RESEARCH ARTICLE

F.U. Vet. J. Health Sci.
2020; 34 (3): 147 - 151
http://www.fusabil.org

Ameliorating Effects of Rutin on Nephrotoxicity Caused by Cisplatin in Rats

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Cisplatin treatment in patients with solid tumor is common, but a major factor limiting its use is nephrotoxic effects. Thus, this study was performed to investigate the effects of rutin on oxidative stress, apoptotic protein caspase-3 and anti-apoptotic protein Bcl-3 levels in nephrotoxicity caused by cisplatin in rats. The rats were randomly separated into three groups (7 rats in each group). Group I was treated with physiological saline. Group II was treated with cisplatin intraperitoneally on the tenth day of the study. Group III was treated with rutin 150 mg/kg orally for 14 days plus cisplatin 10 mg/kg intraperitoneally on the tenth day of the study. The kidneys and blood samples were collected for the measurement of urea, creatinine, malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), caspase-3 and B-cell lymphoma protein 3 (Bcl-3) levels. Single dose cisplatin treatment in the rats caused the kidney injury. Rutin alleviated nephrotoxicity by significantly increasing antioxidant activity, and decreasing MDA, caspase-3 and Bcl-3 levels ($P<0.001$). The present study suggested that rutin could decrease oxidative stress by enhancing antioxidant activity and nephrotoxicity caused by cisplatin by decreasing apoptotic pathways.

Key Words: *Cisplatin, nephrotoxicity, apoptosis, oxidative stress*

Ratlarda Sisplatinden Kaynaklanan Nefrotoksisite Üzerine Rutinin İyileştirici Etkileri

Solid tümörü olan hastalarda sisplatin tedavisi yaygındır, fakat kullanımını sınırlandıran önemli bir faktör nefrotoksik etkileridir. Dolayısıyla bu çalışma ratlarda sisplatinden kaynaklanan nefrotoksisitede oksidatif stres, apoptotik protein kaspaz-3 ve antiapoptotik protein Bcl-3 düzeyleri üzerine rutinin etkilerini değerlendirmek amacıyla yapıldı. Ratlar rastgele 3 gruba ayrıldı (her grupta 7 adet rat). Grup I'e serum fizyolojik uygulandı. Grup II'ye çalışmanın 10. gününde periton içi sisplatin uygulandı. Grup III'e 14 gün boyunca 150 mg/kg ağızdan rutin ve çalışmanın 10. gününde 10 mg/kg periton içi sisplatin uygulandı. Üre, kreatinin, malondialdehit (MDA), glutatyon (GSH), süperoksit dismutaz (SOD), katalaz, glutatyon peroksidaz (GSH-Px), kaspaz-3 ve B-hücre lenfoma protein 3 (Bcl-3) düzeylerinin ölçülmesi için böbrekler ve kan örnekleri toplandı. Ratlarda tek doz sisplatin uygulanması böbrek hasarına neden oldu. Rutin, antioksidant aktiviteyi artırarak ve MDA, kaspaz-3 ve Bcl-3 düzeylerini azaltarak nefrotoksisiteyi önemli düzeyde azalttı ($P<0.001$). Mevcut çalışma rutinin antioksidant aktiviteyi artırarak oksidatif stresi ve apoptotik yolakları azaltarak sisplatinden kaynaklanan nefrotoksisiteyi hafifletebileceğini gösterdi.

Anahtar Kelimeler*: Sisplatin, nefrotosisite, apoptoz, oksidatif stres*

Introduction

Cisplatin is widely used for the treatment of lung, head and neck, breast, ovarian and testicular cancer in patients (1-6). Cisplatin is highly effective chemotherapeutic drug in the treatment of the various cancer types (7). Cisplatin exerts its effects on tumors via DNA damage, oxidative stress, and inflammation (8). However, the major adverse effect of the cisplatin treatment is acute kidney injury. Cisplatin nephrotoxicity has been reported in 20-40% of the patients treated with cisplatin (9). Cisplatin accumulation in the S3 segment of the renal proximal tubule results in intracellular toxication, tubular necrosis, and mitochondrial vacuolization (10). Kidney injury induced by cisplatin is involved with tubular cell death due to apoptosis and necrosis, vascular dysfunction, and inflammatory reactions of proximal and distal tubules. In cisplatin nephrotoxicity, decrease in cytochrome C oxidase activity and in complex IV protein expression causes the production of the reactive oxygen species (ROS) (11) and decrease in mitochondrial manganese superoxide dismutase (MnSOD) (12), and glutathione (13). The occurrence of mitochondrial dysfunction, inflammation, apoptosis, and necrosis in the kidney resulted from cisplatin treatment causes loss of renal function and tendency toward acute renal failure (14).

Rutin (3,3',4',5,7-pentahydroxyflavone-3rhamnoglucoside), a flavonoid glycoside, consists of flavonol quercetin and disaccharide rutinose. Many plant species such as passion flower, buckwheat, tea and apple contain rutin (15). Rutin consumed in the daily diet is an important flavonoid (16). Rutin has many pharmacologic activities with potent antioxidant and anti-inflammatory effects. Ameliorative effects of rutin on hepatic, renal, reproductive, neurologic, and cardiac diseases have been demonstrated in many studies (17-22).

Received : 17.07.2020
Accepted : 01.09.2020

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Few studies evaluated the protective effects of rutin against cisplatin-induced nephrotoxicity. However, there is no studies on anti-apoptotic protein Bcl-3 levels in cisplatin nephrotoxicity to the best of the author's knowledge. Thus, this study investigated the effects of rutin on oxidative stress, the levels of apoptotic protein caspase-3 and anti-apoptotic protein Bcl-3 in nephrotoxicity caused by cisplatin in rats.

Materials and Methods

Chemicals: Cisplatin (100 mg/100 mL; Onco-Tain, Hospira Inc., Lake Forest, IL, USA) and rutin (rutin hydrate, HPLC ≥ 94; Sigma Chemical Co., St. Louis, MO, USA) were supplied from a pharmacy store. All other chemicals with analytical grade were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals: The study consisted of 21 male Sprague-Dawley rats, 230-250 g weight, and eight weeks old. The animals were obtained from Atatürk University, Experimental Animal Unit, Erzurum, Turkey, and kept under standard conditions (at 24±1 °C temperature and 45±5% humidity and in 12 h light and dark cycle). The rats were fed ad libitum. They were acclimatized for 1 week prior to the experimental process. This study carried out on laboratory animals was approved by Local Ethics Committee of Atatürk University.

The rats were randomly separated into three groups, including 7 rats in each group. Physiological saline was administered to Group I for 14 days. Group II was treated with cisplatin on the tenth day of the study. Group III was treated with rutin 150 mg/kg orally for 14 days (17) plus cisplatin 10 mg/kg intraperitoneally on the tenth day of the study.

Blood and Kidney Samples Collection: Blood samples taken to the tubes without any anticoagulant were allowed to coagulate for 15 min at room temperature and centrifuged at 800 g for 5 min at 4 °C. The serum samples were allocated to the Eppendorf tubes and kept at -20 °C.

The rat kidneys were removed after euthanasia under sevoflurane anesthesia. They were washed with physiological saline and stored at -20 °C for further analyses.

Biochemical Analyses: The commercial kits were used for the analyses of the serum urea and creatinine concentrations (Diasis Diagnostic Systems, Istanbul, Turkey). In the, kidney, malondialdehyde (MDA) levels (23), catalase activity (24), protein concentration in the supernatant (25), superoxide dismutase (SOD) activity (26), glutathione (GSH) content (27), and glutathione peroxidase activity (GSH-Px) (28) were analyzed. In addition, caspase 3 and B-cell lymphoma protein 3 (Bcl-3) levels were determined according to the ELISA kit procedures.

Statistical Analysis: The variables of this study had normal distribution according to Kolmogorov-Smirnov test. Accordingly, the data were analyzed with one-way ANOVA and Tukey HSD tests using SPSS package program. The data were presented as the mean ± standard error of means (SEM). Statistical significance level was determined as $P<0.05$ (29).

Results

Assessment of Renal Function: The serum urea and creatinine concentrations were significantly increased in cisplatin group than control group. The kidney function markers such as serum urea and creatinine concentrations were significantly decreased in cisplatin plus rutin treated group compared to cisplatin group. However, these concentrations were still high in cisplatin plus rutin treated group compared to control group (Table 1).

Table 1. Effects of rutin on serum renal function markers, and on oxidants, antioxidants, apoptotic and anti-apoptotic parameters in rats with cisplatin nephrotoxicity

Parameters	Group I Control group	Group II Cisplatin-treated group	Group III Cisplatin-plus-rutin-treated group	P
Urea (mg/dL)	7.46±0.22^{c}	19.36±0.36^{a}	14.19±0.39^{b}	*
Creatinine (mg/dL)	0.62±0.01^{c}	1.96±0.05^{a}	1.29±0.04^{b}	*
Malondialdehyde (nmol/g tissue)	65.03±0.71^{a}	95.27±0.89^{a}	80.64±0.54^{b}	*
Glutathione (nmol/g tissue)	4.22±0.03^{a}	2.35±0.07^{c}	3.10±0.05^{b}	*
Catalase (katal/g protein)	56.44±0.46^{a}	41.92±0.42^{c}	48.09±0.39^{b}	*
Glutathione peroxidase (U/g protein)	32.72±0.65^{a}	22.04±0.38^{c}	27.83±0.37^{b}	*
Superoxide dismutase (U/g protein)	29.20±0.58^{a}	20.45±0.45^{c}	24.55±0.32^{b}	*
Caspase-3 (ng/g tissue)	31.22±0.42^{c}	61.40±0.46^{a}	44.68±0.51^{b}	*
B-cell lymphoma protein 3 (ng/g tissue)	256.19±2.95^{c}	416.44±4.26^{a}	361.44±5.29^{b}	*

Notes : All the values are presented as the mean ± SEM of seven rats in each group.
* : $P<0.001$
^{a,b,c} : Mean values with different superscripts within a row have a significant difference.

MDA Levels: The kidney MDA levels were significantly elevated in cisplatin group than control group. The kidney MDA levels were significantly reduced in cisplatin plus rutin treated group than cisplatin group. However, the decrease in MDA levels in cisplatin plus rutin treated group was still significantly higher than control group (Table 1).

Evaluation of Antioxidants: The kidney GSH level, catalase, GSH-Px, and SOD activities were significantly reduced in cisplatin group than control group. The kidney GSH level, catalase, GSH-Px, and SOD activities were significantly increased in cisplatin plus rutin group than cisplatin group, but were still lower than control group (Table 1).

Evaluation of Apoptosis-Related Parameters: The kidney caspase-3 and Bcl-3 levels were significantly elevated in cisplatin group than control group. The kidney caspase-3 and Bcl-3 levels were significantly reduced in cisplatin-plus-rutin-treated group than cisplatin group. However, these levels were still higher in cisplatin-plus-rutin-treated group than control group (Table 1).

Discussion

In this study, it was focused on the evaluation of the effects of rutin against cisplatin-induced kidney injury with the kidney function tests (serum urea and creatinine), oxidative stress parameter (MDA), antioxidants (catalase, SOD, GSH), caspase-3, and Bcl-3 parameters. This study demonstrated the oxidative stress, increased caspase-3 and Bcl-3 levels in the kidney injury induced by cisplatin treatment. Similarly, several studies have demonstrated that the administration of cisplatin to the rats causes nephrotoxicity by elevated inflammatory markers such as NF-κB, TNF-α, and IL-1β and oxidative stress marker MDA levels and decreased antioxidants such as GSH levels, catalase, and SOD activities (30). In this study, single dose of 10 mg/kg cisplatin established the kidney injury determined by the kidney dysfunction as demonstrated by increased serum urea and creatinine concentrations, increased MDA levels, and increased caspase-3 levels.

In the pathogenesis of cisplatin-induced kidney injury, the mitochondrial dysfunction caused by reactive oxygen species (ROS) with increase in oxidants such as superoxide anion, hydrogen peroxide and decrease in SOD activities were noted (31, 32). Another study also confirmed the role of increased ROS, and apoptotic pathway proteins such as caspase-3, NF-κB, and TNF-α in cisplatin related kidney injury (33). Similarly, another study showed that cisplatin induced the release of proinflammatory cytokines such as NF-κB, TNF-α, IL-1β, IL-6, IL-33, iNOS and COX-2 and decreased the kidney SOD, catalase and GSH-Px activities and GSH levels (34).

In this study, the increased kidney MDA levels revealed that cisplatin could cause lipid peroxidation in the kidney epithelial cells via decrease in the kidney SOD, catalase and GSH-Px activities and GSH levels, and could activate apoptotic protein pathways as revealed by increased caspase-3 levels. This suggests the activation of caspases and the induction of apoptosis. Caspases are activated by the release of cytochrome C to cytosol (35). Malik et al. (35) have showed that cisplatin causes increase in caspase expression and apoptosis in the kidney cells.

In this study, rutin pretreatment prevented significantly apoptotic protein caspase-3 increase in cisplatin plus rutin group. Thus, rutin pretreatment provided decrease in anti-apoptotic protein Bcl-3 due to alleviation of apoptosis in the kidney of cisplatin plus rutin group. This suggests that rutin has significant anti-apoptotic effects against cisplatin nephrotoxicity in the rat kidney tissue.

Several studies have demonstrated that the antioxidant compounds such as rutin (33), tangeretin (36), embelin (30), melatonin (367) prevent oxidative stress and inflammation on cisplatin-induced kidney injury.

Rutin, an active citrus flavonoid compound, exerts its effects via potent antioxidant properties. Rutin can scavenge radicals and activate intrinsic antioxidant defense systems (17, 19, 38).

Cisplatin can lead to the depletion of GSH and other antioxidants (34). In this study, rutin pretreatment reversed the kidney GSH depletion due to cisplatin treatment by increasing significantly GSH contents in the kidney. In addition, rutin pretreatment reversed oxidative stress induced by cisplatin as demonstrated by increased GSH levels, SOD, catalase, and GSH-Px activities, and decreased MDA levels.

B-cell lymphoma protein 3 (Bcl-3) is described as a negative regulator of transcription factor NF-κB (39, 40). Poveda et al. (41) have revealed that Bcl-3 is upregulated in acute kidney injury and supports to block inflammatory and lethal responses related to inflammatory stimuli in tubular cells. In addition, cleaved caspase-3 is increased by Bcl-3 silencing in inflammatory condition. Accordingly, rutin pretreatment in cisplatin nephrotoxicity provided the reduction of kidney Bcl-3 levels due to alleviation of oxidative stress, the reduction of the apoptotic protein caspase-3, and increasing of GSH levels and other antioxidants such as SOD, catalase and GSH-Px activities in the rat kidneys.

This study demonstrated that rutin pretreatment given at a dose of 150 mg/kg alleviated cisplatin nephrotoxicity in the rats by decreasing serum urea and creatinine concentrations, the kidney MDA and the kidney caspase-3 levels, and by increasing the kidney SOD, catalase and GSH-Px activities, and GSH levels.

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