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Effects of Huperzin-A on the Beta-amyloid accumulation in the brain and skeletal muscle cells of a rat model for Alzheimer's disease

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ABSTRACT

Aims: Alzheimer's Disease (AD) is characterized by a loss of cognitive function and also the accumulation of β-amyloid peptide (βAP) in the brain parenchyma, which plays an important role in this disease. However, it is often also associated with the non-cognitive symptoms such as loss of muscle function (Inclusion-Body Myositis-IBM).
Main methods: Sprague-Dawley rats (13 weeks-n = 68) were randomly assigned into five groups: Group C: Control; Group D: D-galactose; Group O + D: Bilateral oophorectomy + D-galactose; Group O: Bilateral oophorectomy; Group O + D + H: Bilateral oophorectomy + D-galactose + Hup-A. Tissue fixation was performed with the perfusion method. The Compound Muscle Action Potential (CMAP) and mechanical muscle activity were recorded using the standard electro-biophysical techniques. Immune staining was performed with specific antibodies, and the pathological changes were examined. RNA was obtained from brain tissue samples with the Trizol Method. Then, the expression data of mature-miRNAs (*rno-miR-9-5p, rno-miR-29a-3p, rno-miR-106a-5p, rno-miR-107 and rno-miR-125a-3p*), which may be effective in AD, were taken with Real-Time PCR.
Key findings: Impairments occurred in behavioral tests of the rats in the O + D group. βAP accumulation and AChE activity increased significantly in the forebrain in the O + D group compared to the C group. It was seen that Huperzine-A (Hup-A) reduced AChE activity and destructed βAP accumulation. There was a significant decrease in the maximum contractile force at different frequencies in the O + D group and in the O group compared to the C group.
Significance: It was found that Hup-A contributed to the healing process in rats for damage occurring both in the brain and in the neuro-muscular system.

1. Introduction

Alzheimer's Disease (AD) leads to neuronal death due to the excessive accumulation of β-amyloid peptide (βAP) in the extracellular tissue and of neurofibrillary tangles in the intracellular tissue as a result of the loss of the cholinergic neurons in the brain's frontal cortex and the hippocampus [1–2].

Another pathological finding in AD except for cognitive impairments is the loss of muscle functions. Kuo et al. [3] found for the first time that there was an increase in the aggregation of Aβ40 and Aβ42 peptide forms in the temporal muscles in patients with AD dementia compared to healthy controls. Thus, they showed that the changes in β-amyloid-precursor protein and βAP metabolism might also occur in peripheral tissues [3]. In the studies conducted in patients with Inclusion-Body Myositis (IBM), which is considered to share the same etiology with AD, the presence of βAP accumulation and phosphorylated tau protein was shown in both muscle biopsy and brain tissue [4–5]. In a study performed using conventional electro-biophysical techniques to investigate the effect of βAP and its mechanisms on the resting membrane potential of skeletal muscle fibers of the frog, it was shown that βAP disrupted the resting membrane potential of skeletal muscle fibers by leading to a marked depolarization on skeletal muscle plasma membrane and also this was due to both the inhibition of Na+/K+-ATPase and the formation of βAP-pores. The formation of βAP-pores leads to an increase in the membrane permeability. It is considered that the depolarization of skeletal muscle plasma membrane induced by βAP can disrupt significantly the functions of skeletal muscle [6–8].

MicroRNAs (miRNA)'s contribution to sporadic AD is not clear. It was found that several miRNAs are significantly altered in AD. Expression

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levels of genes appeared to have decreased in the brain tissue of AD patients [9].

It is known that Hup-A serves as a cholinesterase inhibitor which enhances neurotransmitter levels in the brain [10]. There is still a need for new studies on this subject. Huperzine-A shows the effect on the peripheral and central nervous system by blocking acetylcholinesterase. After it is rapidly absorbed from the blood-brain barrier, it distributes to all body [11]. This effect suggests that it can be effective on the expression of the down-regulated miRNAs in the brain.

In the literature, although there are many studies about the deterioration of the electrical mechanism of skeletal muscle fiber membranes due to AD, there is no any study investigating the effect of AD on the mechanical activity of skeletal muscle.

The purpose of this study is to examine effects of AD on contractility of skeletal muscle and to contribute to the information available on the mechanisms of skeletal muscle dysfunction and motor disturbances due to AD with the obtained results. Furthermore, we hypothesize that Hup-A, with potentially disease-modifying qualities in AD, may have an important role in skeletal muscle mechanical activity in AD modeling. It developed as based on a long-term administration of D-galactose following the formation of menopause. Hup-A is a potential drug which is used for AD treatment and it was applied in the rat model used in this study and then their effects on the brain and skeletal muscle functions were examined. Moreover, the relationship between Hup-A and the expression levels of miRNAs was investigated, and its role in the mechanism of miRNA during the treatment period was shown.

2. Materials and methods

2.1. Animals

Sixty-eight healthy adult female Sprague-Dawley rats (13 weeks old and average body weight 180–200 g) were used in this study. The rats were obtained from the Experimental Animal Center, Guinea-Pig Laboratory of Ankara, Turkey. The study was approved by the Research and Ethical Committee of the University of Mersin. The rats were housed in polycarbonate boxes (three or four rats per box) with steel wire tops and rice husk bedding. They were maintained in a controlled atmosphere of 12 h dark/light cycle, at 22 ± 2 °C temperature, and at 50–70% humidity, with free access to pelleted feed and fresh tap water. The animals were supplied with dry food pellets commercially available. The animals were randomly assigned into five groups: Group C (the Control Group-12 rats), Group D (D-galactose, 100 mg/kg/day-14 rats), Group O + D (bilateral oophorectomy-(OVX), D-galactose 100 mg/kg/day-14 rats), Group O (bilateral oophorectomy-(OVX)-14 rats) and Group O + D + H (bilateral oophorectomy-(OVX), D-galactose 100 mg/kg/day, Huperzine-A 0,1 mg/kg/day-14 rats).

2.2. Experimental protocol

After the rats in the O + D + H group underwent bilateral ovariectomy, D-galactose (100 mg/kg/day in 9% saline, i.p. injection every day) was applied for 10 weeks; and Hup-A (0,1 mg/kg/day in 9% saline, i.p. injection every day) was applied for 3 weeks from the 8th week following one recovery week. The same experimental procedure except for Hup-A injection in the O + D + H group was performed in the rats in the O + D group. The incision was opened and closed on the *linea alba* without excising the ovaries of the rats in the D group and D-galactose; and 9% saline (i.p.) injection were applied as in the O + D + H group following recovery. After the rats in the O group underwent bilateral ovariectomy, 9% saline (i.p.) injection was applied. The rats in the C group were given D-galactose and 9% saline (i.p.) injection within Hup-A every day for 11 weeks.

2.3. Behavioral tests

The open field and the Morris water maze tests were performed respectively in 27 weeks to evaluate spatial memory ability and learning and locomotor activity. The procedures for these behavioral tests were made as previously reported and briefly defined below [12–14]. All experiments were recorded by the Noldus Ethovision XT software with the computer system and the digital camera.

2.3.1. Open field test

Locomotor activity of the rats was observed in the area where there is bright light (100 cm diameter closed by a wall 40 cm high). The rats were placed in the center of the open-field and the test was started. The distance (cm) covered in five minutes on the floor by them and their movement speeds (cm/s) were recorded.

2.3.2. Morris water maze testing

The Morris water maze ($R = 150$ cm diameter, $h = 60$ depth) was used to examine the effects of the applications on learning and spatial memory. In short, it was observed with the computer system and the digital camera that the rats were looking for the platform in any time period. Four attempts were made every day for four consecutive days. The platform was removed on the 5th day and each rat was allowed to swim freely for 60 s and then the probe test was completed. At the end of this period, the time spent by them and their swimming speeds at the quadrant where the platform was formerly were recorded.

2.4. Tissue preparation

After the behavioral tests, each group was divided in two for electrobiophysics and histopathological examinations. They were anesthetized by applying 80 mg/kg Ketamine HCl (Ketalar® flakon, Parke-Davis, ESA) and 10 mg/kg xylazine HCl (Rompun flk, Bayer, Turkey) i.p. They were placed in the supine position on the electrically heated AOT0801-DC Animal Operating Table (DC-Heated Animal Operating Table-MAY QOT08019). Rectal temperature was allowed to keep at 37–38 °C for electrical records to be taken from the EDL muscle of the left leg. The EDL muscle of the right leg was removed from mechanical records. One end was connected to isometric force transducer (FDT 05 Force Displacement Transducer) with 2–0 silk sutures bound to the tendons of the isolated EDL muscle, and the other end was connected by placing in the organ bath which contained 10 ml of Krebs solution and was kept at 37 °C; and then it was maintained under these conditions until the record was received. A mixture of 4% paraformaldehyde and 0.05% glutaraldehyde within 9% cold saline was perfused into the rats for immunohistochemical examinations. The brain tissues including the cerebral cortex and the hippocampus and also the EDL muscle were removed. The tissues were allowed to be fixed in formaldehyde for immunohistochemical examinations [13]. The brain tissues were stored at −80 °C for genetic analysis.

2.5. Electro-biophysical tests

2.5.1. Electrical activity properties of EDL muscles

The Compound Motor Action Potentials (CMAP) were recorded in all groups using the standardized nerve conduction study techniques [15]. The data were collected by means of a BIOPAC® MP 100 acquisition system (Santa Barbara, USA). Bipolar surface electrodes (Medelec® small bipolar nerve electrodes, 6984 T, Oxford, UK) were used for stimulation. Surface disc electrodes (Medelec®, number 017 K006, Oxford, UK) were used for CMAP recordings from the EDL muscle. The ground electrode was placed on the thigh on the side of stimulation. The supramaximal stimulus consisted of single square pulse (duration 0,5 ms). BIOPAC Acknowledge Analysis software® (ACK 100 W) was used to measure CMAP peak-to-peak amplitude, peak latency, total duration, depolarization duration, 50% repolarization duration, amplitude and area.