速　報

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Protective effects of Kampo medicines against cis-diammine-dichloroplatinum (II) －induced nephrotoxicity and bone marrow toxicity in mice

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Abstract

The protective effect of 15 Kampo medicines on the nephrotoxicity and bone marrow toxicity induced by cis-diamminedichloroplatinum (II) (c-DDP) was investigated. These medicines have been used traditionally in the treatment of kidney diseases or for patients with anemia, anorexia or fatigue. To evaluate the effect we measured blood urea nitrogen (BUN), serum creatinin, number of white blood cells (WBC) and platelet (PLT) in blood, and also measured the body weight in an animal model with ddY mice. Combined pre-and post-administration of 10-fold clinical dosage of Juzen-taiho-to (1728 mg/kg), Hochu-ekki-to (1426 mg/kg), Toki-shakuyaku-san (805 mg/kg) and Hachimi-jio-gan (1057 mg/kg) to the mice, greatly protected the increase in BUN and serum creatinin level, the decrease in WBC and PLT counts and body weight loss caused by 3 mg/kg of c-DDP *i.p.* administration of 9 times, without reducing the antitumor effect of c-DDP on Sarcoma 180 (S-180) cells in ddY mice inoculated subcutaneously. In contrast, diuretics, Chorei-to (584 mg/kg), Gorei-san (474 mg/kg), Ryo-kei-jutsu-kan-to (315 mg/kg), Boi-bukuryo-to (401 mg/kg) or furosemide (20 mg/kg) reduced the antitumor effect of c-DDP as well as the toxicity. The radical scavenger, α-tocopherol, did not prevent the decrease in WBC counts and body weight loss, but, α-tocopherol protected the increase in BUN and serum creatinin level. The nephrotoxicity was not influenced by Sho-saiko-to, which has been known to have anti-inflammatory effects. These results suggest that Juzen-taiho-to, Hochu-ekki-to, Toki-shakuyaku-san and Hachimi-jio-gan prevent the nephrotoxicity and bone marrow toxicity of c-DDP, and the protective effects of these Kampo medicines may be produced by multiple mechanisms.

Key words cisplatin, nephrotoxicity, bone marrow, Juzen-taiho-to, Toki-shakuyaku-san, furosemide, blood urea nitrogen.

Abbreviations Boi-bukuryo-to (Fang-Yi-Fu-Ling-Tang), 防已茯苓湯; Boi-ogi-to (Fang-Yi-Huang-Qi-Tang), 防已黄耆湯; BUN, blood urea nitrogen; c-DDP, cis-diammine-dichloroplatinum (II); Chorei-to (Zhu-Ling-Tang), 猪苓湯; Gorei-san (Wu-Ling-San), 五苓散; Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan), 八味地黄丸; Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang), 補中益気湯; Inchin-ko-to (Yin-Chen-Hao-Tang), 茵蔯蒿湯; Juzen-taiho-to (Shi-Quan-Da-Bu-Tang), 十全大補湯; Oren-gedoku-to (Huang-Lian-Jie-Du-Tang), 黄連解毒湯; PLT, platelet; Rikkunshi-to (Liu-Jun-Zi-Tang), 六君子湯; Ryo-kei-jutsu-kan-to (Ling-Gui-Shu-Gan-Tang), 苓桂朮甘湯; Ryutan-shakan-to (Long-Dan-Xie-Gan-Tang), 竜胆瀉肝湯; S-180, Sarcoma 180; Shimbu-to (Zhen-Wu-Tang), 真武湯; Sho-saiko-to (Xiao-Chai-Hu-Tang), 小柴胡湯; Toki-shakuyaku-san (Dang-Gui-Shao-Yao-San), 当帰芍薬散; WBC, white blood cell.

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Introduction

c-DDP is an important anticancer agent, widely used against ovarian, testicular and urinary bladder carcinomas.[1,2] However, c-DDP has severe toxic side effects, notably to the kidney, gastrointestinal tract and bone marrow[3] Especially, nephrotoxicity is the dose-limiting factor in humans.[4,5] Many attempts have been made to improve the therapeutic value of c-DDP with candidate protective agents.[6-11] However, it is still difficult to get sufficient protective effect against c-DDP-induced toxicity.

Kampo medicine has a history of approximately 2000 years, during which the fundamental concept for the remedy of diseases has changed little. For the treatment of diseases, Kampo medicine stresses supplementing resistance against diseases rather than elimination of the cause of the disease. In fact, Kampo medicines, which are mixtures of crude drugs, have been recently utilized for the treatment of cancer patients, with a view toward improving the patient's immunological function and maintaining the quality of life.[12] Consequently, it has been gradually clarified that Kampo medicines compensate for the shortcoming of anticancer agents.[13] In the present experiment, we have, therefore, investigated the effects of Kampo medicines on c-DDP-induced nephrotoxicity and bone marrow toxicity and also investigated the effect of co-application of these drugs on the antitumor effect of c-DDP in ddY mice bearing S-180 cells.

In the present experiment, we tested 15 Kampo medicines which have been used traditionally as a remedy for kidney diseases or for patients with anemia, anorexia or tatigue.[14] The Kampo medicines used are as follows : Boi-bukuryo-to, Boi-ogi-to, Chorei-to, Gorei-san, Hachimijio-gan, Hochu-ekki-to, Inchin-ko-to, Juzen-taiho-to, Oren-gedoku-to, Rikkunshi-to, Ryo-keijutsu-kan-to, Ryutan-shakan-to, Shimbu-to, Sho-saiko-to, and Toki-shakuyaku-san.

Material and Methods

Animals : Five-week-old, male, ddY mice (average weight, 25 g), were obtained from Japan SLC, Inc., Shizuoka, Japan, and kept in rooms with controlled temperature (23±0.5°C), humidity (50±5 %), and 12 h light-dark cycles. They were fed with a commercial mouse chow (MF ; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water ad libitum, and were used after one week of acclimation (average weight, 30 g).

Chemicals : c-DDP was kindly provided by Nippon Kayaku Co., Ltd., Tokyo. The Solution of c-DDP was prepared one week before injection in sterile 0.9 % saline at a concentration of 0.5 mg/ml.[15,16] Furosemide and α-tocopherol (D, L-α-tocopherol) were obtained from Wako Pure Chemical Industries, Ltd., Tokyo.

Kampo medicines : Crude drugs for the formulation of the Kampo medicines were purchased from Yamamoto Yakuhin Kogyo, Co., Ltd., Tokyo. They were cut into small pieces and blended according to the recipe (Table I).[14] The blend was then extracted with boiling water for 30 min. After cooling, the extracts were filtered, and then lyophilized. The lyophilized materials were dissolved in water immediately before use. Kampo medicines were given to the mice at a dose of 10-fold the clinical dose as shown in Table I.

Treatment of animals : The design of this study is shown in Fig. 1. c-DDP (3 mg/kg) was given *i.p.* to the mice on days 1, 2, 3, 4, 5, 6, 8, 9 and 10. Kampo medicines were perorally given to the mice on days −5, −4, −3, −2, −1, 1, 3, 5, 6, 8, 10, 12 and 13. The control group was treated with water instead of Kampo medicines. On day 15, mice were anesthetized with ether, and blood was collected from the inferior vena cava using a heparinized syringe, and the number of WBC and PLT was immediately counted. After the centrifugation of the remaining blood, the serum was analyzed for BUN and creatinine.

Measurement of functions : WBC and PLT counts were made on a Celltac 4150 (Nihon Koden, Ltd., Tokyo). BUN and creatinine were measured on a COBAS FARA (Baxter, Ltd., Tokyo) spectrometrically using assay kits for urea nitrogen-HR II and creatinine-HA test Wako (Wako Pure Chemical Industries, Ltd.),

Table I Experimental dose and recipe of Kampo medicines.

Kampo medicines	Dose[a] (mg/kg)	Recipe (g)
Juzen-taiho-to	1728	Angelicae Radix (3), Hoelen (3), Glycyrrhizae Radix (2), Ginseng Radix (3), Astragali Radix (3), Cinnamomi Cortex (3), Atractylodis Rhizoma (3), Paeoniae Radix (3), Cnidii Rhizoma (3), Rehmanniae Radix (3)
Hochu-ekki-to	1426	Angelicae Radix (3), Glycyrrhizae Radix (2), Ginseng Radix (4), Astragali Radix (4), Atractylodis Rhizoma (4), Zingiberis Rhizoma (1), Aurantii Nobilis Pericarpium (2), Cimicifugae Rhizoma (1), Zizyphi Fructus (2), Bupleuri Radix (1)
Rikkunsi-to	805	Hoelen (4), Glycyrrhizae Radix (1), Ginseng Radix (4), Atractylodis Rhizoma (4), Zingiberis Rhizoma (1), Aurantii Nobilis Pericarpium (2), Zizyphi Fructus (2), Pinelliae Tuber (4)
Toki-shakuyaku-san	1421	Angelicae Radix (3), Hoelen (4), Atractylodis Rhizoma (4), Paeoniae Radix (4), Cnidii Rhizoma (3), Alismatis Rhizoma (4)
Hachimi-jio-gan	1057	Hoelen (3), Cinnamomi Cortex (1), Rehmanniae Radix (5), Dioscoreae Rhizoma (3), Alismatis Rhizoma (3), Moutan Cortex (3), Aconiti Tuber (1), Corni Fructus (3)
Chorei-to	584	Hoelen (3), Polyporus (3), Alismatis Rhizoma (3), Talcum (3), Asini Gelatinum (3)
Gorei-san	474	Hoelen (5), Polyporus (5), Alismatis Rhizoma (6), Cinnamomi Cortex (3), Atractylodis Rhizoma (5)
Ryo-kei-jutsu-kan-to	315	Hoelen (6), Glycyrrhizae Radix (2), Cinnamomi Cortex (4), Atractylodis Rhizoma (3)
Boi-bukuryo-to	401	Hoelen (5), Glycyrrhizae Radix (2), Cinnamomi Cortex (3), Astragali Radix (5), Sinomeni Caulis et Rhizoma (5)
Ryutan-shakan-to	1847	Angelicae Radix (5), Glycyrrhizae Radix (2), Bupleuri Radix (3), Rehmanniae Radix (5), Akebiae Caulis (2), Alismatis Rhizoma (3), Gentianae Scabrae Radix (2), Plantaginis Semen (3), Scutellariae Radix (3), Gardeniae Fructus (2)
Boi-ogi-to	1012	Glycyrrhizae Radix (2), Astragali Radix (5), Atractylodis Rhizoma (3), Zizyphi Fructus (3), Zingiberis Rhizoma (3), Sinomeni Caulis et Rhizoma (5)
Shimbu-to	432	Hoelen (5), Atractylodis Rhizoma (3), Paeoniae Radix (3), Zingiberis Rhizoma (3), Aconiti Tuber (1)
Oren-gedoku-to	295	Coptidis Rhizoma (2), Scutellariae Radix (3), Phellodendri Cortex (2), Gardeniae Fructus (2)
Sho-saiko-to	932	Glycyrrhizae Radix (2), Ginseng Radix (3), Bupleuri Radix (7), Zingiberis Rhizoma (4), Zizyphi Fructus (3), Scutellariae Radix (3), Pinelliae Tuber (5)
Inchin-ko-to	304	Artemisiae Capillaris Herba (4), Gardeniae Fructus (3), Rhei Rhizoma (1)
Furosemide	20	
α-Tocopherol	500	

[a] Test samples were perorally administered to mice 30 min before c-DDP.

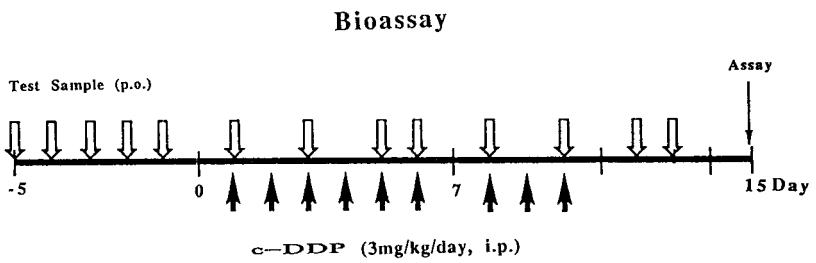

Fig. 1 Experimental design for examining the effect of Kampo medicines on c-DDP-induced toxicity. c-DDP ; cis-diamminedichloroplatinum (II).

respectively.

Evaluation of Antitumor Activity : The effect of Kampo medicines on the antitumor activity of c-DDP against a solid type tumor was examined in ddY mice which were inoculated with 10^6 S-180 tumor cells in the left thigh subcutaneously on the day −1. Kampo medicines and c-DDP were given as already described. The experiment was terminated on day 15 and the excised tumor was weighed.

Statistics : Student's *t* test was used to evaluate the significance of difference between experimental groups.

Results

Effects of Kampo medicines on nephrotoxicity

The effects of Kampo medicines on BUN and serum creatinine levels were examined on day 15, when these parameters reached the maximum levels. BUN and creatinine levels increased significantly to about 4-fold and 2-fold of the control level, respectively, when using the treatment with 3 mg/kg of c-DDP *i.p.* administration of 9 times. Kampo medicines significantly diminished these increments except for Rikkunsi-to, Shimbu-to, Sho-saiko-to and Inchin-ko-to. When furosemide or α-tocopherol was administered in combination with c-DDP, BUN and serum creatinine levels remained near the control level (Table II).

Each Kampo medicine alone did not exert any significant effect on BUN and serum creatinine levels (data not shown).

Effects of Kampo medicines on bone marrow toxicity

Administration of c-DDP alone decreased WBC counts to 39 % of the control values. Of the 15 Kampo medicines tested, 9 significantly prevented the decrease in the WBC counts. Especially, the treatment with Juzen-taiho-to or Hochu-ekki-to kept the WBC counts to near the control level. However, furosemide and α-tocopherol did not exert any significant effect on the decreased WBC counts (Table III).

A decrease in PLT counts to 18 % of the control value by administration of c-DDP was also inhibited by 10 of the 15 Kampo medicines tested. The protective effect was strongly shown in the cases of Juzen-taiho-to, Hochu-ekki-to, Toki-shakuyaku-san and Hachimi-jio-gan. On the contrary, for Rikkunshi-to, Shimbu-to and Inchin-ko-to, the PLT counts decreased to less than that of c-DDP alone group. Furosemide and α-tocopherol also strongly protected the decrease in PLT counts (Table III). Each Kampo medicine alone did not exert any significant effect on WBC and PLT counts (data not shown).

c-DDP alone did not change in red blood cell counts compared to the control values, because their half-life is longer than that of WBC and PLT (data not shown).

Effect of Kampo medicines on the antitumor effect of c-DDP

The antitumor effects of c-DDP / Kampo medicine combination in ddY mice, inoculated with S-180 cells, are shown in Table IV. The combined use of Chorei-to, Gorei-san, Ryo-kei-jutsu-kan-to or Boi-bukuryo-to with c-DDP produced a remarkable decrease in the antitumor

Table II The influence of Kampo medicines on the nephrotoxicity of c-DDP in ddY mice.

Kampo medicines	c-DDP (3 mg/kg)	BUN (mg/dl)	Creatinine (mg/dl)
Control	−	28.9 ± 1.3***	0.54 ± 0.02***
c-DDP alone	+	107.7 ± 9.8	0.98 ± 0.14
Juzen-taiho-to	+	30.1 ± 1.6***	0.44 ± 0.02***
Hochu-ekki-to	+	34.4 ± 3.7***	0.47 ± 0.03***
Rikkushi-to	+	75.0 ± 6.8	0.59 ± 0.07
Toki-shakuyaku-san	+	39.9 ± 6.8***	0.50 ± 0.10***
Hachimi-jio-gan	+	31.3 ± 2.5***	0.42 ± 0.02***
Chorei-to	+	32.6 ± 1.6***	0.42 ± 0.06***
Gorei-san	+	36.4 ± 3.3***	0.55 ± 0.04***
Ryo-kei-jutsu-kan-to	+	41.8 ± 6.5***	0.50 ± 0.04***
Boi-bukuryo-to	+	47.4 ± 5.0***	0.50 ± 0.01***
Ryutan-shakan-to	+	43.7 ± 7.6***	0.46 ± 0.20***
Boi-ogi-to	+	69.4 ± 10.4*	0.50 ± 0.03***
Shimbu-to	+	84.4 ± 9.4	0.72 ± 0.05
Oren-gedoku-to	+	44.2 ± 10.0***	0.57 ± 0.04**
Sho-saiko-to	+	74.2 ± 10.5	0.60 ± 0.05
Inchin-ko-to	+	104.5 ± 8.2	0.60 ± 0.05
Furosemide	+	37.7 ± 3.4***	0.54 ± 0.02**
α−Tocopherol	+	33.5 ± 3.7***	0.56 ± 0.03**

Kampo medicines, furosemide and α-tocopherol were administered *p.o.* 30 min before c-DDP. BUN and creatinine in serum were measured on day 15. Each value represents the mean ± S.E. of 10 mice. Significantly different from c-DDP alone group, $*p<0.05$, $**p<0.01$, $***p<0.001$.

Table III The influence of Kampo medicines on the bone marrow toxicity of c-DDP in ddY mice.

Kampo medicines	c-DDP (3 mg/kg)	WBC ($\times10^4$/mm^3)	PLT ($\times10^5$/mm^3)
Cotrol	−	0.61 ± 0.07***	8.45 ± 0.36***
c-DDP alone	+	0.24 ± 0.02	1.48 ± 0.11
Juzen-taiho-to	+	0.61 ± 0.03***	7.15 ± 0.41***
Hochu-ekki-to	+	0.59 ± 0.04***	6.99 ± 0.42***
Rikkunshi-to	+	0.31 ± 0.05	0.59 ± 0.26
Toki-shakuyaku-san	+	0.50 ± 0.08***	7.00 ± 0.92***
Hachimi-jio-gan	+	0.51 ± 0.06***	6.58 ± 0.55***
Chorei-to	+	0.45 ± 0.04***	4.39 ± 0.48**
Gorei-san	+	0.37 ± 0.02*	5.28 ± 0.51**
Ryo-kei-jutsu-kan-to	+	0.46 ± 0.04***	4.44 ± 0.65**
Boi-bukuryo-to	+	0.41 ± 0.03**	4.68 ± 0.64**
Ryutan-shakan-to	+	0.31 ± 0.08	3.76 ± 0.31*
Boi-ogi-to	+	0.21 ± 0.01	1.73 ± 0.42
Shimbu-to	+	0.25 ± 0.03	0.23 ± 0.14
Oren-gedoku-to	+	0.46 ± 0.05***	3.75 ± 0.87*
Sho-saiko-to	+	0.28 ± 0.03	1.72 ± 0.64
Inchin-ko-to	+	0.23 ± 0.04	0.34 ± 0.20
Furosemide	+	0.33 ± 0.04	6.25 ± 0.62***
α−Tocopherol	+	0.37 ± 0.03	6.39 ± 0.76***

Kampo medicines, furosemide and α−tocopherol were administered *p.o.* 30 min before c-DDP. WBC and PLT were measured on day 15. Each value represents the mean ± S.E. of 10 mice. Significantly different from c-DDP alone group, $*p<0.05$, $**p<0.01$, $***p<0.001$.

Table IV The influence of Kampo medicines on the antitumor effect of c-DDP in ddY mice inoculated with S 180 cells.

Kampo medicines	c-DDP (3 mg/kg)	Tumor weight (g)	% Inhibition (vs. c-DDP alone)
Control	−	1.12 ± 0.12***	
c-DDP alone	+	0.23 ± 0.05	−
Juzen-taiho-to	+	0.20 ± 0.03	13.0
Hochn-ekki-to	+	0.20 ± 0.03	13.0
Rikkunshi-to	+	0.26 ± 0.05	−13.0
Toki-shakuyaku-san	+	0.21 ± 0.03	8.7
Hachimi-jio-gan	+	0.23 ± 0.02	0.0
Chorei-to	+	0.47 ± 0.10*	−104.3
Gorei-san	+	0.33 ± 0.06	−43.5
Ryo-kei-jutsu-kan-to	+	0.36 ± 0.07	−56.5
Boi-bukuryo-to	+	0.30 ± 0.05	−30.4
Ryutan-shakan-to	+	0.26 ± 0.04	−13.0
Boi-ogi-to	+	0.24 ± 0.04	−4.3
Shimbu-to	+	0.25 ± 0.05	−8.7
Oren-gedoku-to	+	0.23 ± 0.05	0.0
Sho-saiko-to	+	0.27 ± 0.04	−17.4
Inchin-ko-to	+	0.26 ± 0.05	−13.0
Furosemide	+	0.66 ± 0.10**	−187.0
α−Tocopherol	+	0.23 ± 0.07	0.0

S−180 cells (10^6) were subcutaneously inoculated in the left thigh on day −1. Kampo medicines, furosemide and α−tocopherol were administered *p.o.* 30 min before c-DDP. The tumor was excised and weighed on day 15. Each value represents the mean±S.E. of 10 mice. Significantly different from c-DDP alone group, $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Table V The influence of Kampo medicines on the decrease in body weight induced by c-DDP in ddY mice.

Kampo medicines	c-DDP (3 mg/kg)	Final body weight (g)	Recovery rate (%)
Control	−	36.6 ± 0.9***	
c-DDP alone	+	20.6 ± 0.5	
Juzen-taiho-to	+	33.3 ± 0.6***	79.4
Hochu-ekki-to	+	31.7 ± 0.6***	69.4
Rikkunshi-to	+	22.7 ± 1.0	13.1
Toki-shakuyaku-san	+	33.5 ± 0.7***	80.6
Hachimi-jio-gan	+	32.6 ± 0.4***	75.0
Chorei-to	+	31.9 ± 1.0***	70.6
Gorei-san	+	31.6 ± 0.9***	68.8
Ryo-kei-jutsu-kan-to	+	29.6 ± 1.1***	56.3
Boi-bukuryo-to	+	27.8 ± 0.8**	45.1
Ryutan-shakan-to	+	27.5 ± 0.9**	43.1
Boi-ogi-to	+	25.4 ± 0.6*	30.0
Shimbu-to	+	21.4 ± 0.9	5.0
Oren-gedoku-to	+	26.5 ± 1.4*	36.9
Sho-saiko-to	+	24.1 ± 0.8*	21.9
Inchin-ko-to	+	22.6 ± 0.7	12.5
Furosemide	+	28.4 ± 1.5**	48.8
α−Tocopherol	+	25.6 ± 0.8*	31.3

Six-week-old, ddY mice, initially weighing 30 g, were used on day −5, and the final body weight of the mice was measured on day 15. Kampo medicines, furosemide and α−tocopherol were administered *p.o.* 30 min before c-DDP. Each value represents the mean±S.E. of 10 mice. Significantly different from c-DDP alone group, $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

effect by approximately 30 to 100 % compared with c-DDP alone, and the decrease was particularly significant in the case of Chorei-to. A similar effect was also observed when the diuretic, furosemide, was administered (approximately 200 % compared with c-DDP alone). On the other hand, regarding Juzen-taiho-to, Hochu-ekki-to and Toki-shakuyaku-san, the antitumor effect of c-DDP alone was increased by approximately 10 % (the difference was not significant). Each Kampo medicine alone had no obvious antitumor effect on S-180 (data not shown).

Effect of Kampo medicines on body weight

As shown in Table V, c-DDP alone showed a 43.7 % loss in final body weight of mice compared with the control value. Administration of all Kampo medicines tested protected the weight loss induced by c-DDP. In particular, restoration of weight loss was strongly observed when using Juzen-taiho-to, Hochu-ekki-to, Toki-shakuyaku-san, Hachimi-jio-gan, Chorei-to and Gorei-san with a 70-80 % approximate recovery rate. The recovery rate was calculated by the following formula :

$$\text{Recovery rate (\%)} = \frac{B - C}{A - C} \times 100,$$

where A, B and C are the final body weight of the control, test samples and c-DDP alone, respectively. Furosemide or α-tocopherol treatment also resulted in significant protection in the weight loss, albeit less pronounced than with the Kampo medicines (48.8 % and 31.1 %, respectively).

Discussion

The present experiment demonstrated Kampo medcines protected nephrotoxicity and bone marrow toxicity induced by c-DDP, in line with the earlier report [13] that Juzen-taiho-to depressed the lethal toxicity and the nephrotoxicity (indicated by an increase in BUN). This previous study, however, applied c-DDP (13.5 mg/kg, *s.c.*) to ICR mice as a single treatment, in contrast to the present investigation where a multiple treatment (9×3 mg/kg/day, *i.p.*) was employed. In the present experiment, treatment of only c-DDP to mice caused severe nephrotoxicity (represented by an increase in BUN and creatinine levels) and bone marrow toxicity (represented by a decrease in WBC and PLT counts). These observations are in basic agreement with several prior studies utilizing c-DDP in animal models [17, 18] or in patients. [3]

Many attempts have been made to demonstrate the molecular mechanism of c-DDP-induced nephrotoxicity, but the actual mechanism is still unknown. [19-22] Radical scavengers, such as metallothionein [9] and α-tocopherol [23, 24] are known to reduce the nephrotoxicity caused by c-DDP. In order to assess any involvement of active oxygen in c-DDP-induced toxicity in this model, the effect of a radical scavenger, α-tocopherol, was investigated. In this experiment, α-tocopherol (500 mg/kg/day, *p.o.*) reduced the increase in BUN and creatinine levels to near the control level without reducing the antitumor effect of c-DDP, suggesting that a part of the c-DDP-induced nephrotoxicity may be caused by a radical reaction. [20, 25] A similar effect was obtained when Juzen-taiho-to, Hochu-ekki-to, Toki-shakuyaku-san or Hachimi-jio-gan was administered in combination with c-DDP. These results indicate that the radical scavenging effect may contribute to producing the protective effect against nephrotoxicity in these Kampo medicines. On the other hand, α-tocopherol did not protect the decrease in WBC counts, though Juzen-taiho-to, Hochu-ekki-to, Toki-shakuyaku-san and Hachimi-jio-gan did protect against any decrease. This might suggest that these Kampo medicines may contribute to the protective effect through some other mechanism, such as the effect of enhancing the number of colony-forming units in the spleen. [26, 27]

Diuretics are known to reduce the antitumor effect of c-DDP as well as its toxicity. [28, 29] Actually, furosemide reduced both the antitumor effect and the toxicity of c-DDP in this study. Similar results were obtained when Chorei-to, Gorei-san, [30] Ryo-kei-jutsu-kan-to or Boi-Bukuryo-to, which have traditionally been used as diuretics, [14] were administered in combination with c-DDP. This observation might suggest that the diuresis effect of the Kampo medicines may play an important role in eliciting the protective effect of

these medicines against c-DDP-induced toxicity. On the other hand, Toki-shakuyaku-san and Hachimi-jio-gan, which contain crude drugs with diuresis effects such as Hoelen and Alismatis Rhizoma,[31] did not reduce the antitumor effect of c-DDP. In addition, the diuretic, Shimbu-to,[14] did not show any protective effect against the c-DDP-induced toxicity. These results suggest that the mechanism or the ability of the diuresis effect of these Kampo medicines may be different for each of them or the protective effect of some of these Kampo medicines may not be produced by the diuresis effect.

Sho-saiko-to is known to have an antinephritic effect[32] and an anti-inflammatory effect.[33] In this study, however, the c-DDP-induced nephrotoxicity was not influenced by the treatment with Sho-saiko-to, presumably because c-DDP-induced nephrotoxicity was very serious in our model system[34] or the c-DDP-induced kidney damage in our medel system was a type different from that previously reported.[32]

Juzen-taiho-to and Hochu-ekki-to have traditionally been used for patients with anorexia, anemia or fatigue,[14] and are known to enhance antitumor effects.[35-37] In this study, however, the antitumor effect of c-DDP against S-180 was slightly influenced by the treatment with these medicines. Further study is needed to precisely evaluate the combined effect of these medicines with c-DDP, in terms of the administration amount of the Kampo medicines or c-DDP.[38]

The Kampo medicines tested comprise a mixture of 3 to 10 crude drugs (Table I). Juzen-taiho-to, Hochu-ekki-to and Toki-shakuyaku-san, which contain Angelicae Radix[31] as a common constituent crude drug, and Hachimi-jio-gan, Chorei-to, Gorei-san, Ryo-kei-jutsu-kan-to and Boi-bukuryo-to, which contain Hoelen[31] as a common constituent crude drug, showed a significant protective effect, suggesting that these constituent crude drugs might play a protective role against c-DDP-induced toxicity. We are now investigating the role of these constituent crude drugs regarding the protective action against c-DDP-induced toxicity.[39]

In conclusion, the present examination indicates that Juzen-taiho-to, Hochu-ekki-to, Toki-shakuyaku-san and Hachimi-jio-gan could significantly protect against c-DDP-induced nephrotoxicity and bone marrow toxicity without reducing its antitumor activity. Futher studies to clarify the multiple mechanisms on the protective effect against c-DDP-induced nephrotoxicity and bone marrow toxicity by these Kampo medicines are being conducted in our laboratory.[40]

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和文抄録

腎疾患あるいは貧血，食欲不振，疲労感を呈する患者の治療に用いる15種の漢方薬のシスプラチンの誘発する腎毒性および骨髄毒性に対する軽減効果を，ddYマウスを用いる実験系において，BUN，クレアチニン，白血球数，血小板数および体重を指標に検討した。臨床10倍量の十全大補湯 (1728 mg/kg)，補中益気湯 (1426 mg/kg)，当帰芍薬散 (805 mg/kg) あるいは八味地黄丸 (1057 mg/kg) は，経口投与で，シスプラチン (3 mg/kg, 9回，*i.p.*) により誘発されるBUMおよびクレアチニンの上昇，白血球数，血小板数および体重の減少を，シスプラチンのSarcoma 180に対する抗腫瘍効果を減弱させることなく顕著に軽減した。一方，利水薬である猪苓湯，五苓散，苓桂朮甘湯，防已茯苓湯あるいはフロセミドは，シスプラチンの毒性に加え，抗腫瘍効果も減弱させた。また，ラジカルスカベンジャーであるα-トコフェロールは，BUNおよびクレアチニンの上昇は軽減したが，白血球数および体重の減少は軽減しなかった。さらに，抗炎症作用を有することが知られている小柴胡湯は，腎毒性をほとんど軽減しなかった。これらの結果は，十全大補湯，補中益気湯，当帰芍薬散あるいは八味地黄丸をシスプラチンと併用することにより，抗腫瘍効果を減弱させることなくシスプラチンの腎毒性および骨髄毒性が軽減され得ること及びこれら漢方薬の軽減効果が，複数の作用機序によって発現している可能性を示唆している。

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