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Thymoquinone enhances cisplatin-induced neprotoxicity in high dose

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ABSTRACT

Background: Cisplatin-induced nephrotoxicity is an important problem of the cancer treatments. The major bioactive component of *Nigella sativa*, thymoquinone (TQ) might limit the nephrotoxic effect of cisplatin in low doses. However, it is not clear how it can affect the kidney as an anti-cytotoxic agent when administered in higher doses or in cisplatin co-treatment. Therefore, we examined the *in vivo* interactions between cisplatin and TQ by measuring serum cystatin C (cys C), creatinine and neutrophil gelatinase-associated lipocalin (NGAL) levels and analyzing the expression status of p53 and NGAL by immunohistochemistry.

Methods: Wistar rats were divided into four groups: Control, TQ treatment (group II; 40 mg/kg i.p. for 5 days), cisplatin treatment (group III; 7 mg/kg, i.p. for at day 3) and TQ and cisplatin co-treatment (group IV).

Results: Administration of 40 mg/kg TQ had no effect on serum kidney parameters. In cisplatin received group's serum creatinine level was insignificant, but serum Csy C and NGAL levels were significantly increased. All serum creatinine, NGAL and Cys C levels were increased in co-treatment of cisplatin and TQ. Additionally, in this group, renal tubular damage was found significantly higher than both control and only cisplatin-treated groups. The kidney immunohistochemistry staining of NGAL and p53 were significantly more intense in group IV rather than the others.

Conclusions: This study showed that the administration of cisplatin and high dose of TQ act synergistically to produce nephrotoxicity and the involvement of apoptotic pathway and proximal tubule damage might be the leading cause of on this effect.

1. Introduction

Cisplatin is a potent chemotherapeutic agent and used for the treatment of a broad spectrum of malignancies. *In vitro* studies show that cisplatin selectively and persistently inhibits the synthesis of deoxyribonucleic acid (DNA) whereas, ribonucleic acid (RNA) and protein synthesis are relatively spared.[1] However, new effects of cisplatin have been discovered with an increasing knowledge in pathological mechanisms of cancer. These effects are related to the inhibition of DNA synthesis and repairment that might result in cell cycle arrest at the G1, S, or G2-M phase, therefore apoptosis is induced.[2] The major limiting factors in the use of cisplatin are its side effects which include general cell-damaging effects, such as nausea and vomiting, myelosuppression

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(neutropenia and thrombocytopenia) and immunosuppression.[3] Additionally, nephrotoxicity is regarded as the most common and serious side effect of cisplatin.[4] In the rat animal models, cisplatin primarily damages the proximal tubule, especially the S3 segment.[5] The histopathological characteristics of cisplatin-induced nephrotoxicity in rats are massive necrosis and subsequent regeneration of renal proximal tubular cells.[6] The underlying mechanism of cisplatin-induced nephrotoxicity is not completely understood; however, several mechanisms, including hypoxia, free radicals, inflammation, and apoptosis are thought to be involved.[7] The major benefits of the combination therapies are to reduce the development of drug resistance while using the drugs effectively in cancer treatment and each drug can be given at its optimal dose without intolerable side effects. Therefore cisplatin is currently used in combination with other antineoplastic drugs in *in vitro* cancer models and clinical trials.[8]

Thymoquinone (TQ) is one of the major bioactive components (30–48%) of *Nigella sativa* L. plant which is named as black seed or black cumin.[9] It's steam-distillated essential oil was investigated for its antioxidant and anti-inflammatory properties.[10] It has also been shown that TQ have anti-neoplastic activity against a variety of cancer cell lines.[11] TQ has enhanced the cytotoxic effect of cisplatin in cancer. Also, TQ has been reported to protect the normal cells against cisplatin nephrotoxicity when it is administrated orally in small doses (50 mg/lt in drinking water).[12–15] Moreover, Sagit et al. have been reported that 40 mg/kg TQ has a protective effect on female rats with cisplatin-induced hearing dysfunction.[16] However, there are only a few reports in the literature about the toxicity of TQ. Therefore, it is necessary to study the toxicity of this constituent in animal models. It has been reported that when it is given intraperitoneally, the toxic doses of TQ are varied from 10 mg/kg to 57.5 mg/kg in the rats.[17,18] For the aim of evaluating anti-cancer effect, TQ was given 5–20 mg/kg intraperitoneally in the studies.[12,19] But unfortunately these studies have not been focused on the kidney effect of the agent. Furthermore, the adverse effect of anti-cancer doses of TQ on kidney is unknown. In addition, the impact of administration of TQ over the reno-protective doses concomitantly with a nephrotoxic drug, such as cisplatin on kidney is also ambiguous.

We therefore designed this study to investigate the mechanisms of the possible effect of TQ against *in vivo* cisplatin-induced renal damage in a rat nephrotoxicity model. For this purpose, serum creatinine, Cys C and NGAL levels were measured. Histological changes were evaluated and the expression status of p53 and NGAL were analyzed by immunohistochemistry.

2. Experimental methods

2.1. Animals

The study has the permission of Animal Ethical Committee of Adnan Menderes University (ADU-HADYEK 64583101/2013/028) and the guidelines for the Care and Use of Laboratory animals were strictly followed. Female Wistar rats (n = 28, 8–10wk-old) were kept in an environmentally controlled room at constant temperature (21 ± 1 °C) and humidity (75 ± 5%) under a 12 h light/dark cycle. The animals were acclimatized for 1 week before the study and had free access to standard laboratory feed and water ad libitum.

2.2. Experimental protocol

The rats were divided into four groups. The experimental groups were as follows: *Group I (control)*: The rats in this group were administered intraperitoneally (i.p.) %1 ethanol concentration for 5 days and served as a healthy animal group. *Group II*: TQ (Sigma–Aldrich, Alfagen, Izmir) -treated; 40 mg/kg b.w, i.p. for 5 days.[16] *Group III*: Cisplatin (Platinol®, Bristol-Myers Squibb, Istanbul) -treated; 7 mg/kg b.w., i.p.[14] *Group IV*: TQ (40 mg/kg b.w., i.p. for 5 days) and cisplatin (7 mg/kg b. w., i.p.) co-treated. TQ administration was started two days before the single i.p. injection of cisplatin. TQ was dissolved in ethanol. Final ethanol concentration was 1%. On the day 6 (72 h after the cisplatin treatment), anesthesia induced by a single i.p. injection of Ketamine (Ketalar®, Pfizer, Istanbul, Turkey) and Xylasine (Rompun®, Bayer, Istanbul, Turkey) (50 mg/kg and 5 mg/kg, respectively). Blood samples were taken by

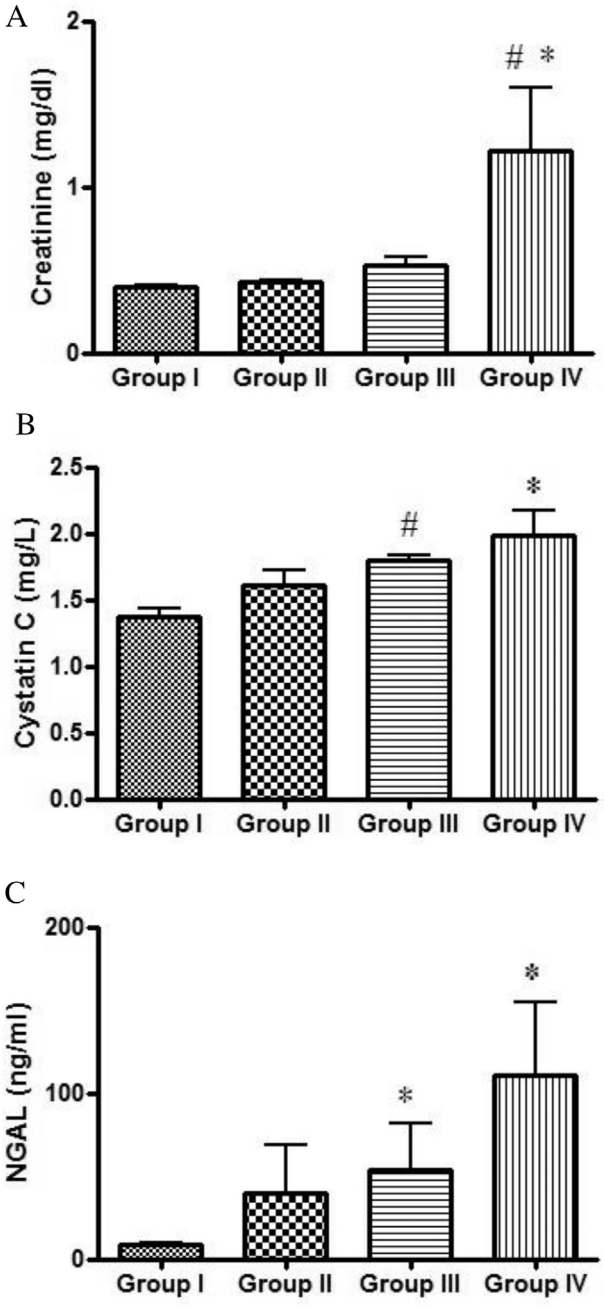

Fig. 1. The effect of thymoquinone on renal functions in cisplatin-induced nephrotoxicity. Values are mean ± SEM (n = 7–8) **A**. Serum creatinine level.*P < 0.01 *vs*. group I (control); #P < 0.05 vs group II (TQ). **B**. Serum CyS C level. *P < 0.01 *vs*. group I; #P < 0.05 *vs*. group I. **C**. Serum NGAL level. *P < 0.01 *vs*. group I (control).