Protective effects of resveratrol on aging-induced cognitive impairment in rats

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Resveratrol, a polyphenol phytoalexine, has been shown to play a neuroprotective role in the neurodegenerative process in Alzheimer’s disease (AD) and improve memory function in dementia. However, the in vivo effect of resveratrol in normal aging models of learning and memory has not yet been evaluated. Therefore, the present neurobehavioral study was undertaken to evaluate the effect of resveratrol on cognitive impairment induced by aging in passive avoidance and Morris water maze (MWM) tests. Male Wistar albino rats were divided into four groups: young control (4 month), young resveratrol (4 month + RESV), old control (24 month) and old resveratrol (24 month + RESV). Resveratrol (50 mg/kg/day) was given to the 4 month + RESV and 24 month + RESV groups orally for 12 weeks. There was no significant difference between the groups for the first day of latency, while in aged rats, the second day of latency was significantly shortened compared to the young group in the passive avoidance test (p < 0.05). Additionally, in the MWM test, the results showed a decrease in the time spent in the escape platform’s quadrant in the probe test in aged rats (p < 0.05). The administration of resveratrol at 30 mg/kg/day increased the retention scores in the passive avoidance test and the time spent in the escape platform’s quadrant in the MWM task (p < 0.05). Furthermore, resveratrol attenuated the protein levels of TNFα and IL1β in the 24-month group. These findings indicate that aging impairs emotional and spatial learning-memory and resveratrol reverses the effect of age-related learning and memory impairment. The results of this study suggest that resveratrol is effective in preventing cognitive deficit in aged rats by inhibiting the production of inflammatory cytokines.

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1. Introduction

Normal aging generally induces cognitive impairment, with older people often considered to be less efficient than younger people in using memory, attention, visuospatial capacities or executive function (Tromp, Dufour, Lithfous, Pebaye, & Despres, 2015). There is profound evidence of increased inflammation, oxidative damage and deficient antioxidative defense mechanisms in different regions of the brain in the aging process (Butcher & Lord, 2004; Gemma, Vila, Bachstetter, & Bickford, 2007, chap. 15). Several studies have found that inflammatory markers, such as tumor necrosis factor-α (TNFα) and interleukin-1 β (IL-1β) are elevated in the elderly and are associated with age-related cognitive impairment and decline (Dimopoulos et al., 2006; Engelhart et al., 2004; Trollor et al., 2011; Zuliani et al., 2008) as well as Alzheimer’s Disease (Mucke, 2009; Vetrivel & Thinakaran, 2010). The deterioration of brain function in the physiological process of aging causes a decrease in learning and memory skills. In age-related neuronal disorders, free radicals, oxidative stress and inflammatory cytokines are known to be the candidates responsible for producing cellular changes in such diseases (Cantuti-Castelvetri & Shukitt-Hale, 2000; Harman, 1994).

The learning and memory deficits associated with aging may be alleviated using numerous contents within dietary fruits and vegetables exhibit anti-aging activities in various systems, among which one component, resveratrol, is a promising candidate (Baur & Sinclair, 2006). Resveratrol is a polyphenol phytoalexine found in grape skins and red wine (Jang et al., 1997; Vinson, 1998). Accumulating evidence has shown that resveratrol can prevent or slow the progression of a variety of diseases, including...
cancer, cardiovascular diseases, ischemic injuries and Alzheimer's disease (Baur & Sinclair, 2006; Bradamante, Barenghi, & Villa, 2004). Resveratrol has various biological properties, including antioxidant, anti-inflammation and neuroprotective effects (Baur & Sinclair, 2006; Saiko, Szakmary, Jaeger, & Szekerés, 2008). Recently, a number of studies have focused on the neuroprotective effects of resveratrol, such as diminishing the toxicity induced by the amyloid beta peptide (Anekonda, 2006; Han, Zheng, Bastianetto, Chabot, & Quiron, 2004) and kainic acid (Wang, Yu, Simonyi, & Rottinhaus, 2005), preventing cerebral ischemic damage (Wang, Xu, & Rottinhaus, 2002). The neuroprotective effects of resveratrol are attributable to its antioxidant activity (Poulose, Thangthaeng, Miller, & Shukitt-Hale, 2015). Furthermore, resveratrol has been shown to improve cognitive function in an age-accelerated mouse (SAMP8) model of Alzheimer’s disease (Porquet et al., 2012). Other studies show that resveratrol could be a useful therapeutic agent for Alzheimer’s disease (Ono et al., 2008; Turner et al., 2015). Similarly, previous studies in our laboratory have shown that resveratrol has a beneficial effect on cognitive deficit induced by scopolamine (Gacar et al., 2011). In addition, resveratrol has been shown to improve memory function and reverse the effects of acetylcholinesterase in streptozotocin-induced models of dementia (Sharma & Gupta, 2002).

Therefore, in this study we aimed to evaluate whether resveratrol improves the age-related spatial and emotional cognitive impairment using a water maze and a passive avoidance task and decreases inflammatory cytokines, respectively.

2. Materials and methods

2.1. Animals

Young (4 months old, 200–250 g, n = 30) and aged (24 months old, 550–600 g, n = 20) male Wistar-albino rats (Kocaeli University, Experimental Medical Research and Application Center, Kocaeli, Turkey) were kept in an animal colony with approximately 5–6 per cage for 2 weeks prior to the experiments. All experiments were conducted between 9:00 A.M. and 12:00 P.M. under standard laboratory conditions (22 ± 2 °C room temperature; 12-h light/dark cycle with lights on at 7:00 A.M.). Tap water and food pellets were provided ad libitum. All animals used in this study were naive to the experimental tests, and different rat groups were used in each experiment.

The experiments reported in this study were conducted in accordance with the Regulation of Animal Research Ethics Committee in Turkey (July 6, 2006, Number 26220). Ethical approval was granted by the Kocaeli University Animal Research Ethics Committee (Project number: HADYEK 28, Kocaeli, Turkey). Animals were divided into five groups (n = 10 per group): young control (4 month), old control (24 month), young resveratrol (4 month + RESV) and old resveratrol (24 month + RESV). Control groups received physiological saline and the second control group received DMSO for 12 weeks. There were no significant differences between data obtained from the rats that received two vehicle solutions. Therefore, the group that received DMSO was accepted as the control for comparison. Resveratrol (50 mg/kg/day) was given to the 4 month + RESV and 24 month + RESV groups orally by lavage for 12 weeks.

2.2. Locomotor activity test

Locomotor activity was measured with a computerized system (40 × 40 × 35 cm box; May Commat, Ankara, Turkey). Total locomotor activity was measured before the behavioral tests over a 5-min period and expressed as the stereotypic, ambulatory, and vertical activities and the total number of movements of animals.

2.3. Passive avoidance test

A one-trial, light–dark passive avoidance apparatus (Ugo Basile model 7551, Italy) was used for the evaluation of emotional memory based on contextual fear conditioning learning. The animal learns to avoid a specific place associated with an aversive event. The reduction of latency was used as a learning index. The apparatus consisted of two compartments, each measuring 22 × 21 × 22 cm. The illuminated white box was connected to the dark box, which was equipped with an electrifiable grid floor. An inescapable electrical shock was delivered to the animal’s feet via a shock generator. The two boxes were separated by a flat-box partition, including an automatically operated sliding door at floor level.

A training trial was carried out as described by Monleon et al. (2002). A preacquisition trial was performed on the first day of training in which the rats were placed individually into the light compartment and allowed to explore the boxes. The door between the two boxes was opened after 30 s and the animal was able to move freely into the dark compartment. Fifteen minutes after the preacquisition trial, an acquisition (training) trial was performed. Rats were again placed in the light compartment of the passive avoidance apparatus. After 30 s of familiarization with the apparatus, the door between the compartments was opened. When the animal entered the dark compartment completely, the sliding door between the chambers was closed automatically and a 3-s electric foot–shock (0.5 mA) was delivered through the grid floor. The time taken to enter the dark compartment was recorded as the training latency. If the animal failed to cross over from the illuminated to the dark compartment within 300 s, it was excluded from the experiment. The animals were then removed from the dark box and put back in their home cages. Both compartments of the box were cleaned thoroughly between each training session to remove any confounding olfactory cues.

Twenty-four hours after the acquisition trial, a retention trial was performed. Recall of this inhibitory stimulus was evaluated by returning the animals to the light compartment and recording their latency to enter the dark compartment (four paws in). No foot shock was applied in this trial. If the animal did not enter the dark compartment within 300 s, it was returned to its home cage and a latency of 300 s was recorded. This latency served as a measure of the retention performance of the passive avoidance response.

2.4. Morris water maze test

The Morris task was assessed in a water tank (150 cm in diameter) as has been previously described (Pothion et al., 2004). The rats underwent three trials during five daily sessions. During the first four days, the platform, which was situated in the center of the southwest quadrant, was submerged 1.5 cm below the surface of water, and small black pieces of plastic were placed on the water’s surface. The plastic was invisible to the rats due to its placement, and it was used to monitor spatial learning. The platform position remained stable over 4 days, and the acquisition of finding the platform was assessed. A trial was started by placing a rat into the pool facing the wall of the tank. Each of three starting positions (north, east, and west) was used once in a series of four randomly ordered trials. Each trial was terminated as soon as the rat had climbed onto the escape platform or when 60 s had elapsed. A rat was allowed to stay on the platform for 20 s. Then, it was taken from the platform and the next trial was started. Rats that did not find the platform within 60 s were placed on the platform by the experimenter and were allowed to stay there for 20 s.
After completion of the 3rd trial, rats were gently dried with a towel, kept warm for an hour and returned to their home cage. Twenty-four hours after the last acquisition session (on day 5), a ‘probe trial’ was used to assess the rat’s spatial retention of the location of the hidden platform. During this trial, the platform was removed from the maze and the rat was allowed to search the pool for 60 s before being removed. During this time, animals should have spent more time searching the quadrant that previously contained the hidden platform than the other three quadrants.

All tests were conducted between 08:00 A.M. and 12:00 P.M.

2.5. Cytokine analysis

For serum and brain hippocampal cytokine measurements, samples were taken in terminally anaesthetized rats and collected in microfuge tubes. Samples were spun down and serum was kept at –80 °C until further use. Quantification of serum TNF-α and IL-1β protein levels were assessed with an ELISA kit (Biosource, Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. The absorbance was measured at 450 nm using a microtiter ELISA reader (VERSAmax Molecular Devices, Sunnyvale, CA, USA). The cytokine results, reported as picograms of the measured molecule per mL of serum (pg/mL), were expressed as the mean values ± SEM. Where indicated, the cytokine amounts were also normalized to the protein content.

2.6. Drugs and treatments

Resveratrol was purchased from Sigma Chemical Co. (St. Louis, USA). For oral administration, fresh resveratrol was dissolved in saline mixed with 5% DMSO and given in a volume of 0.2 ml per 100 g body weight of the rat. Resveratrol (50 mg/kg) was administered daily for 12 weeks. Behavioral testing commenced 60 min after the last drug treatment. In a preliminary experiment, DMSO (orally, 0.2 ml/100 g) and saline (orally, 0.2 ml/100 g) were used as control treatments (data not shown) and the behavioral data did not differ between the rats that received the two vehicle solutions. Therefore, we chose to present only the DMSO control group data for comparison.

2.7. Statistical analysis

All results were expressed as the means ± SEM. Acquisition (1–4 day) latency scores in MWM were measured by two-way ANOVA followed by post hoc Bonferroni’s test. Scores of the time spent in the escape platform’s quadrant in MWM, total locomotor activity scores and first day and retention latencies in the passive avoidance test scores were measured by one-way ANOVA. Further statistical analysis for individual groups was carried out with Bonferroni’s test. The criterion for statistical significance was p < 0.05.

3. Results

3.1. Effects of aging and resveratrol on locomotor activity

Increased locomotor activity may produce behavioral disinhibition and can affect the learning and memory process. To exclude this possibility, the locomotor activity of the animals was assessed by measuring the stereotypic, ambulatory, and vertical movements and the total number of movements over a 5-min period. Statistical analysis of the data showed that there were no significant differences between the groups for any type of locomotor activity (F(3,36) = 1.593; p = 0.2080; Fig. 1a, F(3,36) = 1.516; p = 0.2270; F(3,36) = 1.112; p = 0.342; Fig. 1b, F(3,36) = 3.862; p = 0.1819; Fig. 1c, F(3,144) = 5.183; p = 0.1448; Fig. 1d).

3.2. Effects of the systemic administration of resveratrol on age-induced memory impairment in the passive-avoidance test in rats

During the training session (on day 1) of the light–dark type passive avoidance task, there were no significant differences between any groups (F(3,36) = 3.279, Fig. 2a). However, there was a significant difference between groups in the retention test (F(3,36) = 22.96, Fig. 2b). Aged rats demonstrated significantly lower latency compared to 4-month-old rats during the retention test, which was performed 24 h after the training test (p < 0.001; Fig. 2b). The reduced retention latency indicates the impaired retention of the passive avoidance task. The effect of aging was reversed by 50 mg/kg of resveratrol in the 24 month + RESV group (p > 0.05, vs. control; Fig. 2b), though 50 mg/kg resveratrol had no effect on the 4 month + RESV group (p > 0.05 vs. control; Fig. 2b). Administration of resveratrol at 50 mg/kg/day increased the retention scores in the passive avoidance test.
We found that aging resulted in performance deficits in the water maze tasks. As displayed in Fig. 3a, statistical analysis showed a significant effect of day (two-way ANOVA, effect of day, $F_{(3,144)} = 26.60, p < 0.0001$). In addition, an extremely significant effect of age was demonstrated (two-way ANOVA, effect of treatment, $F_{(3,144)} = 27.73, p < 0.0001$). Further analysis also revealed that the day × age interaction was not significant (two-way ANOVA, day × age, $F_{(9,144)} = 0.53, p = 0.8472$). Post hoc comparison showed that aging caused a significant disruption of learning and memory, indicated by an increase in the escape latency compared to the control animals (two-way ANOVA, effect of treatment, $p < 0.05$, $p < 0.0001$, $p < 0.001$, respectively, Fig. 3a). Bonferroni’s test indicated that resveratrol (50 mg/kg/day, orally) administered for 12 weeks reversed the age-induced impairment in the escape latency in the water maze task (two-way ANOVA, effect of age, $p > 0.05$, 4 month vs. 24 month + RESV; Fig. 3a).

In the Morris water maze test, the results showed a decrease in the time spent in the escape platform’s quadrant in the probe test in aged rats ($F_{(3,36)} = 15.56; p < 0.0001$). Administration of resveratrol at 50 mg/kg/day increased the time spent in the escape platform’s quadrant in the water maze task ($p < 0.05$, 4 month vs. 24 month + RESV; Fig. 3b).

3.4. Effects of systemic administration of resveratrol on age-induced changes in levels of inflammatory cytokines

Plasma TNFα levels (pg/ml) were 23.21 ± 0.70 for the 4 month group, 24.14 ± 3.08 for the 4 month RESV group, 32.50 ± 3.86 for the 24 month group, and 22.26 ± 0.64 for the 24 month RESV group. Plasma IL1β (pg/ml) levels were 38.30 ± 2.66 for the 4 month group, 40.19 ± 1.95 for the 4 month RESV group, 60.29 ± 4.02 for the 24 month group, and 37.60 ± 2.16 for the 24 month RESV group. Brain hippocampal TNFα levels (pg/ml) were 26.95 ± 0.69 for the 4 month group, 23.77 ± 2.04 for the 4 month RESV group, 77.20 ± 10.04 for the 24 month group, and 33.59 ± 2.62 for the 24 month RESV group.

Protein levels of TNFα and IL1β were significantly elevated in 24-month-old rats, but resveratrol treatment reversed the levels to that of 4-month-old controls ($p < 0.05$; one-way ANOVA, Bonferroni’s test).

4. Discussion

In our study, resveratrol significantly improved learning and memory in aged rats. Moreover, resveratrol treatment prevented elevations in plasma TNFα and IL1β levels in aged rats. It is well known that the physiological process of aging involves a progressive cognitive loss caused by the deterioration of brain function, including a decrease in learning and memory skills (Pieramico, Esposito, Cesinaro, Frazzini, & Sensi, 2014). Previous preclinical and clinical studies reported that elevated levels of TNFα and IL1β were associated with dementia in an elderly population (Dimopoulos et al., 2006; Engelhart et al., 2004; Fillit et al., 1991; Tarkowski, Blennow, Wallin, & Tarkowski, 1999; Trollor et al., 2011; Zuliani et al., 2008). In addition, rats with chronic neuroinflammation displayed impaired learning and memory (Belabri et al., 2012). In the present study, resveratrol reversed aging-impaired emotional memory in the passive avoidance task and spatial memory in the MWM tests. This evidence is consistent with our previous data showing that resveratrol treatment reverses age-related vascular dysfunction in aged Fischer-344xBN rats (Gocmez et al., 2011). Therefore, we hypothesized that vascular aging, at least in part, leads to vascular dementia in aging. Additionally, our previous data demonstrated no differences between middle-aged (both 13 months and 18 months old) and aged (24 months old) groups in behavioral and immunohistochemical tests (Bagci, Sarioglu, Yazir, Utkan, & Aricioglu, 2014; Karson, Utkan, Balci, Aricioglu, & Ates, 2012). Therefore, we used 24-month-old rats as the aged group. Thus, it is necessary to test a middle-aged group with young and old groups in further studies. In the present study, young and old rats were treated with either vehicle or resveratrol for 12 weeks. Escape latencies of 24-month-old rats in the MWM test were significantly longer than 4-month-old rats, indicating a deficit in spatial learning. In the 24 month + RESV group, the animals had shorter escape latencies than the 24 month group. The time spent in the escape platform’s quadrant in the probe test was decreased in aged rats, indicating impaired memory retrieval;
however, resveratrol reversed these impairments and restored spatial memory and learning in 24-month-old rats. A similar effect was seen in the passive avoidance test. The effect of aging on retention latency was reversed by resveratrol in the 24 month + RESV group, which indicated improved emotion of behavioral learning and memory. Our results collectively suggest that resveratrol could prevent age-related spatial and emotional cognitive impairment and that a resveratrol-rich diet may be beneficial in preserving cognitive function in aged individuals.

Aging is associated with a general decline of physiological functions. The detrimental effects of aging on the central nervous system result in behavioral and cognitive impairment (Navarro, Sanchez, Gomez, Peralta, & Boveris, 2002). It has been demonstrated that brain aging associated with neuroinflammation is a major risk factor for neurodegenerative diseases, such as Parkinson’s disease and Alzheimer’s disease. Moreover, there is profound evidence of the correlation between increased brain inflammation and decreased behavioral performance in normal aging (Engelhart et al., 2004). In a cross-sectional analysis of clinical populations, a reasonably consistent finding was the association between dementia and higher levels of IL1β, IL6, C-reactive protein (CRP) and TNFα (Alvarez, Cacabelos, Sanpecd, Garcia-Fantini, & Aleixandre, 2007). Belarbi et al. (2008), Bruunsgaard et al. (1999), Dimopoulos et al. (2006), Engelhart et al. (2004), Licastro et al. (2000), Zuliani et al. (2007). In addition, hippocampal neurogenesis and consolidation of hippocampal-dependent memories decrease with increased activity of IL1β, a proinflammatory cytokine produced in the brain by microglia (Abraham & Johnson, 2009). There is growing interest regarding the potential role of IL1β as a cause of cognitive aging, and some studies suggested that increased IL1β in cognitively impaired aged animals is indicative of increased brain inflammation (Blalock et al., 2003; Godbout et al., 2005; Swanson, Vester, Apanavicius, Kirby, & Schook, 2009). Moreover, it was demonstrated that the constitutive expression of IL1β was higher in brains of healthy, old animals than young animals (Chen et al., 2008; Griffin et al., 2006). Studies have recently expanded the body of work on systemic inflammation in pre-dementia syndromes, such as mild cognitive impairment, suggesting that increases in measures of low-grade systemic inflammation are linked to cognitive impairment. Additional studies have demonstrated that increased levels of TNFα are found in mild cognitive impairment patients compared to normal controls (Alvarez et al., 2007; Belarbi et al., 2008; Bruunsgaard et al., 1999; Zuliani et al., 2007). Furthermore, elevated levels of TNFα have been documented in Alzheimer’s disease (Fillit et al., 1991; Tarkowski et al., 1992). Belarbi et al. reported that chronic neuroinflammation induced performance impairment in novel place recognition and spatial learning and memory tests and the inhibition of TNFα synthesis reversed these cognitive deficits in rats (Belarbi et al., 2012). We showed that infliximab, a TNFα inhibitor, improved chronic unpredictable mild stress-induced cognitive deficits in rats (Karson, Demirtas, Bayramgurler, Balci, & Utkan, 2013). It is well known that resveratrol has an anti-inflammatory effect (Svajger, C223, Bayramgurler, Balci, & Utkan, 2013). It is well known that resveratrol has an anti-inflammatory effect (Svajger et al., 2012). Our previous study showed that resveratrol consistently reduced levels of proinflammatory cytokines and improved ileal smooth muscle reactivity in a polymicrobial sepsis model in rats (Gacar et al., 2012). Consistent with these previous findings, we found that IL1β and TNFα levels were increased by aging, and these increases were reversed by resveratrol treatment. Based on these findings, we suggest that the anti-inflammatory effect of resveratrol can be protective and reduce cognitive deficits in normal aging.

A number of studies demonstrate that resveratrol can cause cognitive enhancement with other effects, such as neurogenesis and angiogenesis, which directly improve brain function (Kodali et al., 2014). Furthermore, it was shown that resveratrol exerts beneficial cognitive effects via the upregulation of brain-derived neurotrophic factor (BDNF) in the hippocampus, which is important in synaptic plasticity and declarative memory (Phelps, 2004; Roozendaal, McReynolds, & McCaugh, 2004; Figuero, Pozzo-Miller, Olafsson, Wang, & Lu, 1996; Kang & Schuman, 1995). BDNF plays a pivotal role in age-related memory impairments and is associated with age-related atrophy of the hippocampus. Furthermore, in previous studies, reduced serum levels of BDNF have been documented in Alzheimer’s Disease, mild cognitive impairment and major depressive disorder (Gezen-Ak et al., 2013; Karege et al., 2002; Peng, Wu, Mufson, & Fahnstock, 2005). Previously, Liu et al. showed that resveratrol upregulated BDNF levels in the hippocampus and amygdala in rats exposed to chronic mild unpredictable stress (CUMS), suggesting that it can improve cognition. This is consistent with our previous study, which demonstrated that resveratrol improves BDNF levels in hippocampal CA1 and CA2 in rats exposed to CUMS (Yazir, Utkan, Gacar, & Aricioglu, 2015). Additionally, resveratrol has been shown to improve learning and memory function in normal aged mice through the microRNA-CEB-BDNF pathway (Zhao et al., 2013). Based on these findings, we speculate that the improved cognitive performance of aged animals receiving resveratrol may be due to the effects of resveratrol on BDNF levels.

5. Conclusion

The current study showed that aging increased the levels of TNFα in the hippocampus and the resultant chronic neuroinflammation impaired spatial learning and memory (including Morris water maze performance) and hippocampus-independent passive avoidance performance. These cognitive deficits were reversed with the chronic administration of resveratrol, which returned the cytokine level to normal. Our data are consistent with a large body of literature demonstrating that a resveratrol-rich diet is generally beneficial for human health and additionally suggest a new strategy for the therapeutic intervention of human diseases associated with memory impairment (Zhao et al., 2013).

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References


