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Protective Effects of Vitamin C on Cisplatin Nephrotoxicity

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Cis-dichlorodiammineplatinum(II)(cisplatin) is one of the most effective antitumor agents currently available for cancer therapy. However, its clinical use has been limited by its severe side effects, especially nephrotoxicity. To evaluate the effect of radical scavengers on cisplatin nephrotoxicity in rats, cisplatin and Vitamin C were given intraperitoneally. Remarkable protective effects of Vitamin C against nephrotoxicity of cisplatin were observed when Vitamin C was administered to rats 1hr before cisplatin injection. Hepatotoxicity induced by combination treatment of cisplatin and Vitamin C was evaluated by measuring serum glutamic pyruvate transaminase(sGPT) and serum glutamic oxalate transaminase(sGOT). Combination treatment did not affect the levels of sGPT and sGOT, and any combination treatment did not induce metallothionein biosynthesis in kidney. Vitamin C which has radical scavenging effect directly reduced nephrotoxicity of cisplatin *in vivo*. Thus, it seems that free radical is the cause of cisplatin nephrotoxicity. Also, combination treatment did not reduce anticancer activity of cisplatin. The present results indicate that Vitamin C, when it is given with cisplatin, may provide protection against cisplatin nephrotoxicity without reducing anticancer activity.

Key words: Cisplatin, Nephrotoxicity, Vitamin C, BUN, Creatinine, Hepatotoxicity, Metallothionein, Anticancer activity

INTRODUCTION

Cis-dichlorodiammineplatinum(II)(cisplatin), first synthesized in 1845, was known for many decades as Peyron's chloride. Its planar structure was deduced in a classic paper by Alfred Werner in 1893 that also discussed the *trans* isomer.

Cisplatin is an inorganic complex formed by a central atom of platinum surrounded by chlorine and ammonia atoms in the cis position in the horizontal plane.

Antibiological activity of cisplatin was reported by Rosenberg in 1965 (Rosenberg *et al.*, 1965), and several platinum compounds were then tested in experimental tumor systems. Among them, cisplatin was found to possess the greatest antitumor activity (Rosenberg *et al.*, 1965; Rosenberg, 1973).

Cisplatin is one of the most effective anticancer drug, widely used against various tumors (Prestayko *et al.*, 1980) such as testicular tumor, brain tumor, ovary tumor, bladder carcinoma, colon cancer, etc. However, its clinical use has been limited by nephrotoxicity, ototoxicity(tinnitus, hearing loss), gastrointestinal disturbances(nausea, vomiting), myelosuppression (leukopenia, thrombocytopenia, anemia) and allergic reactions(eczema, dermatitis) (Von Hoff *et al.*, 1979; Madias and Harrington, 1978; Gringier *et al.*, 1988). Of these toxicities, dose-related and cumulative nephrotoxicity is the major dose-limiting factor.

Acute tubular necrosis is a prominent feature of cisplatin nephrotoxicity, and cisplatin nephrotoxicity is clinically manifested by elevations in blood urea nitrogen(BUN), serum creatinine, proteinuria and hyperuricemia (Madias and Harrington, 1978). Electrolyte disturbances have also been described in treated patients and may be related to impaired renal tubular reabsorption.

Metabolites of the cisplatin complex, rather than the platinum atom, mediate the nephrotoxicity of this drug (Rosenberg, 1975). Indeed, biotransformation of cisplatin has been suggested by the *in vitro* lability of the chloride ligands of the complex in aqueous media. It has become increasingly evident that chemically induced cytotoxicity may be related to the generation of reactive metabolite which bind covalently to tissue macromolecules such as protein, lipid or nucleic acid. Such an electrophilic complex may bind to essential macromolecules of the kidney, resulting in nephrotoxicity. Macromolecule binding of reactive metabolites

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of cisplatin may account for the persistent and prolonged retention of platinum in kidney tissue (Goldstein and Mayor, 1983).

It has been also reported that lipid peroxide levels in kidney tissue are elevated by administration of cisplatin (Sodzuka *et al.*, 1991; Sugihara *et al.*, 1987) and cisplatin induced nephrotoxicity may be related to the generation of oxygen free radical by stimulating immune cells such as neutrophil (Choi and Choung, 1992).

Reducing the side effect of cisplatin, especially nephrotoxicity, is important in clinical aspects. Several attempts have been made to decrease the nephrotoxicity of cisplatin (Walker and Gale, 1981). (a) Various platinum analogues have been developed, which are less nephrotoxic than cisplatin. Carboplatin is indeed less nephrotoxic, but severe bone marrow toxicity limit its clinical use (Rose and Schurig, 1985). (b) Hydration and induction of chloruresis provide some protection against cisplatin nephrotoxicity (Ozols *et al.*, 1984). (c) Several agents have been tested for their ability to protect against cisplatin nephrotoxicity in animals (Uozumi *et al.*, 1983; Bodenner *et al.*, 1986), but until now none of them has led to an improved therapeutic index of cisplatin in patients.

The present study focuses on free radical, which have been reported to induce cisplatin nephrotoxicity mediated by neutrophil. Thus, we have examined the influence of Vitamin C, which has radical scavenging effect, on cisplatin induced nephrotoxicity and antitumor activity.

MATERIALS AND METHODS

Laboratory Animals

Female SD rats and ICR mice were obtained from the You-Han Central Institute, and maintained on a conventional diet and water, *ad libitum*. Rats weighing 200 g and mice weighing 20 g were used in experiments.

Tumors

Sarcoma 180 tumor cells were obtained from the Institute of Kyung-Hee Medical Center. The tumor cells were maintained by weekly passages in ICR mice. Cells were counted with hemacytometer.

Kidney Function

Body weight was examined daily, and blood for measurement of BUN and serum creatinine was obtained by heart puncture anesthetized with diethyl ether on day4. BUN and serum creatinine were measured spectrometrically using the urea nitrogen reagent kit and the creatinine reagent kit from Young-Dong Pharm. Co. of Korea.

Liver Function

sGPT and sGOT were measured spectrometrically using the GPT reagent kit and GOT reagent kit from Young-Dong Pharm. Co..

Effect on Metallothionein Induction in Kidney

Vitamin C was administered intraperitoneally to examine the effect on metallothionein induction in SD rats. 1hr after Vitamin C administration, rats were administered with a single i.p. dose of cisplatin (5 mg/kg) and sacrificed on day4. Metallothionein was measured by Cd-hem saturation method (Onosaka and Chrian, 1982). Control group was treated with physiological saline instead of cisplatin.

Evaluation of Antitumor Activity

The influence of Vitamin C on the antitumor activity of cisplatin was examined in ICR mice, i.p. inoculated with 10^6 sarcoma 180 tumor cells (Day0). After 24 hr, the mice were treated with a single i.p. dose of cisplatin (5 mg/kg). The influence of Vitamin C was assessed by injecting Vitamin C intraperitoneally 1hr prior to cisplatin. Control groups were treated with physiological saline instead of cisplatin. Mice were examined daily for occurrence of tumor. The experiments were terminated on day42 and MSTs were calculated.

Statistics

Student's t test was used to evaluate the significance

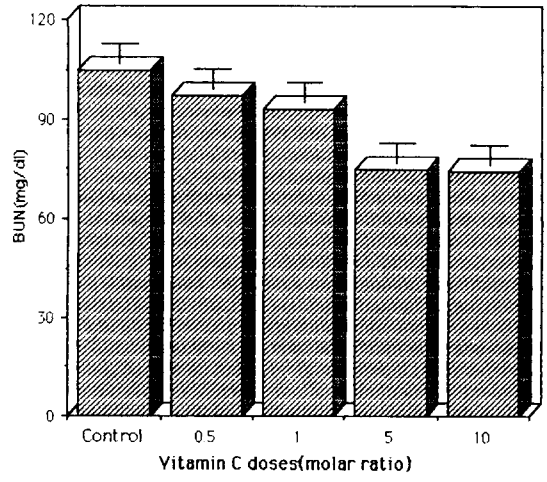

Fig. 1. Effects of Vitamin C doses on BUN levels at 4 days after cisplatin injection. Vitamin C was administered simultaneously with cisplatin (5 mg/kg). Control animals were given injection of cisplatin alone. Data are given as means± S.E. (n=6).

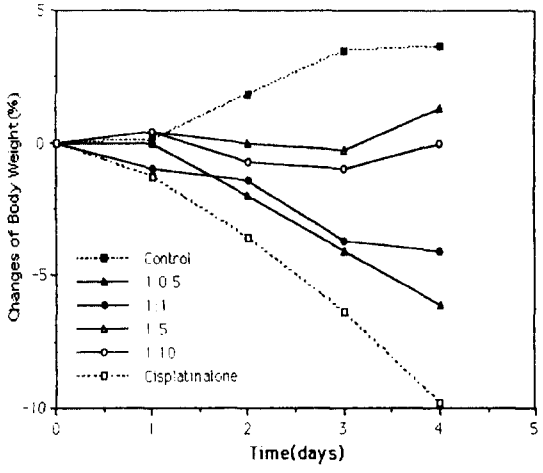

Fig. 2. Effects of Vitamin C doses on time course changes of body weight after cisplatin administration to rats. Rats received an intraperitoneal injection of cisplatin (5 mg/kg). Vitamin C was administered simultaneously with cisplatin. Control animals were given saline injection.

of differences between experimental groups.

RESULTS

Effects of Vitamin C Doses on Cisplatin Nephrotoxicity

Vitamin C, which decreased the cytotoxicity of cisplatin in primary cultured rabbit kidney proximal tubular cells *in vitro* (data not shown), was administered in dose range of 0.5-10 molar ratio of cisplatin, and cisplatin (5 mg/kg) was administered simultaneously i.p..

Administration of Vitamin C decreased BUN levels at all Vitamin C doses, especially 1 : 10 of molar ratio (Fig. 1). BUN levels have been known as a good index for early renal lesions produced by cisplatin (Ward and Fauve, 1976).

Time course changes of body weight were measured to compare cisplatin alone with combination treatment of cisplatin and Vitamin C, as shown in Fig. 2. In group given i.p. Vitamin C, weight loss was less than that in group given cisplatin alone. Especially, the group given high dose of Vitamin C showed remarkable increase of body weight.

Serum creatinine levels, another index for nephrotoxicity of cisplatin, were also measured when Vitamin C was administered simultaneously with cisplatin (Table I). All doses of Vitamin C decreased serum creatinine levels, and molar ratio 1 : 10 of Vitamin C significantly reduced serum creatinine levels of cisplatin.

sGPT and sGOT levels were also measured to observe the induction of hepatotoxicity according to combination treatment of cisplatin and Vitamin C (Table I). Any dose of Vitamin C did not cause a significant increase in sGPT or sGOT levels in SD rats at day4. Thus, Vitamin C at any dose did not cause liver damage.

These results indicate that Vitamin C is a protective agent for the renal toxicities of cisplatin without liver damage.

Effects of Vitamin C Treatment Time on Cisplatin Nephrotoxicity

Vitamin C (molar ratio 1 : 10), which showed protective effect of cisplatin nephrotoxicity, was administered to SD rats 1hr prior to or after cisplatin. Administration of Vitamin C 1hr prior to cisplatin did significantly decrease BUN levels (Fig. 3), which are similar to those of control group.

The group given Vitamin C 1 hr prior to cisplatin showed a similar pattern of body weight change to control group without weight loss (Fig. 4).

Serum creatinine levels were also markedly decreased when Vitamin C was administered 1hr prior to cisplatin (Table II).

Also, administration of Vitamin C 1hr prior to or later cisplatin did not cause a significant increase in sGPT or sGOT levels. Thus, Vitamin C at any treatment time did not cause liver damage.

These results indicate that Vitamin C is a protective agent for the nephrotoxicity of cisplatin without liver damage when it is given prior to cisplatin.

Table I. Effects of Vitamin C doses on nephrotoxicity (creatinine), hepatotoxicity (sGPT, sGOT) of cisplatin in SD rats[a]

Mole-fold	Creatinine (mg/dl)	sGPT (Karmen unit)	sGOT (Karmen Unit)
Control	0.09± 0.04	19.5± 1.1	30.5± 5.3
Cisplatin alone	2.12± 0.30	14.1± 0.3	33.3± 4.8
Cisplatin+Vitamin C(1 : 0.5)	1.28± 0.28	15.0± 0.2	10.2± 0.8
Cisplatin+Vitamin C(1 : 1)	1.89± 0.11	15.6± 0.2	8.0± 0.9
Cisplatin+Vitamin C(1 : 5)	1.43± 0.00	15.0± 0.1	5.0± 0.0
Cisplatin+Vitamin C(1 : 10)	0.57± 0.00	12.2± 2.2	4.0± 0.0

[a] Rats received intraperitoneal injection of cisplatin (5 mg/kg). BHA was administered simultaneous injection of cisplatin. Control animals were given saline. Data are given as means± S.E. (n=6).

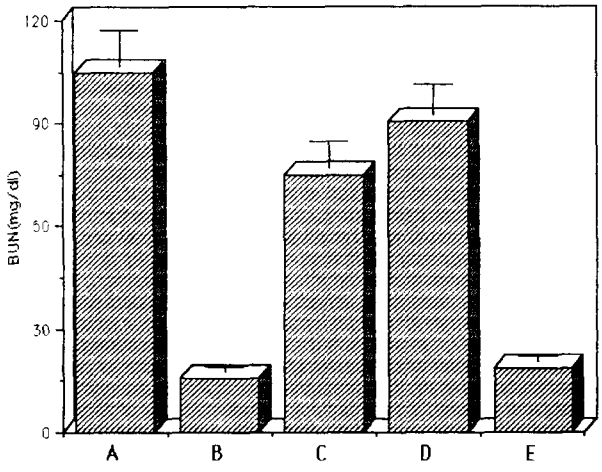

Fig. 3. Effects of Vitamin C treatment time on BUN levels at 4 days after cisplatin administration to rats. Rats received an intraperitoneal injection of cisplatin (5 mg/kg). Control animals were given saline. Data are given as means± S.E. (n=6). A: Cisplatin alone, B: Pretreatment, C: Simultaneous treatment, D: Posttreatment, E: Control.

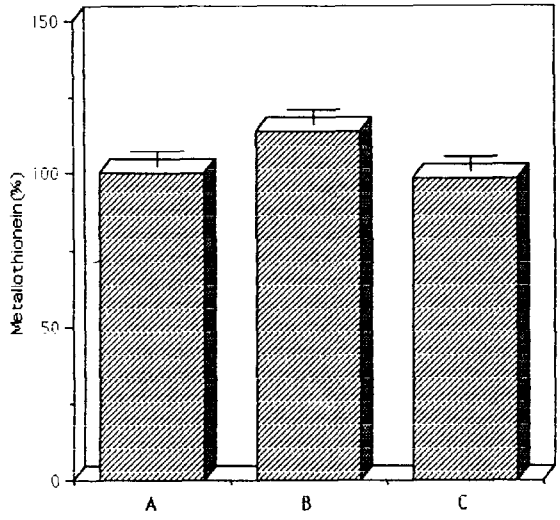

Fig. 5. Effects of Vitamin C on induction of metallothionein in cisplatin treated rats. Rats received intraperitoneal injection of cisplatin (5 mg/kg) and Vitamin C. Control animals were given saline. Data are given as means± S.E. (n=6).
A: Control, B: Cisplatin alone, C: Cisplatin+Vitamin C.

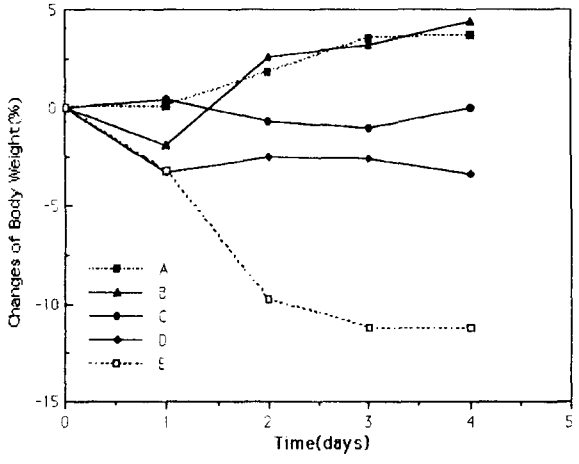

Fig. 4. Effects of Vitamin C treatment time on changes of body weight after cisplatin administration to rats. Rats received an intraperitoneal injection of cisplatin (5 mg/kg). Control animals were given saline.
A: Control, B: Pretreatment, C: Simultaneous treatment, D: Posttreatment, E: Cisplatin alone.

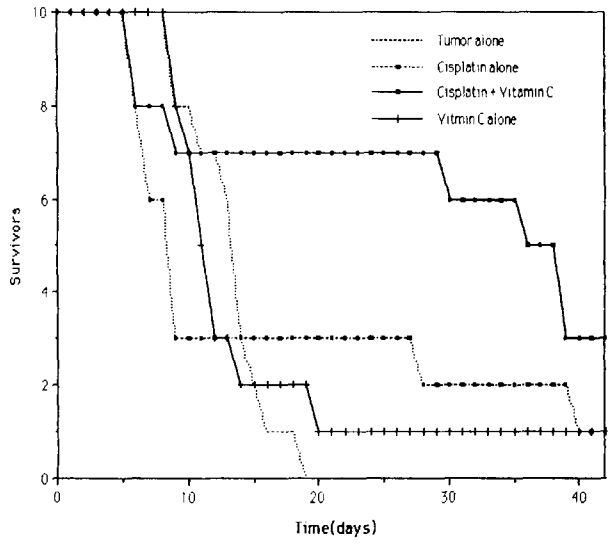

Fig. 6. Effects of Vitamin C on antitumor activity of cisplatin in ICR mice inoculated with sarcoma 180 tumor cells (n=10). Rats received intraperitoneal injection of cisplatin and Vitamin C. Control animals were given injection of saline.

Effect of Vitamin C on Induction of Metallothionein in Kidney

As shown in previous data, Vitamin C given 1hr prior to cisplatin showed remarkable protective effect of cisplatin nephrotoxicity without inducing hepatotoxicity.

In a subsequent study, metallothionein was measured to examine whether the effect of protection against cisplatin nephrotoxicity was resulted from the direct radical scavenging effect of Vitamin C itself or the induction of metallothionein, which might inactivate the cisplatin or remove free radicals (Fig. 5). There was no significant increase of metallothionein. Thus, this result indicate that the protective effect of Vitamin C was due to the direct removal of free radicals, which might be the cause of cisplatin nephrotoxicity (Choi and Choung, 1992).

Effect of Vitamin C on the Antitumor Activity of Cisplatin

The antitumor activity of cisplatin and Vitamin C

Table II. Effects of Vitamin C treatment time on levels of creatinine, sGPT, sGOT in cisplatin treated rats[a]

	Creatinine (mg/dl)	sGPT (Karmen unit)	sGOT (Karmen unit)
Control	0.09± 0.04	19.5± 1.1	30.5± 5.3
Cisplatin alone	2.12± 0.30	14.1± 0.3	33.3± 4.8
Cisplatin+Vitamin C			
Pretreatment	0.20± 0.01	7.9± 0.4	54.3± 2.4
Simultaneous treatment	0.57± 0.00	12.2± 2.2	4.0± 0.0
Posttreatment	1.03± 0.11	10.2± 0.7	45.3± 2.6

[a] Rats received intraperitoneal injection of cisplatin (5 mg/kg). Control animals were given saline. Data are given as means± S.E. (n=6).

Table III. Effects of Vitamin C on antitumor activity of cisplatin in ICR mice inoculated with sarcoma 180 tumor cells (n=10)[a]

	Survival[b] (%)	Incidence[b] Tumors (%)	MST (days)	T/C (%)
Tumor alone	80	75	13.4± 1	100
Cisplatin alone	30	0	>16.3± 4	>122
Cisplatin+Vitamin C	70	0	>29.1± 5	>217
Vitamin C alone	80	75	15.0± 3	112

[a] Rats received intraperitoneal injection of cisplatin (5 mg/kg). Data are given as means± S.E. (n=10).
[b] Survival and incidence tumors were measured at day 9.

combination treatment in ICR mice, inoculated with sarcoma 180 tumor cells, is shown in Fig. 6 and Table III.

High dose of cisplatin was administered to induce the nephrotoxicity in rats. Thus, the MST of cisplatin was decreased because of the nephrotoxicity. Almost complete inhibition of the tumor cell growth was observed in the mice received cisplatin (5 mg/kg) at day 9. There was remarkable increase in MST of mice treated with the combination treatment of cisplatin and Vitamin C. This suggested that antitumor activity of cisplatin was not masked by 1 hr pretreatment of Vitamin C. Combination treatment of cisplatin and Vitamin C inhibited growth of tumor cells and prolonged the life span of sarcoma 180 tumor cells inoculated mice.

The present results indicate that Vitamin C markedly protected the nephrotoxicity of cisplatin, but did not reduce the antitumor activity of cisplatin.

DISCUSSION

Cisplatin is an effective antitumor agent currently available for cancer therapy. However, its clinical use has been limited by its severe side effects, especially nephrotoxicity.

The protective effect of Vitamin C was dose-related and especially molar ratio 1 : 10 markedly reduced the nephrotoxicity of cisplatin without elevation in sGPT or sGOT levels. To reduce the nephrotoxicity of cisplatin, high dos of Vitamin C was needed. This may be concerned that Vitamin C is the material which is needed in all tissues of body. Thus, high dose of Vitamin C was needed to decreased the nephrotoxicity of cisplatin.

Furthermore, Vitamin C given 1hr prior to cisplatin significantly protected the nephrotoxicity of cisplatin than simultaneous treatment or 1hr later treatment without elevation in sGPT or sGOT levels. Remarkable effect of pretreatment may be concerned that free radicals, which may be produced according to cisplatin administration (Choi and Choung, 1992), was removed effectively by Vitamin C which may be distributed to target site before cisplatin distribution. But, posttreatment or simultaneous treatment was not as effective as pretreatment because Vitamin C administered after cisplatin administration did not effectively remove the free radicals produced by cisplatin. Thus, 1hr pretreatment of Vitamin C effectively protected the nephrotoxicity of cisplatin without elevation in sGPT or sGOT levels.

Serum creatinine levels, another index of cisplatin nephrotoxicity, showed similar pattern to BUN levels.

The result from the quantification of metallothionein, there was no significant increase of metallothionein. Thus, It is thought that Vitamin C did not induce metallothionein in kidney but directly removed free radicals produced by cisplatin.

If Vitamin C also reduce the cytotoxicity of cisplatin to tumor cells, the antitumor activity of cisplatin may also be decreased by Vitamin C. Therefore, the effect of Vitamin C on the antitumor activity of cisplatin was examined. Combination treatment of cisplatin and Vi-

tamin C did not decrease the MST but increased than that of cisplatin alone. Thus, combination treatment of cisplatin and Vitamin C inhibited growth of tumor cells and prolonged the life span of mice.

The present findings indicate that Vitamin C remarkably decrease the nephrotoxicity of cisplatin without reducing the antitumor activity of cisplatin.

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