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Original Article:

Ubiquinone [Q10] and Dentate Gyrus Ischaemia Reperfusion Injury in a Rat Model of Brain Ischaemia

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Abstract

Introduction: This research was conducted to evaluate the effect of ubiquinone following ischemia/reperfusion on dentate gyrus in Wistar rats.

Materials and Methods: Twenty-four Rats were randomly assigned to four groups [n=6] . Common carotid arteries were bilaterally closed for 20 minutes in order to perform ischemic model. Four days later, all rats were slaughtered and hippocampal tissue was examined by Nissl staining method.

Results: The data showed that ubiquinone had a neurotrophic effect on dentate gyrus cells of hippocampal region in ischemia/reperfusion model.

Conclusion: Our findings adduce the argument that ubiquinone can be used to treat brain lesions following ischemia.

Keywords: Dentate gyrus, Ubiquinone, Wistar rat

1. Introduction

As an important cause of disability and death among adults, ischemia is one of the biggest problems of today's world. Additionally, given the potential of cerebral ischemia to create long-term irreversible seizures, it is a suitable area for scientific research to prevent consequences as much as possible. Cerebral ischemia leads to sensory-motor disorders, aphasia - visual impairment, and apraxia as well as agnosia. In cerebral ischemia, due to blood flow reduction and consequently fall in brain oxygen, the brain metabolites decreases, and hence the death of body tissues [1-3]. The hippocampus plays a key role in the formation of new memory and the analysis of spatial information [4]. This area is supplied by the anterior carotid artery from the internal carotid branch. This artery is prone to thrombosis due to its anatomical structure and its thin wall [5]. New cells develop in two different zones in the hippocampus which are called the subventricular zone and dentate gyrus. They proliferate due to damage to the hippocampal region with their neurogenic capacity

for repair [6-8]. So far, the protective effects of more than 100 therapeutic agents on programmed cell death has been proven in laboratory models [9]. Unlike the promising results obtained from animal models in preventing programmed cell death, no effective pharmacological strategy has unfortunately been established to approach ischemia. This may be as a result of inefficiency and side effects of ubiquinone [10]. The use of ubiquinone has recently been considered a new and appropriate approach with a neuroprotective role [11-14]. Coenzyme Q10 [a Special lipophilic anti-oxidant] is one of the essential components of the mitochondrial electron transport chain [15]. Coenzyme Q10 is also associated with the production of adenosine triphosphate [ATP] and also improved maintenance of cognitive tasks. Following cerebral ischemia, the release of free oxygen radicals occurs [16]. The formation or distribution of these radicals must be reduced in order to repair the adverse neurological consequences of neuronal damage. The use of coenzyme Q10, which is resistant to oxidative stress and promotes the brain's bioenergy along with reperfusion after ischemic brain injury, seems logical. In animal studies, it has been shown that coQ10 [it reduces the amount of caspase-3 [cpp32] is a neuroprotective agent with an antiapoptotic function [17]. Antioxidants such as coenzymes Q10 can be effective in reducing the cytosolic activity of LDH and $Ca2^{+}$ levels, which increases following ischemia/ perfusion [18]. There are many reports on the protective effects of coQ10 on the tissue surface. Yet, little research has been done on the neurotrophic effect of this substance so far. Given that, this study evaluated the effect of ubiquinone on the dentate gyrus cells in male Wistar rats in the model of pervasive ischemia.

2. Materials and Methods

In this study, twenty-four male Wistar rats [220-250g, 6 weeks old] were used. The animals, provided by Pasteur Institute in Tehran, were housed under normal conditions. Cages were exposed to a standard 12:12 hour light:dark cycle. Temperatures ranged from 22 to 24 degrees Celsius, with a humidity of 45 % to 5%. Food and drink were provided in sufficient quantities for rats.

Groups and Designs

Animals were randomly divided into four groups [n=6] as follows:

1. Control group: Animals were kept in standard laboratory conditions and were sacrificed after four days.

2. Ischemia group: ischemia/reperfusion were inducted for 20 Minutes by bilateral closure of common carotid arteries followed by reperfusion.

3. The experimental group received Q10 [100 mg/kg] via gavage for five days, and then on the fifth day, ischemia followed by reperfusion was inducted. Then, the rats received the coenzyme Q10 again for three consecutive days, and on the fourth day, the mice were killed and their brains were prepared for Nissl staining. CoQ10 was dissolved in soybean oil as a vehicle

4. Vehicle group: Rats received only the solvent of coenzyme Q10, soybean oil, for the first five days and underwent ischemia for 20 minutes followed by reperfusion in the same manner as other groups. They were again soaked in soybean oil for three days.

Surgical procedure

Midline incision was exerted on the neck after anesthetizing by Pentobarbital sodium [40 mg/kg] [IP], then bilateral carotid arteries were isolated and detached from the vagus nerve and closed by microsurgical clamps. After 20 minutes, the clamps were removed and the blood flow was restored and withdrawn blood re-infused [19]. The body temperature of animals were maintained at around 37°C with a heating pad and monitored by a rectal thermometer throughout the procedure. At the end of the ischemia, the incision was sewn by suture thread and the animals were kept under observation until they were awake and were then kept in separate cages.

Histopathology studies

Nighty-six hours after ischemia, rats were killed and perfused with PBS, followed by four percent paraformaldehyde at 7.4 pH. For three days the brains, before being embedded in paraffin, were postfixed at 4°C. Coronal Sections were obtained by using a rotary microtome and mounted onto gelatin-coated glass slides with a thickness of 10 μm and a distance of 2.3 to 5 mm from the posterior of the bregma .After fixing and preparation coronal sections were stained using the Nissl technique. An optical microscope with an amplification of 400 was utilized to examine the samples. Neurons with a distinct nucleolus and nucleus were only deemed healthy and alive. Eight photomicrographs were randomly picked with a minimum distance of 40 mm from each sample.

Image Tools 2 software was used to count granular

neurons in the dentate gyrus area, and the mean counts were calculated.

Statistical analysis

All the data were analyzed using SPSS 23 software. One-way ANOVAs were utilized to compare the differences between the means of the groups followed by post hoc Tukey's. The data are expressed as the mean ± SD. Significance was defined as $p < .05$

Ethical considerations

All experimental animals were treated in accordance with institutional guidelines and the *Guide for the Care and Use of Laboratory Animals* recommended by Islamic Azad University of Tehran medical Sciences. [Approval number: IR.IAU.TMU.REC. 1397.239].

3. Results

Effects of ubiquinone on dentate gyrus area of hippocampus [Nissl staining]

Coronal sections of brain in different groups stained with cresyl violet [Nissl] were measured, and the number of neurons of hippocampal dentate gyrus was evaluated [Table1, chart 1]. Many damaged neurons with diminished cytoplasm and pyknotic nuclei were observed in I/R and I/R + soybean oil groups [Figure 1B, D] while no apparent morphological changes in the control group was observed [Figure 1 A]. Integrity of neurons within the dentate gyrus was maintained by administration of ubiquinone [Figure 1 C]. The number of intact neurons in I/R group compared with the control group was remarkably lowered. Treatment with ubiquinone considerably prevented the neuron loss compared with that in I/R and I/R+ soybean oil groups.

Table 1. Mean data in each group

Group	Mean	N	Std. Deviation
Control	133.83	6	17.938
Ischemia	78.50	6	10.445
Experimental	131.17	6	8.841
Vehicle	82.67	6	6.772
Total	106.54	24	28.757

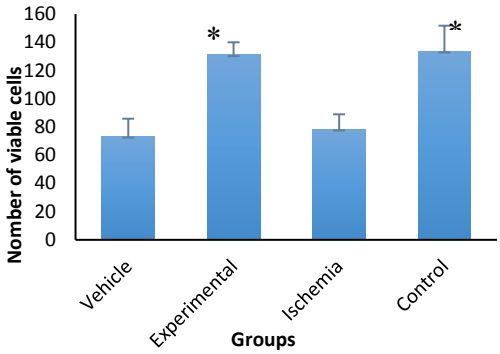

Chart 1. Mean number of viable cells in each group

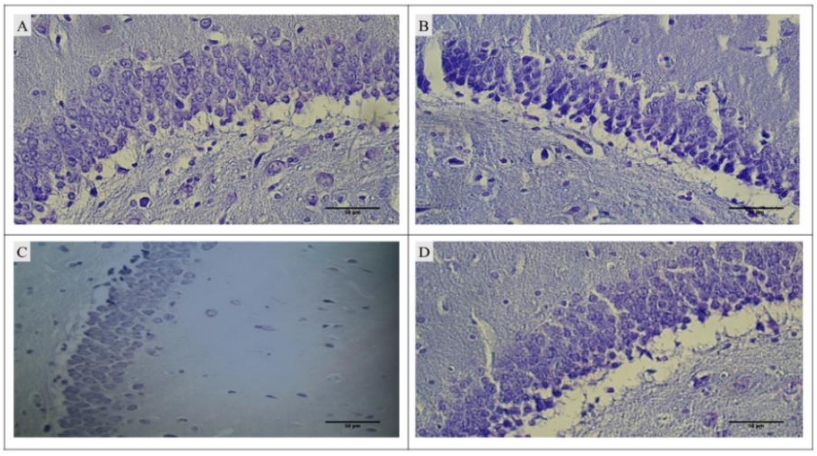

Figure 1. Dentate gyrus area of A: Control group, B:Ischemic group, C:Ubiquinone group, D: Vehicle group [Magnification 40X], Nissl staining

4. Discussion

Given the importance of ischemia in the world and its both short-term and long-term consequences, this study attempted to investigate the neurotrophic effect of ubiquinone on neurons in the dentate gyrus, which is one of the few areas of the brain that has its own neurogenesis. It was observed that the number of viable neurons was noticeably reduced following the development of perfusion ischemia in the dentate gyrus area of the rat hippocampus. In compliance with this research, cerebral blood flow is limited during cerebral ischemia, and since most of this energy is spent on ionic balance on both sides of the cell membrane, the process of electrolyte imbalance begins with ischemia. Eventually, neurons try to supply ATP through anaerobic glycolysis, which produces lactic acid and lowers the PH within the neurons, resulting in more damage [20]. Damage to this area is not only due to ischemia but also to the creation of reperfusion by releasing free radicals intensifying this damage. This will also lead to apoptosis, necrosis and an inflammatory response due to the return of white blood cells to the ischemic area [21]. Ubiquinone [Coenzyme Q10] is a fat-soluble antioxidant in plasma membranes and lipoproteins produced inside cells through pathways containing mitochondrial multi-proteins. The coenzyme Q10 consists of a benzoquinone ring and a polyisoprenoid tail with about 6 to 10 subtypes that vary in species and maintain stability in the molecule between the phospholipid bilayers. This substance is one of the substances in cellular organs, especially mitochondria that play a significant role in energy production by ATP. It also exerts its antioxidant role directly by reacting with free radicals and indirectly by raising the amount of ascorbic acid and tocopherol [22]. Coenzyme Q10 induces electron transfer, which is involved in the synthesis of adenosine triphosphate. As a result, due to the need for the vast majority of the body's functions for adenosine triphosphate, ketone plays a decisive role in maintaining tissue function and [23] Also, given the fact that in ischemia the disruption of electrolytes on both sides of the cell membrane associated with ATPase causes ATP not to be supplied and burned in the usual way and lactic acid is produced, receiving coenzyme Q10 improves this defect during ischemia. Zinc, on the other hand, has been shown to reduce the tissue level of endogenous coenzyme Q10 following transient cerebral ischemia [24]. In fact, the biochemical role of coenzyme Q10 and its positive effects on brain tissue have been proven in our study. This coenzyme is one of the most prominent lipid antioxidants preventing the formation of free radicals and also the damage caused by ischemic reperfusion such as apoptosis. Apart from that, its neuroprotective properties have been pointed out in a number of studies. In our study, coenzyme Q10 caused a significant decrease in the number of damaged neurons in the recipient group following ischemia-perfusion. In a study conducted by Salehpour et al in 2019, rats developed global ischemia, and after receiving coenzyme Q10 and Photobiomodulation [PBM], it was shown that cognitive impairment after global ischemia was improved both in combination with these two drugs and individually [25]. This study agrees with the results of our study on the neurotrophic futures of coenzyme Q10. A 2019 study by Nasoohi et al showed that coenzyme Q10 intake improved ischemic defects, even in mice with statin toxicity [26]. A study by Soleimani et al in 2018 on the positive effect of coenzyme Q10 on the damaged hippocampus of mice demonstrated that coagulation of Coenzyme Q10 at a dose of 10 mg / kg twice a day for two weeks had positive effects on hippocampal damage in mice, which confirms our findings [27]. Xu et al In 2017 studied the influence of coenzyme Q10 on cognitive impairment caused by sevoflurane for anesthesia and showed that coenzyme Q10 reminded this cognitive disorder [28]. In the present study, the neurotrophic properties of coenzyme Q10 were confirmed. Study of Lu et al in 2017 on the effects of coenzyme Q10 on improving brain injury due to reperfusion ischemia in mice with hyperglycemia indicated that pretreatment with coenzyme Q10 can improve the damage caused by cerebral ischemia and reperfusion in patients with hyperglycemia [29]. This study confirms our findings.

5. Conclusion

The findings of the present study support ubiquinone [coenzyme Q10] as a neurotrophic substance as well as adjunctive therapy in patients with ischemic stroke.

Ethical Considerations

Compliance with ethical guidelines

All experimental animals were treated in accordance with institutional guidelines and the Guide for the Care and Use of Laboratory Animals recommended by Islamic Azad University of Tehran medical Sciences. [Approval number: IR.IAU.TMU.REC.1397.239].

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Author's contributions

Conceptualization and Supervision: Zahra Nadia Sharifi; Methodology: Shabnam Movassaghi; Investigation, Writing and editing: All authors; Data collection and Data analysis: Nilofar Talebi Tadi and Mohammad Mahdi Nazarnejad.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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