Effect of α-Tocopherol on Lipid Peroxidation Caused by Cisplatin in Rat Kidney

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ABSTRACT

Cisplatin (CDDP) is one of the most commonly used antineoplastic agents in current clinical practice. The major toxicities of CDDP are nonhaematological as nephrotoxicity and ototoxicity. Free oxygen radicals are known to play major role in CDDP-induced acute renal failure in rats. α-tocopherol is one of the well-known antioxidant agents. This study was designed to investigate the role of α-tocopherol pretreatment against CDDP-induced lipid peroxidation in rat kidney. Male Wistar rats were divided into three groups and treated as follows: control (saline intraperitoneally), CDDP (10 mg/kg, intraperitoneally), α-tocopherol (200 mg/kg, plus CDDP, intraperitoneally). Rats were sacrificed on third day of the treatment, and kidney tissues were obtained and analyzed. CDDP-treated rats showed high malondialdehyde (MDA) levels ($p< 0.05$). In the CDDP plus α-tocopherol group, renal MDA levels were not significantly different from the controls. These data suggest that α-tocopherol may be used to prevent CDDP-induced lipid peroxidation.

Key Words: Cisplatin, α-Tocopherol, Malondialdehyde.

ÖZET

α-Tokoferolün Sıçan Böbreğinde Sisplatinin Yol Açtığı Lipid Peroksidasyonuna Etkisi

Sisplatin (CDDP) günlük klinik pratikte en çok kullanılan anti-neoplastik ajanlardandır. CDDP'nin en önemli toksisiteleri, nefrotoksisite ve ototoksisitedir. Sıçanlarda CDDP'ye bağlı, akut renal yetmezlikte serbest oksijen radikallerinin önemli rol oynadığı bilinmektedir. α-tokoferol iyi bilinen antioksidan ajanlardandır. Bu çalışma α-tokoferolün sıçan böbreğinde CDDP'ye bağlı lipid peroksidasyonu önlemedeki rolünü araştırmak için yapılmıştır. Erkek Wistar sıçanları 3 gruba bölünmüştür. 1. kontrol (periton için salin), 2. CDDP (periton için

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10 mg/kg), 3. α-tokoferol (200 mg/kg) + CDDP, periton içi. Tedavinin üçüncü günü sıçan böbrek dokuları incelenmiştir. CDDP verilen sıçanlarda malonildialdehid (MDA) düzeyleri yüksek bulunmuştur ($p< 0.05$). CDDP artı α-tokoferol alan sıçanlarda ise kontrol grubuna göre fark saptanmamıştır. Bu sonuçlar α-tokoferolün CDDP'ye bağlı lipid peroksidasyonu önlemede kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Sisplatin, α-Tokoferol, Malonildialdehid.

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INTRODUCTION

Cisplatin (CDDP, cis-diamminedichloroplatinum) is a planar platinum complex consisting of two chloride leaving groups in the cis-position around platinum. CDDP has a broad spectrum of activity against many different solid tumours such as lung, ovary, testis, bladder and head and neck cancers and is also an effective agent in treatment of some haematological malignancies such as refractory lymphomas[1]. The adverse effects of CDDP include nausea and vomiting, nephro-, oto- and, neurotoxicities and myelosupression. The renal damage of CDDP is cumulative to glomeruli and tubules[2,3]. The exact mechanism of CDDP-induced nephrotoxicity is insufficiently known. It was suggested that the toxic effect of CDDP might be related to free radicals that may cause oxidative damage in the kidney[4,5].

Biochemical studies indicate that heavy metals can react with free sulphydryl (SH) groups. It was shown that after administration of CDDP, there was a significant decrease in SH groups per gram wet weight in rats[6,7]. Additionally, CDDP can inhibit the activity of antioxidant enzymes and increase the lipid peroxidase content in renal tissue[8]. Anti-oxidant therapies may be effective in prevention of the tissue damaging effects of various cytotoxic agents. The concentration of vitamin E in the renal tissue of adults rats was shown to decrease significantly after the administration of CDDP[9]. It is known that, some antioxidants as vitamin C, α-tocopherol, amifostine and asetylcystein may prevent the damage induced by free oxygen radicals and reduce the incidence of cumulative nephrotoxicity of CDDP. To our knowledge, there are few studies on the interaction of CDDP and vitamin E in the literature[10-14]. The objective of this study was to investigate the effect of α-tocopherol against CDDP-induced lipid peroxidation in rat kidney tissues.

MATERIAL and METHODS

CDDP (Cisplatinum-Onko) was obtained from TR Onko as 10 kg/10 mL flacons. Vitamin E (Evigen) was obtained from Aksu Farma Ltd. Şti. as 300 mg/2 mL ampules. Male Wistar rats, weighing from 190-220 g, were divided into three groups and treated as follows:

1. Controls (saline, 1 cc, intraperitoneally);

2. CDDP (10 mg/kg, intraperitoneally);

3. CDDP plus α-tocopherol (200 mg/kg, intraperitoneally).

Rats were fed on normal rhythm and standard breeding food and water according to the Institutional Animal Care and Use Committee and National Institute of Health Guidelines for animals care. All were sacrificed on third day of drug administration after 12 hours of fasting (overnight). The kidneys were obtained and washed with cold saline solution and were placed into Petri dishes containing ice-cold isolation medium (125 M KCl, 10 mM Tris, pH 7.4). They were stored at -20°C until analysed. Briefly the tissues were thawed and homogenised with Ultra-Turax in 0.15 M KCl solution. Each mL of the homogenate were mixed with 1.5 mL thiobarbituric acid (TBA), 1.5 mL acetic acid (pH: 3.5) and 0.2 mL sodium dodecyl sulphate. A set of MDA standards was also freshly prepared. After co-

upling, all samples and standards were heated at 100°C for one hour. The samples and standards were cooled on ice and MDA was extracted by adding 5 mL 1-butanol pyridine (15:1) to each sample. Each tube was centrifuged at 200 RPM for 10 minutes to separate the aqueous and organic phases. The absorbency of 1-butanol phase was measured at 532 nm (Shimahatsu VV 1601 Spectrophotometer) and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption[15]. Specimen concentration was calculated as (specimen absorption/standard absorption) x standard concentration. TBA was purchased from Sigma Chemical Cooperation.

All results were expressed as the mean ± the standard error of the mean (SEM). Statistical analyses were performed by Kruskall Wallis and Mann-Whitney U tests. p values of less than 0.05 were considered as significant.

RESULTS

MDA levels of all groups are shown in Figure 1. MDA levels were 2.61 ± 0.66 nmol/g wt in th control group. CDDP increased the MDA levels to 3.87 ± 0.70 nmol/g wt ($p< 0.05$). So tissue MDA levels were increased significantly in the CDDP group. In the CDDP plus α-tocopherol group, renal MDA levels were not different (2.26 ± 0.70 nmol/g wt.) as compared to the control levels ($p> 0.05$).

DISCUSSION

CDDP is a widely used antineoplastic drug with prominent nephrotoxicity. The exact cellular mechanism of CDDP-induced nephrotoxicity has not been elucidated, but some preclinical studies in rats have demonstrated that lipid peroxidation was involved[12,16]. Sugihara et al showed that the toxic effect of CDDP might be related to free oxygen radical induced damage[13,16]. During incubation with ct-DNA in vitro CDDP have generated superoxide anions[17]. An increment in levels of MDA, a lipid peroxidation product, has been observed in renal cortical slices following in vitro CDDP incubation[18]. Additionally, the concentration of α-tocopherol may decrease in the renal tissue after administration of CDDP and, pretreatment with vitamin E is likely to be therapeutically useful[9]. We observed that the tissue MDA levels were increased by CDDP. But in the α-tocopherol plus CDDP group MDA levels were not increased. In our study, renal tissue MDA levels were higher in the CDDP group than CDDP plus α-tocopherol group. The tissue MDA levels in the CDDP plus α-tocopherol group was similar to the control group (Figure 1).

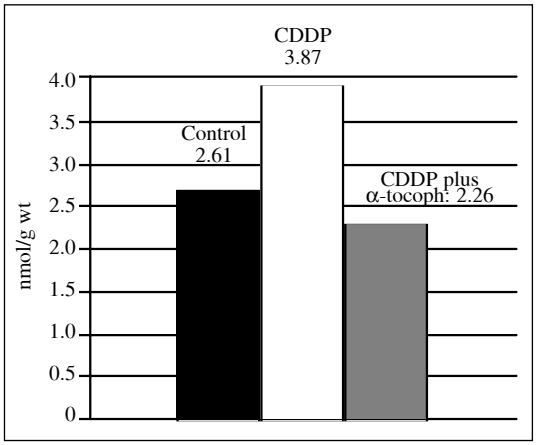

Figure 1. Renal tissue MDA levels.

Renal toxicity is one of the most common nonhaematological toxicities that limit the clinical use of CDDP treatments in a variety of cancers[2]. Many agents have been used to protect the kidney tissues from CDDP-induced free radical damage[4-7,11-14]. An important observation was that α-tocopherol-treated patients did not have the risk of CDDP toxicity in a former study[19]. In our study; MDA levels were not increased in kidney tissues of the rats in the CDDP plus α-tocopherol group compared to controls. According to our point of view, α-tocopherol may be protective against deleterious effects of CDDP in rat kidney tissues.

In conclusion, CDDP-induced renal toxicity was closely associated with increased lipid peroxidation.

This study showed that, the increment of MDA, a lipid peroxidation product related with free oxygen radicals induced kidney damage, could be prevented by α-tocopherol treatment before CDDP. We suggest that the relationship in between CDDP nephrotoxicity and α-tocopherol should be further evaluated by well-designed preclinical and clinical studies.

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