Protective Effects of Co-Enzyme Q10 on Thioacetamide-Induced Acute Liver Damage and Its Correlation With Behavioral, Biochemical, and Pathological Factors

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Received 2015 April 14; Revised 2015 August 29; Accepted 2015 September 26.

Abstract

Background: Acute liver damage may be followed by biochemical, behavioral, and pathological alterations, which can result in serious complications and even death.

Objectives: In this experimental study we determined whether coenzyme Q10 (CoQ10), a common supplementary medicine known to have protective, antioxidative, and anti-inflammatory effects in cells, has any protective effect against thioacetamide (TAA)-induced liver damage and its related neurobehavioral alterations in rats.

Materials and Methods: In this experimental study forty-eight Wistar rats were divided randomly into four groups (n = 12): C1 was the control group; C2 received a single-dose of TAA (350mg/kg; intraperitoneally) without any other treatment; E1 received TAA + 5 mg/kg CoQ10 (intraperitoneally); and E2 received TAA + 10 mg/kg CoQ10. After sacrificing the rats, liver enzymes and plasma-ammonia (NH4) were measured and histopathological analyses of the livers were carried out. Elevated-plus-maze, open-field, and forced-swimming tests were also performed to investigate behavioral correlations.

Results: The serum levels of alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), and NH4 show significant increases (P < 0.05). The groups treated with CoQ10 were shown to have significantly lower clinical grade of encephalopathy (P = 0.001), higher locomotor activity (P = 0.000), and lower levels of depression (P = 0.000). Furthermore, it was also shown that CoQ10 treatment may lead to significant decreases in scores of centrilobular necrosis, apoptosis, inflammatory cell infiltration, vacuolization, and liver necrosis (P < 0.05).

Conclusions: Overall, CoQ10 was determined to have positive effects on liver injury and its related behavioral and biochemical changes.

Keywords: Coenzyme Q10, Thioacetamide, Acute liver Failure, Behavioral Symptoms, Hyperammonemia

1. Background

Acute liver damage (ALD) leads to malfunctioning of detoxifying pathways of the body and therefore may be followed by neurobehavioral and functional complications. Neurobehavioral complications, specifically acute hepatic encephalopathy (AHE), which occurs in 25% of patients suffering from acute liver failure (ALF), is still one of the most important concerns of medical researchers (1). AHE is a neuropsychiatric syndrome that may induce a range of neuropsychiatric problems. These range from subtle abnormalities, such as depression, decrease in motion and attention, and disorders of executive functioning leading to impairment of attention, response inhibition, and memory, which are apparent only by performing psychometric testing, to more clinically apparent neurological defects such as confusion, stupor and coma, reduced locomotor activity, depression, and anxiety (2). Moreover, hepatic encephalopathy (HE) also includes cytotoxic brain edema, especially swelling of the astrocytes (3), which can exacerbate liver failure and can lead to increase in intracranial pressure, which may cause cerebral herniation, a major cause of mortality in ALD.

Ammonium intoxication, which is used widely both for diagnosis and as a guide for treatment when hepatic en-
cerebrospinal fluid (CSF) production (1). CoQ10 administration has been suggested to protect brain neurons, reduce the release of brain-derived neurotrophic factor (BDNF) in the hippocampus, and ameliorate AHE (2). The present study was designed to evaluate the protective influence of CoQ10 against thioacetamide (TAA)-induced liver damage and its related pathological, biochemical, and behavioral changes, i.e., depression, anxiety, and locomotor activity, in laboratory rats.

2. Objectives

In the present study, we determined the protective effect of CoQ10 against thioacetamide (TAA)-induced liver damage and its related pathological, biochemical, and behavioral changes, i.e., depression, anxiety, and locomotor activity, in laboratory rats.

3. Materials and Methods

3.1. Animals and Drug Administration

This experimental study was carried out in August and September of 2014. Forty-eight adult male Wistar rats weighing 220 ± 20 grams were obtained from Animal House, Shiraz University of Medical Sciences, Shiraz, Iran. The health status of all animals was checked by an expert veterinarian before involving them in the experiments; any animal with any diseases such as infections or movement abnormalities was replaced. Rats were kept in standard cages at 25 ± 3°C temperature, 40-50% humidity, with a 12 h light-dark cycle and access to standard food and water ad libitum during the study and for at least a week before the experiments. The rats were randomly (simple randomization method) divided into four groups (n = 12) as follows: control group 1 (C1), which received 1 mL normal saline intraperitoneally every 24 hours starting from day 1; control group 2 (C2), which received 1 mL normal saline intraperitoneally every 24 hours starting from day 1; experimental group 1 (E1), which was treated with 5 mg/kg (IP) of CoQ10 every 24 hours from day 1; experimental group 2 (E2), which received 10 mg/kg of CoQ10 (Tishcon Corp., Westbury, NY, USA) dissolved in 1 mL of normal saline (IP) every 24 hours from day 1 up to the end of the study. The C2, E1, and E2 groups all received a single dose of TAA (Sigma-Aldrich, St. Louis, MO, USA), dissolved in sterile normal saline solution, was injected intraperitoneally as a single dose of 350 mg/kg to all of the experimental groups (day 3) (14). Twenty-four hours later all animals were injected (subcutaneously) with 1 mL solution of 0.45% NaCl, 5% dextrose and 0.2% KCl in order to prevent hypervolemia, hypokalemia, and hypoglycemia (15). The last day of the study was set as the day in which more than half of the rats in any group reached grade IV encephalopathy based on the neurobehavioral test scores (day 10 in this case).

The entire study was carried out in Animal House, Shiraz University of Medical Sciences, Shiraz, Iran. The experimental protocol was approved (Code: 9101044509) by the medical ethics committee of Shiraz University of Medical Sciences, Shiraz, Iran, and all criteria for taking care of laboratory animals outlined in the “guide for the care and use of laboratory animals” were applied.

3.2. Clinical Grading of Encephalopathy State

The behavioral manifestations of encephalopathy caused by liver failure in the rats receiving IP injection of TAA may evolve through different stages. In this study, the scoring method described in Table 1 was used to compare the groups (16). To avoid possible bias during observations, testing was done every other day by the same observer who was blind to the group designations.

3.3. Open Field Test

Locomotor activity of the rats was assessed in five open field test sessions (days 1, 3, 5, 7, and 9) after training the animals according to the method used by Bengtsson et al. (17) by using a 50 × 50 cm² field divided into 25 equal squares. Rats were placed individually in the center of the field facing a random direction and were observed for 5 minutes.
Table 1. Clinical Grading Scores of the Animals’ Behavior

| Clinical Grade | Definition                                                                 |
|----------------|---------------------------------------------------------------------------|
| 0              | Normal behavior                                                           |
| 1              | Mild lethargy                                                             |
| 2              | Decreased motor activity, poor gesture control, diminished pain perception |
| 3              | Severe ataxia, no spontaneous righting reflex                             |
| 4              | No righting reflex, no reaction to pain stimuli                           |

Locomotor activity was documented by counting the number of lines crossed by the rat’s front legs. The total number of crossings in the first session was set as the baseline motor activity (100%) and subsequent results were stated as total number of crossings in the session/baseline motor activity (total number of crossings in the first session) × 100.

3.4. Elevated Plus Maze

The elevated plus maze was used for assessing the anxiety level of the rats according to a previously reported method (18). The instrument consisted of two open arms 50 cm in length, 11.5 cm in width, and 2 cm in depth and two closed arms 50 cm in length, 11.5 cm in width, and 11.5 cm in depth. The maze was elevated to a height of 100 cm from the floor. In three test sessions (days 1, 5, 9), the rats were placed individually in the central platform facing an open arm, and the following measures were collected: time spent in the central field, in the open arm, and in the closed arm. An arm entry was defined as passing the front legs over the red line at the beginning of each arm. Animals were allowed to explore freely for 5 minutes. The percentage of time spent in the open arms [time in open arms/(time in open + closed arms)] × 100 was computed as an indicator of anxiolytic-like activity (19).

3.5. Forced Swimming Test

To determine the depression level of the animals, the forced swimming test was performed four times (days 1, 3, 6, 9; the first session determined the base swimming time) according to a reported method (20). In this procedure, rats were placed in cylinders filled with water at 23-25°C, 30 cm in depth. They were removed from the cylinder 5 seconds after they stopped swimming. The test was conducted and recorded by two observers who were blind to the animal groups. Results were reported as duration of swimming in the session/base swimming duration (the swimming duration of the first session of the test) × 100.

3.6. Biochemical Assessment

In order to evaluate the effect of treatment on liver function and plasma ammonia, at the end of the study, blood samples were taken from the animals via cardiac puncture. The samples were sent to an outside lab and checked for the following factors: aspartate aminotransferase/alanine aminotransferase (AST/ALT), total bilirubin (TBili), alkaline phosphatase (AlkP), and level of plasma ammonia (NH4+) using clinical test kits (Randox, Randox Laboratories Ltd., UK) and AutoAnalyzer Technicon RA-1000 (Technicon Co, Oakland, CA, USA).

3.7. Histopathological Investigation of the Livers

After sacrificing the rats, portions of liver were taken from the left lateral lobes and washed with ice-cold normal saline, cut into small pieces, and fixed immediately in 10% phosphate-buffered formalin for 2 days. Sections were then stored in 70% ethyl alcohol until processed. Slides 5 µm thick were prepared using a tissue processor (ThinPrep Processor 2000, USA) and microtome (Mikrotom model: 1212, Ernst Leitz GMBH, Wetzlar, Germany) from the liver sections and stained with hematoxylin and eosin (H&E) for histological examination under a light microscope (Olympus, Japan). Five H&E-stained slides randomly selected from each group (twenty in all) were examined and scored for centrilobular necrosis, apoptosis, vacuolization, and inflammatory cell infiltration. The extent of liver necrosis was estimated semi-quantitatively and lesions were scored as multifocal necrosis. Scoring was as follows: 0, no lesion; 1, minimal, only occasionally found lesions in any lobule; 2, mild, less than one-third of lobular structure affected; 3, moderate, between one-third and two-thirds of lobular structure affected; 4, severe, greater than two-thirds of lobular structure affected; 5, most severe, damage to most of the parenchyma of the liver (21). Slides were scored by an expert pathologist who was unaware of the slide codes.

3.8. Data Analysis

Laboratory results are expressed as mean ± standard deviation (SD) and also as median (med) and interquartile range (IQR) for non-normal data according to the Kolmogorov-Smirnov test; points in the figures are also expressed as mean ± standard deviation. For statistical analyses, due to the non-parametric nature of the data, the Kruskal-Wallis test was used to compare the groups and the Mann-Whitney U test plus exact methods were used to compare every two groups. All analyses were carried out using SPSS® software (20.0, IBM®, USA), and P value ≤ 0.05 was considered statistically significant.
4. Results

4.1. Biochemical Study

Baseline levels of the measured biochemical markers (C1) are shown in Table 2. Overall, the measured values of ALT, AST, NH4 (but not TBili) tended to show lower amounts in the E1 and E2 groups compared with group C2, although the significance of the responses to the treatment of each individual marker varied and was < 0.05.

Table 2 shows the mean and standard deviation of the values. As shown, the plasma level of NH4 was significantly lower in C2 (med: 1191.05, IQR: 117.51) compared with E2 (med: 867.01, IQR: 117.51; P = 0.006) and E1 (med: 943.55, IQR: 117.15; P = 0.011). ALT levels in both E1 (med: 510.01, IQR: 79.75) and E2 (med: 467.02, IQR: 66.52) showed a significant decrease in contrast with group C2 (med: 786.56, IQR: 271.15; P = 0.011 and P = 0.006, respectively). Moreover, CoQ10 significantly influenced AST levels in E2 (med: 467.24, IQR: 155.47; P = 0.006) and E1 (med: 912.17, IQR: 115.74; P = 0.011) in comparison to C2 (med: 1258.74, IQR: 145.53). An insignificant decrease in AlkP levels was observed in groups E1 (med: 705.18, IQR: 649.89; P = 0.394) and E2 (med: 1191.05, IQR: 117.51; P = 0.201) when compared with C2, but in contrast to group C1 (med: 43.03, IQR: 43.59), these values were noticeably high (P ≤ 0.05). Unexpectedly, TBili plasma concentrations were insignificantly increased in experimental groups in comparison to the C2 group (Table 2).

4.2. Neurobehavioral Assessment

4.2.1. Clinical Grading of Encephalopathy State

As shown in Table 3, groups E1 and E2 received CoQ10. In spite of the increase in clinical grades in contrast with control group C1, the grades in experimental groups E1 and E2 are noticeably lower in comparison to the non-treated C2 group with TAA-induced AHE (P = 0.019 and P = 0.010, respectively).

4.2.2. Locomotor Activity, Anxiety and Depression Level

As demonstrated in Figure 1, in the third session of the open field test, a significant difference was shown between control group C1 and the TAA-injected groups, E1 (P = 0.003), E2 (P = 0.001), and C2 (P = 0.01). In the fourth open field test session, the locomotor activity of group C2 decreased significantly compared to E1 (P = 0.003) and E2 (P = 0.012). In the fifth session, this difference was seen in E1 and E2 versus both C1 and C2 (P ≤ 0.01) (Figure 1).

Anxiety level, according to the outcomes of the elevated plus maze test (Figure 2), in the second session in group C2 differed considerably from E1 (P = 0.007), E2 (P = 0.001) and C1 (P = 0.05); however, in the third session of the test, the anxiety levels in the experimental groups became more similar to group C2 with a noticeable difference with C1 (P < 0.001). Measurement of depression levels by the forced swimming test (Figure 3) showed that although the experimental groups and group C1 had lower levels of depression shown by higher swimming times in the third session of the training, the percentages of swimming time (percent of baseline time) were significantly lower than for C1 but insignificantly higher than C2 by E1 (P = 0.002 and P = 0.21, respectively) and E2 (P = 0.016 and P = 0.051, respectively). In the fourth session, noticeable differences were observed in groups E1 (P = 0.05) and E2 (P = 0.001) in comparison with C2; moreover, E1 still differed from C1 (P = 0.002).
**Table 2.** Effect of CoQ10 on T Bilir (Total Bilirubin), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), AlkP (Alkaline Phosphatase), and NH4 (Plasma Ammonia) in the Animal Model of Acute Liver Injury Induced by TAA

| Groups  | T Bilir (mg/dL) | AST (IU/L) | ALT (IU/L) | AlkP (IU/L) | NH4 (µg/dL) |
|---------|----------------|------------|------------|-------------|-------------|
| C1      | 0.17 ± 0.51    | 109.79 ± 53.21 | 129.01 ± 58.97 | 51.21 ± 30.74 | 198.98 ± 59.09 |
| C2      | 0.20 ± 0.84b   | 1262.67 ± 83.04b | 778.33 ± 136.78b | 898.51 ± 256.49b | 1222.85 ± 83.66b |
| E1      | 0.32 ± 0.15b   | 924.75 ± 61.62b | 507.25 ± 41.45b | 716.75 ± 347.28b | 938.71 ± 61.61b |
| E2      | 0.22 ± 0.08b   | 775.59 ± 98.59b | 478.81 ± 45.10b | 685.21 ± 174.12b | 885.04 ± 79.11b |

Values are presented as mean ± SD.

1b P = 0.05 versus C1 group.

1c P ≤ 0.05 versus C2 group.

**Table 3.** Effect of CoQ10 on Clinical Grade of Encephalopathy in the Animal Model of Acute Liver Damage Induced by TAA

| Groups  | Clinical Grade |
|---------|----------------|
| C1 group | 0              |
| C2 group | 3.40 ± 0.55    |
| E1 group | 2.67 ± 0.53b   |
| E2 group | 2.58 ± 0.49b   |

Values are presented as mean ± SD.

1b P ≤ 0.05 versus C2 and C1 groups.

**Figure 3.** Effect of CoQ10 on Depression (Forced Swimming Test) of Laboratory Rats with TAA-Induced Acute Liver Damage

The first test, session 1, was considered the baseline and values of the following sessions were calculated as the duration of swimming in the session/baseline swimming duration × 100. A, P < 0.05 for E1 versus E2; B, P < 0.05 for E1 and E2 versus C1 and for E2 versus C2; C, P < 0.05 for E1 versus C1 and for E1 and E2 versus C2.

4.3. Histopathological Analyses of the Livers

Histopathological findings (shown in Table 4) demonstrated that in groups E1 and E2, centrilobular necrosis was decreased in contrast with group C2 (P = 0.018). Vacuolization and inflammatory cell infiltration were decreased in groups E1 and E2 in contrast with C2, but only in E2 was the difference statistically significant (P = 0.043 and P = 0.01, respectively). Comparison of apoptosis scores showed no statistically significant discrepancies between groups E1 and E2 versus C2. CoQ10 significantly affected liver necrosis score, as groups E1 and E2 had lower scores compared to group C2 (P = 0.01 and P = 0.001, respectively). None of the groups receiving CoQ10 had histopathological parameter similarities with group C1 (P < 0.05).

5. Discussion

CoQ10 is a necessary cofactor that participates in electron transferring in the mitochondrial oxidative respiratory chain where production of adenosine triphosphate takes place (22). CoQ10 is extensively available in various formulations and is very well tolerated with minimal complications (23). The most common side effect is mild gastrointestinal discomfort, and this has been observed in less than 1% of patients (23). CoQ10 was shown to be able to cross the blood-brain barrier in animal models (24), and as a dietary supplement it has recently been noted for its neuroprotective potential in neurodegenerative diseases such as Huntington’s disease, Alzheimer’s disease, and Parkinson’s disease, mostly through its antioxidative and anti-inflammatory effects, which lead to a reduction of neuronal loss and improvement in functions of the nervous system (22). It has been strongly suggested that mitochondrial dysfunction and oxidative stress are major factors in the pathogenesis of neurodegenerative diseases (25, 26). CoQ10 is a lipid-soluble antioxidant that is synthesized endogenously and prevents oxidation of proteins, lipids and DNA (27-30). Previous studies have evaluated the role of CoQ10 as a neuroprotective factor against injury by reactive oxygen species (ROS) and apoptotic cell death. It has been reported that CoQ10 may function by stabilizing the mitochondrial membrane once neuronal cells are exposed to oxidative stress (31). Moreover, pre-treatment with CoQ10 has been shown to preserve mitochondrial membrane potential throughout oxidative stress and has decreased the amount of mitochondrial ROS production.
(31). Another study revealed that administration of CoQ10 can also improve brain energy metabolism (32); its depletion, which is caused by some substances like statins, could induce fatigue, myalgia and neurocognitive disorders such as concentration and memory disturbances (33).

As demonstrated by the results of the present study, CoQ10 administration led to diminishing plasma ammonia levels, a major indicator of progression of liver malfunction to neurobehavioral alterations. Although the exact molecular mechanisms of ammonia-induced neurotoxicity have not yet been determined, oxidative stress is considered an important factor in the pathophysiology of ammonia toxicity (34). Norenberg et al. consequently reported that protein peroxidation as well as lipid peroxidation may occur in astrocytes treated with ammonia (35). A study utilizing a portacaval shunt model of HE in rats also revealed that ammonia enhances mRNA levels of heme-oxygenase-1 (HO-1), one of the main markers of oxidative stress (36). Clinically, previous studies have shown that N-acetylcysteine, which is an antioxidant and anti-inflammatory agent, is of use in the treatment of patients with ALF (37), and agents such as mannitol and sodium benzoate, which are currently used in the management of ALF, have also been demonstrated to possess antioxidant effects (38).

Hence, a potential approach in prevention and treatment of neurobehavioral disturbances may be administration of agents with antioxidant effects such as CoQ10. According to the results of the current study, concentrations of liver enzymes (ALT, AST, and AlkP) as indicators of liver damage were reduced in groups treated with CoQ10, mostly in group E2, which may be a sign of a dose-dependent hepatoprotective effect of this agent. Histopathological analyses also supported the hepatoprotective effect of CoQ10. Indeed, CoQ10 reduced the infiltration of inflammatory cells and vacuolization as well as necrosis of the liver tissue. In support of this result, Fouad et al. showed that CoQ10 protects rat’s liver against acute acetaminophen-induced hepatotoxicity, most probably through its antioxidant, anti-inflammatory, and anti-apoptotic effects (39). Another study carried out on schistosomiasis-induced liver injury disclosed that CoQ10 not only reduces liver fibrosis but can also improve liver function through significant reduction in oxidative stress markers and preservation of antioxidant factors (40). Outcomes of the present study also demonstrated that CoQ10 consumption can slow the development of behavioral changes and encephalopathy caused by liver malfunction and the corresponding increase of toxins in circulation according to clinical grades of the AHE state and behavioral examinations. Depression and motor activity, which are affected by encephalopathy, were also improved to some extent by treatment with CoQ10; however, the outcome of the elevated plus maze test demonstrated that CoQ10 did not significantly improve the anxiety level of the rats since the time spent in open arms of the maze by groups E1 and E2 was closer to that of group C2. Correspondingly, Sinatra et al. suggested that the increased locomotor activity based on the results of the open field test observed in CoQ10-treated mice may be the result of psychomotor stimulation or decreased anxiety after CoQ10 treatment (41). Likewise, it has been supposed that nutritional supplements acting on mitochondrial metabolism, such as CoQ10, could improve depression (42).

One of the limitations of the present study was that we did not evaluate the pathological changes of the brains in our laboratory rats. Because the method used in this study was an established method of producing AHE, we assumed that the behavioral changes observed in our tests were the preliminary signs of developing AHE in rats; however, these alterations could be due to toxicity and biochemical disturbances. The behavioral examinations were carried out in accordance with previous studies in this case; however, some errors in examinations are unpreventable, usually due to insufficient training of the animals and examiner error.

In conclusion, the results of this experimental study showed that CoQ10, as a medicine with minimal side effects, can be an effective agent for treating patients with acute liver injuries, especially those at risk of advancing to AHE. Thus, further investigations are needed to assess the adverse effects and exact mechanisms of action of this supplement.

### Table 4. Effect of CoQ10 on Histopathological Changes of the Liver in the Animal Model of Acute Liver Damage Induced by TAA

| Group | Centrilobular Necrosis | Vacuolization | Apoptosis | Inflammatory Cell Infiltration | Necrosis Score |
|-------|------------------------|---------------|-----------|-------------------------------|---------------|
|       | 0 ± 0.00b              | 0.4 ± 0.46b   | 0 ± 0.00b | 0 ± 0.00b                     | 0 ± 0.00b     |
| C2 group | 1.4 ± 0.23            | 2.5 ± 0.23    | 2.0 ± 0.23 | 2.2 ± 0.23                   | 2.1 ± 0.23    |
| E1 group | 1.2 ± 0.21            | 2.3 ± 0.23    | 2.1 ± 0.21 | 2.1 ± 0.21                   | 2.1 ± 0.21    |
| E2 group | 1.2 ± 0.21            | 2.3 ± 0.23    | 2.1 ± 0.21 | 2.1 ± 0.21                   | 2.1 ± 0.21    |

a Data are expressed as the individual scores (left column) and the mean ± SD (right column).
b P ≤ 0.05 versus C2 group.

c P ≤ 0.01 versus C2 group.

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Ashkani-Esfahani S et al. Iran Red Crescent Med J. 2016; 18(8):e29166.
agent. Moreover, more clinical trials should also be done to shed light on the possible efficacy and therapeutic impact of this type of coenzyme on humans. Such research could result in enormous benefits for patients by slowing the disease’s progression, especially the advance to AHE, and providing additional time for patients waiting to receive liver transplants.

Acknowledgments

The authors thank Dr. M. Vahedi, Mr. Sarayi and all the personnel of the stem cell and Transgenic technology research center, Ms. Keivanshokooh and the research improvement center of Shiraz University of Medical Sciences, Shiraz, Iran.

Footnote

Funding/Support: This study is from the doctorate thesis (Project no. 91-01-01-4509) of Soheil Ashkani-Esfahani and was supported by Shiraz University of Medical Sciences, Shiraz, Iran.

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