Coenzyme Q10 Protects Hippocampal Neurons against Ischemia/ Reperfusion Injury via Modulation of BAX/Bcl-2 Expression

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Article info:
Received: 4 December 2012
First Revision: 15 January 2012
Accepted: 31 August 2012

Key Words:
CoQ10,
Hippocampus,
Ischemia-Reperfusion,
Neuroprotective Effect.

ABSTRACT

Introduction: Preliminary studies have confirmed reduction in cell death following treatment with antioxidants. According to this finding we study the relationship between consumption of CoQ10 and expression of Bax and Bcl2 in hippocampus following ischemia/reperfusion as proteins involved in cell programmed death or apoptosis.

Methods: We studied the protective role of CoQ10 against ischemia-reperfusion. Experimental design includes four groups: intact, ischemic control, sham control and treatment group with CoQ10. The mice were pre-treated with CoQ10 for a week, then ischemia was induced by common carotid artery ligation and following the reduction in inflammation (a week) the mice was treated with CoQ10. Nissl staining was applied for counting the necrotic cells of hippocampus and the western blot was performed to measure the Bax and Bcl2 expression.

Results: Cell death was significantly lower when mice were treated with CoQ10. Bax expression was significantly high in the ischemic group but low in the treatment group, and the bcl2 expression was lower in the ischemic group than the treatment and the vehicle groups.

Discussion: Ischemia for 15 minutes induced cell death in hippocampus with more potent effect on CA1. CoQ10 intake significantly reduced cell death and prevented the expression of Bax while inducing an increase in expression of bcl2.

1. Introduction

Cerebral ischemia is a critical clinical issue with no satisfactory cure that can be fatal and disabling, requiring long-term treatment and heavy financial expenses (Aliev et al, 2008; Camarata et al, 1994; David et al, 2002).

Hippocampus is involved in memory formation and spatial information processing and is among the first areas of the brain affected by ischemia/reperfusion and hypoxic conditions.

Ischemia and hypoxia can cause cells to have anaerobic respiration to survive (Zhang et al, 1994), but may also lead to acidosis and lactate accumulation and cell death. During reperfusion, release of oxygen free radicals and other oxidative may cause damage to cells, with even greater intensity than the ischemia. Rapid medical intervention may reduce cell death (Turunen et al, (2004).

Antioxidants are substances that remove free radicals, preventing damage to cell membranes, DNA, and cell death. Coenzyme Q10 (CoQ10) is a lipid-soluble cofactor found naturally in the inner mitochondrial membrane

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of human cells (Lehninger et al, 1971) as a component of the mitochondrial electron transport chain that is known as a potent antioxidant and membrane stabilizer.

CoQ10 can be used to improve neurological outcomes and preventing neuronal damage by reducing the production of free radicals of oxygen induced by ischemia and reperfusion. Various studies have shown that CoQ10 is efficient in the treatment of ischemia-reperfusion injury (Portakal et al, 1999; Ostrowski et al, 1999). CoQ10 has also been found to prevent lipid peroxidation, a major cause of damage by free oxygen radicals, in addition, transient cerebral ischemia leads to decrease in tissue levels of CoQ10 (Mellors et al, 1966). Furthermore, it has also been reported that CoQ10 administration could lessen the diameter of ischemic lesions in animal models (Takayanagi et al, 1980; Lemke et al, 1990; Piotrowski et al, 1998).

The present study was done to test whether oral supplementation of coenzyme Q10 could protect against hippocampus ischemia-induced cell death in an experimental model of brain stroke.

2. Methods

2.1. Animals

Twenty eight adult BALB/c mice, weighting 30-35 g, were obtained from Iranian Razi Institute. Mice were maintained in one colony room at temperature of 21 ± 1C (50 ± 10% humidity) on a 12-h light/12-h dark cycle with access to water and food ad libitum.

2.2. Experimental Design

The mice were assigned as follows:

1. Intact group (n=7)
2. Ischemia group (n=7)
3. Vehicle group (n=7)
4. Treatment group (n=7)

Ischemia induction: Ischemia was induced by clamping common carotid arteries for 15 min. CoQ10 Administration: CoQ10 (Sigma) was prepared as powder and sesame seed oil (Sigma) was used as a solvent. In order to evaluate the protective effect of CoQ10 pretreatment, it was administrated during a week prior to ischemia induction (450 mg/kg daily) and seven days of administration after reduction of inflammation on ischemic zone at day 7 following the induction of ischemia.

Nissl staining: The animals were sacrificed and brain tissue perfused with paraformaldehyde 4% (Sigma) prior to autopsy, followed by fixation with paraformaldehyde 4% for one week. Sections were deparaffinized in xylene and then hydrated and rinsed in distilled water and stained with 0.1% cresyl violet solution for 3-10 minutes, followed by quick rinse in distilled water and dehydrated in alcohol and cleared in xylene and mounted with permanent mounting medium.

2.3. Western Blotting

Following the ischemia reperfusion, animals were sacrificed and hippocampus tissues were removed and washed with saline and homogenized in ripa buffer (Sigma) and anti-protease cocktail for 60 seconds and then were incubated in the same buffer and cocktail for 30 min and vortex-mixing every 10 minutes. All steps were carried at 4oC. The homogenate was filtered and centrifuged at 1200 RPM for 20 minutes (Universal 16L, Hettich, Germany). The resulting supernatants (lysates) were frozen at - 80oC for further investigation. The protein concentration was determined by Bradford's method, using bovine serum albumin as a calibrator.

Twenty micrograms of cell lysate proteins were mixed with 5 µL of loading buffer (50 mmol/L tris, 20g/L sodium dodecyle sulphate, 100 mL/L glycerol, 100 mmol/L β -mercaptoethanol and 0.05% bromephenol blue solution,(pH 6.8)) and were boiled for 5 minutes and separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The gels were transferred to nitrocellulose membranes in Tris-glycine buffer (25 mmol/L Tris, 192 mmol/L glycine, 200 mL/L methanol, pH 7.4) for 5 hours at 60 V.

The nitrocellulose sheets were washed and free binding sites were saturated with 50g/L bovine serum albumin in Tris-buffered saline buffer (50 mmol/L tris, pH 7.5, 150 mmol/L NaCl, 2 mmol/L EDTA) for 1 hour at room temperature. Then, the membranes were incubated with phosphate buffer saline and mouse monoclonal anti-rat Bcl-2 antibody was diluted 1:300 (by volume) overnight at 4ºC and then rabbit anti-mouse IgG alkaline phosphatase conjugate was diluted 1:500 for 90 min at room temperature.

Finally, the membranes were incubated with BCIP/NBT (nitro-blue tetrazolium chloride) / (5-bromo-4-chloro-3'-indolyphosphate p-toluidine salt) alkaline phosphatase substrate solution (at room temperature) until the developed bands were of desired intensity. Then the reaction was stopped by 200 mL of 0.5 mol/L

EDTA (pH 8) and 50 mL of phosphate buffered saline. Bax and Bcl-2 protein bands were identified by comparing with the molecular weight marker.

2.4. Data Analysis

Data are expressed as means ± SEM. Comparison of data was performed by one-way ANOVA. $P < 0.05$ was considered to be statistically significant.

3. Results

In this study we used the Nissl staining to compare the necrotic and normal cells and western blotting to measure the expression of Bax and bcl2.

3.1. Cresyl Violet Staining

Call death was less in animals treated with CoQ10 compared to the vehicle and ischemia groups (Figure 1). CoQ10 significantly increases cell density compared to the ischemic group (Figure 2). Our finding suggested that CoQ10 might protect hippocampal CA1 cells against ischemia/reperfusion.

3.2. Western Blotting

Western blotting analysis revealed that pretreatment of CoQ10 could significantly ($P<0.05$) down-regulate Bax expression, a pro-apoptotic protein. Oral consumption of CoQ10, significantly up-regulated Bcl2 (antiapoptotic) expression in the treatment group compared to the ischemic group (figure 3).

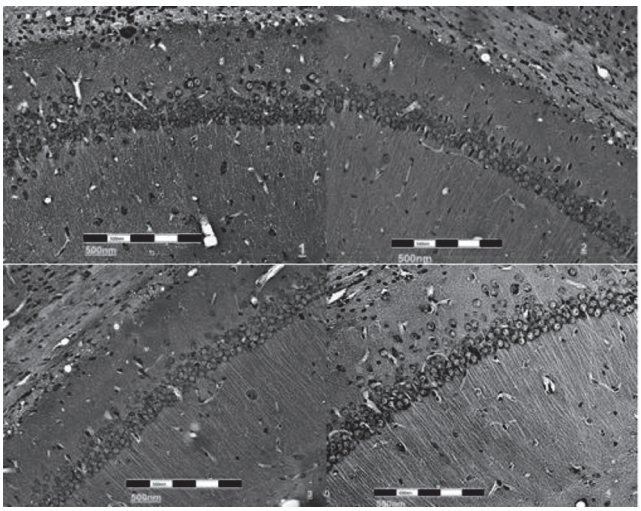

Figure 1. the nissl staining result in 4 groups. In this staining method the necrotic cells indicated with dark and compact nucleus. 1. The intact group without necrotic cell, 2. The ischemic group with a lot of necrotic cells 3. The vehicle group with less necrotic cell, 4. The treatment group showed infrequent necrotic cell.

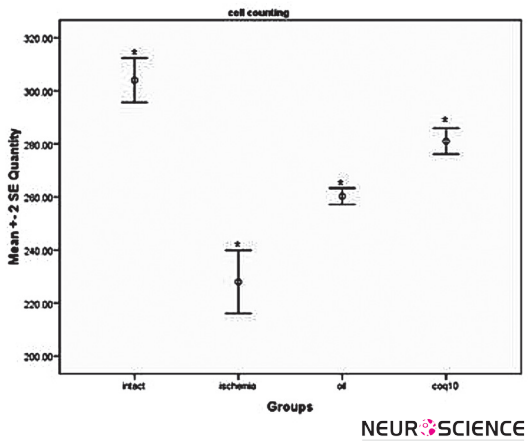

Figure 2. Comparison of the density of healthy cells in the CA1 region of hippocampus. Cell density of the treatment groups is significantly different than ischemic group. ($P<0.05$) *

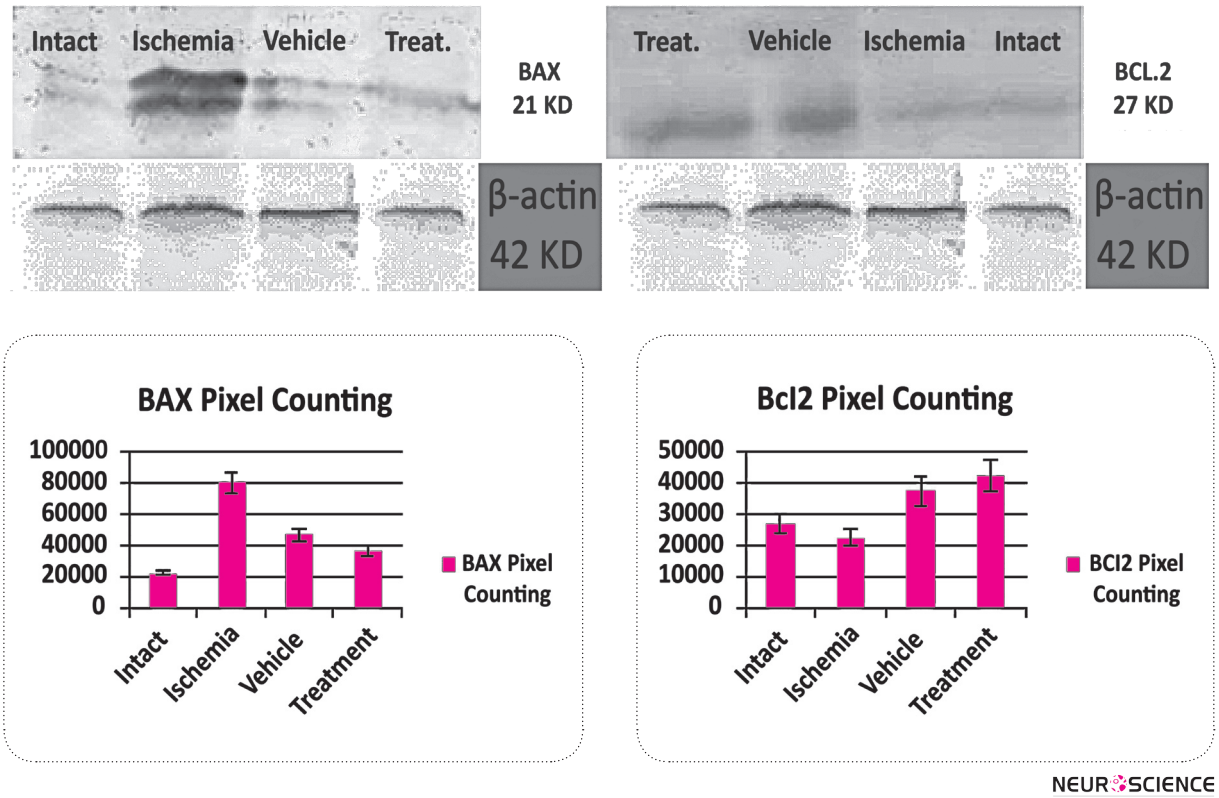

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Figure 3. Western blotting for Bax and Bcl2 protein expression. Bax (pro-apoptotic) expression in the treatment group was significantly down-regulated and Bcl2 (antiapoptotic) expression in the treatment group was significantly up-regulated.

4. Discussion

Our results demonstrated that CoQ10 pretreatment and treatment significantly reduced cell death.

In addition, the present study confirmed that oral consumption of CoQ10 could increase anti-death protein Bcl2 expression while lowering expression of proapoptotic protein Bax.

Ren and colleagues (Ren et al,1994) investigated the effects of CoQ10 on dogs that suffered deep hypothermic circulatory arrest in a model of clinical bypass procedure. The results showed that dogs pre-treated with CoQ10 had higher levels of ATP and greater protection of the cortex from structural damage.

In agreement to our findings, it has already been proved that the model of global cerebral ischemia leads to neurodegenerative lesions in CA1 area of hippocampus. It is also known that global cerebral ischemia can cause neuronal death in CA1 pyramidal hippocampus and reduce the spatial learning and memory in rats (McBean et al, 1998).

Furthermore, endothelin models of cerebral ischemia in rats demonstrated the neuroprotective effects of CoQ10 on lactate acidosis, ATP production, oxidized and reduced glutathione ratio, and super oxide dismutase activities after induced cerebral ischemia (Ostrowski et al, 1998). Previous studies suggest that CoQ10 may be neuroprotective. CoQ10 has been described to inhibit oxidative stress in mice and in vitro, and lessen damage to the hippocampus following carotid ligation or toxic injury (Piotrowski et al, 2001).

Our results showed that CoQ10 pretreatment might reduce neuronal loss in hippocampus following ischemia reperfusion, but Li et al. reported that post-treatment of CoQ10 via intraperitoneal injection fails to protect brain against focal and global ischemia in rats (Li et al, 2000). In an experimental model of diabetes and ischemia, pretreatment with CoQ10, 10 mg/kg IP for 7 days led to significant decrease in neuronal loss due to ischemia and reduced activity of a key enzyme (CPP32) involved in apoptotic cell death (Piotrowski et al, 2001).

CoQ10 has been shown to be protective against damage in various tissues (Zhipeng et al, 2007; Ostrowski et

al, 2000; Premkumar et al 2008; Littarru et al, 2005). It is also demonstrated that CoQ10 consumption reduces brain ischemic mortality and protects neurons from harmful agents and thus reduces ischemic complications (Geng et al, 2007; Gainotti et al, 2004; Shults et al 2004). More recently, it was found that coenzyme Q10, when administrated during reperfusion after ischemic brain injury, increases resistance to oxidative stress and improves brain bioenergetics (Horecký et al, 2011).

Here, we measured the levels of Bcl2 expression, an anti-apoptotic protein, and Bax, a pro-apoptotic protein, for evaluating the protective effects of CoQ10 pretreatment. A statistically significant reduction ($p < 0.05$) in the cell death was observed in the group administered CoQ10, a week prior to ischemia induction, compared to the group to which only ischemia was applied.

These findings suggest that CoQ10 is a neuronal protector against oxidative damage in ischemia/reperfusion induced brain injury.

From the results of our histomorphological assessment, it is concluded that administration of CoQ10 before ischemic brain injury in mice, reduces neuronal loss, nuclear pyknosis, and nuclear hyperchromasia in a statistically significant manner when compared to the ischemic groups.

5. Conclusion

Pretreatment and treatment with CoQ10 can be a pharmaceutical approach to lessen the effects of ischemia reperfusion on hippocampus.

Acknowledgment

We want to thanks the research council of Tehran University of Medical Sciences for financial support of the present work.

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