Effects of Juzen-taiho-to in reducing the side effects of cisplatin

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Abstract

The effects of oral treatment with Kampo formulations on the toxicity caused by *i.p.* administration of 3.0 mg/kg/day of cisplatin (CDDP) 9 times (on days 3,4,5,6,7,8,10,11 and 12) were examined in ddY mice which inoculated *s.c.* with sarcoma 180 cells on day 1. Co-treatment with 1.7 g/kg/day (10 fold the usual daily dose) of a water extract of Juzen taiho to (Shi-Quan Da-Bu Tang, 十全大補湯) once a day 12 times (on days 3,4,5,6,7,8,10,11,12,13,14 and 15) prevented the nephrotoxicity, bone marrow toxicity, hepatic toxicity and gastrointestinal toxicity of CDDP without reducing the antitumor activity of CDDP.

Juzen-taiho-to consists of 10 herbs. Effects of these herbs on the CDDP-induced toxicity were examined. Angelicae radix (Dang Gui, Toki) showed the strongest protective effect on the toxicity among the ingredients without reducing the antitumor activity of CDDP. Bioassay directed fractionation of a water extract of Angelicae radix resulted in isolation of a constituent having protective effects on the nephrotoxicity : sodium L-malate was found to exhibit protective effects on the nephrotoxicity (ED_{50} ; 0.17 mg/kg/day, *p.o.*), without reducing the antitumor activity of CDDP. The content of sodium malate was determined by HPLC : 2.33 % in Angelicae radix and 0.98 % in Juzen taiho-to. From these contents and their activity, sodium malate seems to be an important constituent in the protective action of Angelicae radix and Juzen taiho to.

The pharmacokinetics of platinum (Pt) have been studied in the combined administration of CDDP and sodium malate. The administration of CDDP in combination with sodium malate selectively reduced renal accumulation of Pt and prolonged the half-life of non-protein-bound Pt in plasma. A derivative diamminoplatinum(II) malate (DPM), which seems to be formed in the body following the administration of CDDP and sodium malate, had an antitumor effect and marrow toxicity comparable to those of CDDP but had no nephrotoxicity. This derivative was also detected in blood, using HPLC. These results suggest that sodium malate, an ingredient of Juzen-taiho-to, binds to a portion of the administered CDDP in the body, to yield DPM with less nephrotoxicity. Sodium malate seems to selectively reduce the renal toxicity of CDDP in this way.

Key words Juzen taiho to, cisplatin, nephrotoxicity, sodium malate, carboplatin, Anglicae radix, diamminoplatinum (II) malate.

Introduction

Cisplatin (CDDP, Fig. 1) is a platinum (Pt) complex discovered incidentally by Rosenberg *et al.* in 1965.[1] It has a broad spectrum antitumor effect and has often been used to treat various solid type cancers, such as testicular, ovarian, bladder and uterine cancers.[2,3] This drug has a serious disadvantage, however, in that it is associated with strong side effects such as renal toxicity, vomiting and nausea.[4-6] These side effects limit continuous treatment with this agent. In an attempt to reduce these side effects, large amounts of intravenous fluids and/or diuretics are

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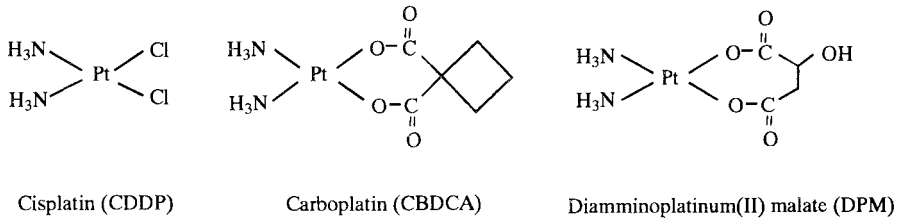

Fig. 1 Structures of antitumor platinum compounds.

often used in combination with cisplatin, but these measures have not yielded satisfactory results.[7,8]

The present study was undertaken to search for Kampo medicines which would reduce the side effects of CDDP through systematic study of the efficacy, active ingredients, mechanisms of action and roles of combinations of herbs.

I. Animal model and efficacy assessment

1. Establishment of animal model

In clinical application of CDDP, patients were given from 10 to 50 mg/m^2/day of CDDP twice or more times.[9,10] Acute toxicity of this drug can be reduced considerably by using large amounts of intravenous fluids and/or diuretics. However, its subacute toxicity[11] has not been reduced with satisfactorily and has become a serious problem. Thus, we attempted to establish a mouse model for the study of the subacute toxicity of CDDP.

Fig. 2 shows the model we established. The ddY mice with implanted Sarcoma 180 (S-180) were daily given a dose of CDDP (3 mg/kg/day) for 9 consecutive days. Biochemical and histopathological studies of these animals revealed symptoms resembling those clinically known as toxic effects of CDDP.[12] Of markers of nephrotoxicity,[13] the blood urea nitrogen (BUN) and creatinine levels showed a 4-fold and a 2-fold increase over the normal levels, respectively, and urinary volume decreased by 42 %. Of the markers of marrow toxicity,[5] the white blood cell count (WBC), the platelet count (PLT), the relative spleen weight and the relative thymus weight decreased to 29, 27, 60 and 24 % of their normal levels, respectively. Of the markers of hepatic toxicity, sGOT and sGPT rose to approximately 3 times and 5 times the normal level, respectively. In addition, the body weight and the food consumption decreased to 65 % and 31 % of the normal level, respectively. The stomach weight (including the weight of gastric contents) increased to 4 times the normal level.[14]

2. Efficacy assessment of Kampo formulations

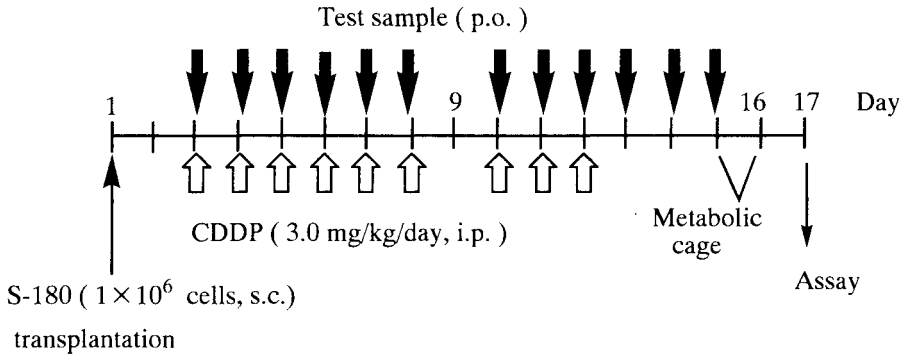

Fig. 2 Experimental design for examining the effect of Kampo medicines on CDDP induced toxicity.
Five week old, male, ddY mice (average weight, 30 g) were used (n 10). Mice were inoculated with sarcoma 180 (S-180) cells in the left thigh *s.c.* on day 1. On day 17, blood was collected from the inferior vena cava, and biochemical data were measured. CDDP ; cis diamminedichloroplatinum (II).

Table I Prescription and experimental dose of Kampo formulations.

Kampo formulation	Dase[a)]	Prescription (g)
Juzen-taiho-to (十全大補湯)	1.7	Angelicae Radix(3), Hoelen(3), Glycyrrhizae Radix(2), Ginseng Radix(3), Astragali Radix(3), Cinnamomi Cortex(3), Cnidii Rhizoma(3), Rehmanniae Radix(3), Paeoniae Radix(3), Atractylodis Rhizoma(3)
Toki-shakuyaku-san (当帰芍薬散)	1.4	Angelicae Radix(3), Hoelen(4), Atractylodis Rhizoma(4), Paeoniae Radix(4), Cnidii Rhizoma(3), Alismatis Rhizoma(4)
Hochu-ekki-to (補中益気湯)	1.4	Angelicae Radix(3), Glycyrrhizae Radix(2), Ginseng Radix(4), Astragali Radix(4), Zingiberis Rhizoma(1), Zizyphi Fructus(2), Bupleuri Radix(1), Atractylodis Rhizoma(4), Aurantii Nobilis Pericarpium(2), Cimicifugae Rhizoma(1)
Hachimi jio gan (八味地黄丸)	1.1	Hoelen(3), Cinnamomi Cortex(1), Rehmanniae Radix(5), Dioscoreae Rhizoma(3), Alismatis Rhizoma(3), Moutan Cortex(3), Aconiti Tuber(1), Corni Fructus(3)
Rikkunshi-to (六君子湯)	0.8	Hoelen(4), Glycyrrhizae Radix(1), Ginseng Radix(4), Atractylodis Rhizoma(4), Zingiberis Rhizoma(1), Aurantii Nobilis Pericarpium(2), Zizyphi Fructus(2), Pinelliae Tuber(4)
Chorei-to (猪苓湯)	0.6	Hoelen(3), Polyporus(3), Alismatis Rhizoma(3), Talcum(3), Asini Gelatinum(3)
Gorei-san (五苓散)	0.5	Hoelen(5), Polyporus(5), Alismatis Rhizoma(6), Cinnamomi Cortex(3), Atractylodis Rhizoma(5)
Ryo-kei-jutsu-kan-to (苓桂朮甘湯)	0.3	Hoelen(6), Glycyrrhizae Radix(2), Cinnamomi Cortex(4), Atractylodis Rhizoma(3)
Boi-bukuryo-to (防己茯苓湯)	0.4	Hoelen(5), Glycyrrhizae Radix(2), Cinnamomi Cortex(3), Astragali Radix(5), Sinomeni Caulis et Rhizoma(5)
Boi-ogi-to (防己黄耆湯)	1.0	Glycyrrhizae Radix(2), Astragali Radix(5), Atractylodis Rhizoma(3), Zizyphi Fructus(3), Zingiberis Rhizoma(3), Sinomeni Caulis et Rhizoma(5)
Shimbu-to (真武湯)	0.4	Hoelen(5), Atractylodis Rhizoma(3), Paeoniae Radix(3), Zingiberis Rhizoma(3), Aconiti Tuber(1)

Each experimental dose for animal was calculated on the basis of each yield of the water extract of Kampo formulations. a) Ten times the usual clinical dose (g/kg/day)

Because serious toxic effects of CDDP occur in the kidneys, marrow and gastrointestinal organs, we selected those Kampo formulation[15] known to be effective on the urinary system or to have tonifying effects and assessed the effects of these formulations (Table I).

Fig. 3 shows the results of oral treatment with each formulation at 10 times the usual daily dose. Each formulation was prepared by extraction in hot water and subsequent freeze-drying. The BUN, a marker of nephrotoxicity, showed an approximately 4-fold increase after treatment with CDDP alone, compared to the control level. This indicates the onset of severe renal disorder. When CDDP was administered in combination with Juzen-taiho-to (Shi-Quan-Da-Bu Tang, 十全大補湯), Toki-shakuyaku-san (Dang-Gui-Shao-Yao-San, 当帰芍薬散), Hochu-ekki-to (Bu-Zhong-Yi-Qi-Tang, 補中益気湯), Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan, 八味地黄丸), Chorei-to (Zhu-Ling-Tang, 猪苓湯), Gorei-san (Wu-Ling-San, 五苓散), Ryo-kei-jutsu-kan-to (Ling-Gui-Shu-Gan-Tang, 苓桂朮甘湯) or Boi-bukuryo-to (Fang-Yi-Fu-Ling-Tang, 防己茯苓湯), the increase in BUN over the control level was prevented almost completely. The effect of these Kampo formulations in reducing the nephrotoxicity of CDDP was comparable to that of furosemids, a frequently used diuretic.[7,8]

The antitumor effect of CDDP was slightly increased by the combined use of tonifying Kampo formulations (Juzen-taiho-to, Toki-shakuyaku-san, Hochu-ekki-to), while it was reduced by the combined use of Kampo formulations acting on the urinary system (Chorei-to, Gorei-san, Ryo-kei-jutsu-kan-to, Boi-bukuryo-to). Furosemide reduced the antitumor effect of CDDP more markedly. These results suggest that the mechanism of reducing the

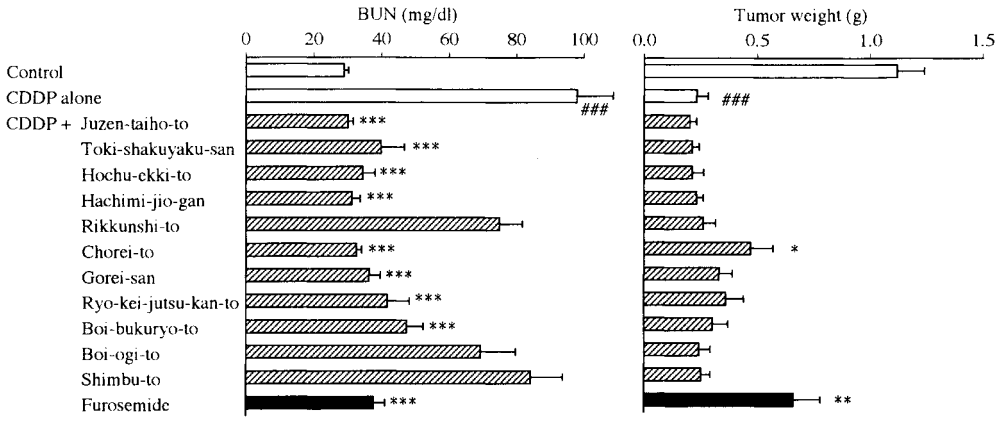

Fig. 3 Effects of Kampo formulations on toxicity and antitumor effect of CDDP.
Experimental protocol is shown in Fig. 2. Kampo formulations (10 times the usual daily dose /day) or furosemide (20 mg/kg/day) was administered *p.o.* to mice once a day 30 min before CDDP *i.p.* injection. Each value is the mean ± S.E.(n=10). Significant difference from the control group, ### : $p < 0.001$. Signifficant difference from the CDDP alone group, * : $p < 0.05$, ** : $p < 0.01$, *** : $p < 0.001$.

toxicity of CDDP differs between tonifying Kampo formulation and Kampo formulation acting on the urinary system. The latter type of Kampo formulations seem to reduce the toxicity of CDDP by promoting the urinary elimination of CDDP through its diuretic action,[16] similar to the mechanism of the action of furosemide.[16-18] Tonifying Kampo formulations seem to reduce the toxicity of CDDP by some other mechanisms.

II. Effects of Juzen-taiho to in reducing the toxicity of CDDP

Fig. 4 shows the dose response curve of Juzen-taiho to. Oral treatment with Juzen-taiho to (freeze-dried extracts) at a dose level of 1.7 g/kg/day (10

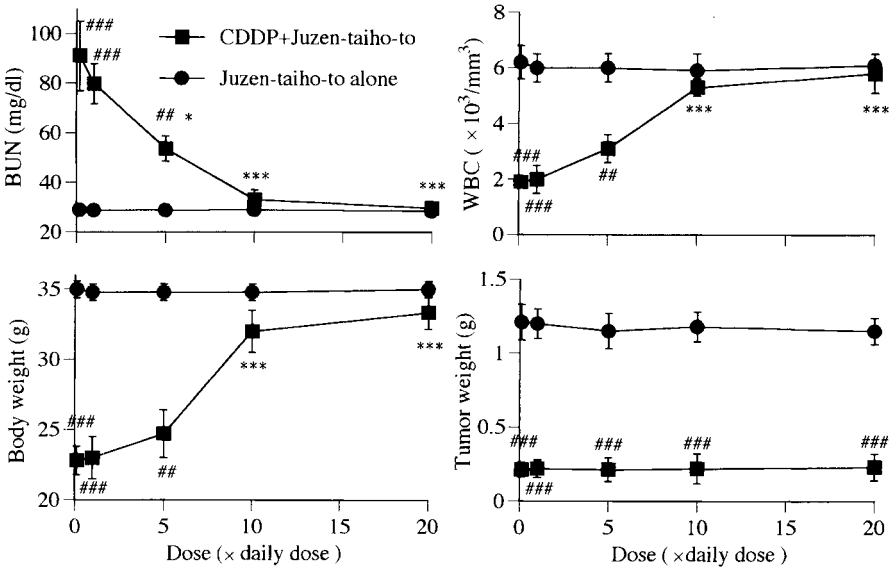

Fig. 4 Relationship berween the dose of Juzen taiho to and its effect in reducing the toxicity of CDDP.
Juzen taiho to (0.17, 0.85, 1.7 and 3.4 g/kg/day) was administered *p.o.* to mice once a day 12 times. Each result is the mean ± S.E. (n=10). Significant difference from the Juzen taiho to alone group, ## : $p < 0.01$, ### : $p < 0.001$. Significant difference from the CDDP alone group, * : $p < 0.05$, *** : $p < 0.001$.

Table II Pathological changes in kidneys (HE Staining).

Pathological changes	Groups:	Normal				CDDP				CDDP+JTT				JTT			
	(Animal No):	(n=9)				(n=8)				(n=9)				(n 9)			
	Grade:	−	±	+	++	−	±	+	++	−	±	+	++	−	±	+	++
Cortex																	
	tubular degeneration/necrosis:	2	7	0	0	0	1	4	3	7	2	0	0	9	0	0	0
	tubular dilation/cast formation:	3	5	1	0	0	0	5	3	6	0	3	0	8	1	0	0
	changes of glomerulus:	9	0	0	0	8	0	0	0	9	0	0	0	9	0	0	0
Medulla																	
	degenertation of Henle's loop:	9	0	0	0	7	0	1	0	9	0	0	0	9	0	0	0
	tubular dilation/cast formation:	7	2	0	0	4	2	1	1	8	1	0	0	9	0	0	0
Interstitium																	
	edema:	9	0	0	0	6	0	2	0	9	0	0	0	9	0	0	0
	cell increase:	9	0	0	0	8	0	0	0	3	0	6	0	4	2	3	0

Experimental protocol is shown in Fig. 2. Mice were sacrificed on day 17. Kidneys were removed, weighed, and processed for light microscopy, by routine histology.
−: negative or within the borderline of normal variation, ±: slightly positive, +: positive, ++: strongly positive.

times the usual daily dose) resulted in approximately normal BUN (a marker of nephrotoxicity), WBC (a marker of marrow toxicity) and body weight. The other markers of toxicity were also improved markedly by treatment with Juzen-taiho-to at a daily dose more than 10 times the usual dose. The antitumor effect of CDDP was not affected by this Kampo formulation at any dose.

Table II shows histopathological changes of the kidneys. Treatment with CDDP alone resulted in moderate to severe degeneration and necrosis of the renal cortex and tubules, accompanied by ureteral dilation and cast formation in a relative narrow area. These changes are identical to those reported clinically.[11,19] When CDDP was combined with Juzen-taiho-to, these changes were prevented almost completely.

Fig. 5 shows the effects of Juzen-taiho-to in reducing the toxicity of CDDP administered in higher doses. When mice were treated daily with 3.0 mg/kg of CDDP, their death was first noted on the 19 th day, and the survival rate on the 25 th day was 60 %. When the same dose of CDDP was combined with Juzen-taiho-to at a daily dose level 20 times the usual dose, all mice were alive on the 25 th day. When the dose of CDDP was increased to 4.5 mg/kg/day, while keeping the Juzen-taiho-to dose unchanged, all mice were alive on the 25 th day. When the CDDP dose level was further increased to 6.0 mg/kg/day, 50 % of mice were alive on the 25 th day. At a CDDP dose level over

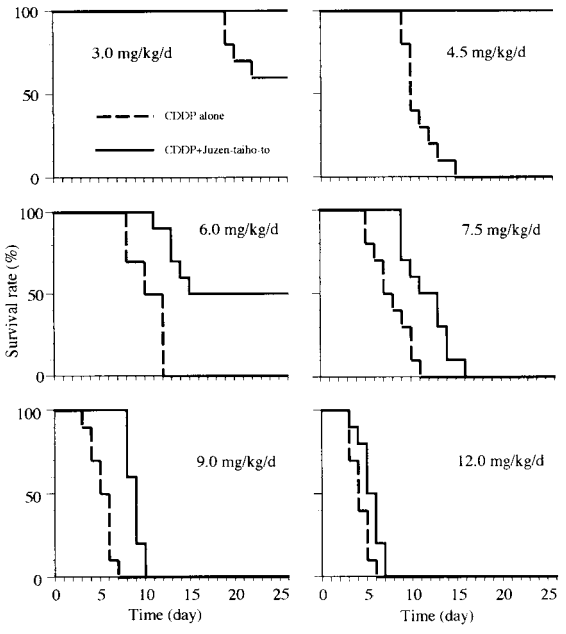

Fig. 5 Effect of Juzen taiho to on lethal toxicity of CDDP. Juzen taiho to (3.4 g/kg/day) was administered *p.o.* to mice inoculated with S 180 cells 30 min before CDDP (3.0 12.0 mg/kg/day, *i.p.*) injection once a day during the experimental period. All mice which received CDDP alone at doses of 4.5, 6.0, 7.5, 9.0 and 12.0 died within 15,12,11, 7 and 6 days after the initial CDDP injection, respectively. All mice that received Juzen-taiho-to and 3.0 and 4.5 mg /kg/day of CDDP were alive 25 days after the initial CDDP injection.

7.5 mg/kg/day, the combined use of Juzen-taiho-to did not prolong the survival period of animals any more. The daily dose 3.0 mg/kg is close to the maximum clinical dose used for continuous CDDP therapy. Juzen-taiho-to was found to be capable of reducing the toxicity of CDDP even when the CDDP dose is twice the maximum clinical dose.

Fig. 6 shows the effects of Juzen-taiho-to on different days after the inoculation of S-180 cells. Nephrotoxicity of CDDP appeared on the 8 th day. Marrow toxicity began to be noted immediately after the start of treatment. Weight loss began on the 8 th day. When CDDP was combined with Juzen-taiho-to, no change in BUN, WBC or body weight was seen at any point of assessment during the treatment period, suggesting that Juzen-taiho-to prevents the toxic

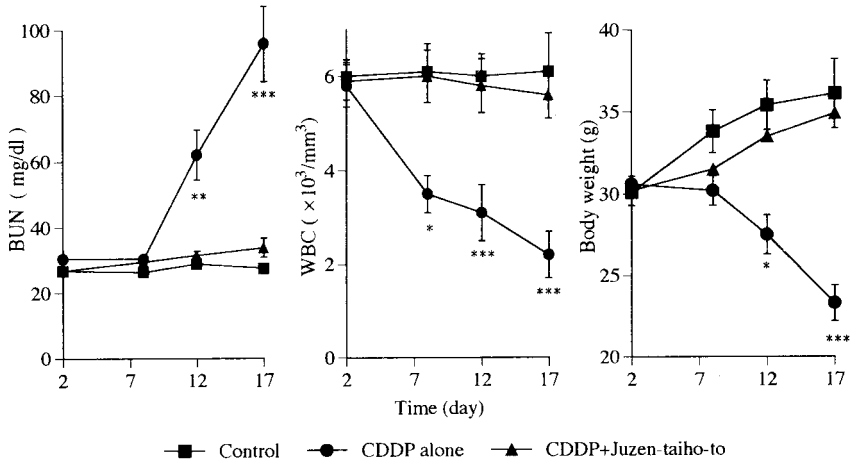

Fig. 6 Time course of effect of Juzen-taiho-to on CDDP-induced toxicity.
Juzen-taiho-to (1.7 g/kg/day, *p.o.*) and CDDP (3.0 mg/kg/day, *i.p.*) were administered to mice with S-180 cells once a day. Each value is the mean±S.E. (n=10). Significant difference from the control group, * : $p<0.05$, ** : $p<0.01$, *** : $p<0.001$.

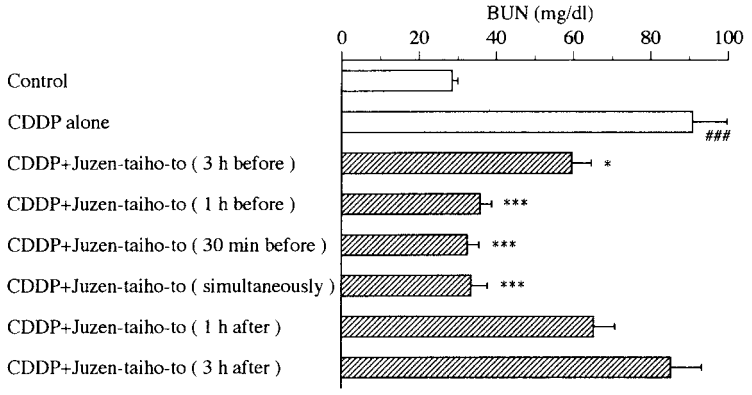

Fig. 7 Relationship between the timing of Juzen-taiho-to treatment and its effect in reducing the toxicity of CDDP.
Juzen-taiho-to (1.7 g/kg/day, *p.o.*) was administered to mice with S-180 cells after (1 h or 3 h) or simultaneously with or prior to (3 h, 1 h or 30 min) CDDP treatment. Each value is the mean±S.E. (n=10).
Significant difference from the CDDP alone group, * : $p<0.05$, *** : $p<0.001$. Significant difference form the control group, ### : $p<0.001$.

effects of CDDP.

Fig. 7 shows the relationship between the timing of Juzen-taiho-to treatment and its effect in reducing the toxicity of CDDP. When this Kampo formulation was administered simultaneously with or prior to CDDP treatment, the toxicity of CDDP was reduced. However, when the Kampo formulation was administered after CDDP treatment, the toxicity almost remained unchanged. The antitumor effect of CDDP was not affected by Juzen-taiho-to irrespective of the timing of its administration (data not shown).

Fig. 8 shows the effects of Juzen-taiho-to in reducing the myelosuppression of carboplatin (Fig. 1), a derivative of CDDP with reduced nephrotoxicity. Marrow toxicity, which limits the dose levels of carboplatin,[20,24] was reduced by the combined use of Juzen-taiho-to at a daily dose 10 times the usual dose, while the antitumor effect of carboplatin was not affected by this Kampo formulation. The other toxic effects of carboplatin were also reduced markedly by Juzen-taiho-to.[25]

We have undertaken a comparative study of the effect of Juzen-taiho-to and some other drugs on the CDDP toxicity. Furosemide (a diuretic) and sodium thiosulfate (a neutralizing agent)[26] reduced both the toxicity and antitumor effect of CDDP. Metalloth-

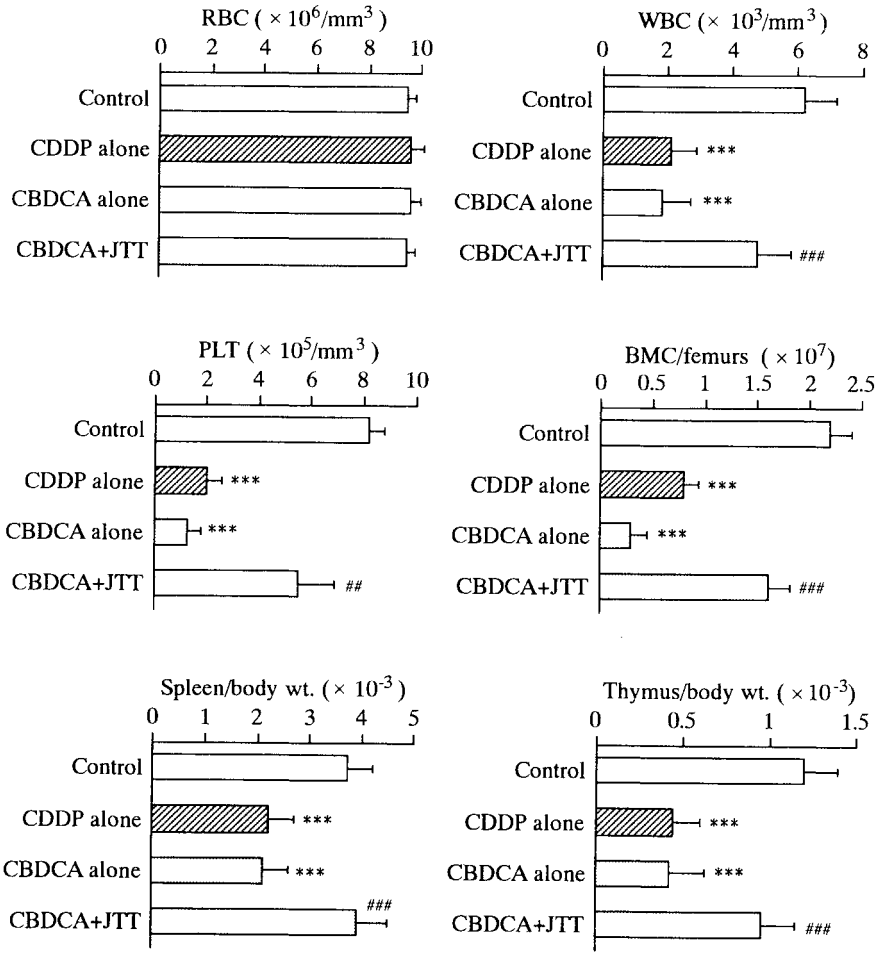

Fig. 8 Effect of Juzen-taiho-to (JTT) on CBDCA-induced myelosuppression.
JTT (1.7 g/kg/day × 12) was administered *p.o.* to mice inoculated with S-180 cells 30 min before CBDCA (15 mg/kg/day) *i.p.* injection 9 times. All parameters were determined on day 17. Each value is the mean ± S.E. (n-10). Significant difference from the control group, *** : $p<0.001$. Significant difference from the CBDCA alone group, ## : $p<0.01$, ### : $p<0.001$. BMC : bone marrow cells.

ionein inducers zinc chloride[27] and bismuth subnitrate,[28] an antioxidant vitamin E[29] and lysosome membrane stabilizers fosfomycin[30] and urinastain[31] reduced the toxicity of CDDP less markedly than Juzen-taiho-to (data not shown).

Fig. 9 shows the effects of Juzen-taiho-to on P388 cells. The average survival period for the untreated control (8 days) was not prolonged by treatment with Juzen-taiho-to alone (at a daily dose 10 times the usual dose), while it was prolonged up to 22 days by treatment with CDDP alone. Treatment with a combination of CDDP and Juzen-taiho-to further prolonged the survival period to 33 days. Since the survival period was not prolonged by Juzen-taiho-to alone, the prolonged survival period following the combined use of CDDP and Juzen-taiho-to seems to be attributable to the reduction of the side effects of CDDP by the use of Juzen-taiho-to.

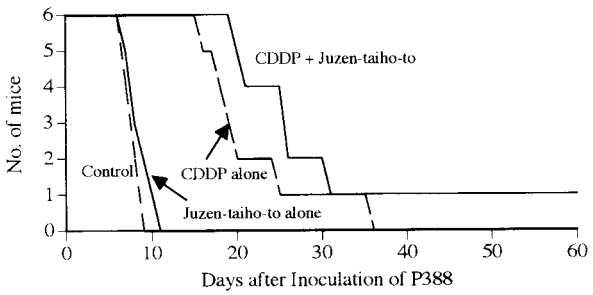

Fig. 9 Effect of Juzen-taiho-to on lethal toxicity of CDDP in BDF_1 mice inoculated with P388 cells.
P388 cells (10^6 cells/mouse) were inoculated *s.c.* on day 1. Juzen-taiho-to (1.7 g/kg/day, *p.o.*) and/or CDDP (3.0 mg/kg/day, *i.p.*) was administered to BDF_1 mice once a day (n 6).

These results suggest that Juzen-taiho-to prevents the toxic effects of CDDP, and that this Kampo formulation is expected to exert the most favorable effects when it is administered at a daily dose more than 10 times the usual dose simultaneously with or prior to CDDP treatment.

III. Analysis of active ingredients

Juzen-taiho-to is composed of 10 herbs. Fig. 10 shows the effects of each of these herbs (freeze dried extracts) in reducing the nephrotoxicity of CDDP. Angelicae radix (Toki), Ginseng (Ninjin) and Hoelen (Bukuryo) reduced the nephrotoxicity, and the effect was comparable to that of Juzen-taiho-to when these herbs were administered in amounts equal to those

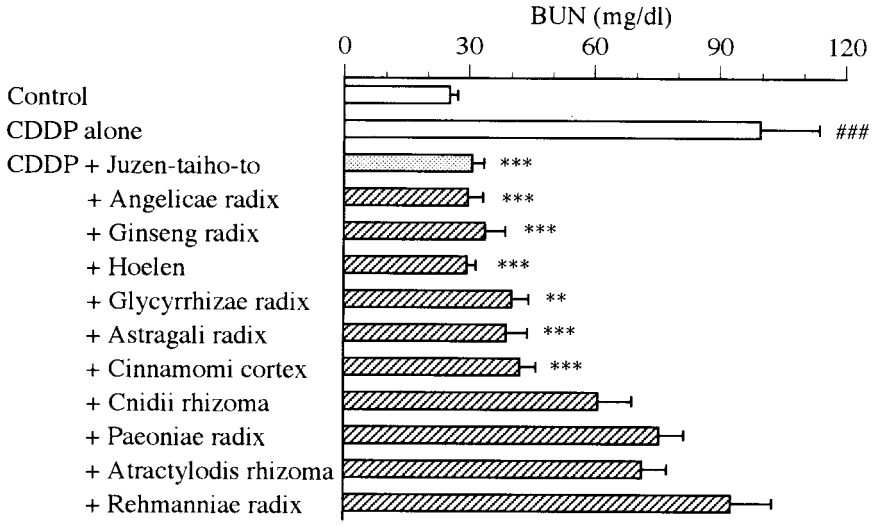

Fig. 10 Effects of ingredients of Juzen-taiho-to on CDDP induced nephrotoxicity.
All samples tested were administered *p.o.* to mice at a dose of 10 times the usual daily dose 30 min before CDDP (3 mg/kg/day, *i.p.*) injection. The control group was treated with water (*p.o.*) and saline (*i.p.*). S-180 cells were inoculated *s.c.* on day 1 and nephrotoxicity was determined on day 17. Each value is the mean±S.E. (n 10). Significant difference from the control group, ### : $p<0.001$. Significant difference from the CDDP alone group, ** : $p<0.01$, *** : $p<0.001$.
Experimental dose : Juzen-taiho-to ; 1.7 g/kg/d, Angelicae radix ; 165 mg/kg/d, Ginseng radix ; 167 mg/kg/d, Rehmanniae radix : 242 mg/kg/d, Astragali radix ; 128 mg/kg/d, Glycyrrhizae radix ; 87 mg/kg/d, Atractylodis rhizoma ; 175 mg/kg/d, Cnidii rhizoma ; 123 mg/kg/d, Paeoniae radix ; 93 mg/kg/d, Cinnamomi cortex ; 28 mg/kg/d, and Hoelen ; 7 mg/kg/d.

contained in the Juzen-taiho-to. We made a general assessment of the effects of individual herbs in reducing marrow, hepatic and gastrointestinal toxicity and of their antitumor effects. This assessment revealed that Toki plays a more significant role than any other herb of Juzen-taiho-to in reducing the toxicity of CDDP (data not shown).

Fig. 11 shows the procedure to isolate an active constituent from Toki. Using various methods of isolation, we obtained pure sodium malate (5.2 mg, Fig. 12) from 2 kg of Toki, with a recovery of 0.0003 %.[32]

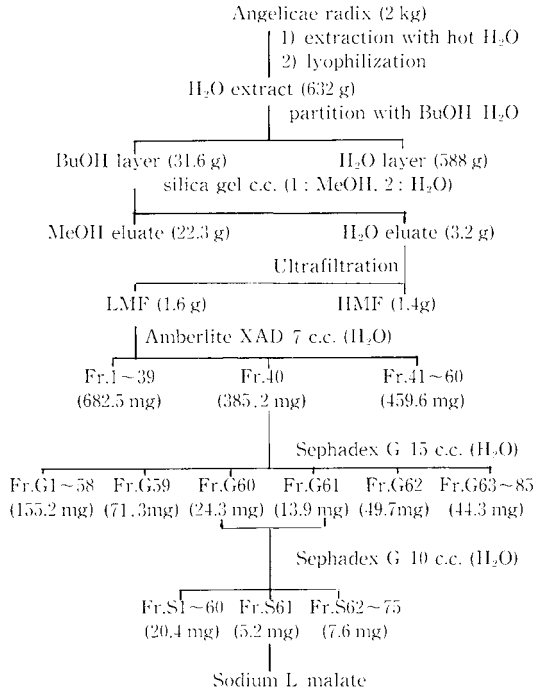

Fig. 11 Isolation procedure for sodium L-malate from Angelicae Radix. Isolation was performed, guided by BUN measurements in mice. The yield of each fraction obtained from 2 kg of Angelicae Radix is designated in parentheses. LMF and HMF indicate low-molecular weight fraction (MW < 10,000) and high-molecular-weight fraction (MW > 10,000), respectively.

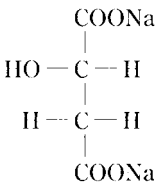

Fig. 12 Structure of sodium L-malate.

When sodium malate (*p.o.*) and CDDP (*i.p.*) with a molar ratio over 0.5 : 1 was used, the renal, hepatic and gastrointestinal toxicity of CDDP were suppressed almost completely, without affecting the antitumor effect of CDDP. The effect of sodium malete in reducing the renal, hepatic and gastrointestinal toxicity was comparable to that of Juzen-taiho-to, while its effect in reducing the marrow toxicity of CDDP was weaker (Fig. 13). These results combined with the results of quantitative analysis of sodium malate contained in Juzen-taiho-to (0.98%), as determined using high performance liquid chromatography (HPLC) indicate that sodium malate plays an important role for Juzen-taiho-to to reduce nephrotoxicity of CDDP.

Ikehara and Yamada *et al.*[33] found that the unsaturated fatty acids, contained in Juzen-taiho-to,

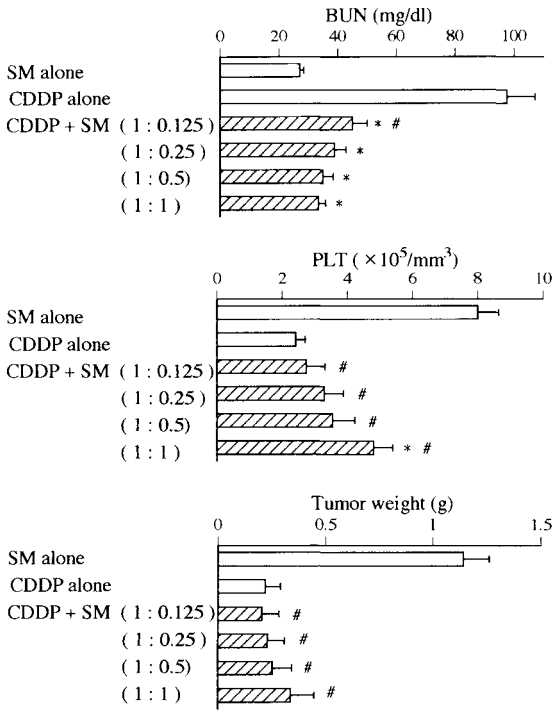

Fig. 13 Effect of sodium malate on CDDP induced toxicity and antitumor effect.
Sodium malate (SM, *p.o.*) and CDDP (*i.p.*) with a molar ratio over 0.125 1 : 1 were administered to mice with inoculated S-180 cells. Each value is the mean+S.E. (n 10). Significant difference from the SM alone group, # : $p < 0.05$. Significant difference from the CDDP alone group, * : $p < 0.05$.

stimulate the proliferation of marrow stem cells. We also demonstrated that a macromolecule (polysaccharide with molecular weight over 5000), contained in Juzen-taiho-to, promoted the proliferation of marrow stem cells (CFU-meg and CFU-GM), and that this substance originates from Atractylodis rhizoma (Byakujutsu) or Atractylodis lanceae rhizoma (Sojutsu). Therefore, the effect of Juzen-taiho-to in reducing the marrow toxicity of CDDP involves these substances rather than sodium malate.

IV. Mechanism of suppression of nephrotoxicity by Juzen-taiho-to

It is thought that the toxic actions of CDDP on cells undergoing rapid mitosis (*i.e.*, the antitumor effects, marrow toxicity, *etc.* of CDDP) are induced by the binding of CDDP to DNA through cross-linking to the N-7 positions of the adjacent guanines.[31-39] The toxic actions of CDDP on the cells of the kidney and some other organs, however, cannot be fully explained by its binding to DNA and involve many unresolved questions. CDDP in blood is eliminated via the glomerulus and the proximal tubule. At the same time, CDDP can be reabsorbed into blood from the proximal tubule.[40] Enzymes in the proximal tubule, exposed to high concetrations of CDDP, lose their activity through binding to CDDP. Inactivation of ATPase,[41] glutathione and its related enzymatic systems,[42,43] cytochrome P-450,[44] and Na^+-dependent transport systems,[45] *etc.* can lead to the necrosis of cells, leading to severe renal damage.[46]

Some derivatives of CDDP with reduced nephrotoxicity have been used clinically, including carboplatin. These derivatives have a lower potential to bind to protein, compared to CDDP.[20,47-49] Taking note of the finding that sodium malate, an active ingredient of Juzen-taiho-to, is a dicarboxylic acid which resembles the ligand of carboplatin, we assumed that sodium malate binds to CDDP *in vivo* to yield a less toxic derivative of CDDP. To test the validity of this assumption, we conducted two experiments. First, we analyzed the pharmacokinetics of Pt following the combined administration of CDDP and sodium malate. This experiment revealed that the administration of CDDP in combination with sodium malate relectively reduced renal accumulation of Pt (Fig. 14) and prolonged the half-life of non-protein-bound Pt (Fig. 15). These kinetics of Pt were similar to those of carboplatin (data not shown). We subsequently synthesized a derivative of CDDP, diammineplatinum (II) malate (DPM, Fig. 1),[50,51] which seems to be formed in the body following the administration of CDDP and sodium malate, and assessed its effects. DPM had an antitumor effect and a marrow toxicity comparable to those of CDDP but had no nephrotox-

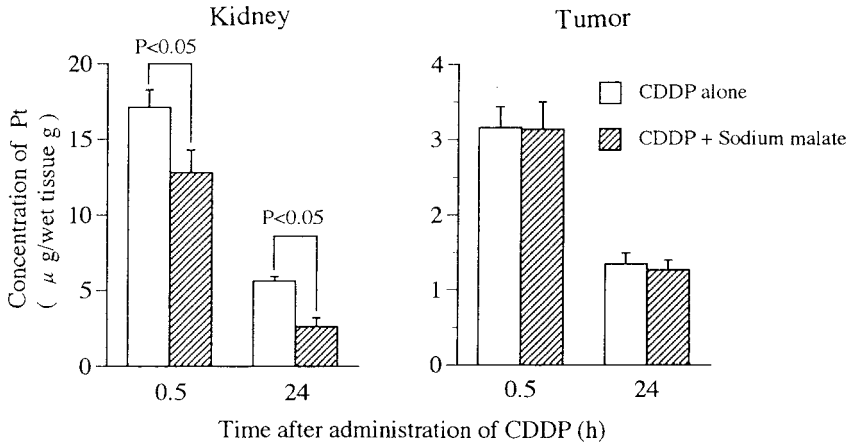

Fig. 14 Accumulation of Pt in kidneys and tumor 30 min or 24 h after administration of CDDP. S-180 cells (10^6 cells/mouse) were inoculated *s.c.* to ddY mice on day 1. On day 14, sodium malate (14.8 mg/kg) was administered to the mice 30 min prior to CDDP (12.5 mg/kg) treatment. The concentration of Pt was detected by use of an inductively coupled plasma spectrometer (ICP)(Seiko SPS 1200A). Each value is the mean±S.E. (n 10).

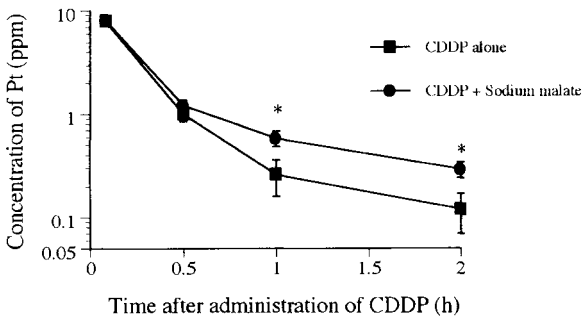

Fig. 15 Filterable Pt levels in plasma of mice after treatment with CDDP and/or sodium malate.
S-180 cells (10^6 cells/mouse) were inoculated *s.c.* to ddY mice on day 1. On day 14, sodium malate (14.8 mg/kg) was administered to the mice 30 min prior to CDDP (12.5 mg/kg) treatment. Five, 30, 60 and 120 min after, blood was collected, and plasma was ultrafiltered through Centricon 10 (Amicon Co., Ltd.). Pt was measured by a SPS 1200A. Significant difference from the CDDP alone group, * : $p<0.05$.

icity (Fig. 16). This derivative was also detected in blood, using HPLC.

These results suggest that sodium malate, an ingredient of Juzen-taiho-to, binds to a portion of the administered CDDP in the body, to yield DPM with less nephrotoxicity. Sodium malate seems to selectively reduce the renal toxicity of CDDP in this way.

V. Role played by herbs constituting Juzen-taiho to

Following the isolation of sodium malate, an active ingredient of Juzen-taiho-to involved in reducing the renal toxicity of CDDP, we examined the role played by various herbs contained in this Kampo formulation, using sodium malate as an index. The usual daily dose of Juzen-taiho-to (freeze-dried extracts) contained 79.5 mg (0.98 %) of sodium malate.

Table III Contents of sodium malate in Juzen-taiho to and its ingredients.

	Clinical dose (Herbs) (g)	Content of Sodium malate (mg)	(%)
Juzen taiho to	29	79.5	0.98
Angelicae radix	3	23.1	2.33
Ginseng radix	3	14.8	1.34
Rehmanniae radix	3	10.7	0.74
Astragali radix	3	9.2	1.20
Glycyrrhizae radix	2	6.0	0.77
Atractylodis rhizoma	3	6.0	0.81
Cnidii rhizoma	3	4.8	0.46
Paeoniae radix	3	4.5	0.81
Cinnamomi cortex	3	1.1	0.67
Hoelen	3	0.3	0.79

Contents of sodium malate in Kampo medicines were determined by HPLC : column ; TSK ODS 80 Ts (4.6×250 mm), column temp. ; 40°C, eluent ; 0.05M KH_2PO_4/0.05M H_3PO_4 (1 : 1), flow rate ; 0.5 ml/min, detection ; UV 210 nm, injection ; 10 μl (50 mg/ml). Retention time of sodium malate was 8.2 min.

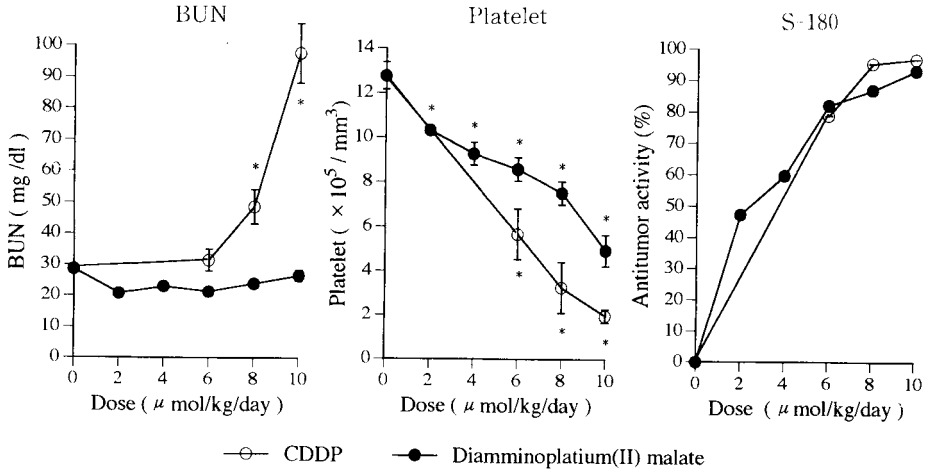

Fig. 16 Toxicity and antitumor effect of diamminoplatinum (II) malate. S 180 cells (10^6 cells/mouse) were inoculated *s.c.* in the left thigh on day 1. Diamminoplatinum (II) malate (2,4,6,8 and 10 μmol/kg/day) and CDDP (6,8 and 10 μmol/kg/day) were administered *i.p.* on days 3,4,5,6,7, 8,10,11 and 12. BUN, platelet count and tumor weight were measured on day 17. Each vale is the mean±S.E. (n=10). Significant difference from the control group, * : $p<0.05$.

Of all herbs (freeze-dried extracts) which are contained in Juzen-taiho-to, Toki was found to be more active than any other herb and it contained larger amounts of sodium malate (23.1 mg, 2.33 %) as compared to the other herbs. The amounts of sodium malate contained in the other herbs were also considerably high (Table III).

Fig. 17 shows the roles played by individual herbs in the expression of the effects of Juzen taiho-to, as assessed on the basis of the amount of sodium malate contained and the degree of reduction in nephrotoxicity. The actual measurement of the effect of Juzen-taiho-to in reducing renal toxicity was about 1/4 of its theoretical magnitude and did not correlate with the amount of sodium malate contained (79.5 mg). On the other hand, the actual magnitude of this effect of Toki was approximately equal to its theoretical magnitude and correlated well with the amount of sodium malate contained (23.1 mg). Rehmanniae radix (Jukujio) reduced renal toxicity only very slightly although it contained large amounts of sodium malate (10.7 mg). When the activity of Juzen-taiho-to was assessed after Jukujio was removed from this formulation, the actual activity agreed with the theoretical activity. The other herbs showed a good correlation between activity and the amount of sodium malate contained, resembling the relationship seen for Toki. These results indicate that sodium malate is the most important ingredient involved in the activity of Juzen-taiho-to, and that Jukujio, another ingredient of this

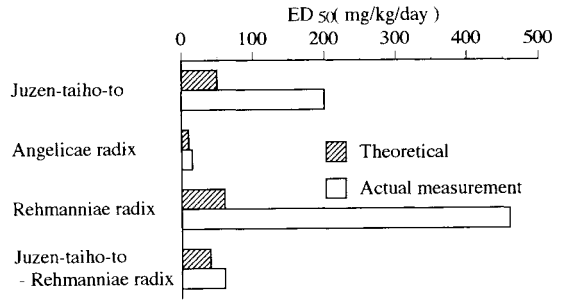

Fig. 17 Comparison of the experimental ED_{50} with calculated ED_{50} of Kampo formulation on CDDP induced nephrotoxicity.
Experimental protocol is shown in Fig. 2. Theoretical ED_{50} was calculated on the basis of the amount of sodium malate contained and the degree of reduction in nephrotoxicity (BUN).

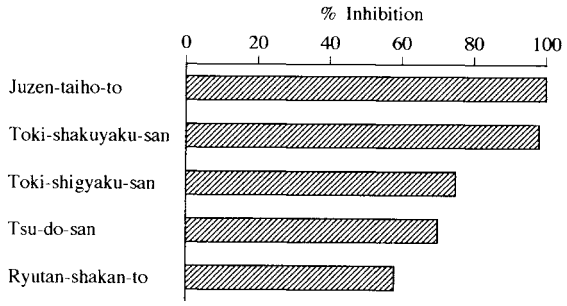

Fig. 18 Effects of Kampo formulations contained a similar amount of Angelicae radix (Toki) on CDDP induced nephrotoxicity.
Experimental protocol is shown in Fig. 2. Kampo formulations were administered *p.o.* to mice at a dose of 10 times the usual daily dose. BUN was measured on day 17.

Kampo formulation, markedly suppresses the action of sodium malate.

Each Kampo formulation is composed of various herbs and its effects are derived from the mixture of these herbs. So far as the effect of Juzen-taiho-to in reducing the renal toxicity of CDDP is concerned, Toki can be regarded as playing a major role and Jukujio controls the action of Toki. The addition of the actions of the other herbs to the actions of these two leads to the manifestation of the overall effects of Juzen taiho-to. These elaborate interactions among defferent herbs are a characteristic of Kampo formulations. Fig. 18 shows the data yielded by our recent study, in which the effect in reducing nephrotoxicity was compared among 5 different Kampo formulations, each of which contained a similar amount of Toki. The effect differed among different formulations ; that is, nephrotoxicity was reduced less by formulations indicated for "excess syndrome". This suggests that the toxicity of CDDP pertains to "deficient syndrome", and provides a key to clarifying why the same herb is used in different Kampo formulations with defferent indications.

Conclusion

In Kampo medicine, Qi (気), blood and water are understood to be essential components of the body, indispensable for life. Individuals are healthy when

these three components perform their functions well. Illness causes dysfunction of these components. Kampo medicine treats diseases by causing these 3 components to resume their functions and eliminating the cause of diseases. This is called the "Fu Zheng-Qu Xie (扶正祛邪)" rule.

Qu Xie (祛邪) means elimination of pathogenic factors. This concept is close to the concept of treatment used in Western medicine. Fu Zheng (扶正) means resumption of the proper functioning of Qi, blood and water, and is the most important concept in Kampo medicine. Western medicine has also concepts corresponding to Fu Zheng, but these concepts are not so clearly defined and are not supported by the actual methods used. The author believe that if the concept of Fu Zheng is incorporated into Western medicine, we may be able to make up for the weak points that exist in the present forms of medicine and achieve satisfactory therapeutic effects.

We had studied the Kampo formulations used for the purpose of Fu Zheng (*i.e.*, tonifying Kampo formulations) to provide scientific evidences to the concept Fu Zheng and to translate the concept into scientific terms. To these ends, we have conducted systematic studies, including the extablishment of animal models, evaluations of efficacy, analyses of active ingredients, clarification of the mechanisms of action, and analyses of the roles played by constituent herbs.[52,53] The present study of Juzen-taiho-to was conducted within this framework. The present study revealed that the effect of Juzen-taiho-to in reducing the toxicity of CDDP is not attributable to any single ingredient or herb but is due to composite actions of nephrotoxicity-reducing substances (carboxylic acids such as sodium malate), substances controlling the actions of these substances, and substances promoting hemopoiesis (polysaccharides). Present study suggests the possibility of new applications of Kampo formulations and provided scientific evidences for the efficacy of Kampo formulations.

Acknowledgments

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和文抄録

第1日目 (day 1) に，予め Sarcoma-180 を皮下移植した ddY マウスに，シスプラチン (CDDP) 3 mg/kg/day を9回腹腔内投与 (days 3,4,5,6,7,8,10,11 及び 12) して毒性を誘発させる実験系で，種々の漢方薬の毒性軽減効果を経口投与で評価した。その結果，十全大補湯水抽出エキス 1.7 g/kg/day (臨床 10 日量) の併用 (days 3,4,5,6,7,8,10,11,12,13,14 及び 15) により，CDDP の抗腫瘍効果を減弱させることなく，腎毒性，骨髄毒性，肝毒性及び消化器毒性を軽減できることが明らかになった。

十全大補湯は 10 種の生薬から構成されている。次に，これら配合生薬の毒性軽減効果を検討した。その結果，配合生薬の中で，当帰に最も良好な軽減効果が認められた。そこで，生物検定法を指標に，当帰水抽出エキス中の有効成分を検索した。その結果，リンゴ酸ナトリウム (L 体) が腎毒性軽減物質として単離された。本物質は，CDDP の抗腫瘍効果には影響を及ぼさず腎毒性を軽減し，その ED_{50} は経口投与で 0.17 mg/kg/day であった。さらに，HPLC でリンゴ酸ナトリウムを定量した結果，当帰水抽出エキス中には 2.33 %，十全大補湯水抽出エキス中には 0.98 % 含まれることが明らかになった。このリンゴ酸ナトリウムの含有量と当帰及び十全大補湯の毒性軽減効果より，リンゴ酸ナトリウムはこれら漢方薬の作用発現において重要な役割を担っていることが示唆された。

さらに，リンゴ酸ナトリウム併用時の白金 (Pt) の体内動態を検討した。その結果，リンゴ酸ナトリウム併用により，腎臓内 Pt の蓄積量が減少し，血漿中の蛋白非結合型 Pt の半減期が延長することが明らかとなった。次に，体内において，リンゴ酸ナトリウムと CDDP との反応によって，生成が予想される成績体 diammino-platinum (II) malate (DPM) の作用を検討した。その結果 DPM は，腎毒性はほとんど有さず，抗腫瘍効果と骨髄毒性は CDDP とほぼ同等の化合物であることが明らかになった。また，DPM が血中に存在することが，HPLC を用いて明らかにされた。これらの結果より，リ

ンゴ酸ナトリウムは体内でCDDPの一部と反応し，腎毒性の低い成績体DPMを生成し，CDDPの腎毒性を軽減しているものと考えられた。

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