Original Article

Protective Effect of Coenzyme Q10 on Methamphetamine-Induced Apoptosis in Adult Male Rats

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Received: 20 December, 2016; Accepted: 07 May, 2017

Abstract

Background: The negative consequence of methamphetamine abuse is due to neuropathologic changes in the brain, which reduces dopaminergic neurons and result in damage to different brain areas. Neurotoxicity induced by methamphetamine increases the oxidative stress and associated with neuronal apoptosis. The role of the antioxidant coenzyme Q10 probably produces its neuroprotective effects. Therefore, the purpose of the present study was to examine the protective effect of coenzyme Q10 on methamphetamine-induced apoptosis in adult male rats.

Materials and Methods: Fifty Wistar eight-week adult rats randomly divided into 5 groups: Healthy control, methamphetamine injection (Meth), methamphetamine injection and CoQ10 5mg/kg treatment (Meth+Post CoQ10 5mg/kg), methamphetamine injection and CoQ10 10mg/kg treatment (Meth+Post CoQ10 10mg/kg), methamphetamine injection and CoQ10 20mg/kg treatment (Meth+Post CoQ10 20mg/kg). Methamphetamine with a purity of 96% with a dosage of 20 mg/kg was injected Intraperitoneal. Coenzyme Q10 for three treatment groups was injected intraperitoneally for 14 days in a dosage of 5, 10 and 20 mg/kg/day. The protein expressions of Baxand Bcl2 were evaluated by western blotting technique.

Results: Bax protein expression was significantly lower in Meth+Post CoQ10 5mg/kg (p=0.010) and so Meth+Post CoQ10 10mg/kg (p=0.004) comparing to Meth group. In addition, Bcl2 protein expression was significantly higher in Meth+Post CoQ10 5mg/kg comparing to Meth group (p=0.018). However, there were no significant differences between control and CoQ10 treatment groups. Bax/Bcl2 ratio was significantly lower in Meth+Post CoQ10 5mg/kg (p=0.005), Meth+Post CoQ10 10mg/kg (p=0.008) and Meth+Post CoQ10 20mg/kg (p=0.044) comparing to Meth group.

Conclusion: We suggest that CoQ10 reduces the methamphetamine-induced apoptosis in the striatum of the rats through the reduction of apoptotic factors and increase of anti-apoptotic pathways.

Keywords: Addiction, Apoptosis, Coenzyme Q10, Methamphetamine

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Please cite this article as: Gholipour F, Shams J, Zahiroddin A. Protective Effect of Coenzyme Q10 on Methamphetamine-Induced Apoptosis in Adult Male Rats. Novel Biomed. 2017;5(3):127-32.

Introduction

Addiction and substance abuse have negative effects on human's life, including physical and mental health, and social, legal, and emotional status[1]. Methamphetamine is an addictive psychoactive drug which has been proven to have strong stimulant effects on CNS. Its popularity is increasing in US and other parts of the world because of low price[2]. Methamphetamine abuse is becoming a public health problem in global level and it is estimated that there are 15-16 million methamphetamine abuser around the world, which is making it the second abused

substance[3]. Euphoria caused by methamphetamine is accompanied by loss of appetite and body temperature, paranoia, aggression, and increased sense of joy[4]. In humans, methamphetamine abuse has cognitive and neurologic damages and cardiovascular complications[5]. Methamphetamine can pass through blood-brain barrier and is probably able to damage dopaminergic neurons. Methamphetamine injection can cause prolonged release of dopamine in humans and animals[6].

Oxidative stress, mitochondrial disruption, apoptosis, neural inflammation, and toxicity mediated by NMDA receptors are mechanisms generally mentioned in neurotoxicity of dopaminergic neurons[7]. Chronic abuse of methamphetamine causes decrease in dopamine transporters in cortex, caudate and putamen, and decrease in serotonin transporters[8], as well as microglial activation in different areas of brain[9] and morphologic changes like a decrease in gray matter and hypertrophy of white matter[10]. In many studies there are convincing evidences in support of methamphetamine damage to different areas of brain and neuron loss[10-12]. Evidences suggest that methamphetamine neurotoxicity involves reactive oxygen species (ROS), reactive nitrogen species (RNS) and lower-hand oxidative stress mechanisms activation[13]. Methamphetamine enters dopaminergic neurons via dopamine transporters and shifts dopamine vesicle. Moved amines can be oxided in enzyme and non-enzyme forms and makes reacting Quoinons and ROS and increase oxidative stress[14]. Thus, ROS causes death of neurons due to damage to cellular parts including DNA, RNA, and proteins. Also mitochondrial disruption in neural destruction caused by methamphetamine has been noticed[15]. Methamphetamine is lipophilic cation molecule that can infiltrate into mitochondria and stays there. In fact, methamphetamine reduces mitochondrial membrane potential and level of I, III, and VI complex of electron transport chain in primary human cells[16] which is followed by decreased ATP in brain[17]. Pro apoptotic proteins like Bax, Bad and Bid are increased and anti-apoptotic proteins like Bcl-2 and Bcl-XL are decreased after injection of methamphetamine[17]. Plus, methamphetamine not only causes cellular death via apoptosis, but also via necrotic mechanisms[18].

Studies suggest that medication modalities in order to prevent and treat destructive effects of methamphetamine need attention to pathways that form substrates of methamphetamine-induced toxicity. Co-enzyme Q10 is necessary enzyme in the electron transport chain and an effective agent for neuroprotection in neurodegenerative diseases which increases plasma levels of Q10 co-enzyme and prevents decrease in dopamine when added to diet[19]. In recent years use of co-enzyme Q10 has been in center of attention for treatment of neurodegenerative diseases.co-enzyme Q10 probably acts as a neuroprotective agent by its anti-oxidant and free radical neutrizing effects. Plus that, co-enzyme Q10 is an electron receptor in complex I of electron transport chain[20]. Co-enzyme Q10 protects neurons from oxidative stress and prevents reduction in membrane potential of mitochondrial membrane[21]. Studies have shown that co-enzyme Q10 reduces neural damage of hippocampus in regions CA1, CA2, and CA3. Clinical studies also showed that co-enzyme Q10 can protect dopaminergic system of striatum and slows progression of disability in Parkinson disease[22]. Since apoptosis caused by methamphetamine-induces neurotoxicity can damage various parts of brain, especially dopaminergic part, and there are findings on protective effects of co-enzyme Q10 on neurologic system, in this survey, we study the protective effect of co-enzyme Q10 on apoptosis caused by injection on methamphetamine on adult male rat.

Methods

This is an experimental-interventional study on heads of 50 adult male 8-weeks old wistar rats, weighing 200±20 gr. Rats were housed in standard cages and controlled conditions of light (12 hr darkness, 12 hr light), temperature 22±3℃ and humidity around 45%, with free access to food and water. All steps were done by supervision of Ethics committee of Iran University of Medical Sciences and according to the moral protocol of experiments on lab animals. Samples are divided randomly into these five groups: Control, methamphetamine injection (meth), methamphetamine injection + post 5mg/kg co-enzyme Q10, methamphetamine injection + post 10mg/kg co-enzyme Q10, and methamphetamine injection + post 20mg/kg co-enzyme Q10.

Methamphetamine was obtained from police and had 96% purity. It was solved in physiologic serum and was injected totally 20 mg/kg within 2 days (10 mg each day, 5 mg 2 times a day with 12hr intervals) intraperitoneally[3]. For verification of injection, mobility test was performed. When injected, if animal passed 10 times around a 30cm basket, an injection would be

considered effective. Then the Co-enzyme Q10 injection as a post-treatment was starting after one day from the last injection of Methamphetamine. Co-enzyme Q10 provided from Sigma Co (St. louis, MO, USA) and solved with concentration of 100mg/cc in sesame oil. The solution of Co-enzyme Q10 was injected within 14 days in 5, 10, 20 mg/kg/day intraperitoneally for three post-treatment groups. The control group just received physiologic serum without Methamphetamine but the same volume to Meth group.

For the purpose of studying of expression of Bax and Bcl2 proteins in striatal region, the animals was sacrificed for provide brain tissue. All of the animals anesthetized by intraperitoneal injection of ketamine and xylazine in 8:1 proportion and after cutting their head and removing their brain, striatal part was dissected and washed by normal saline immediately and put striatal parts in microtubes and freeze in liquid nitrogen and then into a refrigerator with -80℃ and stored until western blotting them. Process had to done in less than 4 minutes after cutting head for each rat. For studying expression of Bax Bcl2 proteins, it was first derived and after that, using western blotting technique, protein bands removed from SDS-PAGE gel via electrophoresis were moved to a nitrocellulose membrane and in next step, were detected using primary and secondary antibodies[23]. For Quantitation of protein bands TotalLab Version 1.10 software was used. Data analysis was done using SPSS software. All data are reported with medium ± standard deviation. Kolmogrof-smirnof test was done to evaluate normal distribution of data and if distribution was normal, then parametric tests were used. Difference between groups was studied using unilateral variance analysis test and Tukey. Dunnett`s T3post hoc tests was done to compare the groups. The level of significance was set at $p \leq 0.05$.

Results

As shown in figure 1, expression of Bax protein in control group (p=0.012), meth +post 5mg/kg (p=0.010), meth + post 10mg/kg (p=0.004) was significantly less than Meth group. But there is no significant difference between meth+ post 20mg/kg and Meth (p=0.056). There is also no significant difference between groups which were treated with co-enzyme Q10 and control group (p>0.05).

Expression of Bcl2 protein in meth + post 5mg/kg co-enzyme Q10 group (p=0.018) was significantly higher

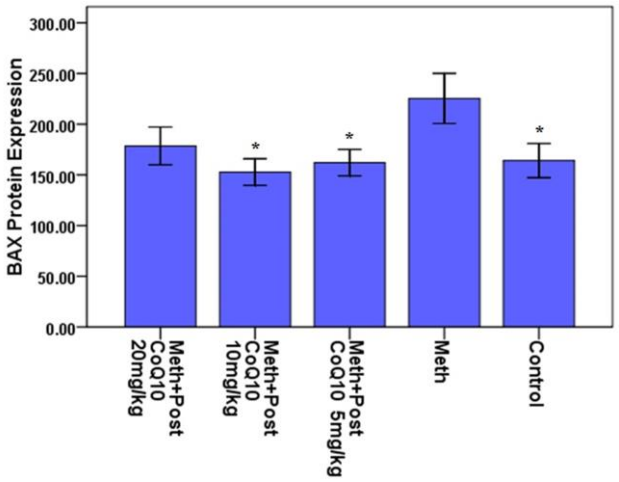

Figure 1. Expression of Bax protein in between groups,* significant

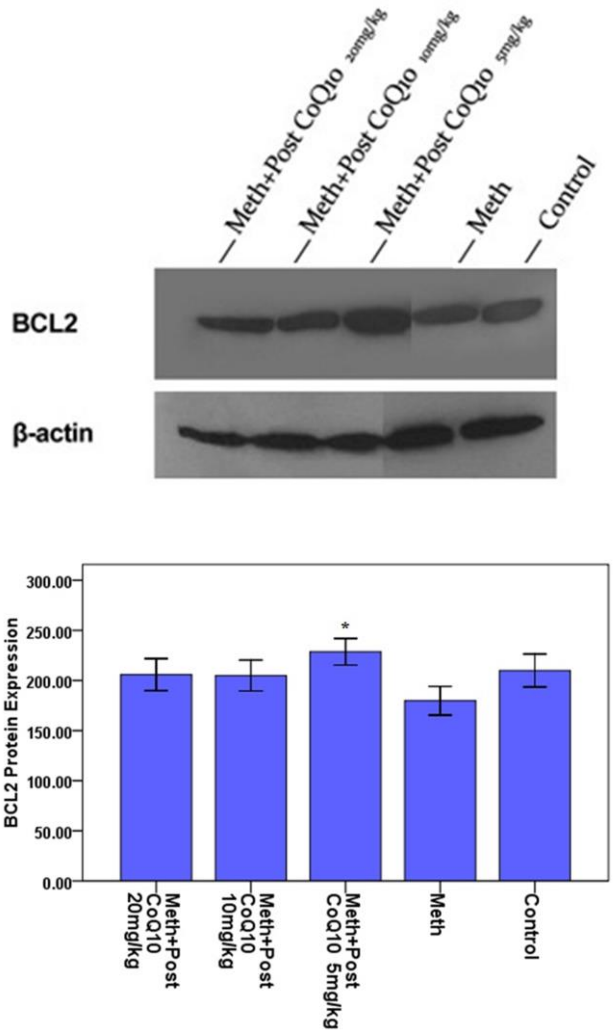

Figure 2. Expression of BCL2 protein in between groups.*

than meth group, but there was no significant difference between groups treated by co-enzyme Q10 and control group (p>0.05, figure 2).

Ratio of Bax/Bcl2 in the control group (p=0.014), meth +post 5mg/kg co-enzyme Q10 (p= 0.005), meth + post 10mg/kg co-enzyme Q10 (p=0.008), meth + post 20mg/kg co-enzyme Q10 (p=0.044) was significantly lower than Meth group, but there was no significant difference between groups treated by co-enzyme Q10 and control group (p>0.05).

Discussion

Deep stimulation of the brain is another modality for treatment of addiction and various parts of brain including nucleus accumbens, subthalamic nucleus, dorsal striatum, medial prefrontal cortex, and other parts of the brain are targeted[24]. How the methamphetamine increased apoptosis induction in striatum? There are convincing evidences that negative psycho-neural consequences of methamphetamine abuse is partly because of pathologic neurological changes in brain of abusers[25]. Dopamine release caused by methamphetamine is oxidized by monoamine oxidase and dihydroxyphenil acetic acid and hydrogen peroxide are produced[26]. Hydrogen peroxide interacts with metal ions and produces highly toxic hydroxyl free radical via phenton reaction. Thus, reacting oxygen species (ROS) including hydroxyl free radicals cause neuron death via damage to cellular parts like DNA, RNA and proteins. Methamphetamine is a lipophilic cation molecule which can diffuse into mitochondria and stays there. Methamphetamine also decreases the activity of complex 2 and 4 of electron transport chain swiftly which is accompanied by a decrease of ATP in brain[17]. In this study methamphetamine injection caused apoptosis in striatum which was consistent with previous studies[27]. This apoptosis might be because of various factors like oxidants, oxygen radicals and nitric oxide, which are originated from excessive release of glutamate and dopamine[28]. It is shown that methamphetamine causes an increase in pro apoptotic proteins like Bax, Bad and Bid, but decreases anti apoptotic proteins like Bcl-2 and Bcl-Xl[17]. In this study, compatible with previous surveys, we demonstrated that methamphetamine induces apoptotic factors like Bax in rats. We also showed that induction of these apoptotic factors is reduced by co-enzyme Q10 anti-oxidant. So there is probability that apoptosis of dopaminergic neurons in striatum of rats is reduced. How Co-enzyme Q10 reduced apoptosis induction? Co-enzyme Q10 is a necessary enzyme in the electron transport chain and an effective neuroprotective agent in neurodegenerative disease. Adding co-enzyme Q10 to the diet increases its plasma level and prevents dopamine decrease[19]. It also acts as important antioxidants in mitochondria and lipid membranes. There is a remarkable interest in using co-enzyme Q10 in neurodegenerative diseases.co-enzyme Q10 protects neurons from oxidative stress and prevents reduction in mitochondrial membrane potential[21]. In animal models of Parkinson disease has protective function[29]. Co-enzyme Q10 also protects dopaminergic neurons from mitochondrial membrane depolarization and rotenone-induced death[30]. A mechanism of co-enzyme Q10 neuroprotective effects is acting as an uncoupling proteins co-factor[31].

Co-enzyme Q10 suppresses apoptosis caused by oxidative stress and mitochondrial permeability[32]. It also stops Bax connection with mitochondria and cytochrome C release from mitochondria[33]. Co-enzyme Q10 significantly reduces indices of lipid peroxidation in plasma, erythrocytes, liver and brain of rats[34]. So there is probability that in this study, co-enzyme Q10 supplementation protects against methamphetamine-induced dopaminergic apoptosis by increasing anti-oxidant activity and neutralizing free radicals. It has also has been shown that co-enzyme Q10 reduces cocaine and methamphetamine induced neurotoxicity[23].

In this study, we demonstrated induction of apoptosis in striatum after acute injections of methamphetamine, because levels of Bax protein in Meth group were increased. Although treatment with doses of 5 and 10 of co-enzyme Q10 after injection of methamphetamine caused a decrease in Bax protein levels in comparison with Meth group. Probably protective effects of co-enzyme Q10 after injection of methamphetamine is due to its anti-apoptotic mechanisms. In addition, to mentioned apoptotic factors, higher levels of Bcl-2 protein in treatment with dose 5 of co-enzyme Q10 after injection of methamphetamine was observed. Also ratio of Bax/Bcl2 was significantly lower in groups of treatment with dose 5, 10 and 20 of co-enzyme Q10 in comparison with Meth group which is a reflection of an increase in anti-apoptotic status.

Conclusion

Overall, the findings of this study suggest that co-enzyme Q10 reduces apoptosis induced by methamphetamine injection in striatum of rats via reduction in apoptotic factors like Bax and increase in anti-apoptotic pathways. We noticed that co-enzyme Q10 reduces apoptosis induction after injection of methamphetamine in male rats. We hope that treatment with co-enzyme Q10 after addiction to methamphetamine be helpful in addicted patients.

Acknowledgment

This survey has been done by the financial support of Behavioral Science Research Center Shahid Beheshti University of medical sciences.

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