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ARAŞTIRMA / RESEARCH

Protective effect of resveratrol on cisplatin induced damage in rat kidney

Resveratrolün sıçan böbreğinde cisplatine bağlı hasar üzerindeki koruyucu etkisi

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Abstract

Purpose: The aim of this study was to evaluate the protective effect of resveratrol on cisplatin induced damage in rat kidney.
Materials and Methods: 30 female Wistar-Albino rats were allocated to form three groups: In group 1 (control group), 1 mL of 0.9% NaCl (saline) was administered intraperitoneally for 3 days. In group 2 (cisplatin group), 7.5 mg / kg intraperitoneal cisplatin was given for 3 days. In group 3 (cisplatin + resveratrol group) 7.5 mg / kg cisplatin and 10 mg / kg resveratrol were given via intraperitoneal route. Right kidneys were surgically extirpated in all groups. Malondialdehyde (MDA) levels and activities of catalase (CAT) and superoxide dismutase (SOD) were measured in both blood and tissues. Also, toxicity markers such as vascular congestion, hemorrhage, tubule degeneration and glomerular damage were assessed by examining the slides prepared from kidney tissue with microscopy.
Results: Tissue damage was significantly higher in group 2 than other groups. The MDA levels were significantly higher and the activities of SOD, and CAT were lower in group 2 than other groups.
Conclusion: According to our short term findings, resveratrol might be an effective molecule to prevent the harmful effect of cisplatin in rat kidney.
Keywords:. Cisplatin, resveratrol, rat, kidney, toxicity

Öz

Amaç: Bu çalışmanın amacı, resveratrolün sıçan böbreğinde cisplatin kaynaklı hasar üzerine koruyucu etkisini değerlendirmektir.
Gereç ve Yöntem: 30 adet dişi Wistar-Albino sıçan üç gruba ayrıldı: Grup 1'de (kontrol grubu) 3 gün boyunca 1 mL %0.9 NaCl (salin) intraperitoneal olarak uygulandı. Grup 2'de (cisplatin grubu) 3 gün süreyle 7.5 mg/kg intraperitoneal sisplatin verildi. Grup 3'te (cisplatin + resveratrol grubu) 7,5 mg/kg cisplatin ve 10 mg/kg resveratrol intraperitoneal olarak verildi. Sağ böbrekler tüm gruplarda cerrahi olarak ekstirpe edildi. Hem kanda hem de dokularda malondialdehit (MDA) seviyeleri ve katalaz (CAT) ve süperoksit dismutaz (SOD) aktiviteleri ölçüldü. Ayrıca böbrek dokusundan hazırlanan slaytlar ışık mikroskobu ve immünohistokimya ile incelenerek damar tıkanıklığı, kanama, tübül dejenerasyonu ve glomerüler hasar gibi toksisite belirteçleri değerlendirildi.
Bulgular: Grup 2'de doku hasarı diğer gruplara göre anlamlı derecede yüksekti. Grup 2'de diğer gruplara göre MDA düzeyleri anlamlı olarak yüksek, SOD ve CAT aktiviteleri daha düşüktü.
Sonuç: Kısa dönem bulgularımıza göre resveratrol sıçan böbreğinde cisplatinin zararlı etkisini önlemede etkili bir molekül olabilir.

Anahtar kelimeler: Cisplatin, resveratrol, sıçan, böbrek, toksisite

INTRODUCTION

Cisplatin has been utilized for many years in gynecologic cancers. However, its' toxic effects such as damages of kidney, ovary, esophagus, blood, and liver were diminished the widespread usage[1]. Numerous mechanisms could lead to kidney damage. Nuclear and mitochondrial DNA injury occurs due

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to cisplatin accumulation[2]. Thus, cisplatin constitutes a complex with DNA and this formation is responsible for the cytotoxic effect[3]. Reactive oxygen species (ROS) and free radicals formed during oxidative stress due to cisplatin. These elevated free radicals and ROS levels deteriorate the cellular anatomy and triggers the apoptosis. Also, cisplatin causes oxidative stress on cellular organels especially mitochondrion. Calcium uptake into the cell is reduced, mitochondrial protein-SH level decreases and as a result the function of mitochondrial membrane deteriorates p53 gene is elevated due to DNA damage[4]. The elevated p53 gene leads to DNA repair and cell death by apoptosis[5].

Resveratrol is a polyphenolic substance that is found in fruits such as blueberry, grape and peanut. It has been shown that resveratrol could be useful for prevention of vascular diseases, metabolic syndrome, coronary heart diseases, and stress. Den Hartogh et al reported that resveratrol has beneficial effects against kidney diseases. The possible underlying mechanisms were diminishing oxidative stress, decreasing inflammation, and enhancing antioxidant activity[6]. Also, prior studies showed that resveratrol exhibits cytoprotective effect and improves endothelial activities[7].

Therefore, we hypothesized that resveratrol might improve the harmful effect of cisplatin. There is no study demonstrating the behaviour of resveratrol against cisplatin induced nephrotoxicity, yet. Thus, this study was planned to assess the efficacy of resveratrol on due to toxicity of cisplatin on renal tissue.

MATERIALS AND METHODS

Cisplatin and resveratrol were purchased from a drug store (Kirsehir, Turkey). Cisplatin was administered in terms of the prior reported article[8]. Resveratrol was given in relation with a treatment protocol reported by Bao et al[9]. Resveratrol was given via intraperitoneal route.

Animals

A total of 30 female adult Wistar-Albino rats were utilized in this study. Rats were kept between 20 and 22 °C under a 12 h light/12 h dark cycle. The rats fed with Ad libitum. The study was approved by the Ethical Committee of Kirikkale University Medical Faculty. The date and protocol number of the ethics committee aproval were 18.06.2020 and 2020/03/16, respectively. All animals were allocated randomly into three groups. In group 1 (control group), intraperitoneal 1 mL of 0.9% NaCl (saline) was administered for 3 days. In group 2 (cisplatin group), 7.5 mg / kg intraperitoneal cisplatin was given for 3 days. In group 3 (cisplatin + resveratrol group) 7.5 mg / kg cisplatin and 10 mg / kg resveratrol were given via intraperitoneal route.

Ketamine hydrochloride (45 mg/kg, Ketalar, Eczacibasi, Istanbul, Turkey) and xylazin hydrochloride (5 mg/kg, Rompun, Bayer, Leverkusen, Germany) were utilized for anesthesia. All rats were sacrificed via cervical dislocation when the drug doses were finished. Then their kidneys were surgically removed.

Histopathological examination

The tissue samples were stored in 10% formaldehyde and then embedded in paraffin. The tissues were cut at 4 μm and stained with hematoxylin-eosin. The hematoxylin-eosin dying was performed according to the method reported by Lillie et al[10]. Additionally, immunohistochemical p53 staining was performed. p53 staining was performed in relation with the method of Bradford[11]. Histopathological findings were evaluated under a light microscope (Olympus CX41 microscope) (Olympus Corp., Tokyo, Japan) by a pathologist who was unaware of the experimental groups. 10 fields for each slide was analyzed and evaluated for severity of changes. Histopathological scoring was done by determining the highest area. Four categories (0: None 1: Minimal 2: Mild 3: Moderate 4: Severe) were determined by making semi-quantitative analysis and the parameters were scored accordingly. 'Tubular dilatation, proteineous material accumulation in the tubule, and tubular epithelial cell change' parameters were used to determine the degree of tubular damage. Glomerular damage was evaluated by measuring the fibrosis/ atrophy/thrombosis. Interstitial damage was assessed according to the degree of fibrosis, congestion, hemorrhage, and mononuclear inflammatory cell infiltration.

Immunohistochemistry

p53 expression levels were graded using the 0-3 + range. (p53; 0: no staining, 1: less than 10% nuclear staining in renal tubular epithelial cells, 2: 10-30% nuclear staining ,3: more than 30% nuclear staining).

Biochemistry

Both tissue and blood samples were examined. Malondialdehyde (MDA) levels and superoxide dismutase (SOD) and catalase (CAT) activities were measured by calculating the absorbance of the samples in a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). Thiobarbituric acid test was used to calculate the MDA levels[12]. SOD enzyme activity was calculated in relation with the method reported by Marklund et al[13]. CAT activity was assessed in relation with the method of Aebi[14].

Statistical analysis

Statistical Package for the Social Sciences (22.00 SPSS Inc., Chicago, IL) was used for statistical analyses. Power analysis was used and the sample size was calculated as at least 8 for each group with 80% accuracy. One-way ANOVA test was used for levels of tissue and blood MDA, and activities of SOD and CAT. Tissue damage scores were compared by nonparametric chi square test. p value < 0.05 was accepted as statistically significant.

RESULTS

Tissue MDA and SOD levels were demonstrated in Table 1. The MDA level was significantly lower in cisplatin + resveratrol group compared to cisplatin group ($p < 0.05$). SOD and CAT activities were found to be significantly higher in cisplatin + resveratrol group than cisplatin group, too ($p < 0.05$). Blood MDA levels and SOD and CAT activities were distributed similarly.

Table 1. Tissue levels of malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) in experimental groups.

Groups (n = 10)	MDA (nmol/mg)	SOD (U/mg)	CAT (U/mg)
Control	6.41 ± 0.17	47 ± 3.8	92 ± 6.7
Cisplatin (7.5 mg/kg)	11.54 ± 0.38*	21 ± 2.4*	30 ± 3.2*
Cisplatin+resveratrol (7.5 mg/kg+10 mg/kg)	8.23 ± 0.29*	33 ± 2.9*	67 ± 5.2*

MDA means malondialdehyde, SOD means superoxide dismutase, CAT means catalase Data are presented as mean ± SD.
* Significant difference ($p < 0.05$) between groups 2 and 3.

Table 2. Distribution of histopathologic findings.

Groups (n = 10)	Tubular Dilatation	Hemorrhage	Fibrosis	Glomerular atrophy	Tubular cell degeneration
Control	0	0	0	0	0
Cisplatin (7.5 mg/kg)	2*	3*	2*	2*	4*
Cisplatin+resveratrol (7.5 mg/kg+10 mg/kg)	1*	1*	1*	1*	2*

* Significant difference ($p < 0.05$) between groups 2 and 3.
Histopathological scoring was done by determining the highest area. Four categories (0: None 1: Minimal 2: Mild 3: Moderate 4: Severe) were determined by making semi-quantitative analysis and the parameters were scored accordingly.

There was no difference between the cisplatin and cisplatin+resveratrol groups according to the macroscopic features of the kidney tissue. The scores demonstrating histopathologic damage were significantly higher in cisplatin group than cisplatin+resveratrol group ($p < 0.05$). All of these damage scores were shown in Table 2.

The morphological appearance of the tissues in the control group was normal and ordinary cellular architecture (Figure 1A). Glomerular atrophy and interstitial hemorrhage, mononuclear inflammatory cell infiltration, and interstitial fibrosis were seen in cisplatin-administered rats (Figure 1B). Also, massive tubular cell degeneration and interstitial hemorrhage were detected. In the cisplatin+resveratrol group, the kidney tissue damage scores such as interstitial hemorrhage, fibrosis, and mononuclear cell infiltration were less than cisplatin group. Glomerular compartment and tubulo- interstitial areas were better preserved and morphologically similar to control tissue (Figure 1C).

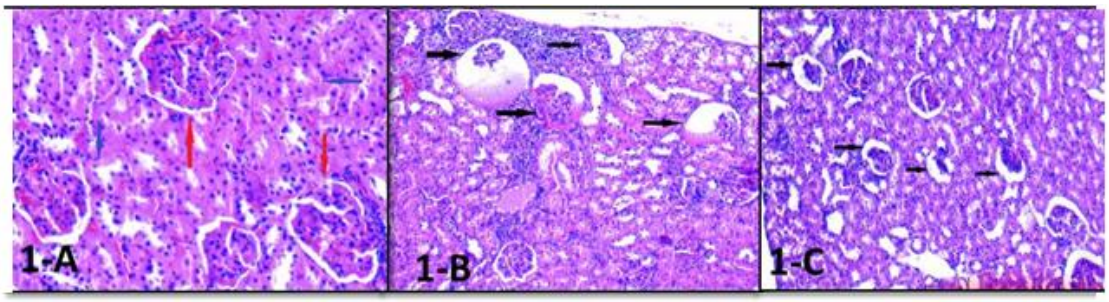

Figure 1. Light microscopic appearance of kidney. (A) Histological appearance of normal glomeruli (red arrow) and tubular structures (blue arrow) in the kidney of the rats from the control group (H&E, x200). (B) In the cisplatin group, kidney sections showed pronounced glomerular atrophy (black arrow) (H&E, x100). (C) In the cisplatin+resveratrol group, glomerular atrophy was not as pronounced as in the cisplatin group (black arrow) (H&E, x100).

In p53 immunohistochemical staining of rats, the distribution of the groups was similar. Glomerular atrophy and interstitial inflammatory cell infiltration were pronounced in cisplatin group than cisplatin+resveratrol group, but the difference was not statistically significant (Figure 2A, B, C).

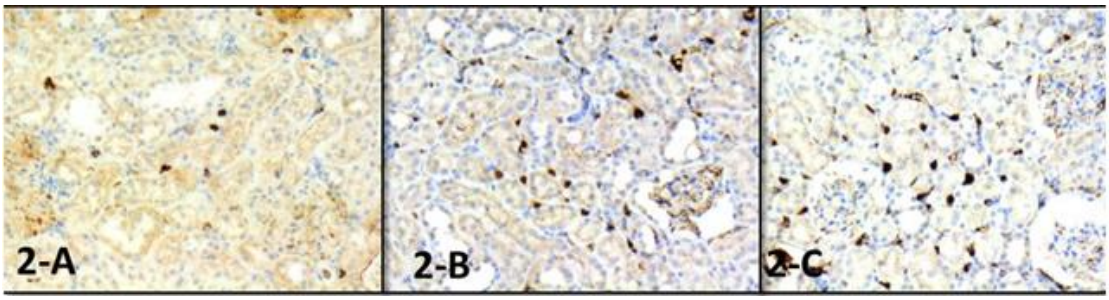

Figure 2. (A, B, C). In p53 immunohistochemical staining of rats belonging to the control, cisplatin and cisplatin+resveratrol groups, respectively, similar and weak staining prevalence were observed in focal areas (x200) (x200) (x200).

DISCUSSION

The effect of resveratrol on cisplatin induced nephrotoxicity was studied in this prospective randomized controlled rat trial. To the best of our knowledge, this is the first experimental study to evaluate the renoprotective effect of resveratrol on cisplatin-induced nephrotoxicity. At the end of the study, tissue MDA levels were lower and SOD and CAT activities were found to be higher in cisplatin group than cisplatin+resveratrol group, and this differences were appeared to be statistically significant ($p < 0.05$). Also, resveratrol usage reversed the harmful histopathological effects of cisplatin.

Although the cisplatin is a potent and widespreadly used chemotherapeutic agent the clinicians should pay attention about the toxicity of this molecule[15]. One of the organs in which cisplatin is most toxic is the kidney. Despite many theories were alleged about the damage the exact mechanism still remains a challenge. For example, cisplatin could exhibit a direct cytotoxicity due to DNA formation[16]. Satoh et al claimed that the increased lipid peroxidation and the elevated free radicals and reactive oxygen species (ROS) due to cisplatin contributes the toxicity[17]. They reported that especially elevated MDA levels were responsible from the cellular damage. In the present study, the levels of MDA were significantly increased in cisplatin group than other groups.

Many antioxidant chemicals such as quercetin, selenium, and curcumin were used to decrease the adverse effects caused by cisplatin[18, 19]. Resveratrol is a natural component of phenol. Hascalik et al reported that resveratrol reduced the lipid peroxidation on ischemia-reperfusion damage of the ovaries in a rat model[20]. Gocmen et al demonstrated that resveratrol showed cardioprotective effects in hypercholesterolemic rats[21]. It has been suggested that resveratrol had beneficial effect on many diseases, from diabetes to hypertension. Various theories have been suggested about resveratrol's mechanisms of action. These are antioxidant features, antiinflammatory activities and related to its ability to modulate many molecules such as vascular endothelial growth factor, cytokines, and caspases[22]. Thus, we thought that resveratrol could be effective for decreasing the toxic renal injury due to cisplatin.

Measuring the expression of proteins associated with apoptosis and oxidative stress can provide insight into the extent of stress-induced damage[23]. Therefore, we wanted to assess p53 expression with immunohistochemical staining.

In present study, the addition of resveratrol enhanced the activities of SOD and CAT and reduced the MDA levels. The preventive effect of resveratrol was confirmed also histopathologically. The damage scores such as hemorrhage, fibrosis and inflammatory cell infiltration were significantly lower in cisplatin+resveratrol group than cisplatin group. Eventhough there was no significant difference with immunohistochemical staining, the renal damage was more prominent in cisplatin group than cisplatin+resveratrol group.

The small number of subjects and the possibility of variation when the study was adapted to humans are the limitations of our study.

In conclusion, resveratrol seems to be effective to prevent the renal injury due to cisplatin. Nevertheless, large randomized and controlled clinical trials are required to assess the efficacy of resveratrol on cisplatin induced renal toxicity.

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