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Ubiquinone Ameliorates Hippocampus Injury Induced by Cerebral Ischemia/Reperfusion

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Abstract

Background: Apoplexy is known as a critical issue all over the world and certain parts of the brain are more sensitive to Ischemia/cerebral reperfusion such as the hippocampus. Coenzyme Q10 is a powerful anti-oxidant, which helps in cells membrane durability.

Aim: This study attempts to find the effect of coenzyme Q10 on the change of hippocampal area texture after cerebral reperfusion/Ischemia.

Methods: Twenty-four male Wistar rats were organized into 4 groups of six including control, Ischemia, vehicle and experimental groups, with 100 mg /Kg of coenzyme Q10. Coenzyme Q10 was given to the rats 5 days before and 3 days after Ischemia/reperfusion induction. Ischemia was done for 20 minutes by reciprocal blocking of carotid arteries. The rat's brains were removed and stained by applying the chrysalis fast violet method. The number of viable cells of the hippocampal regions of all 4 groups was counted by Imaging-Pro-Plus software. Statistical analysis of the data was then accomplished by one-way ANOVA and Tukey's test.

Results: Findings revealed that the number of viable cells in CA2 and CA3 area reduced following ischemia induction. Whereas, there was no notable change between the control and experimental groups in terms of cells numbers. Besides, there was no remarkable change between the control, experimental and ischemia groups in terms of the number of cells within CA4 area.

Conclusion: The results support the use of coenzyme Q10 as a neurotrophic substance and as an adjunctive therapy in patients at risk for ischemic stroke.

Conflicts of Interest: The Authors declare no conflicts of interest.
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Introduction

Leading cause of cerebral ischemia is stroke. Cerebral Ischemia is the third main cause of death in western countries after myocardial infarction and cancer (2). Cerebral ischemia leads to sensory-motor disorders, speech and visual disturbances, neuropsychiatric, cognitive and spatial learning disorders (3-5). Specific regions of the brain and specific types of neurons are more vulnerable to cerebral ischemia (6-9). Pyramidal neurons in CA2-CA3-CA4 regions of the hippocampus and striatum are among this type of neurons. In these areas, specific damage to glial cells is also activated, which leads to intensification of neuronal damage (10). This area is involved in memory and depression and stress. Nowadays, the occurrence of neurogenesis in this area has been considered to have an important role in short-term memory.

Journal of Otorhinolaryngology and Facial Plastic Surgery 2023;9(1):1-8.
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So far, the protective effect of many substances on programmed cell death has been proven in laboratory models (12), but unfortunately, contrary to the results obtained from animal models in preventing this type of cell death, there is no effective pharmacological approach for the cure of ischemia. This could be on account of drug's deficiency or side effects (13). Recently, the use of ubiquinone as an appropriate and new approach in the role of a neuroprotective has been considered (14-17). Coenzyme Q10 (a specific lipophilic antioxidant) is a key element of the mitochondrial electron transport chain (18), which acts as a strong scavenger of free radicals in lipid and mitochondrial membranes (19). It plays an essential role in regulating blood pressure, helping to treat heart failure, ischemic heart disease, and preventing statin myopathy. It is involved in the production of adenosine triphosphate (ATP) and is associated with improved cognitive function. Since cerebral ischemia results in the release free oxygen radicals, the formation or distribution of these radicals must be reduced in order to repair the neurological adverse outcomes and neuronal inhibition (20). It makes sense to use coenzyme Q10 (Co-Q10), since it promotes oxidative stress resistance and improves brain energy during reperfusion after ischemic brain injury. The reduction of caspase 3 as a key enzyme in apoptosis by CoQ10 in animal studies also suggests the use of coQ10 as a neuroprotective in the role of an anti-apoptotic (21). Antioxidants such as coenzyme Q10 can be effective in reducing the cytosolic activity of LDH and Ca^{2+} levels that increase following reperfusion ischemia (22). Since so little research has been done on the neurotrophic effect of this CoQ10, in this study the effect of ubiquinone on the CA2-CA3-CA4 areas of the hippocampus of male Wistar rats following global transient ischemia has been examined.

Methods

Twenty-four adult male Wistar rats (weighing 250-300 g) were purchased from Iran's Pasteur Institute and kept in standard laboratory conditions (12 hours of light - 12 hours of darkness) at a temperature of 22-24. Adequate water and food were available for the animals. Rats were haphazardly organized into 4 groups of 6:

1. Control group: Rats were kept in standard laboratory conditions.
2. Ischemia group: After anesthesia with ketamine / xylazine (50/5 mg/kg, IP), carotid arteries were closed and ischemia was induced for 20 followed by reperfusion.
3. Vehicle group: Animals received only soybean oil (coenzyme Q10 solvent) for 5 days, then ischemia/reperfusion was done. Soybean oil was given again for three consecutive days.
4. Experimental group: The group received the CoQ10 (100mg/kg) for 5 days before and 3 days after ischemia/ reperfution

All rats were killed on the fourth day after ischemia and their brains were prepared for Nissl staining

Preparation of coenzyme Q10 for gavage

Since coenzyme Q10 (Co-Q10) is a fat-soluble substance, the recommended dose with the use of suitable solvent (soybean oil) was prepared. Due to the low stability of ubiquinone in long-term storage and its sensitivity to light, the material used for gavage (ubiquinone dissolved in soybean oil and centrifuged) was made daily and fed to animals according to weight.

Surgical process and induction of ischemia/reperfusion

Common carotid artery occlusion (CCAO) was done to induct cerebral I/R injury. Briefly, after a vertical incision in the anterior region of the neck**,** the sternocleidomastoid muscle was aside after anesthesia by intraperitoneal injection of ketamine/xylazine (50/5 mg/kg, IP). The common carotid arteries were exposed and were closed with a microsurgical clamp for 20 minutes followed by reperfusion. The animal's rectal temperature was frequently recorded using a thermometer and a heat lamp was set at

Journal of Otorhinolaryngology and Facial Plastic Surgery 2023;9(1):1-8.
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370.5 ° C, during operation. After Suturing, the animals were kept under constant observation until they regained awareness and were able to be stabilized.

Nissl staining

Neuronal cell loss was examined by using a cresyl violet stain. Only cells consistent of a distinct nucleus and nucleolus are deemed alive and healthy in the Nissl technique. In this method, paraffin sections 10-µm in thickness were directly mounted onto gelatin-coated glass slides and air-dried according to standard protocols, then stained with 5% Cresyl violet, dehydrated and cover-slipped with Entellan. Eight photomicrographs were made from each animal (at a level of 2.3–5 mm posterior to the bregma fortune, in accordance with the Paxinos atlas).

Three of them were chosen and counted by a blind examiner under 400x magnification in a light microscope. Images were taken at×400 magnification with a microscope (OlympusAX-70, Japan) and examined by applying Imaging-Pro-Plus (LEICA DMLB, Germany) software.

Statistical analysis

The data was evaluated by using One-way ANOVA and post hoc Tukey for comparing groups. The significance level was set at P<0.05.

Ethical considerations

Animals were housed in regular laboratory circumstances (light for 12 hours and darkness for 12 hours), with a temperature of 22 to 24 degrees Celsius and a humidity of 50%. The rodents had access to plenty of water and food. This initiative is based on the Islamic Azad University, Tehran Medical Sciences, approved methodology for keeping and working with laboratory animals, as well as current scientific solutions.

Results

Data revealed that clamping the common carotid arteries for 20 minutes may decrease the number of viable neurons in the CA2 and CA3 regions of hippocampus, so there was remarkable deference between the control and ischemia groups. (p<0.05), (p<0.05) Also, the mean number of viable cells in the CA2 and CA3 areas of the hippocampus were similar in the vehicle and ischemia groups. While this reduction was markedly improved by administering ubiquinone (coenzyme Q 10 at a dose of 100 mg / kg) in the experimental group. So that no significant difference between the control and experimental groups was seen (p=0.576), (P=0.321) (Chart 1, 2 and Figure 1, 2).

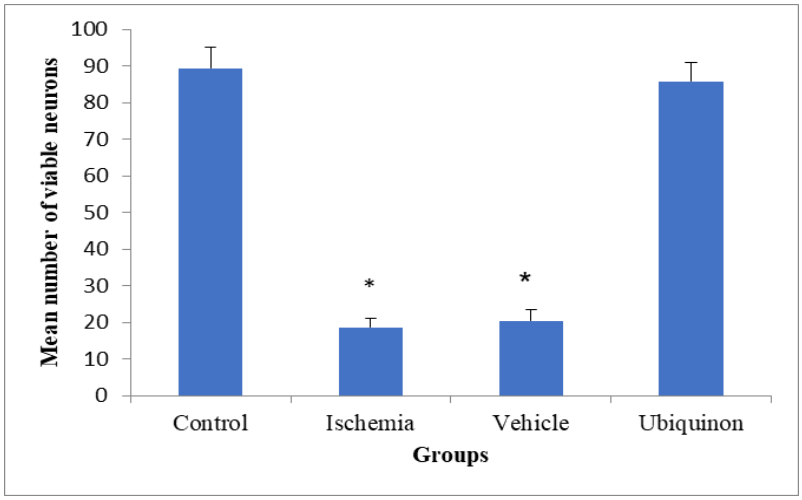

Chart 1. Quantitative analysis of Nissl staining showed that in the ischemic group, the number of viable cells in the CA2 area was significantly decreased in comparison with control group. The resulting ubiquinone also

Journal of Otorhinolaryngology and Facial Plastic Surgery 2023;9(1):1-8.
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ameliorated this reduction in the experimental group. Data are presented as mean ± standard deviation. * P<0.05 vs. control group.

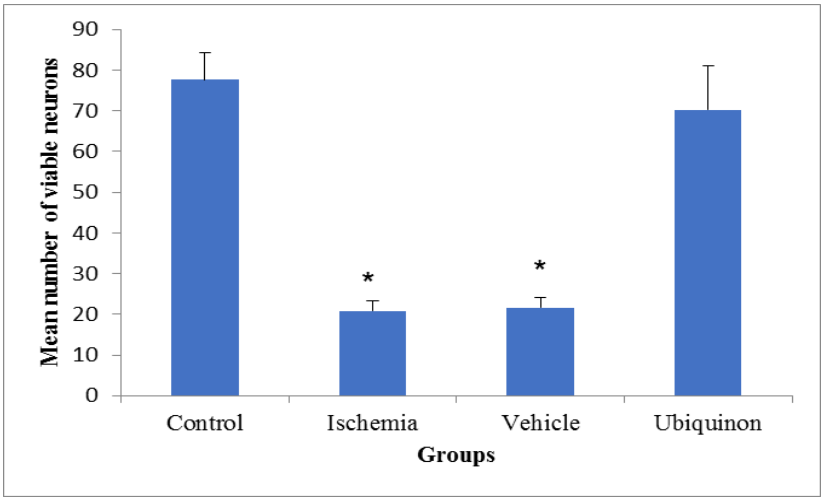

Chart 2. The quantitive analysis of Nissl staining showed that, in the Ischemia group, the number of viable cells of CA3 region, was significantly reduced in comparison with control group. Besides, received ubiquinone ameliorated this reduction in experimental group. Data are presented as mean± standard deviation. * P<0.05 vs. control group.

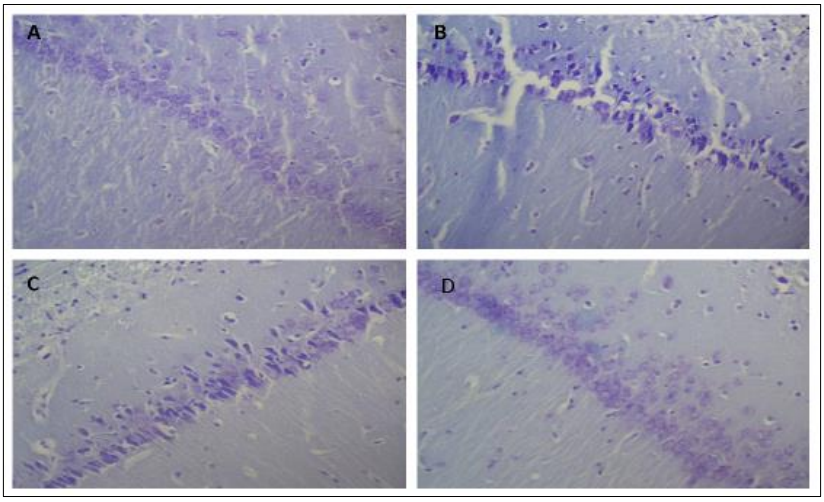

Figure 1. Photomicrograph of CA2 cells in different groups, Nissel staining with X400 magnification (A: control B: Ischemia C: Vehicle D: ubiquinon groups).

Journal of Otorhinolaryngology and Facial Plastic Surgery 2023;9(1):1-8.
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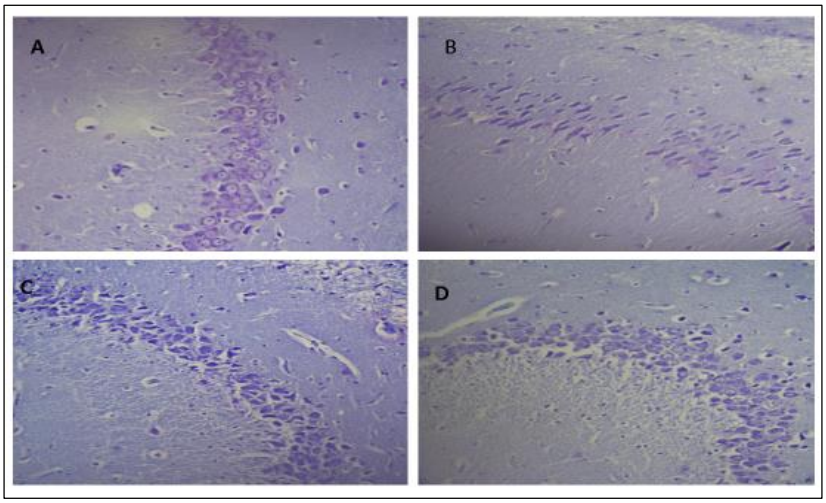

Figure 2. Photomicrograph of CA3 cells in different groups, Nissel staining with X400 magnification (A: Control B: Ischemia C: Vehicle D: ubiquinol groups).

This result also indicated that ischemia could not cause a marked reduction in the number of viable neurons in the hippocampal CA4 area. So, there was no remarkable statistically deference between the four groups (p=0.103), (Chart 3 and Figure 3).

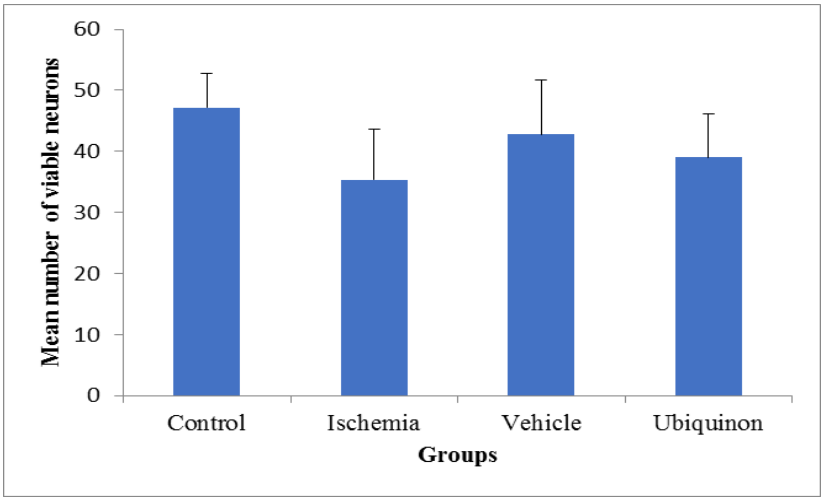

Chart 3. Quantitative analysis of Nissl staining showed that ischemia did not cause a notable reduction in the number of surviving neurons in the hippocampal CA4 region. There were no remarkable statistical differences among the four groups. Data are presented as mean ± standard deviation.

Journal of Otorhinolaryngology and Facial Plastic Surgery 2023;9(1):1-8.
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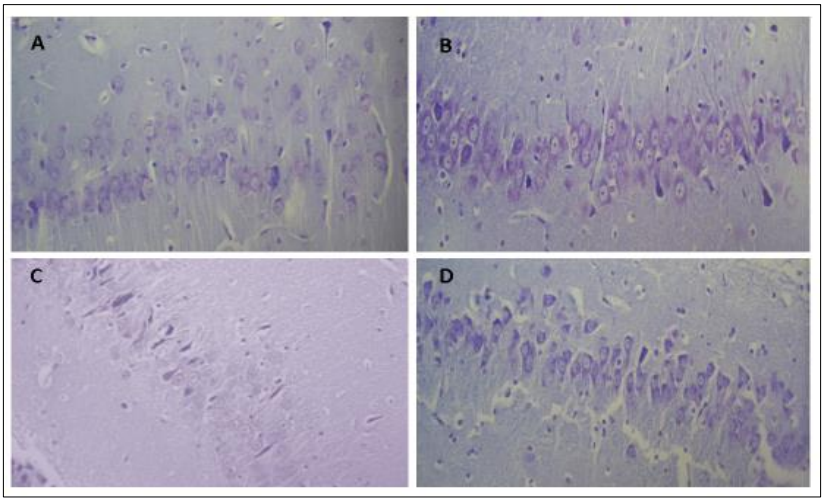

Figure 3. Photomicrograph of CA4 cells in different groups, Nissel staining with X400 magnification (A: Control B: Ischemia C: Vehicle D: Ubiquinone groups).

Discussion

This study revealed that cerebral ischemia / reperfusion reduces the number of pyramidal cells in the CA2, CA3 areas of hippocampal tissue with no effect on cells in the CA4 region. Our data also showed that administration of ubiquinone at a dose of 100 mg / kg attenuated the effects of transient global I/R injury. Coenzyme Q10 is one of the substances that play a crucial role in the production of cellular organs, especially mitochondria, and its protective antioxidant role, both directly through the reaction with free radicals and indirect role by increasing the levels of ascorbic acid and tocopherol (23, 24). It was proved that CoQ10 can prevent lipid peroxidation, reduce lactate accumulation, protect against adenosine triphosphate removal, and regulate cytoplasmic calcium homeostasis by modulating energy production (25-27). Reduction of caspase-3 (as a key enzyme in apoptosis), with coenzyme Q10 as an anti-apoptotic agent was proved (28). In studies by Kalayci et al., administration of CoQ10 after brain trauma, it significantly reduces the increased level of malonyl di aldehyde (as a product of lipid peroxidation). It should also be noted that the biochemical roles and mechanisms mentioned about coenzyme Q10 justify its positive effects on brain tissue and confirm our results on the positive effect of coenzyme Q10. Decreased tissue levels of coenzyme Q10 androgen following transient cerebral ischemia have been proved (29). Numerous studies have been performed on the role of coenzyme Q10 in ameliorating the biochemical changes resulting from cerebral reperfusion ischemia. However, histological studies on the effect of coenzyme Q10 following ischemia/reperfusion are not numerous.

According to several studies that have been carried out on the role of coenzyme Q10 in several neurodegenerative diseases, this study inquired the effect of coenzyme 010 following cerebral ischemia/reperfusion. The important issue that can play an essential role in the recovery of brain lesions is the timing of drug administration. Since the brain lesions caused by ischemia reach their maximum within 2-3 days after ischemia induction (30, 31).

Due to the onset of action of coenzyme Q10 after 6-8 hours, its secondary plasma peak occurred after 24 hours due to intestinal-hepatic cycle (32), coagulation of coenzyme Q10 in mice, 5 days before and 3 days after CoQ10 after induction of ischemia and perfusion.

In biochemical research on the effect of coenzyme Q10 on ameliorating the changes induced by ischemia /reperfusion, coenzyme Q10 was administered only before the time of

Journal of Otorhinolaryngology and Facial Plastic Surgery 2023;9(1):1-8.
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induction of ischemia/reperfusion (33, 34). But Delayed death of neurons due to ischemia/reperfusion as well as onset of effect and secondary plasma peak of coenzyme Q10 also needs to be considered.

Conclusion

This study showed that ischemia / cerebral reperfusion reduces the number of pyramidal cells in the CA2, CA3 areas of hippocampal tissue, but has no effect on cells in the CA4 region. It was also revealed that administration of ubiquinone at a dose of 100 mg / kg attenuated the effects of transient global I/R injury. The results support the use of coenzyme Q10 as a neurotrophic substance and as an adjunctive therapy in patients at risk for ischemic stroke.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Ethics

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References

1. Chuang DM, Chen RW, Chalecka- Franaszek E, Ren M, Hashimoto R, Senatorov V, Kanai H, Hough C, Hiroi T, Leeds P. Neuroprotective effects of lithium in cultured cells and animal models of diseases. Bipolar disorders. 2002 Apr;4(2):129-36.
2. Amarenco P, Bogousslavsky J, Caplan LR, Donnan GA, Hennerici MG. Classification of stroke subtypes. Cerebrovascular diseases. 2009;27(5):493-501.
3. Bokura H, Robinson RG. Long-term cognitive impairment associated with caudate stroke. Stroke. 1997 May;28(5):970-5.
4. Brådvik B, Sonesson B, Holtås S. Spatial impairment following right hemisphere transient ischaemic attacks in patients without carotid artery stenosis. Acta neurologica scandinavica. 1989 Nov;80(5):411-8.
5. Godefroy O, Rousseaux M, Pruvo JP, Cabaret M, Leys D. Neuropsychological changes related to unilateral lenticulostriate infarcts. Journal of Neurology, Neurosurgery & Psychiatry. 1994 Apr 1;57(4):480-5.
6. Pulsinelli WA, Brierley JB. A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke. 1979 May;10(3):267-72.
7. Zola-Morgan S, Squire LR, Amaral DG. Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. Journal of Neuroscience. 1986 Oct 1;6(10):2950-67.
8. Amaral DG, Scharfman HE, Lavenex P. The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). Progress in brain research. 2007 Jan 1;163:3-790.
9. Saab BJ, Georgiou J, Nath A, Lee FJ, Wang M, Michalon A, Liu F, Mansuy IM, Roder JC. NCS-1 in the dentate gyrus promotes exploration, synaptic plasticity, and rapid acquisition of spatial memory. Neuron. 2009 Sep 10;63(5):643-56.
10. Cameron HA, Mckay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. Journal of comparative neurology. 2001 Jul 9;435(4):406-17.
11. Gould E, Tanapat P, McEwen BS, Flügge G, Fuchs E. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proceedings of the National Academy of Sciences. 1998 Mar 17;95(6):3168-71.
12. Gold BG, Udina E, Bourdette D, Navarro X. Neuroregenerative and neuroprotective actions of neuroimmunophilin compounds in traumatic and inflammatory neuropathies. Neurological research. 2004 Jun 1;26(4):371-80.
13. Gold BG. Neuroimmunophilin ligands: evaluation of their therapeutic potential for the treatment of neurological disorders. Expert opinion on investigational drugs. 2000 Oct 1;9(10):2331-42.
14. Dumont FJ. FK506, an immunosuppressant targeting calcineurin function. Current medicinal chemistry. 2000 Jul 1;7(7):731-48.
15. Gummert JF, Ikonen T, Morris RE. Newer immunosuppressive drugs: a review. Journal of the American Society of Nephrology. 1999 Jun 1;10(6):1366-80.
16. Giordani F, Benetolli A, Favero-Filho LA, Lima KC, Cestari Junior L, Milani H. Tacrolimus (FK506) reduces ischemia-induced hippocampal damage in rats:

Journal of Otorhinolaryngology and Facial Plastic Surgery 2023;9(1):1-8.
https://doi.org/10.22037/orlfps.v9i1.43937

a 7-and 30-day study. Brazilian journal of medical and biological research. 2003 Apr;36(4):495-502.
17. Gold BG, Zeleny-Pooley M, Wang MS, Chaturvedi P, Armistead DM. A nonimmunosuppressant FKBP-12 ligand increases nerve regeneration. Experimental neurology. 1997 Oct 1;147(2):269-78.
18. Benetoli A, Paganelli RA, Giordani F, Lima KC, Fávero Filho LA, Milani H. Effect of tacrolimus (FK506) on ischemia-induced brain damage and memory dysfunction in rats. Pharmacology Biochemistry and Behavior. 2004 Mar 1;77(3):607-15.
19. Sasaki T, Hamada J, Shibata M, Gotoh J, Araki N, Fukuuchi Y. FK506 abrogates delayed neuronal death via suppression of nitric oxide production in rats. Brain research. 2004 May 29;1009(1-2):34-9.
20. Tokime T, Nozaki K, Kikuchi H. Neuroprotective effect of FK506, an immunosuppressant, on transient global ischemia in gerbil. Neuroscience letters. 1996 Mar 15;206(2-3):81-4.
21. Chwiej J, Janeczko K, Marciszko M, Czyzycki M, Rickers K, Setkowicz Z. Neuroprotective action of FK-506 (tacrolimus) after seizures induced with pilocarpine: quantitative and topographic elemental analysis of brain tissue. JBIC Journal of Biological Inorganic Chemistry. 2010 Feb;15(2):283-9.
22. Nishinaka Y, Sugiyama S, Yokota M, Saito H, Ozawa T. Protective effect of FK506 on ischemia/reperfusion-induced myocardial damage in canine heart. Journal of cardiovascular pharmacology. 1993 Mar 1;21(3):448-54.
23. Lenaz G, Fato R, Formiggini G, Genova ML. The role of Coenzyme Q in mitochondrial electron transport. Mitochondrion. 2007 Jun 1;7:S8-33.
24. Niklowitz P, Sonnenschein A, Janetzky B, Andler W, Menke T. Enrichment of coenzyme Q10 in plasma and blood cells: defense against oxidative damage. International Journal of Biological Sciences. 2007;3(4):257.
25. Mellors AA, Tappel AL. The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol. J Biol Chem. 1966 Oct 10;241(19):4353-6.
26. Aliev G, Palacios HH, Walrafen B, Lipsitt AE, Obrenovich ME, Morales L. Brain mitochondria as a primary target in the development of treatment strategies for Alzheimer disease. The international journal of biochemistry & cell biology. 2009 Oct 1;41(10):1989-2004.
27. Jassem W, Fuggle SV, Rela M, Koo DD, Heaton ND. The role of mitochondria in ischemia/reperfusion injury. Transplantation. 2002 Feb 27;73(4):493-9.
28. Amarenco P, Bogousslavsky J, Caplan LR, Donnan GA, Hennerici MG. Classification of stroke subtypes. Cerebrovascular diseases. 2009;27(5):493-501.
29. Mancuso M, Orsucci D, Volpi L, Calsolaro V, Siciliano G. Coenzyme Q10 in neuromuscular and neurodegenerative disorders. Current drug targets. 2010 Jan 1;11(1):111-21.
30. Sarter B. Coenzyme Q10 and cardiovascular disease: a review. Journal of Cardiovascular Nursing. 2002 Jul 1;16(4):9-20.
31. Turunen M, Olsson J, Dallner G. Metabolism and function of coenzyme Q. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2004 Jan 28;1660(1-2):171-99.
32. Sohal RS, Kamzalov S, Sumien N, Ferguson M, Rebrin I, Heinrich KR, Forster MJ. Effect of coenzyme Q10 intake on endogenous coenzyme Q content, mitochondrial electron transport chain, antioxidative defenses, and life span of mice. Free Radical Biology and Medicine. 2006 Feb 1;40(3):480-7.
33. Choi DW. Excitotoxic cell death. Journal of neurobiology. 1992 Nov;23(9):1261-76.
34. Benetoli A, Dutra AM, Paganelli RA, Senda DM, Franzin S, Milani H. Tacrolimus (FK506) reduces hippocampal damage but fails to prevent learning and memory deficits after transient, global cerebral ischemia in rats. Pharmacology Biochemistry and Behavior. 2007 Nov 1;88(1):28-38.