Life Sciences 217 (2019) 25–33

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Cannabinoid type 2 receptor agonist JWH-133, attenuates Okadaic acid induced spatial memory impairment and neurodegeneration in rats

Murat Çakır[a,*], Suat Tekin[b], Züleyha Doğanyiğit[c], Yavuz Erden[d], Merve Soytürk[b], Yılmaz Çiğremiş[e], Süleyman Sandal[b]

[a] Faculty of Medicine, Department of Physiology, University of Bozok, Yozgat 66200, Turkey
[b] Faculty of Medicine, Department of Physiology, University of Inonu, Malatya 44280, Turkey
[c] Faculty of Medicine, Department of Histology and Embryology, University of Bozok, Yozgat 66200, Turkey
[d] Department of Molecular Biology and Genetics, Faculty of Science, Bartın University, Bartın 74100, Turkey
[e] Department of Medical Biology and Genetics, Faculty of Medicine, Inonu University, Malatya 44280, Turkey

ARTICLE INFO

Keywords:
Alzheimer's disease
Okadaic acid
Cannabinoid type 2 receptor
JWH-133

ABSTRACT

Aim: Cannabinoid system has various physiological roles such as neurogenesis, synaptic plasticity and emotional state regulation in the body. The presence of cannabinoid type 2 receptor (CB2), a member of the cannabinoid system, was detected in different regions of the brain. CB2 receptor plays a role in neuroinflammatory and neurodegenerative processes. We aimed to determine the possible effect of CB2 agonist JWH-133 in Okadaic acid (OKA)-induced neurodegeneration model mimicking Alzheimer's Disease (AD) through tau pathology.

Materials and methods: In this study, 40 Sprague Dawley male rats were divided into 4 groups (Control, Sham, OKA, OKA + JWH-133). Bilateral intracerebroventricular (icv) injection of 200 ng OKA was performed in the OKA group. In the OKA + JWH-133 group, injection of JWH-133 (0.2 mg/kg) was performed intraperitoneally for 13 days different from the group of OKA. Morris water maze test was used to evaluate the spatial memory. Levels of caspase-3, phosphorylated tau (ser396), amyloid beta (Aβ), tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) levels in brain cortex; and the hippocampus regions were examined by immunohistochemical methods.

Key findings: In the OKA group, caspase-3, phosphorylated tau (ser396), Aβ, IL-1β levels were higher in the cortex and hippocampus than in the other groups. The implementation of the JWH-133 reversed the increments in these parameters, and also prevented spatial memory impairment.

Significance: In this study, we found that the administration of the CB2 receptor agonist JWH-133 in this study reduced neurodegeneration, neuroinflammation, and spatial memory impairment in the OKA-induced Alzheimer's Disease model.

1. Introduction

Alzheimer's Disease (AD) is the most common cause of dementia, especially in people over 65 years of age. The number of AD cases is estimated to reach 100 million in 2050. This disease is characterized by loss of progressive and irreversible cognitive functions; and is a significant problem for the patients in terms of their self-care, their daily lives and their health expenditures [9,38].

In the pathophysiology of the disease; cognitive function loss is accompanied by neurofibrillary tangles (NFT) and amyloid beta (Aβ) plaque formation, neuroinflammation, oxidative stress and cholinergic neuron loss [2]. There is a correlation between the amount of NFT and the disorder in cognitive functions in AD. The underlying cause of NFT formation in brains of the people with AD is abnormal tau hyperphosphorylation [16]. Protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) are the most important serine/threonine phosphatases found in mammalian brain. These phosphatases are a potential target in research on tau pathology [17]. Inactivation of PP1 and PP2A leads to hyperphosphoryl tau formation and consequently results in NFT formation. In addition, the expression and activity of PP1 and PP2A in the brain were found to be low in AD patients [26].

Okadaic acid (OKA) is a potent serine/threonine PP1 and PP2A inhibitor. This molecule has been shown to cause tau hyperphosphorylation *in vivo* and *in vitro* studies [23]. In many experimental studies, tau hyperphosphorylation has been demonstrated in a manner similar to AD by intracerebroventricular (icv) application of OKA [1].

* Corresponding author at: Faculty of Medicine, Department of Physiology, Bozok University, Yozgat 66200, Turkey.
E-mail address: murat.cakir@bozok.edu.tr (M. Çakır).

https://doi.org/10.1016/j.lfs.2018.11.058
Received 10 October 2018; Received in revised form 14 November 2018; Accepted 27 November 2018
Available online 28 November 2018
0024-3205/ © 2018 Published by Elsevier Inc.

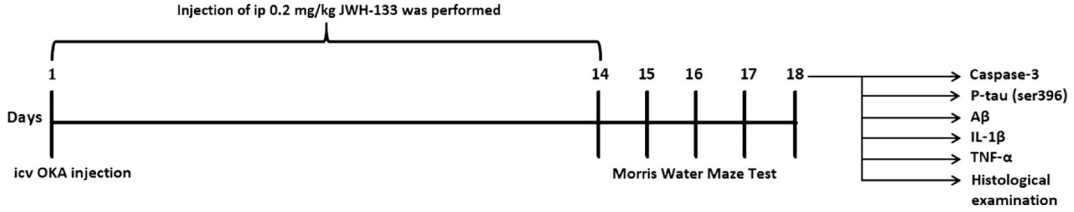

Fig. 1. Scheme of experimental procedure.

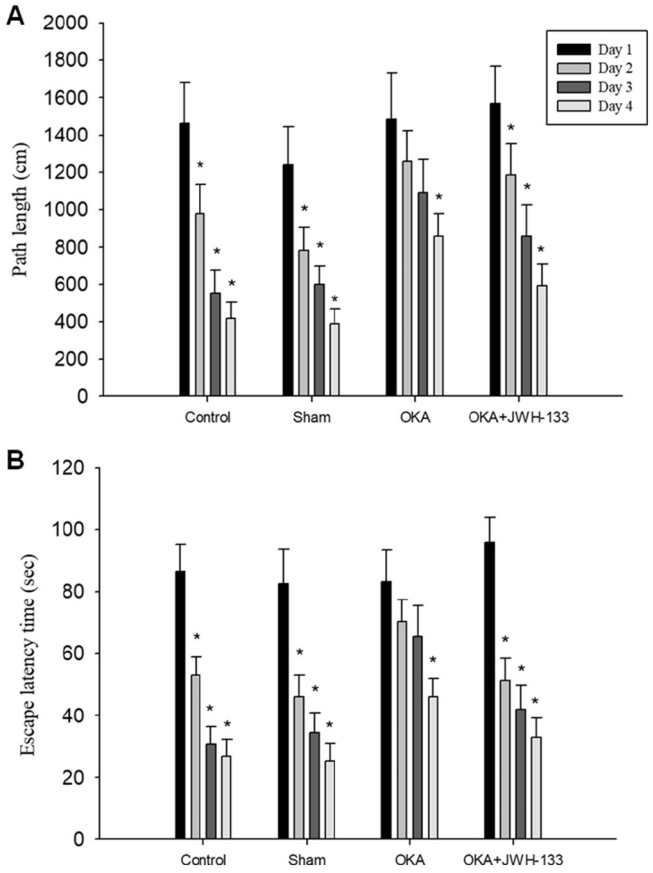

Fig. 2. Treatment with JWH-133 attenuated spatial memory deficits induced by OKA icv injection in rats. JWH-133 significantly reduced the latency time significantly on OKA-induced spatial memory impairment in rats (B). JWH-133 significantly reduced the path length significantly on OKA-induced spatial memory impairment in rats (A). *$p < 0.05$ different from day 1. Data expressed as mean ± SD ($n = 10$).

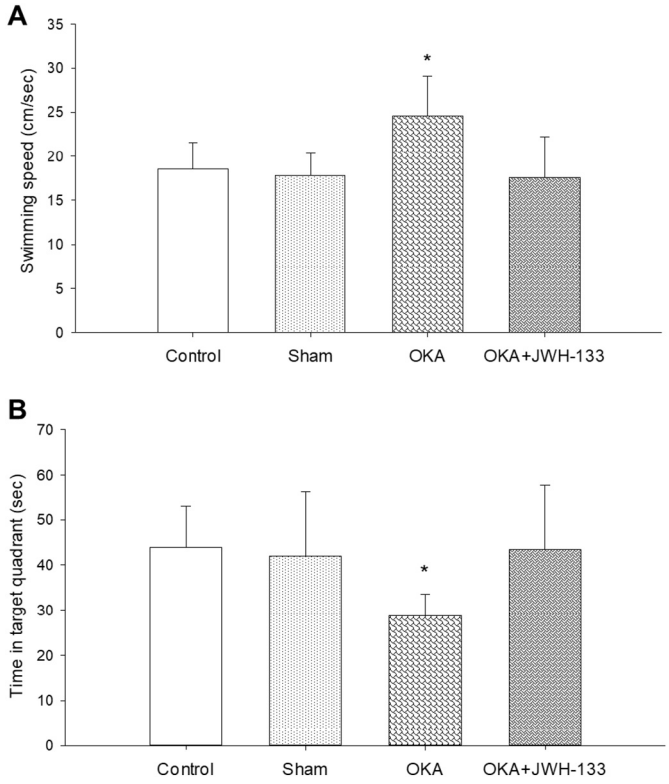

Fig. 3. Effect of JWH-133 on swimming speed and time in the target quadrant. The JWH-133 application reduced the increase in swimming speed caused by OKA (A). The JWH-133 application increased the time spent in the target quadrant (B). *$p < 0.05$ different from Control, Sham, OKA + JWH-133 groups. Data expressed as mean ± SD ($n = 10$).

Icv OKA has been shown to induce neuroinflammation, oxidative stress and Aβ formation in the brain with cognitive function impairment in rats [10,21,22,36,41].

Cannabinoids are substances that activate cannabinoid receptors. In the body, cannabinoid system has various physiological roles such as neurogenesis, synaptic plasticity and emotional state regulation [27]. The presence of the cannabinoid type 2 receptor (CB2), a component of the cannabinoid system, was detected in different regions of the brain and was found, especially in astrocytes and microglia cells [4]. CB2, which has been shown to play an active role in neuroinflammatory and neurodegenerative processes, has also been reported to increase microglial migration and infiltration [15,39]. Increased levels of CB2 have been shown in brains of Alzheimer's patients [7,35]. JWH-133 is a selective cannabinoid type 2 receptor agonist [8]. In recent years, studies have shown that JWH-133 administration in transgenic AD models prevents neuroinflammation, Aβ accumulation, and impairment of cognitive function [3,28]. In this study, we investigated the effect of CB2 agonist JWH-133 on OKA-induced neurodegeneration.

2. Materials and methods

2.1. Animals

In this study, 40 male Sprague-Dawley rats weighing 250–350 g obtained from Inonu University Experimental Animal Production and Research Center were used. All the applications in this study were carried out in accordance with the experimental protocol approved by the Ethics Board of the Inonu University Medical Faculty Experimental Animal Research (2017/A-42). The animals were housed in a ventilated environment at 20–22 °C, with 12 h of light and 12 h of darkness cycle. The rats were fed *ad libitum* with standard pelleted rat diet and served with tap water. The animals used in the experiment were randomized into 4 groups of 10 rats each. Prior to the experiment all animals were housed in single cages; and were kept in single cages throughout the experiment.

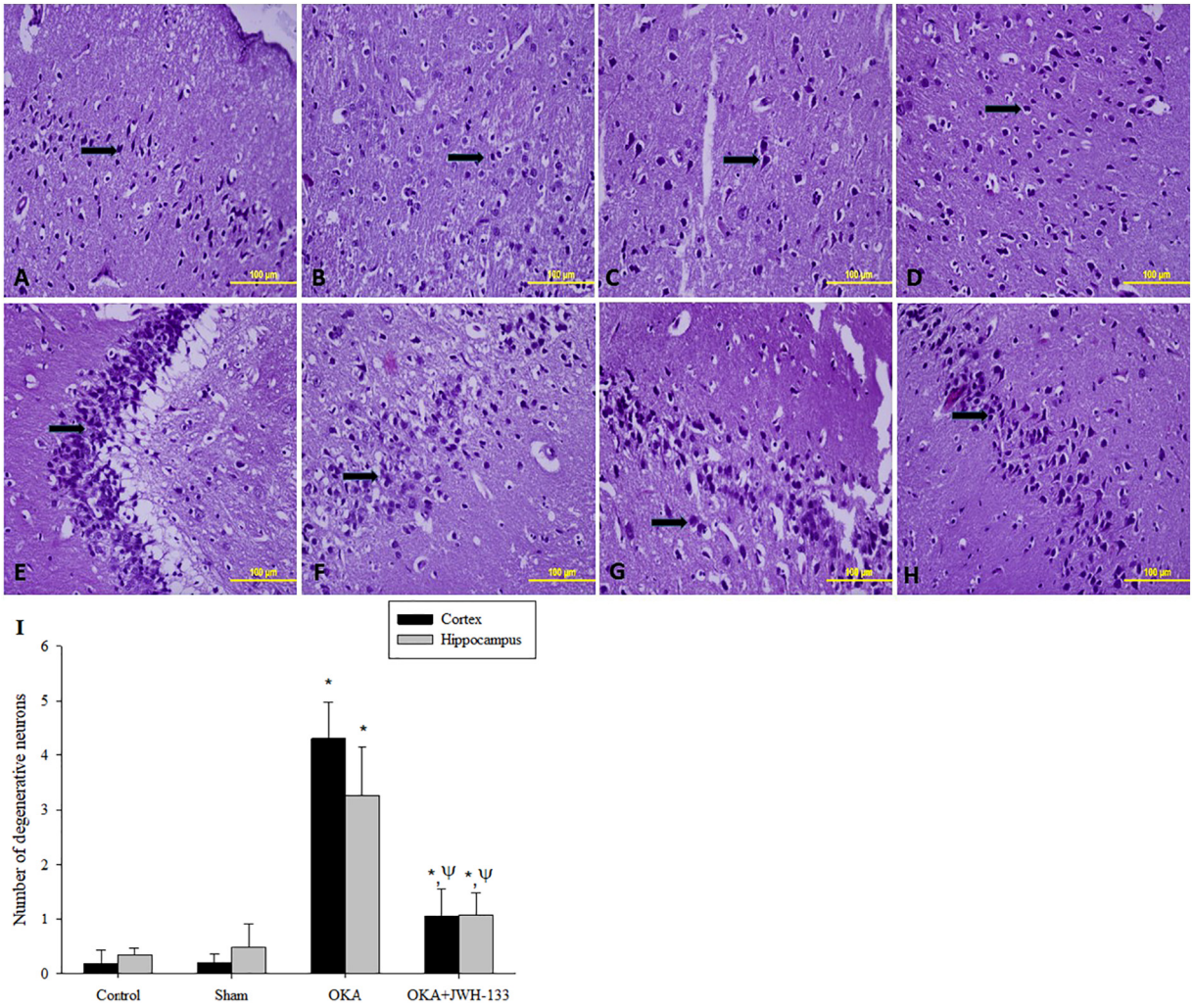

Fig. 4. JWH-133 ameliorated cortex and hippocampal neurons damage induced by OKA. Cerebral cortex: Control (A), Sham (B) and OKA + JWH-133 (D) groups notice normal pyramidal cell (arrows) OKA (C) the view of eosinophilic degeneration of pyramidal cells. Hippocampus: Control (E), Sham (F) and OKA + JWH-133 (H) groups showing normal hippocampus. OKA (G) the view of eosinophilic degeneration of pyramidal cells H-E; ×40. The number of degenerated neurons was calculated using the ×40 objective in 100 different field in the cerebral cortex and hippocampus. $^{*}p < 0.05$ different from Control, Sham, OKA + JWH-133 groups. $^{\Psi}p < 0.05$ different from OKA group. Data expressed as mean ± SD ($n = 10$).

2.2. Surgical applications

Rats-except for the control group-were anesthetized with intraperitoneally (ip) 70 mg/kg ketamine and 8 mg/kg xylazine. The head skin area to be incised was shaved and cleaned with povidone iodine solution. After the animals were fixed to the stereotactic device (Rodent Stereotaxic Instruments, Harvard Apparatus, the USA), the skull skin was cut in the middle line with the scalpel showing the bone structure. Dental burr holes were drilled in the skull on both the sides over the lateral ventricles using the stereotaxic coordinates: 0.8 mm posterior to bregma, 1.4 mm lateral to sagittal suture, and 4.8 mm beneath the surface of the brain [32].

2.3. Experiment groups

The formation of the groups and the applications were made as follows (Fig. 1):

Control group: No injection or surgical procedure was performed. At the end of the learning experiment, the animals were decapitated.

Sham group: Artificial cerebrospinal fluid (aCSF) injections were performed in 5 μL volumes bilaterally in the lateral ventricles of the animals. Subsequently, the vehicle was injected ip for 13 days.

OKA group: Injection of 200 ng OKA (Santa Cruz Biotechnology, California, the USA) injected bilaterally into the lateral ventricles in 5 μL volumes was performed as icv. [37]. Subsequently, the vehicle was injected ip for 13 days.

OKA + JWH-133: Bilateral injection of 200 ng OKA dissolved in 5 μL volumes in aCSF was performed in the lateral ventricles as icv [37]. Subsequently, JWH-133 (Biorbyt, California, the USA) was dissolved in DMSO and diluted to 99% in phosphate buffer. An injection of 0.2 mg/kg JWH-133 was administered ip for 13 days.

On the 14th day, all animals were taken into Morris water maze test and their spatial memories were evaluated.

2.4. Administration of Morris water maze test

The Morris water maze test is a test constructed by Richard G. Morris designed to measure spatial memory in rodents [30]. The water tank with a diameter of 150 cm and a height of 60 cm was filled with water up to a height of 40 cm. The tank surface was virtually divided into 4 equal quadrants (Northeast, northwest, southeast and southwest). The Northwest quadrant was identified as the target quadrant. A platform with a diameter of 10 cm was placed on the target quadrant at a distance of 30 cm from the edge of the pool, 1 or 2 cm below the

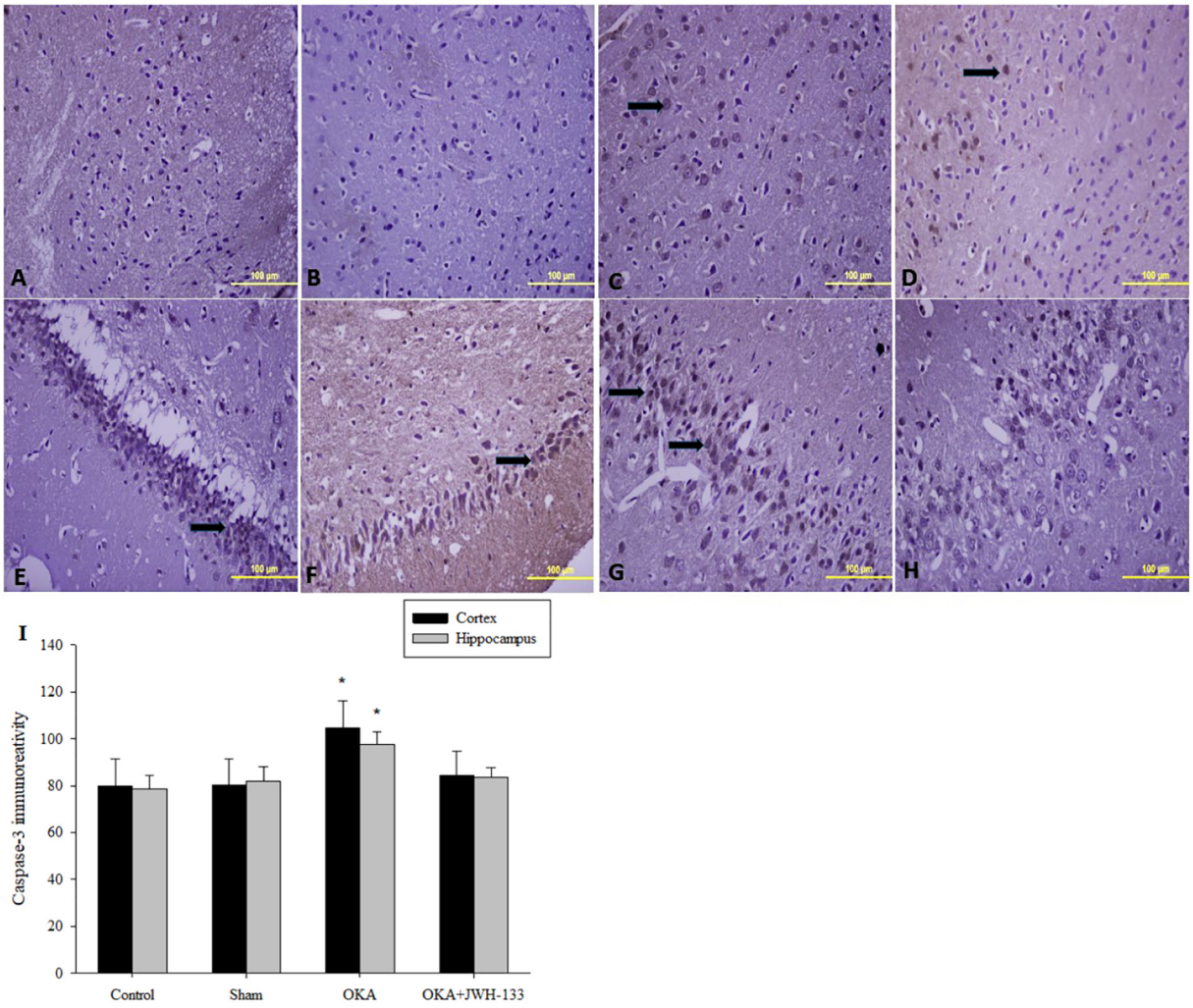

Fig. 5. JWH-133 administration reduced the level of OKA-induced caspase-3 immunoreactivity in the hippocampus and cortex (I). Cerebral cortex: Control (A), Sham (B) and OKA (C) and OKA + JWH-133 (D) caspase (+) immunoreactivity are observed in cerebral cortex (arrows) caspase; ×40. Hippocampus: Control (E), sham (F) and OKA (G) OKA + JWH-133 (H) caspase (+) immunoreactivity are observed in hippocampus (arrows) caspase; ×40. The number of caspase-3 (+) immunoreactivity was calculated using the ×40 objective in 100 different field in the cerebral cortex and hippocampus. *$p < 0.05$ different from Control, Sham, OKA + JWH-133 groups. Data expressed as mean ± SD ($n = 10$).

surface of the water. The platform was kept stationary for 4 days. The water in the pool was painted with non-toxic food stain (Mixol, Germany) so that the rats could not see the platform when they were released into the water; and the water temperature was maintained at 26 ± 2 °C. In the first 4 days of the trial, the trials were applied 4 times at 20-min intervals every day. In each trial, the rats were left in the water in the tank from four different directions. Rats were allowed to swim for 120 s to find the platform. Within 120 s, the rat, which failed to locate the platform, was placed on the platform. They waited on the platform for 30 s to see and learn the clues around them. During the 4-day learning period, escape latency times of the rats were measured, and the path length of the platform was measured. On the 5th day of the learning experiment, in the probe trial test, the platform inside the tank was removed. The animals were allowed to swim for 120 s. During this time, the time and speed of the animals spent in the quadrant where platform was placed were measured. A computerized video camera system (Ethovision, Noldus) was used to record and evaluate the movements of the rat in the tank.

2.5. Histological examination

At the end of the Morris water maze test, the rats under the anesthesia were decapitated. The brain tissue of the rats was removed. Care was taken to make this process happen quickly. Brain samples were fixed for 48 h in 10% neutral formalin. Then, after being washed with water, these samples were dehydrated following a graded alcohol series. After cleaning with xylol, they were then immersed in paraffin. The 5 μm-thick brain sections were taken from the paraffin blocks. These were stained with hematoxylin-eosin for evaluating the brain structure [10]. The sections were studied using an Olympus BX-51 Photomicroscope. Number of degenerated neurons (degenerated neurons were defined as acidophilic cytoplasm) was calculated using the X40 objective in 50 different field in the cerebral cortex and hippocampus.

2.6. Immunohistochemistry analysis

The Avidin-Biotin-peroxidase method was applied immunohistochemically to determine caspase-3, p-tau (ser396), Aβ, TNF-α and IL-1β immunoreactivity in the brain tissue. The sections of paraffin blocks with a thickness of 5 μm were incubated at 60 °C for 1 night. Following this procedure, sections were passed through xylene and descending grades of alcohol. The distillate was then washed with water and then treated with citrate buffer for antigen recovery. After washing with PBS, application of hydrogen peroxide was performed. Subsequent operations were performed using the Large Volume

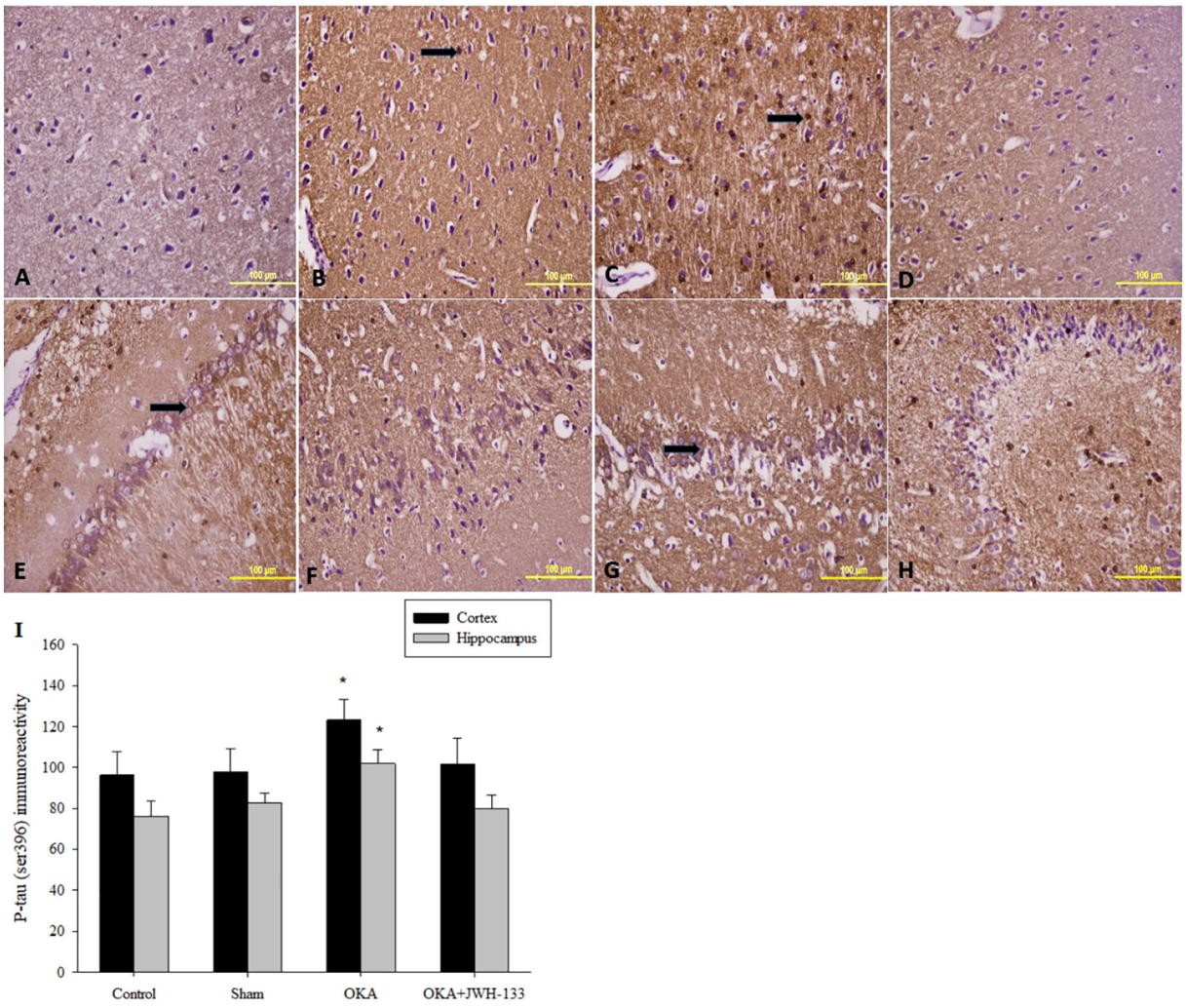

Fig. 6. JWH-133 administration reduced the level of OKA-induced p-tau (ser396) immunoreactivity in the hippocampus and cortex regions (I). Cerebral cortex: Control (A), Sham (B), OKA (C), and OKA + JWH-133 (D) p-tau (ser396) (+) immunoreactivity are observed in cerebral cortex (arrows) p-tau (ser396); ×40. Hippocampus: Control (E), Sham (F), OKA (G) and OKA + JWH-133 (H) p-tau (ser396) (+) immunoreactivity are observed in hippocampus (arrows) p-tau (ser396); ×40. The number of p-tau (ser396) (+) immunoreactivity was calculated using the ×40 objective in 100 different field in the cerebral cortex and hippocampus. *$p < 0.05$ different from Control, Sham, OKA + JWH-133 groups. Data expressed as mean ± SD ($n = 10$).

Detection System kit. Afterwards, 5 min serum block were applied to samples. Following this procedure, Primer Antibodies caspase-3, p-tau (ser396), Aβ, TNF-α, IL-1β (Santa Cruz Biotechnology, Texas, the USA) were applied to the samples overnight at +4 °C. Antibodies were stained from different slides. Antibodies were stained by the same procedure. Biotinylated secondary streptavidin-HRP and DAB chromogens were applied after primer antibody application and then stained with Gill Hematoxylin. Finally, it gradually passed through increasing alcohol series and xylene, and was closed with entellan [10]. The sections were examined under 40× objective magnification using an Olimpus BX51 light microscope. Image-J program was used to measure immunoreactivity from images taken from the slides. For measurements, 100 different areas were evaluated from each group.

2.7. Statistical analysis

SPSS 22 package program was used for statistical analysis. The homogeneous distribution of the data was determined by the Shapiro-Wilk test. The One-Way ANOVA test was used as a statistical method and the Post-Hoc Tukey test was used in multiple comparisons. Unlike other data, in the statistical analysis of the learning experiment of the first 4 days, repeated measurements ANOVA test was used. Values were expressed as mean ± standard deviation (SD). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of JWH-133 on the cognitive deficit of OKA injected rats

In the trial test, escape latency times (Fig. 2B) in the control and sham groups were significantly decreased on the second, third, and fourth days compared to the first day ($p < 0.05$). When we compared the escape latency times of the OKA group, it was seen that only the escape latency times on the fourth day was significantly shorter than on the first day ($p < 0.05$). Compared to escape latency times in OKA + JWH-133 group, there was a statistically significant decrease in the second, third and fourth day ($p < 0.05$).

In the comparison of the Path Length (Fig. 2A) in the control and sham group in the trials test, it was observed that the distances decreased significantly on the second, third and fourth days compared to the first day ($p < 0.05$). When the Path Length of the OKA group was compared, it was observed that the distance decreased significantly only on the fourth day compared to the first day ($p < 0.05$). In the path length comparison of the OKA + JWH-133 group, there was a

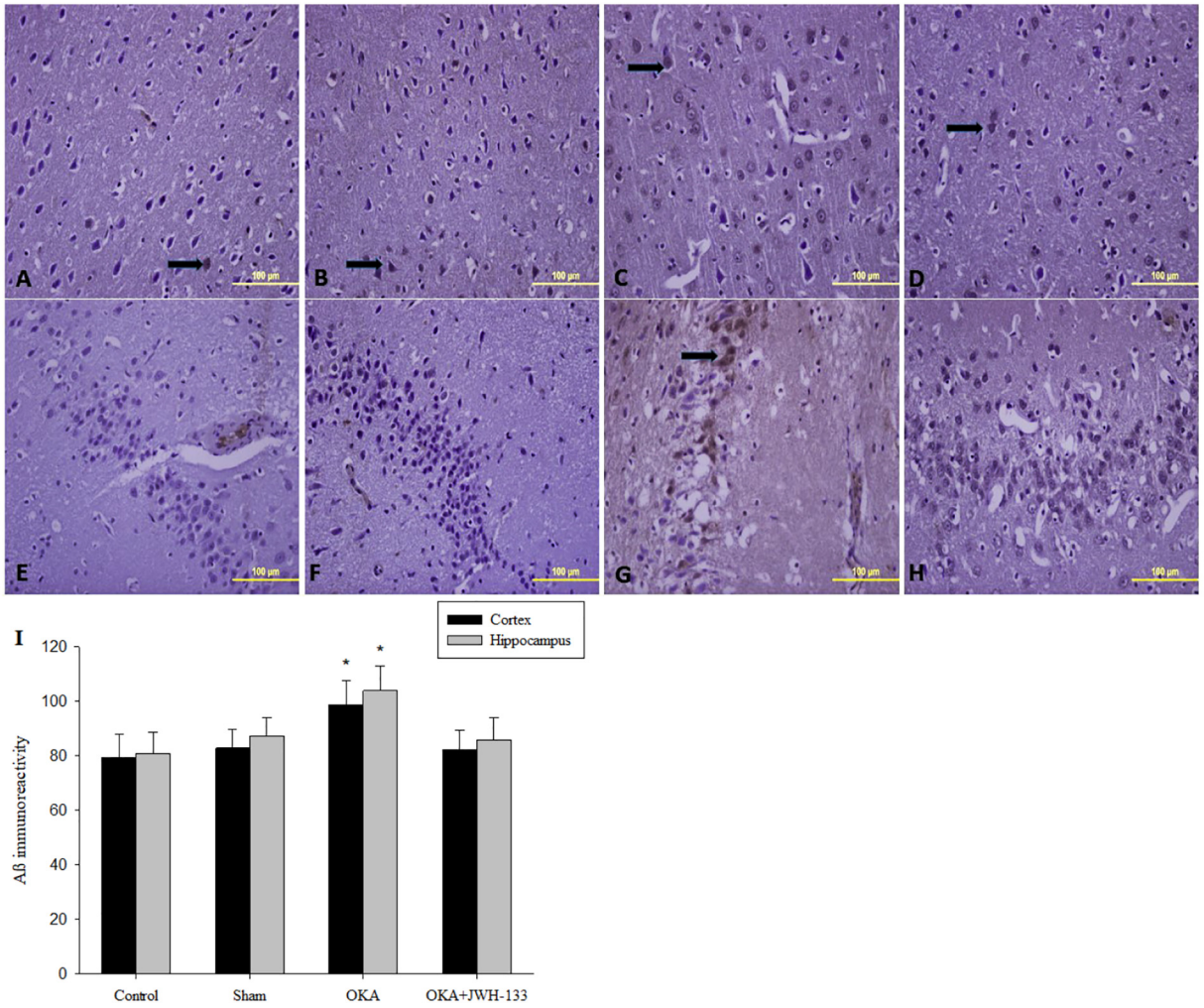

Fig. 7. JWH-133 administration reduced the Aβ immunoreactivity caused by OKA in the cortex and hippocampus regions (I). Cerebral cortex: Control (A), Sham (B), OKA (C) and OKA + JWH-133 (D Aβ (+) immunoreactivity are observed in cerebral cortex (arrows) Aβ × 40. Hippocampus: Control (E), Sham (F), OKA (G) and OKA + JWH-133 (H) Aβ (+) immunoreactivity are observed in hippocampus (arrows) Aβ × 40. The number of Aβ (+) immunoreactivity was calculated using the ×40 objective in 100 different field in the cerebral cortex and hippocampus. *$p < 0.05$ different from Control, Sham, OKA + JWH-133 groups. Data expressed as mean ± SD ($n = 10$).

statistically significant shortening of distance on the second, third and fourth day compared to the first day ($p < 0.05$).

In the Probe Trial test, the time and swimming speed (Fig. 3A) of the animals on the target quadrant (Fig. 3B) were evaluated. Compared with the control and sham group, the time spent in the target quadrant in the OKA group was OKA group was shorter ($p < 0.05$). In the OKA + JWH-133 group, the time spent in the target quadrant was significantly higher than in the OKA group ($p < 0.05$). When the swimming speed of animals was compared in the control and sham group, the swimming speed of the animals in the OKA group increased ($p < 0.05$). The swimming speed of animals in OKA + JWH-133 group was less than that of OKA group ($p < 0.05$).

3.2. Effect of JWH-133 on the neuropathologic alterations of rats with OKA

The cortex and hippocampus tissue were found to have normal histological structure in the sections stained with hematoxylin and eosin in the Control and Sham groups (Fig. 4A, B, E, F). The number of eosinophilic cells was significantly increased in the OKA and OKA + JWH-133 groups compared to the Control and Sham groups ($p < 0.001$) (Fig. 4C, D, G, H). In the OKA + JWH-133 group, the number of eosinophilic cells in the cortex and hippocampus tissue decreased significantly compared to the OKA group ($p < 0.001$) (Fig. 4D,H). In Control, Sham and OKA + JWH-133 groups, regular cells are indicated by arrows, while eosinophilic cells are shown by arrows in OKA group.

The immunoreactivity of brain cortex areas is given in Figs. 5, 6, 7, 8, and 9. The levels of caspase-3, p-tau (ser396), Aβ, IL-1β immunoreactivity in the cerebral cortex were increased at a statistically significant level in the OKA group compared to the control and sham groups ($p < 0.05$). The level of immunoreactivity of caspase-3, p-tau (ser396), Aβ, IL-1β showed a statistically significant decrease in the OKA + JWH-133 group compared to the OKA group ($p < 0.05$).

The immunoreactivity of the hippocampus areas is given in Figs. 5, 6, 7, 8, and 9. In the OKA group, the levels of caspase-3, p-tau (ser396), Aβ, IL-1β and TNF-α immunoreactivity in the hippocampus regions were statistically higher than control and sham groups ($p < 0.05$). The immunoreactivity of Caspase-3, p-tau (ser396), Aβ, IL-1β and TNF-α decreased statistically in the OKA + JWH-133 group compared to the OKA group ($p < 0.05$).

4. Discussion

OKA is a selective inhibitor of the PP1 and PP2A enzymes. It can easily pass through the cell membrane. It leads to inhibition of PP1 and PP2A in neurons that results in hyperphosphorylation of tau proteins

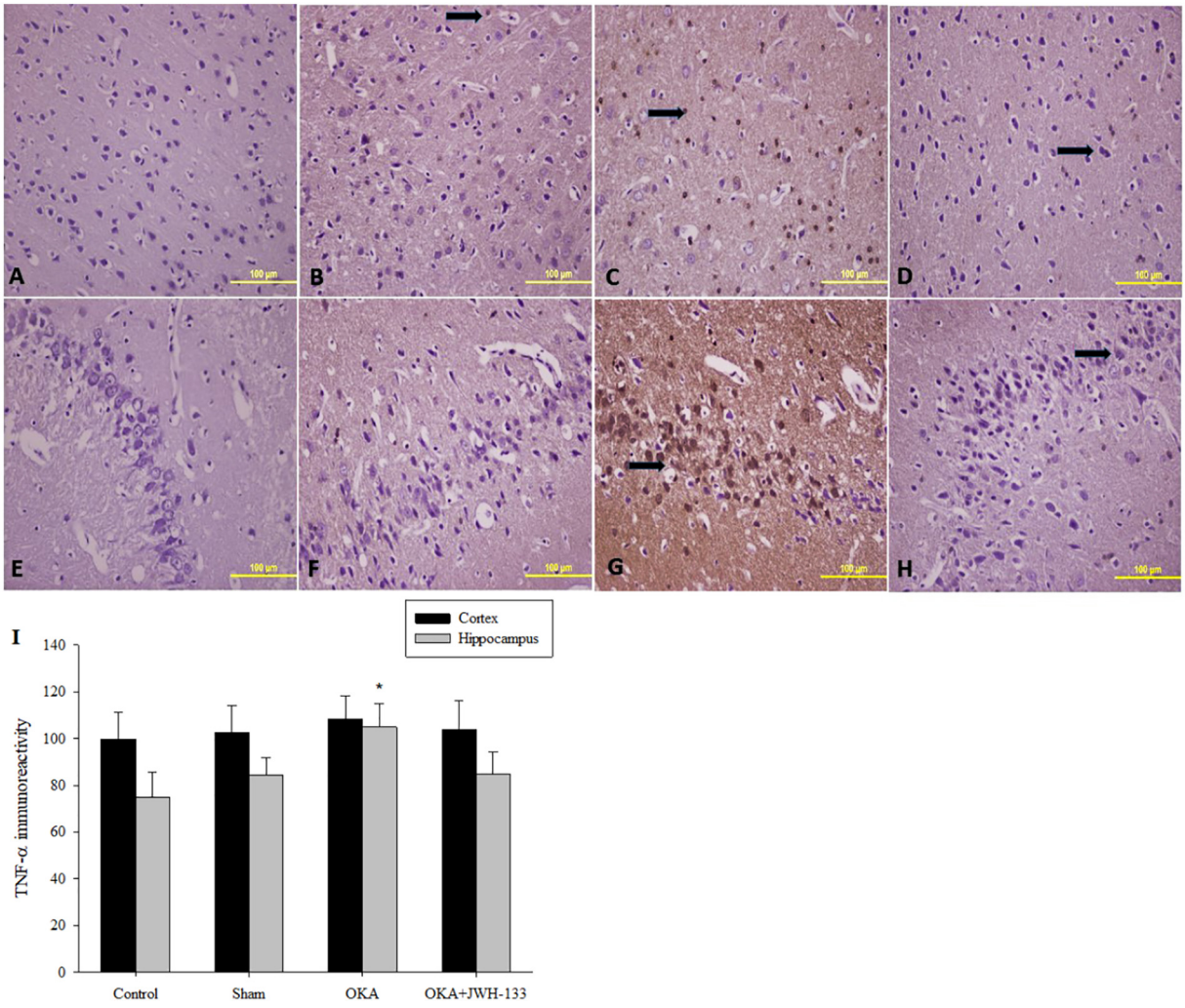

Fig. 8. JWH-133 administration reduced the level of OKA-induced TNF-α immunoreactivity in the hippocampus region (I). Cerebral cortex: Control (A), Sham (B), OKA (C) and OKA + JWH-133 (D) groups TNF-α (+) immunoreactivity are observed in cerebral cortex (arrows) TNF-α × 40. Hippocampus: Control (E), Sham (F), OKA (G) and OKA + JWH-133 (H) TNF-α (+) immunoreactivity are observed in hippocampus (arrows) TNF-α × 40. The number of TNF-α (+) immunoreactivity was calculated using the ×40 objective in 100 different field in the cerebral cortex and hippocampus. *$p < 0.05$ different from Control, Sham, OKA + JWH-133 groups. Data expressed as mean ± SD ($n = 10$).

that provide microtubule stability. The tau protein, which is hyperphosphorylated, causes the microtubule structure to deteriorate. As a result, NFT formation occurs in the brain. It has been found that the level and activity of PP1 and PP2A, the most important serine/threonine phosphatase in mammalian brain, is low in brains of patients with AD [26]. Many experimental studies have demonstrated that tau hyperphosphorylation is similar to AD after OKA has been administered as icv [1]. Icv OKA-induced rats show memory deterioration and increased microglial activation in the brain, neuroinflammation, oxidative stress, and increased accumulation of Aβ [21,22,41]. In our previous study, rats were subjected to the learning test 14 days after injection of icv 200 ng OKA bilaterally, and then the animals were sacrificed. At the end of the experiment, tau phosphorylation in the cortex and hippocampus regions of the brains of animals was accompanied by impairment of spatial memory formation and increased levels of Aβ, TNF-α and IL-1β [10].

The cannabinoid system is composed of two different receptors activated by these ligands and *N*-arachidonoyl ethanolamide (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG) ligands known as endocannabinoids [31]. Cannabinoid type 1 receptor (CB1) and CB2 receptors have been detected in many regions of the brain [11]. The level of CB2 receptors has been shown to increase in various pathological conditions such as AD, multiple sclerosis and amyotrophic lateral sclerosis [7,40]. It has been reported that there is a correlation between Aβ-42 level in AD brains and senile plaque score with CB2 level. [35]. Studies in the transgenic AD model have shown increased levels of Aβ and phosphorylated tau in mice with CB2 receptor deficiency. [25]. Köfalvi et al. showed that the level of endogenous cannabinoid AEA in brain tissue is reduced in TgAPP-2576 mice in their studies [24]. Aso reported et al. had been reported that administration of 0.2 mg/kg CB2 agonist JWH-133 in APP/PS1 mice decreased tau phosphorylation, microglial activation, pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, and IFN-γ) and cognitive function impairment [5]. In another study, administration of JWH-133 (0.2 mg/kg) to TgAPP-2576 mice was reported to decrease the levels of Aβ, TNF-α and IL-1β [28]. The dose of JWH-133, which was administered by Aso et al. and by Martin-Moreno et al., was the same as the dose in our study [5,28]. Tg2576 mouse model and APP/PS1 mouse model are young type genetic AD model based on pathogenesis resulting from Aβ overproduction [19]. Genetically derived AD accounts for about 5–10% of all AD cases [6]. Whereas Sporadic AD has nearly 90–95% prevalence. Therefore, research on sporadic AD is of great importance [18]. Our study was the first to examine the effect of CB2 receptor activation directly on tau pathology.

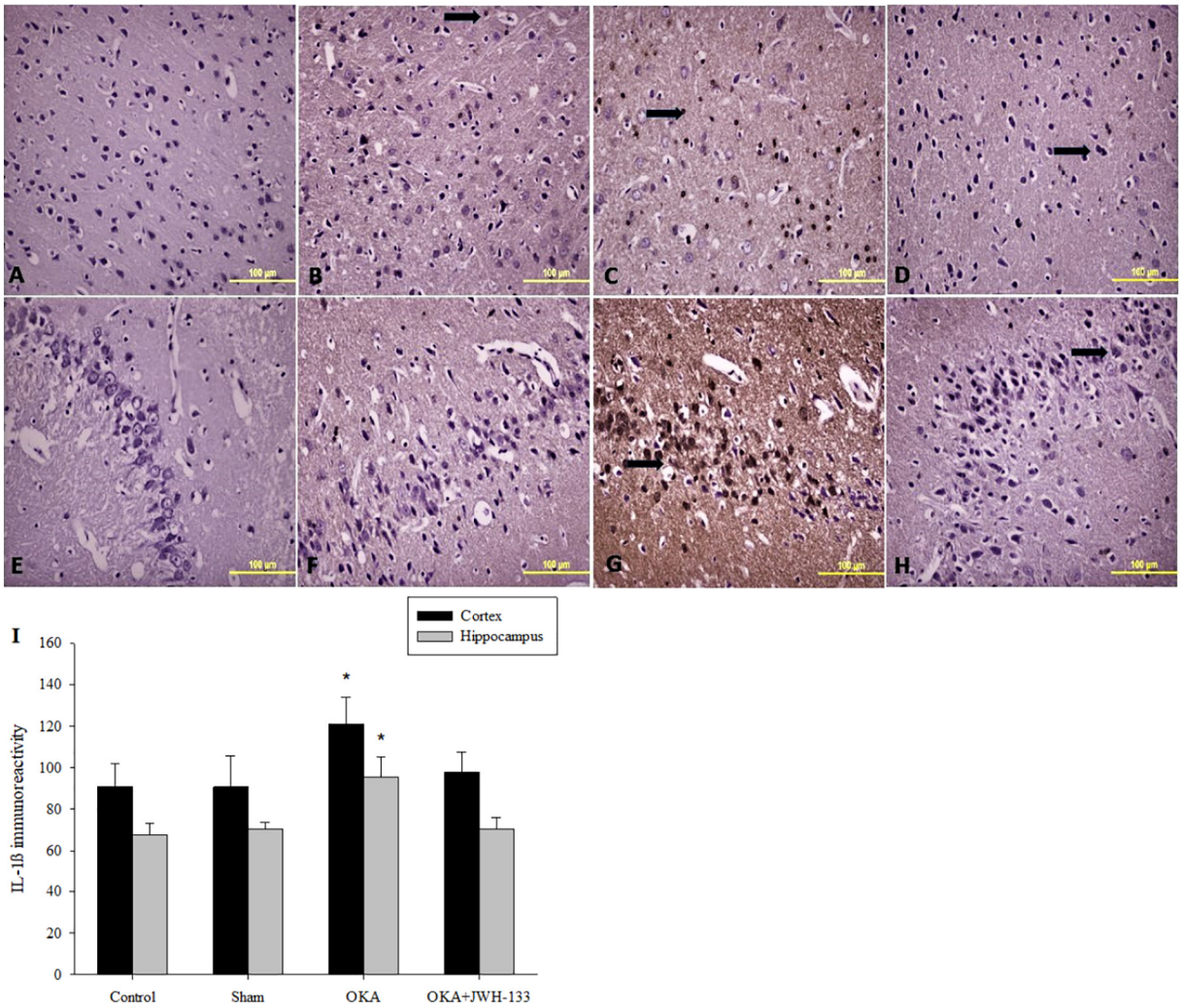

Fig. 9. The application of JWH-133 reduced IL-1β immunoreactivity, which was increased due to OKA, in the hippocampus and cortex regions (I). Cerebral cortex: Control (A), Sham (B), OKA (C) and OKA + JWH-133 (D) groups IL-1β (+) immunoreactivity are observed in cerebral cortex (arrows) IL-1β × 40. Hippocampus: Control (E), Sham (F), OKA (G) and OKA + JWH-133 (H) IL-1β (+) immunoreactivity are observed in hippocampus (arrows) IL-1β × 40. The number of IL-1β (+) immunoreactivity was calculated using the ×40 objective in 100 different field in the cerebral cortex and hippocampus. *$p < 0.05$ different from Control, Sham, OKA + JWH-133 groups. Data expressed as mean ± SD ($n = 10$).

Morris Water Maze is a test used in AD studies to assess the cognitive functions in rodents [13] In our study, icv OKA implementation impaired spatial memory formation. In the OKA group, escape latency times and path length decreased significantly on day 4 compared to day 1 only. In the probe trial test, the time spent in the target quadrant in the OKA group was significantly lower than in the control and sham groups. At the same time, the swimming speed in the OKA group increased significantly compared to the control and sham group. The JWH-133 application reversed the impairment in spatial memory formation. In the JWH-133 group, escape latency times, path length, as in the control group, decreased significantly on days 2, 3, and 4 compared to day 1. The time spent in the target quadrant increased significantly in the JWH-133 group compared to the OKA group. The JWH-133 application significantly reduced swimming speed significantly compared to the OKA group. Swimming speed is an anxiety indicator in Morris water maze test [29].

Neuroinflammation is associated with neurodegenerative diseases such as Alzheimer's disease, multiple sclerosis, Parkinson's disease, and amyotrophic lateral sclerosis. Clinical and experimental studies have been reported to increase the production of cytokines such as TNF-α, IL-1β in the brains of Alzheimer's patients. Astrocytes as microglial cells, are also proinflammatory cytokine-releasing cells. Neuroinflammation is a process that increases neuronal damage more [42].

Caspase-3 is a mediator that plays a key role in neuronal apoptosis. The signal cascade, which is caused by caspase-3 activity in many neurodegenerative diseases such as AD, has been shown to be associated with neuronal damage [12,34].

Aβ accumulation is the cause of neurotic plaque formation, which is characteristic of AD pathology. As Aβ accumulation in AD causes tau phosphorylation, tau phosphorylation also causes Aβ accumulation [20].

In our study, OKA administration increased the immunoreactivity of TNF-α, IL-1β, caspase-3, Aβ and p-tau (ser396) in the cortex and hippocampus. This suggests that neurodegeneration develops as stated in the literature above. JWH-133 administration in animals reduced proinflammatory cytokine, caspase-3, Aβ and p-tau (ser396) levels in the cortex and hippocampus. JWH-133 can help protect the spatial memory of animals by reducing neurodegeneration in animals. These results suggest that CB2 receptor agonist JWH-133 has therapeutic potential in sporadic AD.

Increased levels of TNF-α, IL-1β, p-tau, Aβ occurring in AD may be both a cause and result of pathological changes because neuroinflammation may cause an increase in Aβ and p-tau levels. Increasing levels of Aβ and p-tau may also cause neuroinflammation [42]. There is

a similar relationship between Aβ and tau hyperphosphorylation. The increase in Aβ levels causes tau hyperphosphorylation. At the same time, increased tau hyperphosphorylation leads to an increase in Aβ levels [14]. In our study, the JWH-133 administration decreased proinflammatory cytokine, Aβ and p-tau (ser396) levels. We thought that the reductions in these parameters affected each other and thus reduced neurodegeneration.

5. Conclusions

Despite all the advances in the field of medicine, no definitive treatment of AD was found. The treatments applied are mostly treatments to slow the progression of the disease and its symptoms [33]. In this study, we found that JWH-133 decreased the levels of Aβ and p-tau (ser396), which were increased with OKA administration. JWH-133 alleviated the inflammatory response caused by OKA by reducing IL-1β and TNF-α levels. It also reduced caspase-3 levels, which activated neuronal apoptosis. The JWH-133 application improves impairment in the formation of spatial memory caused by the OKA effect. The CB2 receptor agonist JWH-133 may be a novel therapeutic agent for neurodegenerative diseases such as AD associated with tau hyperphosphorylation; however, further studies are needed on the subject.

Acknowledgements

This study was supported by the Department of Scientific Research Projects of Bozok University (Project no: 6602c-TF/17-139). Authors declare no conflict of interest.

References

[1] T. Arendt, M. Holzer, M. Brückner, C. Janke, U. Gärtner, The use of Okadaic acid in vivo and the induction of molecular changes typical for Alzheimer's disease, Neuroscience 85 (1998) 1337–1340.
[2] R.A. Armstrong, What causes Alzheimer's disease, Folia Neuropathol. 51 (2013) 169–188.
[3] E. Aso, P. Andres-Benito, M. Carmona, R. Maldonado, I. Ferrer, Cannabinoid receptor 2 participates in amyloid-beta processing in a mouse model of Alzheimer's disease but plays a minor role in the therapeutic properties of a cannabis-based medicine, Journal of Alzheimer's Disease: JAD. 51 (2016) 489–500.
[4] E. Aso, I. Ferrer, CB2 cannabinoid receptor as potential target against Alzheimer's disease, Front. Neurosci. 10 (2016) 243.
[5] E. Aso, S. Juves, R. Maldonado, I. Ferrer, CB2 cannabinoid receptor agonist ameliorates Alzheimer-like phenotype in AbetaPP/PS1 mice, Journal of Alzheimer's Disease: JAD. 35 (2013) 847–858.
[6] E. Bagyinszky, Y.C. Youn, S.S. An, S. Kim, The genetics of Alzheimer's disease, Clin. Interv. Aging 9 (2014) 535–551.
[7] C. Benito, E. Nunez, R.M. Tolon, E.J. Carrier, A. Rabano, C.J. Hillard, et al., Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains, J. Neurosci. 23 (2003) 11136–11141.
[8] T. Bisogno, S. Oddi, A. Piccoli, D. Fazio, M. Maccarrone, Type-2 cannabinoid receptors in neurodegeneration, Pharmacol. Res. 111 (2016) 721–730.
[9] R. Brookmeyer, E. Johnson, K. Ziegler-Graham, H.M. Arrighi, Forecasting the global burden of Alzheimer's disease, Alzheimer's & Dementia: The Journal of the Alzheimer's Association. 3 (2007) 186–191.
[10] M. Cakir, H. Duzova, S. Tekin, E. Taslidere, G.B. Kaya, Y. Cigremis, et al., ACA, an inhibitor phospholipases A2 and transient receptor potential melastatin-2 channels, attenuates Okadaic acid induced neurodegeneration in rats, Life Sci. 176 (2017) 10–20.
[11] L. Callen, E. Moreno, P. Barroso-Chinea, D. Moreno-Delgado, A. Cortes, J. Mallol, et al., Cannabinoid receptors CB1 and CB2 form functional heteromers in brain, J. Biol. Chem. 287 (2012) 20851–20865.
[12] M. D'Amelio, V. Cavallucci, F. Cecconi, Neuronal caspase-3 signaling: not only cell death, Cell Death Differ. 17 (2010) 1104–1114.
[13] R. D'Hooge, P.P. De Deyn, Applications of the Morris water maze in the study of learning and memory, Brain Res. Brain Res. Rev. 36 (2001) 60–90.
[14] Y. Duan, S. Dong, F. Gu, Y. Hu, Z. Zhao, Advances in the pathogenesis of Alzheimer's disease: focusing on tau-mediated neurodegeneration, Translational neurodegeneration. 1 (2012) 24.
[15] J. Fernandez-Ruiz, M.R. Pazos, M. Garcia-Arencibia, O. Sagredo, J.A. Ramos, Role of CB2 receptors in neuroprotective effects of cannabinoids, Mol. Cell. Endocrinol. 286 (2008) S91–S96.
[16] C.X. Gong, I. Grundke-Iqbal, K. Iqbal, Targeting tau protein in Alzheimer's disease, Drugs Aging 27 (2010) 351–365.
[17] C.X. Gong, T. Lidsky, J. Wegiel, L. Zuck, I. Grundke-Iqbal, K. Iqbal, Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain. Implications for neurofibrillary degeneration in Alzheimer's disease, J. Biol. Chem. 275 (2000) 5535–5544.
[18] N.V. Gulyaeva, N.V. Bobkova, N.G. Kolosova, A.N. Samokhin, M.Y. Stepanichev, N.A. Stefanova, Molecular and cellular mechanisms of sporadic Alzheimer's disease: studies on rodent models in vivo, Biochemistry (Mosc) 82 (2017) 1088–1102.
[19] A.M. Hall, E.D. Roberson, Mouse models of Alzheimer's disease, Brain Res. Bull. 88 (2012) 3–12.
[20] H.C. Huang, Z.F. Jiang, Accumulated amyloid-beta peptide and hyperphosphorylated tau protein: relationship and links in Alzheimer's disease, Journal of Alzheimer's Disease: JAD. 16 (2009) 15–27.
[21] P.K. Kamat, S. Tota, S. Rai, S. Swarnkar, R. Shukla, C. Nath, A study on neuroinflammatory marker in brain areas of Okadaic acid (ICV) induced memory impaired rats, Life Sci. 90 (2012) 713–720.
[22] P.K. Kamat, S. Tota, G. Saxena, R. Shukla, C. Nath, Okadaic acid (ICV) induced memory impairment in rats: a suitable experimental model to test anti-dementia activity, Brain Res. 1309 (2010) 66–74.
[23] P.K. Kamat, S. Tota, R. Shukla, S. Ali, A.K. Najmi, C. Nath, Mitochondrial dysfunction: a crucial event in Okadaic acid (ICV) induced memory impairment and apoptotic cell death in rat brain, Pharmacol. Biochem. Behav. 100 (2011) 311–319.
[24] A. Kofalvi, C. Lemos, A.M. Martin-Moreno, B.S. Pinheiro, L. Garcia-Garcia, M.A. Pozo, et al., Stimulation of brain glucose uptake by cannabinoid CB2 receptors and its therapeutic potential in Alzheimer's disease, Neuropharmacology 110 (2016) 519–529.
[25] J. Koppel, V. Vingtdeux, P. Marambaud, C. D'Abramo, H. Jimenez, M. Stauber, et al., CB2 receptor deficiency increases amyloid pathology and alters tau processing in a transgenic mouse model of Alzheimer's disease, Mol. Med. 20 (2014) 29–36.
[26] R. Liu, J.Z. Wang, Protein phosphatase 2A in Alzheimer's disease, Pathophysiology: The Official Journal of the International Society for Pathophysiology/ISP. 16 (2009) 273–277.
[27] H.C. Lu, K. Mackie, An introduction to the endogenous cannabinoid system, Biol. Psychiatry 79 (2016) 516–525.
[28] A.M. Martin-Moreno, B. Brera, C. Spuch, E. Carro, L. Garcia-Garcia, M. Delgado, et al., Prolonged oral cannabinoid administration prevents neuroinflammation, lowers beta-amyloid levels and improves cognitive performance in Tg APP 2576 mice, J. Neuroinflammation 9 (2012) 8.
[29] R.K. McNamara, R.W. Skelton, Diazepam impairs acquisition but not performance in the Morris water maze, Pharmacol. Biochem. Behav. 38 (1991) 651–658.
[30] R. Morris, Developments of a water-maze procedure for studying spatial learning in the rat, J. Neurosci. Methods 11 (1984) 47–60.
[31] D. Parolaro, N. Realini, D. Vigano, C. Guidali, T. Rubino, The endocannabinoid system and psychiatric disorders, Exp. Neurol. 224 (2010) 3–14.
[32] G. Paxinos, W. Charles, The Rat Brain in Stereotaxic Coordinates, 6 edn., Academic Press, London, 2007.
[33] C. Reitz, R. Mayeux, Alzheimer disease: epidemiology, diagnostic criteria, risk factors and biomarkers, Biochem. Pharmacol. 88 (2014) 640–651.
[34] S. Snigdha, E.D. Smith, G.A. Prieto, C.W. Cotman, Caspase-3 activation as a bifurcation point between plasticity and cell death, Neurosci. Bull. 28 (2012) 14–24.
[35] M. Solas, P.T. Francis, R. Franco, M.J. Ramirez, CB2 receptor and amyloid pathology in frontal cortex of Alzheimer's disease patients, Neurobiol. Aging 34 (2013) 805–808.
[36] X.Y. Song, J.F. Hu, S.F. Chu, Z. Zhang, S. Xu, Y.H. Yuan, et al., Ginsenoside Rg1 attenuates Okadaic acid induced spatial memory impairment by the GSK3beta/tau signaling pathway and the Abeta formation prevention in rats, Eur. J. Pharmacol. 710 (2013) 29–38.
[37] X.Y. Song, Y.Y. Wang, S.F. Chu, J.F. Hu, P.F. Yang, W. Zuo, et al., A new coumarin derivative, IMM-H004, attenuates Okadaic acid-induced spatial memory impairment in rats, Acta Pharmacol. Sin. 37 (2016) 444–452.
[38] H.V. Vinters, Emerging concepts in Alzheimer's disease, Annu. Rev. Pathol. 10 (2015) 291–319.
[39] L. Walter, A. Franklin, A. Witting, C. Wade, Y. Xie, G. Kunos, et al., Nonpsychotropic cannabinoid receptors regulate microglial cell migration, J. Neurosci. 23 (2003) 1398–1405.
[40] Y. Yiangou, P. Facer, P. Durrenberger, I.P. Chessell, A. Naylor, C. Bountra, et al., COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord, BMC Neurol. 6 (2006) 12.
[41] S.Y. Yoon, J.E. Choi, J.H. Yoon, J.W. Huh, D.H. Kim, BACE inhibitor reduces APP-beta-C-terminal fragment accumulation in axonal swellings of Okadaic acid-induced neurodegeneration, Neurobiol. Dis. 22 (2006) 435–444.
[42] F. Zhang, L. Jiang, Neuroinflammation in Alzheimer's disease, Neuropsychiatr. Dis. Treat. 11 (2015) 243–256.