 mg/L TAU (40 min) and further exposed to 10 mM PTZ (20 min) to analyze the seizure-like behaviors. As a positive control, another group was previously treated with 75 μM diazepam (DZP). Afterwards, biochemical experiments were performed. All TAU concentrations tested decreased seizure intensity in the first 150 s. Importantly, 150 mg/L TAU attenuated seizure-like behavioral scores, decreased seizure intensity, reduced the frequency of clonic-like seizures (score 4), and increased the latency to score 4. TAU (150 mg/L) also prevented oxidative stress in PTZ-challenged fish by decreasing lipid peroxidation and protein carbonylation and preventing changes on nonprotein thiol levels. No significant changes were observed in MTT assay and LDH activity. Differently than observed in DZP group, TAU did not affect the overall swimming activity of fish, suggesting different mechanisms of action. Collectively, we show that TAU attenuates PTZ-induced seizure-like behaviors and brain oxidative stress in zebrafish, suggesting the involvement of antioxidant mechanisms in neuroprotection.

Keywords Epilepsy · Seizure-like behaviors · Neuroprotection · Pentylenetetrazole · Oxidative stress · Zebrafish

Introduction

Epilepsy is a common neurological disorder characterized by recurrent unprovoked seizures that influence behavioral and neurobiological functions [1, 2]. Approximately 65 million people suffer with epilepsy [3], which can lead to psychosocial disorders and even death [4–6]. The occurrence of sudden and abnormal neuronal discharges disrupts cellular metabolism and increases reactive oxygen species (ROS) formation triggering oxidative stress, mechanisms usually associated to neurodegeneration in epileptic patients [7].

Experimental models of epilepsy-related pathogenesis represent interesting strategies to investigate seizure-related phenotypes and their underlying neurochemical mechanisms. Accordingly, the use of neurotoxins (e.g., pentylenetetrazole (PTZ) and kainic acid) represents chemical models that impair GABAergic and glutamatergic neurotransmission, promoting excitotoxicity and seizures [2, 8–11]. PTZ acts in the central nervous system (CNS) as a $GABA_A$ receptor antagonist [12],

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and acute PTZ exposure has been considered a suitable protocol to assess seizure-like behaviors and possible therapeutic approaches [13]. Since one-third of epileptic patients do not respond to antiepileptic drugs (AEDs) and experience important side effects (e.g., cognitive deficit, sedation, increased aggression, and impulsivity) [14, 15], novel pharmacological strategies aiming to attenuate seizures are needed.

Taurine (TAU) is an amino sulfonic acid obtained from the diet and also synthesized in the CNS from sequential cysteine oxidation reactions [16]. This molecule has a pleiotropic role since it regulates Ca^{2+} metabolism, osmoregulation, and membrane potential and acts as antioxidant and inhibitory neuromodulator [17–21]. Although TAU positively modulates $GABA_A$ and glycine receptors, the existence of specific TAU receptors has been postulated [22]. Interestingly, TAU counteracts excitatory synaptic activity in the dentate gyrus of rodents, a CNS region involved in seizure initiation [23–26]. Thus, assessing the neuroprotective effects of TAU may provide important data regarding new alternatives to prevent seizures.

In a translational neuroscience perspective, the use of zebrafish (*Danio rerio*) has grown exponentially during the last decade, offering a real possibility for high-throughput screens [27–30]. Despite the neuroanatomical differences in comparison to the human CNS, zebrafish presents numerous brain areas with homologous functions [31–33]. The evolutionarily conserved physiological responses and the well-characterized behavioral repertoire [29] make zebrafish a suitable model organism to investigate the neurochemical mechanisms underlying the protective roles of TAU in vertebrates [21]. Although the seizure-related behavioral scores following PTZ exposure in adult zebrafish have been characterized previously [2], there are no data regarding the effects of PTZ on oxidative stress-related parameters and a potential protective role of TAU in PTZ-challenged fish. Thus, considering the positive effects of TAU as antioxidant and inhibitory neuromodulator in the CNS, we investigate whether TAU pretreatment prevents PTZ-induced behavioral and neurochemical changes in adult zebrafish.

Materials and Methods

Animals

Subjects were adult *short-fin* zebrafish (*Danio rerio*) (4–6 months old, ~ 50: 50 male to female ratio, weighing 0.250–0.400 g) obtained from a local distributor (Hobby Aquariums, Santa Maria, RS). Fish were acclimated in the laboratory for 15 days in 50 L tanks with a maximum density of 2 animals/L containing dechlorinated water kept under constant aeration, mechanical and chemical filtration at 25 ± 2 °C, pH = 7.1. The water chemical conditions were monitored using commercial kits for determining pH, nitrite, and ammonia (Alcon Basic®, Alcon, Brazil). Illumination was provided by fluorescent lamp tubes adjusted to a 14/10 h light/dark photoperiod cycle (lights on at 7:00). Fish were fed twice daily with commercial flake food (Alcon Basic®, Alcon, Brazil) and maintained in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals. All experimental procedures were approved by the Ethics Commission on Animal Use of the Federal University of Santa Maria (protocol number 8707070316).

Pharmacological Manipulations

Before PTZ exposure, animals were pretreated with TAU (42, 150, and 400 mg/L) for 40 min. These concentrations were chosen based on previous reports, which showed positive effects of TAU on zebrafish behavior [20, 34–36]. As a positive control, another cohort was exposed to 75 μM diazepam (DZP) for 40 min [2], while the control (CTRL) group was kept in dechlorinated water for the same period. The induction of seizure-like behaviors was further performed using 10 mM PTZ (20 min) in a 1-L tank. Both exposure period and PTZ concentration were chosen to allow the quantification of seizure-related behavioral scores without fish mortality [2]. Figure 1 shows a schematic drawing explaining the pharmacological manipulations and the experimental groups.

Seizure-Like Behavioral Scores

During PTZ exposure, seizure-like behaviors were manually computed by two observers blind to the experimental condition (inter-rater reliability > 0.90) using the scores depicted in Table 1 as described previously [2]. Seizure intensity was estimated by the area under the curve (AUC) of the scores obtained for each cohort across the 20-min exposure period. The latency to reach score 4 represents the time in which animals displayed clonic seizure-like behavior, while score 4 events represent the frequency of these episodes. Behavioral activities were recorded using the ANY-maze™ software (Stoelting CO, USA) at 30 frames/s.

Spatiotemporal Reconstructions of Swimming Behavior

Spatiotemporal reconstructions of the swimming pattern were performed using the spatial coordinates (*x* center and *y* center) across fractions of seconds [20, 34, 37, 38]. For each experimental group, track data were analyzed based on their similarities by two trained observers (inter-rater reliability > 0.85), on a consensus basis [20, 39, 40]. Results were further exported into separated spreadsheets and the representative swimming traces were depicted as scatter plots (Graphis 3D graphing software™). The *x* center (horizontal position), *y* center (vertical position), and time were plotted on the X, Y, and Z axes, respectively. A spectrum of colors (blue to red) was used to represent the position of fish in a 5-min period (0–300 s).

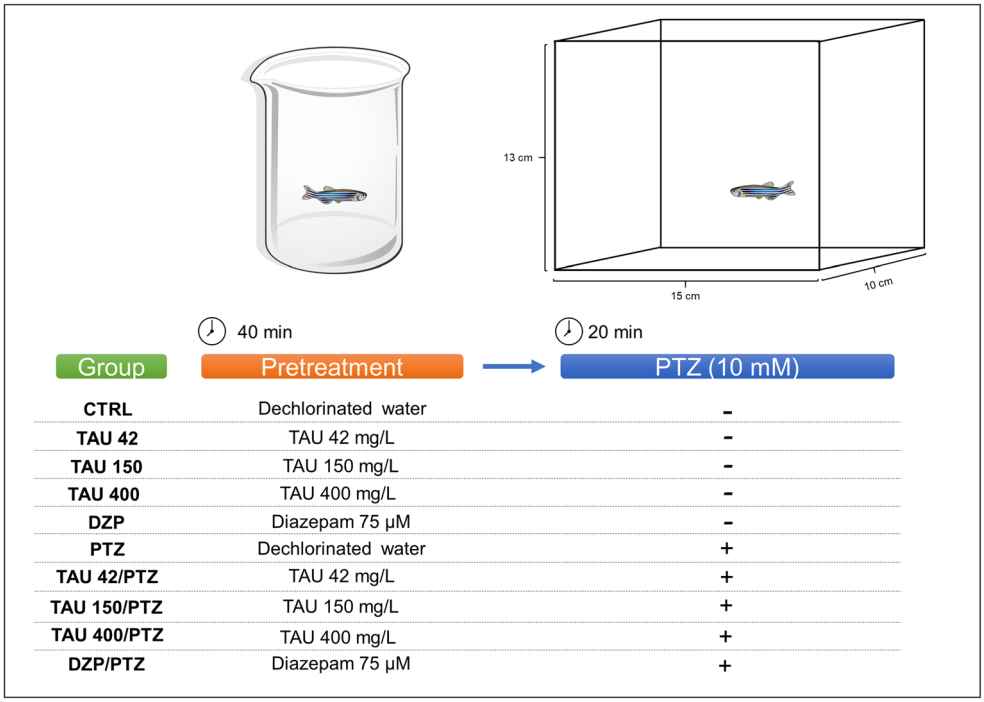

Fig. 1 Representative illustration of the experimental design. Animals were pretreated for 40 min (green and orange column) and further exposed to PTZ (blue column) for 20 min. Seizure-like behavioral scores were evaluated each 30 s

Sample Preparation

To assess enzymatic and non-enzymatic antioxidant defenses and oxidative stress-related parameters, brain samples were prepared as described previously [34]. After PTZ exposure, fish were immediately euthanized, and the brains were further dissected and homogenized in 1 mL of phosphate buffered saline (PBS), pH 7.4, containing 137 mM NaCl, 10.1 mM Na_2HPO_4, and 1.76 mM KH_2PO_4. Four brains were pooled per sample, homogenates were further centrifuged at 700×*g* for 5 min at 4 °C to remove cell debris, and the supernatants were used for the experiments. To determine cell viability and LDH activity, intact brain tissues were transferred to 24-well culture plates containing 0.5 mL of 10 mM HBSS-HEPES buffer (pH 7.4) after euthanasia. All plates were maintained at 37 °C throughout the experiment [41].

Table 1 Seizure-like behavioral scores in PTZ-challenged zebrafish

Scores	Behavioral endophenotypes
0	Swimming in the bottom area
1	Increased swimming activity and opercular movements
2	Erratic movements and burst swimming
3	Circular swimming in the top of the tank
4	Clonic seizure-like behavior (abnormal muscular contraction, corkscrew swimming)
5	Tonic seizure-like behavior (loss of body posture in the bottom of the tank)

Table adapted from Mussulini et al. [2]

Determination of Antioxidant Enzyme Activities

Superoxide dismutase (SOD) activity was measured using a colorimetric assay to detect adrenaline oxidation rate at 480 nm [42]. Supernatants from zebrafish brain tissue (20–30 μg protein) were mixed with glycine buffer (50 mM, pH 10.2) following substrate addition (1 mM adrenaline). SOD activity was expressed as U SOD/mg protein [34]. Catalase (CAT) activity was assessed by measuring the decrease in the absorbance of hydrogen peroxide at 240 nm [43]. Results were expressed as μmol/min/mg protein using the conditions described previously for zebrafish (50 mM potassium phosphate buffer, pH 7.0, 0.3 M H_2O_2, and 20–30 μg protein) [34]. Glutathione-S-transferase (GST) activity was determined as reported previously [44]. Supernatants from zebrafish brain tissue (40–60 μg protein) were mixed with potassium phosphate buffer (20 mM, pH 6.5) and reduced glutathione (10 mM). Later, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) diluted in ethanol was added as substrate and samples were read at 340 nm. GST activity was expressed as nmol of S-(2,4-dinitrobenzyl) glutathione (GS-DNB)/min/mg protein.

Non-enzymatic Antioxidant Defenses

Nonprotein thiol levels (NPSH) were measured as described elsewhere [45–47]. Briefly, supernatants from zebrafish brain tissue were mixed with 10% trichloroacetic acid (TCA) and centrifuged at 3000×*g* for 10 min at 4 °C. Supernatants (60–80 μg protein) were then mixed with 10 mM 5,5-dithio-bis-2-

nitrobenzoic acid (DTNB) dissolved in ethanol. The resultant yellow complex was measured at 412 nm after 1 h. Results were expressed as nmol SH/mg protein.

Oxidative Stress-Related Parameters

Lipid peroxidation was evaluated by measuring thiobarbituric reactive substances (TBARS) as described previously [34, 48]. Supernatants from zebrafish brain tissue (80–100 μg protein) were added to 0.16 mL of 15% trichloroacetic acid and further centrifuged at 10,000×*g* for 10 min. Supernatants were further mixed with 0.1 mL of 0.67% thiobarbituric acid and boiled for 30 min. TBARS levels were determined at 532 nm using malondialdehyde (MDA) as standard. Results were expressed as nmol MDA/mg protein. Protein carbonylation was estimated by protein precipitation in the presence of trichloroacetic acid and dinitrophenylhydrazine (DNPH) [49]. Protein samples (200 μL) were mixed with 0.15 mL of 10 mM DNPH and incubated for 1 h. Later, 0.125 mL of SDS (3.0%), 0.5 mL of heptane (99.5%), and 0.5 mL of ethanol (99.8%) were added to samples and mixed for 30 s. Samples were centrifuged at 1000×*g* for 15 min and the supernatant was discarded. Finally, the pellet was homogenized in 0.25 mL of 3% SDS and the amount of carbonylated proteins was determined at 370 nm. Results were expressed as nmol carbonyl/mg protein and calculated using a molar extinction coefficient of 22.000 M/cm.

MTT Assay

MTT assay was measured by the conversion of intracellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide to purple formazan by mitochondrial succinate dehydrogenases, which is a standard protocol that predicts cell viability [50, 51]. After euthanasia, whole zebrafish brains were immediately immersed in 0.5 mg/mL of MTT solution in a covered water bath shaker for 20 min at 37° [41, 52]. After the incubation period, 300 μL dimethyl sulfoxide (DMSO) was added per sample and kept overnight under constant homogenization to allow the solubility of formazan crystals in order to quantify the product formation. Lastly, 200 μL of extracted formazan was added to 96-well plates and cell viability was assessed at 560 and 650 nm. Results were expressed as a percentage of control [41].

Lactate Dehydrogenase Activity

Lactate dehydrogenase (LDH) activity, a parameter that estimates cell survival [53], was measured as described elsewhere [41]. Briefly, after incubation of intact zebrafish brain tissues in HBSS-HEPES buffer for 10 min, the medium was removed and used to measure the extracellular conversion of lactate to pyruvate. Thus, 1,10-phenanthroline was dehydrogenated to a colored complex by reacting with NADH, which was measured at 490 nm [41, 54]. Total LDH activity was estimated in lysates of brain tissue. Results were expressed as percentage of total LDH activity per sample.

Protein Quantification

Total protein amount was measured according to Bradford [55] using bovine serum albumin as standard.

Statistics

Normality of data and homogeneity of variances were analyzed by Kolmogorov-Smirnov and Bartlett's tests, respectively. Nonparametric data (seizure scores across 20 min) were expressed as median ± interquartile range, and the area under curve was calculated to estimate seizure intensity. The cumulative frequency was expressed as the percentage of animal that reached each score across the 20-min observation period. Parametric data were expressed as means ± standard error of mean (S.E.M) and further analyzed using one- or two-way analysis of variance (ANOVA), followed by Tukey's post hoc test. The level of significance was set at $p \leq 0.05$.

Results

Seizure Related-Phenotypes

The occurrence of seizure-related behavioral phenotypes and the percentage of animals that reach each score were assessed in a 20-min PTZ exposure period (Fig. 2). PTZ elicited seizure-like behaviors and zebrafish displayed corkscrew swimming and immobility, which closely resemble tonic/clonic seizures (scores 4 and 5). As expected, DZP attenuated PTZ-induced changes in behavior and 150 mg/L TAU showed a similar response (Fig. 2a). Furthermore, while 100% of PTZ-exposed fish reached score 4 after 210 s, the maximum percentage of fish reached score 4 in TAU 150, and DZP groups after 600 and 450 s, respectively (Fig. 2b).

Figure 2c shows the seizure intensity in initial (0–150 s), intermediary (150–300 s), and final (300–1200 s) periods of test estimated by the area under curve. One-way ANOVA yielded significant differences in initial ($F_{4,109} = 28.68$, $p < 0.001$), intermediary ($F_{4,109} = 13.78$, $p < 0.0001$), and final ($F_{4,109} = 11.29$, $p < 0.0001$) periods. In the initial time, all TAU and DZP pretreated groups had decreased seizure intensities when compared to PTZ-exposed fish. However, in the other segments of tests, the seizure was less intense in TAU 150- and DZP-pretreated groups.

Figure 3a shows representative heat maps of the individual scores of zebrafish across the PTZ exposure period. Basically,

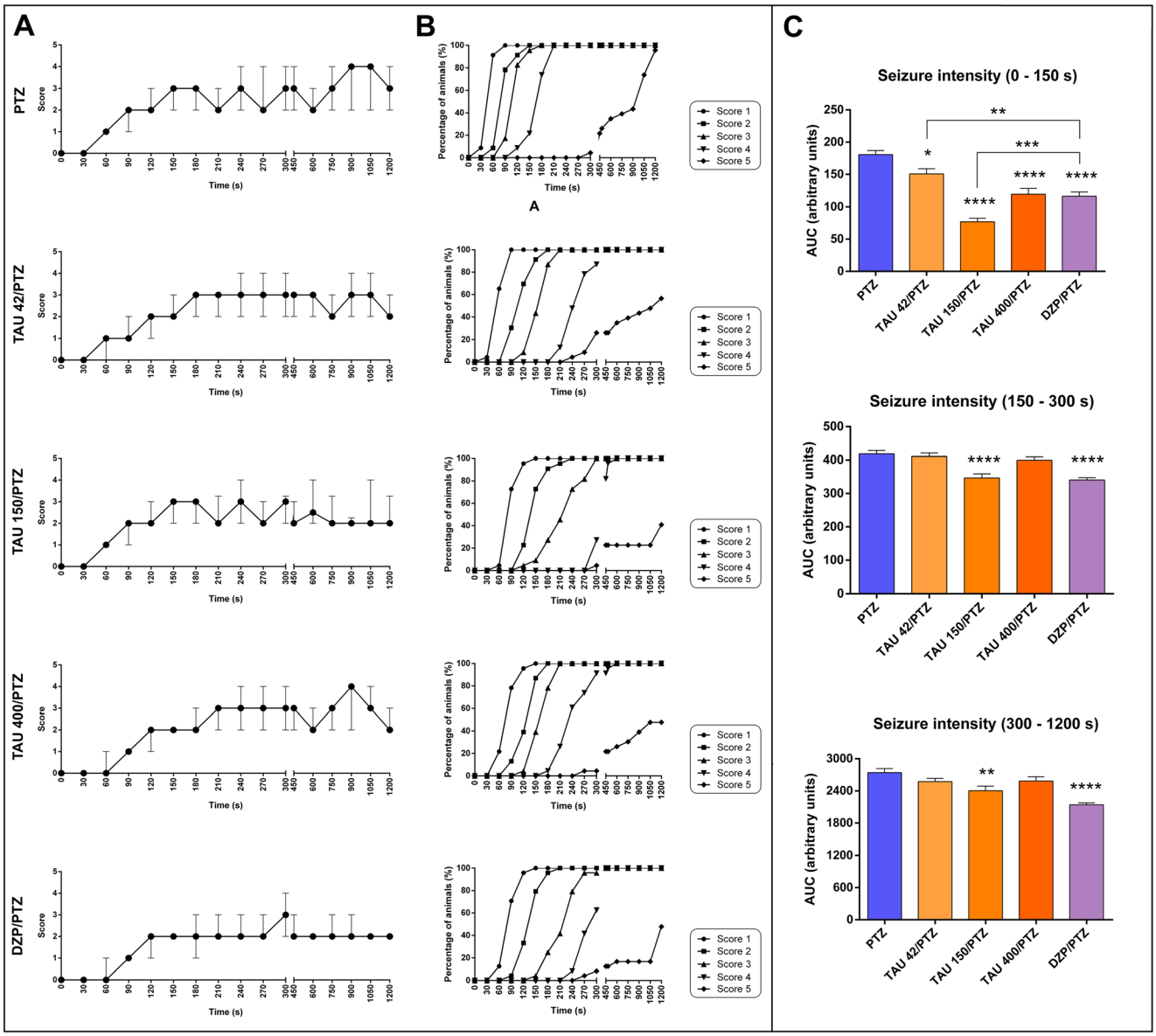

Fig. 2 Effects of TAU and DZP pretreatment in PTZ-challenged zebrafish. **a** Seizure-like behavioral scores across time (data were represented as median ± interquartile range). **b** Percentage of animals (%) that reached each score across time. **c** Seizure intensity at distinct time periods (0–150, 150–300, and 300–1200 s) evaluated by the area under curve (AUC) for each treatment. Seizure intensity was represented as mean ± S.E.M and analyzed by one-way ANOVA followed by Tukey's test. Asterisks above bars express significant differences compared to the PTZ group, while asterisks above brackets indicate statistical differences compared to the DZP group (n = 20–24 animals per group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

the latency to reach score 4 increased in TAU 150-, TAU 400-, and DZP-pretreated groups ($F_{4,109} = 19.17$, $p < 0.001$) (Fig. 3b). Meanwhile, the number of score 4 events was significantly reduced in fish pretreated with 150 mg/L TAU and DZP ($F_{4,109} = 10.32$, $p < 0.001$) (Fig. 3c).

Reconstruction of Zebrafish Swimming Traces

Figure 4 shows the swimming traces across the first 5 min of exposure, a period in which animals reached score 4. Representative spatiotemporal reconstructions of behavior depict different patterns of activity, where the PTZ group showed aberrant swimming in the surface of the tank. Both TAU 42/PTZ and TAU 150/PTZ groups had a similar profile in comparison to CTRL, showing an initial swimming in the bottom area followed by increased activity in the top. TAU 400/PTZ group showed a distinct pattern of activity, exploring the bottom and top area proportionally across the test. Additionally, DZP-pretreated group presented different swimming traces, since animals swam preferentially in the bottom area of the tank.

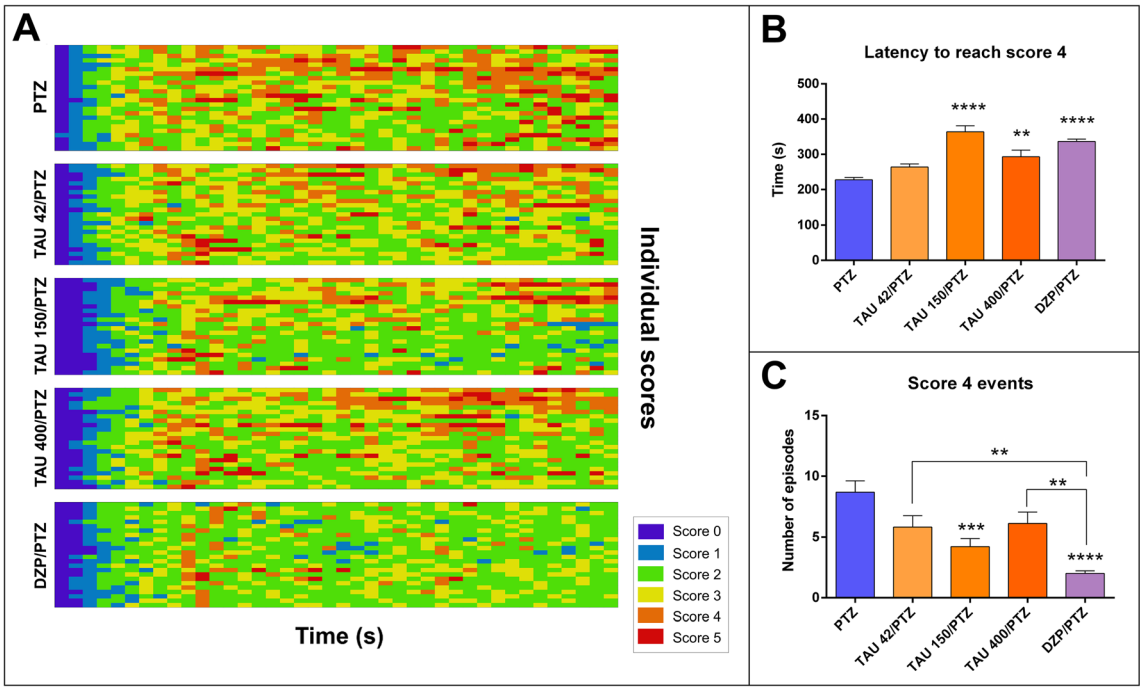

Fig. 3 TAU and DZP attenuate PTZ-induced changes in seizure-like behavioral scores. **a** Representative heat maps showing the individual scores every 30 s across the 20-min exposure period (X axis) of each individual fish (Y axis). **b** Latency to reach score 4. **c** Frequency of score 4. Latency and frequency of score 4 were represented as mean ± S.E.M and analyzed by one-way ANOVA followed by Tukey's test. Asterisks above bars express significant differences compared to the PTZ group, while asterisks above brackets indicate statistical differences compared to the DZP group (n = 20–24 animals per group; $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$)

Enzymatic and Non-enzymatic Antioxidant Defenses

Figure 5 shows the effects of PTZ and TAU on enzymatic and non-enzymatic antioxidant defenses. Concerning the enzymatic antioxidant defenses, no significant differences were observed in SOD and GST assays. Regarding CAT activity, two-way ANOVA using treatment and PTZ as factors showed significant effects of treatment ($F_{4,54} = 18.82$, $p < 0.001$). Basically, PTZ increased CAT activity similarly to TAU 42 and TAU 150 alone and TAU 42/PTZ groups. Moreover, two-way ANOVA showed a significant effect of interaction ($F_{4,54} = 3.69$ $p < 0.0099$), where all PTZ-exposed groups had decreased NPSH levels, except TAU 150/PTZ.

Oxidative Damage and Cell Viability-Related Parameters

Figure 6 depicts the effects of TAU and PTZ on oxidative damage- and cell viability-related parameters. Concerning lipid peroxidation, two-way ANOVA showed a significant effect of treatment ($F_{4,54} = 91.04$, $p < 0.0001$) and a treatment x PTZ interaction ($F_{4,54} = 4.21$, $p < 0.0052$). PTZ increased TBARS levels, whereas pretreatment with 42 mg/L TAU, 150 mg/L TAU, and DZP prevented this effect. PTZ increased carbonylated protein levels and a preventive effect was observed in TAU 150/PTZ and DZP/PTZ groups ($F_{4,46} = 8.69$, $p < 0.0001$ for interaction term, and $F_{1,46} = 10.63$, $p < 0.0001$ for treatment, respectively). No significant differences were observed in MTT assay and LDH activity among groups.

Discussion

In this study, we report for the first time a preventive effect of TAU on neurobehavioral parameters in PTZ-exposed zebrafish. Our data showed that similarly to DZP, TAU attenuates seizure-like behaviors since TAU pretreatment increases the latency to reach score 4 (clonic-like behaviors) and decreases seizure frequency and intensity. Moreover, depending on the concentration tested, TAU abolished the effects of PTZ on NPSH levels, lipid peroxidation, and protein carbonylation. Overall, we suggest a protective role of TAU against PTZ-induced behavioral and neurochemical changes in zebrafish.

The occurrence of seizure-like behaviors after a single PTZ exposure has been extensively described in zebrafish [2, 3, 6, 9, 56, 57]. Exposure to 10 mM PTZ for 20 min elicits all seizure-like behavioral phenotypes, in which 95% animals reached score 5 and frequently exhibited tonic/clonic seizure scores [2]. In the CNS, PTZ antagonizes $GABA_A$ receptors, thereby modifying excitatory/inhibitory tonus, which culminates in acute seizures [58–60]. Importantly, corkscrew swimming (a behavioral phenotype observed in score 4) occurs simultaneously to electroencephalogram (EEG) abnormal discharges in zebrafish exposed to 10 mM PTZ [6].

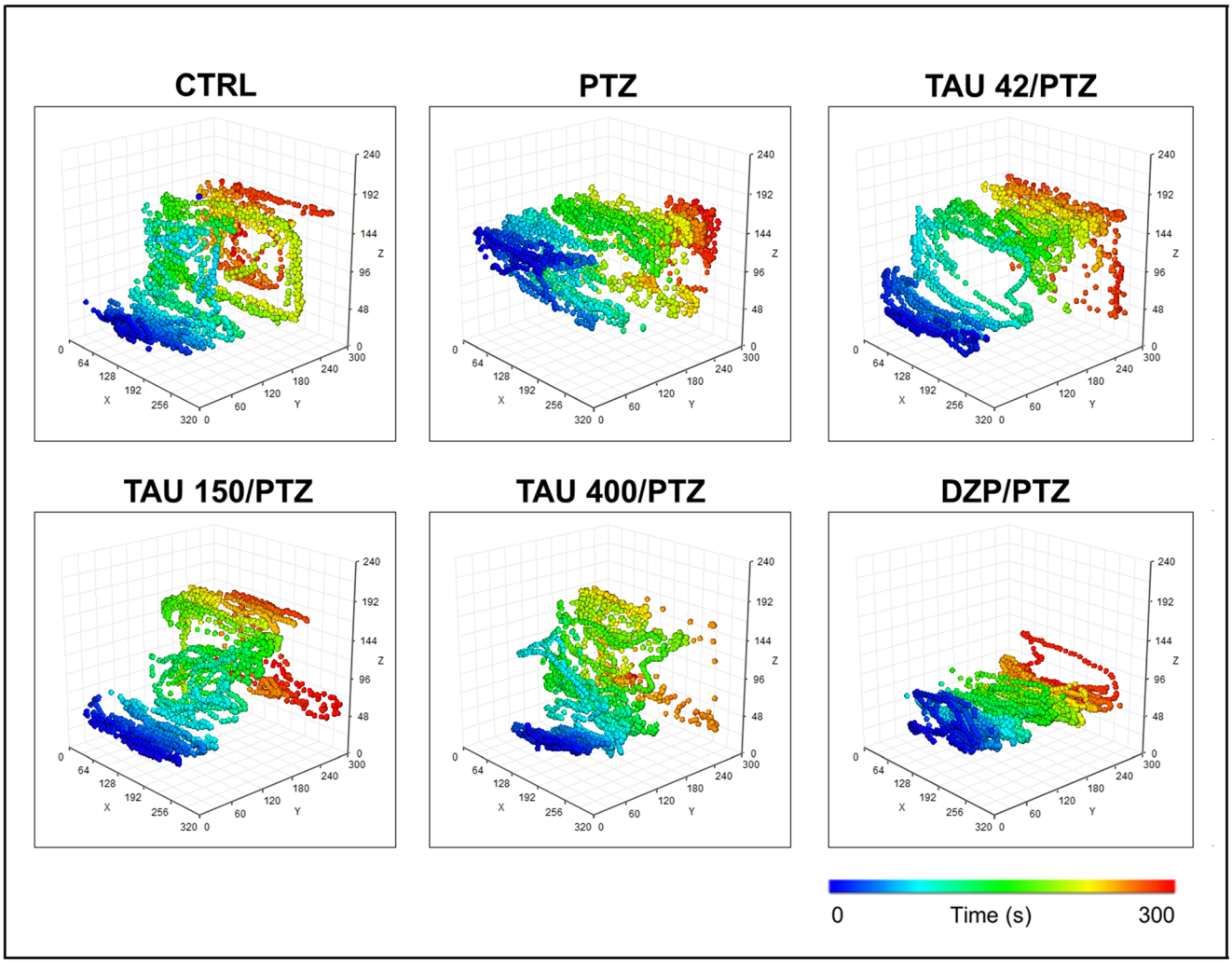

Fig. 4 Representative swimming traces of the experimental groups during the initial periods of PTZ exposure (5 min). Reconstructions were obtained by plotting animal coordinates (X and Z axes) across time (Y axis). The segments of test (0–300 s) were represented by a color scale gradient, indicating the beginning (blue) and the end of trial (red)

As an endogenous neuromodulator, TAU plays a role in controlling the inhibitory/excitatory synapses since it may act as a $GABA_A$ receptor agonist [61–63]. TAU can also prevent excitotoxicity by inhibiting the reverse mode of Na^+–Ca^{2+} exchanger decreasing intracellular Ca^{2+} levels, suggesting an interaction between TAU and N-methyl-D-aspartate (NMDA) receptor [64, 65]. All TAU concentrations tested decreased the seizure intensity at the first 150 s, whereas only 150 mg/L TAU showed a protective effect from 150 to 1200 s, similarly to DZP. Additionally, 150 mg/L TAU pretreatment increased the latency to reach stage 4 and decreased the frequency of clonic-like behaviors. Since TAU promotes hyperpolarization and inhibits neuronal firing [66], these data could be associated to a modulatory role in the CNS. Interestingly, TAU levels increase in the brain following seizure episodes in both animal models [67] and human serum [68–70]. Thus, TAU may counteract glutamatergic excitotoxicity and attenuate seizure episodes in PTZ-challenged animals, which is in accordance with the individual heat maps of scores and the representative spatiotemporal reconstructions of behavior depicted here.

Spatiotemporal reconstructions of swimming activity serve as useful tools for assessing the behavioral neurophenotypes of zebrafish after pharmacological treatments [39, 40]. In general, zebrafish has a tendency to swim in the bottom section and gradually increase the activity in upper sections of a tank [29, 71, 72]. PTZ-exposed animals swam mainly in the top area, showing a disrupted swimming activity. These changes could be a consequence of impaired neurochemical signaling pathways, with culmination in aberrant swimming activity [2, 73]. Interestingly, TAU 42/PTZ and TAU 150/PTZ exhibited similar swimming traces when compared to CTRL group. These data may reflect protective effects of TAU in attenuating seizure-like behaviors, since TAU alone does not affect swimming activity [20, 35, 36]. Conversely, DZP group spent more time in the bottom area, which could reflect sedative effects due

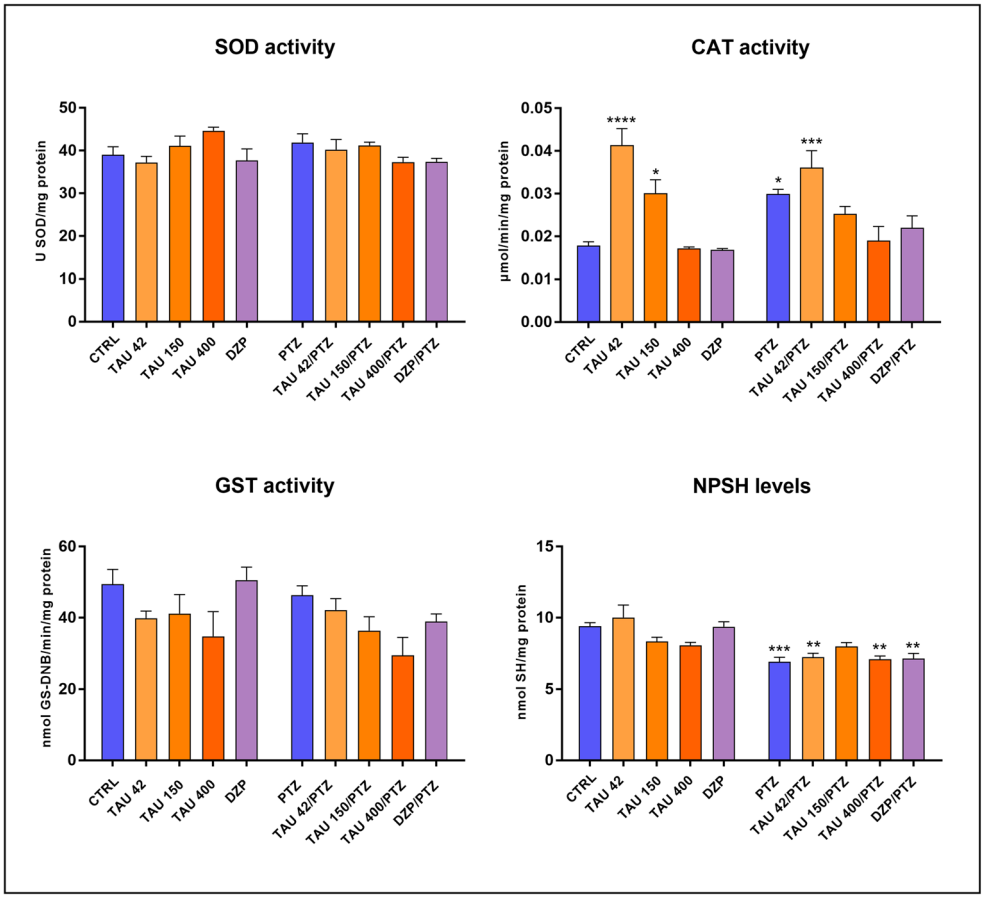

Fig. 5 Enzymatic and non-enzymatic antioxidant defenses in zebrafish brain. Data were expressed as mean ± SEM and analyzed by two-way ANOVA followed by Tukey's test using treatment and PTZ as factors. Asterisks express significant differences compared to control (CTRL) ($n = 6$ biological preparations per group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$)

to its interaction with $GABA_A$ receptors [74, 75]. This sedative side effect is common in epileptic patients and impairs several daily activities [76, 77]. Moreover, AEDs, such DZP, are not effective for many epileptic conditions, in which 5–10% of patients are resistant for first- and second-generation of AEDs [78]. These data highlight the importance for screening new therapeutic molecules to prevent seizures. Due to its pleiotropic role in the CNS [17, 21, 64, 79], TAU probably attenuates seizures via different molecular pathways compared to DZP, modulating seizure-like behaviors without altering the overall swimming pattern.

Seizures may lead to brain insults due to excitotoxicity, which culminates in an increased production of reactive oxygen/nitrogen species (ROS/RNS) [80]. ROS and RNS play a role in seizure-induced neurodegeneration [81–83]. PTZ may induce oxidative stress by increasing free radical production [84] and/or decreasing antioxidant defenses [85]. Since the brain has an increased demand for oxygen consumption and is enriched with polyunsaturated fatty acids, it is more susceptible to lipid peroxidation and protein carbonylation [7]. PTZ-exposed fish showed decreased NPSH content, as well as increased TBARS levels and protein carbonylation. We observed that 150 mg/L TAU prevented PTZ-induced changes in NPSH, TBARS, and carbonylated protein levels suggesting antioxidant activity. TAU may protect against glutamate excitotoxicity by inhibiting extracellular calcium influx and calcium release from the internal pools, which reflect putative mechanisms of TAU antioxidant activity [64, 86]. Furthermore, TAU has antioxidant properties by modulating oxidative stress-related parameters in vivo [34, 87]. Despite the neuromodulatory role of TAU in extracellular milieu, TAU may interact directly with some oxidant radicals that cause lipid peroxidation, acting as scavenger at physiological intracellular concentrations [88]. Interestingly, CAT activity increased substantially in both TAU 42 and TAU 42/PTZ groups, reflecting a complex modulatory effect of TAU on enzymatic antioxidant defenses. Vasodilator molecules may increase CAT activity, which represents a possible mechanism of protection [89]. Since TAU may decrease blood pressure in experimental models [21], the increased CAT activity could reflect a vasodilator action, not necessarily indicating alterations in a specific response to oxidative stress. Conversely, the increased CAT activity in PTZ-exposed animals could reflect a compensatory mechanism to stimulate enzymatic antioxidant defenses in PTZ-exposed fish. Thus, although CAT activity in both TAU 42 and PTZ groups showed a similar result, they may indicate different physiological responses. Additionally, DZP showed protective effects against PTZ-induced lipid peroxidation and

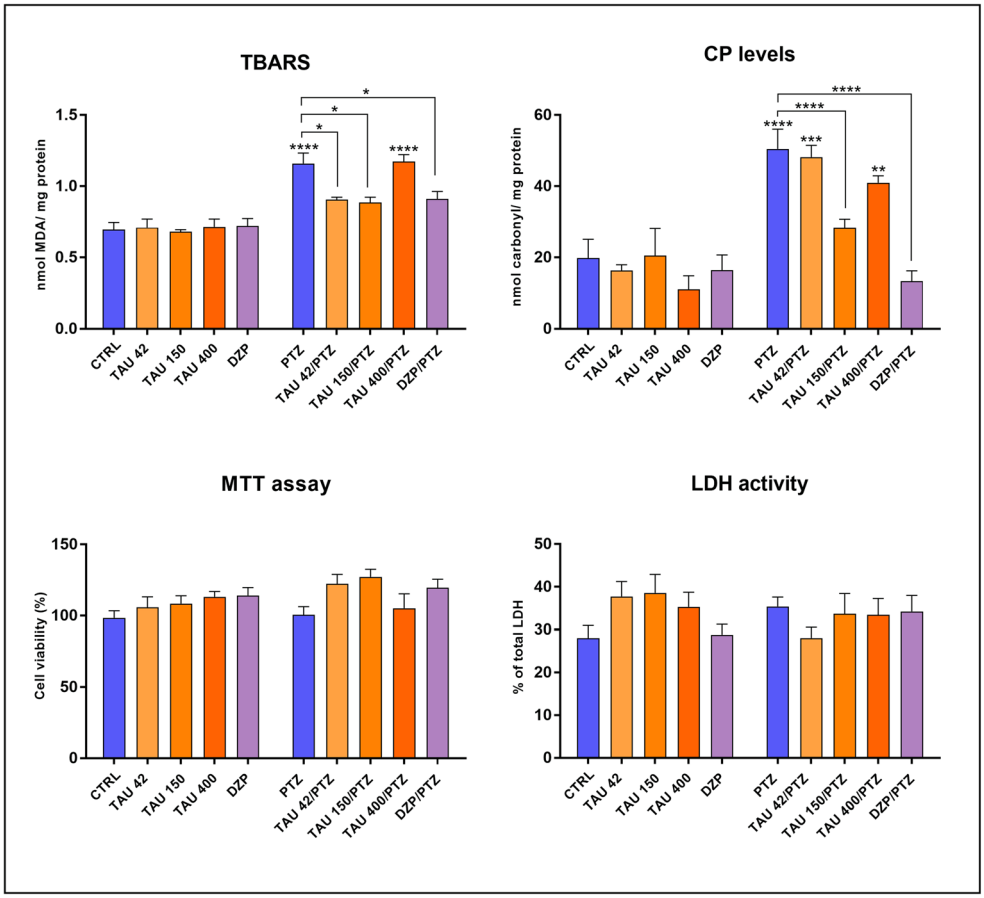

Fig. 6 Effects of TAU and DZP pretreatment on TBARS, carbonylated protein (CP) levels, and cell death-related parameters in brain tissue of PTZ-challenged zebrafish. Data were expressed as mean ± SEM and analyzed by two-way ANOVA followed by Tukey's test using treatment and PTZ as factors. Asterisks above bars express significant differences compared to control (CTRL), while asterisks above brackets indicate statistical differences compared to the PTZ group ($n = 6$ biological preparations per group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$)

protein carbonylation. Accordingly, previous studies showed that enhanced GABAergic neurotransmission decreases oxidative stress by counteracting glutamatergic excitotoxicity in mice [90]. We did not verify significant effects on MTT and LDH assays probably due to the lack of seizure recurrence in the acute PTZ exposure model [91]. Notably, the effects of TAU on PTZ-induced toxicity seem not to be concentration-dependent in biochemical or behavioral analyses. Although the precise mechanisms underlying TAU actions in zebrafish brain have not been fully elucidated, this biphasic response suggests that TAU may act on different receptors or signaling transduction pathways in the CNS [21, 92]. Nonetheless, our data show positive effects of TAU in attenuating seizure-like behaviors and preventing oxidative stress in PTZ-challenged zebrafish.

Conclusion

In summary, our novel data show a protective role of TAU against PTZ-induced behavioral changes and oxidative stress in zebrafish. TAU prevents lipid peroxidation and protein carbonylation following PTZ exposure in zebrafish brain, suggesting that antioxidant mechanisms are involved in neuroprotection. Importantly, TAU does not affect swimming activity, a common effect observed after DZP treatment. Since TAU presents beneficial effects in the CNS, further studies are needed to elucidate the mechanisms underlying neuroprotection, as well as its long-term efficacy in recurrent seizure episodes.

Author Contributions All authors contributed to the preparation of the manuscript and approved the final version.

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Compliance with Ethical Standards

All experimental procedures were approved by the Ethics Commission on Animal Use of the Federal University of Santa Maria (protocol number 8707070316).

Conflict of Interest The authors declare that they have no conflict of interest.

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