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The effect of intracerebroventricular amyloid beta 1–42 application on cognitive functions in aged rats supplemented with taurine and the change of peroxisomal proteins in this process

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ABSTRACT

Objective: The aim of our study is to investigate the change of peroxisomal proteins in the neurodegenerative and oxidative process caused by the neurotoxicity of Aβ 1–42 in aged rats supplemented with taurine and to show the possible positive effects of taurine in this process.
Methods: 30 Wistar albino rats were randomly divided into 5 groups as control, sham, Aβ 1–42, taurine, and Aβ 1–42+taurine. Taurine administration continued for 6 weeks (1000 mg/kg/day with drinking water). Stereotaxic surgery was applied to all groups (intracerebroventricular per lateral ventricle needle only or 5 μl, PBS, or Aβ 1–42). Spatial learning and memory performances of the animals were evaluated with Morris water maze and elevated plus maze. The levels of MDA and GSH were measured as oxidative stress parameters in the cerebral cortex and hippocampus. Expressions of CAT, PEX14, PMP70 of peroxisomal membrane proteins were indicated by Western blot analysis.
Results: Our results showed that injection of Aβ 1–42 decreased the spatial learning and memory performance, cortex CAT and hippocampus PEX14, PMP70 and GSH levels, and increased cortex and hippocampus MDA levels ($p < 0.05$). Although the administration of taurine partially ameliorated the adverse effects of Aβ 1–42 injection, a significant difference was found only at the hippocampus GSH levels ($p < 0.05$). Also, taurine caused anxiety at this dose ($p < 0.05$).
Discussion: In conclusion, decreased peroxisomal proteins and antioxidant capacity in neurodegenerative and oxidative processes induced by intracerebroventricular Aβ 1–42 injection showed that peroxisomes may play a role in this process and taurine supplementation may have positive effects especially in increasing antioxidant capacity.

1. Introduction

Alzheimer's disease (AD) occurs with neuron and synapse losses in the central nervous system. It is a progressive neurodegenerative disease especially characterized by decreased cognitive functions and mental dysfunction (Gilman, 1997; Lleo and Greenberg, 2006). The most likely factor in the onset and progression of AD is known as amyloid beta (Aβ). According to the amyloid cascade hypothesis, increasing Aβ 1–42 level is more in line with the onset of AD (Hardy and Selkoe, 2002). Synthetic Aβ species are injected into different parts of the brain of rodents to create an experimental AD model (Cetin and Dincer, 2007; Eskandari-Roozbahani et al., 2019). It has been observed that Aβ types distort memory abilities in experimental models. Aβ directly stimulates neurotoxicity and causes cholinergic destruction in the basal forebrain. Depending on the accumulation of Aβ, intercellular communication is impaired, cell death increases, and as a result, the tissue becomes dysfunctional (Hardy and Selkoe, 2002; Sadigh-Eteghad et al., 2015).

Having more than fifty enzymes, peroxisomes (Wanders and Waterham, 2006a, 2006b) have many important roles such as oxidation of long-chain fatty acids, biosynthesis of bile acids, destruction of toxic substances with catalase (CAT) enzyme, converting H_2O_2 (hydrogen peroxide) to H_2O and O_2 (Wanders et al., 2010), biosynthesis of plasmalogens for the normal function of the brain, and neutralizing free oxygen radicals. Peroxisomes function like a pretreatment-center for

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mitochondria. At the same time, peroxisomes have enzymatic components that prevent oxidative and nitrosative stress (Deori et al., 2018). Due to its close relationship with mitochondria and its effectiveness on oxidative stress, peroxisomes are highly effective on neurodegenerative diseases, but their role in AD pathogenesis is not yet fully known. It has also been discovered over the past decade that mammalian peroxisomes are not only metabolic organelles but can also serve as signalling platforms that modulate various physiological and pathological processes, including inflammation, innate immunity, and cell fate. Abnormalities that occur in these metabolic processes or any of the signalling functions can directly or indirectly cause genetic disorders or age-related diseases (such as neurodegenerative diseases and cancer) (Fransen et al., 2017).

Taurine (2-aminoethanesulfonic acid), a broad spectrum cytoprotective agent, is a sulphur-containing amino acid with high antioxidant properties in the adult hippocampus, cerebellum (Shivaraj et al., 2012) and in the hypothalamus (Huxtable, 1992). Taurine allows neural progenitor cells to proliferate and supports synapse formation in brain regions required for long-term memory (Shivaraj et al., 2012). Taurine levels in the brain decrease significantly with age. This has led to studies investigating the potential neuroprotective effects of taurine (Anan-chaipatana-Auitragoon et al., 2015; Taranukhin et al., 2012). Many studies are showing the beneficial effects of taurine on oxidative stress and neurodegenerative diseases (Cetin et al., 2012; Gebara et al., 2015; Jia et al., 2016). However, it is known that it has harmful and toxic effects according to dosage and mode of administration (Chen et al., 2019; Curran and Marczinski, 2017; El Idrissi et al., 2009; Neuwirth et al., 2013). Therefore, studies are needed to determine the appropriate administration type and appropriate dose range of taurine. Also, the study on the effect of taurine on peroxisomal functions is very limited in the literature.

In this study, the effect of peroxisomes, one of the most important organelles related to oxidative and nitrosative stress, and taurine, which is a strong antioxidant, was investigated in AD model created with Aβ 1–42 neurotoxicity.

2. Methods

2.1. Animals

Thirty adult (28 ± 4 months-old) male Wistar rats were used in the following groups: control (n = 6), sham (n = 6), Aβ 1–42 (n = 6), taurine (n = 6), and Aβ 1–42+taurine (n = 6). The animals were purchased from Gazi University Laboratory Animals and Experimental Research Center and housed under standard laboratory conditions with a 12 h alternating light/dark cycle (08:00–20:00 h with a light period) at 23 °C and were fed on commercial rat chow and water ad libitum.

2.2. Taurine supplementation

Taurine (2-aminoethanesulfonic acid) (BioShop Canada Inc., TAU303.1) was added to the drinking water of animals at a dose of 1000 mg/kg/day for 6 weeks (Groups taurine and Aβ 1–42+taurine) (Kim et al., 2014).

2.3. Intracerebroventricular injection of Aβ 1–42

Aβ 1–42 (Sigma-Aldrich USA, A9810) was diluted in 10 mM sterile PBS (pH: 7.4) (BioShop Canada Inc., PBS408.500) and incubated for one week at 37 °C. After taurine administration, the rats were anesthetized with the combination of intraperitoneal 90 mg/kg ketamine and 10 mg/kg xylazine, and stereotaxic applications were performed. In the Aβ 1–42 and Aβ 1–42+taurine groups, a 10 μL dose (5 μL to each lateral ventricle) was injected with icv Aβ 1–42 and the same amount of PBS to the sham and taurine groups. The skull was opened with the help of a thin-tipped driller according to the coordinates determined using stereotaxic coordinate atlas (Paxinos and Watson, 2007: 456) (0.8 mm posterior from Bregma, 1.5 mm lateral from the sagittal suture, 3.8 mm in depth). Icv Aβ 1–42 injection was done with Hamilton micro injector. Aβ 1–42 or PBS injection was infused for 6 min, and after the infusion, the syringe was kept at the injection site for 5 more minutes for the absorption of the chemical (Giovannini et al., 2002). Only the injector was immersed in the control rats using the same procedure. The animals were taken to the individual cages after the surgery.

2.4. Behavior tests

After the surgery, the healing process of the scalp was checked daily. This process took 7 days in our study. After the healing process, behavioral testing practices for animals started. Cognitive and behavioral function tests were performed every day at the same time (10:00−12:30), at the same room temperature (22 ± 4 °C) and humidity (50–70 %).

2.4.1. Morris water maze (MWM) test

After the 7-day recovery period, rats were included in the experimental protocol in MWM to evaluate their learning ability and spatial memory functions. MWM consists of a plexiglass pool filled with water and a removable escape platform labyrinth below the water level. The escape platform was held constant in the same quadrant for all tests. The water tank, made of plexiglass material in black color, 150 cm in diameter and 65 cm in depth, was filled with brown food coloring (non-toxic) mixed water up to approximately 45−50 cm. The temperature of the water was maintained at 20 ± 2 °C. The tank was covered with black curtains. The pool was divided into four parts (northeastern, northwestern, southeastern, and southwestern) with two imaginary lines passing through the middle of the pool. Visual clues were kept constant during the experiment.

Escape latency: Rats were subjected to escape latency test, where the duration of finding the hidden platform was evaluated for 4 days. Reach time of the rats that were dropped into the water from four different poles was recorded. The time spent was evaluated to determine the learning capacity of animals (McDonald et al., 2004; Morris, 1984). The time from being released into the water to finding the platform was determined to be 90 s. Rats who could not find the platform during this period were placed on the platform by the researcher and allowed to stay on the platform for 30 s and recognize the surrounding clues.

Probe test: On the 5th day, the platform was removed from the water. Reference memory was tested. A 90-second time was given for rats to search for the location of the platform. The preference of the subjects to search in the section where the platform was previously (time spent in the target quadrant) accepted as the acquisition of the reference memory function (Fig. 1).

2.4.2. The elevated plus maze (EPM) test

EPM has two open arms measuring 50 cm × 10 cm and two closed arms measuring 50 cm × 50 cm × 10 cm. The test setup made of black plexiglass consists of four arms. The arms are placed on the floor and at right angles to them. The EPM test application was continued for 2 days. On the first day, the animals were allowed to explore the system for 5 min. The second day was the day of the experiment (Jung and Kim, 2019). After each experiment, the medium was wiped with 65 % ethanol and dried. At the end of each experiment, a 15-minute break was given to dry and ventilate the environment. The behavior of the subjects was recorded at the experimental stage and then analyzed. In the experiment, the time and percentage on the open arm, the time and percentage on the closed arm, and the number of passes between the open and closed arms were manually examined by a trained observer (Fig. 1). Experiments were recorded using a video camera and video files were stored for analyses.

Rats were sacrificed after behavioral testing. The cerebral cortex and hippocampus tissues were isolated (according to the Atlas of Paxinos and Watson). The isolated tissues were quickly frozen in liquid nitrogen

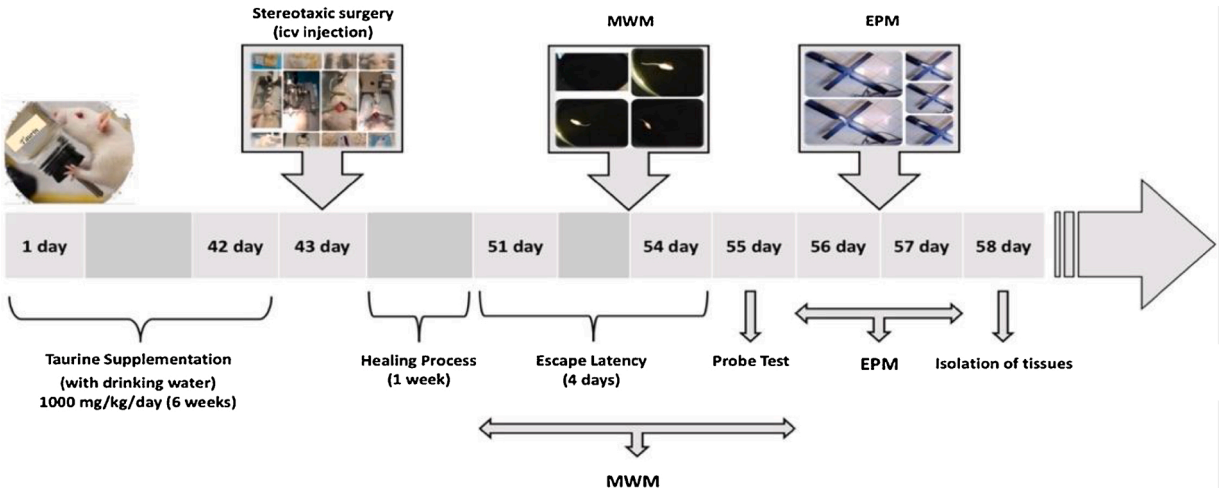

Fig. 1. Timeline for animal experiments.
Groups 4 and 5 were supplemented with taurine. Stereotaxic surgery and behavior analysis tests were performed on all groups (Group 1: control, group 2: sham, group 3: Aβ 1–42, group 4: taurine, and group 5: Aβ 1–42+taurine) (icv: Intracerebroventricular, MWM: Morris water maze, EPM: Elevated plus maze).

and stored at -80 °C until the relevant parameters were studied. The rats were treated according to the guidelines of the European Convention ETS 123, and all the methods used in the current study were approved by the Experimental Animal Ethics Committee of Gazi University (G.U.ET-66332047-604.01.02-E.116581).

2.5. Measurements of the tissue lipid peroxidation and GSH levels

Tissue samples were homogenized in ice-cold trichloroacetic acid (40 mg tissue plus 0.72 mL TCA (10 % w/v)) in a tissue homogenizer and tissue homogenates were centrifuged at 4000 rpm for 15 min. The formation of thiobarbituric acid reactive substances (TBARS) was used to measure the lipid peroxidation as described by Casini et al. (Casini et al., 1986). After centrifugation, butylated hydroxytoluene (BHT) (1% w/v) and thiobarbituric acid (TBA) (0.67 % w/v) was added to the supernatants, and the mixture was heated at 100 °C for 15 min. After cooling, the absorbance at 532 nm was measured immediately and lipid peroxidation levels were expressed as malondialdehyde (MDA) equivalents (nmol/g tissue) using an extinction coefficient of 1.56×105 mol^{-1} cm^{-1}. The glutathione (GSH) level was determined by using a modified Ellman method (Aykaç et al., 1985). Briefly, the supernatants of tissue homogenates were added to 0.3 M Na_2HPO_4 and DTNB solution (0.4 mg/mL 1% sodium citrate). Absorbance was read spectrophotometrically at 412 nm and the GSH levels (μmol/g tissue) were calculated using an extinction coefficient of 13,000 mol^{-1} cm^{-1}.

2.6. Detection of PMP70, PEX14 and CAT expression by western blotting

For western blotting, the total protein concentration of each cerebral cortex and hippocampal tissue lysates was determined by the Bradford method (Bradford, 1976) and equal amounts of protein extracts (20 μg) were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis SDS-PAGE (10 %) and then transferred to nitrocellulose membrane (GE Healthcare Lifesciences, Germany). The membrane was blocked with 3 % (w/v) bovine serum albumin (BSA) (Bioshop, Canada) dissolved in Tris Buffered Saline (TBS) containing 0.1 % Tween 20 (TBST) for overnight at +4 °C. The blocking was followed by incubation with primary antibodies (PMP70 (Sigma-Aldrich, USA), PEX14 (Elabscience Biotechnology, USA) and CAT (Sigma-Aldrich, USA)), and then horseradish peroxidase (HRP) conjugated secondary antibodies (Advansta, USA) for 2 h at room temperature. The antibodies were diluted in TBST with 3% (w/v) BSA at the suggested dilution. Washings were done in TBST at each step, 3 times for 10 min/wash. Following the last washing, the membranes were incubated with enhanced chemiluminescence (ECL) detection reagents (Thermo Fisher Scientific, USA). Target protein bands were visualized using the imaging system (Kodak Gel Logic, 2200 Pro) and recorded in TIFF and JPEG formats. The bands were quantified by using Image J software (Windows version of NIH Image, http://rsb.info.nih.gov/nih-image/) and normalized to β-actin (Cell Signaling Technology, The Netherlands) expression levels.

2.7. Statistical analysis

All quantitative results are presented as mean ± standard deviation (SD). The non-parametric Kruskal Wallis and Mann Whitney *U* test was used in probe test analysis of behavior tests, CAT, PEX14, PMP70 western blot analysis, and comparison of MDA, GSH levels and Bonferroni correction was applied. Friedman and Wilcoxon test were used to compare escape latency test times. A Paired-sample *t*-test was used to evaluate the relationship between cortex and hippocampus tissues. Pearson r was calculated. $p < 0.05$ was set as the level of statistical significance.

3. Results

3.1. Behavioral tests

3.1.1. Spatial learning and memory performance in the Morris water maze

As of the second day, a decrease in the time to reach the platform area started in all groups according to the learning performance curves of the rats. A decrease in the time of finding the platform on the 4th day was statistically significant for all groups ($p < 0.05$). When the escape latency performances were examined, the longest platform finding time was observed in the Aβ 1–42 group. The taurine group performed best in terms of platform discovery time (Fig. 2A). Taurine supplementation showed improvement effects on the learning performance ($p < 0.05$). According to the probe test results, when compared with the control group, a statistically significant difference was found only in the Aβ 1–42 group. There was a significant decrease in spatial learning and memory performance in Aβ 1–42 group compared to the control group ($p < 0.05$). However, there was no significant difference between the other groups (control, sham, Aβ 1–42, taurine and Aβ 1–42 + taurine group; 37.6 ± 6 s, 28.8 ± 1.5 s, 22.1 ± 3.7 s, 32.37 ± 6.2 s and 28.1 ± 4.2 s, respectively) ($p > 0.05$) (Fig. 2B). According to the probe test results, although there was a tendency to improve the cognitive functions of animals supplemented with taurine, the difference was not statistically

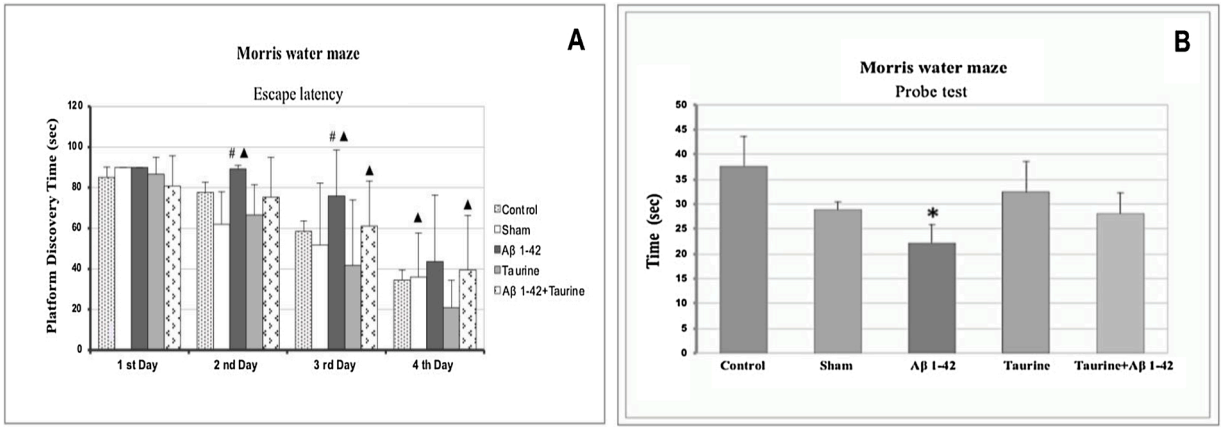

Fig. 2. A-Mean escape latency to find the invisible platform in a Morris water maze, B- Probe test results of the groups in Morris water maze (n = 6 per group) (Group 1: control, group 2: sham, group 3: Aβ 1-42, group 4: taurine, and group 5: Aβ 1-42+taurine) (significant at *; $p < 0.05$ compared to control, #; $p < 0.05$ compared to sham, ▲; $p < 0.05$ compared to taurine).

significant ($p > 0.05$). The decrease in the time spent by the rats in the Aβ 1–42 group in the target quadrant is an indicator of the decrease in spatial learning and memory performance.

3.1.2. *Emotional learning and anxiety findings in elevated plus maze*

When compared with the control group, there was no significant difference in the time spent in the open and closed arms in the other groups. However, when the times spent in both open and closed arms were examined, a significant difference was found between the groups receiving taurine treatment compared to the Aβ 1–42 group. Time spent on the open arm in the EPM was found as 40 ± 21.09 s, 39.5 ± 14.72 s, 86.67 ± 72.23 s, 17.33 ± 6.28 s and 24.29 ± 13.88 s in the control, sham, Aβ 1–42, taurine and Aβ 1–42+taurine groups, respectively. The time spent in the open arm decreased significantly in the taurine and Aβ 1–42+taurine groups compared to Aβ 1–42 group ($p < 0.05$). The time spent in the closed arm in the groups was found 260 ± 21.09 s, 260.5 ± 14.72 s, 213.33 ± 72.23 s, 282.67 ± 6.28 s, 275.72 ± 13.88 s, respectively. The time spent in the closed arm increased significantly in the taurine and Aβ 1–42+taurine groups compared to the Aβ 1–42 group ($p < 0.05$) (Fig. 3A).

The number of entries to the open arm in the groups was found as 1,5 ± 0,84, 1,83 ± 0,75, 6,33 ± 2,73, 1 ± 0 and 3.83 ± 2.23, respectively. The number of closed arm entries in the groups was found 2.33 ± 2.80, 3.33 ± 1.51, 5.83 ± 2.48, 2 ± 0 and 5.33 ± 3.01, respectively. The number of open and closed arm entries in the Aβ 1–42 group increased significantly compared to the control group ($p < 0.05$) (Fig. 3B). In addition, a statistically significant increase was observed in the Aβ 1–42 + taurine group compared to the taurine group in both open and closed arm entry numbers ($p < 0.05$). All the animals tried to explore the maze. However, the locomotor activity of the Aβ 1–42 group was significantly increased, the number of passes between open and closed arms was significantly higher, and the group fell from the maze the most among the groups. There was no distinction between open and closed arms for this group. Taurine significantly increased the duration of the animals in the closed arm. And the animals in these groups (taurine and taurine + Aβ 1–42 group) freezed in the closed arm at different times. These results show that taurine causes emotional learning accompanying anxiety and anxiety.

3.2. *Injection of Aβ 1–42 in elderly rats reduced expression of peroxisomal proteins*

According to the results obtained by western blot analysis, CAT expression in cortex tissue decreased significantly in the Aβ 1–42 group compared to the control group (control, sham, Aβ 1–42, taurine and Aβ 1–42 + taurine group; 1.87 ± 0.24, 1.70 ± 0.26, 1.21 ± 0.32, 1.85 ± 0.22 and 1.59 ± 0.21 fold respectively) ($p < 0.05$). CAT expression increased in the taurine supplemented group. However, this increase was not statistically significant ($p > 0.05$) (Fig. 4A). In the hippocampus tissue, there was no statistically significant difference between the groups ($p > 0.05$) (Fig. 4B). According to the results obtained by western blot analysis, there was no statistically significant difference between

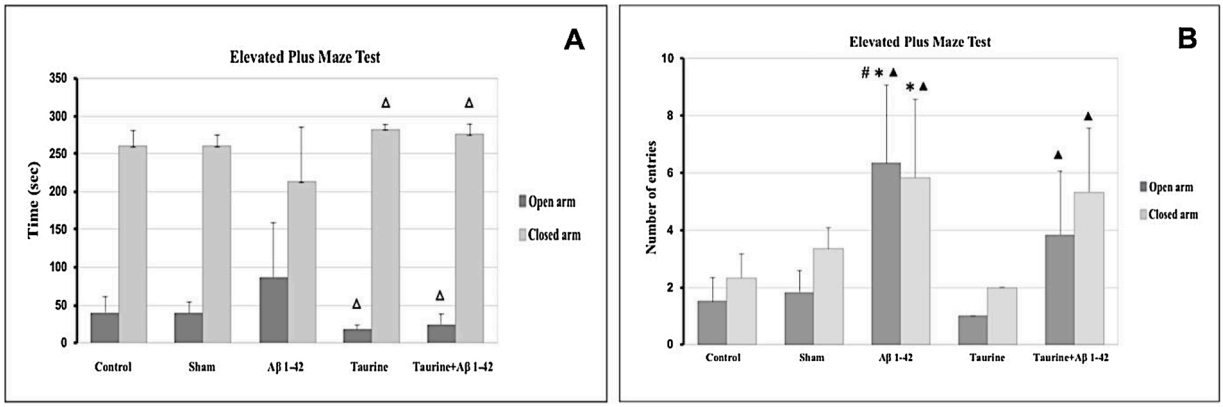

Fig. 3. A- Time spent on open and closed arms in the elevated plus maze, B- The number of open and closed arm entries in the elevated plus maze (n = 6 per group) (Group 1: control, group 2: sham, group 3: Aβ 1-42, group 4: taurine, and group 5: Aβ 1-42+taurine) (significant at *; $p < 0.05$ compared to control, #; $p < 0.05$ compared to sham, △; $p < 0.05$ compared to Aβ 1-42, ▲; $p < 0.05$ compared to taurine).

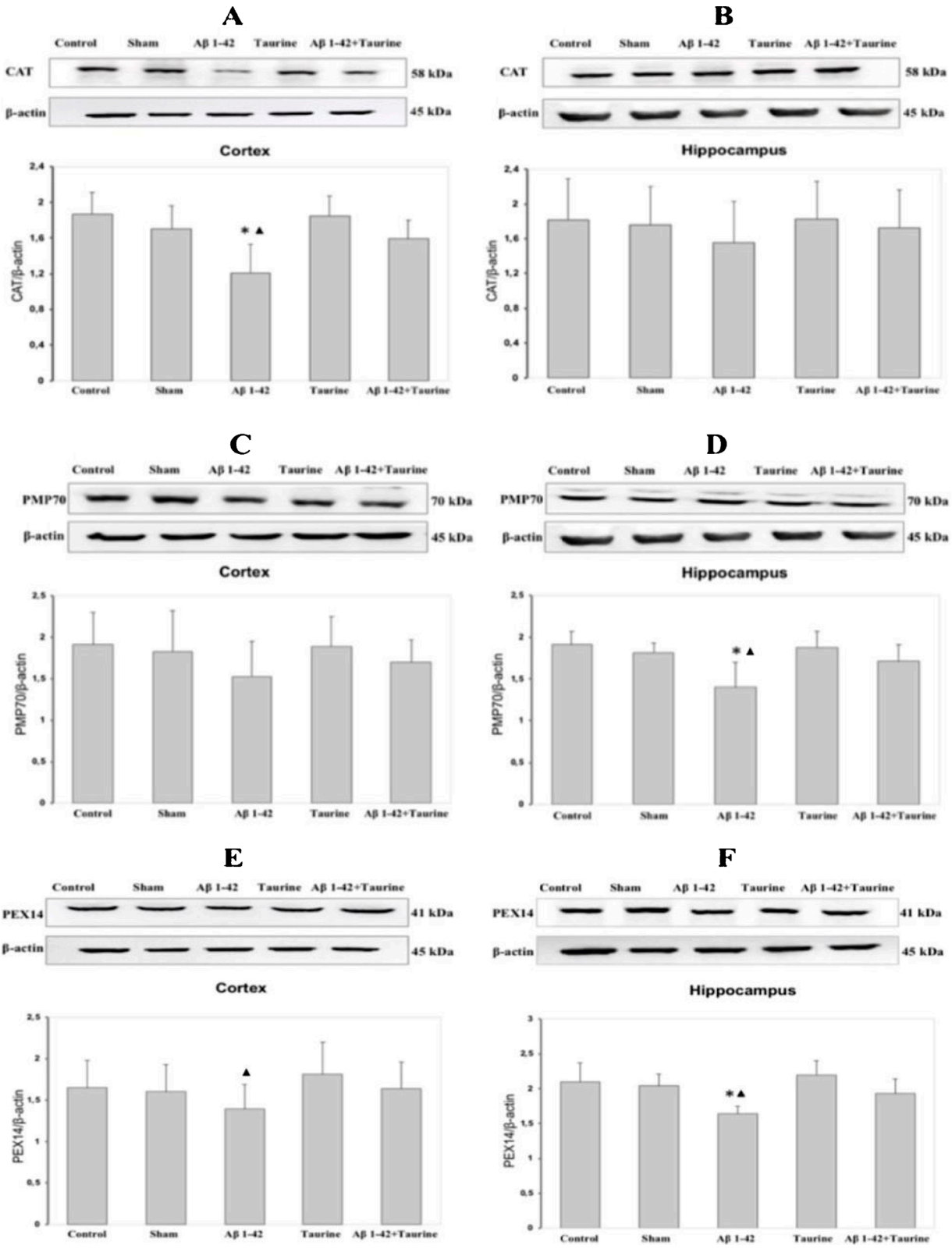

Fig. 4. Representative western blot in the hippocampus and cortex of rats (A) and (B) CAT protein expression levels, (C) and (D) PMP70 protein expression levels, (E) and (F) PEX14 protein expression levels. The protein expression levels were evaluated by western blotting and normalized to β-actin expression level and calculated as fold change about the expression level of the control group (n = 6 per group) (Group 1: control, group 2: sham, group 3: Aβ 1-42, group 4: taurine, and group 5: Aβ 1-42+taurine) (significant at *; $p < 0.05$ compared to control, ▲; $p < 0.05$ compared to taurine).

the groups in terms of PEX14 and PMP70 expression in cortex tissue ($p > 0.05$) (Fig. 4C and E). In the hippocampus tissue, PEX14 and PMP70 expression decreased significantly in the Aβ 1–42 group compared to the control group. It was observed that taurine administration reduced the decrease in PEX14 and PMP70 expression that occurred with Aβ 1–42 injection. However, this increase was not statistically significant ($p > 0.05$) (Fig. 4D and F). In addition, according to western blot analysis results, there was no correlation between cortex and hippocampus CAT levels ($r = 0.217$; $p = 0.224$), but there was a positive correlation between PEX14 and PMP70 levels ($r = 0.463$; $p = 0.05$ and $r = 0.5$; $p = 0.005$). We observed a decrease in CAT expression in both cortex and hippocampus tissue of the Aβ 1–42 group. However, the decrease in the hippocampus was not statistically significant. Although the number of peroxisomes has been shown to increase functionally to adapt to the situation in neurodegenerative diseases, we found that both PMP70 and PEX14 expression in the Aβ 1–42 group significantly decreased in the cortex and hippocampus. Chronic taurine supplementation also did not cause a significant difference on CAT, PMP70 and PEX14 expressions. This may lead us to think that taurine supplementation, which is highly associated with Alzheimer's disease, is not effective on peroxisomal proteins.

3.3. Changes in MDA and GSH levels

It was found that MDA levels increased significantly in the cortex and hippocampus with Aβ 1–42 administration ($p < 0.05$). Taurine reduced MDA levels, but this effect was not statistically significant ($p > 0.05$) (Fig. 5A and B). In terms of GSH levels in the cortex, there was no statistically significant difference between the groups ($p > 0.05$) (Fig. 5C). There was no statistically significant difference in the hippocampus GSH level in the Aβ 1–42 group compared to the control group ($p > 0.05$). In the taurine-treated group, it was observed that GSH level increased significantly compared to sham and Aβ 1–42 group ($p < 0.05$) (Fig. 5D). In terms of MDA levels, a positive correlation was found between both brain regions ($r = 0.707$; $p = 0.05$). However, no correlation was found between the two brain regions in terms of GSH levels ($r = 0.173$; $p = 0.361$). Taurine supplementation showed a decreasing effect on MDA levels increased with Aβ 1–42 administration. In addition, GSH levels, which indicate antioxidant capacity, were found to be low in the hippocampus in the group that we applied Aβ 1–42, and taurine supplementation significantly increased GSH levels in the hippocampus. These data we obtained reveal that taurine especially has an effect on reducing oxidative stress.

4. Discussion

According to the amyloid cascade hypothesis, increasing Aβ 1–42 level in the onset and progression of AD is known as the most likely factor (Hardy and Selkoe, 2002). Different doses of synthetic or naturally secreted Aβ peptides are widely used to create an experimental AD model in rats. It has been reported that acute or chronic infusion of the Aβ fragment, primarily icv or intrahippocampal, may cause neurodegeneration, as well as learning and memory disorders (Bagheri et al., 2013; Ghofrani et al., 2015; Kasza et al., 2017).

In the experimental AD model, which we created by intracerebroventricular injection of Aβ 1–42 with a dose of 5 μL into the lateral ventricles in elderly rats, the MWM experiment probe test results showed that rats in the Aβ 1–42 group had reduced time in the target quadrant. This is an indicator of the decrease in spatial learning and memory performance of animals (Fig. 2). The absence of open and closed arm separation in rats in the group with Aβ 1–42 applied in the EPM test indicates that these animals emotional learning performance also decreased. Chronic taurine supplementation did not significantly improve spatial learning and memory performance in elderly rats, according to the MWM test results. However, the increased time spent on the closed arm in the EPM test suggests that emotional learning accompanying anxiety has occurred (Fig. 3). Injection of Aβ 1–42 significantly reduced the expression of peroxisomal proteins in the

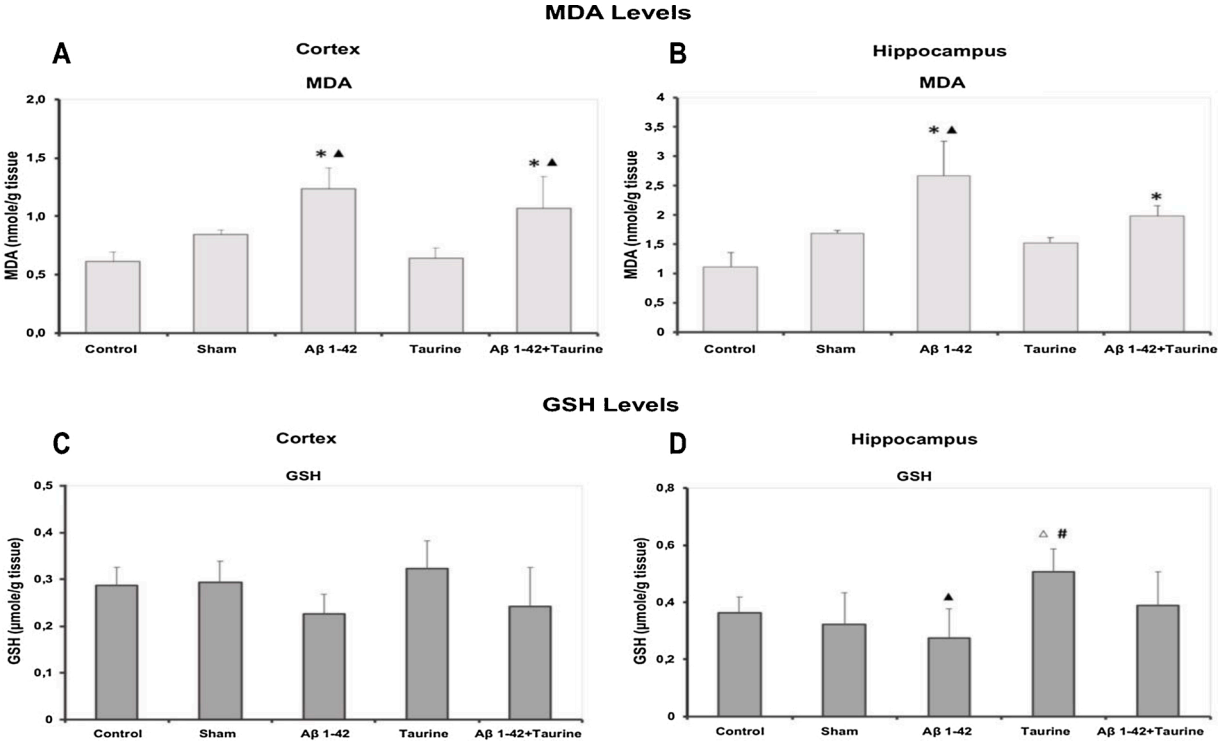

Fig. 5. Changes in MDA (A and B) and GSH (C and D) levels. MDA levels measured in cortex and hippocampus tissues are given as nmole/g tissue and GSH levels are given as μmole/g tissue (n = 6 per group) (Group 1: control, group 2: sham, group 3: Aβ 1-42, group 4: taurine, and group 5: Aβ 1-42+taurine) (significant at *; $p < 0.05$ compared to control, #; $p < 0.05$ compared to sham, △; $p < 0.05$ compared to Aβ 1-42, ▲; $p < 0.05$ compared to taurine).

elderly rats, especially in the hippocampus. Although this decrease tended to increase in the groups in which we applied taurine, the difference was not statistically significant. If more animals are used, the difference is likely to be significant (Fig. 4). Aβ 1–42 injection caused a significant increase in MDA levels in both cortex and hippocampus. It also caused a decrease in CAT in the cortex and GSH levels in the hippocampus. This indicates that the AD model we created triggered oxidant damage. Chronic taurine supplementation increased decreased CAT levels with Aβ 1–42 injection in the cortex, and GSH levels in the hippocampus. This indicates that taurine increases antioxidant capacity (Fig. 5).

MWM is one of the most preferred models in the field of behavioral neuroscience to evaluate spatial learning and memory performances due to the hippocampus (Reeta et al., 2017). In our study, we observed that Aβ 1–42 application affects spatial learning and memory functions in accordance with literature, according to the findings we obtained from the behavioral tests between 7–14 days following the healing process (Eskandari-Roozbahani et al., 2019). On the 4th day, the group with the shortest time to reach the platform is the taurine supplementation group, and the group with the longest time to reach is the Aβ 1–42 group (Fig. 2). According to the probe test results, although the taurine supplemented animals tended to improve cognitive functions, and the difference was not statistically significant.

EPM is widely used to evaluate learning and memory functions, especially anxiety-like behaviors (Jung and Kim, 2019; Kim et al., 2014; Vuralli et al., 2019). The prolonged time on the closed arm, the increase in the freezing time, the decrease in the number of open arm entry, and the decrease in the duration of the navigation in the central area indicate anxiety. In this test, passive avoidance refers to the subject's learning to stay still to avoid punishment (Gargiulo et al., 1996). In our study, we used the EPM test to examine the effects of both Aβ 1–42 neurotoxicity and taurine on learning and memory functions, especially anxiety. All animals tried to explore the maze. However, the locomotor activity of the Aβ 1–42 group was significantly increased, the number of passes between the open and closed arms was significantly higher than all groups, and it was the group that fell from the maze the most. There was no distinction between open and closed arms for this group. Taurine, on the other hand, significantly increased the duration that the animals stayed in the closed arm. Also, the animals in these groups (taurine and Aβ 1–42+taurine group) froze at different times in the closed arm. These results show that taurine causes anxiety and emotional learning accompanying anxiety. Taurine caused anxiety at 1000 mg/kg/day when administered chronically for 6 weeks. This result we obtained is similar to a few studies in the literature (El Idrissi et al., 2009).

Taurine, one of the most abundant endogenous amino acids in the central nervous system, has recently been proposed as a cognitive enhancing therapeutic agent for neurological disorders (Akande et al., 2014; Gebara et al., 2015; Jia et al., 2016). However, in the literature, besides these beneficial effects of taurine, there are also studies showing its harmful effects (El Idrissi et al., 2009; Neuwirth et al., 2013) and even toxic effects (Chen et al., 2019). These different effects of taurine can probably vary depending on the dosage, the type of animal to be administered (mouse, rat or guinea pig), the mode of administration (such as drinking water, intraperitoneal) or duration (whether acute or chronic) (Dincer and Aslan Karakelle, 2019). In a study using a 7-month transgenic AD model mouse, animals were given taurine at a dose of 1000 mg/ kg/day with drinking water for 6 weeks. Administration of taurine to mice has been shown to increase spatial memory capacity based on the results of the Y-maze (Kim et al., 2014). In another study, the AD model was created by injecting 5 μL of Aβ intracerebroventricularly in 6-week old mice. In this study, taurine was administered orally as 250 mg/kg/day from day 3 to day 7 following injection of Aβ, and it was found that taurine significantly increased spatial memory (Jang et al., 2017). In our study, the administration of taurine to rats for 6 weeks at a dose of 1000 mg/kg/day orally with drinking water did not produce a statistically significant difference in spatial learning and memory performance in animals. It also caused anxiety. This may be because the rats we used in the experiment were much older than those used in other studies were.

In a study, both anxiolytic and anxiogenic effects of taurine have been observed. In open field (open-field) testing, chronic taurine supplementation increased locomotor activity, while acute taurine injection suppressed locomotor activity. In the EPM, acute taurine injection suppresses anxiety while chronic taurine supplementation has an anxiogenic effect. In light of these findings, two conclusions can be reached. First, these results show that taurine can play a role in the modulation of anxiety and locomotor activity, as well as cognitive functions such as learning and memory. Second, acute taurine injection and chronic taurine supplementation have opposite effects (El Idrissi et al., 2009).

The exposure of chronic taurine has been shown to cause more fear. It was also revealed that once negative learning was awakened, mice became hypersensitive to new environments. It is claimed that taurine levels in the brain increase in response to stressors with a neuroprotective mechanism to prevent hyperexcitability. There are studies in the literature showing that chronic taurine supplementation increases somatostatin expression by increasing cysteamine accumulation, as a result of the decomposition of fear inhibition by the amygdala and periaqueductal gray matter, thus exaggerated freezing response is observed (Neuwirth et al., 2013; Suge et al., 2007).

The relationship between mitochondria and peroxisomes has a highly important role in oxidative stress. (Cipolla and Lodhi, 2017; Schrader et al., 2015). Cellular oxidative stress and peroxisomal (along with mitochondria) activity are important for cell survival and health (Bonekamp et al., 2009; Schrader and Fahimi, 2006). Studies examining the relationship of oxidative stress with mitochondria, one of the most important causes of multifactorial AD, have been increasing recently in the literature (De La Monte and Wands, 2006; Wang et al., 2014). However, the role of peroxisomes in this process has always been ignored alongside mitochondria. It has also been found that over the past decade, mammalian peroxisomes are not only metabolic organelles, but can also serve as signalling platforms that modulate various physiological and pathological processes, including inflammation, innate immunity, and cell fate.

Age-related decrease in activity peroxisome, reduced CAT activity, low plasmalogen synthesis (Goodenowe et al., 2007), and increased sensitivity to cell death, especially necrosis (Manivannan et al., 2012) containing aging and dementia-related disorders of peroxisomal function can trigger neurodegenerative diseases such as AD (Terlecky et al., 2006). The first evidence of the potential role of peroxisomes in AD development has been shown on rat hippocampal neuron cultures. In these cells, peroxisomal proliferation induced by WY-14.463, a potent PPAR agonist, has been shown to protect against cell death induced by Aβ peptide (Santos et al., 2005).

In our study, we analyzed PMP70, PEX14, and CAT expression, which are peroxisomal proteins, using western blot analysis. CAT, which is an important peroxisomal enzyme and associated with aging in recent studies, decreases in aging and neurodegenerative diseases (Fanelli et al., 2013). Our results are consistent with the studies in the literature. We observed that the expression of CAT decreased in both cortex and hippocampus tissue of the Aβ 1–42 group. However, the decrease in the hippocampus was not statistically significant (Fig. 4). No statistically significant difference was observed in the expression of CAT in chronic taurine supplemented groups. The results we obtained from the expressions of PMP70 and PEX14, which are structural proteins of peroxisomes and are considered as important biomarkers for the amount and function of peroxisomes, contradict the limited number of studies in the literature. Studies in the literature show that it increases the number of peroxisomes to functionally adapt to the situation in aging and neurodegenerative diseases, and therefore PMP70 and PEX14 expression increases (Fanelli et al., 2013). However, in our study, we found that both PMP70 and PEX14 expression in the Aβ 1–42 group decreased in the cortex and hippocampus. This decrease was not statistically

significant in the cortex. We think that the old animals we used in our study could not increase the number and function of peroxisome to adapt to the situation. Chronic taurine supplementation also did not cause a significant difference in PMP70 and PEX14 expressions. In the literature, the relationship of taurine with peroxisomes has been evaluated only on PPARs. Moreover, these studies have established a relationship based on oxidative stress parameters. It has been shown that taurine supplementation increases PPAR level while oxidative stress decreases PPARs (Bai et al., 2016). In this context, although taurine did not significantly alter PMP70 and PEX14 expression, it has an enhancing effect on PPAR.

The release of free radicals leads to lipid peroxidation and oxidative damage to the cell membrane and proteins (Repetto et al., 1999; Smith et al., 2005). In our study, MDA levels increased significantly in both cortex and hippocampus in the groups we applied Aβ 1–42. These results we obtained, i.e. the increase of free radical damage products, were found to be compatible with the results of other researchers (Reeta et al., 2017). Taurine supplementation had a decreasing effect on increasing MDA levels with Aβ 1–42 application. Also, GSH levels showing antioxidant capacity were found to be low in the hippocampus in the group we applied Aβ 1–42, and taurine supplementation significantly increased GSH levels in the hippocampus. This data shows that taurine effects especially in reducing oxidative stress (Fig. 5).

Peroxisomes have a highly important role in aging, oxidative stress, and neurodegenerative diseases that occur with aging. Studying peroxisomal functions in detail could be a new and effective strategic approach to the diagnosis and treatment of Alzheimer's disease. Studies showing the effectiveness of taurine in this process are very few in the literature. We think that acute and chronic effects of taurine and its relationship with peroxisomal functions should be investigated in further studies with different types of animals, different ages, and different dose trials.

Author contributions

Nida Aslan Karakelle: Conceptualization, Software, Visualization, Writing-Reviewing and Editing. Atiye Seda Yar Sağlam: Methodology, Data curation. Sibel Dinçer: Writing-Original draft preparation, Investigation, Supervision, Validation.

Declaration of Competing Interest

The authors report no declarations of interest.

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