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Antitumour Activity and Side Effects of Combined Treatment with Chitosan and Cisplatin in Sarcoma 180-Bearing Mice

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Abstract

We examined the possible modulation by chitosan of the antitumour effects and side effects of cisplatin (cis-diaminedichloroplatinum, CDDP). The study showed that CDDP had potent antitumour activity when administered orally as well as intraperitoneally. We also compared the antitumour activity and side effects of orally administered CDDP plus orally administered chitosan versus intraperitoneally administered CDDP plus orally administered chitosan in sarcoma 180-bearing mice.

When CDDP ($1{\cdot}25\,mg\,kg^{-1} \times 2\,day^{-1}$) was intraperitoneally administered to sarcoma 180-bearing mice, myelotoxicity (the reduction of leucocyte and platelet numbers), nephrotoxicity (the increase of blood nitrogen urea level), immunotoxicity (the reduction of spleen and thymus weight) and a reduction in body weight resulted. These intraperitoneally administered CDDP-induced side effects were not prevented by oral administration of chitosan ($150\,mg\,kg^{-1} \times 2\,day^{-1}$ and $750\,mg\,kg^{-1} \times 2\,day^{-1}$) for 14 consecutive days. On the other hand, the side effects such as the reductions of body and spleen weights induced by orally administered CDDP ($1{\cdot}25\,mg\,kg^{-1} \times 2\,day^{-1}$) were prevented by the oral administration of chitosan ($150\,mg\,kg^{-1} \times 2\,day^{-1}$ and $750\,mg\,kg^{-1} \times 2\,day^{-1}$).

From these results, we conclude that the orally administered chitosan plus CDDP might be useful for the prevention of body weight reduction and immunotoxicity (the reduction of spleen weight) induced by the orally administered CDDP without diminishing antitumour activity.

Chitin and chitosan are polymers containing more than 5000 acetylglucosamine and glucosamine units, respectively, and their molecular weights are around 1000 kDa. Although chitin is widely distributed in natural products such as the protective cuticles of crustaceans and insects, and the cell walls of some fungi and microorganisms, it is usually prepared from the shells of crabs and shrimps. Chitin is converted to chitosan by alkaline hydrolysis with 45% NaOH at 100°C for 2 h. Previously, we reported that chitosan reduced the blood pressure elevation caused by NaCl intake (Kato et al 1994), augmented the natural killer activity of mouse lymphocytes (Zhou et al 1994), prevented the hyperlipidaemia and fatty liver induced by a high-fat diet (Han et al 1999) and prevented side effects induced by the cancer chemotherapy drug 5-fluorouracil (5-FU) (myelotoxicity, gastrointestinal toxicity, immunocompetent organ toxicity and reduction in body weight) without interfering with the antitumour activity of 5-FU (Kimura & Okuda 1999). Among cancer chemotherapy drugs, cisplatin (*cis*-diaminedichloroplatinum, CDDP) has been used extensively in the treatment of certain types of cancer (Asano et al 1998; Muso 1998; Caponigro et al 1999; Okuda et al 1999; Shirasaka et al 1999; Uchida et al 1999); however, CDDP induces severe spells of nausea and vomiting (Beppu et al 1998; Satoh et al 1998; Tsavaris et al 1998), nephrotoxicity (Bardary et al 1997; Huang et al 1997; Nishikawa et al 1998; Ochiai et al 1998; Ueda et al 1998; Takayama et al 1999) and myelotoxicity (Haim et al 1999; Langer 1999; Lippe et al 1999). CDDP is mainly

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administered by intravenous injection. In this study we examined the antitumour and side effects (e.g. myelotoxicity, immunocompetent organic toxicity, nephrotoxicity and reduction in body weight) of CDDP administered orally and intraperitoneally, and the administration of oral or intraperitoneal CDDP plus chitosan in solid-type sarcoma 180-bearing mice.

Materials and Methods

Materials

Chitosan was supplied by Fuji Bio Co. (Shizuoka, Japan). Chitosan was converted to the chloride salt, and the intrinsic viscosity was about 113 cP. The average molecular weight was determined as ~500 to 700 kDa based on the viscosity, and the degree of acetylation was 14%. Chitosan was suspended in 0·9% NaCl solution, adjusted to about pH 6·0 with 0·1 N HCl. CDDP was supplied by Nihon Kayaku Co. (Tokyo, Japan) and dissolved in 0·9% NaCl. Other chemicals were of reagent grade. Sarcoma 180 cells were maintained in the laboratory of the Second Department of Medical Biochemistry, School of Medicine, Ehime University, Japan.

Animals

Male ICR strain mice (6 weeks old) were obtained from Clea Japan (Osaka, Japan). ICR mice were housed for 1 week in a room maintained at $25 \pm 1^{\circ}C$ with 60% relative humidity and given free access to food and water. The room was illuminated for 12 h per day starting at 7·00 a.m.

Measurement of antitumour activity and side effects induced by CDDP in sarcoma 180-bearing mice

Solid-type sarcoma 180 was prepared by subcutaneous transplantation of $3{\cdot}0 \times 10^6$ cells into the right abdomen of mice on day 0. As the experimental protocol for the intraperitoneal administration of CDDP, CDDP (1·25 mg kg^{-1} body weight) or CDDP (1·25 mg kg^{-1} body weight) plus chitosan (150 mg kg^{-1} body weight or 750 mg kg^{-1} body weight) dissolved and suspended in 0·9% NaCl and adjusted to pH 6·0 with 0·1 N HCl, respectively, were administered intraperitoneally twice daily (7.00 a.m. and 7.00 p.m.) for 14 consecutive days, starting 12 h after the implantation of tumour cells. As the experimental protocol for the oral administration of CDDP, CDDP (1·25 mg kg^{-1} body weight) or CDDP (1·25 mg kg^{-1} body weight) plus chitosan (150 mg kg^{-1} body weight or 750 mg kg^{-1} body weight) was administered orally on the same schedule. Control mice were also given 0·9% NaCl solution alone on the same schedule. The tumour volume was determined by direct measurement with callipers and calculated by the formula $(\text{length} \times \text{width}^2)/2$, every 2 to 3 days. On day 15, blood was obtained by venous puncture under anaesthesia with diethyl ether, and then the tumour, small intestine, liver epididymal adipose tissue, spleen and thymus were removed and weighed for evaluation of antitumour activity and side effects. The blood samples were chilled in test-tubes containing heparin, and the numbers of leucocytes, red cells and platelets, and the haemoglobin content were measured using a Coulter Counter (Japan Scientific Instruments Co. Ltd, Tokyo, Japan). Blood urea nitrogen (BUN) was measured using Wako BUN-B-Test kits.

Data and statistical analysis

Data are expressed as means ± standard error (s.e.m.). Statistical analysis was performed with the Dunnet test to determine significance ($P < 0{\cdot}05$) using Super ANOVA Software.

Results

Oral administration of CDDP or oral administration of CDDP plus chitosan in sarcoma 180-bearing mice

Figures 1A and 2A show that oral administration of CDDP (1·25 mg kg^{-1} body weight) and CDDP plus chitosan (150 mg kg^{-1} body weight and 750 mg kg^{-1} body weight) significantly reduced the tumour growth and final tumour weight compared with the control group (sarcoma 180-bearing mice). There was no significant difference between the CDDP-treated group and the CDDP plus chitosan-treated (150 mg kg^{-1} body weight and 750 mg kg^{-1} body weight) group. These results indicate that chitosan did not interfere with the antitumour activity of CDDP. As shown in Figures 3A and 4, chitosan (150 mg kg^{-1} body weight and 750 mg kg^{-1} body weight) prevented the reduction in body weight and spleen weight induced by CDDP. CDDP plus chitosan had no effect on the numbers of leucocytes, platelets or red cells (Table 1). The weights of the liver, kidney, small intestine, adipose tissue and thymus, and the contents of haemoglobin and BUN were also not affected by the oral administration of CDDP or CDDP plus chitosan (Tables 2 and 3). These results indicate

that chitosan did not cause side effects when co-administered with CDDP, and prevented the CDDP-induced reduction in body weight and spleen weight without interfering with the anti-tumour activity induced by CDDP.

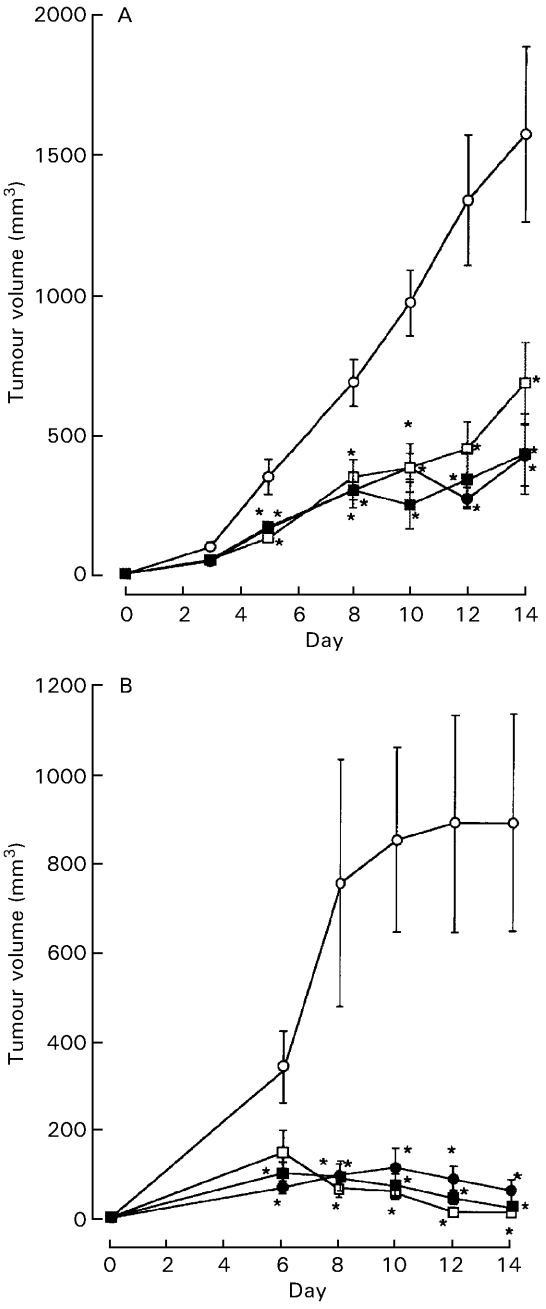

Figure 1. Inhibitory effects of orally administered CDDP (A) or intraperitoneally administered CDDP (B) plus chitosan on tumour growth in sarcoma 180-bearing mice. Results are expressed as mean ± s.e.m., for 10 mice in each group. (A) ○, sarcoma 180-bearing mice (control); ●, CDDP ($1{\cdot}25\,mg\,kg^{-1}$ body weight $\times$ $2\,day^{-1}$, po); □, CDDP + chitosan ($150\,mg\,kg^{-1}$ body weight $\times$ $2\,day^{-1}$, po); ■, CDDP + chitosan ($750\,mg\,kg^{-1}$ body weight $\times$ $2\,day^{-1}$, po). (B) ○, sarcoma 180-bearing mice (control); ●, CDDP ($1{\cdot}25\,mg\,kg^{-1} \times 2\,day^{-1}$, ip); □, CDDP (ip) + chitosan ($150\,mg\,kg^{-1}$ body weight $\times$ $2\,day^{-1}$, po); ■, CDDP (ip) + chitosan ($750\,mg\,kg^{-1}$ body weight $\times$ $2\,day^{-1}$, po). $^{*}P < 0{\cdot}05$ was considered significantly different from sarcoma 180-bearing mice (control).

Intraperitoneal administration of CDDP or intraperitoneal administration of CDDP plus oral administration of chitosan in sarcoma 180-bearing mice

As shown in Figures 1B and 2B , intraperitoneal administration of CDDP ($1{\cdot}25\,mg\,kg^{-1}$ body weight $\times$ $2\,day^{-1}$) or CDDP plus oral administration of chitosan ($150\,mg\,kg^{-1}$ body weight $\times$ $2\,day^{-1}$ or $750\,mg\,kg^{-1}$ body weight $\times$ $2\,day^{-1}$) also strongly reduced tumour growth. Body weight was also markedly reduced after day 8 by the intraperitoneal administration of CDDP; however, body weight reduction induced by intraperitoneal administration of CDDP was not retrieved by the oral administration of chitosan ($150\,mg\,kg^{-1}$ body weight $\times$ $2\,day^{-1}$ and $750\,mg\,kg^{-1}$ body weight $\times$ $2\,day^{-1}$) (Figure 3B). As shown in Figures 5 and 6, the immunocompetent organic toxicity (the reduction of spleen and thymus weights) and the myelotoxicity (the reduction of leucocyte and platelet numbers) induced by intraperitoneal CDDP administration were not retrieved by the oral administration of chitosan. The oral administration of chitosan did not inhibit nephrotoxicity with the elevation of the BUN level induced by intraperitoneal CDDP administration (Figure 7).

Discussion

In this study we found that CDDP had potent antitumour activity when administered orally as well as intraperitoneally. We also compared the antitumour activity and side effects of orally administered CDDP plus orally administered chitosan with intraperitoneally administered CDDP plus orally administered chitosan in sarcoma 180-bearing mice.

In the intraperitoneal administration of CDDP in sarcoma 180-bearing mice, the myelotoxicity (the reduction of leucocyte and platelet numbers), nephrotoxicity (the increase of BUN), immunotoxicity (the reduction in spleen and thymus weights) and body weight reduction induced by the intraperitoneal administration of CDDP were not relieved by the oral administration of chitosan. Thus, the side effects induced by the intraperitoneal administration of CDDP could not be prevented by the oral administration of chitosan. On the other hand, the oral administration of chitosan did not cause side effects when orally co-administered with CDDP, and prevented the orally administered

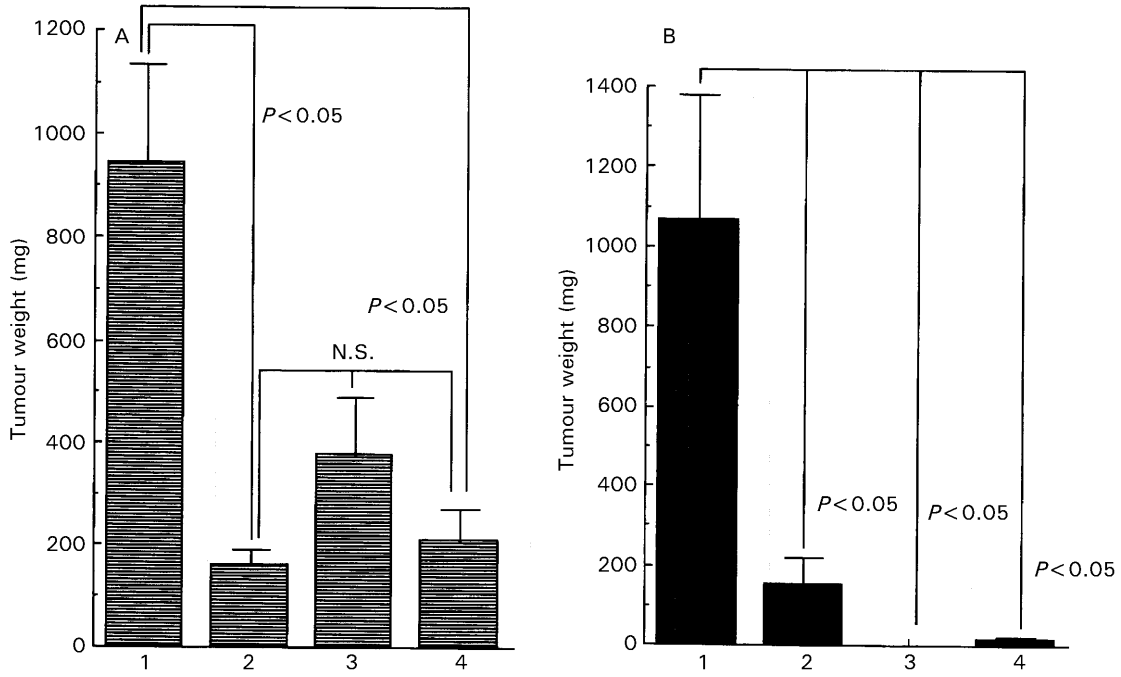

Figure 2. Reduction in tumour weight by orally administered CDDP (A) or intraperitoneally administered CDDP (B) plus chitosan in sarcoma 180-bearing mice. Results are expressed as mean ± s.e.m., for 10 mice in each group. A. 1, Sarcoma 180-bearing mice (control); 2, CDDP (1·25 mg kg^{-1} body weight × 2 day^{-1}, po); 3, CDDP (po) + chitosan (150 mg kg^{-1} body weight × 2 day^{-1}, po); 4, CDDP (po) + chitosan (750 mg kg^{-1} body weight × 2 day^{-1}, po). $P < 0{\cdot}05$, significantly different from control; not significantly different from CDPP alone; N.S., not significant. B. 1, Sarcoma 180-bearing mice (control); 2, CDDP (1·25 mg kg^{-1} body weight × 2 day^{-1}, ip); 3, CDDP (ip) + chitosan (150 mg kg^{-1} body weight × 2 day^{-1}, po); 4, CDDP (ip) + chitosan (750 mg kg^{-1} body weight × 2 day^{-1}, po). $P < 0{\cdot}05$, significantly different from control.

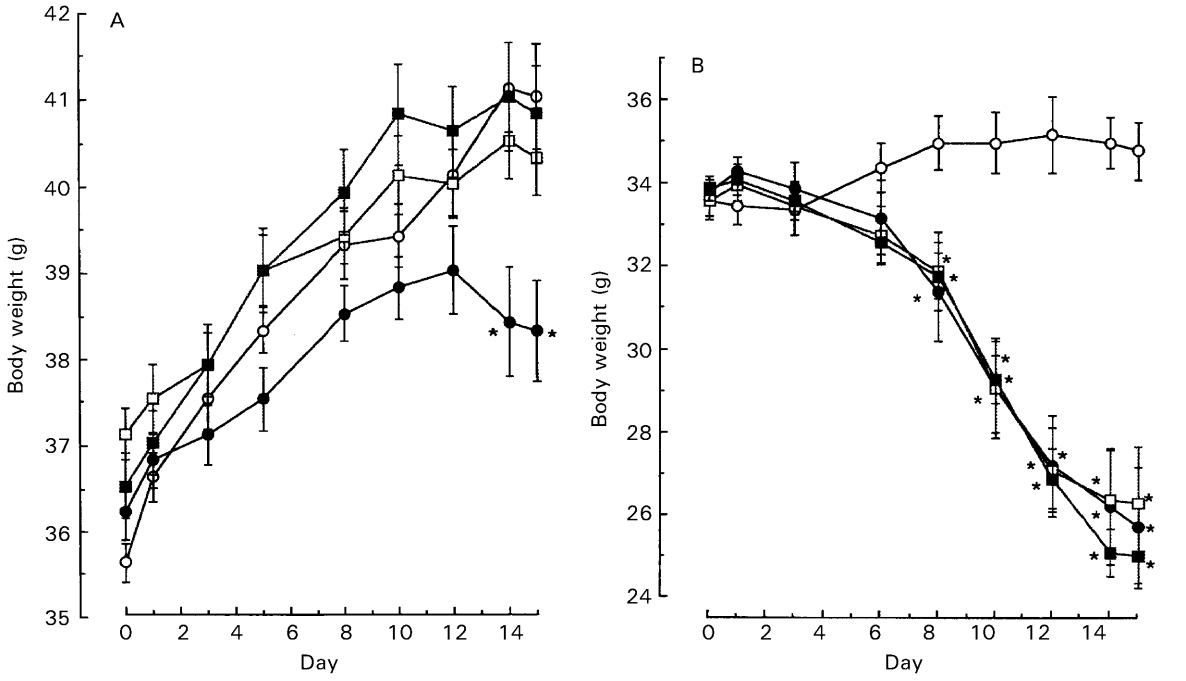

Figure 3. Body weight changes in sarcoma 180-bearing mice treated with orally administered CDDP (A) or intraperitoneally administered CDDP (B) plus chitosan. Results are expressed as mean ± s.e.m., for 10 mice in each group. Significantly different from sarcoma 180-bearing mice (control) (*$P < 0{\cdot}05$). (A) ○, sarcoma 180-bearing mice (control); ●, CDDP (1·25 mg kg^{-1} body weight × 2 day^{-1}, po); □, CDDP (po) + chitosan (150 mg kg^{-1} body weight × 2 day^{-1}, po); ■, CDDP (po) + chitosan (750 mg kg^{-1} body weight × 2 day^{-1}, po). (B) ○, sarcoma 180-bearing mice (control); ●, CDDP (1·25 mg kg^{-1} body weight × 2 day^{-1}, ip); □, CDDP (ip) + chitosan (150 mg kg^{-1} body weight × 2 day^{-1}, po); ■, CDDP (ip) + chitosan (750 mg kg^{-1} body weight × 2 day^{-1}, po).

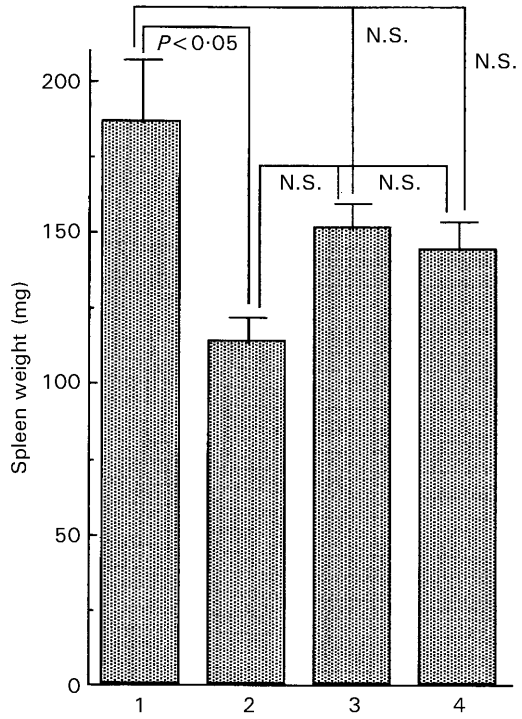

Figure 4. Effects of orally administered CDDP plus chitosan on spleen weight in sarcoma 180-bearing mice. Results are expressed as mean ± s.e.m., for 10 mice in each group. 1, Sarcoma 180-bearing mice (control); 2, CDDP (1·25 mg kg^{-1} body weight × 2day^{-1}, po); 3, CDDP (po) + chitosan (150 mg kg^{-1} body weight × 2 day^{-1}, po); 4, CDDP (po) + chitosan (750 mg kg^{-1} body weight × 2 day^{-1}, po); N.S., not significant. $P < 0·05$, significantly different from control.

CDDP-induced reduction in body weight and spleen weight without interfering with the antitumour activity induced by orally administered CDDP.

We previously reported that platinum levels in the blood of mice were about 490 ng mL^{-1} and 70 ng mL^{-1}, respectively, at 5 min after the intraperitoneal and oral administration of CDDP (2·5 mg kg^{-1} body weight) and then decreased rapidly. It was found that the blood platinum level (C_{max} and AUC) after the oral administration of CDDP was lower than that after the intraperitoneal administration of CDDP.

There are a number of reports that chitosan can be used as an absorption-enhancer drug (Schipper et al 1996, 1997; Miekka et al 1998; Tozaki et al 1997). Sugimoto et al (1998) reported that ampillicin absorption by poly(vinylalcohol) gel spheres was enhanced by the chitosan combination, and that poly(vinylalcohol) gel spheres prepared with chitosan prolonged the small intestinal transit time more than poly(vinylalcohol) gel spheres alone. Singh & Udupa (1998) reported that the antitumour activity in Ehrlich ascites tumour-bearing mice given methotrexate-loaded chitosan microspheres was better when compared with plain methotrexate on oral administration, and the plasma methotrexate levels were more sustained. It seems likely that the oral administration of CDDP may be adsorbed on the cationic polymer chitosan and consequently the release rate of CDDP from the CDDP–chitosan complex is slow so that the small intestinal transit time is prolonged. It is therefore suggested that the maintenance of a lower concentration of CDDP after the oral co-administration of CDDP plus chitosan prevented side effects such as the reduction in body weight and spleen weight induced by orally administered CDDP alone.

Generally, CDDP has been clinically administered by intravenous injection and shown to have potent antitumour activity, but orally administered CDDP is not used clinically, nor is it likely to be. From these experiments, orally administered CDDP was shown to have potent antitumour activity and caused side effects (body weight reduction and immunotoxicity). The side effects induced by orally administered CDDP were weaker than those of intraperitoneally administered CDDP. The orally administered chitosan prevented the reduction in body weight and immunotoxicity induced by the orally administered CDDP without interfering with

Table 1. Effects of CDDP plus chitosan on the numbers of leucocytes, red cells and platelets in sarcoma 180-bearing mice.

	Blood cell numbers		
	Leucocytes ($\times 10^3\ \mu L^{-1}$)	Red cells ($\times 10^6\ \mu L^{-1}$)	Platelets ($\times 10^3\ \mu L^{-1}$)
Sarcoma 180-bearing mice (control)	8·21 ± 0·74	8·11 ± 0·52	520·5 ± 33·7
CDDP (po, 1·25 mg kg^{-1} body weight × 2 day^{-1})	10·0 ± 1·44	7·31 ± 0·16	353·0 ± 70·0
CDDP (po) + chitosan (150 mg kg^{-1} body weight × 2 day^{-1})	9·84 ± 1·11	7·62 ± 0·18	342·5 ± 37·9
CDDP (po) + chitosan (750 mg kg^{-1} body weight × 2 day^{-1})	9·78 ± 1·05	8·37 ± 0·20	521·7 ± 57·3

Results are expressed as mean ± s.e.m., for 10 mice in each group.

Table 2. Effects of CDDP plus chitosan on the weights of liver, kidney, small intestine, adipose tissue and thymus in sarcoma 180-bearing mice.

	Liver ($g\,100\,g^{-1}$ body weight)	Kidney ($g\,100\,g^{-1}$ body weight)	Small intestine ($g\,100\,g^{-1}$ body weight)	Adipose tissue (mg)
Sarcoma 180-bearing mice (control)	6·98 ± 0·08	2·01 ± 0·036	3·55 ± 0·20	603·6 ± 25·6
CDDP (po, 1·25 mg kg^{-1} body weight × 2 day^{-1})	6·60 ± 0·150	2·02 ± 0·039	3·27 ± 0·13	611·8 ± 54·2
CDDP (po) ± chitosan (150 mg kg^{-1} body weight × 2 day^{-1})	6·45 ± 0·093	1·90 ± 0·048	3·39 ± 0·23	778·0 ± 48·0
CDDP (po) + chitosan (750 mg kg^{-1} body weight × 2 day^{-1})	6·46 ± 0·132	2·06 ± 0·088	3·55 ± 0·16	661·2 ± 49·7

Results are expressed as mean ± s.e.m., for 10 mice in each group.

Table 3. Effects of CDDP plus chitosan on the thymus weight, haemoglobin and blood urea nitrogen (BUN) contents in sarcoma 180-bearing mice.

	Thymus (mg)	Haemoglobin (mg dL^{-1})	BUN (mg dL^{-1})
Sarcoma 180-bearing mice (control)	50·7 ± 4·77	13·7 ± 0·27	23·8 ± 0·79
CDDP (po, 1·25 mg kg^{-1} body weight × 2 day^{-1})	40·0 ± 5·50	13·5 ± 0·29	22·7 ± 1·21
CDDP + chitosan (po, 150 mg kg^{-1} body weight × 2 day^{-1})	51·5 ± 5·03	13·9 ± 0·25	22·7 ± 0·72
CDDP + chitosan (po, 750 mg kg^{-1} body weight × 2 day^{-1})	47·6 ± 3·02	14·9 ± 0·29	22·0 ± 0·89

Results are expressed as mean ± s.e.m., for 10 mice in each group.

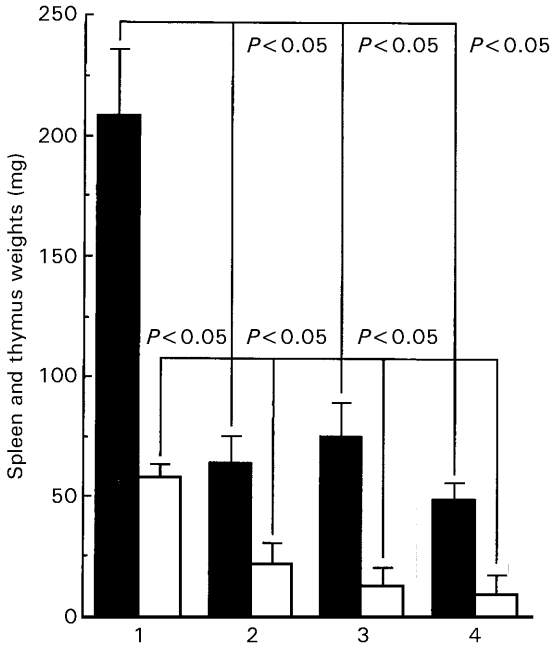

Figure 5. Effects of intraperitoneally administered CDDP plus orally administered chitosan on spleen (■) and thymus (□) weights in sarcoma 180-bearing mice. Results are expressed as mean ± s.e.m. for 10 mice in each group. 1, Sarcoma 180-bearing mice (control); 2, CDDP (1·25 mg kg^{-1} body weight × 2 day^{-1}, ip); 3, CDDP (ip) + chitosan (150 mg kg^{-1} body weight × 2 day^{-1}, po); 4, CDDP (ip) + chitosan (750 mg kg^{-1} body weight × 2 day^{-1}, po). $P < 0·05$ was considered significantly different from control.

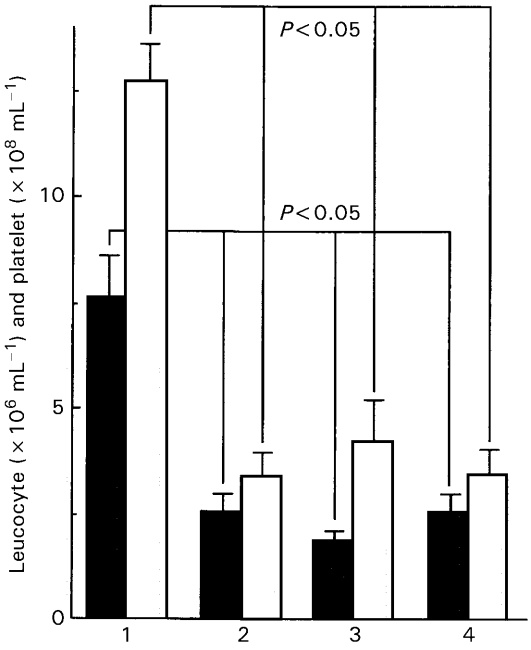

Figure 6. Effects of intraperitoneally administered CDDP plus orally administered chitosan on leucocyte (■) and platelet (□) numbers in sarcoma 180-bearing mice. Results are expressed as mean ± s.e. for 10 mice in each group. 1, Sarcoma 180-bearing mice (control); 2, CDDP (1·25 mg kg^{-1} body weight × 2 day^{-1}, ip); 3, CDDP (ip) + chitosan (150 mg kg^{-1} body weight × 2 day^{-1}, po); 4, CDDP (ip) + chitosan (750 mg kg^{-1} body weight × 2 day^{-1}, po). $P < 0·05$ was considered significantly different from control.

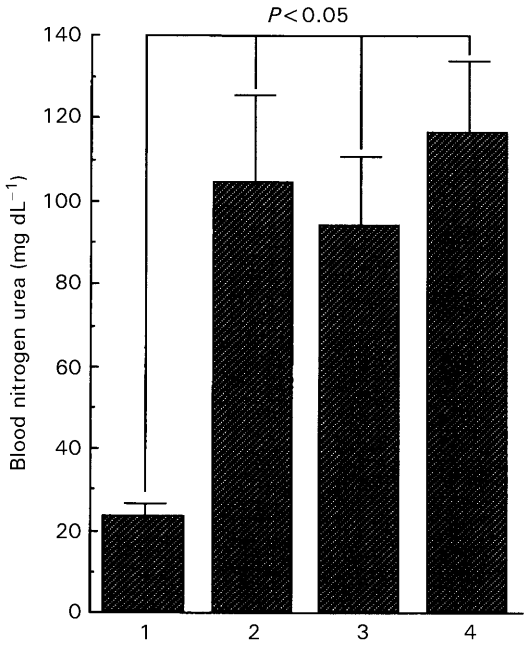

Figure 7. Effects of intraperitoneally administered CDDP plus orally administered chitosan on blood nitrogen urea in sarcoma 180-bearing mice. Results are expressed as mean ± s.e.m. for 10 mice in each group. 1, Sarcoma 180-bearing mice (control); 2, CDDP (1·25 mg kg^{-1} body weight × 2 day^{-1}, ip); 3, CDDP (ip) + chitosan (150 mg kg^{-1} body weight × 2 day^{-1}, po); 4, CDDP (ip) + chitosan (750 mg kg^{-1} body weight × 2 day^{-1}, po). $P < 0{\cdot}05$ was considered significantly different from control.

the antitumour activity. Further work is needed to clarify the clinical significance of the oral combined administration of CDDP plus chitosan in cancer chemotherapy.

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