

Protective effect of coenzyme Q10 in paclitaxel-induced peripheral neuropathy in rats

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ABSTRACT

الأهداف: دراسة الأثر الوقائي المحتمل لتميم الأنزيم في الأمراض العصبية لدى الفئران.

الطريقة: أجريت دراسة مخبرية في قسم علوم الأدوية، كلية الطب، أنقرة، تركيا خلال الفترة من يناير ومارس 2012م. قمننا بتقسيم 40 فأر إلى 4 مجموعات المجموعة الأولى (الشاهد)، المجموعة الثانية (باكليتاكسيل)، المجموعة الثالثة (شاهد مع تميم الأنزيم)، المجموعة الرابعة (باكليتاكسيل مع تميم الأنزيم). تلقت المجموعة الثانية والرابعة عقار باكليتاكسيل جرعة 2 ملغ/كلغ بالحقن الوريدي في اليوم 0، 2، 4، 6، 8، 10، 14، 16، 19، 39 و 41 للفئران الذين لم يتلقوا عقار باكليتاكسيل وتميم الأنزيم تلقتوا سواغات غذائية. إضافة إلى ذلك تم إجراء اختبارات آلام الجلدية لجميع الحيوانات في اليوم 0، 2، 4، 6، 8، 10، 14، 16، 19، 39، 41 باستخدام خيوط فون فري.

النتائج: في اليوم الأول كانت عتبة الانسحاب الميكانيكية متشابهة في جميع المجموعات. في اليوم الثاني انخفضت عتبة مجموعة باكليتاكسيل. كان باكليتاكسيل وتميم الأنزيم مرتفع بشكل إحصائي مقارنة بمجموعة باكليتاكسيل من اليوم العاشر ولكنها أقل من مجموعة الشاهد. كانت قيم العتبة لدى مجموعة الشاهد وتميم الأنزيم مشابهة خلال فترة الدراسة.

خاتمة: يخفف عقار تميم الأنزيم من شدة الأمراض العصبية المحيطية الناتجة من استخدام عقار باكليتاكسيل في الفئران.

Objective: To investigate the possible protective effect of coenzyme Q10 (CQ10) on neuropathy in rats.

Methods: Experiments were conducted in the Department of Pharmacology, Faculty of Medicine, Hacettepe University, Ankara, Turkey between January and March 2012. Forty rats were divided into 4 groups: group 1 (control), group 2 (paclitaxel), group 3 (control + CQ10), and group 4 (paclitaxel + CQ10). Group 2 and 4 rats received paclitaxel (2

mg/kg, intraperitoneally, on days 0, 2, 4, 6). Group 3 and 4 rats were treated with CQ10 (10 mg/kg, intraperitoneally, on days 0, 1, 2, 3, 4, 5, 6, 7, 8, 9). The rats that did not receive paclitaxel or CQ10 received vehicle. Mechanical allodynia tests were performed for each animal on day 0, 2, 6, 8, 10, 14, 16, 19, 39 and 41 for all groups with von Frey filaments.

Results: At day 0, mean mechanical withdrawal thresholds were similar among all groups. Starting from day 2, the threshold of the paclitaxel group decreased. Starting from day 10, paclitaxel+CQ10 treated rats had significantly higher thresholds compared with the paclitaxel group, but these values were still significantly lower than that of the controls. Control and control + CQ10 rats had similar threshold values during the protocol.

Conclusions: The CQ10 treatment decreased the degree of paclitaxel-induced peripheral neuropathy in rats.

Neurosciences 2013; Vol. 18 (2): 133-137

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Received 19th December 2012. Accepted 10th March 2013.

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Disclosure. The authors declare no conflicting interests, support or funding from any drug company. This study was supported by Hacettepe University Scientific Research Unit (Grant No. 5755).

The major dose-limiting side effect of many chemotherapeutic agents is chemotherapy induced peripheral neuropathy (CIPN), which is common and occurs mostly due to axonal degeneration induced by chemotherapeutics.^{1,2} Chemotherapy-induced peripheral neuropathy is a very painful disease, which diminishes the quality of life of cancer patients dramatically.³ Paclitaxel is a commonly and effectively used chemotherapeutic against solid tumors including breast, lung, cervical, ovarian, and testicular cancer. Chemotherapy-induced peripheral neuropathy due to paclitaxel is characterized by pain, numbness, tingling, and a burning sensation, which usually develops within 1-3 days of paclitaxel administration.⁴ Pain has commonly been treated with nonsteroidal anti-inflammatory drugs, acetaminophen, or opioids, or both. None of these drugs were shown to prevent or treat CIPN. Previous studies investigated the mechanism of CIPN and mitochondrial dysfunction during paclitaxel-induced peripheral neuropathy, but the exact mechanisms remain to be elucidated.^{1,5,6}

Coenzyme Q10 (ubiquinone, CQ10) is a very essential molecule, which plays an important role in electron transfer in the mitochondrial respiratory chain. The CQ10 also functions as a free radical scavenger, membrane stabilizer, and cofactor in the production of adenosine triphosphate by oxidative phosphorylation. Free radicals play a role in pathogenesis of CIPN, which is characterized by neuronal injury due to chemotherapeutics. Antioxidants, like alpha-lipoic acid and phenyl N-tert-butyl nitron, were shown to prevent mitochondrial damage and neuropathy in experimental paclitaxel-induced neuropathy,^{1,6} but the protective effect of CQ10 in experimental or human paclitaxel-induced peripheral neuropathy has not been investigated previously. In this study, we investigated the possible protective effect of CQ10 administration on experimental paclitaxel-induced neuropathy in rats.

Methods. Forty male Sprague-Dawley rats weighing 200-250 g, obtained from the Institutional Animal Breeding Unit were used. The animals were housed in communal cages with food and water available ad libitum under a 12/12 hour light (7:00 am-7:00 pm) and dark cycle (7:00 pm-7:00 am) for 45 days. The Institutional Animal Care and Use Ethics Committee approved the procedures. Experiments were conducted in accordance with the Institutional Animal Care Guidelines. Experiments were conducted in the Department of Pharmacology, Faculty of Medicine, Hacettepe University, Ankara, Turkey between January 2012 and March 2012.

Rats were randomly divided into 4 groups: group 1 (control), group 2 (paclitaxel), group 3 (control + CQ10) and group 4 (paclitaxel + CQ10). Paclitaxel and CQ10 were purchased from Sigma Chemical Company (St. Louis, MO, USA). Group 2 and 4 rats received intraperitoneal (i.p.) paclitaxel (2 mg/kg for each dose on days 0, 2, 4, and 6) to induce peripheral neuropathy. Paclitaxel was dissolved in saline to a final concentration of 2 mg/ml. Group 1 and 3 rats received i.p. saline treatment on days 0, 2, 4, and 6 in equal volume to paclitaxel administered to other groups. Group 3 and 4 rats were treated with i.p. CQ10 (10 mg/kg on days 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9).⁷ The CQ10 was dissolved in Tween 80 and distilled water to a final concentration of 10 mg/ml. Rats in groups 1 and 2 received vehicles in equal volume to CQ10 administered to the other groups.

Rats were weighed frequently (at least once a week) during the experimental protocol to follow the weight gain in each group. Rats were also examined daily to detect clinical signs of paclitaxel toxicity such as hind limb weakness and bleeding. A blind investigator without prior knowledge of the study design, and experimental groups conducted behavioral tests. The same investigator performed these tests for every animal in each group during the experimental protocol. The behavioral tests measured were foot mechanical withdrawal thresholds in response to varying degrees of mechanical stimuli applied to the left and right hind mid plantar surface of paws with von Frey filaments.⁸ Rats were placed in a chamber with a wire mesh bottom and allowed to acclimatize for at least 15 minutes before testing. The mechanical stimuli were administered to the plantar surface of the paws. Mechanical sensitivity was assessed by using a series of Von Frey filaments (Stoelting Co., Wood Dale, IL, USA), as previously described.⁸ Mechanical withdrawal thresholds were determined by the up-down method by using a set of von Frey filaments (filaments values: 0.45, 0.74, 1.26, 2.04, 3.31, 5.5, 8.32, 14.45 g). A von Frey filament was applied to the plantar surface with sufficient force to bend the filament slightly for 3-4s. During stimulation or after stimulus removal, a withdrawal of the foot was considered a positive response. The first stimulus was always the 2.04 g, and when there was a positive response, the next lower filament was used. When there was no response, the next higher filament was applied. This test pattern continued until responses to the sixth von Frey stimuli from the first change of response were measured. Mechanical allodynia tests were performed for each animal on day 0, 2, 6, 8, 10, 14, 16, 19, 39, and 41 for all groups.

Data were expressed as mean \pm standard error of the mean. All measurements were analyzed with two-way ANOVA and post-Hoc Bonferroni Multiple Comparisons Test by using GraphPad Prism 5 (GraphPad Prism®, La Jolla, CA, USA). For all data sets, $p < 0.05$ was accepted to be statistically significant.

Results. Animal's general health. The administration of paclitaxel or CQ10 was well tolerated by the rats. Mortality or general toxicity was not observed in any group during the experiments. The body weights of animals at the beginning of the experiments were similar in all groups (219.1 ± 5.21 g in the control group, 226.1 ± 6.01 g in the paclitaxel group, 214.2 ± 6.94 g in the control + CQ10 group, and 225.5 ± 10.13 g in the paclitaxel + CQ10 group). Except from one rat in the paclitaxel group, which lost 29 g during the protocol, all rats gained weight during the experimental protocol. Each group of rats had similar average gains of body weight (55.2 ± 4.83 g in the control group, 37.5 ± 9.11 g in the paclitaxel group, 51.1 ± 4.31 g in the control + CQ10 group, and 42.9 ± 2.30 g in the paclitaxel + CQ10 group in 41 days) (Figure 1). Body weights of rats during the experimental protocol were similar in all groups ($p = 0.35$).

Paclitaxel-induced neuropathic pain model in rats. At day 0, the mean mechanical withdrawal thresholds were similar among all groups (13.79 ± 0.73 g in the control group, 14.13 ± 0.70 g in the paclitaxel group, 14.11 ± 0.51 g in the control + CQ10 group, and 14.11 ± 0.51 g in

the paclitaxel + CQ10 group, $p = 0.41$). Starting from day 2, the mechanical withdrawal threshold of the paclitaxel group decreased, which became significantly lower at day 8 and further, compared with the controls ($p = 0.03$ for day 8, $p = 0.001$ for day 10, $p = 0.0007$ for day 14, $p = 0.0006$ for day 16, $p = 0.0006$ for day 19, $p = 0.0005$ for day 39, $p = 0.0005$ for day 41) (Figure 2). The greatest decrease in mechanical withdrawal threshold in the paclitaxel group was observed between days 8 and 10. The paclitaxel + CQ10 treated rats had a similar mechanical withdrawal threshold values compared with paclitaxel group until day 8. Starting from day 10, the paclitaxel + CQ10 treated rats had significantly higher mechanical withdrawal thresholds compared with the paclitaxel group ($p = 0.0008$ for day 10, $p = 0.0008$ for day 14, $p = 0.009$ for day 16, $p = 0.001$ for day 19, $p = 0.0002$ for day 39, $p = 0.0001$ for day 41) (Figure 2) until the end of the experimental period, but these values were still significantly lower than that of the controls. The control and control + CQ10 rats had similar mechanical withdrawal threshold values during the protocol ($p = 0.51$). These results suggest that CQ10 treatment has a partial protective effect in paclitaxel-induced peripheral neuropathy in rats.

Discussion. Our study results show that CQ10 has a partial effect in terms of preventing paclitaxel-induced peripheral neuropathy. Paclitaxel-induced peripheral neuropathy is a common side effect of therapy, and is

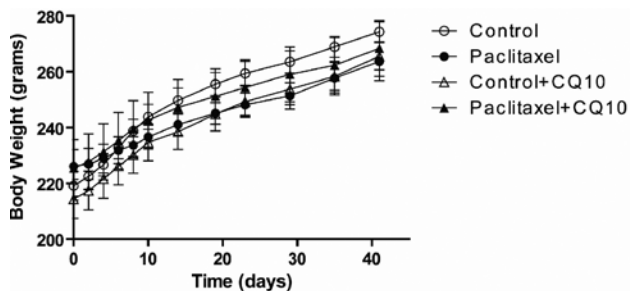


Figure 1 - The time courses of body weights in the experimental groups. Control group received vehicles, paclitaxel group received paclitaxel and vehicle, control + CQ10 group received vehicle and CQ10, paclitaxel + CQ10 group received paclitaxel and CQ10. Data were presented as mean \pm standard error of mean. CQ10: coenzyme Q10. Body weight trends of groups were similar ($p = 0.41$)

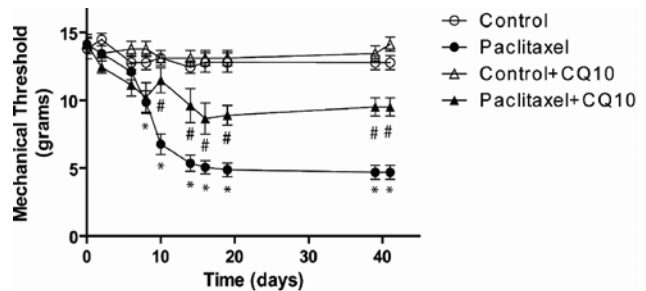


Figure 2 - The time courses of mechanical withdrawal threshold in experimental groups. Control group received vehicles, paclitaxel group received paclitaxel and vehicle, control + CQ10 group received vehicle and CQ10, paclitaxel + CQ10 group received paclitaxel and CQ10. Data were presented as mean \pm standard error of mean. CQ10: coenzyme Q10, * $p < 0.05$ compared to control group ($p = 0.03$ for day 8, $p = 0.001$ for day 10, $p = 0.0007$ for day 14, $p = 0.0006$ for day 16, $p = 0.0006$ for day 19, $p = 0.0005$ for day 39, $p = 0.0005$ for day 41), # $p < 0.05$ compared to paclitaxel group ($p = 0.0008$ for day 10, $p = 0.0008$ for day 14, $p = 0.009$ for day 16, $p = 0.001$ for day 19, $p = 0.0002$ for day 39, $p = 0.0001$ for day 41).

increasing in frequency. Typically, patients describe this type of peripheral neuropathy with symmetrical, painful numbness, paraesthesia and loss of sensory function especially on the feet and hands. Some studies used larger cumulative doses of paclitaxel than used here. The higher doses of paclitaxel damaged sensory fibers in the rats; but lower doses (2mg/kg on 4 alternate days, total of 8 mg/kg) resulted in peripheral neuropathy in the distal extremities. Also, many studies have shown that high doses of paclitaxel impaired animal health and caused peripheral nerve degeneration.^{9,10} In our study, the administration of 8 mg/kg paclitaxel i.p. (cumulative doses) to adult rats produced a peripheral neuropathy. Paclitaxel produced a painful peripheral neuropathy without impairment of the animals' general health. The paclitaxel-induced neuropathic pain sensations began within days and lasted for weeks.

The mechanisms of CIPN are not yet fully understood. Free radicals play a role in the pathogenesis of CIPN, which is characterized by neuronal injury due to chemotherapeutics. Some investigators have reported the useful effect of antioxidants. Acetyl-L-carnitine may be useful for prevention and/or reduction of paclitaxel-induced peripheral neuropathy.^{11,12} Kim et al⁶ indicates that free radical scavengers are potential candidates for the treatment of chemotherapy induced neuropathic pain, and showed that phenyl N-tert-butyl nitron, a free radical scavenger, prevented paclitaxel-induced neuropathic pain in rats.⁶ As an antioxidant, CQ10 scavenges free radicals and acts to inhibit lipid and protein peroxidation. We expect that it will protect neurons from oxidative damage. Also, free radicals and oxidative stress play major roles in the pathogenesis of many neurodegenerative diseases.¹³

Flatters et al^{5,11} suggested that paclitaxel-induced abnormality in the axonal mitochondria of sensory nerves contributes to paclitaxel-induced pain. Coenzyme Q10, which is a potent intracellular antioxidant was shown to prevent mitochondrial damage in the mitochondria of cardiac myocytes, and thus, prevented the development of anthracycline-induced cardiomyopathy.¹⁴ Coenzyme Q10 has also been used in the treatment of mitochondrial disorders.¹⁵ The mitochondria play a central role in apoptotic cell death and mitochondrial dysfunction appears to have a certain impact on the pathogenesis of several neurodegenerative diseases.¹⁶

Folkers et al¹⁷ reported that blood levels of CQ10 were reduced in cancer patients. A nutritional supplement with CQ10 can supplement deficiency and provide extra benefits to cancer patients. Although CQ10 is

found in foods such as meat, fish, fish oils, and the germ of all grains, CQ10 is also available as a nutritional supplement. Coenzyme Q10 is well-tolerated, and no serious adverse effects of CQ10 in humans have been associated with its use, including epigastric discomfort, appetite suppression, nausea, and diarrhea.¹⁸

A limitation of our study is that CQ10 was used only in a dose of 10 mg/kg per day, and the effects of different doses were not investigated. Also, it is unclear whether any significant benefit will remain over long-term follow up and repetitive chemotherapeutic administration. Another limitation of our study is the lack of usage of molecular methods (Western Blot, etc). The prophylactic long-term administration and analgesic effects of CQ10 and different CQ10 doses in a paclitaxel-induced peripheral neuropathy model need to be investigated in future studies.

In conclusion, our results show that CQ10 treatment with paclitaxel decreases the degree of paclitaxel-induced peripheral neuropathy, while a similar effect was not observed in rats that were not under paclitaxel treatment.

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ILLUSTRATIONS, FIGURES, PHOTOGRAPHS

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