

L-Carnitine Protection Against Cisplatin Nephrotoxicity In Rats: Comparison with Amifostin Using Quantitative Renal Tc 99m DMSA Uptake

L-Karnitin'in Siçanlarda Sisplatin Nefrotoksisitesine Karşı Koruyuculuğu: Kantitatif Böbrek Tc 99m DMSA Uptake'ini Kullanarak Amifostinle Karşılaştırma

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Abstract

Objective: In this study, we aimed to investigate the cytoprotective effect of L-carnitine against cisplatin-induced nephrotoxicity and to compare its efficacy with that of amifostin by quantitative renal Tc 99m DMSA uptake.

Material and Methods: Male Wistar rats were randomly divided into six groups of six animals each. 1) Control (saline; 5 ml/kg intraperitoneally); 2) L-carnitine (CAR; 300 mg/kg intraperitoneally); 3) Amifostine (AMI; 200 mg/kg intraperitoneally); 4) Cisplatin (CIS; 7 mg/kg intraperitoneally); 5) Cisplatin plus L-carnitine (CIS + CAR); 6) Cisplatin plus amifostine (CIS + AMI). L-carnitine and amifostine were injected 30 minutes before cisplatin in Group 5 and 6. Tc 99m DMSA, 7.4 MBq/0.2 ml, was injected through the tail vein 72 hours after the drug administration. Rats were killed and kidneys removed by dissection 2 hours after the injection of the radiopharmaceutical. The percentage of the injected dose per gram of kidney tissue (%ID/g) was calculated. Renal function was monitored by measuring BUN and plasma levels of creatinine. Lipid peroxidation and glutathione content were determined by measuring malondialdehyde (MDA) and reduced glutathione (GSH) in kidney tissue homogenates.

Results: Tc 99m DMSA uptake per gram tissue of the kidney as %ID/g was 29.54 ± 4.72 , 29.86 ± 7.47 and 26.37 ± 4.54 in the control, CAR and AMI groups respectively. %ID/g was the lowest of all the groups, 11.60 ± 3.59 ($p < 0.01$), in the cisplatin group. Carnitine or amifostine administration 30 minutes before cisplatin injection resulted a significant increase in %ID/g, 21.28 ± 7.73 and 18.97 ± 3.24 respectively, compared to those of cisplatin-treated rats ($p < 0.002$). A marked increase in plasma BUN and creatinine indicating nephrotoxicity and acute renal failure was observed in the cisplatin-treated group. MDA and GSH levels were concordant with cisplatin-induced oxidative stress in the kidney tissue.

Conclusion: The results showed that L-carnitine significantly attenuates the cisplatin-induced nephrotoxicity as amifostin. (*MIRT 2011; 20: 1-6*)

Key words: Spermatic cord torsion; radionuclide imaging; cryptorchidism; inguinal hernia

Özet

Amaç: Bu çalışmanın amacı sisplatinin neden olduğu nefrotoksisiteye karşı L-karnitinin koruyucu etkisini ve bu etkiyi kantitatif renal Tc 99m DMSA uptake'ini kullanarak amifostinin etkisiyle karşılaştırmaktır.

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Gereç ve Yöntemler: Erkek Wistar sıçanlar her biri 6 hayvandan oluşan 6 gruba ayrıldı: 1) Kontrol grubu (serum fizyolojik, 5 ml/kg); 2) L-karnitin (CAR; 300 mg/kg intraperitoneal); 3) Amifostin (AMI; 200 mg/kg intraperitoneal); 4) Sisplatin (CIS; 7 mg/kg intraperitoneal); 5) Sisplatin artı L-karnitin (CIS + CAR); 6) Sisplatin artı amifostin (CIS + AMI). Grup 5 ve 6'da L-karnitin ve amifostin sisplatininden 30 dakika önce enjekte edildi. İlaçlar verildikten sonraki 72. saatte kuyruk veninden 7.4 MBq/0.2 ml Tc 99m DMSA enjekte edildi. Radyofarmasötik enjeksiyonundan 120 dakika sonra sıçanlar öldürüldü ve böbrekleri çıkarıldı. Enjekte edilen dozun her bir böbrek dokusu gramındaki oranı (%ID/g) hesaplandı. Böbrek fonksiyonu BUN ve plazma kreatinin düzeyi ölçümü ile değerlendirildi. Böbrek dokusu homojenatlarında malondialdehid (MDA) ve glutatyon (GSH) ölçümü ile lipid peroksidasyonu ve glutatyon seviyesi belirlendi.

Bulgular: Kontrol, CAR ve AMI gruplarında %ID/g olarak Tc 99m DMSA uptake'i sırasıyla 29.54 ± 4.72 , 29.86 ± 7.47 and 26.37 ± 4.54 olarak hesaplandı. Sisplatin grubunda tüm gruplar içinde en düşük %ID/g, (11.60 ± 3.59) ($p < 0.01$), değeri gözlemlendi. Sisplatin uygulamasından 30 dakika önce L-karnitin veya amifostin verilmesi sadece sisplatin verilen gruba kıyaslandığında %ID/g'de anlamlı artışa (sırasıyla, 21.28 ± 7.73 ve 18.97 ± 3.24) neden oldu ($p < 0.002$). Sisplatin verilen grupta plazma BUN ve kreatinin düzeylerinde nefrotoksisite ve akut renal yetmezliği gösteren belirgin artışlar gözlemlendi. MDA ve GSH düzeyleri sisplatinin böbrek dokusunda neden olduğu oksidatif stres ile uyumlu idi.

Sonuç: Bulgular L-karnitinin amifostine benzer şekilde sisplatinin neden olduğu nefrotoksisiteyi anlamlı düzeyde azalttığını göstermektedir. (*MIRT 2011; 20: 1-6*)

Anahtar kelimeler: Karnitin, amifostin, sisplatin, nefrotoksisite, radyofarmasötik, Tc 99m DMSA

Introduction

Cisplatin is an antineoplastic agent in the treatment of many types of human tumors including testicular, head and neck, ovarian, cervical, nonsmall cell lung carcinoma. Its cytotoxic activity is mediated through DNA inter and intrastrand cross linking and adduct formation resulting in defective DNA templates and arrest of DNA synthesis and replication (1) Cisplatin induces significant ototoxicity, neurotoxicity and nephrotoxicity. The side effects of cisplatin, particularly dose-related nephrotoxicity, have been the major factor that restricts its optimal use in cancer therapy. Nephrotoxicity was observed in almost one-third of patients undergoing cisplatin treatment. Although the precise molecular mechanism of cisplatin nephrotoxicity is not known, recent research reveals that it is a complex multifactorial process involving necrosis, apoptosis, inflammation, ischemia and oxidative stress. It is known that renal tubules are the major sites of damage resulting in acute renal failure. Numerous agents have been tested to prevent cisplatin-induced nephrotoxicity (2,3,4) However, effective methods for clinical use has not been established yet.

L-carnitine is a low-molecular-weight, natural compound obtained from the diet and also biosynthesized in vivo from the essential amino acids lysine and methionine. It is a cofactor required for transport of long-chain fatty acids into the mitochondrial matrix for energy production. However, biological functions of L-carnitine go far beyond its role in the transport of fatty acids. Carnitine and its acetylated derivatives facilitate the β -oxidation and improve energy metabolism, minimize the toxic effects of free forms of long-chain fatty acids in and around mitochondria, protect the mitochondrial membranes and prevent permeability transitions, therefore, suppresses the release of free electrons that generate free radicals and cytochrome c that activates caspases.

L-carnitine protects against oxidative stress and reduces the apoptosis rate (5,6,7,8,9).

Amifostine (Ethyol), a pro-drug that forms an activated free thiol through dephosphorylation by membrane-bound alkaline phosphatase in normal cells. AMI selectively protects normal cells from toxic effects of antineoplastic agents by scavenging free radicals, donating hydrogen ions, binding to active metabolites antineoplastic agents. There is a growing amount of evidence of its ability to selectively protect almost all normal tissues except central nervous system, but not neoplastic tissues from the cytotoxic effects of some of chemotherapeutic agents and radiation therapy. Amifostine was recommended in the American Society of Clinical Oncology Clinical Practice Guidelines for the Use of Chemotherapy and Radiotherapy Protectants for the prevention of cisplatin-related toxicity (10-13) Tc 99m DMSA, meso-2,3-dimercaptosuccinic acid, is used for functional imaging of the proximal renal tubular mass. It is accumulated in the cytoplasm of the proximal tubular cells by peritubular uptake which is mediated by the organic anion transport system. Its uptake depends on the renal blood flow and proximal tubular cell membrane transport function (14,15,16). Tc 99m DMSA scintigraphy has been used clinically to diagnose and monitor ifosfamid-induced tubular dysfunction (17) and cisplatin and cisplatin/ifosfamid nephrotoxicity in children (18)

Because, oxidative stress has been recognized as a major factor in the mechanism of cisplatin nephrotoxicity, lipid peroxidation and glutathione content were determined by measuring malondialdehyde and reduced glutathione in kidney tissue homogenates.

In this study, we aimed to investigate the role of L-carnitine in the protection of cisplatin-induced nephrotoxicity and to compare its efficacy with that of cytoprotective agent amifostin by using quantitative Tc 99m DMSA uptake measurements and biochemical parameters.

Materials and Methods

Male Wistar albino rats, weighing 190-260 g, were provided by the Experimental Surgery and Research Laboratories of Ege University. Rats were housed in standard laboratory conditions and 12-hour light-dark cycle and had free access to commercial food and tap water. The study protocol was approved by the Institutional Animal Review Committee of Ege University (Ref no: 2005-04) and all animals have received human care in compliance with the Guide for the Care and Use of Laboratory Animals.

Rats were randomly divided into six groups. Each group consisted of six rats. 1) Control (saline; 5 ml/kg intraperitoneally); 2) L-carnitine (CAR; 300 mg/kg intraperitoneally); 3) Amifostine (AMI; 200 mg /kg intraperitoneally); 4) Cisplatin (CIS; 7 mg/kg intraperitoneally); 5) Cisplatin plus L-carnitine (CIS + CAR); 6) Cisplatin plus amifostine (CIS + AMI). (Table 1). Amifostine and L-carnitine were injected 30 minutes before cisplatin in Group 5 and 6. Cisplatin dose is determined according to the cisplatin model of acute renal failure in rats described previously (19,20). Rats were killed by heart puncture under intense ether atmosphere 72 hours after the injections.

Cisplatin (DBL, 10 mg/10ml vial, FH Faulding & Co Limited Australia), amifostine (Ethyol, 500 mg, TR Erkim Ltd Sti) and L-carnitine (Carnitene, 1 g, Sigma Tau, Italy) were purchased commercially. Amifostine was dissolved in 9.7 ml of double distilled water.

Tc 99m, as sodium pertechnetate, was eluted from a 99Mo/Tc 99m generator (Monrol A.S. Istanbul, Turkey) just before the radiolabelling procedure. DMSA (TechneScan, Mallinckrodt Medical B.V Holland) was purchased as a freeze-dried commercial kit containing 1.2 mg dimercaptosuccinic acid, 0.3 mg stannous chloride dehydrate and 30 mg inositol. Tc 99m DMSA was prepared by adding 740 MBq of Tc 99m pertechnetate to 3 ml of saline to the kit. Quality control procedures were performed according to the manufacturer's instructions after 15 minutes' incubation at room temperature. Labeling efficiency was greater than 95%.

Quantitative Tc 99m DMSA Uptake

Tc 99m DMSA, 7.4 MBq/0.2 ml, was administered through the tail vein. Rats were killed and the kidneys were removed by dissection 2 hours after the radiopharmaceutical injection. Samples of kidneys were weighed in pre-weighed containers. Radioactivity in each organ sample was counted using a Cd (Te) detector equipped with a RAD 501 single-channel analyzer. The activity was expressed as a percentage of the injected dose per gram of tissue (%ID/g). The %ID/g activity was calculated by dividing the activity in each kidney by total activity injected and the mass of the organ.

Biochemical Assays

The blood samples from the treatment groups were collected in heparinized vials and centrifuged at 3500 rpm for 15 min to isolate serum. BUN and creatinine levels in serum were measured with an autoanalyser using commercial diagnostic kits (Sigma-Aldrich Chemie, Germany). One of the kidneys of each animal was washed with cold saline and kept in deep freezer (-80 °C) until homogenization. The homogenates were centrifuged at 3500 rpm and supernatants were collected and used for the determination of malondialdehyde (MDA) and reduced glutathione (GSH). MDA level was determined by a method based on the reaction with thiobarbituric acid (TBA) at 90-100 °C (21). GSH content in tissue supernatants was measured according to the method of Beutler et al (22).

Statistical Analysis

Data are expressed as the mean±SD. The nonparametric Kruskal-Wallis test was performed on the data of radiopharmaceutical uptake and biochemical variables to examine the differences among groups. Mann-Whitney U test was used to compare two independent samples. Statistical differences were considered significant when P value was smaller than 0.05 with two sided probability.

Table 1. Study groups and experimental design

| Groups | Administered agents (intraperitoneally) |
|---------------------|--|
| Group 1 (Control) | Saline (0.9% NaCl) 5 ml/kg |
| Group 2 (CAR) | L-carnitine 300 mg/kg |
| Group 3 (AMI) | Amifostine 200 mg/kg |
| Group 4 (CIS) | Cisplatin 7 mg/kg |
| Group 5 (CIS + CAR) | Cisplatin 7 mg/kg plus L-carnitine 300 mg/kg (30 min before CIS) |
| Group 6 (CIS + AMI) | Cisplatin 7 mg/kg plus amifostine 200 mg/kg (30 min before CIS) |

AMI: Amifostine, CAR: L-carnitine, CIS: Cisplatin

Table 2. Effect of cisplatin (CIS)

| Groups | BUN, mg/dl | Creatinine, mg/dl | MDA, nmol/g | GSH, $\mu\text{mol/g}$ |
|------------------|--------------------|-------------------|------------------|------------------------|
| Control (saline) | 43.56 \pm 2.8 | 0.39 \pm 0.03 | 364.4 \pm 113 | 3.29 \pm 1.5 |
| CIS | 118.42 \pm 23.5* | 2.35 \pm 0.74* | 609.2 \pm 217* | 1.34 \pm 0.5* |
| CAR | 43.51 \pm 4.7 | 0.37 \pm 0.03 | 347.1 \pm 123 | 4.22 \pm 1.3 |
| AMI | 48.92 \pm 6.3 | 0.44 \pm 0.12 | 434.7 \pm 75 | 3.81 \pm 0.8 |
| CIS+CAR | 54.46 \pm 8.3 | 0.54 \pm 0.09 | 325.4 \pm 110 | 3.02 \pm 0.7 |
| CIS+AMI | 59.74 \pm 7.1 | 0.59 \pm 0.23 | 359.6 \pm 132 | 2.89 \pm 0.9 |

L-carnitin (CAR), amifostine (AMI) and their combination on the levels of blood urea nitrogen (BUN), plasma creatinine, malondialdehyde (MDA) and reduced glutathione (GSH). Values are means \pm SD. *Significantly different from control group ($p < 0.05$)

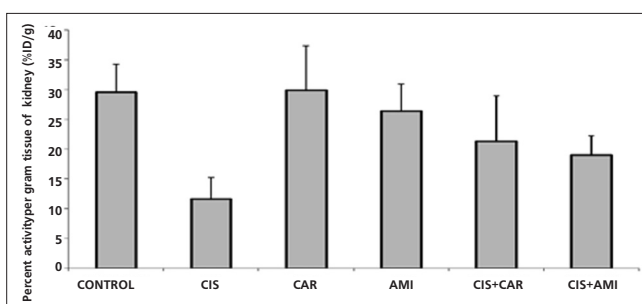


Figure 1. The percentage of the injected Tc 99m DMSA dose per gram of kidney tissue (%ID/g) in Control, CIS, CAR, AMI, CIS+CAR and CIS+AMI groups are shown.

Results

Intraperitoneal injection of cisplatin at a single dose of 7 mg/kg body weight resulted in acute renal failure as indicated by significant increase in plasma BUN and creatinine levels compared with controls ($p < 0.001$). L-carnitine and amifostine administered 30 minutes before cisplatin prevented the cisplatin-induced elevations in BUN and creatinine. Cisplatin induced a significant increase in the MDA content of the kidneys and caused depletion of GSH as compared to the control group ($p < 0.01$). No significant changes were observed in MDA and GSH when carnitine or amifostin was administered prior to cisplatin (Table 2).

The percentage of the injected dose per gram of kidney tissue in Control, CIS, CAR, AMI, CAR+CIS and AMI+CIS groups are shown in Fig 1. Tc 99m DMSA uptake per gram tissue of the kidney as %ID/g was 29.54 \pm 4.72, 29.86 \pm 7.47 and 26.37 \pm 4.54 in the control, CAR and AMI groups respectively ($p > 0.05$). Cisplatin led to a significant decrease in tubular uptake of Tc 99m DMSA. %ID/g was the lowest of all the groups, 11.60 \pm 3.59 ($p < 0.01$), in the cisplatin group. Carnitine or amifostine administration 30 minutes before cisplatin injection resulted a significant increase in the uptake of DMSA as %ID/g (21.28 \pm 7.73 and 18.97 \pm 3.24 respectively) compared to those of cisplatin-treated rats ($p < 0.002$). There was no statistically significant difference between the CAR only and AMI only groups (Figure 1).

Discussion

Nephrotoxicity prevents the application of appropriate therapeutic schedules with higher doses of cisplatin and limits its treatment success significantly. Renoprotective agents have been studied extensively because they are important in terms of preventing cisplatin-induced nephrotoxicity and improving its therapeutic potential. Numerous agents have been tested for renoprotection against cisplatin toxicity (3).

L-carnitine is a natural compound whose biological functions go far beyond its role in the transport of fatty acids. Carnitine inhibits oxidative stress, apoptosis and inflammation (5). There are many experimental studies reporting the protective effect of carnitine against the toxicity of irradiation and antineoplastic agents including doxorubicin and cisplatin (23,24,25,26,27,28,29,30,31,32). In the studies investigating the potential renoprotective effect of carnitine against cisplatin-induced nephrotoxicity, it has been demonstrated that carnitine successfully prevented renal dysfunction and injury (23,24,25) But, a recent study concludes that neither carnitine nor amifostin has protective effect against cisplatin induced nephrotoxicity (33). Carnitine doses of 100-500 mg/kg were used in various types of experimental studies (23). We used an average dose within this range.

In the present study, intraperitoneal administration of a single dose of 7 mg/kg cisplatin resulted in acute renal failure as indicated by the increased BUN and serum creatinine measured at 72 hour. Oxidative stress and lipid peroxidation is strongly related to cisplatin-induced nephrotoxicity (34,35) Malondialdehyde is an indicator of lipid peroxidation and oxidative stress. Glutathione is a marker of antioxidant status. Cisplatin induced significant elevation in the renal tissue MDA and decreased the GH content of the rat kidneys compared to the controls. Both L-carnitine and amifostine significantly inhibited MDA elevation and GH depletion induced by cisplatin ($p < 0.05$).

Cisplatin induced a significant decrease in the uptake of Tc 99m DMSA in the rats' kidneys compared to the control group ($p < 0.001$) consistent with tubular injury and

nephrotoxicity. Renal function was monitored by measuring BUN and plasma levels of creatinine. In the groups which rats were pretreated with 300 mg/kg L-carnitine or 200 mg/kg amifostine before cisplatin injection, no significant decrease in the uptake of Tc 99m DMSA was observed, which is concordant with the protective effect of both L-carnitine and amifostine. Although there is no significant difference between the renal DMSA %ID/g of CIS+CAR and CIS+AMI groups, L-carnitine seems to have higher protective effect than amifostine. Our results confirmed the previous experimental studies indicating the protective effect of L-carnitine against cisplatin-induced toxicity.

Because, scintigraphic imaging with measurement of renal activity as counts per pixel is nearly equivalent of this quantitative technique, it is possible to determine the grade of renal toxicity and the potential protective effect of the agents tested, to compare different agents and combinations simply and accurately without sacrificing animals.

The methods for the evaluation of cisplatin-induced nephrotoxicity in experimental studies are based on the measurements of biochemical markers of renal function and indicators of oxidative stress and histopathological findings of renal injury. In this study, we demonstrated the cisplatin nephrotoxicity and the protective effect of L-carnitine by using quantitative renal Tc 99m DMSA uptake.

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