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Etanercept improves aging-induced cognitive deficits by reducing inflammation and vascular dysfunction in rats

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ABSTRACT

Normal aging may lead to cognitive deficits, which is associated with endothelial dysfunction and neuroinflammation. Dysregulation of TNFα expression contributes to vascular aging and dementia. In this study, we investigated the effects of etanercept, which is a TNFα inhibitor, on cognitive and endothelial function in aged rats. Male Wistar albino rats were divided into 3 groups: Young (4 month), aged (24 month) aged + ETA (24 month + etanercept). Etanercept (0.8 mg/kg/weekly) was given to the aged + ETA group subcutaneously for 8 weeks. Then passive avoidance test (PAT) and the Morris water maze test (MWMT) were used to evaluate cognitive functions of rats. After the behavioral tests, the rats were subjected to systolic blood pressure (SBP) measurement, and then endothelial function of thoracic aorta was evaluated by isolated organ bath system. Thoracic eNOS expression, hippocampal BDNF expression and serum and hippocampal TNF levels were also measured. In aged rats, it was shown that cognitive performances in MWMT and PAT were abolished whereas SBP unchanged. Furthermore, aging resulted in endothelial dysfunction, decreased expressions of thoracic eNOS and hippocampal BDNF, and increased level of TNF in serum and hippocampus. In contrast, ETA improved age-related cognitive deficits and endothelial dysfunction. In addition, ETA reversed changes in protein expression in aged rats. The results of this study indicate that ETA prevents cognitive deficits, endothelial dysfunction, peripheral and neuro-inflammation and decreament of neurotrophin expression in aged rats. These findings suggest that ETA may be beneficial with neuroprotective and vasculoprotective effects in elderly patients.

1. Introduction

As the percentage of elderly individuals in the general population rises, the incidence of Alzheimer's disease (AD) and other dementias increases with physiological aging. It's well-known that AD and vascular dementia (VaD) are the most common forms of dementias [1]. Initially, vascular dementia is assumed to develop cognitive dysfunction due to vascular diseases, whereas AD is suggested to cause impairment of cognition by the neurodegenerative processes. However, in recent years, it has been shown that these two forms of dementia are connected. Cardiovascular risk factors such as diabetes, hypertension, dyslipidemia, and metabolic syndrome have been indicated to be associated not only with VaD but also with AD in current studies [2, 3, 4]. It is suggested that most forms of dementia may have developed in the continuation of vascular disease [5].

Aging is a major risk factor for most chronic diseases including neurodegenerative disorders such as dementia and cardiovascular diseases. [6,7]. Epidemiological studies have shown that cardiovascular risk factors originated from physiological aging are also associated with cognitive dysfunction [6]. Another condition caused by aging is immune dysregulation, which is leading to increased blood levels of proinflammatory markers, including IL-1β and TNFα [8]. In addition to the increased proinflammatory markers in the blood, anti-inflammatory-proinflammatory balance in the brain has been shown a shift toward the proinflammatory state and thus have a significant impact on neural and behavioral processes [9]. It is well known that proinflammatory cytokines, which increase with aging, are one of the underlying causes of cardiovascular diseases (CVD) such as

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atherosclerosis, as well as impairing cognitive functions by causing neuroinflammation in the brain [10–15].

A number of epidemiological studies show that inflammation is a risk factor for CVD [8,16]. It is well known that aging is a major risk factor for endothelial dysfunction, and recent studies support that inflammation is one of the underlying mechanisms [11–15,17]. There is growing evidence indicates that chronic inflammation and inflammageing is a risk factor across traditionally viewed as patophysiologically unrelated diseases, such as CVD, diabetes, depression, and dementia [8,16,18,19,20]. Inflammageing, which defines a chronic, low-grade inflammation process in advanced age, is an important risk factor for CVD and dementia [8,10,14]. However, there is no treatment available to reverse or delay both cognitive and vascular dysfunction related to aging.

Anti-TNF treatments are used in inflammatory diseases such as RA. CVD risk has been shown to be reduced after these treatments [8,21,22]. Besides, anti-TNF treatment has been tried and found to be successful in the treatment of AD [23]. But the link between TNFα, age-related cognitive impairment, and vascular dysfunction has not been investigated.

Based on the previous studies, the present study was to designed to evaluate spatial and emotional cognitive performances using a water maze and passive avoidance tasks, respectively, in aged rats which were treated with etanercept, an anti-TNF agent. Moreover, we aimed to investigate blood pressure and vascular endothelial function after etanercept treatment in the VD with cognitive dysfunction as a result of increased inflammation during normal physiological aging.

2. Materials and methods

2.1. Animals

Male Wistar-albino rats (ages from 4 months old, 250–300 g (young) to 24 months old, 400–450 g (aged)) purchased from Kocaeli University, Experimental Medical Research and Application Center, Kocaeli, Turkey; were used in the experiments. Rats were housed in an animal colony with 5–6 per cage for 2 weeks prior to beginning experiments. All behavioral tests were performed between 9:00 AM-12:00 PM under standard laboratory conditions (22 ± 2 °C room temperature and 12:12 h light: dark cycle (07:00 to 19:00 hr)) with water and food pellets available ad libitum. In this study, the animals used were naive to experimental tests and each experiment was exerted with different groups of rats.

The experiments reported in this study were performed in accordance with the Regulation of Animal Research Ethics Committee in Turkey (July 6, 2006, Number 26,220). All animal use protocols were approved by the Kocaeli University Animal Research Ethics Committee (Project number: KOU HADYEK 11/14–2019, Kocaeli, Turkey).

Aged rats were randomly divided into two groups with one receiving etanercept treatment (Aged + ETA, 0.8 mg/kg, s.c., $n = 10$) and the other receiving saline treatment (Aged, 0.1 mL/100 g body weight, s.c., $n = 10$) once a week for 8 weeks. Etanercept dose was selected on the basis of our previous study [24]. Young control rats were given saline (Young, 0.1 mL/100 g body weight, s.c., $n = 10$) once a week for 8 weeks.

At the end of 8 weeks treatment Schedule, the behavioral tests of rats were performed by locomotor activity, Passive Avoidance Test and the Morris Water Maze Test. After the behavioral tests, the rats were subjected to systolic blood pressure measurement and then sacrificed. Thoracic aorta, hippocampus and blood samples were collected to evaluate isolated organ bath, immunohistochemical and biochemical studies.

2.2. Locomotor activity test

Locomotor activity was measured over a 5-min period before the behavioral tests in a locomotor activity cage with a computerized system (May Commat, Ankara, Turkey) and expressed as the total distance moved of animals.

2.3. Passive avoidance test (PAT)

The emotional memory based on contextual fear conditioning learning of the animals was assessed by a one-trial, light-dark passive avoidance apparatus (Ugo Basile model 7551, Italy) according to a previous study [25]. In this test, the animals learn to avoid a specific location associated with an aversive event. The learning index used in this test is the reduction of latency to avoid. The light and dark box compartments were combined to form the apparatus and these compartments were separated by a automatically operated sliding door.

The illuminated light box was connected to the dark box with an electrifiable grid floor. The animals were applied an inescapable electrical foot shock via a shock generator. A training of PAT included two days trials. On day 1, rats were placed into the light compartment to explore the boxes in the pre-acquisition trial, individually. The animals were able to enter and to move into the dark box without an electric foot-shock in the pre-acquisition trial. Fifteen minutes after pre-acquisition trial, an acquisition trial was performed. The rats were placed into the light box and then the door between light and dark boxes was opened after 30 s. The door was closed automatically when the rats completely entered the dark box and an electric foot-shock (0.5 mA) of 3 s duration was given to the animals through the grid floor. The time taken for animals to enter the dark compartment was recorded as the training latency. The cut-off time was accepted as 300 s, when the rats did not enter light to dark compartment within 300 s. At the end of the acquisition trial, the animals were removed from dark box and put back into their cages. On day 2, the retention trial was performed 24 h after the acquisition trial. In this trial, the animals were placed in the light box again. Their latencies to enter the dark box was used for evaluating the recall of the inhibitory stimulus and served as a measure of the retention performance of the passive avoidance response.

2.4. Morris water maze test (MWMT)

MWMT was performed for the assessment of spatial learning and memory of rats as previously described [26]. The water maze pool, which was a black circular tank with 150 cm diameter and 30 cm in depth, was used for the measurement of animals MWM performance. The MWMT was applied 5 consecutive days for each animal. MWM tank filled with water (20–24 °C) contained a hidden platform (submerged 1.5 cm below the surface of the water). The water tank was divided into four quadrants and the platform was placed in the center of the southwest quadrant during the first 4 days of the test. The animal's acquisition of finding platform after training were assessed. Small black pieces of plastic covered the water surface and prevented the rats recognition of the platform. The time to find the platform with the 60 s cut-off time was named escape latency and used as quantify spatial learning. The rats underwent 3 trials during each of the first 4 daily sessions. A trial was started by placing a rat into the water tank with three starting positions (north, east, and west), facing the wall of the tank. The escape latency value was recorded in each trial. After the rat climbed onto the hidden platform, it was allowed to stay on there for 20 s. The next trial was started after the rat was removed from the platform. If the rat did not find the platform within 60 s, it was placed on the platform and allowed to stay there for 20 s. On the 5th day, a “probe trial” was used to assess the spatial retention performance. The platform was removed from the southwest quadrant, the rats were put into the center of the tank and then allowed to search for the escape platform for 60 s during the probe test. The time spent in the target quadrant that previously contained the hidden platform was recorded (for 60 s) and used as an index of retrieval (memory). If the animals learned the task, it was expected to spend more time in the target

quadrant.

2.5. Systolic blood pressure (SBP) measurement

At the end of the behavioral tests, indirect SBP was recorded using by the tail-cuff plethysmography (MAY-COM BPHR 200; Commat Iletisim, Ankara, Turkey) as previously described [24]. The rats were placed in a restraining holder to perform this procedure, consciously. Their tails were protruded from this holder and warmed with an infrared light bulb for vasodilatation, locally. A cuff and pulse sensor were placed around the tail, and the cuff was inflated until the pulse disappeared. When the cuff was deflated, the point of the reappearance of the pulse was used as the value of SBP. Three sequential inflation-deflation cycles of the cuff were carried out to obtain the average value of SBP in each rat.

2.6. Organ bath studies

Endothelial function was assesed as previously described [24]. Following the measurement of SBP, the rats were sacrificed and the thoracic aorta was excised surgically. The isolated thoracic aortas were immediately placed in Krebs Solution (KS) and 3–5 mm long rings were prepared with dissection. The rings were placed in the 20-mL organ bath chambers filled with KS (for composition see below). Tissue baths were aerated with the carbogen (95% O2 and 5% CO2) and maintained at 37 °C, pH 7.4. Each ring was connected to a force-displacement transducer (MAY-COM FDT 10A; Commat Iletisim) for the measurement of isometric force, which was displaced continuously and recorded on-line on a personal computer by using a four-channel transducer data-acquisition system (MP30B-CE; Biopac Systems, Santa Barbara, CA, USA), using software (BSL Pro 3.7; Biopac Systems) to analyze the data.

At the beginning of the experiments, all tissues were alllowed to equilibrate to a 1 g resting tension by repeated adjustment and not altered during the experiment. The tissue bath solution was changed every 15 min during this period. After the equilibration, the aortic rings were exposed to 80 mM KCl to test the viability of the preparation for 5 min. Then, the tissues were washed with KS and allowed to equilibrate for 30 min. Before the relaxant responses to carbachol, sodium nitroprusside, and papaverine, each ring was precontracted with 10^{-6} or 10^{-5} M concentration of phenylephrine, which was shown to produce 90% of maximal response to phenylephrine in preliminary studies. After the phenylephrine-induced precontraction had reached plateau in thoracic aorta rings, cumulative concentration responses of carbachol (10^{-8}–10^{-5} M), sodium nitroprusside (SNP; 10^{-9}–10^{-4} M), or papaverine (10^{-4} M) were obtained by addition of one of these agents to the bath at 30 min intervals, cumulatively. The agonist concentration was increased after the response to the previous concentration reached completely a plateau. After a concentration-response curve was completed, tissues were rinsed with fresh buffer and allowed to return basal tension for 30 min.

2.7. Biochemical analysis for TNFα

At the end of the experiment, serum, and hippocampus samples were collected and tissues were homogenized with a tissue homogenizer in PBS (1/10; weight/volume). After centrifugation, the supernatant was collected and kept in −80 °C until further analyses. The total protein content of tissue samples was quantified by the modified Lowry's method. Enzyme-linked immunosorbent assay (ELISA) results were given as a percentage of the total protein concentration of the sample.

Serum and tissue TNFα levels were determined by ELISA kit (Biosource, Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The absorbance was measured at 450 nm using a microtiter ELISA reader (VERSAmaxMolecularDevices, Sunnyvale, CA, USA).

2.8. BDNF and e-NOS immunohistochemistry

Following sacrification, thoracic aorta and brain tissues belonging to all groups ($n = 8$ in each group) were excised and immersed in 10% neutral buffered formalin solution for fixation. After dehydration with graded series of ethanol and toluene, the tissues were embedded in paraffin. We examined hippocampus region of the brains in 3 μm-thick sections. For immunohistochemical staining (BDNF for hippocampus, e-NOS for thoracic aorta), sections were deparaffinized in fresh toluene, then treated with descending series of ethanol for rehydration and rinsed with tap water, followed by two rinses of distilled water. To perform heat-induced antigen retrieval, the slides were kept in citrate buffer (pH = 6,0) in a microwave. After cooling, 3% hydrogen peroxide (H_2O_2) was used to block endogenous peroxidase activity. The sections were washed in phosphate-buffered saline (PBS, pH = 7.6) containing 0.1% Triton X (T-PBS) twice. The epitopes were stabilized by the protein block solution (ExposeRabbitSpecific HRP/DAB detection IHC kit, Abcam, Cambridge, UK). Then anti-BDNF (sc-20,981, Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:300 dilution and anti-e-NOS (ab119292, Abcam, Cambridge, UK; 1:100) polyclonal primary antibodies were applied to brain and thoracic aorta sections, respectively. After −4 °C overnight incubation, biotinylated secondary antibody (Goatantirabbit HRP Conjugate, Abcam,) was applied for 20 min at room temperature. 3,3′-diamino benzidine (DAB) solution(DAB Substrate, Abcam, Cambridge, UK) was performed as a chromogen. Mayer's hematoxylin (Invitrogen, Carlsbad, CA, USA)was used for counterstaining. The specimens were mounted with Entellan (Merck Milipore, Darmstadt, Germany). All sections were evaluated with an optical microscope (Leica DM 1000) and monitored with an attached digital camera (Leica DMC 2900). Percentage of BDNF and e-NOS positive cells were semiquantitatively scored according to the following criteria: 0, < 5%; 1, 6–15%; 2, 16–50%; 3, 51–80%; and 4, > 80% positive cell.

2.9. Drugs and solutions

Etanercept was purchased from Wyeth (Münster, Germany) and it was dissolved in physiologic (0.9%) saline. The ionic composition of Krebs solution (mM) was as follows: NaCl 118, KCl 4.71, $MgCl_2$ 1.05, NaH_2PO4 1.33, $NaHCO_3$ 25, $CaCl_2$ 2.7, and glucose 5.6. In the high K^+ solution, NaCl was exchanged for equimolar amounts of KCl. Fresh solutions were prepared fresh on the day of testing. Phenylephrine hydrochloride, carbachol chloride, SNP and papaverine hydrochloride were used (all obtained from Sigma Aldrich). Drugs were prepared fresh every day in distilled water and kept on ice during experiments.

2.10. Statistical analysis

All results were presented as mean ± standard error of the mean (SEM). The relaxant responses to carbachol and SNP were expressed as the percentage of the precontraction response to Phe. Differences between experimental groups were assessed using one-way ANOVA followed by Bonferroni's post hoc test except 1–4 day latency score of MWM test. Two-way ANOVA followed by Bonferroni's test was only used to measure the significancy of 1–4 day latency score of the MWM test. Kruskal-Wallis test following Dunn's multiple comparison test was used for evaluation of immunoreactivity scores. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of aging and ETA on locomotor activity

Locomotor activity of the rats was assessed for the exclusion of the probability of behavioral disinhibition which may affect the learning memory test performance. Total distance traveled for each rat was

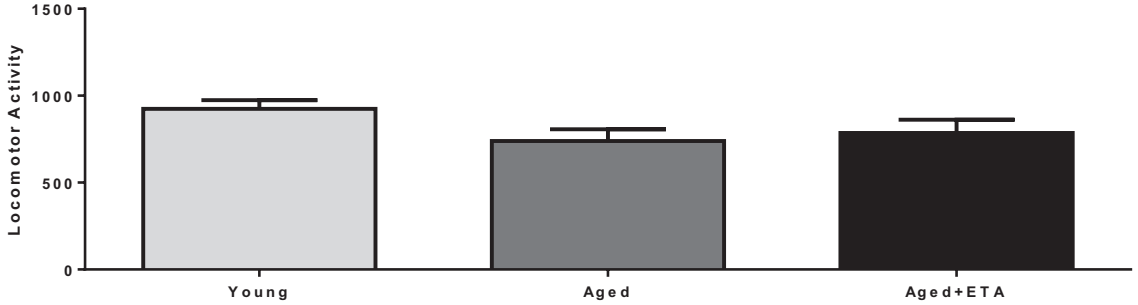


Fig. 1. Total locomotor activity score for young, aged and aged + ETA groups. Values are expressed as mean ± *S*.E.M. $n = 10$ for each group.

measured for 5 min. The total locomotor activity was not affected by aging or etanercept treatment (one-way ANOVA, $F(2,27) = 2.180$, $p = 0.1325$; Fig. 1). This data suggested that motor disabilities were shown in neither aging nor etanercept treatment.

3.2. Effects of aging and ETA on age-induced memory impairment in the PAT in rats

In the first day training session of the passive avoidance task, there were no significant differences between groups (one-way ANOVA, $F(2,27) = 0.6205$, $p = 0.5451$; Fig. 2a). However, the retention latencies of aged rats were significantly lower than the young rats, which demonstrates the impaired retention of memory (one-way ANOVA, $F(2,27) = 15.29$, $p < 0.001$; Fig. 2b). This impairment of retention memory (passive avoidance task performance) of aged rats was reversed by etanercept treatment.

3.3. Effects of aging and ETA on age-induced memory impairment in the MWMT in rats

We found that significantly decreased acquisition latency time in young rats from day 1 to 4 reflecting the normal learning ability and aging resulted in performance deficits in the MWM task. A significant effect of the day was demonstrated as displayed in Fig. 3a (two-way ANOVA, effect of the day, $F(3108) = 12.91$, $p < 0.0001$). In addition, age had extremely significant effect in the dataset (two-way ANOVA, effect of treatment, $F(2108) = 69.46$, $p < 0.0001$; Fig. 3a). Further analysis revealed that the day X treatment interaction was not significant (two-way ANOVA, day X treatment, $F(6108) = 2.170$, $p = 0.0513$; Fig.3a). It was shown that aging caused performance deficits in 1–4 days of this test (Two-way ANOVA, effect of treatment, $p < 0.01$, $p < 0.0001$, $p < 0.001$, $p < 0.0001$, respectively, Fig. 3a). Posthoc comparison indicated that administration of etanercept (0.8 mg/kg/week, orally) for 8 weeks prevented the age-induced impairment in the escape latency in the water maze task (two-way ANOVA, effect of age, $p > 0.05$, young vs aged + ETA; Fig. 3a). In the probe test, the young rats spend more time

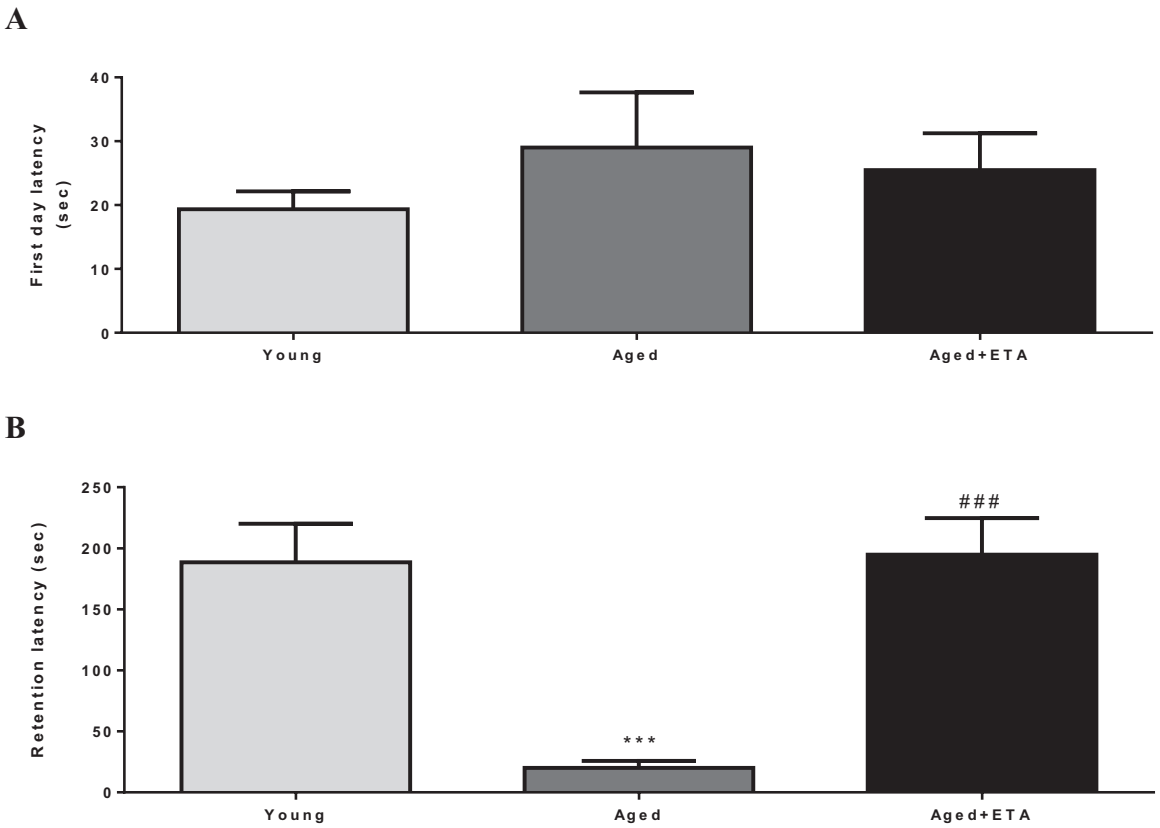


Fig. 2. First day (A) and retention latencies (B) in passive avoidance test for young, aged and aged + ETA groups. Values are expressed as mean ± *S*.E.M. *** $p < 0.001$ compared with young group, ### $p < 0.001$ compared with aged group. $n = 10$ for each group.

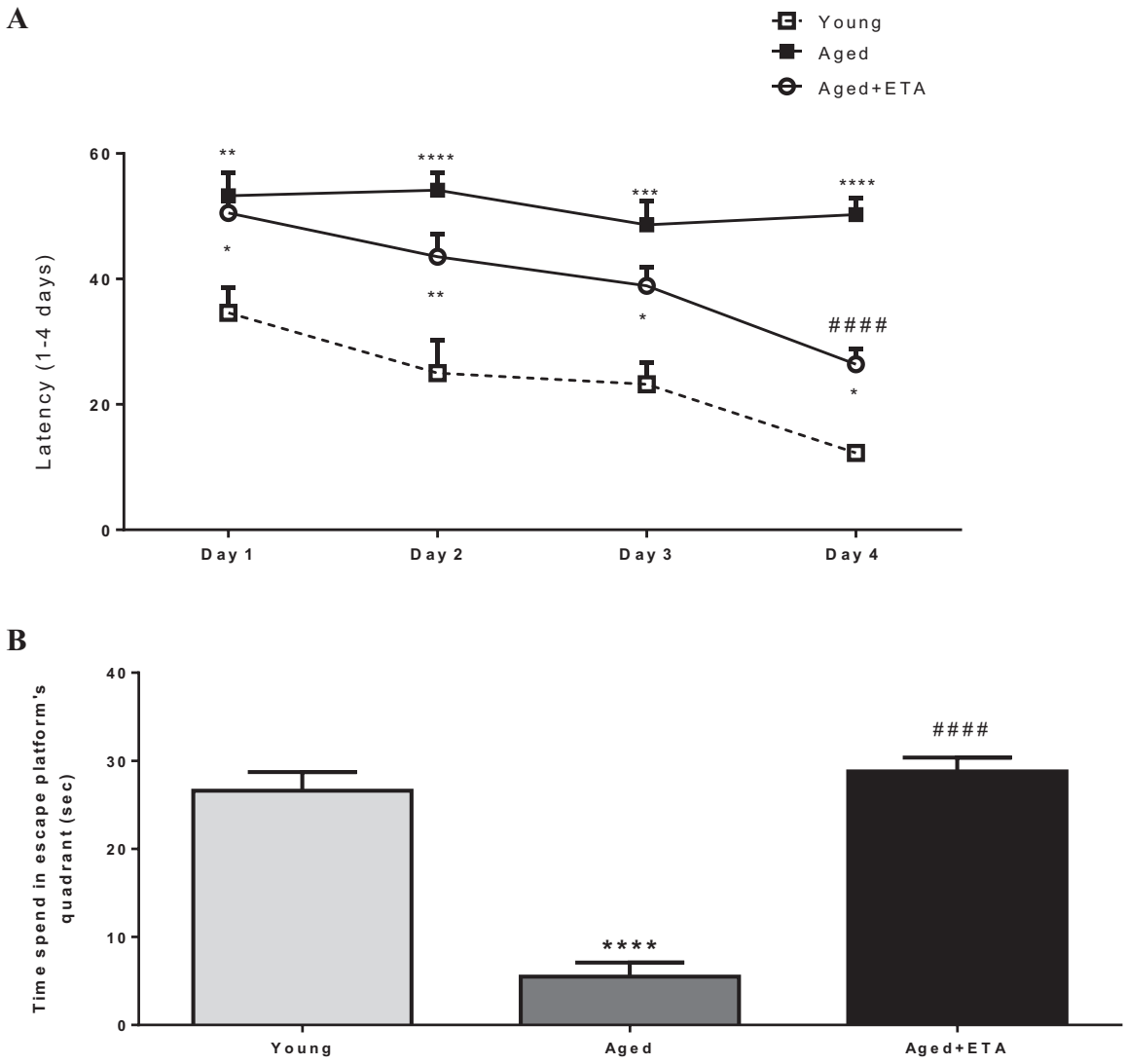


Fig. 3. Escape latency (*sec*) (A) and time spend in escape platform's quadrant (*sec*) (B) in Morris Water Maze test for young, aged and aged + ETA groups. Values are expressed as mean ± *S*.E.M. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with young group; $^{\#\#\#\#}$ $p < 0.0001$ compared with aged group. $n = 10$ for each group.

in the target quadrant on day 5, reflecting normal retrievel (memory) as well (one-way ANOVA, Fig. 3b). It was demonstrated that the time spent in the escape platform's quadrant was decreased in aged rats (one-way ANOVA, $F(2,27) = 57.41$; $p < 0.0001$; Fig. 3b), whereas reversed to the young controls after etanercept treatment ($p > 0.05$, young vs aged + ETA; Fig. 3b).

3.4. Effects of aging and ETA on SBP and vascular reactivity in rats

There was no significant difference in the mean arterial blood pressure according to the SBP measurement (Fig. 4). The endothelium-dependent relaxation in response to carbachol and -independent relaxation in response to SNP were determined in thoracic aorta rings precontracted with phenylephrine. The precontractile tone in the thoracic aorta rings obtained by submaximal concentration (10^{-5} M) of phenylephrine was similar in all groups (data not shown). In the old control group, relaxation response to carbachol was decreased ($p < 0.05$; Fig. 5a) whereas was not changed for SNP relaxation (Fig. 5b). The impairment of endothelium-dependent relaxation was returned to the young control group after etanercept treatment (Fig. 5a). In addition, there were no significant differences among the relaxation responses to papaverine (data not shown) and the contractile responses to 80 mM KCl in any group (Fig. 6).

3.5. Effects of aging and ETA on serum and hippocampal levels of TNFα in rats

TNFα levels (ng/mL) were increased in the aged group as compared with young group in both serum and brain hippocampal region ($p < 0.05$; Fig. 7a and b). They were reversed to the young control levels after etanercept treatment (Fig. 7a, Fig. 7b).

3.6. Effects of aging and ETA on expression of BDNF and eNOS protein in rats

In the young group, BDNF and eNOS immunoreactivity were detected in the neurons in the CA1 and CA3 regions of hippocampus and the cytoplasm of the endothelial cells of thoracic aorta respectively (Fig. 8, Fig. 9). BDNF expressions both in the CA1 and CA3 regions of the hippocampus were significantly decreased by aging ($p < 0.05$, Fig. 8). This immunreactivity in the hippocampus was similar to young group in the aged + ETA group. Similarly, the levels of eNOS protein in the thoracic aorta were decreased in aged rats compared to young group ($p < 0.05$, Fig. 9), whereas it was reversed to the control level

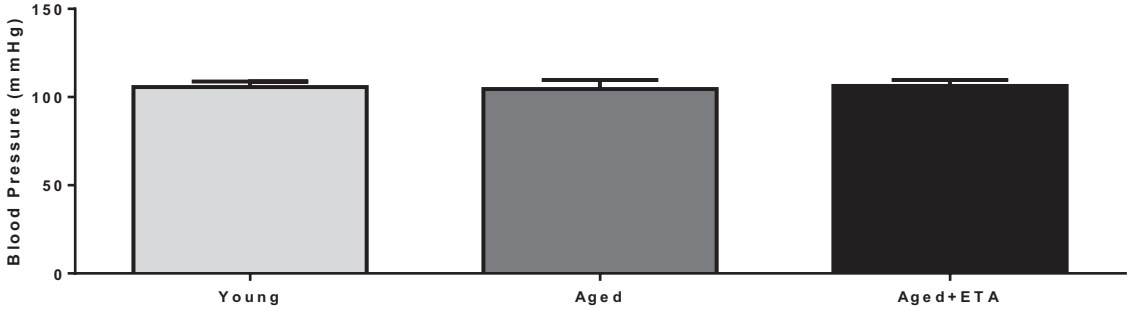
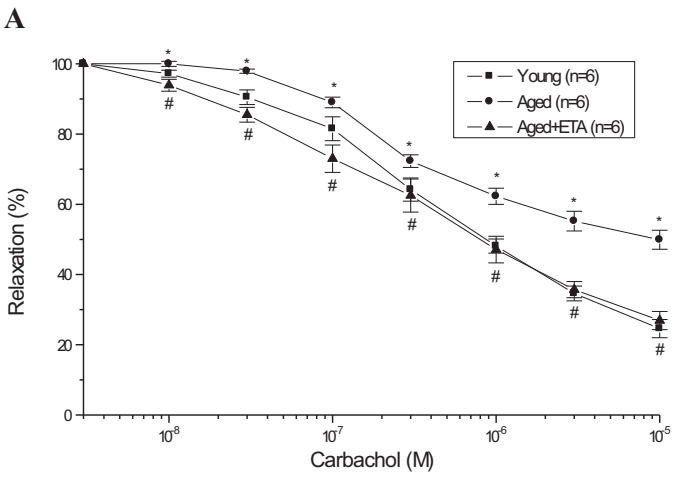


Fig. 4. SBP of rats in young, aged and aged + ETA groups. Values are expressed as mean ± *S*.E.M. $n = 10$ for each group.



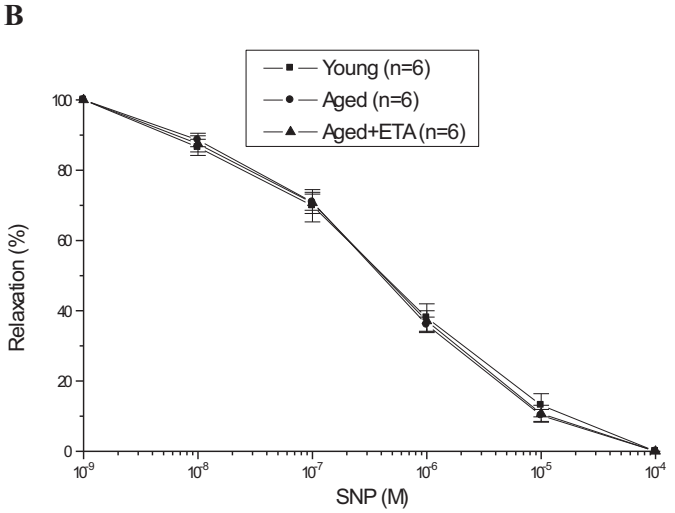


Fig. 5. Carbachol (A) and SNP (B) concentration-response curves in isolated thoracic aorta precontracted with phenylephrine (10^{-5} M) for young, aged and aged + ETA groups. Values are expressed as mean ± *S*.E.M. * $p < 0.05$ compared with young group; # $p < 0.05$ compared with aged group. $n = 10$ for each group.

after etanercept treatment.

4. Discussion

In the present study, etanercept treatment significantly reversed the impairment of learning memory abilities associated with decreased

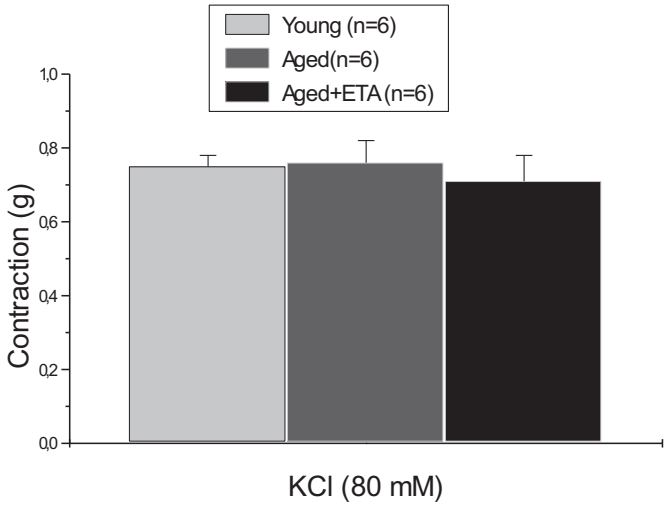


Fig. 6. 80 mM KCl-induced contractions in isolated thoracic aorta for young, aged and aged + ETA groups. Values are expressed as mean ± *S*.E.M. $n = 10$ for each group.

neurotrophic factors such as BDNF in aged rats. In addition to the beneficial effect of the treatment of etanercept on cognitive function, etanercept reversed vascular endothelial dysfunction, which co-exists with aging. Moreover, the elevations of TNFα levels in serum and hippocampus in aged rats were prevented by etanercept treatment.

Our findings in accordance with the previous studies have demonstrated that endothelial nitric oxide synthase (eNOS) expression, eNOS derived NO production and endothelium-dependent relaxation in vascular tissue decline with increasing age [27,28]. NO, which level decreases with aging, is an important vasodilator that modulates vascular function. The decreased NO bioavailability is dependent on inactivation by superoxide anion and/or reduction of its production during aging [11,29]. It's suggested that TNFα, is a proinflammatory cytokine, decreases the eNOS expression and induces the activity of NADPH oxidase, which is a major source of superoxide anion in the vasculature [30–32]. The levels of proinflammatory cytokines such as TNFα in blood and brain are increased with aging [8,9,33–36]. There are some studies demonstrated that an increase in TNFα level in advanced aging contributes to endothelial dysfunction in various vascular beds [11–13,15]. It is reported that anti-TNF therapies with etanercept may improve inflammation-induced endothelial dysfunction in animal models of biological aging, depression, and diabetes mellitus [13,26,37]. It is also shown that eNOS expression was increased with chronic etanercept treatment whereas the expression of NADPH oxidase was decreased after etanercept in estrogen-deficient rats [38,39]. In addition, some clinical studies have shown that anti-TNF treatment with infliximab improved endothelial function in rheumatoid arthritis and systemic vasculitis patients [40,41]. Consistent with previous studies, we found that etanercept prevented impairment of endothelium-

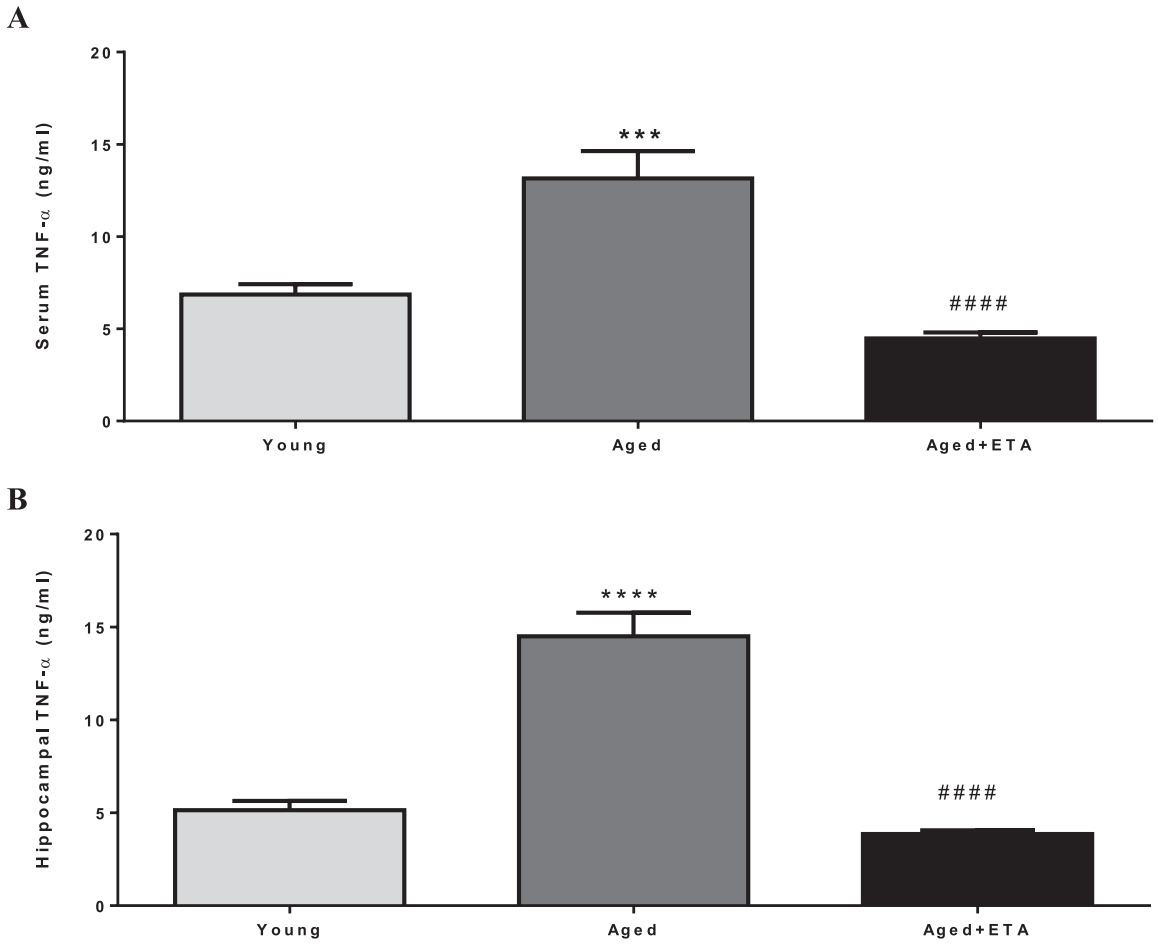


Fig. 7. TNFα levels in serum (A) and hippocampus (B) for young, aged and aged + ETA groups. Values are expressed as mean ± S.E.M. *** $p < 0.001$, **** $p < 0.0001$ compared with young group; #### $p < 0.0001$ compared with aged group. $n = 10$ for each group.

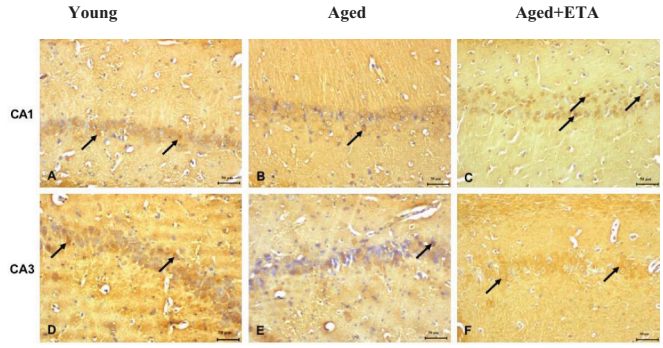


Fig. 8. Representative images illustrating BDNF expression in hippocampal regions. Young (A), aged (B) and aged + ETA (C) groups in CA1 region, and young (D), aged (E) and aged + ETA (F) groups in CA3 region. (Scale bars: 50 μm. Arrows indicate BDNF expression).

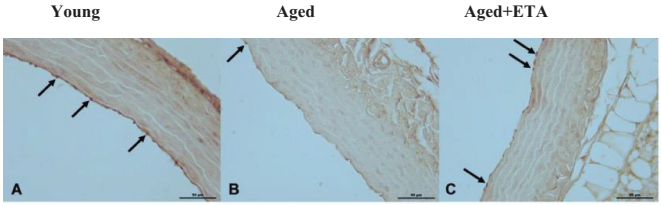


Fig. 9. Representative images illustrating eNOS expression in thoracic aorta. Young (A), aged (B) and aged + ETA (C) groups in the endothelium of aorta. (Scale bars: 50 μm. Arrows indicate eNOS expression).

dependent relaxations and enhanced eNOS expression in the vascular tissue to young control values in the aged rats. Our findings suggest that there is a link between aging, TNFα, and endothelial dysfunction and that anti-TNFα therapy may be vasculoprotective in the elderly.

Behavioral and cognitive impairments are associated with normal aging [42]. Previous studies have shown that the levels of proinflammatory cytokines are elevated in elderly humans with dementia and also neuroinflammation is a major risk factor for various neurodegenerative diseases including AD [14,20,35,43]. Correlation between elevated blood level of TNFα and IL1b and impaired learning memory performance in physiological aging was also supported with strong evidence in previous clinical studies [6,36,43]. Consistently, animal studies have shown that increased hippocampal TNFα level was found cognitive impairment in rats [44–46]. In line with previous studies by us and others, we demonstrated emotional and spatial memory impairments coexisting with increased levels of TNFα in the hippocampus in old animals in the present study. On the other hand, the current study has also demonstrated that elevated levels of TNFα in blood contributed to systemic inflammation because of aging. This finding was in accordance with the previous studies suggesting that increase in systemic inflammation is linked to cognitive disabilities [47,48]. Our previous study supports the hypothesis that anti-TNFα therapies with infliximab may have a beneficial effect on learning memory deficits in chronic unpredictable mild stress-induced depression model of rat [49]. Furthermore, it was shown that another anti-TNFα treatment with etanercept may prevent DM-induced cognitive impairment in which inflammation has a pivotal role [26]. Based on previous and our findings, the decrease in TNFα levels to young controls in the blood and brain

following chronic ETA treatment in old animals suggest that the anti-inflammatory effect of ETA may be protective for age-related cognitive deficits. Present findings also suggested that cognitive deficits may be caused by endothelial dysfunction, whereas ETA treatment could be protective through its vasculoprotective effect.

Brain-derived neurotrophic factor (BDNF), is one of the most studied neurotrophins in the brain, has a pivotal role in synaptic plasticity and memory [50]. Previous clinical and preclinical studies have reported that aging causes a reduction of BDNF levels in both brain and serum coexisting with impaired memory abilities [51,52,53]. Moreover, there are only few studies that have shown the link between decreased BDNF levels and neuroinflammation process in the brain during aging [54]. Studies on diabetes- and stress-induced cognitive impairments in animals have recently shown that anti-TNF treatments improve learning memory deficits, at least in part, by elevation of hippocampal BDNF levels [26,55]. Consistent with these studies, in the present study, it was shown that BDNF levels in the hippocampus decreased in elderly rats with decreased cognitive functions and these decreases were blocked by etanercept treatment. These findings also suggested that cognitive deficits in aged rats may be caused by deterioration effect of neuroinflammation on BDNF level in the brain.

Conclusion

The present study show that aging leads to peripheral and neuroinflammation with increased serum and hippocampal TNFα levels. Dysregulation of TNFα levels may result in endothelial dysfunction and impairment of neurotrophin expression and by this way contribute to impaired spatial and emotional memory. Anti-TNF treatment, which reversed cognitive deficits of aged rats through anti-inflammatory, neuroprotective and vasculoprotective synergistic effects, may be beneficial in elderly people.

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