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Protective Effect of Brazilin on Cisplatin Nephrotoxicity

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Abstract—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. Therefore, brazilin, which has a radical scavenging effect, was given intraperitoneally to evaluate the effect on cisplatin nephrotoxicity in rats. Remarkable protective effects against nephrotoxicity of cisplatin were observed when brazilin was administered to rats simultaneously with cisplatin. Hepatotoxicity induced by combination treatment of cisplatin and brazilin was evaluated by measuring serum glutamic pyruvate transaminase and serum glutamic oxalate transaminase. Combination treatment did not affect the levels of sGPT and sGOT, and any combination treatment did not induce metallothionein in kidney. Brazilin which has radical scavenging effect directly reduced nephrotoxicity of cisplatin *in vivo*. Thus, it seems that nephrotoxicity of cisplatin was caused by free radicals. The present results indicate that brazilin, when it is given with cisplatin, may provide protection against cisplatin nephrotoxicity in rats.

Keywords □ cisplatin, nephrotoxicity, brazilin, weight loss, hepatotoxicity, metallothionein.

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 cisplatin 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 binds 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 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. Brazilin, which has been used as the natural dyes, was examined to reduce the nephrotoxicity of cisplatin. Brazilin has antilipidperoxidative effect (Lea, 1944; Moon *et al.*, 1987), protective effect on hepatotoxicity (Moon and

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Ha, 1984). Brazilin showed the inhibitory effects on both enzymatic (NADPH-dependent) and nonenzymatic (ascorbate-induced) lipidperoxidation pathways (Moon *et al.*, 1987).

The present study focuses on free radicals, which have been reported to induce cisplatin nephrotoxicity and release from cisplatin stimulated neutrophil. Thus, we have examined the influence of brazilin, which has radical scavenging effect, on cisplatin induced nephrotoxicity

Materials and Methods

Laboratory animals

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

Animal treatment

SD rats were divided randomly 5 groups. Each groups had 6 rats.

Brazilin was administered *i.p.* in dose range of 0.1~5 molar ratio and cisplatin (5 mg/kg) was administered *i.p.* simultaneously.

Thereafter brazilin was administered *i.p.* 1 hr prior to or after cisplatin (5 mg/kg) administration.

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 day 4. 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

Brazilin was administered *i.p.* to examine the effect on metallothionein induction in SD rats. Rats were administered with a single *i.p.* dose of cisplatin (5 mg/kg) and sacrificed on day 4. Metallothionein was measured by Cd-hem saturation method (Onosaka and Chrian, 1982). Control group was treated with physiological saline instead of cisplatin.

Statistics

Student's t test was used to evaluate the significance of differences between experimental groups.

Results

Effects of brazilin doses on cisplatin nephrotoxicity

Administration of brazilin decreased BUN levels at all brazilin doses, especially 1 : 0.5 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 brazilin, as shown in Fig. 2. In group given *i.p.* brazilin, weight loss was less than that in

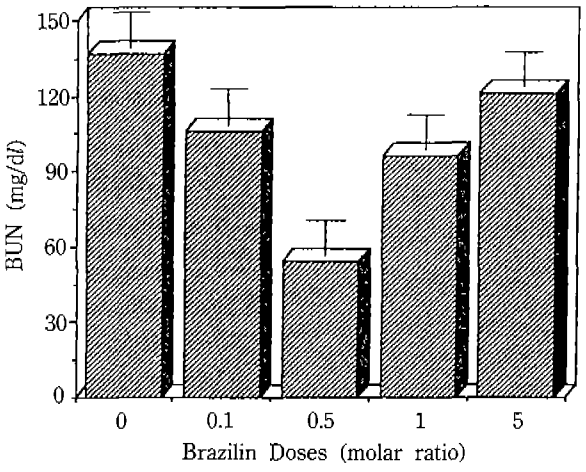

Fig. 1. Effects of brazilin doses on BUN levels at 4 days after cisplatin injection. Brazilin was administered simultaneously with cisplatin (5 mg/kg). Control animals were given injection of saline. Data are given as means ± S. E. ($n=6$).

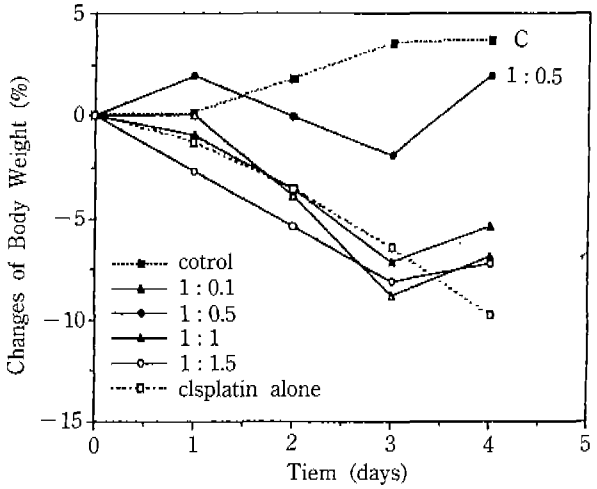

Fig. 2. Effects of brazilin doses on time course changes of body weight after cisplatin administration to rats. Rats received an intraperitoneal injection of cisplatin (5 mg/kg). Brazilin was administered simultaneously with cisplatin. Control animals were given saline injection.

Table I. Effects of brazilin doses on nephrotoxicity and hepatotoxicity of cisplatin in SD rats

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+Brazilin (1 : 0.1)	0.87± 0.51	10.3± 2.5	47.0± 3.3
Cisplatin+Brazilin (1 : 0.5)	0.66± 0.17	8.8± 0.6	33.7± 4.7
Cisplatin+Brazilin (1 : 1)	0.83± 0.14	15.2± 2.9	46.8± 1.6
Cisplatin+Brazilin (1 : 5)	1.25± 0.57	13.3± 2.1	63.0± 0.0

*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. ($n=6$).

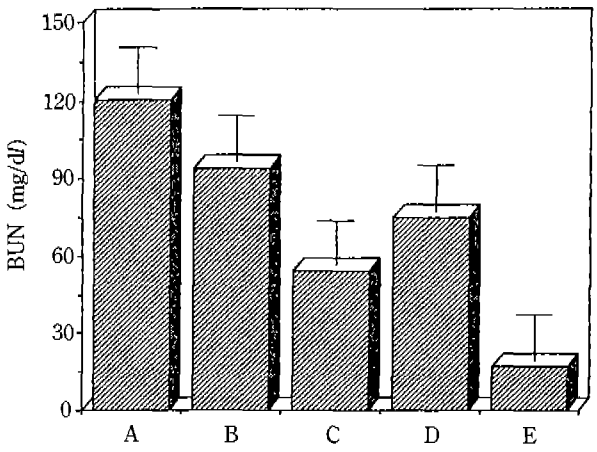

Fig. 3. Effects of brazilin treatment time on BUN levels at 4 days after cisplatin administration to rats. Rats received an intraperitoneal injection of cisplatin (5 mg/kg). Control animals were given saline. Data are given as means ± S. E. ($n=6$). A: Cisplatin alone, B: Pretreatment, C: Simultaneous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

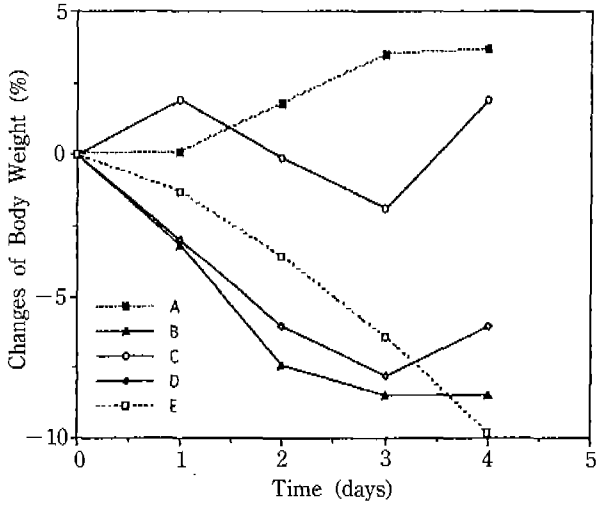

Fig. 4. Effects of brazilin treatment time on changes of body weight after cisplatin administration to rats. Rats received an intraperitoneal injection of cisplatin (5 mg/kg). Control animals were given saline. A: Control, B: Pretreatment, C: Simultaneous treatment, D: Posttreatment, E: Cisplatin alone.

group given cisplatin alone. Especially, the group given 1 : 0.5 molar ratio of brazilin showed similar body weight changes to that of control group at day 4.

Serum creatinine levels, another index for nephrotoxicity of cisplatin, were also measured when brazilin was administered simultaneously with cisplatin (Table I). All doses of brazilin decreased serum creatinine levels, and molar ratio 1 : 0.5 of brazilin significantly reduced serum creatinine levels in cisplatin treated rats.

sGPT and sGOT levels were also measured to observe the induction of hepatotoxicity according to combination treatment of cisplatin and brazilin (Table I). Brazilin except molar ratio 1 : 5 did not cause a significant increase in sGPT or sGOT levels in SD rats at day 4. Thus, brazilin at low dose did not cause liver damage. These results indicate that brazilin is a protective agent for the renal toxicities of cisplatin without liver damage.

Effects of brazilin treatment time on cisplatin nephrotoxicity

Brazilin (molar ratio 1 : 0.5), which showed protective effect of cisplatin nephrotoxicity, was administered to SD rats 1 hr prior to or after cisplatin administration. Administration of brazilin simultaneously with cisplatin showed more effective decrease of BUN levels than any other treatment time (Fig. 3).

Serum creatinine levels were also significantly decreased when brazilin was administered simultaneously with cisplatin. But, brazilin given at any treatment time did not increased the body weight except simultaneous treatment (Fig. 4).

Also, administration of brazilin did not cause a significant increase in sGPT or sGOT levels. Thus, brazilin did not cause liver damage at any treatment time.

Table II. Effects of brazilin treatment time on levels of creatinine, sGPT, sGOT in cisplatin treated rats

	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+Brazilin			
Pretreatment	0.41± 0.11	12.5± 1.1	32.0± 1.4
Simultaneous treatment	0.60± 0.08	5.5± 1.8	20.5± 0.4
Posttreatment	0.64± 0.01	15.3± 3.8	40.0± 6.7

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

These results indicate that brazilin is a protective agent for the nephrotoxicity of cisplatin without liver damage when it is given simultaneously with cisplatin.

Effect of brazilin on induction of metallothionein in kidney

Brazilin given simultaneously with cisplatin showed remarkable protective effect of cisplatin nephrotoxicity without inducing hepatotoxicity.

So, we measured metallothionein levels in kidney to examine whether the effect of protection against cisplatin nephrotoxicity was resulted from the direct radical scavenging effect of brazilin itself or the induction of metallothionein, which might inactivate the cisplatin or remove free radicals (Fig. 5). There was no significant increase of metallothionein. This result indicates that the protective effect of brazilin was due to the direct removal of free radicals, which might be the cause of cisplatin nephrotoxicity.

The present results indicate that brazilin markedly protected the toxicity of cisplatin in rats, but reduced the antitumor activity of cisplatin in mice.

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.

Brazilin, molar ratio 1 : 0.5, markedly reduced the nephrotoxicity of cisplatin without elevation in sGPT or sGOT levels.

Furthermore, brazilin given simultaneously with cisplatin significantly protected the nephrotoxicity of cisplatin than 1 hr prior to or later treatment without elevation in sGPT or sGOT levels.

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

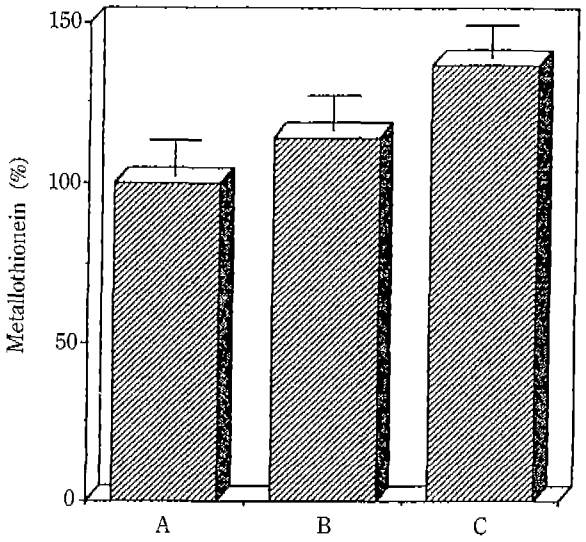

Fig. 5. Effects of brazilin on induction of metallothionein in cisplatin treated rats. Rats received intraperitoneal injection of cisplatin (5 mg/kg) and brazilin. Control animals were given saline. Data are given as means ± S. E. ($n=6$). A: Control, B: Cisplatin alone, C: Cisplatin+Brazilin.

Pretreatment of brazilin was not as effective as simultaneous treatment. This may be related to the induction of microsomal enzyme like BHA (Kong and Choung, 1993). Futhermore, protective effect of brazilin given simultaneously with cisplatin may be thought that the time of free radical production by cisplatin was similar to the time of radical scavenging effect of brazilin in kidney. Thus, simultaneous treatment showed more remarkable protection against cisplatin nephrotoxicity than pretreatment or posttreatment.

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

The present findings indicate that simultaneously treated brazilin remarkably decreases the nephrotoxicity of cisplatin without liver damage in rats.

Acknowlegement

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