



The Protective Effect of Resveratrol on Cisplatin Induced Damage in Rat Liver

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Abstract

Aim: One of the underlying causes of cisplatin-induced hepatotoxicity is oxidative stress. We assessed the effect of an antioxidant, resveratrol, on cisplatin-induced damage in the rat liver.

Methods: The project-starting date was designed as 01.10.2020 and the project-ending date was planned as 01.04.2021. Three groups were created with 30 female Wistar-Albino rats: 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 the intraperitoneal route. The livers were surgically extirpated in all the groups. In both blood and tissues, malondialdehyde (MDA) levels and activities of catalase (CAT) and superoxide dismutase (SOD) were measured. Also, toxicity markers such as hepatocyte damage (cellular changes), inflammation, hemorrhage, congestion, fibrosis, disorganization of the hepatic cords, and necrosis were assessed by examining the preparations prepared from hepatic tissue with light microscopy and immunohistochemistry.

Results: Histopathological tissue damage was significantly higher in group 2 than in other groups ($p < 0.03$). MDA levels were significantly higher and the activities of SOD and CAT were lower in group 2 than in the other groups ($p = 0.04$ and $p = 0.01$, respectively).

Conclusion: According to our short-term findings, resveratrol might be an effective molecule for preventing the harmful effects of cisplatin in the rat liver.

Keywords: Cisplatin, resveratrol, rat, liver, toxicity

Introduction

Cisplatin is a potent chemotherapeutic drug and has been used for malignancies. However, cisplatin can have toxic effects on the kidney and liver even at normal therapeutic doses. Cisplatin constitutes a compound that binds covalently to the DNA bases (1). These crosslinks in the DNA lead to cytotoxic damage in cancer cells. If cisplatin levels rise above the toxic dose, normal cells are affected as well as cancer cells (2). It has been shown that cisplatin toxicity tends to increase in hypochloremic status. At these low chlorine levels, especially harmful compounds such as reactive oxygen species (ROS) and free radicals are released more frequently. The final result of this situation is cell and tissue damage (3). Also, cisplatin causes oxidative stress on cellular organelles, especially mitochondrion.

Calcium uptake into the cell is reduced, mitochondrial protein-SH level decreases, and as a result, the function of the mitochondrial membrane deteriorates (4). Numerous protective mechanisms were developed by the cells to prevent oxidative damage due to cisplatin. Antioxidant effects are increased due to protective enzymatic activities (5,6). Gupta et al. (7) indicated that administration of antioxidants could diminish the extent of the injury.

Resveratrol is a natural antioxidant compound, detected in red wine, grapes, mulberries, and peanuts. It has been shown that resveratrol could be useful for the prevention of vascular diseases, metabolic syndrome, coronary heart diseases, and stress (8). Den Hartogh and Tsiani (9) reported that usage of resveratrol enhanced the injury of renal injury by diminishing oxidative stress,

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decreasing inflammation, and increasing antioxidant activity.

Therefore, it was hypothesized that the addition of an antioxidant such as resveratrol would be useful to prevent the ovarian damage due to cisplatin. There are no studies demonstrating the preservative effect of resveratrol on hepatic injury. In this study, we assessed the protective effect of resveratrol on cisplatin-induced hepatic damage in rats.

Materials and Methods

Compliance with Ethical Standards

This study was approved by the Kirikkale University Animal Experiments Local Ethics Committee (date: 18.06.2020, approval no: 2020/03-16). Cisplatin and resveratrol were obtained from a pharmacy (Kirsehir, Turkey). Cisplatin and resveratrol were given in relation to the treatment protocols reported in previous studies (10,11).

Study Design

A total of 30 female adult Wistar-Albino rats weighing 150-220 g were included in the study. All animals were housed for one week at 24 ± 2 °C. The ad libitum method was performed using a laboratory diet. The ages of the rats were between 8 and 12 weeks. The rats were randomly allocated into three groups (10 rats per group): control (1 mL of 0.9% NaCl), cisplatin (7.5 mg/kg cisplatin), cisplatin + resveratrol (7.5 mg/kg cisplatin+10 mg/kg resveratrol).

Ketamine hydrochloride (45 mg/kg, Ketalar, Eczacibasi, Istanbul, Turkey) and xylazin hydrochloride (5 mg/kg, Rompun, Bayer, Leverkusen, Germany) were used for anesthesia. Cervical dislocation was performed as a sacrifice procedure. Then, the liver was extirpated.

Histopathology

The samples of liver tissue were fixed in a 10% formalin solution and were dehydrated with alcohol. Then the embedding process is completed using paraffin. The tissues were cut at a thickness of $4 \mu\text{m}$ and the sections were stained with hematoxylin and eosin dye (H&E). Additionally, immunohistochemically, bcl-2 and Masson trichrome staining were performed to evaluate fibrosis. Histopathological findings were examined using a microscope (Olympus CX41 microscope, Olympus Corp., Tokyo, Japan) by a pathologist who did not know the experimental groups. A minimum of 10 fields for each kidney slide were analyzed and evaluated for the severity of changes. Histopathological scoring was performed by determining the highest area. The four categories (0: None, 1: Minimal, 2: Mild, 3: Moderate, 4: Severe) were determined by making a semi-quantitative analysis and the parameters were scored accordingly. We

used the parameters of "hepatocyte damage (cellular changes), inflammation, hemorrhage, congestion, fibrosis, disorganization of the hepatic cords, necrosis" to determine the degree of damage.

Immunohistochemistry

The Bcl-2 expression levels were graded using the 0-3+ range (Bcl-2; 0 indicates no staining, 1: hepatocytes with less than 10% cytoplasmic or membranous staining, 2: hepatocytes with 10-30% cytoplasmic or membranous staining, 3: hepatocytes with more than 30% cytoplasmic or membranous staining). A Masson trichrome dye was used to assess fibrosis.

Biochemistry

Both tissue and blood samples were analyzed for MDA levels and SOD and CAT activities using a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). A thiobarbituric acid test was used to calculate the MDA levels (12). SOD enzyme activity was calculated in relation to the method reported by Marklund and Marklund (13). The activity of CAT was assessed in relation to a prior study (14).

Statistical Analysis

The Statistical Package for the Social Sciences (22.0 SPSS Inc., Chicago, IL) was used for statistical analyses. A One-Way ANOVA test was used for levels of tissue and blood MDA and activities of SOD and CAT. Tissue damage scores were compared using the non-parametric chi-square test. A p-value < 0.05 was set as statistically significant.

Results

SOD and CAT activities were lower in the cisplatin group than in the cisplatin + resveratrol group, and the difference was found to be statistically significant ($p=0.01$). The level of MDA was significantly lower in the cisplatin + resveratrol group than in the cisplatin group ($p=0.04$) (Table 1).

There was no difference between the groups in terms of the macroscopic appearance of the tissues. Scores indicating histopathological damage were lower in the cisplatin + resveratrol group than in the cisplatin group ($p=0.03$) (Table 2).

Groups were demonstrated as pie chart (Figure 1). The morphology and structural characteristics of the liver tissue were close to normal in the control group. Hepatic damage parameters such as hepatocyte damage, inflammation, hemorrhage, congestion, fibrosis, disorganization of the hepatic cords, and necrosis, were more prominent in the cisplatin group than in the cisplatin + resveratrol group (Figure 2). Immunohistochemical staining of rats using Masson trichrome dye showed that the hepatic damage was more in the cisplatin group (Figure 3).

Table 1. Distribution 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	5.91±0.12	40±3.2	88±6.0
Cisplatin (7.5 mg/kg)	10.33±0.34*	18±1.9*	27±2.8*
Cisplatin + resveratrol (7.5 mg/kg+10 mg/kg)	7.61±0.21*	29±2.5*	58±4.6*

Data are presented as mean ± SD.
 *Significant difference (p<0.05) between cisplatin group and cisplatin + resveratrol group
 SD: Standard deviation

Table 2. Distribution of histopathologic findings

Groups (n=10)	Hepatocyte damage	Disorganization of the hepatic cords	Inflammation	Congestion	Hemorrhage	Necrosis	Fibrosis	Bcl-2 expression
Control	0	0	0	0	0	0	0	0
Cisplatin (7.5 mg/kg)	1	1	2*	2*	2*	1*	1	1
Cisplatin + resveratrol (7.5 mg/kg+10 mg/kg)	1	1	0*	1*	0*	0*	1	1

*: Significant difference (p<0.05) between cisplatin group and cisplatin+resveratrol group.
 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

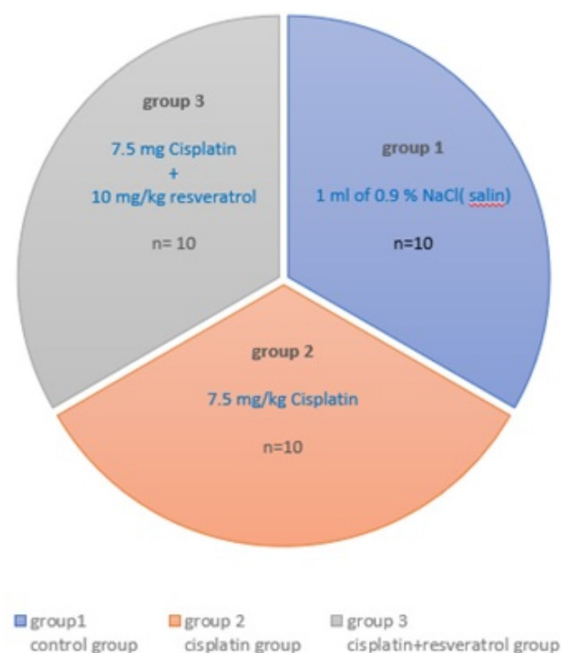
Although the parameters-indicating injury were more prominent, the fibrosis was similar in the cisplatin group and the cisplatin + resveratrol group. Likewise, more damage was observed in the cisplatin group than in the cisplatin + resveratrol group, with bcl-2 staining (Figure 4).

Discussion

Although cisplatin has been widely used for treating cancers, it has severe toxicities such as hepatotoxicity, nephrotoxicity, and ototoxicity. Hepatotoxicity is uncommon, as is nephrotoxicity. However, Zicca et al. (15) demonstrated that when cisplatin was used in high doses, it could have a toxic effect on the liver. There are a few studies on the toxicity of cisplatin in the liver. Therefore, we assessed the preservative effect of resveratrol on cisplatin-induced hepatic damage. To the best of our knowledge, this is the first trial indicating the protective effect of resveratrol on cisplatin-induced hepatotoxicity. Our study has demonstrated that MDA levels tend to increase because of cisplatin. Also, SOD and CAT activities decreased. Additionally, the histopathology of the liver was adversely affected by cisplatin. Resveratrol reduced cisplatin-induced biochemical and histological changes in the rat liver.

Cisplatin could deteriorate liver function by disrupting the balance of oxidants and antioxidants. ROS, hydroxyl radicals, and free radicals formed after oxidative stress might disrupt cellular integrity (16). Koc et al. (17) reported that erdostein reduced cisplatin-induced liver damage by scavenging free radicals and ROS. Cisplatin exhibits toxic effects by binding DNA. Xanthorrhizol could reverse these harmful environments via regulation of DNA-binding activities of transcription factors (18).

SOD and CAT are powerful endogenous enzymatic antioxidants. They reverse oxidative stress by converting H_2O_2 into water and oxygen. Several molecules are used to prevent oxidative stress, such as quercetin, selenium, and curcumin, which were used to decrease the toxicity of cisplatin (19-21). Resveratrol is a natural derivative of phenol. Several theories have been suggested about resveratrol's mechanisms of action. These are antioxidant features, antiinflammatory activities, and related to its

**Figure 1.** Demonstration of the groups as pie chart

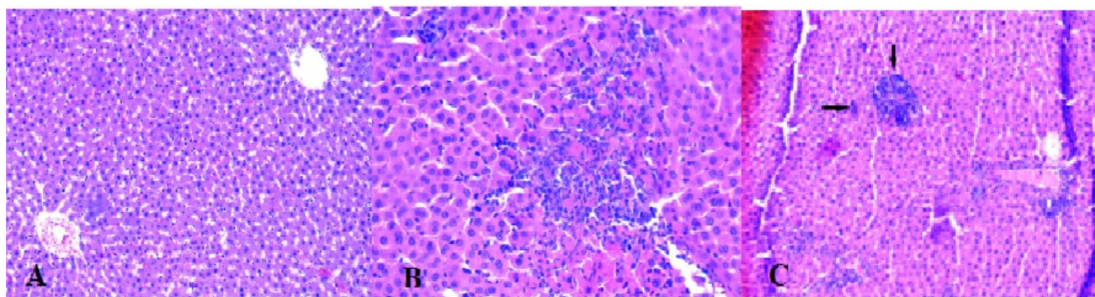


Figure 2. Light microscopic appearance of liver. (A) In the control group, erythrocytes in the sinusoids and the contents of the veins (H&E, X100). (B) In the cisplatin group, focal area of inflammation within the liver parenchyma (H&E, x100). (C) In the cisplatin + resveratrol group, mild mononuclear inflammatory cell infiltration in the central vein and parenchyma (black arrow) (H&E, x100)

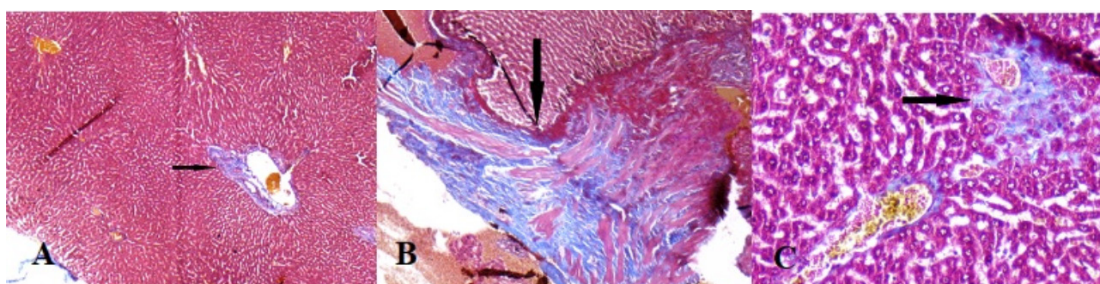


Figure 3. Immunohistochemical staining with Masson trichrome dye. (A) In the control group, a minimal to near-normal increase in connective tissue around the portal area (Masson trichrome, X50). (B) In the cisplatin group, connective tissue increase in the portal area (Masson trichrome, X50). In the cisplatin + resveratrol group, mild fibrosis around the central veins (Masson trichrome, X50)

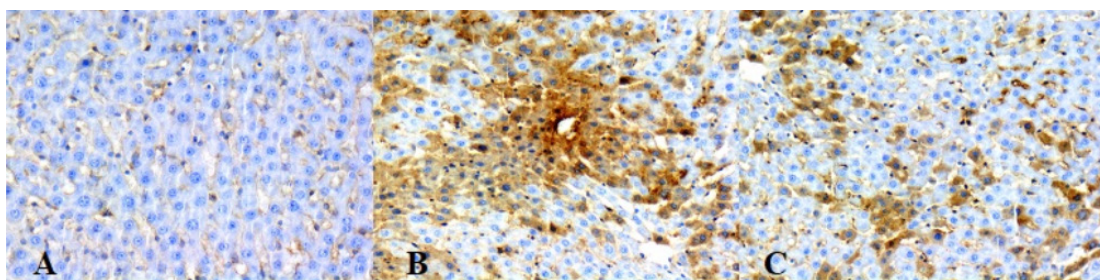


Figure 4. Immunohistochemical staining with bcl-2 dye. (A) In the liver of a rat from the control group, negative immunoreexpression by bcl-2 (x200). (B) In the cisplatin group, cytoplasmic immunoreexpression (x200). (C) In the cisplatin + resveratrol group, more focal cytoplasmic immunoreexpression than cisplatin group (x200)

ability to modulate many molecules such as vascular endothelial growth factor, cytokines, and caspases (22).

Mansour et al. (23) reported that silymarin, an antioxidant flavonoid, ameliorates the hepatotoxicity of cisplatin. They hypothesized that silymarin reversed the oxidative stress by inhibiting lipid peroxidation and enhancing SOD and CAT activities (23). In another study, silymarin and gallic acid were used against cisplatin-induced nephrotoxicity and hepatotoxicity (24). The researchers reported that these molecules reverse the harmful effects of cisplatin. Thus, we thought that a substance with strong antioxidant properties, such as resveratrol, could be useful to prevent the toxic hepatic injury due to cisplatin.

Study Limitations

The small number of subjects and the possibility of variation when the study was adapted to humans are the limitations of our study. The advantage of this study was the widespread use of resveratrol as an antioxidant and the availability of tablets used in many conditions, especially ischemic events.

Conclusion

Decreased MDA levels and increased the activities of SOD and CAT enzymes. Furthermore, an improvement was observed histopathologically too. The parameters demonstrating damage, such as inflammation, congestion,

hemorrhage, and necrosis, were significantly lower in the cisplatin + resveratrol group than in the cisplatin group. In conclusion, resveratrol could be a useful agent in the short-term treatment and prevention of hepatic damage due to cisplatin.

Ethics

Ethics Committee Approval: Approval was obtained from Kirikkale University Animal Experiments Local Ethics Committee (18.06.2020 2020/03, approval no: 16).

Informed Consent: Our study is prospective randomized controlled study.

Peer-reviewed: Externally and internally peer-reviewed.

Authorship Contributions

Concept: O.K., Design: O.K., A.K., Data Collection, or Processing: O.K., Analysis, or Interpretation: A.K., Literature Research: O.K., Writing: O.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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