

Cisplatin Causes Oxidation in Rat Liver Tissues: Possible Protective Effects of Antioxidant Food Supplementation

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Aims: In this study, we aimed to investigate the possible molecular mechanism of cisplatin hepatotoxicity and to establish whether some natural antioxidant foods, namely dried black grape and tomato, may provide protection against cisplatin hepatotoxicity.

Materials and Methods: Twenty-eight rats were used throughout the study. Cisplatin was administered intraperitoneally (i.p.) in a single dose (10 mg/kg). Antioxidant food supplementation was started three days before cisplatin treatment. There were 7 animals in each group (control, cisplatin, cisplatin plus dried black grape and cisplatin plus tomato juice). Rats were sacrificed 72 h after the treatment. The livers were removed and prepared for the biochemical and histopathological investigations. Oxidant and antioxidant parameters were measured in liver tissues of the groups.

Results: Malondialdehyde (MDA) level and xanthine oxidase (XO) activities were higher in the cisplatin group compared with the control values. Catalase (CAT) and glutathione peroxidase (GSH-Px) activities were higher but MDA level lower in the grape-supplemented group compared with the cisplatin group. In the histopathological examination, sinusoidal congestion, hydropic and vacuolar degeneration, extensive disorganization in hepatocytes, and significant fibrosis around central venules and expanded periportal areas were observed in the liver tissues from cisplatin-treated animals. In rats treated with cisplatin and fed with tomato juice, sinusoidal congestion was less in comparison to the cisplatin-treated group and no hepatocyte disorganization or hydropic degeneration was seen. In rats treated with cisplatin and dried black grape, disorganization of hepatocytes was mild in comparison to cisplatin-treated animals. Perivenular and periportal fibrosis was mild.

Conclusions: Results suggest that cisplatin treatment causes significant oxidant load to the liver through both XO activation and impaired antioxidant defense system, which result in accelerated oxidation reactions in the liver tissue. Additionally, cisplatin treatment resulted in significant harmful effects on hepatocytes. We propose that supplementation with some antioxidant foods with high antioxidant power may ameliorate this toxicity.

Key Words: Cisplatin, hepatotoxicity, antioxidant foods, protection

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Sıçan Karaciğer Dokusunda Sisplatinin Neden Olduğu Oksidasyon: Antioksidan Yiyecek Desteğinin Olası Koruyucu Etkileri

Amaç: Bu çalışmada sisplatinin neden olduğu hepatotoksitenin olası moleküler mekanizmalarını ve bazı doğal antioksidan yiyeceklerin özellikle siyah kuru üzüm ve domatesin sisplatin hepatotoksitesine karşı koruyucu etkisini olup olmadığını araştırdık.

Yöntem ve Gereç: Çalışmada 28 adet sıçan kullanıldı. Sisplatin intraperitoneal olarak tek doz (10 mg/kg) şeklinde sıçanlara verildi. Antioksidan yiyecek verilmesine sisplatin uygulamasından üç gün önce başlandı. Her bir grup (kontrol, sisplatin, sisplatin + kuru üzüm ve sisplatin + domates suyu) 7 sıçandan oluşmaktaydı. Sıçanlar tedaviden 72 saat sonra öldürüldü. Sonra karaciğerleri çıkartıldı ve biyokimyasal ve histopatolojik incelemeler için hazırlandı. Oksidan ve antioksidan parametreler karaciğer dokularında çalışıldı.

Bulgular: Malondialdehit (MDA) düzeyi ve Ksantin oksidaz (XO) aktivitesi sisplatin grubunda kontrol grubuna göre yüksekti. Katalaz ve glutatyon peroksidaz aktiviteleri üzüm desteği yapılan grupta sisplatin grubuna göre daha yüksekti, fakat MDA düzeyleri daha düşüktü. Histopatolojik incelemede sisplatin verilen grubun hepatositlerinde sinüsoidal konjesyon, hidropik ve vakuoler dejenerasyon ile yoğun bir şekilde disorganizasyon görülürken, sisplatin ek olarak domates ve siyah kuru üzüm alan grupta bu bulgularda gerileme saptanmıştır.

Sonuç: Veriler göstermektedir ki sisplatin tedavisi karaciğer dokusunda hem ksantin oksidaz aktivitesini arttırarak ve antioksidan savunma sistemini zayıflatarak oksidasyona yol açmış; hem de hepatositlerde morfolojik açıdan önemli düzeyde dejeneratif etkiler oluşturmuştur. Bunun sonucunda da karaciğer dokusunda oksidasyon reaksiyonları hızlanmıştır. Bizim görüşümüz; bazı yüksek antioksidan güce sahip yiyeceklerin (domates ve siyah kuru üzüm gibi..) bu toksisiteyi düzeltebileceği yönündedir.

Anahtar Sözcükler: Sisplatin, hepatotoksitesite, antioksidan yiyecekler, koruma

Received: May 02, 2007
Accepted: February 14, 2008

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Introduction

Cis-diamminedichloroplatinum (cisplatin) is an important anticancer drug used to treat solid tumors. The nephrotoxicity of cisplatin is recognized as the most important dose-limiting factor, but high doses of cisplatin also produce hepatotoxicity (1). Many efforts have been made to improve the therapeutic index of cisplatin using pharmacological strategies, such as the administration of chemoprotectors, intensive hydration and hypertonic saline (2-4). Unfortunately, some of the compounds used as chemoprotectors also inhibit the antitumor activity of cisplatin, while other therapeutic strategies were not completely efficient in reducing the dose-limiting nephrotoxicity. It has been established that lipid peroxidation might participate in the hepatotoxicity in cisplatin-treated animals despite activation of antioxidant enzymes (5). It has been suggested that antioxidant enzymes represent the protective response against cisplatin toxicity in the livers of tumor-bearing animals (1). High doses of cisplatin have been found to produce hepatotoxicity, with apoptosis as the major lesion, and metallothionein protects against cisplatin-induced liver injury (1).

Tomato and grape are the foods with high antioxidant power (6-8). In previous studies, it has been established that both foods may strengthen the antioxidant power of the body and prevent oxidative attacks, thus reducing some oxidant stress-induced health problems (9,10).

The present study aimed to investigate whether antioxidant foods like tomato juice and dried black grape may exert preventive effects on cisplatin-induced hepatotoxicity.

Materials and Methods

Twenty-eight Wistar Albino type female rats (20 weeks old and weighing 220 ± 15 g) were used in the study. Animals were maintained on a 12 h light/dark cycle at room temperature (23 ± 2 °C) and allowed free access to food and water. The animals were randomly divided into 4 groups of 7 rats. One group served as control and the others were treated with a single dose of cisplatin intraperitoneally (i.p.) (10 mg/kg body weight) with or without antioxidant foods. Dried black grape was added into the diet directly and tomato juice into the drinking water. Animals ingested dried black grape and tomato

juice in approximate amounts of 25 g/kg/day and 150 ml/kg/day, respectively. Antioxidant food supplementation was started three days before cisplatin treatment and continued during the study period. Animals were fed laboratory diet and water ad libitum.

The animals of control and cisplatin-treated groups were sacrificed 72 h after the treatment. Livers of the rats were removed by surgical operation, washed with physiological saline solution and cleared of fatty tissue. The tissues were homogenized and prepared for the assays as described previously (11). The upper clear part of the tissue homogenates was used in the experiments. Protein level of the clear supernatants was studied by Lowry's method (12). Malondialdehyde (MDA) levels (nmol/mg protein) and superoxide dismutase (SOD) (U/mg protein), catalase (CAT) (IU/mg protein), glutathione peroxidase (GSH-Px) (mIU/mg protein) and xanthine oxidase (XO) (mIU/mg protein) activities were measured in the supernatant fraction.

MDA level was measured by thiobarbituric acid reactive substances (TBARS) method (13). SOD activity was measured as described (14). One unit for SOD activity was expressed as the enzyme protein amount causing 50% inhibition in nitroblue tetrazolium (NBT) reduction rate. CAT activity was determined by measuring decrease of hydrogen peroxide (H_2O_2) absorbance at 240 nm as described (15). GSH-Px activity was measured by following changes in NADPH absorbance at 340 nm as described (16). XO activity was determined by measuring uric acid formation from xanthine at 293 nm as described (17). In the activity calculations, extinction coefficients of uric acid, H_2O_2 and NADPH were used for XO, CAT and GSH-Px, respectively.

Biopsies from each liver were fixed by 10% formalin, embedded in paraffin and cut at 5 μ m. Sections were stained with hematoxylin-eosin and Masson's trichrome and examined by light microscopy using coded slides by two investigators who were blinded to control and treatment groups. Sinusoidal congestion, disorganization, and hydropic and vacuolar degeneration were evaluated in all groups histopathologically.

Statistics

Mann-Whitney U test was used in the statistical evaluation of the results. The criterion for significance was $P < 0.05$.

Results

As seen from Table 1, MDA level and XO activities were higher in the cisplatin group compared with the control values. CAT and GSH-Px activities were higher but MDA level lower in the grape-supplemented group compared with the cisplatin group. In the tomato group, there were no meaningful changes in this regard.

In the histopathological examination, sinusoidal congestion, hydropic and vacuolar degeneration, extensive disorganization in hepatocytes, and significant fibrosis around central venules and expanded periportal areas were observed in the liver tissues from cisplatin-treated animals. In rats treated with cisplatin and fed with tomato juice, sinusoidal congestion was less in comparison to cisplatin-treated group and no hepatocyte disorganization or hydropic degeneration was seen. Perivenular and periportal fibrosis was mild. In dried black grape-fed rats, results were similar: disorganization was mild in comparison to cisplatin-treated animals. Perivenular and periportal fibrosis was mild. Histopathological results of the liver tissues obtained from the control group were in normal limits.

Discussion

Cisplatin is a widely used chemotherapeutic agent in various cancer treatments. Nephrotoxicity induced by this drug is known but very little information is available on cisplatin-induced hepatotoxicity. The mechanisms of

hepatotoxicity caused by cisplatin are not clear. High doses of cisplatin have been known to produce hepatotoxicity (18). Cisplatin leads to increased XO activity, possibly through conversion reaction of xanthine dehydrogenase to XO (19). Increased activity of XO enzyme is an important risk factor for the oxidant load in the liver tissue since it is the main enzyme producing toxic superoxide radical in vivo (20). On one hand, increased activity of XO enzyme and on the other, decreased activities of antioxidant enzymes, namely GSH-Px and CAT, through as yet unknown mechanism(s), create great oxidant stress, which leads to cellular peroxidation and damage in the tissues.

In an experimental study performed by Yılmaz et al. (21), it was aimed to investigate the effects of caffeic acid phenethyl ester (CAPE), an antioxidant agent, on cisplatin-induced hepatotoxicity through adenosine deaminase (AD), XO, CAT, SOD activities and MDA and nitric oxide (NO) levels in liver tissue of rats. They found that NO level and XO activity increased in the cisplatin group compared to the control group. NO level was found to be decreased whereas CAT increased in the cisplatin + CAPE group in comparison with the cisplatin group. They suggest that CAPE significantly attenuated the hepatotoxicity.

In previous studies, it has been established that both foods used in the present study, namely black grape and tomato, have high antioxidant contents (6,8) and can contribute to the antioxidant power in vivo (9,10).

Table 1. Oxidant and antioxidant parameters of the rat liver tissues in the groups (Mean \pm SD).

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH-Px (U/mg protein)	MDA (U/mg protein)	XO (U/mg protein)
Tomato-Cisplatin (1)	188.9 \pm 25.76	75.1 \pm 7.01	7.23 \pm 2.84	0.97 \pm 0.18	0.36 \pm 0.03
Grape-Cisplatin (2)	162.24 \pm 29.07	107.37 \pm 11.74	11.68 \pm 1.68	0.53 \pm 0.07	0.56 \pm 0.06
Cisplatin (3)	181.8 \pm 18.89	80.4 \pm 5.80	5.91 \pm 2.21	1.17 \pm 0.30	0.33 \pm 0.10
Control (4)	159.2 \pm 25.84	83.1 \pm 3.53	8.90 \pm 3.53	0.46 \pm 0.17	0.17 \pm 0.04
Mann-Whitney U Test					
1-2	n.s.	p<0.01	p<0.05	p<0.05	p<0.05
1-3	n.s.	n.s.	n.s.	n.s.	n.s.
1-4	n.s.	n.s.	n.s.	p<0.05	p<0.05
2-3	n.s.	n.s.	p<0.01	p<0.05	p<0.01
2-4	n.s.	n.s.	n.s.	n.s.	p<0.05
3-4	n.s.	p<0.01	n.s.	p<0.05	p<0.05

n.s.: Non-significant.

However, our results demonstrated that dried black grape can provide significant protection against cisplatin-induced liver damages but tomato juice not much. It is quite possible that the antioxidant constituent of the black grape, namely resveratrol, significantly reduces MDA levels of the animal and that induction of CAT and GSH-Px activities provides protection against oxidant attacks created by cisplatin and/or its metabolites. However, our results show that the antioxidant constituent of tomato, namely lycopene, does not significantly contribute to the antioxidant enzyme activities under these circumstances, and does not prevent oxidant attacks in the liver tissue

very effectively. Histopathologic examination results support this evaluation in part. In this regard, biochemical laboratory results were in good agreement with histological observations in the black grape group, but to some extent, are not in agreement with histological observations in the tomato group, indicating that tomato can provide some protection through way(s) other than the known antioxidant mechanism.

In light of these results, we suggest that supplementation of some antioxidant foods like black grape during the therapy period may play a protective role against cisplatin-induced hepatotoxicity.

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