Journal of Integrative Neuroscience

J. Integr. Neurosci. **2023**; 22(5): 117
https://doi.org/10.31083/j.jin2205117

Original Research

Effect of Physical Exercise on Mitochondrial Dysfunction and Purkinje Cell Survival in the Cerebellum of 3xTg-AD Mice

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Academic Editor: Rafael Franco
Submitted: 19 April 2023 Revised: 18 May 2023 Accepted: 9 June 2023 Published: 14 August 2023

Abstract

Background: The cerebellum is an area of the brain that is prone to damage in individuals with Alzheimer's disease (AD). As a non-pharmacological intervention for AD, exercise training has shown an ameliorating effect on AD pathology; however, the target regions have mostly been the cerebral cortex and hippocampus. The main aim of this study was to explore the influence of 12 weeks of treadmill running on the accumulation of AD-related proteins, dysfunction of mitochondria, and subsequent neuronal cell death in the cerebellum of triple transgenic (3xTg-AD) mice. **Methods**: Four-month-old 3xTg-AD mice were allocated into two groups: an AD control group (AD, n = 10) and an AD exercise group (AD-Exe, n = 10). The AD-Exe mice underwent training on a motorized animal treadmill 5 days a week for 12 weeks. After sacrifice, the cerebellum was collected and biochemically analyzed. **Results**: The AD-Exe mice expressed reduced levels of extracellular β-amyloid plaques and phosphorylated tau (p-tau), and showed improved Purkinje cell survival and mitochondrial function compared with AD mice. **Conclusions**: These findings suggest that engaging in exercise training can offer protection against the progression of AD in the cerebellum by enhancing mitochondrial function and promoting cell survival.

Keywords: Alzheimer's disease; cerebellum; mitochondria; exercise

1. Introduction

Alzheimer's disease (AD), the most prevalent type of dementia, is clinically characterized by a progressive deterioration in intellectual facilities, difficulty in performing basic tasks, and cognitive impairment [1]. These symptoms result from neuroinflammation and neurodegeneration potentially arising from the buildup of extracellular β-amyloid (Aβ) plaques and phosphorylated tau (p-tau) proteins in the AD brain [2]. The toxicity of these proteins may increase neuroinflammation and neuronal loss, subsequently leading to synaptic dysfunction, deficits in synaptic plasticity, and loss of synapses [3]. Most previous studies on AD have focused on the hippocampus because AD symptoms are closely related to cognitive function; however, AD neuropathology extends beyond the hippocampus [4].

The cerebellum is as vulnerable as the hippocampus in AD patients, and recent research has demonstrated Aβ accumulation and its toxic effect in the cerebellum [5]. AD patients with cerebellar lesions exhibit impairments in cognitive behavior and motor functions, as the cerebellum plays a crucial role in the neuronal circuitry [6]. The synaptic plasticity observed in Purkinje cells within the cerebellum is distinct and possesses unique features. These cells repeatedly interact with adjacent afferent climbing fibers, parallel fibers, and granular cells, inducing activity-dependent long-term depression. This activates inhibitory postsynaptic metabotropic glutamate receptors leading to a depression in the input efficacy of nearby cells and regulation of cerebellar functions such as motor coordination [7,8]. In individuals with AD, the presence of Aβ plaques and tau aggregates is observed primarily within the cerebellar Purkinje cell layers, particularly in the vermis [9]. The accumulation of aggregated Aβ and tau tangles in the cerebellum can have detrimental effects, potentially causing cerebellar neuroinflammation and neurodegeneration. This process may ultimately result in the loss of Purkinje cells and subsequent impairment of cerebellar function in AD patients [5,10].

Cerebellar dysfunction involves deficiencies in synaptic plasticity, transmission, and motor performance [4]. The presence of toxic proteins can interfere with mitochondrial structure and function, including mitochondrial dynamics and biogenesis, leading to processes such as mitophagy or neuronal death through apoptosis [11,12]. In the presence of dysfunctional or damaged mitochondria, there is a downregulation of mitochondrial fusion proteins, including optic atrophy-1 (OPA1), phosphorylated dynamin-related protein 1 (Drp1) and Mitofusin 1 (Mfn1). Additionally, there is an increase in the expression of mitochondrial fission proteins, namely Drp1 and Fission Protein 1 (Fis1), as well as biogenesis-related proteins such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1), AMP-activated protein kinase (AMPK), mitochondrial transcription factor A (TFAM), and nuclear respiratory factor (NRF). Changes in morphology and increased mitochondrial biogenesis contribute to the development of

mitochondrial permeability transition pores (PTP) by upregulating the expression of the voltage-dependent anion channel (VDAC), adenine nucleotide translocase (ANT), and Cyclophilin D (CypD). This process leads to the release of Cytochrome C through the pore, ultimately triggering mitophagy and apoptosis [13–16]. Exercise training, as a non-pharmacological intervention and modifiable risk factor for individuals with AD, has indicated beneficial effects in slowing down the progression of AD neuropathology [17]. Furthermore, considering physical inactivity is closely related to AD development, exercise training could be a promising treatment for AD [18]. Extensive research has demonstrated the preventive effects of exercise in AD. Exercise training ameliorates AD neuropathology by reducing Aβ and neurofibrillary tangles, contributing to increased neuronal survival and improved cognitive function [19,20]. Exercise enhances mitochondrial function and Purkinje cell survival by promoting neurogenesis and synaptogenesis. Exercise training, in turn, improves mitochondrial dysfunction and cognitive function in AD patients [21,22]. The present study examines the potential protective effects of exercise on AD using the cerebellum of mice with triple transgenic expression of AD-related genes (3xTg-AD mice), and explores the mechanisms involved.

2. Materials and Methods

2.1 Animals

Ten male wild-type (C57BL/6) mice, aged 4 months, were obtained from Orient Bio (Seongnam, Korea) and 3xTg-AD (B6; 129-Tg (APPSwe, tauP301L) 1Lfa Psen1^{tm1Mpm}/Mmjax) mice carrying human transgenes were originally supplied by Dr. Frank M. LaFerla (Jackson Laboratory, Bar Harbor, ME, USA). The presence of human transgenes in 3xTg-AD mice leads to the progression of amyloid and tau pathologies, as well as cognitive impairment in multiple brain regions, including the cerebellum [4,23]. The mice were bred and housed in a controlled environment under pathogen-free conditions with a 12-hour light and 12-hour dark cycle, and the temperature was maintained at 22 $\pm$ 2 °C. The mice were housed individually in separate cages and had unrestricted access to food and water. All experimental procedures received approval from the Institutional Animal Care and Use Committee of the Sungkyunkwan University School of Medicine.

At four months of age, 3xTg-AD mice were allocated into two groups: an AD control group (AD, n = 10) and an AD exercise group (AD-Exe, n = 10). C57BL/6 mice were employed as wild-type control mice (Con; Fig. 1). The exercise group was given access to a motorized animal treadmill (Columbus Instruments, Inc., Columbus, OH, USA) for running for 40 min per session. The exercise training duration consisted of 5 days per week for a total period of 12 weeks. Prior to the training, the mice were acclimated to the treadmill for 2 days, starting with a 10-min session at a speed of 5 m/min on the first day and increasing to 8 m/min on the second day. Each treadmill session began with a warm-up period for 5 min at a speed of 5 m/min, which was increased to 8 m/min (40–50% VO_2max) for 35 min. Considering cardiovascular adaptations, the running speed was progressively raised by 1 m/min each week, reaching a maximum speed of 15 m/min over 4 weeks [24–26]. Electric shock was not used to minimize the stress associated with treadmill running. Mice were sacrificed at week 12 after anesthesia using a combination of Zoletyl and Rompun before undergoing transcardial perfusion with 1$\times$ phosphate-buffered saline (PBS). For immunohistochemistry, half of the cerebellum was fixed with 4% paraformaldehyde and subsequently treated with 30% sucrose at 4 °C. The remaining half was rapidly frozen using cryogenic nitrogen and stored at –80 °C for subsequent Western blot analysis.

2.2 Western Blot

The half cerebellum was homogenized and protein extracts were prepared. After centrifugation, the resulting supernatants were gathered from the homogenates. The protein concentration in the samples was quantified using the Bradford assay (Bio-Rad, Hercules, CA, USA). A total of 20 µg of protein was boiled in Laemmli sample buffer. The protein samples were then placed onto an SDS/polyacrylamide gel with a concentration range of 7.5% to 15% for electrophoresis. Following electrophoresis, the separated proteins were moved onto a nitrocellulose membrane (Whatman, Dassel, Germany). The membranes were treated with a blocking solution composed of 5% nonfat dry milk and 0.05% Tween in Tris-buffered saline for a duration of 1 hour. The membranes were then incubated with primary antibodies overnight at a temperature of 4 °C. The following antibodies were used: rabbit anti-Amyloid Oligomer (1:1000; ab9234; Merck Millipore, Darmstadt, Germany), mouse anti-Tau (Tau46) (1:1000; #4019; Cell Signaling, Danvers, MA, USA), rabbit anti-Phospho-Tau pThr205 (1:1000; PA5-35757; Thermo Fisher Scientific, Waltham, MA, USA), rabbit anti-β-actin (1:1000; A300-491A; Bethyl, Montgomery, TX, USA), mouse anti-Mfn1 (1:1000; NBP1-71775; Novus Biologicals, Abingdon, UK), rabbit anti-OPA1 (1:1000; 66583-1-lg; Proteintech, Rosemont, IL, USA), rabbit anti-Drp1 (1:1000; sc-32898; Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-p-Drp1 (S616) (1:1000; #4494; Cell Signaling, Danvers, MA, USA), rabbit anti-Fis1 (1:1000; ALX-210-1037; Enzo Life Sciences, Farmingdale, NY, USA), rabbit anti-AMPKα (1:1000; #2532; Cell Signaling), rabbit anti-p-AMPKα (T172) (1:1000; #2531; Cell Signaling), rabbit anti-PGC1α (1:1000; sc-13067; Santa Cruz Biotechnology), rabbit anti-NRF1 (1:1000; ab34682; Abcam, Cambridge, UK), rabbit anti-TFAM (1:1000; ab131607; Abcam), rabbit anti-NRF2 (1:1000; sc-13032; Santa Cruz Biotechnology), rabbit anti-VDAC (1:1000; #4866; Cell Signaling), rabbit anti-ANT (1:1000; PA5-76637; Thermo Fisher Scientific), Rab-

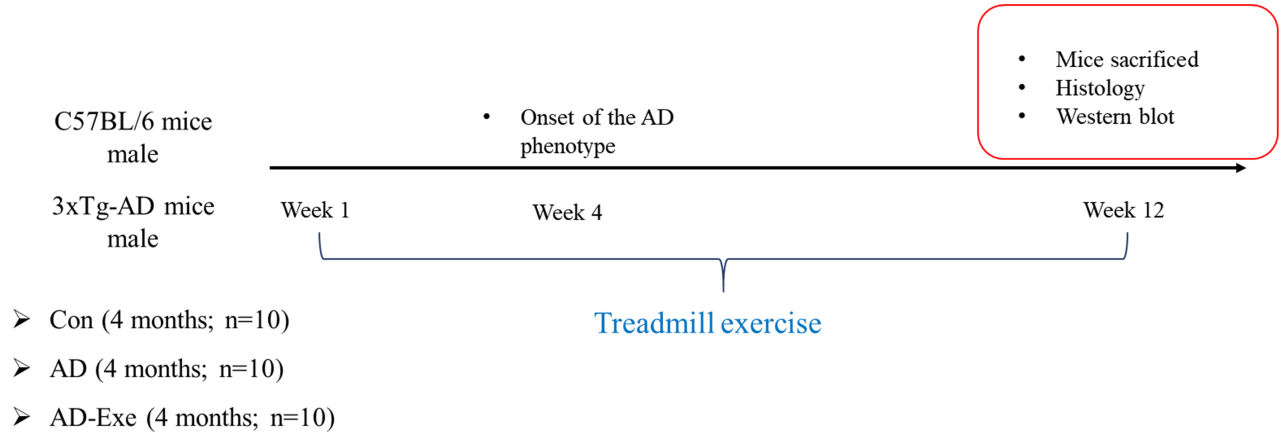

Fig. 1. Study design. Con, control; AD, Alzheimer's disease; Exe, exercise; 3xTg, triple transgenic.

bit Anti-CypD (1:1000; ab110324; Abcam), rabbit anti-Cytochrome C (1:1000; #4272, Cell Signaling), rabbit anti-PINK1 (1:1000; BC-100-494; Novus Biologicals, Abingdon, UK), mouse anti-Parkin (1:1000; sc-32282; Santa Cruz Biotechnology), rabbit anti-p62 (1:1000; #5114; Cell Signaling). Subsequently, the blots were treated with a chemiluminescent horseradish peroxidase (HPR) substrate kit (Millipore, Billerica, MA, USA) to facilitate visualization. The band intensities were quantified using ImageJ version 1.42 (National Institutes of Health, Bethesda, MD, USA).

2.3 Immunohistochemistry

The half cerebellum was cut into 40 µm thick sections using a microtome (CM3050S; Leica Microsystems, Nussloch, Germany). These sections were then preserved in a cryoprotection solution containing 20 mM KH_2PO_4, 80 mM KH_2PO_4, 0.3 g·mL^{-1} sucrose, 154 mM NaCl, 30% v/v ethylene glycol, and 0.01 g·mL^{-1} polyvinylpyrrolidone. The slices of the cerebellum were pretreated with 70% formic acid for 10 min at room temperature, followed by three successive 5-min washes in 1× PBS. The sections were blocked using 5% goat serum for 30 min, followed by immunostaining with a 1:1000 dilution of mouse anti-calbindin D-28k antibody (CB300, Swant, Burgdorf, Switzerland). The incubation was performed overnight at a temperature of 4 °C. Following a subsequent wash to eliminate any excess primary antibody, the sections were incubated with Rhodamine RedTM-X goat anti-mouse IgG (H + L) (1:500; Invitrogen, Waltham, MA, USA) for 2 hours. The sections then underwent three consecutive 5-min washes using 1× PBS. Immunostaining was performed on six sections obtained from the cerebellar vermis of each animal. A blinded specialist examined the stained brain sections, and images were captured utilizing a Zeiss (Oberkochen, Germany) LSM 510 META Duoscan confocal microscope equipped with a 20× objective.

2.4 Statistical Analysis

The recorded values are presented as means with standard deviation (SD). To assess statistically significant disparities in the measurements among the groups of mice, one-way analysis of variance (ANOVA) and post-hoc tests were performed. All statistical analyses were performed using SPSS-PC software (version 21.0, IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1 Treadmill Running Alleviates AD Neuropathology and Promotes the Survival of Purkinje Cells in the Cerebellum of 3xTg-AD Mice

To explore the effects of exercise training on AD neuropathology and subsequent Purkinje cell death, levels of amyloid oligomer, p-tau and Purkinje cell markers were assessed. AD mice had significantly elevated levels of amyloid oligomers ($p < 0.001$) compared with Con mice, but this increase was alleviated in AD-Exe mice ($p = 0.021$; Fig. 2), corresponding to increased Purkinje cell death in AD mice ($p < 0.001$; Fig. 3) compared with Con mice and reduced cell death in AD-Exe mice ($p = 0.018$). These findings indicate that exercise has a protective effect on Purkinje cells in the cerebellar vermis. The level of p-tau, however, was also significantly higher in AD mice ($p < 0.001$) than in control mice; nonetheless, treadmill running did not have any impact on the expression level of p-tau ($p = 0.383$).

3.2 Treadmill Running Mitigates Mitochondrial Dysfunction in the Cerebellum of 3xTg-AD Mice

With respect to mitochondrial dynamics, AD mice showed significantly downregulated expressions of OPA1 ($p = 0.028$) and Mfn1 ($p < 0.001$) compared with Con mice (Fig. 4). The expression level of p-Drp1 was also decreased in AD mice; however, the difference was not statistically significant ($p = 0.309$). Conversely, treadmill running significantly upregulated the expression of Mfn1 ($p < 0.001$) and p-Drp1 ($p < 0.001$) but had no effect on OPA1 ($p =$

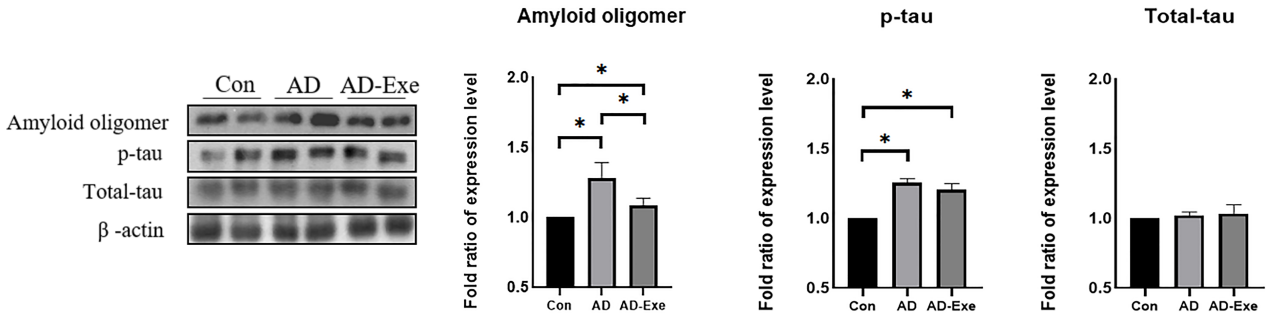

Fig. 2. Effect of treadmill exercise on AD-related pathology in the cerebellum. Representative immunoblot images and quantification of Amyloid oligomer, p-tau, and Total tau levels. Protein levels are expressed as means ± standard deviation; n = 10 per group. * indicates statistical significance at $p < 0.05$. p-tau, phosphorylated tau.

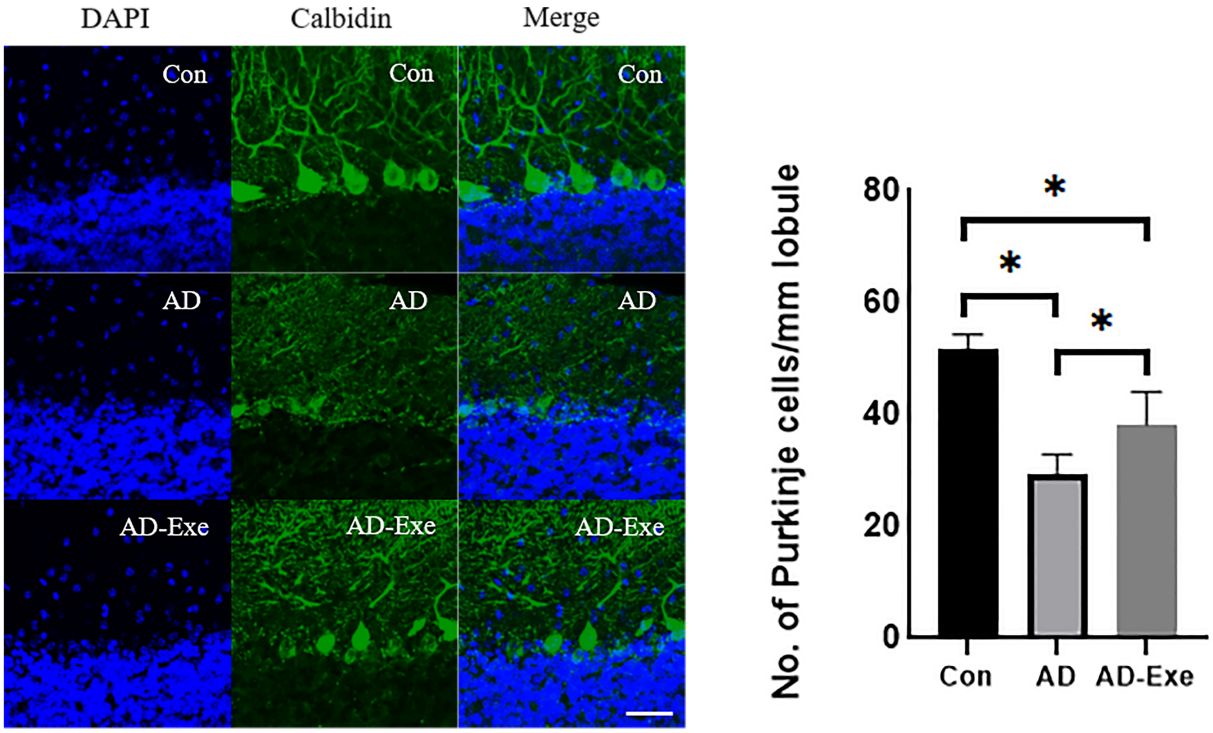

Fig. 3. Effect of treadmill exercise on Purkinje cell survival in the cerebellar vermis. Immunofluorescence staining sections of the cerebellum were imaged, and the count of Purkinje cells was performed. Values are expressed as means ± standard deviation; n = 10 per group. * indicates statistical significance at $p < 0.05$. The scale bar represents 40 μm.

0.822) compared with AD mice. In contrast, the level of the mitochondrial fission protein Drp1 was significantly upregulated in AD mice ($p < 0.001$) compared with that in Con mice but was reduced in AD-Exe mice ($p = 0.025$). Fis1 exhibited significant upregulation in AD mice ($p < 0.001$) in contrast to Con mice; however, no significant difference was observed between AD and AD-Exe mice ($p = 0.832$). These results suggest that exercise training induces mitochondrial fusion.

The mitochondrial PTP markers were assessed for neural apoptosis (Fig. 5). While AD induced significantly enhanced expression levels of VDAC ($p = 0.028$), ANT ($p < 0.001$), CypD ($p < 0.001$), and Cytochrome C ($p < 0.001$) compared with Con mice, treadmill running alleviated the increased levels of Cytochrome C ($p = 0.017$) and ANT ($p < 0.001$) but not VDAC ($p = 0.241$) and CypD ($p = 0.125$), implying that exercise training exerts a protective influence on the formation of PTP in the cerebellar cells.

Alzheimer's disease and exercise training showed no significant influence on the expression of mitochondrial biogenesis (Fig. 6) or mitophagy (Fig. 7) markers. Although exercise induced upregulation of AMPK ($p < 0.001$) and NRF2 ($p = 0.007$) and AD induced downregulation of TFAM ($p < 0.001$), there was no reversing or alleviating effect shown between AD and AD-Exe mice. In terms of mitophagy, the expression levels of Parkin ($p < 0.001$)

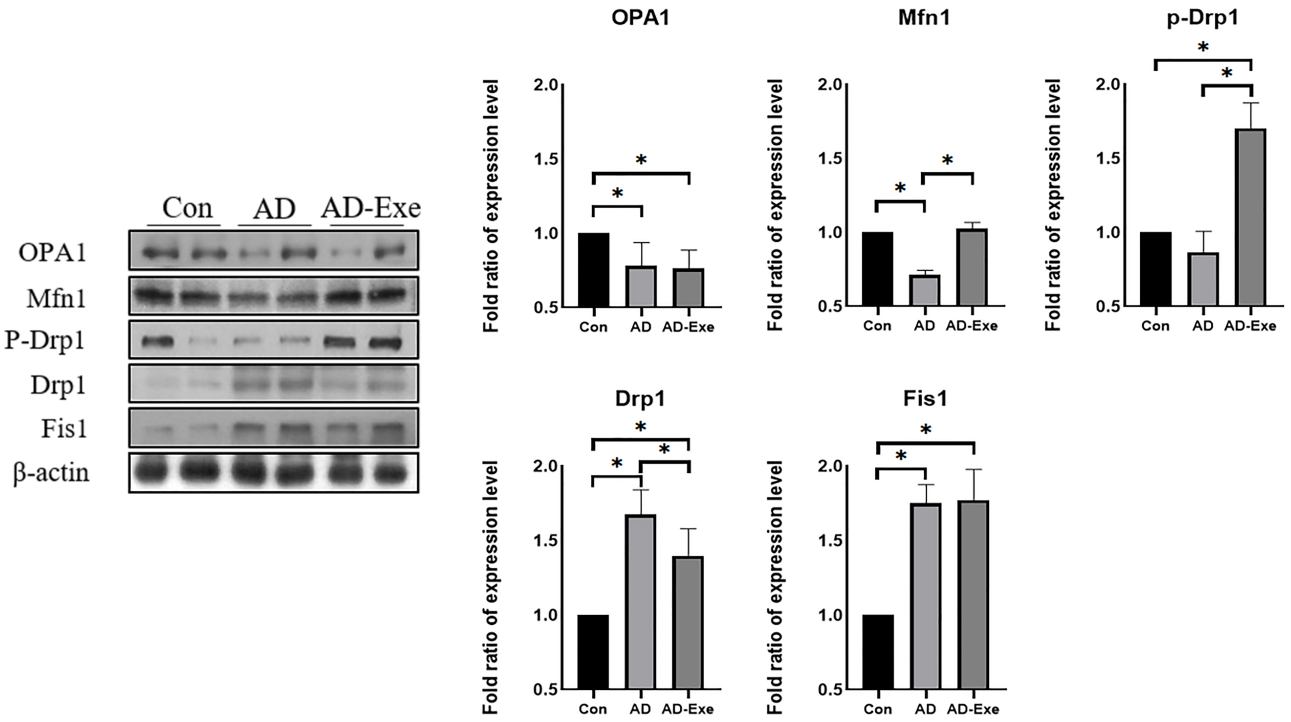

Fig. 4. Effect of treadmill running on mitochondrial dynamics in the cerebellum. Representative immunoblot images and quantification of OPA1, Mfn1, p-Drp1, Drp1, and Fis1 levels. Protein levels are expressed as means ± standard deviation; n = 10 per group. * indicates statistical significance at $p < 0.05$. OPA1, optic atrophy-1; Mfn1, Mitofusin 1; p-Drp1, phosphorylated dynamin-related protein 1; Drp1, dynamin-related protein 1; Fis1, Fission Protein 1.

and PTEN-induced putative kinase 1(PINK1) ($p = 0.022$) significantly increased in AD mice in comparison with Con mice; however, AD-Exe mice showed no significant amelioration of the increased levels of Parkin ($p = 0.346$) and PINK1 ($p = 0.063$).

4. Discussion

We investigated whether 12 weeks of treadmill running alleviated AD neuropathology and mitochondrial damage that could arise from the accumulation of Aβ plaques and neurofibrillary tangles in the cerebellum of 3xTg-AD mice. Our findings indicated that exercise training downregulated the levels of the hallmark proteins, leading to increased survival of Purkinje cells in the cerebellum. Additionally, exercise training decreased mitochondrial fission and alleviated cerebellar apoptosis.

Our findings align with previous studies that provide evidence for accumulated Aβ plaques and p-tau in the cerebellum, as well as the occurrence of Purkinje cell loss in both AD mouse models and patients with AD [10,27,28]. Chaudhari *et al.* [29] reported that patients exhibiting mild cognitive impairment and AD mice exhibiting impaired balance and coordination showed loss of cerebellar Purkinje cells. Russo *et al.* [30] similarly demonstrated that the overexpression of amyloid precursor protein in TgCRND8 mice, which contain human APP695, resulted in a deficit in synaptic flexibility and impairment of noradrenergic modulation at the synapse between parallel fibers and Purkinje cells. Consequently, this impairment of cerebellar circuitry contributed to the observed deficits in balance and coordination. Given that our study exclusively focused on biochemical assays, additional investigation is warranted to explore the effects on balance, motor coordination, or other factors associated with the cerebellar dysfunction in our animal model.

In addition, exercise training has been reported to reduce Aβ deposition and neuropathology in the hippocampus of AD animal models [31–33]. Our results indicate that exercise training alleviated Aβ deposition and possibly led to Purkinje cell survival. In support of this, previous studies showed the effect of exercise training on Purkinje cell survival in diverse animal models. Larsen *et al.* [34] reported that 18 months of treadmill exercise, starting from 5 months of age until 23 months of age, resulted in an 11% increase in the Purkinje cell number and a 9% increase in cell volume in aged rats. Chaturvedi *et al.* [35] reported that 8 weeks of treadmill exercise mitigated cerebellar Purkinje cell loss induced by calpain in a diabetic mouse model.

In our study, no significant decrease in p-tau protein was demonstrated in AD-Exe mice (Fig. 2). This finding corresponds with that of Kim *et al.* [36], indicating no change in p-tau but Aβ deposition after 12 weeks of treadmill exercise in the hippocampus of 3xTg-AD mice. Mankhong *et al.* [37] also reported that AD-like rats in-

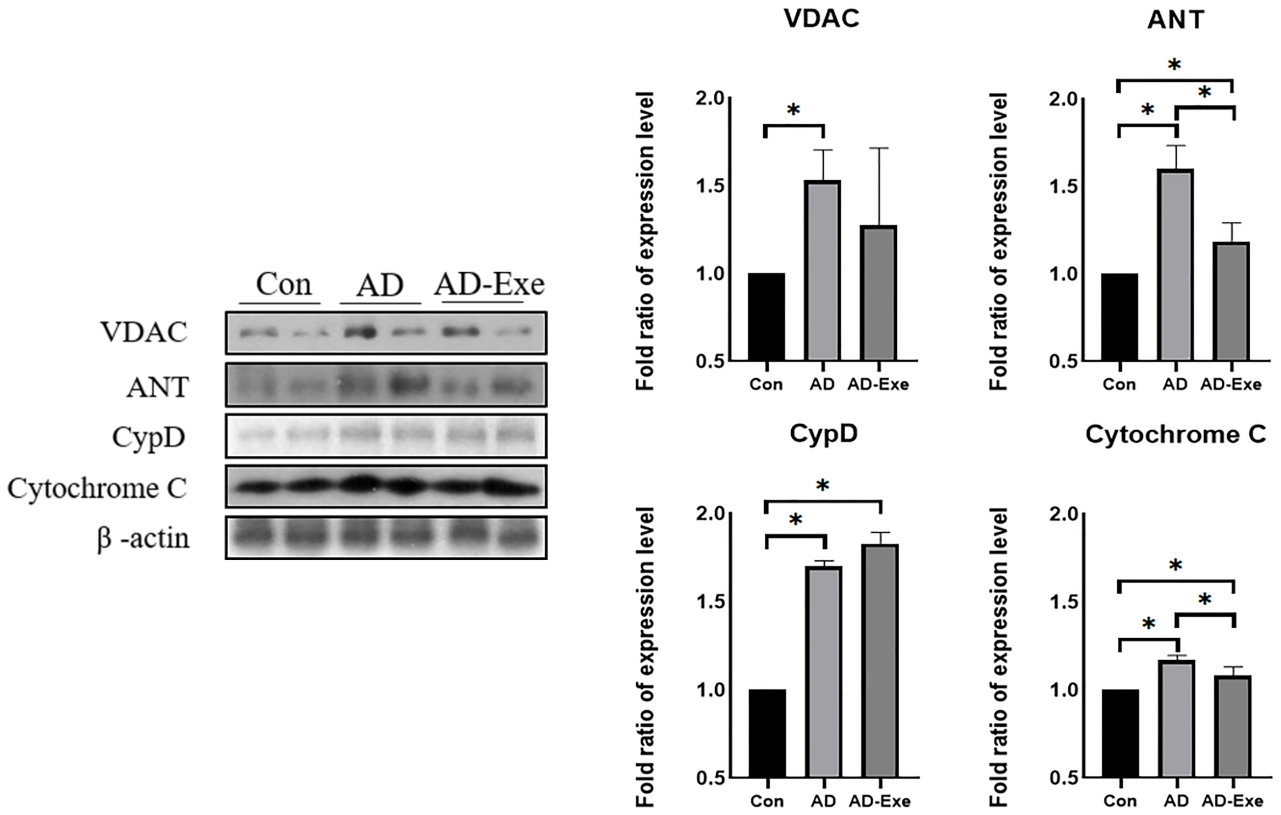

Fig. 5. Effect of treadmill running on apoptosis in the cerebellum. Representative immunoblot images and quantification of VDAC, ANT, CypD, and Cytochrome C levels. Protein levels are expressed as means ± standard deviation; n = 10 per group. * indicates statistical significance at $p < 0.05$. VDAC, voltage-dependent anion channel; ANT, adenine nucleotide translocase; CypD, Cyclophilin D.

duced by middle cerebral artery occlusion stroke showed no significant changes in three p-tau proteins (p-tauS262, p-tauS396, and p-tauS202/T205) in the cortex after 12 weeks of treadmill running. Conversely, Ohia-Nwoko *et al*. [38] and Bayod *et al*. [39] showed decreased levels of p-tau in the cortex and hippocampus of AD mice and rat models after 12 and 36 weeks of treadmill running, respectively. This discrepancy is possibly explained by the findings of Hu *et al*. [40], showing that the cerebellum exhibits the lowest level of tau proteins among various brain regions. Thus, the cerebellum is likely characterized by a slower development of tau pathology when compared with other brain regions.

We further investigated the potential protective effects of exercise training on mitochondrial dynamics and biogenesis in the AD cerebellum. Our findings indicate that AD mice exhibited reduced levels of the mitochondrial fusion proteins Mfn1, OPA1, and p-Drp1, while the expression levels of the fission proteins Fis1 and Drp1 were increased. In contrast, AD-Exe mice exhibited reversed patterns of expression for mitochondrial fusion and fission proteins, suggesting that exercise training induces mitochondrial fusion. This finding aligns with previous research that demonstrated exercise-induced mitochondrial fusion. Yan *et al*. [41] reported that 12 weeks of treadmill exercise improved memory and learning in APP/PS1 mice by increasing the expression of mitochondrial fusion proteins, and balancing mitochondrial fusion and fission in the hippocampus. Marques-Aleixo *et al*. [42] also showed that both treadmill and free-wheel voluntary running improved mitochondrial dynamics and biogenesis in the cortex and cerebellum of Sprague–Dawley rats. However, unlike these findings, we observed significant increases only in AMPK and NRF2 expression in AD-Exe mice, suggesting that exercise had no significant effect on mitochondrial biogenesis (Fig. 6). Possible explanations for this disparity include the AD-induced upregulation of p-tau (Fig. 2) and downregulation of OPA1 (Fig. 4) observed in this study, which did not show significant changes in AD-Exe mice. Jara *et al*. [12] demonstrated that genetic deletion of tau improved mitochondrial biogenesis and brain function, and Caffin *et al*. [43] showed that Opa1+/– mice displayed a defect in mitochondrial biogenesis. Therefore, increased p-tau levels and decreased OPA1 expression may contribute to mitochondrial dysfunction.

Considering the increased levels of mitochondrial fission proteins in AD mice (Fig. 4), mitochondrial fragmentation aligned with Cytochrome C, and fusion protein OPA1 release led to mitophagy and neural apoptosis [44]. There-

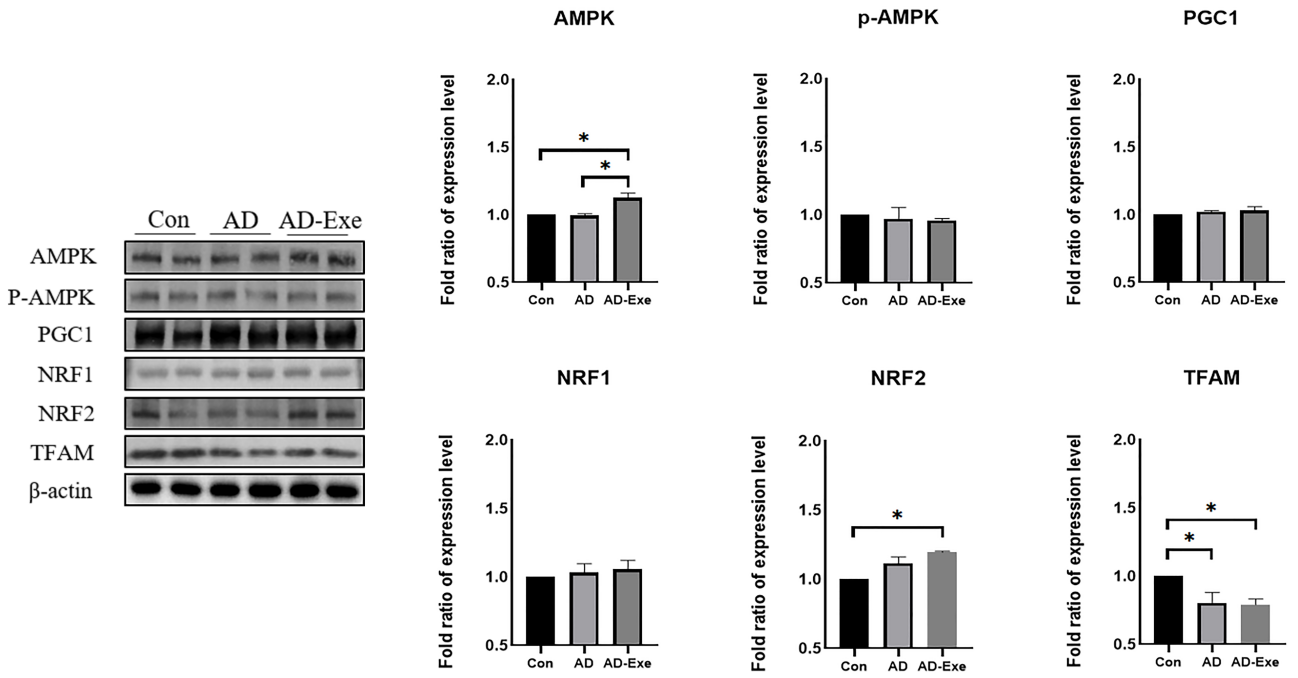

Fig. 6. Effect of treadmill running on mitochondrial biogenesis in the cerebellum. Representative immunoblot images and quantification of AMPK, p-AMPK, PGC1, NRF1, NRF2, and TFAM levels. Protein levels are expressed as means ± standard deviation; n = 10 per group. * indicates statistical significance at $p < 0.05$. AMPK, AMP-activated protein kinase; PGC1, peroxisome proliferator-activated receptor gamma coactivator 1; NRF, nuclear respiratory factor; TFAM, mitochondrial transcription factor A.

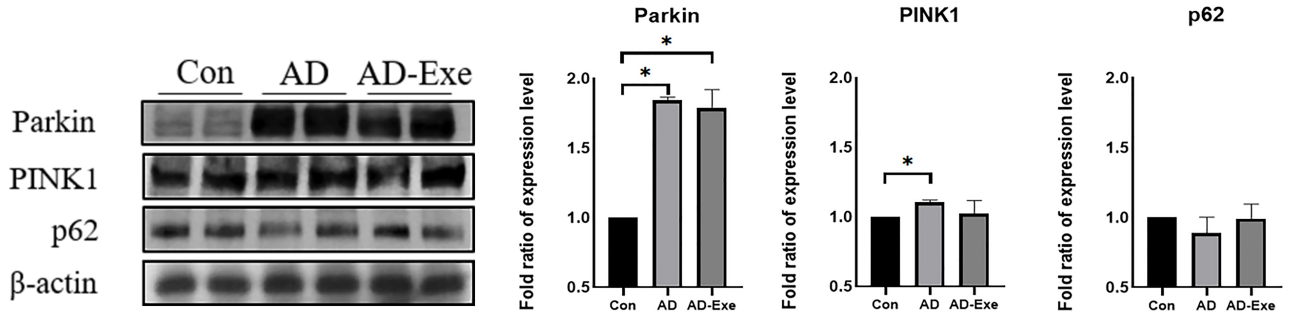

Fig. 7. Effect of treadmill exercise on mitophagy in the cerebellum. Representative immunoblot images and quantification of Parkin, PINK1 and p62 levels. Protein levels are expressed as means ± standard deviation; n = 10 per group. * indicates statistical significance at $p < 0.05$. PINK1, PTEN-induced putative kinase 1.

fore, we investigated the mitochondrial PTP and mitophagy markers in the cerebellum of 3xTg mice. The current findings revealed a significant increase in the expression levels of PTP proteins in AD mice, indicating mitochondrial dysfunction. In contrast, protein expression levels in AD-Exe mice were significantly reduced (Fig. 5). This finding suggests an exercise-induced reduction in apoptosis in the cerebellum of 3xTg mice, which is in accordance with previous studies reporting the regulatory effect of exercise training on PTP formation. Koo *et al.* [45] demonstrated that the brains of transgenic (NSE/APPsw) AD mice subjected to 16 weeks of treadmill exercise showed improved mitochondrial biogenesis and reduced neural apoptosis by suppressing the formation of PTP. Marques-Aleixo *et al.* [42] also showed that both voluntary wheel and treadmill running enhanced resistance to PTP formation and apoptosis with intensified mitochondrial respiratory activity in the brain of Sprague-Dawley rats. With respect to mitophagy, we found a significant increase in the levels of mitophagy activator proteins parkin and PINK1 in AD mice, indicating dysfunctional mitophagy. This has been well documented in previous studies [46,47]. However, exercise training did not affect expression of mitophagy markers, as observed in AD-Exe mice, and there was no significant change in p62 levels among the groups. These findings correspond to those of Kim *et al.* [36] that showed no significant alter-

ations in exercise-induced mitophagy in both the hippocampus and cortex. Marques-Aleixo *et al*. [42] also found no exercise-induced alteration of p62 in the cortex and cerebellum. This feature may have resulted from the toxicity of p-tau [12], which showed no significant change after exercise training in our study, as also reported by Kim *et al*. [36].

Our study had some limitations. First, we did not use the same strains of mice in each group. Thus, it is possible that the diverse phenotypes of the mice may have impacted the results. Second, we did not include a positive control. Hence, the general effect of exercise on mitochondrial activities remains to be verified. Previous research has suggested that muscle contraction stimulates the release of myokines, such as lipids, mRNA, microRNAs, and mtDNA. These molecules are transported to various brain regions through exosomes, subsequently regulating the expression of mitochondrial proteins involved in various mitochondrial activities [48]; hence, third, due to the absence of a behavioral test, we were unable to demonstrate potential behavioral changes that could arise from Purkinje cell survival and mitochondrial dysfunction. Fourth, of the various types of p-tau antibodies available, we used only a single type. It is worth noting that the use of different types of p-tau antibodies could have yielded different results [49]. Last, the utility of the microscopic images we used was limited; future studies that provide visual representation of the protein expression levels related to mitochondrial biogenesis, dynamics, apoptosis, and mitophagy in the cerebellum of AD mice are required.

5. Conclusions

Our study provided evidence that treadmill running resulted in improved survival of Purkinje cells and alleviated the buildup of Aβ plaques in the cerebellum of 3xTg-AD mice. These observations suggest a potential amelioration of mitochondrial dysfunction and a reduction in neural apoptosis. Our findings indicate that exercise training may serve as a beneficial non-pharmacological intervention for early-stage AD patients. Nevertheless, additional investigations are required to examine the histochemistry of mitochondrial proteins at the molecular level.

Abbreviations

AD, Alzheimer's disease; 3xTg-AD mice, triple transgenic Alzheimer's disease mice; PTP, permeability transition pores; Con, control; Exe, exercise; Aβ, β-amyloid; PBS, phosphate-buffered saline; ANOVA, analysis of variance.

Availability of Data and Materials

The data and supportive information are available within the article.

Author Contributions

YJ designed the research study and analyzed the data. TWanK and TWoonK performed the research. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was reviewed and approved by the Sungkyunkwan University School of Medicine Institutional Animal Care and Use Committee in accordance with the AAALAC International Guidelines for animal experiments (SKKUIACUC2021-04-41-3).

Acknowledgment

We would like to extend our appreciation to everyone who assisted us throughout the process of composing this manuscript.

Funding

This work was supported by the Ministry of Education of the Republic of Korea and the National Research Foundation of Korea (NRF-2018R1D1A1B07051291).

Conflict of Interest

The authors declare no conflict of interest.

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