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Original article

Folic Acid and Coenzyme Q10 Ameliorate Cognitive Dysfunction in the Rats with Intracerebroventricular Injection of Streptozotocin

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Abstract

Objective(s)
The present study aimed to investigate the effects of a fat soluble antioxidant, coenzyme Q10 (CoQ10) and folic acid on learning and memory in the rats with intracerebroventricular injection of streptozotocin (ICV-STZ), an animal model of sporadic type of Alzheimer's disease.
Materials and Methods
The lesion groups were injected bilaterally with ICV-STZ (1.5 mg/kg b.wt., in normal saline). In the treated groups, rats received folic acid (4 mg/kg; i.p.) or CoQ10 (10 mg/kg; i.p.), either alone or together, for 21 days. Passive avoidance learning test was used for evaluation of learning and memory.
Results
The results showed that learning and memory performance was significantly impaired in the rats with ICV-STZ ($P< 0.001$), however CoQ10 and folic acid, either alone or together, prevented impairments significantly ($P< 0.001$), as there was not any significant difference between these treated lesion groups and control group.
Conclusion
The present results suggest that CoQ10 and folic acid have therapeutic and preventive effects on cognitive impairments in Alzheimer's disease.

Keywords: Alzheimer disease, Coenzyme Q10, Folic acid, Passive avoidance learning, Streptozotocin.

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Introduction

One of the necessities for survival is learning and memory, and different diseases in human influence these cognitive functions. One of these disorders is Alzheimer's disease (AD), which is a progressive and irreversible disorder of brain with unknown etiology (1). In AD which is characterized by a progressive deterioration of learning and memory (2), there is a reduction in quantity of neurons in different area of the brain, especially in hippocampus that is involved in learning and memory (3). It is estimated that millions of people all over the world are afflicted by AD, which could potentially multiple in the future. Currently there is no conclusive cure for AD, and present treatments just slow down the progression of this disease and manage some of the symptoms (4).

One of the factors which play an effective role in pathogenesis of AD is oxidative stress that is an imbalance between free radicals and antioxidant system (5, 6). Oxygenate radicals can attack proteins, nucleic acids and lipid membranes and accordingly interrupt the integrity and performance of the cell (7). The brain tissue contains a lot of unsaturated fatty acids which are especially vulnerable for free radical attacks (8). Therefore, antioxidant substances can play an important role in prevention and cure of Alzheimer (9, 10).

Co-enzyme Q10 (CoQ10) or ubiquinone, is a fat-soluble, pseudo-vitamin substance that is present primarily in the mitochondria and participates in generating ATP as a component of the electron transport chain (11). It has been demonstrated that CoQ10 acts as a radical scavenger and powerful antioxidant (12) whose effects on improvement of cognitive functions has been shown (13). Also, CoQ10 can help to regenerate other antioxidants (14). CoQ10 is used as an anti-aging substance, and it has been shown that through its antioxidant effects, it is effective in improvement of cognitive disorders in AD (15, 16).

In the body, cells synthesize CoQ10 from the amino acid tyrosine, in an eight-step aromatic pathway, requiring adequate levels of vitamins such as folic acid; and usage of these substances enhances the function of CoQ10 (17). Folic Acid is an essential cofactor for the endogenous synthesis of CoQ10 and any deficiency in that would result in a deficiency in CoQ10 (17,18).

Folic acid or vitamin B9 is a member of vitamin B family that is essential for the production and maintenance of new cells, synthesis and maintenance of DNA synthesis of RNA and remethylation of homocysteine (homocysteine plays an important role in pathogenesis of AD) (19-21). At all ages folic acid is important for the activity of nervous system (22). Also, studies have shown that folic acid is an effective antioxidant with free radical scavenging property (23) that helps maintaining the integrity of neurons and improving the memory status during aging and in AD (24).

There are a number of facts in this study which are as follows: first, folic acid is an essential cofactor for the endogenous synthesis of CoQ10 and usage of it enhances the function of CoQ10 (17), second, reduction of folic acid and its metabolite S-adenosylmethionine in the cerebrospinal fluid of aged people (25) as well in the blood of people suffering from Alzheimer's disease (26), third, the abnormal redox balance of CoQ10 in the cerebrospinal fluid of AD patients (27). Accordingly, the aim of this study was to investigate the effects of CoQ10 and folic acid, either alone or together, on learning and memory in rats with intracerebroventricular injection of streptozotocin (ICV-STZ), as a proper model for studying sporadic type of Alzheimer's disease (28). It has been demonstrated that central application of STZ produces behavioral and neurochemical features that resembled those found in human AD (29).

Materials and Methods

Male Wistar rats (300±20 g; 12 months old; provided by the Pasteur Institute of Iran) were housed four per cage and maintained on a 12 hr light–dark cycle in an air conditioned constant temperature (23±1 °C) room, with food and water made available *ad libitum*. The

Ethic Committee for Animal Experiments at Isfahan University approved the study. Animals were divided into five groups (n= 10-11 in each group): the sham, the lesion, the lesion + folic acid, the lesion + Q10 and the lesion + folic acid + Q10.

The rats were anesthetized with chloral hydrates (400 mg/kg, i.p.) and their heads were fixed in a stereotaxic frame. A heating pad was used to maintain body temperature at 36.5±0.5 °C. The skull was exposed and two small holes were drilled and injection canula was lowered into the lateral ventricles (AP=-0.8 mm; ML= ±1.6 mm; DV= -4.2 mm) (30). Injection canula was connected to a Hamilton syringe attached to a micro-injector unit. The lesion groups received a bilateral ICV injection of STZ (1.5 mg/kg, body weight in saline, 4 µl/injection site) as in previous studies (9). The sham groups underwent the same surgical procedures, but same volume of saline was injected instead of STZ.

From the second day after surgery, rats in different treated groups received folic acid (4 mg/kg, in saline; i.p.) or CoQ10 (10 mg/kg in corn oil; i.p.) or both of them for 21 days. Animals in the sham and the lesion groups received same volume of placebo.

After 3 weeks of intracerebroventricular injection of STZ and treatment, the rats were tested with passive avoidance learning (PAL). The apparatus consists of two separate chambers connected through a guillotine door. One chamber was illuminated, while the other was dark. The floor of both chambers consists of steel grids, used to deliver electric shocks. On the acquisition trial, each rat was placed in illuminated chamber while its back was to the guillotine door. After 10 sec of habituation, the guillotine door separating the illuminated and dark chambers was opened. The guillotine door was closed immediately after the rat enters the dark chamber, and an electric foot shock with 1.5 mA intensity was delivered to the floor grids for 3 sec, then the rat was removed from the dark chamber and returned to its home cage. Twenty four hr and one week later, retention latency time to enter the dark chamber was taken in the same way as in the acquisition trial, but foot shock was not delivered, and the latency time was recorded up to a maximum of 300 sec.

Data were analyzed using the SPSS 16 for Windows. The data were analyzed statistically by repeated measures ANOVA followed by Dunnett's Multiple Comparisons test. The significant level was set at $P< 0.05$. Results are expressed as mean±SEM.

Results

The mean initial latency in the acquisition trial was unchanged among the groups. Results from the retention phase of PAL as measured by mean retention latency time have shown twenty four hr after acquisition phase, mean retention latencies in the lesion group (32.66±27.62 sec) was less than the sham (200.63±20.175 sec; $P< 0.001$), the lesion+folic acid (290.33±31.9 sec; $P< 0.001$), the lesion+Q10 (292±31.9 sec; $P< 0.001$.) and the lesion+folic acid+Q10 (288±31.6 sec; $P< 0.001$) groups (Figure 1A); and one week after acquisition phase, mean retention latencies in the lesion group (20.16±26.82 sec) was less than the sham (205.42±19.58 sec; $P< 0.001$), the lesion+folic acid (274.5±30.97 sec; $P< 0.001$), the lesion+Q10 (264.83±30.97 sec; $P< 0.001$.) and the lesion+folic acid+Q10 (277.16±30.97 sec; $P< 0.001$) groups (Figure 1B). However, the lesion+folic acid, the lesion+Q10 and the lesion+folic acid+Q10 groups comparing to the sham group didn't have any significant difference.

Discussion

The results showed that folic acid and CoQ10, either alone or together prevent learning and memory decline in rats with intracerebroventricular injection of STZ; nevertheless simultaneous application of these two substances did not have better effect than their single application.

Cognitive deficits and biochemical and structural changes in the brain of rats with ICV-STZ mainly were attributed to generating free radicals and altering glucose energy metabolism by depleting ATP synthesis (6, 9, 31).

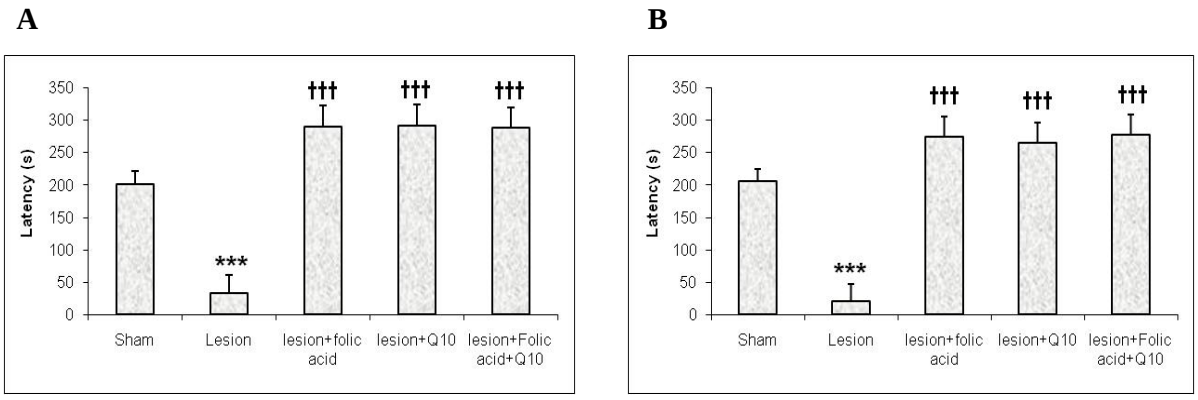

Figure 1. Effects of folic acid and coenzyme Q10 on step-through latency in the rats with intracerebroventricular injection of streptozotocin, 24 hr (A) and 1week (B) after PA acquisition. Data are expressed as mean±SEM (n = 10, 11). *** $P< 0.001$ with respect to the sham group, ††† $P< 0.001$ with respect to the lesion group.

Studies have demonstrated that the normal cellular energy metabolism is necessary for normal functioning of the brain (28), and when availability of ATP is low in the brain, faulty amyloid precursors protein (APP) metabolism and hyperphosphorylation of the tau-protein are high, that induce production of neuritic placques and neurofibrillary tangles, respectively, which are prominent histopathological markers of AD (28). It has been demonstrated that intracerebroventricular injection of STZ causes impairment of neural glucose metabolism leading to reduction of ATP and creatine phosphate formation (32, 33), but it was seen that CoQ10 can restore this impaired glucose energy metabolism effectively in ICV-STZ rats (9).

In addition, it is revealed that through improvement of glucose energy metabolism and production of acetyl CoA, and protection of choline acetyltransferase (ChAT) activity, CoQ10 protects cholinergic neurons in the brain of ICV-STZ infused rats that cholinergic neurons are damaged severely (9,34,35).

Oxidative stress plays a pivotal role in Alzheimer's (36). Oxidative stress damages neuronal membranes lipids and proteins, through generation of free radicals, and therefore damages membrane integrity (37) and reduces the number of nerve cells (38). Because both CoQ10 and folic acid are powerful antioxidant and they have free radical scavenging property, they can reverse the free radical induced damages seen in neurodegenerative diseases and resultant learning and memory defects (23, 39, 40), as seen in our results.

Studies have shown reduction of folic acid as seen in AD results in hyperhomocysteinemia (20, 26, 41, 42). Folic acid deficiency and hyperhomocysteinemia impact neurons by affecting antioxidant defense systems and impairing DNA repair that induces neural cell apoptosis (43, 44). Folic acid supplementation by converting homocysteine into cysteine can increase level of reduced glutathione in all the regions of brain (45). Also, this has beneficial effects in increasing the superoxide dismutase and catalase activities that are protective enzymes against highly reactive free radicals in the brain (46, 47).

Because folic acid and CoQ10 are both reduced in AD patients (26, 27), and folic acid can potentiate endogenous synthesis of CoQ10 (17, 18), therefore, usage of folic acid probably aids to improve AD by increase CoQ10. Hence, administration of folic acid and CoQ10 in AD apparently have same effects, and our results verifies it, because co-administration of CoQ10 and folic acid had same effects on learning and memory as there were in separate administration of them.

Finally, similar to our protocol, other studies have started their intervention, one or two days after ICV-STZ for a period of time (48, 49); and most of them believe that positive effects of their interventions were due

to amelioration of ICV-STZ complications, rather than reduction of STZ effectiveness directly; however we do not reject this possibility.

Conclusion

In conclusion, our findings suggest that CoQ10 and folic acid, either alone or together, protect learning and memory performance in the rats with intracerebroventricular injection of streptozotocin. The data correspond to the possibility that prophylactic treatment with CoQ10 or folic acid can offer protection against Alzheimer's disease.

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