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Effect of coadministration of selenite on the toxicity and antitumor activity of *cis*-diamminedichloroplatinum(II) given repeatedly to mice*

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Summary. The effect of selenite coadministration on the toxicity and antitumor activity of repeated treatment with high doses of *cis*-diamminedichloroplatinum (*cis*-DDP) was examined in mice. Sodium selenite was injected s.c. into separate abdominal sites of mice together with *cis*-DDP at a molar ratio of 1:3.5 (selenite to *cis*-DDP) on day 0. The same amount of selenite was given daily for 4 subsequent days (days 1–4). This fixed administration schedule was repeated weekly for a total of 7 weeks. Under the experimental conditions used, the lethal toxicity, renal toxicity [indicated by an increase in blood urea nitrogen (BUN) and plasma creatinine levels], hepatic toxicity (indicated by an increase in plasma GPT and GOT activity), and myelotoxicity (indicated by a decrease in the numbers of leukocytes and platelets) observed in mice given repeated doses of *cis*-DDP alone (15 or 25 μmol/kg, s.c.) were significantly depressed by the coadministration of sodium selenite. Treatment with *cis*-DDP alone (15, 20, or 25 μmol/kg, s.c.) resulted in some dose-dependent prolongation of the life span of mice transplanted either s.c. with colon adenocarcinoma 38 (colon 38) or i.p. with P388 leukemia (P388) but did not completely depress the tumor growth, and the animals died of either progressive disease or *cis*-DDP-induced toxicity. However, following the coadministration of 7.1 μmol/kg selenite with 25 μmol/kg *cis*-DDP, all of the mice transplanted either s.c. with colon 38 or i.p. with P388 survived for as long as 4 months after the end of the treatment and showed no evidence of malignancy. These results indicate that selenite coadministration enables the use of increasing doses of *cis*-DDP and, consequently, enhances the antitumor effect of *cis*-DDP by depressing its side effects.

Introduction

Among the various anticancer agents developed thus far, *cis*-diamminedichloroplatinum(II) (*cis*-DDP), a platinum coordination complex, has been extensively evaluated for its potent antitumor activity and its broad anticancer spectrum [7, 12, 20]. However, *cis*-DDP produces severe side effects such as renal toxicity, bone marrow toxicity, gastrointestinal toxicity, and ototoxicity [10, 25]. In particular, its renal toxicity is recognized as being dose-limiting [10, 25].

On the other hand, the protective effect of selenium, an essential trace element, against the toxicity of heavy metals such as mercury and cadmium is well known [11]. Selenium compounds interact with these heavy metals to form stable and, consequently, less toxic complexes in animals [14]. It has been reported that selenium compounds selectively reduce the side effects of *cis*-DDP without affecting its antitumor activity [17], and this prominent effect of selenium has been confirmed by several investigators [1, 3, 4, 18, 19]. Satoh et al. [23] have previously demonstrated that the optimal schedule of selenium administration to reduce *cis*-DDP toxicity involves its daily injection for 5 consecutive days (days 1–5) following *cis*-DDP administration on day 1. These studies using experimental animals dealt with the effect of selenium on the toxicity and antitumor activity of a single dose of *cis*-DDP. However, in cancer chemotherapy, *cis*-DDP is generally given repeatedly to patients.

In the present study, we examined the effect of the coadministration of selenite on the toxicity and antitumor activity of repeated treatment with high doses of *cis*-DDP in mice.

Materials and methods

Animals and chemicals. Male ICR mice (22–25 g) were purchased from Charles River Japan, Inc. (Atsugi, Japan). Male C57BL/6, DBA/2, and B6D2F$_1$ mice were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). *cis*-DDP was kindly supplied by Nippon Kayaku Co., Ltd. (Tokyo). Sodium selenite was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo).

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Tumors. Colon adenocarcinoma 38 cells (colon 38) and P388 leukemia cells (P388) were kindly supplied by Dr. T. Tsuruo, Japanese Foundation for Cancer Research (Tokyo). Colon 38 was maintained by s.c. transplantation in the backs of male C57BL/6 mice, and P388 was maintained by i.p. transplantation in male DBA/2 mice. The viability of the tumor cells was tested by trypan blue exclusion.

Treatment with drugs. Selenite was injected s.c. into mice together with *cis*-DDP (s.c.) at a molar ratio of 1:3.5 (selenite to *cis*-DDP) on day 0, and the same amount of selenite was given daily for 4 subsequent days (days 1–4). On day 0, *cis*-DDP and selenite were injected into separate abdominal sites. This administration schedule was repeated weekly for a total of 7 weeks.

Toxic side effects. Lethal toxicity was examined using 10, 15, 20, or 25 μmol/kg *cis*-DDP. On the administration schedule described above, 15 or 25 μmol/kg *cis*-DDP was injected into male ICR mice (22–25 g), and blood samples were collected from animals under ether anesthesia at 1, 3, 5, and 7 weeks after the first dose of *cis*-DDP. The total numbers of leukocytes, erythrocytes, and platelets in the blood were determined using a Coulter counter (ZBI type) as indicators of bone marrow toxicity. Blood urea nitrogen (BUN) and plasma creatinine values as indicators of renal toxicity and the activity of GOT and GPT in plasma as indicators of hepatotoxicity were determined using Urea N-Test reagent, Creatinine-Test reagent, GOT-UV Test reagent, and GPT-UV Test reagent (Wako Pure Chemical Industries, Tokyo), respectively.

Antitumor activity. Colon 38 cells (2×10^6/mouse) were inoculated s.c. into the backs of male B6D2F$_1$ mice (22–26 g) at 15 days prior to the first application of *cis*-DDP (15, 20, or 25 μmol/kg injected s.c. into abdominal sites). P388 cells (1×10^6/mouse) were inoculated i.p. into male B6D2F$_1$ mice (22–26 g) at 1 day prior to the first s.c. injection of *cis*-DDP (15, 20, or 25 μmol/kg). Subsequently, the antitumor activity of *cis*-DDP was evaluated according to the survival of and the solid-tumor volume in mice that had been treated with the drug. For each tumor, two perpendicular diameters were measured once a week. The tumor volume was calculated by the following formula:

$$\text{Tumor volume (mm}^3) = 0.5ab^2,$$

where a represents the maximal tumor diameter and b represents the perpendicular diameter [9].

Results

The effect of coadministration of selenite on the lethal toxicity of repeatedly injected *cis*-DDP (10, 15, 20, or 25 μmol/kg) in mice was examined. All of the mice receiving 25 μmol/kg *cis*-DDP alone died within 6 weeks, and 50% of those given 20 μmol/kg *cis*-DDP alone died within 7 weeks. However, the coadministration of an appropriate dose of selenite together with 20 or 25 μmol/kg *cis*-DDP completely prevented this lethal toxicity (data not shown). The body weight of mice receiving *cis*-DDP showed a marked dose-dependent decrease as compared with that of the control group, but the coadministration of selenite ameliorated this growth suppression (Fig. 1).

Figure 2 shows the effect of coadministration of selenite on the renal toxicity of repeatedly injected *cis*-DDP (15 or 25 μmol/kg). Both BUN and plasma creatinine values markedly increased in a dose-dependent manner in mice receiving *cis*-DDP alone as compared with the control group. The coadministration of selenite significantly depressed the increases in BUN and creatinine values to control levels. Plasma GOT and GPT activity also increased dose-dependently in mice treated with *cis*-DDP

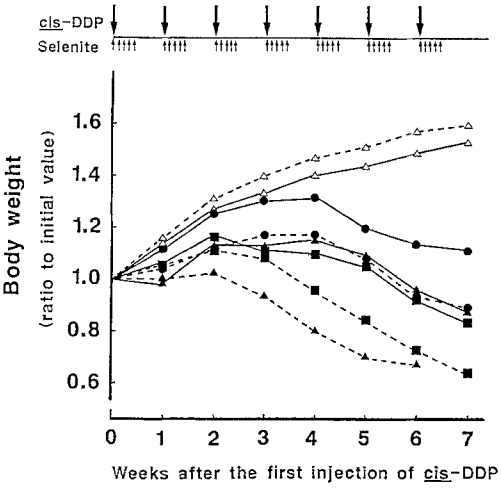

Fig. 1. Effect of selenite on the body weight of mice receiving *cis*-DDP. Control (△----△); selenite, 7.1 μmol/kg (△——△); *cis*-DDP, 15 μmol/kg (●----●); *cis*-DDP, 20 μmol/kg (■----■); *cis*-DDP, 25 μmol/kg (▲----▲); *cis*-DDP, 15 μmol/kg, + selenite, 4.3 μmol/kg (●——●); *cis*-DDP, 20 μmol/kg, +selenite, 5.7 μmol/kg (■——■); *cis*-DDP, 25 μmol/kg, + selenite, 7.1 μmol/kg (▲——▲). Data represent mean values for 10 mice

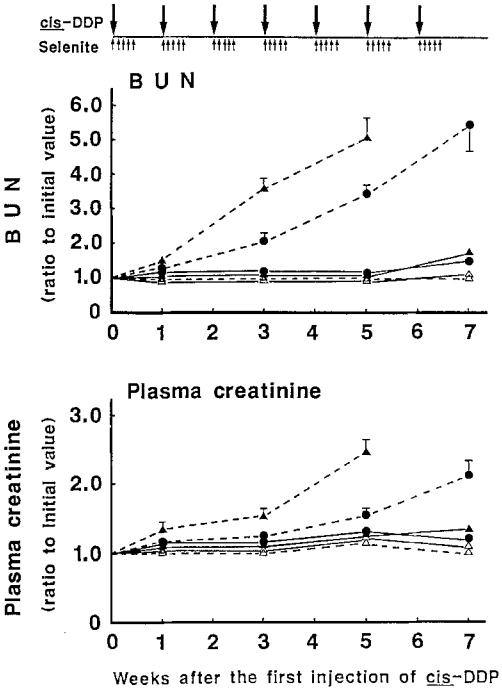

Fig. 2. Effect of selenite on BUN and plasma creatinine values in mice receiving *cis*-DDP. Control (△----△); selenite, 7.1 μmol/kg (△——△); *cis*-DDP, 15 μmol/kg (●----●); *cis*-DDP, 25 μmol/kg (▲----▲); *cis*-DDP, 15 μmol/kg, + selenite, 4.3 μmol/kg (●——●); *cis*-DDP, 25 μmol/kg, + selenite, 7.1 μmol/kg (▲——▲). Data represent mean values ± SD for 4 mice

alone, but both of these values were decreased to control levels by the coadministration of selenite (Fig. 3). Although plasma GOT and GPT activity increased slightly in mice given selenite alone (7.1 μmol/kg), this sign of hepatotoxicity was markedly depressed by the coadministration of *cis*-DDP (Fig. 3). The total numbers of leukocytes and

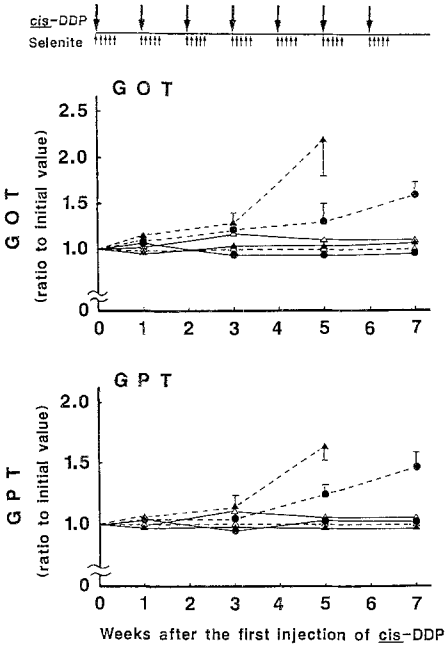

Fig. 3. Effect of selenite on GOT and GPT activity in the plasma of mice receiving *cis*-DDP. Control (△----△); selenite, 7.1 μmol/kg (△——△); *cis*-DDP, 15 μmol/kg (●----●); *cis*-DDP, 25 μmol/kg (▲----▲); *cis*-DDP, 15 μmol/kg, + selenite, 4.3 μmol/kg (●——●); *cis*-DDP, 25 μmol/kg, + selenite, 7.1 μmol/kg (▲——▲). Data represent mean values ± SD for 4 mice

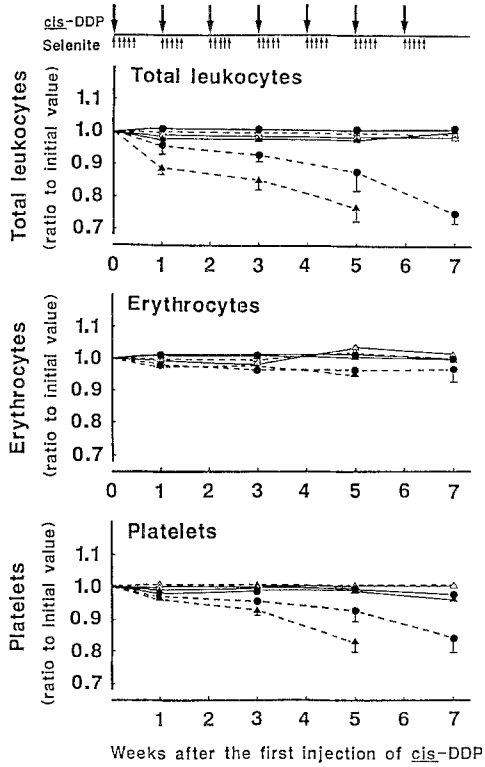

Fig. 4. Effect of selenite on total leukocytes, erythrocytes, and platelets in mice receiving *cis*-DDP. Control (△----△); selenite, 7.1 μmol/kg (△——△); *cis*-DDP, 15 μmol/kg (●----●); *cis*-DDP, 25 μmol/kg (▲----▲); *cis*-DDP, 15 μmol/kg, + selenite, 4.3 μmol/kg (●——●); *cis*-DDP, 25 μmol/kg, + selenite, 7.1 μmol/kg (▲——▲). Data represent mean values ± SD for 4 mice

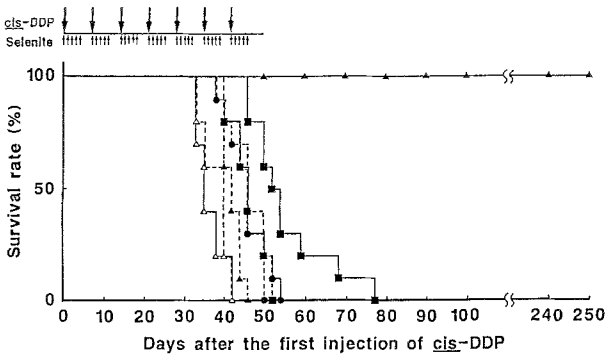

Fig. 5. Effect of selenite on the survival of *cis*-DDP-treated mice inoculated s.c. with colon adenocarcinoma 38 cells. Mice were inoculated s.c. with colon adenocarcinoma 38 cells (2×10^6 cells/mouse) at 15 days prior to the s.c. injection of *cis*-DDP and selenite. Control (△----△); selenite, 7.1 μmol/kg (△——△); *cis*-DDP, 15 μmol/kg (●----●); *cis*-DDP, 20 μmol/kg (■----■); *cis*-DDP, 25 μmol/kg (▲----▲); *cis*-DDP, 15 μmol/kg, + selenite, 4.3 μmol/kg (●——●); *cis*-DDP, 20 μmol/kg, + selenite, 5.7 μmol/kg (■——■); *cis*-DDP, 25 μmol/kg, + selenite, 7.1 μmol/kg (▲——▲). Data represent mean values ± SD for 10 mice

platelets showed a significant dose-dependent decrease following *cis*-DDP administration with time, but these indicators of bone marrow toxicity as well as those of nephrotoxicity and hepatotoxicity, were improved by the coadministration of selenite (Fig. 4). No significant change was observed in the numbers of erythrocytes following the administration of *cis*-DDP (Fig. 4).

Figure 5 shows the effect of coadministration of selenite on the antitumor activity of *cis*-DDP in mice inoculated s.c. with colon 38. All of the control mice died of progressive disease within 39 days of tumor inoculation. On the other hand, repeated administration of *cis*-DDP alone (15, 20, or 25 μmol/kg) prolonged the life span of the animals to some extent as compared with the untreated tumor-bearing mice but failed to depress the tumor growth completely. On this administration schedule, within 55 days the mice receiving low doses of *cis*-DDP died due to disease progression and those receiving high doses of *cis*-DDP succumbed due to the lethal toxicity of the drug (Fig. 5). The coadministration of selenite with 15 or 20 μmol/kg *cis*-DDP did not affect the prolongation of life span observed in mice given the same doses of *cis*-DDP alone (Fig. 5).

In contrast, no death was observed following the administration of 25 μmol/kg *cis*-DDP and 7.1 μmol/kg selenite; indeed, all of the mice were alive at 200 days after the final injection of *cis*-DDP.

Figure 6 illustrates the average tumor volumes measured in surviving mice in each group exhibiting the survival patterns shown in Fig. 5. The tumor volumes in control animals and mice receiving selenite alone increased with time. In mice receiving *cis*-DDP alone, the volume of tumors decreased dose-dependently, indicating the effective antitumor activity of the drug. It seems obvious that the antitumor activity of *cis*-DDP was not compromised by the coadministration of selenite. Furthermore, the tumor volumes in mice treated with 25 μmol/kg *cis*-DDP and 7.1 μmol/kg selenite decreased even after the end of the

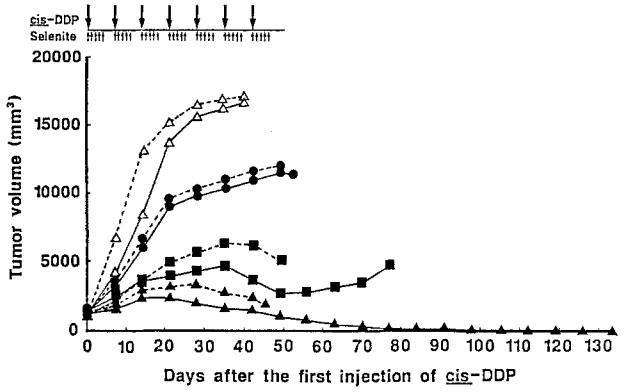

Fig. 6. Effect of selenite on the antitumor activity of *cis*-DDP in mice inoculated s.c. with colon adenocarcinoma 38 cells. Mice were inoculated s.c. with colon adenocarcinoma 38 cells (2×10^6 cells/mouse) at 15 days prior to the s.c. injection of *cis*-DDP and selenite. Control (△----△); selenite, 7.1 μmol/kg (△———△); *cis*-DDP, 15 μmol/kg (●----●); *cis*-DDP, 20 μmol/kg (■----■); *cis*-DDP, 25 μmol/kg (▲----▲); *cis*-DDP, 15 μmol/kg, + selenite, 4.3 μmol/kg (●———●); *cis*-DDP, 20 μmol/kg, + selenite, 5.7 μmol/kg (■———■); *cis*-DDP, 25 μmol/kg, + selenite, 7.1 μmol/kg (▲———▲). Data represent mean values for 10 mice

therapy; the tumors completely disappeared and no subsequent recurrence was observed (Fig. 6). Under the same experimental conditions, no death was observed following the i.p. inoculation of mice with P388 leukemia cells, and the tumors were completely cured by combined treatment with 25 μmol/kg *cis*-DDP and 7.1 μmol/kg selenite (data not shown).

Discussion

cis-DDP exerts superior antitumor activity against a variety of solid tumors and has been used worldwide for clinical therapy. However, in spite of its effectiveness, the clinical use of *cis*-DDP is limited by its severe side effects, mainly renal toxicity. Several trials have been carried out in attempts to improve the efficacy of *cis*-DDP by reducing its renal toxicity. At present, the hydration method using diuretics such as furosemide [6] or mannitol [8] and the hypertonic saline method using 3% salt solutions for injection [2] are applied clinically. However, each of these methods requires large volumes of fluid and takes a long time, which contributes to the patients' discomfort. In the present study, we found that coadministration of selenite dramatically reduced the toxicity arising from repeated dosing of *cis*-DDP in mice without affecting the antitumor activity of the drug.

Previously, we examined the optimal conditions for selenite administration to reduce the toxicity of a single dose of *cis*-DDP in mice. In those experiments, the greatest reduction in the lethal toxicity of *cis*-DDP was obtained when sodium selenite was injected s.c. into mice together with *cis*-DDP at a molar ratio of 1:3.5 (selenite to *cis*-DDP) on the 1st day and the same amount of selenite alone was given daily for 4 subsequent days [23]. In the present study, this fixed administration schedule was repeated weekly for a total of 7 weeks for determination of the effect of selenite on the toxicity and antitumor activity of repeated doses of *cis*-DDP.

Repetition of the selenite/*cis*-DDP coadministration schedule was found to protect the animals against the lethal toxicity, renal toxicity (indicated by an increase in BUN and plasma creatinine levels), hepatic toxicity (indicated by an increase in plasma GPT and GOT activity), and myelotoxicity (indicated by a decrease in the numbers of leukocytes and platelets) of *cis*-DDP that were observed in mice given repeated doses of *cis*-DDP alone. Moreover, following the coadministration of 7.1 μmol/kg selenite and 25 μmol/kg *cis*-DDP, all of the mice transplanted either s.c. with colon 38 or i.p. with P388 survived for as long as 4 months after the end of the treatment and showed no evidence of malignancy. Under the experimental conditions used, treatment with *cis*-DDP alone resulted in some dose-dependent prolongation of the life span but did not completely depress the tumor growth. These results indicate that selenite coadministration enables the use of increasing doses of *cis*-DDP and enhances the antitumor effect of *cis*-DDP by depressing its side effects.

Selenium is an essential trace element for mammals [24]. The most important role of selenium is considered to involve its antioxidative action as a component of glutathione peroxidase [21]. A decrease in selenium intake via the diet has been reported to enhance the toxicity of *cis*-DDP [22]. It is possible that the difference in the sensitivity of patients to *cis*-DDP toxicity observed in clinical cases may be partly due to a discrepancy in dietary selenium intake.

On the other hand, selenium has also been reported to produce toxic effects [5]. However, no selenite toxicity was observed under the present experimental conditions, except for hepatotoxicity. Although slight but significant increases in plasma GOT and GPT activity were observed in mice repeatedly given selenite alone at a daily dose of 7.1 μmol/kg, this hepatotoxicity was depressed by the coadministration of *cis*-DDP (25 μmol/kg). It has been shown that selenium interacts with mercury in animal tissues [11, 13] and that the toxicity of the two substances are depressed by their mutual interaction [15]. It is possible that selenium given as selenite and *cis*-DDP or its metabolites may interact with each other in animal tissues and that the toxicity of these compounds may be mutually reduced as in the case of mercury and selenium. However, the mechanism underlying the protective effect of selenium against *cis*-DDP toxicity remains unclear.

In previous studies on the interaction of selenium with mercury, the tissue distribution of mercury was markedly altered by coadministration of selenite, and the formation of high-molecular-weight complexes containing both mercury and selenium was observed in the blood and the liver [13, 16]. We examined the tissue distribution of both platinum and selenium in mice after the single-agent and combination administration of [^{195m}Pt]-*cis*-DDP and [^{75}Se]-selenite in an attempt to determine the mechanism underlying the protective action of selenite against *cis*-DDP toxicity. As compared with the single-agent administration of *cis*-DDP or selenite, treatment of mice with a combination of the two agents produced hardly any change in the platinum tissue distribution at 24 h after drug administration (data not shown). In contrast, a significant increase in

selenium levels was observed in the liver, kidney, and lung following its coadministration with *cis*-DDP. This enhancement of selenium levels in the tissues may play a role in reducing the toxicity of *cis*-DDP. However, the profile of alterations noted in the tissue distribution of platinum and selenium obviously differs from that observed for the interaction of selenium with mercury [11]. Characterization of the platinum and selenium in the tissue of animals given both *cis*-DDP and selenite is now under way in our laboratory. Further study is needed to develop a safe method for the administration of selenium compounds as an antidote against *cis*-DDP toxicity in clinical cancer chemotherapy.

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