The Effects of Nifedipine on Renal Perfusion Pressure and Kidney During Cisplatin-Induced Nephrotoxicity in Rats*

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SUMMARY

Cisplatin is one of the most effective cancer chemotherapeutic agent used against various solid tumors. Nephrotoxicity is one of the major dose-limiting side effects of cisplatin. It has been known that different mechanisms as oxidative stress may play an important role in cisplatin induced nephrotoxicity resulted with changes in renal haemodynamics. This study was performed to investigate the effect of nifedipine –one of the dihydropyridine calcium antagonist on changes in renal perfusion pressures and kidneys of rats with cisplatin nephrotoxicity. Male wistar albino rats were divided into 3 groups (n=8):1-Control group(1 ml saline. i.p) 2-Cisplatin group (a single dose of cisplatin (5 mg/kg, i.p) 3- A single dose of cisplatin (5 mg/kg, i.p) + Nifedipine (2 mg/kg/day, i.p) for five days. When those pre-treated groups compared with control group, perfusion pressures, serum urea and creatinine levels and tissue MDA levels were found significantly higher in cisplatin group (p<0.001). Histopathological examination showed widespread tubular necrosis and dilatation in cisplatin-treated group versus other groups. In cisplatin + nifedipine pretreated group, perfusion pressures, serum urea and creatinine levels and tissue MDA levels found significantly lower than cisplatin group (p<0. 001) and less tubular dilatation and necrosis was observed. As a result it was demonstrated that Nifedipine has protective effects against cisplatin nephrotoxicity. We suggest that this is partly provided by the beneficial effects of nifedipine on altered renal haemodynamics during cisplatin nephrotoxicity.

Key Words: *Cisplatin, Kidney, Renal Perfusion Pressure, Nifedipine*

Sıçanlarda Sisplatin İle Oluşturulan Nefrotoksisitede Nifedipin'in Renal Perfüzyon Basıncı Ve Böbrek Üzerine Etkileri

ÖZET

Sisplatin kanser kemoterapisinde solid tümörlere karşı oldukça etkin ve geniş kullanıma sahip olan bir kemoterapötik ajandır. Nefrotoksisite en önemli doz-kısıtlayıcı yan etkisidir. Renal hemodinamide değişikliklerle sonuçlanan cisplatin nefrotoksisitesinde oksidatif stres gibi farklı mekanizmaların rol oynadığı bilinmektedir. Bu çalışmada tek doz sisplatin ile nefrotoksisite oluşturulan erkek wistar albino sıçanlarda bir dihidropiridin grubu kalsiyum antagonisti olan nifedipinin renal perfüzyon basıncı ve böbrekler üzerine etkileri araştırıldı. Erkek wistar albino sıçanlar üç gruba ayrıldı (n=8). 1-Kontrol grubu: (1ml salin, i.p) 2-Sisplatin grubu: Tek doz sisplatin (5 mg/kg, i.p). 3-Tek doz sisplatin + 5 gün süre ile Nifedipin (2 mg/kg/gün, i.p). İlaç grupları kontrol grubu ile karşılaştırıldığında perfüzyon basınçları, serum üre, kreatinin ve doku MDA düzeyleri sisplatin grubunda anlamlı olarak yüksek bulundu (p<0.001). Histopatolojik olarak da kontrol grubu ile karşılaştırıldığında, sisplatin grubunda yaygın tubuler nekroz ve tubuler dilatasyon olduğu görüldü. Sisplatin + nifedipin grubu sisplatin grubu ile karşılaştırıldığında perfüzyon basınçları, serum üre, kreatinin ve doku MDA düzeyleri sisplatin grubundan anlamlı olarak daha düşük bulundu (p<0.001) ve histopatolojik olarak daha az tubuler nekroz ve dilatasyon olduğu görüldü. Sonuç olarak nifedipinin sisplatin nefrotoksisitesine karşı koruyucu etkili olduğu görüldü. Biz bu etkiden kısmen, sisplatin nefrotoksisitesi sırasında değişen renal hemodinamikler üzerine nifedipinin yararlı etkilerinin sorumlu olduğunu düşünüyoruz.

Anahtar Kelimeler: *Sisplatin, Böbrek, Renal Perfüzyon Basıncı, Nifedipin*

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INTRODUCTION

Although Cis-dicholorodiammine platinum-II (Cisplatin, CDDP) is a widely used antineoplastic drug in the treatment of ovarian, testicular and bladder carcinomas, head and neck cancers (1,2), its dose-limiting nephrotoxicity is still a major clinical problem. The mechanisms of CDDP nephrotoxicity are still not fully understood. Various studies indicate that oxidative stress play an important role and CDDP exerts its nephrotoxic effects by the generation of free radicals which mediate lipid peroxidation in kidney (3-5).

Oxidative stress may resulted with changes in renal haemodynamics like reduced renal blood flow or increased renal vascular resistance which are important for process of renal damage. Several free radical scavengers or antioxidants have been tested to protect against CDDP-induced nephrotoxicity (6). It has been reported that CDDP is taken up by renal tubular cells and reachs its higher concentrations in the proximal tubular cells and outher medullae especially in S_3 segment which are shown histopathologically that the major sites of CDDP–induced renal damage (7, 8)

Dihidropyridines are the most frequently used calcium antagonists which represent an important group of drugs in the treatment of cardiovascular diseases. Besides their antihyperensive actions, dihidropyridines have beneficial effects on kidney and used in supplemental therapy of kidney diseases (9, 10).

The present study was performed to investigate the effects of nifedipine (NIF) one of the dihidropyridine calcium antagonists on changes in renal perfusion pressures and kidneys of rats with cisplatin nephrotoxicity.

MATERIAL and METHODS

Drugs and Chemicals

Cisplatin (cis-dicholorodiammine platinum-II), Nifedipine, thiobarbituric acid were obtained from Sigma chemical co. (St. Louis, USA).

Animals and Administration

Adult male Wistar albino rats (weighing 250-300 g) obtained from Dicle University Health Sciences Research Center were used in this study. The animals were allowed free access to a standart diet and tap water until the experiment day. All animals received humane care in accordance with the"Guide for the Care and Use of laboratory Animals"(National Institutes of Health publication 85-23, revised 1985). The experimental protocol was approved by the Dicle University Animal Resarch Ethics Committee.

This study was performed in following three groups each consisted of eight rats:

Group 1: Control group (rats received 1ml sterile isotonic saline, i.p).

Group 2:CDDP group (rats received a single dose of CDDP 5mg/kg, i.p)

Group 3:CDDP +NIF group (rats received a single dose of CDDP (5 mg/kg, ip)

+ NIF 2 mg/kg /day, i.p. for five days.

Isolation and Perfusion of Kidneys

On the sixth day, pretreated rats in all groups were anesthetized by ketamine–Xylazine combination (85-15 mg/kg,im) and laparotomy was performed via a midline incision. By dissecting from surrounding tissues, one of the kidneys were removed. After cannulation of renal artery, kidney was isolated. Isolated kidneys were perfused with warmed (37^0C)and aerated (5% CO_2 in O_2) Krebs'-Henseleit solution by a perfusion pump. The composition of Krebs' solution used was as follows(mM): NaCI 112; KCI 5; $CaCI_2$ 2. 5; $NaHCO_3$ 25; $MgCI_2$ 0. 5; NaH_2PO_4 1; D-glucose 11. 5. Perfusion pressure (PP) was continuously recorded on MP30 software (Biopac systems Inc., Santa Barbara. CA, USA). Via a pressure transducer (FDT-10A, Commat iletisim co. Ankara, Turkey). Kidneys were perfused with Krebs' Solution approximately for 60 minutes. After the equilibration period perfusion pressure of isolated kidneys were measured and expressed as mm Hg.

Biochemical Analysis

After isolation of kidney, blood samples were taken for determination of serum urea and creatinine levels. Serum urea and creatinine were measured by an Abbott

Arcitecth C-16000 autoanalyser (Abbott,USA). The other kidneys were separated and used for histopathological examinations and malondialdehyde (MDA) measurement by thiobarbituric acid (TBA) test (11).

Histopathological Examination

The kidneys were transferred to a beaker containing formaldehyde (10%) for light microscopy. After fixing in formaldehyde, tissues were embedded in paraffin, sectioned, stained with hemotoxylin eosin (HE) and evaluated by light microscopy.

Statistical Analysis

All data were expressed as the mean ± standard error of the mean (SEM) and anlysed by Mann-Whitney U test for statistical significance.

RESULTS

After an equilibration period perfusion pressures of kidneys isolated from CDDP pre-treated rats were significantly higher than the control group ($p<0.001$). Perfusion pressure of kidneys isolated from CDDP + NIF pretreated rats were significantly reduced when compared with CDDP group ($p<0.001$). There was also significant difference between control and CDDP+NIF group ($p<0.05$). The results are shown in table 1.

In CDDP group serum urea and creatinine levels were significantly increased above those of control group($p<0.001$). In CDDP + NIF group, serum urea and creatinine levels were significantly decreased when compared with CDDP group ($p<0.001$) but they were also significantly different from control group ($p<0.001$).

An end product of lipid peroxidation, MDA was significantly increased in CDDP group versus control group ($p<0.001$)

Serum urea, creatinine and MDA concentrations are shown in table 1.

Table 1. Renal Perfusion pressure (PP)(mm Hg), serum urea (mg/dl), creatinine (mg/dl) and renal tissue MDA (nmol/gr wet tissue) levels of Control, CDDP and CDDP + NIF groups. Data are the mean ± SEM of eight experiments.

	Control (a)	CDDP (b)	CDDP + NIF (c)	p
PP (mmHg)	47.63 ± 2.32	152.63 ±7.87	58.38 ±3.89	(a-b)(b-c) p<0.001 (a-c) p<0.05
Urea(mg/dl)	33.62 ± 1.34	156.75 ± 7.01	42.37 ±1.32	(a-b) (b-c) p<0.001 (a-c) p<0.001
Creatinine(mg/dl)	0.52 ± 0.02	1.62 ± 0.08	0.68 ± 0.03	(a-b) (b-c) p<0.001 (a-c) p<0.001
MDA (nmol/gr wet tissue)	73.50 ± 5.25	194.30 ± 7.00	94.5 ± 6.00	(a-b)(b-c) p<0.001 (a-c) p<0.05

Renal histology

Histopathological examinations showed widespread tubular necrosis and dilatation in CDDP group versus control group (Fig 1 and 2). Less tubular dilatation and necrosis was observed In CDDP + NIF group (Fig 3).

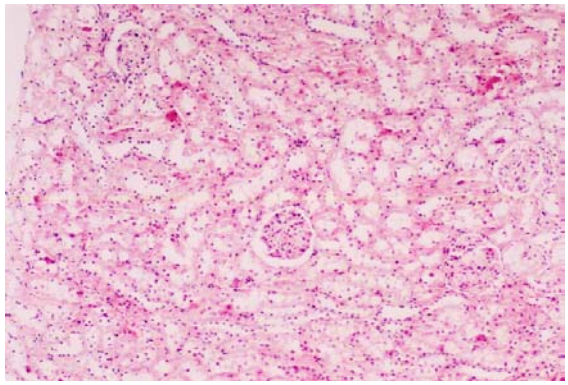


Figure 1. Light micrograph of the corticomedullary region of rat kidneys from the control group. Normal histology. (HE, X41).

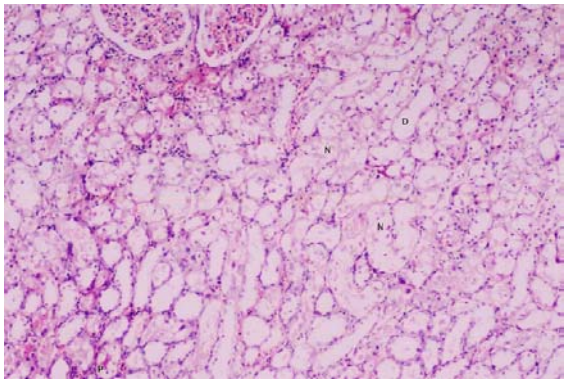


Figure 2. Light micrograph of the corticomedullary region of rat kidneys from the CDDP group. Widespread tubular necrosis and dilatation of the proximal tubules (S_3 segment), protein casts and necrotic cell debris can be seen in the tubular lumina. (N: Tubular necrosis, D: Dilatation, P: Protein casts). (HE, X41).

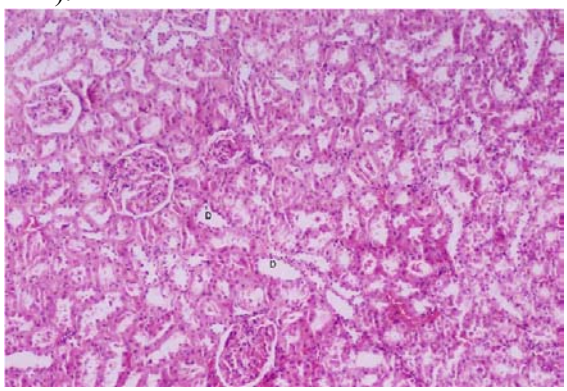


Figure 3. Light micrograph of the corticomedullary region of rat kidneys from the CDDP + NIF group showing less tubular necrosis and less tubular dilatation versus CDDP group. (D:Dilatation). (HE, X41).

DISCUSSION

The present study demonstrates that CDDP causes a specific nephrotoxic effect which is characterised by increasing of renal perfusion pressure and by widespread necrosis and dilatation in the S_3 segment of the proximal tubules and by elevation of serum urea, creatinine and renal tissue MDA levels.

It has been known that free oxygen radicals formation and iron-dependent oxidative damage of biological macromolecules play an important role in CDDP induced nephrotoxicity. Superoxide anion radical, hydroxyl radical and singlet oxygen attack nucleic acids, proteins, lipids (12) and cause an oxidation of these biomolecules which initiate lipid peroxidation. Effecting of membrane structure and functions by lipid peroxidation may resulted with cell death (13, 14).

We found renal tissue MDA (which is an end product of lipid peroxidation)levels significantly higher in CDDP group than control group. It indicates an increased lipid peroxidation and an oxidative tissue injury in kidneys.

Oxidative stress may resulted with changes in renal haemodynamics like reduced renal blood flow or increased renal vascular resistance which are important for progression of renal damage. Similarly in the CDDP group, perfusion pressures were significantly found higher than the control group and it indicates the alterations in renal haemodynamics.

It has been reported that CDDP is taken up by renal tubular cells and reaches its higher concentrations in the proximal tubular cells and outher medullae especially in S_3 segment (7, 8) and CDDP provokes loss of tubular epithelial cells by necrosis and apoptosis followed by inflamatory cell infiltration. Higher number of macrofages was also observed in renal cortices and outher medullae in kidneys of CDDP treated rats which can intensify the cytotoxic effect of reactive oxygen species (6, 15, 16).

In our histopathological examinations we also observed widespread tubular necrosis and dilatation of the proximal tubules especially in S_3 segment and protein casts, necrotic cell debris in the tubular lumina of CDDP group versus control group.

Several free radical scavengers and antioxidants or some therapeutics like calcium antagonists have been used to protect against CDDP-induced nephrotoxicity (6). Calcium antagonists are widely used in the treatment of cardiovascular diseases. Dihidropyridines are the most frequently used calcium antagonists which have important antihypertensive effects. Besides their antihypertensive effects, dihidropyridines have beneficial effects on kidney and used in supplemental therapy of kidney diseases (9, 10).

In a recent study it was suggested that treatment with 10-20 mg/kg NIF (one of the

dihydropyridine calcium antagonists) significantly improved renal dysfunction, renal oxidative stress and prevented the alterations in morphology induced by cyclosporine (17). In an other study, it was suggested that NIF (0. 1 mg/kg/day) treatment for seven days, slightly prevented cyclosporine induced nephrotoxicity (18).

In our study we investigate the effects of NIF on alterations in renal perfusion pressures and kidneys of rats with CDDP nephrotoxicity.

In CDDP group, perfusion pressures, serum urea and creatinine levels and tissue MDA levels were found significantly higher than the control group that indicates the alterations in renal haemodynamics by CDDP administration. In order to see the effects of NIF, we treated the rats(which were injected with a single dose of CDDP) with NIF (2mg/kg/day) for five days.

We observed that perfusion pressure and serum urea and creatinine levels and tissue MDA levels were significantly decreased in CDDP+ NIF group when compared with CDDP group. Less tubular dilatation and necrosis was also observed in CDDP + NIF group. Our all these results confirm the protective effect of NIF on CDDP nephrotoxicity.

As a result, it was concluded that NIF has protective effects against CDDP nephrotoxicity. We suggest that the protection is partly provided by the beneficial effects of NIF on altered renal haemodynamics during CDDP nephrotoxicity.

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