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 1 hr 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), gastrointesti- nal 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
of cisplatin may account for the persistent and prolon-
ged 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 cis-
platin (Sodzuka et al., 1991; Sugihara et al., 1987) and
cisplatin induced nephrotoxicity may be related to the
-generation of oxygen free radical by stimulating im-
mune cells such as neutrophil (Choi and Choung,

Reducing the side effect of cisplatin, especially ne-
phrotoxicity, is important in clinical aspects. Several at-
ttempts have been made to decrease the nephrotoxi-
city 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 (Uo-
sumi 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 antitu-
mor 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 experime-
nts.

Tumors

Sarcoma 180 tumor cells were obtained from the
Institute of Kyung-Hee Medical Center. The tumor ce-
lls 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 obta-
ined 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 exa-
mine 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 cispla-
tin (5 mg/kg). The influence of Vitamin C was assessed
by injecting Vitamin C intraperitoneally 1hr prior to
cisplatin. Control groups were treated with physiologi-
cal 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 simulta-
neously with cisplatin (5 mg/kg). Control animals were given
injection of cisplatin alone. Data are given as means±S.E.
(n=6).](image-url)