Article

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Metallothionein III Decreases the Proliferation of Astrocytes and Enhances Pathological Improvements in a Mouse Model of Alzheimer's Disease

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Background: Alzheimer's disease (AD) affects the brain and causes difficulties with cognition and emotions. At present, there are no viable therapies to halt or slow down the advancement of AD. Metallothionein III (MT-III) exhibits antioxidant and anti-inflammatory characteristics, indicating possible therapeutic benefits. This study aimed to explore the influence of MT-III on AD pathological alterations and cognitive abilities.

Methods: In this research, we employed the universally accepted AD mouse models (3xTg-AD) as test subjects and administrated vehicle or MT-III. The mice were subjected to the Morris water maze test to assess their spatial learning and memory capabilities. Moreover, to evaluate the consequent effects on neuronal groups in the hippocampus, the Nissl staining and neuronal nuclear antigen (NeuN) immunohistochemistry were used to identify the cellular morphology changes and density. Immunohistochemistry was also used to detect β-amyloid (Aβ) and glial fibrillary acidic protein (GFAP) to measure Aβ accumulation and astrocyte growth. Western blot was also used to measure Tau pathology-related PHD finger protein 1 (PHF-1), phosphorylated Tau (AT-8), and total Tau protein.

Results: The administration of MT-III notably enhanced spatial learning and memory function in 3xTg-AD mice, as evidenced by the Morris water maze test ($p < 0.01$). According to immunohistochemistry and the obtained findings, it was observed that brain tissues of mice treated with MT-III showed a notable increase of Nissl bodies and NeuN intensity ($p < 0.01$) while a remarkable decrease in Aβ accumulation and GFAP ($p < 0.01$). Additionally, MT-III largely decreased levels of Tau phosphorylation-related PHF-1 and AT-8 ($p < 0.01$) and slightly reduced the level of Tau 5 ($p < 0.05$).

Conclusion: In summary, our research indicates that MT-III has the capacity to ameliorate pathological alterations in AD mouse models and safeguard their cognitive and emotional abilities. By decreasing β-amyloid accumulation and reducing the intensity of Tau pathology, MT-III protected hippocampal subfield neurons against pathological harm. Furthermore, MT-III reduced inflammation by inhibiting abnormal proliferation of astrocytes. Of utmost importance, MT-III greatly enhanced the cognitive abilities related to spatial learning and memory in mice, suggesting its promising therapeutic properties for AD.

Keywords: AD; MT-III; cognitive function; emotional function

Introduction

Over the last few decades, Alzheimer's disease (AD) has become a significant neurodegenerative condition that poses a grave danger to the well-being of the older population worldwide [1,2]. Its clinical manifestations primarily include memory decline, impaired cognitive function, and emotional fluctuations [3]. Despite thorough investigation into the pathophysiological mechanisms of AD, there is still a lack of effective treatments that can stop or reverse the advancement of this disease [4–6].

AD is characterized by the accumulation of β-amyloid (Aβ), neurofibrillary tangles, and the degeneration of neurons [7]. Aβ deposition is considered a key pathological process in AD, capable of inducing oxidative stress, inflammatory responses, and neuronal apoptosis [8]. Therefore, the primary objective of current research on AD is to hinder the production and accumulation of Aβ, while also reducing the oxidative stress and inflammatory reactions caused by Aβ [9,10].

Metallothionein (MT) is a group of small proteins with high sulfur content that play a vital role in regulating metal ion balance and eliminating harmful free radicals [11]. Previous studies have demonstrated the significant protective effects of MT against oxidative stress and inflammatory responses [12–14]. Thus, utilizing MT to mitigate the pathological impact of Aβ may represent a novel therapeutic strategy for AD [15]. Metallothionein III (MT-III), a unique subtype, is primarily found in the central nervous system among the members of the MT family [11,16]. In addition to having the typical characteristics of other members of the MT family, such as the ability to remove harmful free rad-

icals and regulate metal levels, MT-III also demonstrates distinct neuroprotective qualities. Studies have shown that MT-III can hinder the production and accumulation of Aβ while also reducing the neurotoxic effects caused by Aβ [15,17,18]. Nevertheless, additional inquiry is necessary to elucidate the precise healing impacts and modes of operation of MT-III in AD.

This study aims to examine the healing properties of MT-III in AD by utilizing a transgenic mouse model of AD. It will evaluate the effects of MT-III on Aβ deposition, neurofibrillary tangles, and neuronal loss, along with its ability to protect memory and emotional function. With this study, we aim to offer a fresh possibility for managing AD, particularly by employing MT-III to improve the pathological alterations in AD and safeguard cognitive and emotional well-being.

Methods

Animals

The experiment utilized an AD male mouse model. 5-month-old mice were selected and had the genotype $APP_{swe}/Tau_{P301L}/PS1_{M146V}$ (3xTg-AD). Twenty 3xTg-AD mice were acquired from The Jackson Laboratory (Bar Harbor, ME, USA). All animals were housed in the Sun Protection Factor (SPF) barrier environment of the Experimental Animal Center. The temperature in the room was kept between 20 ℃ and 25 ℃, while the relative humidity ranged from 40% to 60%. The animals experienced a 12-hour alternating light and dark schedule and were provided unrestricted food and water access. Approval for the experimental protocol was granted by the Ethics Committee for the Care and Use of Animals of The First People's Hospital of Jiashan (Approval No. KY2023-056). The mice were weighed and received the Morris water maze test to evaluate their cognitive and recall capabilities. After all mice were proven to have the same levels of cognitive capabilities, they were randomly divided into two groups (Veh group and MT-III group) with 10 mice in each group, and given an intraperitoneal injection of pentobarbital sodium (50 kg/mg; P3761, Sigma-Aldrich, Bellefonte, PA, USA) to anesthetize them before being placed in a stereotaxic apparatus. The lateral ventricle's position was determined based on the stereotaxic coordinates (bregma, posterior 0.6 mm; lateral 1.1 mm) [19]. A cavity was created in the cranium, and a tube was inserted and fastened using dental adhesive. After the surgery, the mice were given a one-week period to recuperate before the commencement of drug treatment. MT-III (PRO-1856, Prospec, Jerusalem, Israel) was dissolved, prepared in physiological saline, and stored at –20 ℃. During the mice's wakeful state, mice in the MT-III group (n = 10) were administered a daily intracerebroventricular injection of 10 mg/kg of MT-III (injection speed: 0.1 mL/min), and mice in the Veh group (n = 10) received a comparable amount of saline solution. The location of the injection point was at –1.0 ± 0.06 mm posterior to bregma, 1.8 ± 0.1 mm lateral to the sagittal suture, and 2.4 mm in depth. The injections were administered continuously for 10 days, and behavioral experiments commenced 10 days after the initiation of drug administration.

Evaluation of Spatial Learning and Memory Using the Morris Water Maze Test

To evaluate the cognitive and recall capabilities of each mouse group, the experiment involving the Morris water maze was carried out [20]. A white plastic tank of 1.5 m in diameter and 50 cm in height was prepared and filled with 22 ± 2 ℃ water to a depth of 40 cm. The platform with the 7-cm diameter was 15 cm above the water surface. The extra-maze visual cues in the room remained in the fixed position. During the initial 5 days, the mice were introduced into the water from a randomly selected quadrant while facing the wall of the tank, and the duration it took for the mice to locate the platform was documented as the escape latency. Each quadrant was repeated twice. In case a mouse was unable to locate the platform within a minute, it was directed toward the platform and permitted to stay there for 15 seconds. After a 48-hour retention time, mice were placed in the tank with no platform from one of the four sections, and their movements while swimming were documented for 60 seconds. The groups' spatial memory abilities were assessed by observing and analyzing the latency of mice reaching the former platform and the number of times they crossed the former platform target quadrant. The behavior of mice was recorded by the Imetronics video-tracking system (Polytrack, Viewpoint, Lyon, France).

Tissue Collection

After the behavioral tests, mice were anesthetized with pentobarbital sodium (50 kg/mg; P3761, Sigma-Aldrich, Bellefonte, PA, USA) via intraperitoneal injection and then euthanized by cervical dislocation. Afterward, the mice's brain tissues (hippocampus and amygdala) were collected and immediately frozen in liquid nitrogen. The frozen tissues were stored at –80 ℃ for subsequent experiments.

Nissl Staining

The repaired brain tissue was placed in containers for embedding, rinsed with flowing water, and subsequently left in 75% alcohol for the night. The tissue was dehydrated in various alcohol concentrations on the second day, including 85% to 95% I, 95% II, 100% I, and 100% II. After clearing, the tissue was embedded in paraffin. Following the embedding process, the tissue underwent consecutive slicing utilizing a microtome, ensuring each slice had a thickness of 4 µm. Following routine deparaffinization, the tissue was stained with methyl violet for Nissl staining. After differentiation, routine dehydration and mounting were performed. An optical microscope (CKX53, Olympus, Tokyo, Japan) was used to observe and photograph the morphol-

ogy of Nissl bodies in the hippocampal region; the quantity was performed by the Image Pro Plus (version 6.0; Media Cybernetics Inc., Rockville, MD, USA) and expressed in densitometric relative units (RU).

Immunohistochemistry

The tissue was rinsed thrice for 1 minute each using phosphate-buffered saline (PBS; 0.01 mol/L; C0221A, Beyotime, Shanghai, Beijing). The endogenous peroxidase activity was deactivated by placing it in a 3% hydrogen peroxide solution for 15 minutes. Afterward, the tissue was rinsed thrice for 1 minute each using PBS (0.01 mol/L). After being blocked with 10% goat serum for 30 minutes, the tissue was incubated at 4 ℃ overnight with the neuronal nuclear antigen (NeuN) primary antibody (1:100 dilution; ab236870, Abcam, Cambridge, MA, USA) for the NeuN immunohistochemistry, or the β-amyloid polyclonal antibody (1:1000 dilution; ab126649, Abcam, Cambridge, MA, USA) for β-amyloid deposition staining, or the glial fibrillary acidic protein (GFAP) polyclonal antibody (1:1000 dilution; PA1-10004, Invitrogen, Carlsbad, CA, USA) for GFAP labeling. Then, the tissue was rinsed thrice for 60 seconds each using PBS (0.01 M). The secondary antibody Goat Anti-Rabbit IgG H&L (1:500 dilution; ab150077, Abcam, Cambridge, MA, USA) was introduced and incubated at ambient temperature for 2 hours. Afterward, the tissue was rinsed thrice for 1 minute each using PBS (0.01 mol/L). The biotin-streptavidin compound (iCell-15140-122, iCell Bioscience Inc., Shanghai, China) was introduced and left at room temperature for 30 minutes. Subsequently, it was rinsed three times with PBS (0.01 mol/L). The diaminobenzidine (DAB; HY-W014212, MedChemExpress, Monmouth Junction, NJ, USA) chromogenic solution was introduced and incubated at room temperature without light for 15 minutes. The tissue was rinsed thrice for 60 seconds each using PBS (0.01 mol/L). The Hematoxylin staining solution (C0107, Beyotime, Shanghai, China) was introduced and left at room temperature for 1 minute; then, the reaction was stopped by rinsing with tap water. A solution containing acidic ethanol was utilized for differentiation, lasting for 2 to 5 seconds, then followed by rinsing with water. After immersing in a bluing solution (G1866, Solarbio, Beijing, China) for 1–2 seconds, the object was rinsed with water. After dehydration, neutral gum was dropped onto the tissue. The mounting medium was covered with a coverslip, and the slide was placed in a fume hood for 24–48 hours to ensure continuous ventilation. All the fluorescence staining was observed using an optical microscope (CKX53, Olympus, Tokyo, Japan); the intensity of staining was performed by the Image Pro Plus (version 6.0; Media Cybernetics Inc., Rockville, MD, USA) and expressed in densitometric relative units (RU).

Enzyme-linked Immunosorbent Assay (ELISA)

According to the manufacturer's instructions, the soluble Aβ 1–40 (PA079, Beyotime, Shanghai, China) and 1–42 (PA082, Beyotime, Shanghai, China) levels in mice brains were determined via ELISA kits. Tissues were mixed with Radio Immunoprecipitation Assay (RIPA) (P0013, Beyotime, Shanghai, China) and homogenized by the homogenizer (CN-41056-98, Cole-Parmer Instrument Company, LLC., Shanghai, China). The lysis was centrifugated, and the total proteins were obtained by collecting the supernatant. In the tissue extracts, the levels of soluble Aβ 1–40 (SIG-38954, Covance Laboratories Inc., Princeton, NJ, USA) and Aβ 1–42 (SIG-38956, Covance Laboratories Inc., Princeton, NJ, USA) were measured.

Western Blot

After the protein extraction described in the ELISA section, the concentration was determined using a bicinchoninic acid (BCA) assay kit (P0012, Beyotime, Shanghai, China). After undergoing sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (P0012A, Beyotime, Shanghai, China), the proteins were subsequently transferred onto a polyvinylidene difluoride PVDF membrane (IPVH00010, Millipore, Billerica, MA, USA). The membrane was obstructed using 5% skim milk for 60 minutes. The primary antibody was introduced and left to incubate overnight at 4 ℃. The details of the antibodies are as follows: Anti-PHD finger protein 1 (PHF-1) (ab184951, Abcam, Cambridge, MA, USA; 1:1000 dilution), anti-AT8 (MN1020, Invitrogen, Carlsbad, CA, USA; 1:1000 dilution), anti-Tau 5 (ab80579, Abcam, Cambridge, MA, USA; 1:1000 dilution), Tubulin (ab6160, Abcam, Cambridge, MA, USA; 1:1000 dilution). Following incubation, the membrane underwent a TBST (ST673, Beyotime, Shanghai, China) wash and subsequent incubation with the horseradish peroxidase (HRP)-conjugated secondary antibody (1:2000 dilution; ab7090, Abcam, Cambridge, MA, USA) at room temperature for 90 minutes. Before being imaged using a gel imaging system, the membranes were incubated with enhanced chemiluminescence (ECL; P0018S, Beyotime, Shanghai, China) solution for 1 minute. ImageJ software (1.48, National Institutes of Health, Rockville, MD, USA) was used to analyze the grayscale values of each band, with Tubulin serving as the internal reference.

Statistical Analysis

The mean $\pm$ standard deviation was used to express all quantitative data, which were then analyzed using the SPSS statistical analysis software (SPSS Inc., Chicago, IL, USA). Statistical analysis was conducted using a *t*-test, with a significance level of $p < 0.05$. The figures were prepared using GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA).

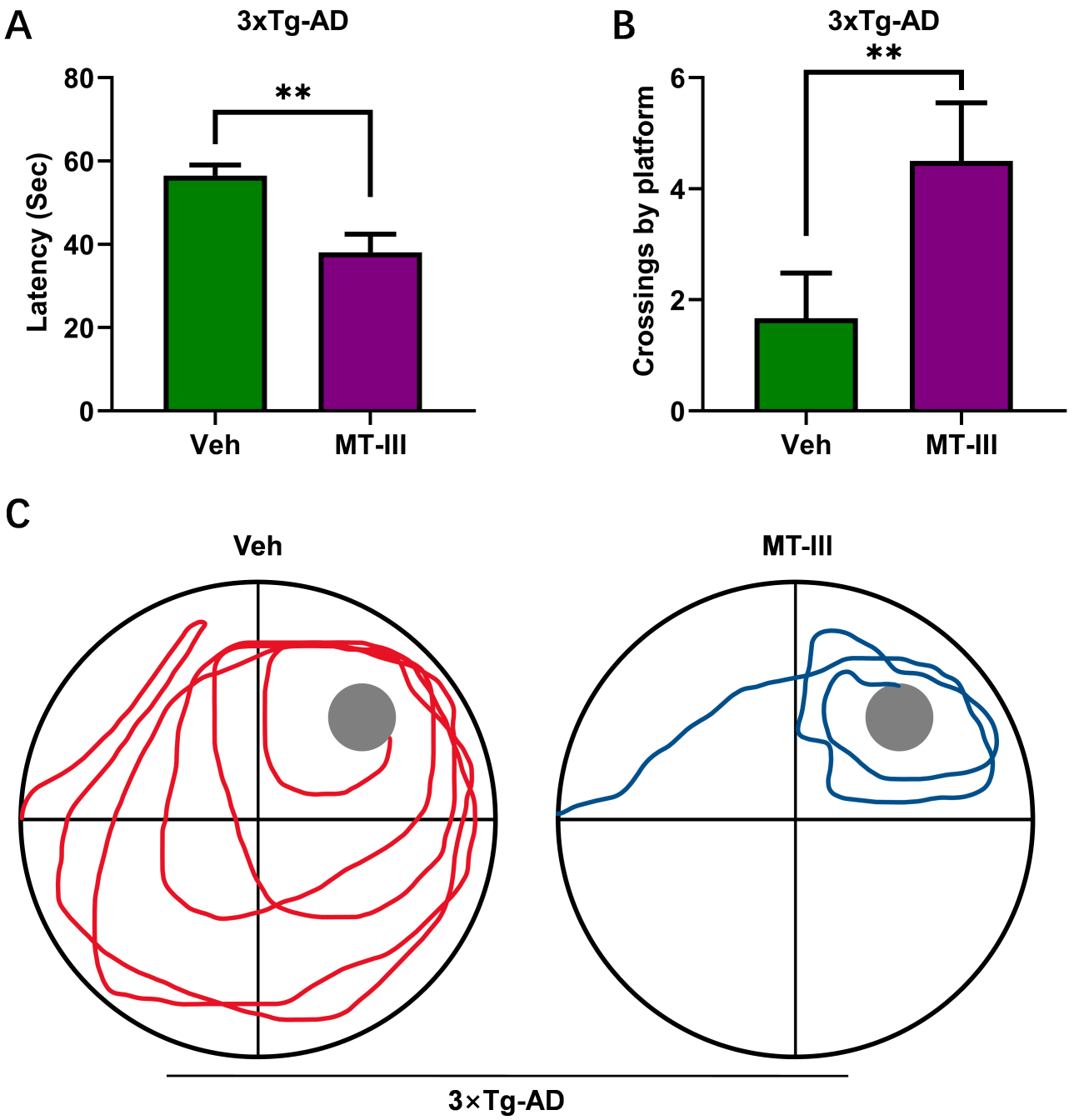

Fig. 1. Spatial learning and memory function are maintained in 3xTg-AD mice by metallothionein III (MT-III). (A) Latency to reach the platform. (B) Number of crossing the platform quadrant. (C) Representative images of the routes of travel during the test. N = 10. $^{**}p < 0.01$. AD, Alzheimer's disease.

Results

Spatial Learning and Memory Function are Maintained in 3xTg-AD Mice by MT-III

The Morris water maze test was employed to evaluate mice's spatial learning and memory function and compare the disparities between the group treated with the vehicle and the group treated with MT-III. The latency period is when it takes the mice to locate the platform within the water maze. Mice with a reduced latency period demonstrate enhanced learning and memory capabilities. The mice in the treatment group of MT-III exhibited a decreased latency period in reaching the platform, suggesting that the administration of MT-III can enhance the spatial learning and memory capabilities of 3xTg-AD mice (Fig. 1A, $p < 0.01$). The crossing count indicates how many times the mice entered various quadrants in the water maze. Mice with fewer crossings demonstrate superior learning and memory capa-

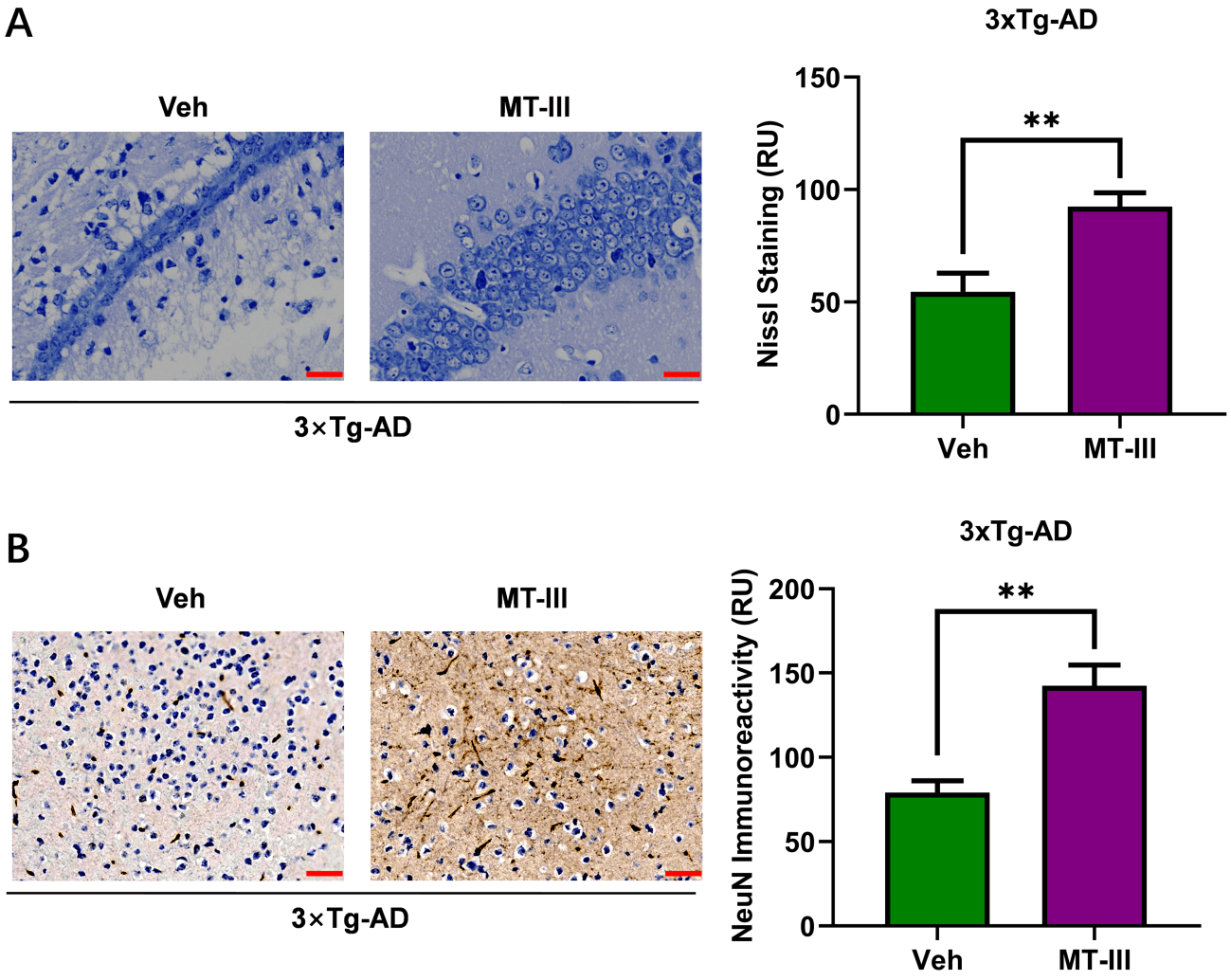

Fig. 2. MT-III safeguards the neuronal population in the subiculum of 3xTg-AD mice. (A) Different treatment conditions were used to perform Nissl staining of the subiculum in 3xTg-AD mice (scale: 50 µm). (B) Representative pictures of neuronal nuclear antigen (NeuN) immunohistochemical evaluation in the subiculum area were compared between the group treated with the vehicle and the group treated with MT-III (scale: 50 µm). N = 10. $^{**}p < 0.01$.

bilities. Fig. 1B shows that the number of crossings was reduced in the MT-III treatment group for the mice ($p < 0.01$). Additionally, Fig. 1C shows the representative images of the route of travel during the test.

MT-III Safeguards the Group of Nerve Cells in the Hippocampal Subregion of 3xTg-AD Mice

The visualization of neuron morphology and quantity was achieved by applying Nissl staining. The current investigation utilized Nissl staining to examine and contrast the neuronal condition in the hippocampal subfield of 3xTg-AD mice subjected to various interventions. The findings suggested that the Nissl staining in the MT-III treated group exhibited superiority compared to the control group, indicating that MT-III might play a role in safeguarding or augmenting the neuronal count (Fig. 2A, $p < 0.01$). Neuronal nuclear antigen (NeuN) is a neuron-specific nuclear antigen commonly used to label and assess neuronal quantity and status. The findings demonstrated that the NeuN staining in the MT-III treated group exhibited superiority over the control group, suggesting that MT-III might also play a role in safeguarding or augmenting the neuronal population (Fig. 2B, $p < 0.01$).

MT-III Improves β-amyloid Deposition in the Assessed Brain Region

The main focus of this study was to evaluate the accumulation of Aβ. Excessive accumulation of β-amyloid in the brains of individuals with AD is a significant pathological characteristic, which ultimately leads to the formation of plaques and causes neuronal damage and death. According to Fig. 3A, the MT-III treatment group exhibited decreased immunoreactivity of Aβ in comparison to the vehicle group ($p < 0.01$), indicating a potential reduction in Aβ deposi-

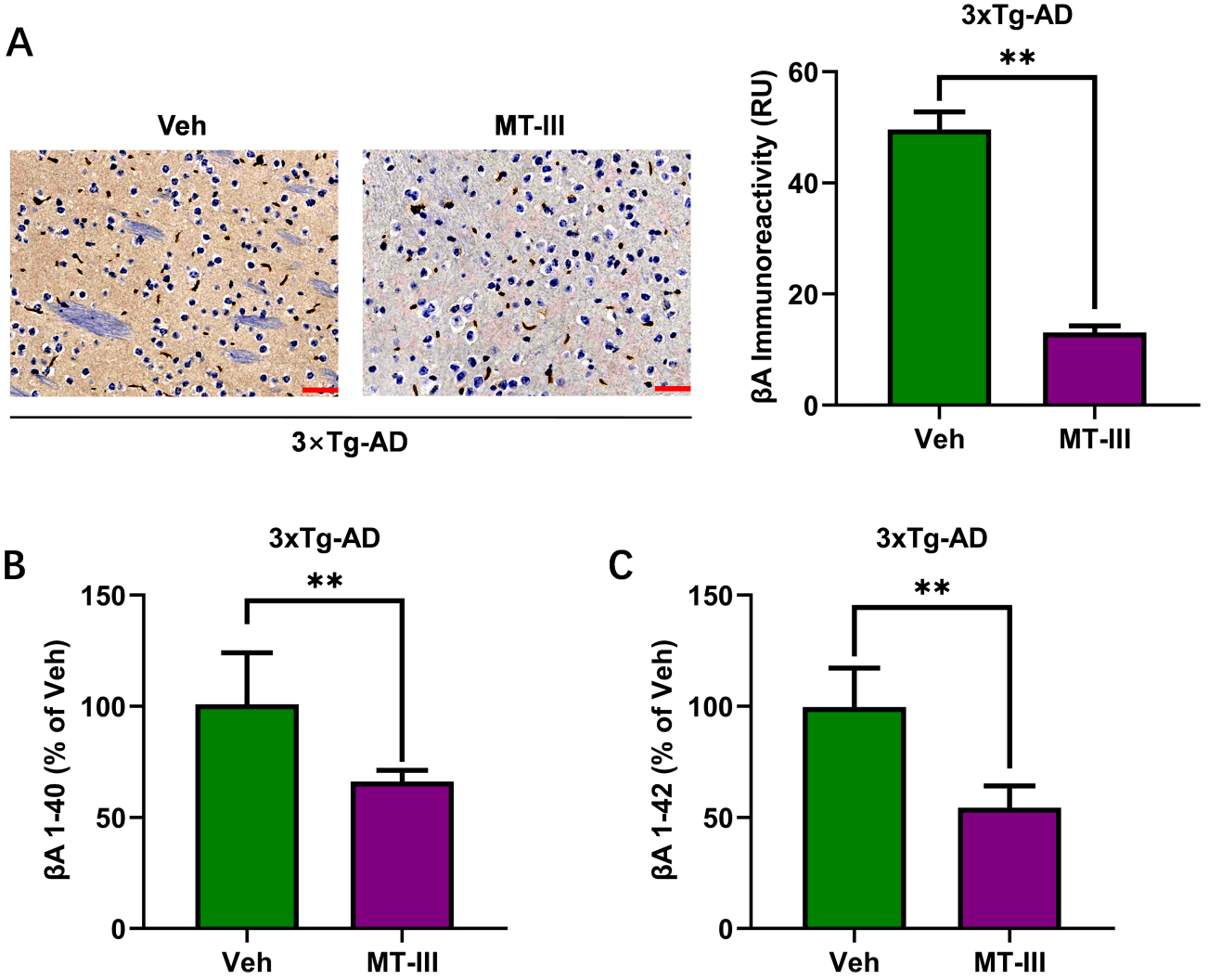

Fig. 3. MT-III improves β-amyloid (Aβ) deposition in evaluated brain regions. (A) The presence of Aβ was observed in 3xTg-AD mice brain tissues (scale: 50 µm). (B,C) Evaluation of the comparative quantities of β-amyloid 1–40 (B) and β-amyloid 1–42 (C) fragments in lysates extracted from the hippocampus. N = 10. $^{**}p < 0.01$.

tion by MT-III. Additional Aβ accumulation was assessed by examining the quantities of Aβ 1–40 and Aβ 1–42 fragments in lysates extracted from the hippocampus. Aβ 1–40 and Aβ 1–42 are the two main forms of Aβ, with Aβ 1–42 being more prone to plaque formation. The trend indicated that the MT-III treatment group had decreased levels of Aβ 1–40 and Aβ 1–42 fragments compared to the vehicle group. This further confirmed MT-III's potential in reducing Aβ deposition (Fig. 3B,C, $p < 0.01$).

In the Brains of AD Mice, MT-III Diminishes Tau Pathology

The phosphorylation status of Tau protein was first assessed using a PHF-1 antibody. The main purpose of utilizing the PHF-1 antibody is to identify atypical phosphorylation of the Tau protein [21]. The immunoreactivity of PHF-1 was decreased in the MT-III treatment group compared to the vehicle group, indicating that MT-III might potentially alleviate the atypical phosphorylation of Tau protein (Fig. 4A, $p < 0.01$). The phosphorylation state of Tau protein was also assessed using phosphorylated Tau (AT-8) and Tau 5 antibodies. The primary application of the AT-8 antibody is to ascertain the phosphorylation state of Tau protein at Serine 202 and Threonine 205 locations. In contrast, the Tau 5 antibody can recognize the total Tau protein [22]. The trend indicated that the immunoreactivity of AT-8 and Tau 5 was decreased in the MT-III treatment group in comparison to the vehicle group, providing additional confirmation of MT-III's potential to diminish abnormal phosphorylation and overall presence of Tau protein (Fig. 4B,C, $p < 0.05$). Since, according to the results in Fig. 4B,C, the extent of the AT-8 decrease was larger than that of the Tau-5 decrease, the proportion of Tau phosphorylation was decreased.

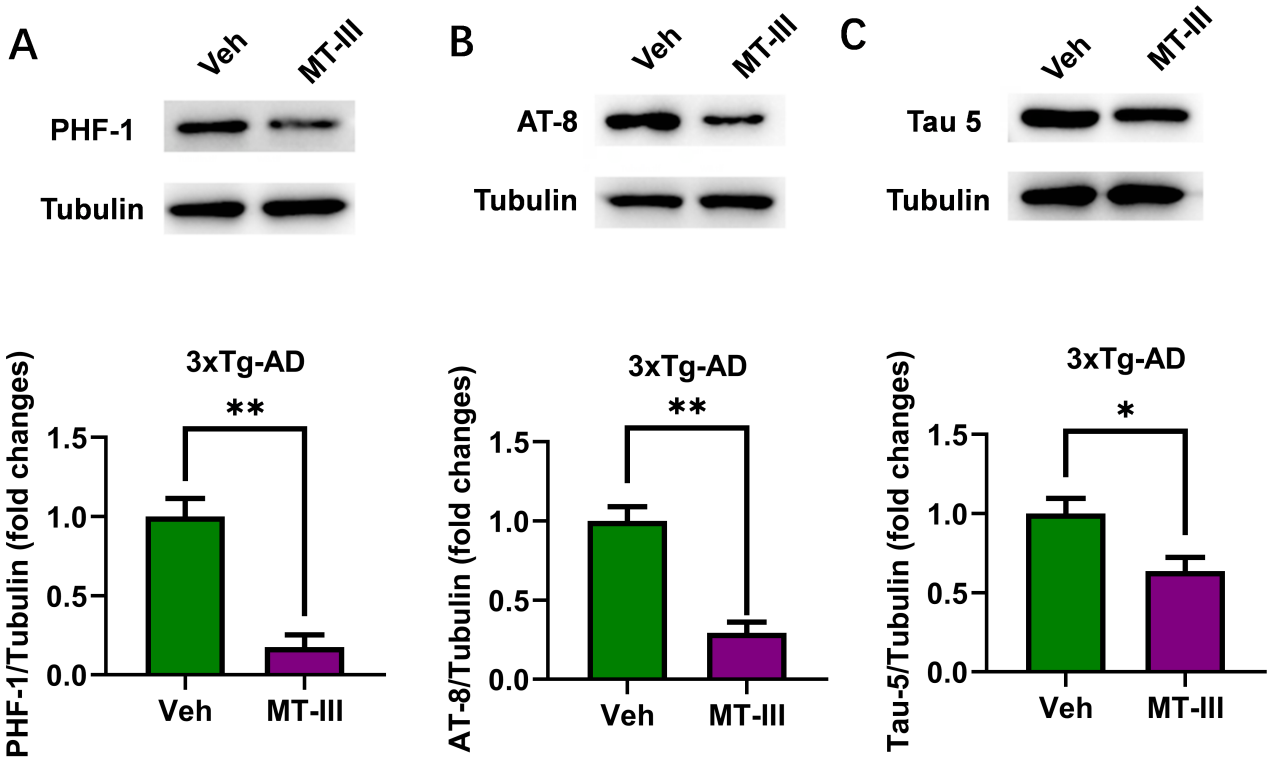

Fig. 4. In the brains of Alzheimer's disease (AD) mice, MT-III diminishes Tau pathology. (A) Representative PHD finger protein 1 (PHF-1) banding patterns for its expression in hippocampal lysates. (B) Detection of phosphorylated Tau (AT-8) protein in lysates from the hippocampus. (C) Detection of Tau 5 protein in lysates extracted from the hippocampus. N = 10. *$p < 0.01$, **$p < 0.01$.

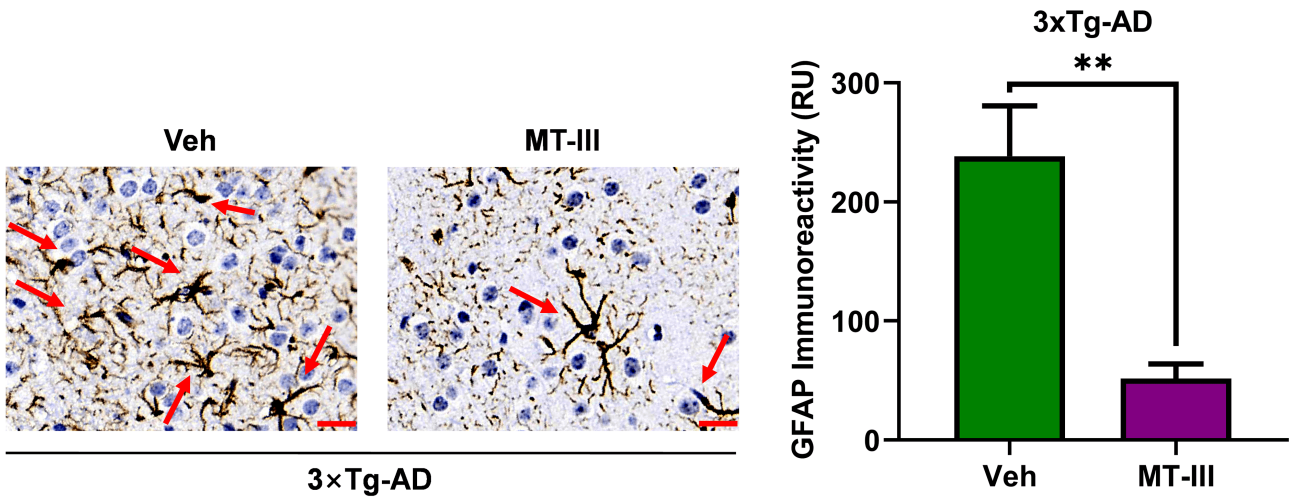

Fig. 5. The use of MT-III decreases the occurrence of astrogliosis in mice with 3xTg-AD. Representative pictures displaying glial fibrillary acidic protein (GFAP) staining in the CA1 area of 3xTg-AD mice following administration of either the control solution or MT-III (scale: 50 µm; red arrows indicated the astrocytes). N = 10. **$p < 0.01$.

MT-III Decreases the Growth of Astrocytes in 3xTg-AD Mice

Astrocytes have a crucial function in assisting and preserving neurons within the brain. Nevertheless, in neurodegenerative disorders like AD, there may be an atypical increase in the growth of astrocytes. GFAP antibody was utilized to evaluate the abundance of astrocytes in the brains of 3xTg-AD mice. In the CA1 area of 3xTg-AD mice, the findings indicated that the levels of GFAP immunoreactivity were elevated in the control group and reduced in the MT-III treated group (Fig. 5, $p < 0.01$). This suggests that administration of MT-III can decrease the excessive growth of astrocytes in the brains of 3xTg-AD mice.

Discussion

AD is a condition that involves the degeneration of neurons, the accumulation of Aβ, and the presence of Tau protein abnormalities in the brain [23]. Identifying novel therapeutic approaches is crucial in addressing the ongoing challenge of treating AD, as it is essential to mitigate the pathological alterations in AD and safeguard cognitive and emotional well-being [24,25]. This study aims to evaluate the effectiveness of MT-III in elderly mice with a triple transgenic model of AD. According to initial results, MT-III has shown various positive impacts, such as safeguarding the neuronal population in the hippocampal subregion, enhancing Aβ accumulation in the brain, lowering Tau pathology, reducing astrocyte proliferation, and maintaining spatial learning and memory function. The findings indicate that MT-III could potentially be beneficial in treating AD.

MT-III is a protein with antioxidant and anti-inflammatory properties [11]. Numerous studies have highlighted the crucial role of MT-III in neuroprotection, safeguarding neurons from damage caused by free radicals and other harmful substances [18,26,27]. MT-III has been found to exhibit potent antioxidant activity, clearing free radicals within the body and protecting cells from oxidative stress [28]. Additionally, MT-III has been observed to inhibit inflammatory responses, alleviating cell damage induced by inflammation [29]. Despite significant progress in the research of MT-III, its specific mechanisms of action and clinical applications require further investigation. In this study, we found that MT-III safeguards the neuronal populace in the subregion of the hippocampus, which implies that it could ameliorate AD pathology by diminishing neuronal depletion. AD severely impacts the hippocampus, a brain area closely linked to cognitive function and memory acquisition [30]. Hence, the safeguarding impact of MT-III might aid in upholding and recovering cognitive abilities in individuals with AD, verified by the Morris water maze test results that the MT-III improved AD mice's spatial learning and memory function. The cognitive abilities were always impaired by AD [31], and the MT-III improving cognitive abilities suggested its potential to alleviate AD. Moreover, reducing Aβ buildup in the brain indicates that MT-III could potentially alleviate AD pathology by decreasing Aβ deposition. Aβ deposition is a major characteristic of AD, closely associated with neuronal damage and inflammatory responses, and anti-Aβ therapy has been proven to be an effective, feasible, and promising approach in the optimization of AD prevention and treatment [32]. In this study, MT-III was found to reduce Aβ deposition, especially the most possibly deposited ones of Aβ 1–40 and Aβ 1–42, thus inhibiting the Aβ plaque formation and protecting the nervous system from the toxic effects of Aβ. MT-III's potential to improve AD pathology by controlling Tau protein's metabolism and phosphorylation status is indicated by the decrease in Tau pathology observed in the brains of AD mice. Abnormal phosphorylation and Tau protein aggregation are other important pathological features of AD, closely related to neuronal loss and decline in cognitive function [33]. By controlling Tau protein's phosphorylation status and aggregation, MT-III can potentially reduce harm to the nervous system. We also found that compared with the suppressive effect on the Tau protein aggregation, the MT-III had a more inhibitive effect on the phosphorylation of Tau proteins, indicating its effectiveness in alleviating AD since one of the main characteristics of AD is the hyperphosphorylated Tau [34].

MT-III reduces astrocyte proliferation, suggesting its potential to ameliorate the pathological changes in AD by decreasing neuroinflammatory responses and neurodegenerative alterations. Activation and proliferation of astrocytes, the primary immune cells in the central nervous system, are strongly linked to inflammation and neuronal harm [35]. MT-III may mitigate neuroinflammation by inhibiting astrocyte activation and proliferation, thereby protecting the nervous system from inflammatory damage.

Conclusion

The findings of this research indicate that MT-III can improve pathological alterations and maintain cognitive and emotional abilities in elderly triple transgenic AD mouse models. Nevertheless, despite the encouraging results of this investigation, additional research is imperative to authenticate these findings and explore the mechanisms by which MT-III operates, as well as its potential therapeutic significance in the treatment of AD. Furthermore, further clinical investigations are required to establish the safety and effectiveness of MT-III in individuals with AD. In summary, MT-III may emerge as a promising therapeutic approach for AD, but further research is required to confirm its clinical applicability.

Availability of Data and Materials

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Author Contributions

JL: Conception, Design, Materials, Data Collection, Analysis, Literature Review, Writing. FW: Design, Supervision, Materials, Data Collection, Analysis, Literature Review, Writing. TW: Design, Supervision, Materials, Data Collection, Writing. YS: Supervision, Materials, Data Collection, Analysis, Writing. All the listed authors in the study carried out the experiments, participated in the design of the study and performed the statistical analysis, conceived of the study, and helped to draft the manuscript. All authors contributed to the article and approved the submitted version. All authors agree to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study has been approved by the Ethics Committee for the Care and Use of Animals of The First People's Hospital of Jiashan (Approval No. KY2023-056).

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Conflict of Interest

The authors declare no conflict of interest.

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