Synergistic effects of Kampo medicines and cis-dichlorodiammineplatinum (II) on Meth-A fibrosarcoma in BALB/c mice

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(Received March 9, 1991. Accepted July 10, 1991.)

Abstract

The effects of combined treatment of Kampo medicines and cis-dichlorodiammineplatinum (II) (CDDP) were investigated using a mouse model with transplanted Meth-A fibrosarcoma. Eight Kampo medicines, which are used in treatment of kidney diseases or are known to have immunomodulatory activities, were tested by oral administration in a range of 0.25 to 1.0 g/kg/day. The combined use of CDDP (0.5 and 1.5 mg/kg) with Sairei-to or Inchin-gorei-san resulted in the significant augmentation of the antitumor activity compared with CDDP alone. In the case of 3.0 mg/kg CDDP, Sairei-to restored the toxic effects of CDDP in terms of blood nitrogen urea and survival time in addition to the enhancing effect on the antitumor activity of CDDP. These results suggest that Sairei-to is useful in the treatment of cancer patients in combination with CDDP.

Key words Kampo, Chinese traditional drugs, CDDP, Meth-A, antitumor, kidney toxicity, BUN.

Abbreviations CDDP, cis-dichlorodiammineplatinum ; BUN, blood urea nitrogen.

Introduction

Cis-Dichlorodiammineplatinum (II) (CDDP) has strong antitumor activity and has been used in the treatment of various human tumors since its spectrum of activity is broad. Many clinical studies, however, have shown that CDDP has severe side effects, and the major dose-limiting factor in man is the nephrotoxic effect of CDDP.[1-5] In order to ameliorate the renal toxicity, various trials have been performed, typically administering diuretics with CDDP.[6-8]

In the present paper, the effects of the simultaneous use of Kampo medicines with CDDP on Meth-A fibrosarcoma were investigated in BALB/c mice. We have tested 8 Kampo medicines which are known to be used in therapy against kidney diseases and to have immunomodulatory effects. The Kampo medicines used are as follows : Gorei-san, Moku-boi-to, Gosya-jinki-gan, Sairei-to, Inchin-gorei-san, Sho-saiko-to, Hochu-ekki-to, Juzen-taiho-to.

Materials and Methods

Animals : Specific pathogen-free female BALB/c mice (6 weeks old when used) were purchased from Charles River Japan, Inc. (Atsugi, Japan). The mice were kept in plastic cages and allowed at least 1 week for acclimation before the beginning of the experiment. Seven mice per group were used in all experiments.

Tumor : Meth-A cells, originated from the fibrosarcoma induced with methylcholanthrene in BALB/c mice, were maintained by intraperitoneal injection using BALB/c mice. Viable cells were counted in a hemocytometer with trypan blue.

Reagents : CDDP was obtained from Nippon Kayaku Co., Ltd. (Tokyo, Japan). All Kampo

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Journal of Medical and Pharmaceutical Society for WAKAN-YAKU 8, 89－95, 1991

medicines, consisting of spray-dried hot water extracts of a mixture of certain medicinal plants, were obtained from the Ibaraki Plant of our company (Tsumura & Co., Tokyo). CDDP and Kampo medicines were dissolved in physiological saline and distilled water, respectively.

Assay for antitumor activity : Meth-A tumor cells (1×10^6) were transplanted into the right abdomen of BALB/c mice. Kampo medicines were administered orally for 7 consecutive days from the following day after the transplantation of Meth-A tumor and CDDP was injected intraperitoneally for 6 consecutive days from day 2. Antitumor activity was assessed by weighing tumors excised on day 14 after the transplantation. In the case of a high dose of CDDP (3 mg/kg), the survival time of tumor-bearing mice was determined as a parameter of antitumor activity.

Measurement of blood urea nitrogen (BUN) and creatinine : BUN and creatinine were assayed with an automatic analyzer (Toshiba TBA-380) using assay kits of urea nitrogen-TA test Wako and creatinine-TA test Wako (Wako Pure Chemical Industries, Tokyo), respectively.

Statistical analysis : Comparisons of tumor weight and survival time between experimental groups were performed using Student's t-test and χ^2-test, respectively.

Results

Combined effect of each Kampo medicine and CDDP

Table I presents the combined effect of each Kampo medicine (0.5 g/kg) and CDDP (0.5 mg/kg) on the growth of the Meth-A tumor. When Sairei-to or Inchin-gorei-san was administered in combination with CDDP, the inhibition rate of tumor growth was 30 % higher than that by CDDP alone (the difference was significant). Each Kampo medicine alone had no obvious antitumor activity on Meth-A fibrosarcoma (data not shown).

Administration doses of Sairei-to and Inchin-gorei-san

The varying doses (0.25, 0.5 and 1.0 g/kg) of Sairei-to and Inchin-gorei-san were tested in combination with 0.5 mg / kg CDDP (Table 2). CDDP alone showed the inhibition of tumor growth by 23.3 %. The combined use of Sairei-to or Inchin-gorei-san with CDDP at all doses tested produced an increase in antitumor effect by approximately 20 to 30 % compared with CDDP alone, and the increase was particularly significant at doses of 0.5 and 1.0 g/kg. Likewise, a similar effect was obtained when the dose of CDDP was increased to 1.5 mg/kg (Table 3). In

Table I Effect of combined treatment of CDDP and Kampo medicines on transplanted Meth-A fibrosarcoma in BALB/c mice.

Kampo medicines (0.5 g/kg)	CDDP (0.5 mg/kg)	Tumor weight (g)	Inhibition rate (%)
		Mean±S.D.	Versus CDDP alone
None	−	2.04±0.61	
None	+	1.34±0.33	−
Sho-saiko-to	+	1.24±0.35	7.5
Gorei-san	+	1.38±0.32	-3.0
Moku-boi-to	+	1.06±0.29	20.9
Hochu-ekki-to	+	1.22±0.34	9.0
Juzen-taiho-to	+	1.09±0.26	18.7
Gosha-jinki-gan	+	1.17±0.28	12.7
Sairei-to	+	0.91±0.10*	32.1
Inchin-gorei-san	+	0.93±0.33*	30.6

Meth-A tumor cells (1×10^6) were transplanted into the right abdomen of BALB/c mice. Tumor weight was determined by weighing tumors excised on day 14 after the transplantation. Values are represented as the mean±S.D. of 7 determinations. *Significantly different from CDDP alone with $p<0.05$.

Table II Effect of combined treatment of CDDP (0.5 mg/kg) and Sairei-to or Inchin-gorei-san on transplanted Meth-A fibrosarcoma in BALB/c mice.

Kampo medicines (g/kg)	CDDP (0.5 mg/kg)	Tumor weight (g) Mean ± S.D.	Inhibition rate (%) Versus CDDP(−)	Inhibition rate (%) Versus CDDP(+)	Increase of body weight (g)
None	−	1.60±0.28	−		0.43
None	+	1.18±0.17	26.3	−	0.68
Sairei-to (0.25)	+	0.95±0.27	40.6	19.5	0.81
Sairei-to (0.5)	+	0.91±0.20*	43.1	22.9	1.31
Sairei-to (1.0)	+	0.96±0.06*	40.0	18.6	0.59
Inchin-gorei-san (0.25)	+	0.92±0.35	42.5	22.0	0.77
Inchin-gorei-san (0.5)	+	0.85±0.30*	46.9	28.0	1.37
Inchin-gorei-san (1.0)	+	0.81±0.27*	49.4	31.4	0.98

Experiment was performed as described in Table I. The increase of body weight on day 14 after the transplantation of tumor cells was represented as the difference from that on day 0.
* Significantly different from CDDP alone with $p<0.05$.

Table III Effect of combined treatment of CDDP (1.5 mg/kg) and Sairei-to or Inchin-gorei-san on transplanted Meth-A fibrosarcoma in BALB/c mice.

Kampo medicines (g/kg)	CDDP (1.5 mg/kg)	Tumor weight (g) Meam ± S.D.	Inhibition rate (%) Versus CDDP (−)	Inhibition rate (%) Versus CDDP (+)	Increase of body weight (g)
None	−	1.60±0.28	−		0.43
None	+	0.97±0.19	39.4	−	-0.40
Sairei-to (0.25)	+	0.75±0.12*	53.1	22.7	0.41
Sairei-to (0.5)	+	0.69±0.17*	56.9	28.9	0.66
Sairei-to (1.0)	+	0.68±0.27	57.5	29.9	0.33
Inchin-gorei-san (0.25)	+	0.76±0.19	52.5	21.6	0.58
Inchin-gorei-san (0.5)	+	0.68±0.11**	57.5	29.9	0.65
Inchin-gorei-san (1.0)	+	0.78±0.10	51.3	19.6	0.06

Experiment was performed as described in Table I.
*, ** Significantly different from CDDP alone with $p<0.05$ and $p<0.01$, respectively.

Table IV Effect of combined treatment of CDDP (3.0 mg/kg) and Sairei-to or Inchin-gorei-san on survival time of tumor-bearing mice.

Kampo medicines (g/kg)	CDDP (3 mg/kg)	>35 days Survivors/Total	Survival Days Mean±S.E.	Range (day)	ILS (%) Versus CDDP alone
None	−	0/7	28.9±1.2	25-35	
None	+	0/7	21.0±4.5	8-35	−
Sairei-to (0.25)	+	3/7	28.6±5.2	10-46	36
Sairei-to (0.5)	+	4/7	29.1±5.9	10-46	39
Sairei-to (1.0)	+	5/7*	37.1±3.3*	19-47	77
Inchin-gorei-san (0.25)	+	1/7	20.0±4.4	10-40	-5
Inchin-garei-san (0.5)	+	1/7	21.4±5.1	11-42	2
Inchin-gorei-san (1.0)	+	4/7	30.9±3.7	13-39	47

Meth-A tumor cells (1×10^6) were transplanted into the abdomen of BALB/c mice and the survival time of tumor-bearing mice was observed.
* Significantly different from CDDP (−) and CDDP (+) groups with $p<0.05$.

Table V Effect of combined treatment of CDDP (1.5 mg/kg) and Sairei-to on transplanted Meth-A fibrosarcoma in BALB/c mice.

Treatment with Sairei-to (0.5 g/kg)	CDDP (1.5 mg/kg)	Tumor weight (g) Mean±S.D.	Inhibition rate (%) Versus CDDP alone	Increase of body weight (g)
None	−	1.03±0.32		1.73
None	+	0.85±0.14	−	0.38
Pre-Sairei-to [a]	+	0.56±0.17**	34.1	0.79
Post-Sairei-to[b]	+	0.59±0.19*	30.6	1.38
Pre- and Post-Sairei-to[c]	+	0.50±0.19***	41.2	1.10

[a] Pre-Sairei-to : Sairei-to was administered *p.o.* for days -7～-1.
[b] Post-Sairei-to : Sairei-to was administered *p.o.* for days 1～7,
[c] Pre- and Post-Sairei-to : Sairei-to was administered *p.o.* for days -1～-7 and 1～7.
CDDP : CDDP was administered *i.p.* for days 1～7.
*, **, *** Significantly different from CDDP alone with $p<0.05$, $p<0.01$ or $p<0.005$, respectively.

addition, administration of Sairei-to or Inchin-gorei-san restored the weight loss induced by 1.5 mg/kg CDDP.

Effect of combined use on survival time

Table 4 presents the effect of the combined use of Sairei-to or Inchin-gorei-san and CDDP (3 mg/kg) on the survival time of tumor-bearing mice. The mean survival time of tumor-bearing mice without any treatment was 28.9±1.2 days. Administration of CDDP alone at 3 mg/kg resulted in the earlier death of tumor-bearing mice and the reduction of the mean survival time to 21.0± 4.5 days, and all mice tested died by day 35 after the transplantation of tumor cells. When Sairei-to (all doses tested) or Inchin-gorei-san (1.0 g/kg) was administered in combination with CDDP, the survival time was prolonged by 36 to 77 % compared with CDDP, alone. In particular, in the case of 1.0 g/kg Sairei-to, the prolongation of the mean survival time (37.1±3.3 days) was significantly different from that in mice without any treatment or treated with CDDP alone, and 5 of the 7 mice survived for more than 35 days.

Administration regimens of Sairei-to

The combined effect of Sairei-to (0.5 g/kg) and CDDP (1.5 mg/kg) regarding antitumor activity was investigated by changing the administration regimens of Sairei-to (Table 5). Sairei-to was administered for 7 days before, after, or before and after the transplantation of tumor cells. Regardless of the administration regimen for Sairei-to, the tumor growth was significantly inhibited at a similar rate (34 to 41 %).

Effect of Sairei-to on BUN and creatinine

As shown in Fig. 1, CDDP induced an increase in BUN in mice, and the maximum value of BUN was seen on day 12 (5 days after the final injection of 1.5 mg/kg CDDP) after the transplantation of tumor cells. Fig. 2 presents the effect of Sairei-to on BUN. Administration of Sairei-to at all doses tested significantly reduced the BUN. With respect to creatinine, no change was observed following the injection of CDDP.

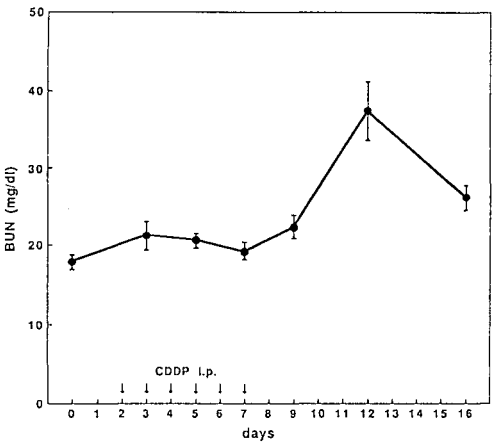

Fig. 1 Time course of plasma BUN in mice treated with CDDP. Mice were transplanted with Meth-A tumor cells (1×10^6) at day 0 and 1.5 mg/kg CDDP was injected intraperitoneally for 6 days from day 2. The BUN in plasma was measured at the days indicated.

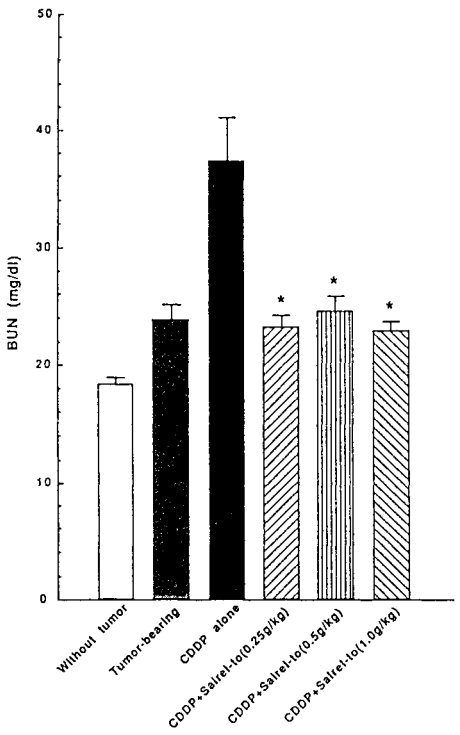

Fig. 2 Effect of Sairei-to on a rise in BUN caused by CDDP. Experiment was performed as described in Fig. 1. The BUN in plasma was measured on day 12 after the transplantation of tumor cells (5 days after the final injection of CDDP). *Significantly different from CDDP alone with $p<0.05$.

Discussion

Recently, there have been many reports that various Kampo medicines have beneficial effects in enhancing the activity of anticancer agents and reducing the side effects of anticancer agents or radiation.[9-13] This work was performed to determine the combined effect of Kampo medicines and one of the most commonly used anticancer agents, CDDP, in tumor-bearing mice.

Kampo medicine alone did not show any antitumor activity. However, Sairei-to and Inchin-gorei-san, among the Kampo medicines tested, significantly augmented the antitumor activity when each of them was used in combination with CDDP. These Kampo medicines also prevented the weight loss of tumor-bearing mice treated with CDDP. In the case of 3.0 mg/kg of CDDP, the survival time of tumor-bearing mice was also prolonged by the combined use of Sairei-to and CDDP. In this experiment, the survival time of tumor-bearing mice treated with CDDP (3.0 mg/kg) alone was shorter than that of the nontreatment group, suggesting that the animals died due to the toxicity of CDDP rather than due to the tumor. Therefore, Sairei-to seems to protect the mice from the toxic effects of CDDP. This is also supported by the effect of Sairei-to on BUN in which Sairei-to clearly inhibited a rise in BUN caused by CDDP. This may indicate the possibility that Sairei-to enables us to use higher clinical doses of CDDP in the therapy of cancer patients. The reducing effect of Sairei-to on CDDP-induced toxicity may be one mechanism for enhancing the apparent antitumor activity of CDDP. On the other hand, Sairei-to also revealed the stimulatory effect on the antitumor activity of CDDP of 0.5 mg/kg, at which little toxic effect was observed. Therefore, Sairei-to may contribute to the enhanced antitumor activity through some other mechanism. According to our unpublished data, Sairei-to showed various effects on the production of interleukins. We are now investigating the effects of Sairei-to on the immune system in combination with CDDP.

CDDP is known to induce an increase in BUN, which is one parameter of kidney damage, as well as in creatinine. Sugihara *et al.*[14,15] have reported that the kidney damage by CDDP correlated with the production of active oxygen. Therefore, Sairei-to may reduce the CDDP-induced kidney toxicity by its scavenger effect since Sairei-to has been reported to inhibit the production of active oxygen.[16] Alternatively, Sairei-to may reduce the uptake of CDDP by renal tubular cells by inducing an increase in the tubular flow rate and a decrease in the renal concentration of the drug since Sairei-to is originally known as a diuretic.[17,18] In general, diuretics. however, are known to reduce the antitumor effect of CDDP as well as the toxicity. In addition, Sairei-to contains saponin; therefore, the saponin may be

associated with anti-inflammatory effect such as that exerted by steroids.[19, 20] These possibilities are now under investigation.

On the other hand, no increase in plasma concentration of creatinine was observed after administration of CDDP in this study. This may be because increase in plasma concentration of creatinine does not occur until the damage of kidney becomes very severe since the excretion of creatinine continuously occurs. In this study, 1.5 mg/kg CDDP may not be high enough to cause the increased plasma concentration of creatinine in mice.

In conclusion, our results suggest that Kampo medicines, especially Sairei-to, enhance the antitumor activity of CDDP and decrease the toxicity of kidney. Therefore, Sairei-to may allow us to administer much higher doses of CDDP in clinical therapy.

和文抄録

BALB/c マウスに Meth-A 腫瘍を移植する実験系を用いて，漢方エキス末とシスプラチンの併用効果を検討した。シスプラチン (0.5 あるいは 1.5 mg/kg, 腹腔内投与) に加えて，腎疾患の治療に用いられる 5 処方あるいは免疫系に作用を示す 3 処方を経口投与 (0.25～1.5 g/kg/day) した結果，柴苓湯あるいは茵蔯五苓散を併用した場合に腫瘍増殖抑制の有意な増強が認められた。シスプラチンの高投与量 (3 mg/kg) の場合においても，柴苓湯を併用した時に抗腫瘍効果増強作用が得られた。またこの時，柴苓湯併用により，担癌動物の延命が見られ，更に腎障害の指標の一つである血中尿素窒素値の上昇が有意に抑制された。以上，シスプラチンと共に柴苓湯を併用することにより，抗腫瘍作用の増強並びに腎障害の軽減効果がみられ，これらの結果から，柴苓湯が臨床において癌患者の治療に有用であることが示唆される。

References

1) Ogawa, M. and Inagaki, J.: Cisplatin. *Farumashia* **16**, 407-409, 1980.
2) Blachley, J.D. and Hill, J.B.: Renal and electrolyte disturbances associated with cisplatin. *Ann. Intern. Med.* **95**, 628-632, 1981.
3) Goldstein, S. and Mayor, G.H.: The nephrotoxicity of cisplatin. *Life. Sci.* **32**, 685-690, 1983.
4) Weiner, M.W. and Jacobs, C.: Mechanism of cisplatin nephrotoxicity. *Fed. Proc.* **42**, 2974-2978, 1983.
5) Litterst, C.L.: Cisplatinum: A review, with special reference to cellular and molecular interactions. *Agents Actions* **15**, 520-524, 1984.
6) Umeki, S., Watanabe, M., Yagi, S. and Soejima, R.: Supplemental fosfomycin and /or steroids that reduce cisplatin-induced nephrotoxicity. *Am. J. Med. Sci.* **295**, 6-10, 1988.
7) Salgo, L. and Szabo, A.: γ-Glutamyl transpeptidase activity in human urine. *Clin. Chemica. Acta* **126**, 9-16, 1982.
8) Hayes, M.D., Cvitkovic, E., Golby, B.R., Scheiner, E., Helson, L. and Krakoff, H.I.: High dose cis-platinum diammine dichloride. Amelioration of renal toxicity by mannitol diuresis. *Cancer* **39**, 1372-1381. 1977.
9) Nabeya, K. and Ri, S.: Effects of oriental herbs on the restoration of the human body before and after operation. *Proc. Symp. WAKAN-YAKU* **16**, 201-206, 1983.
10) Adachi, I.: Juzen-Taiho-To as a supporting therapy in advanced breast cancer. *BIOTHERAPY* **3**, 782-788, 1989.
11) Kurokawa, T., Imai, J. and Tamakuma, S.: Clinical and immunological examination of Juzen-Taiho-To (TJ-48) for cancer. *BIOTHERAPY* **3**, 789-795, 1989.
12) Kawamura, H., Maruyama, H., Takemoto, N., Komatsu, Y., Aburada, M., Ikehara, S. and Hosoya, E.: Accelerating effect of Japanese kampo medicines on recovery of murine haematopoietic stem cells after administration of mitomycin C. *Int. J. Immunotherapy* **5**, 35-42, 1989.
13) Ohnishi, Y., Yasumizu, R., Fan, H., Liu, J., Takao-Liu, F., Komatsu, Y., Hosoya, E., Good, R.A. and Ikehara, S.: Effects of Juzen-taiho-to (TJ-48), a traditional oriental medicine, on hematopoietic recovery from radiation injury in mice. *Exp. Hematol.* **18**, 18-22, 1990.
14) Sugihara, K. and Gemba, M.: Modification of cisplatin toxicity by antioxidants. *Japan. J. Pharmcol.* **40**, 353-355, 1986.
15) Sugihara, K., Nakano, S. and Gemba, M.: Effect of cisplatin on in vitro production of lipid peroxidase in rat kidney cortex. *Japan. J. Pharmacol.* **44**, 71-76, 1987.
16) Hattori, T., Ito, M. and Suzuki, Y.: Studies on antinephritic effects of Japanese kampo medicine in rats (4). Effects of Sairei-to and the production of reactive oxygen species scavengers in puromycin aminonucleoside nephrosis in rats. *J. Med. Pharm. Soc. WAKAN-YAKU* **7**, 12-17, 1990.
17) Haranaka, R., Watanabe, S., Kohahi, R., Hidaide, K., Makiyama, I., Okada, M., Takahashi, G. and Kobayashi, M.: The effect of the Chinese herb diuretics (Goreisan, Choreito, Saireito) in growing rats: Part I. *Proc. Symp. WAKAN-YAKU* **14**, 105-110, 1981.
18) Watanabe, S., Haranaka, R., Kohashi, R., Ueno, K., Maruta, J., Okada, M. and Kobayashi, M.: The effect

of the Chinese herb diuretics (Goreisan, Choreito, Saireito) in growing rats : Part II. *Proc. Symp. WAKAN-YAKU* **14**, 111-116, 1981.

19) Tanizawa, H., Numano, H., Odani, T., Takino, Y., Hayashi, T. and Arichi, S.: Study of the saponin of Panax ginseng C.A. Meyer. I. Inhibitory effect on adrenal atrophy, thymus atrophy and the decrease of serum K concentration induced by cortisone acetate unilateral adrenalectomized rats. *YAKUGAKU-ZASSHI* **101**, 169-173, 1981.

20) Ogihara, Y.: On the possibility of reducing side effects of steroids by Kanpo-Hozai simultaneously. *Biomedicine & Therapeutics* **10** (supple), 118-128, 1983.