Protective Effect of Juzen-taiho-to against Carboplatin-Induced Toxic Side Effects in Mice

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The effect of an oral treatment with the Kampo formulation Juzen-taiho-to on the toxicity caused by the intraperitoneal administration of 15 mg/kg carboplatin (CB Dana) 9 times (on days 3, 4, 5, 6, 7, 8, 10, 11 and 12) was examined in ddY mice, which were subcutaneously inoculated with sarcoma 180 (S-180) cells on day 1. White blood cell counts, platelet counts, bone marrow cell counts, relative spleen and thymus weight, food intake and body weight decreased significantly, to about 29%, 13%, 14%, 59%, 36%, 42% and 72% of the control levels, respectively, and serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase and relative stomach weight increased significantly, to about 4, 6 and 3 times the control levels, respectively, by the treatment with CB Dana. However, the blood urea nitrogen and serum creatinine were only slightly increased compared to the control value. Co-treatment with 1.7 g/kg of a lyophilized water extract of Juzen-taiho-to once a day 12 times (on days 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14 and 15) prevented both the decreases and increases caused by CB Dana to near the control levels without reducing the antitumor activity of CB Dana against S-180.

The inhibitory effect of Juzen-taiho-to against CB Dana-induced myelosuppression was similar to that against 3.0 mg/kg cisplatin (CDDP) 9 times, while CB Dana-induced myelosuppression was more serious in comparison with CDDP. Therefore, these findings indicate that Juzen-taiho-to could be an effective drug for protecting against the side effects induced by CB Dana in the clinic as well as by CDDP.

Key words carboplatin; cisplatin; Juzen-taiho-to; myelosuppression; nephrotoxicity; platelet

cis-Diamminedichloroplatinum(II) (CDDP) is an important anticancer agent with a broad spectrum of antitumor activity. However, the clinical use of CDDP is limited by its severe nephrotoxicity. Many CDDP derivatives have been synthesized to improve the therapeutic value of CDDP. Among these derivatives, cis-diammine(1,1-cyclobutaneedicarboxylato)platinum(II) (carboplatin; CB Dana), shown in Fig. 1, has been introduced into clinical practice, because CB Dana causes little or no nephrotoxicity in comparison with CDDP and a similar spectrum of antitumor activity to that of CDDP. However, myelosuppression, especially thrombocytopenia, is regularly seen as the dose-limiting toxicity instead of the nephrotoxicity.

While the exact molecular mechanisms in the toxicity induced by CDDP and CB Dana remain unknown, the major ultimate CB Dana-DNA adduct is thought to be chemically similar to the CDDP-DNA adduct, which is important not only to their antitumor activity but also to their side effects. This suggests that a detoxifying agent against CDDP may possess a preventive effect against the toxicity induced by CB Dana.

We previously demonstrated that the Kampo formulation, Shi-Quan-Da-Bu-Tang (Juzen-taiho-to), prevented CDDP-induced nephrotoxicity and myelosuppression without reducing the antitumor activity of CDDP. We have therefore undertaken a comparative study of the effect of Juzen-taiho-to on CB Dana-induced toxicity.

MATERIALS AND METHODS

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

Chemicals CDDP and CB Dana were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The solutions of CDDP and CB Dana were prepared with sterile 0.9% saline at concentrations of 0.5 mg/ml and 2.5 mg/ml, respectively.

Preparation of Juzen-taiho-to The ingredients of the formulation of Juzen-taiho-to were purchased from Yamamoto Yakuin Kogyo Co., Ltd., Tokyo. A usual daily dose of a water extract of Juzen-taiho-to was prepared as follows: Angelicae Radix (3 g), Hoelen (3 g), Glycyrrhizae Radix (2 g), Ginseng Radix (3 g), Astragali Radix (3 g), Cinnamomi Cortex (3 g), Atractylodis Rhizoma (3 g), Paeoniae Radix (3 g), Coicis Rhizoma (3 g), and Rehmanniae Radix (3 g) were blended and then extracted with 400 ml of boiling water for 60 min. After cooling, the extract was filtered and then lyophilized. The yield of the water extract was 8.5 g (29.3%). The lyophilized extract was dissolved in water just before use.

Treatment of Animals Animals were treated by a modification of the method reported previously. Briefly,

Fig. 1. Chemical Structures of Cisplatin and Carboplatin

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the animals in the test groups, each comprised of 10 mice, were inoculated with sarcoma 180 (S-180) cells (10⁶/mouse) in the left thigh subcutaneously on day 1. CDDP (3.0 mg/kg) and CBDDA (15.0 mg/kg) were given intraperitoneally (i.p.) to the mice once a day on days 3, 4, 5, 6, 7, 8, 10, 11, and 12. The lyophilized water extract of Juzen-taiho-to was given orally (p.o.) to the mice 30 min before the CBDDA injection once a day on days 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14 and 15. The mice in the control group were treated with water (p.o.) and 0.9% saline (i.p.). On day 17, the mice were anesthetized with ethyl ether, then blood was collected from the inferior vena cava using a heparinized syringe, and the number of red blood cells (RBC), white blood cells (WBC) and platelets (PLT) was immediately counted. After centrifugation of the remaining blood, the serum was analyzed for blood urea nitrogen (BUN), serum creatinine, serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT). The liver, kidneys, spleen, thymus, stomach (organ and contents) and tumor were resected and weighed. The femora were excised, flushed of bone marrow cells using a 23-gauge needle, and suspended in 0.9% saline. The cells were centrifuged, washed twice with 0.9% saline, and counted. Twenty-four-hour urine volume and 24-h intake of diet were measured on day 14.

**Measurement of Functions** RBC, WBC and PLT counts were made on a Celltac 4150 (Nihon Koden, Ltd., Tokyo). The bone marrow cells (BMC) were counted in a hemocytometer. BUN, serum creatinine, SGOT and SGPT were measured on a COBAS FARA (Baxter, Ltd., Tokyo) spectrophotometrically using assay kits for urea nitrogen-HR II, creatinine-HA test Wako, GOT-FTA test Wako and GPT-FTA test Wako (Wako Pure Chemical Industries, Ltd., Tokyo), respectively.

**Antitumor Activity** The inhibitory percent of the antitumor effect was calculated by using the following formula:

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\text{Inhibition (\%) = } (1 - B/A) \times 100
\]

where \( A \) is the mean tumor weight of the control group and \( B \) is that of the drug-treated groups.

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

**RESULTS**

**Myelosuppression** Figure 2 shows the effects of Juzen-taiho-to on CBDDA-induced myelosuppression. WBC, PLT and BMC were markedly decreased to about 29%, 13% and 17% of the control levels, respectively, by the i.p. treatment with 15.0 mg/kg CBDDA alone once a day 9 times, and these decreases were much higher than those of 3.0 mg/kg CDDP alone 9 times. These decreases were significantly prevented by 68%, 61% and 68%, respectively, as a result of the oral co-treatment with 1.7 g/kg of the lyophilized water extract of Juzen-taiho-to once a day 12 times. The inhibitory effects of Juzen-taiho-to against CBDDA-induced myelosuppression were similar to those against CDDP. CBDDA alone did not change in RBC count compared to the control value, because its half-life is longer than that of WBC and PLT.

**Nephrotoxicity** Figure 3 shows the effects of Juzen-taiho-to on 15.0 mg/kg CBDDA-induced nephrotoxicity. A zero or slight increase in BUN and serum creatinine and a decrease in 24-h urinary volume was observed in comparison with CDDP, by the treatment with CBDDA alone, while these parameters increased markedly by the treatment with CDDP alone. The slight increase in BUN was inhibited to near the control level by co-treatment with 1.7 g/kg of the lyophilized water extract of Juzen-taiho-to.

Treatment with 1.7 g/kg of the extract of Juzen-taiho-to alone did not significantly change any of the parameters measured (data not shown).

**Hepatic Toxicity** Figure 4 shows the effects of Juzen-taiho-to on 15.0 mg/kg CBDDA-induced hepatic toxicity. A greater increase in SGOT and SGPT was
observed in comparison with CDDP, by the treatment with CBDCAlone. These increases were prevented to near the control levels by co-treatment with 1.7 g/kg of the lyophilized water extract of Junzen-taiho-to. The inhibitory effects of Junzen-taiho-to against CBDCAl-induced hepatic toxicity were similar to those against CDDP.25

Other Toxicity Treatment with 15.0 mg/kg CBDCAlone 9 times decreased the body weight and food intake to 72% and 42% of the control values, respectively, and increased the relative stomach weight to about 3 times the control value. Both the decreases and the increase induced by CBDCAlone were prevented to near the control levels by co-treatment with 1.7 g/kg of the lyophilized water extract of Junzen-taiho-to 9 times (Fig. 5). The inhibitory effects of Junzen-taiho-to against these CBDCAl-induced toxicities
were similar to those against CDDP. Treatment with 1.7 g/kg of the lyophilized water extract of Juzen-taiho-to alone did not significantly change any of the parameters measured (data not shown).

**Antitumor Activity** Treatment with CBDDA alone dose-dependently inhibited the growth of S-180 cells, and the inhibitory rate reached about the highest level at a dose of 15.0 mg/kg (Fig. 6). The antitumor activity nearly reached the highest level in mice treated with 3.0 mg/kg CDDP. Co-treatment with 1.7 g/kg of the lyophilized water extract of Juzen-taiho-to did not show any significant effect on the antitumor activity of CBDDA (Table 1).

Treatment with 1.7 g/kg of the extract of Juzen-taiho-to alone also did not inhibit the growth of S-180 cells (data not shown).

**DISCUSSION**

Previously, we demonstrated that co-treatment with more than 1.7 g/kg of a lyophilized water extract of Juzen-taiho-to 12 times prevented all the toxicity induced by 3.0 mg/kg (0.01 mmol/kg) CDDP without reducing its antitumor activity against S-180 cells. Moreover, we found that oral treatment with 3.4 g/kg of a lyophilized water extract of Juzen-taiho-to alone 12 times did not exhibit any toxicity. In this study, we examined the effect of 1.7 g/kg of the lyophilized water extract of Juzen-taiho-to on 15.0 mg/kg (0.04 mmol/kg) CBDDA-induced toxicity, because the amount of CBDDA needed to obtain the same level of antitumor activity was 4 times as high as that of CDDP (Fig. 6). Treatment with 15.0 mg/kg CBDDA alone 9 times to mice caused severe myelosuppression, but only slight nephrotoxicity (Figs. 2 and 3). These observations are in basic agreement with several prior studies. The present findings demonstrate clearly the protective effect of Juzen-taiho-to against the toxicity of CBDDA, including the myelosuppression, without reducing its antitumor activity.

The mechanism(s) of the protective effect of Juzen-taiho-to against CBDDA-induced toxicity cannot be explained at present, but we presume that the synergistic and/or additive effects of various ingredients in Juzen-taiho-to account for its protective effect against CBDDA.

Kawamura et al. found that the oral administration of Juzen-taiho-to to mice treated with mitomycin C resulted in a significant increase in colony-forming units in the spleen (CFU-S) and in granulocyte-macrophage colony-forming cells. Ohnishi et al. demonstrated that the administration of Juzen-taiho-to to irradiated mice for 7 d did not reduce the loss of mature hemopoietic cells or progenitor cells, but significantly increased the number and size of day-14 CFU-S. Moreover, recent studies reported its function as a biological response modifier.

Besides its immune-system-enhancing action, Juzen-taiho-to possesses a number of pharmacological effects that may help account for the observed prevention against CBDDA-induced toxicity. These include antioxidant action, accelerated blood circulation, improved nutritre and a cholevetic effect. In addition, animal studies have shown that treatment with Juzen-taiho-to prevented toxicities induced by mitomycin C, suggesting that the detoxifying effect of Juzen-taiho-to is not specific for antitumor platinum compounds such as CBDDA and CDDP.

Juzen-taiho-to has been used traditionally for patients with anorexia, anemia and/or fatigue, and its toxicity is very low. Therefore, Juzen-taiho-to could be an effective detoxifying agent for CBDDA- and CDDP-induced toxicity. Additional studies are currently underway to elucidate the mechanism(s) for the detoxifying effects produced by Juzen-taiho-to.

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