

## Full Paper

**Paradoxical Effect of 2,3-Dimercapto-1-propanesulfonic Acid (DMPS) on Enhancing Antitumor Activity of Cisplatin in Ascites Sarcoma 180 Cells**Toshihiro Sato<sup>1</sup>, Migiwa Okubo<sup>1</sup>, Kohei Sawaki<sup>1</sup>, Hiroshi Maehashi<sup>2,#</sup>, and Mitsuru Kawaguchi<sup>1,\*</sup><sup>1</sup>Department of Pharmacology, Tokyo Dental College, 1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan<sup>2</sup>Department of Pharmacology, Matsumoto Dental University, 1780 Gobara, Hirooka, Shiojiri, Nagano 399-0781, Japan

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**Abstract.** We investigated the enhancing effect of two metal-chelating compounds, 2,3-dimercapto-1-propanesulfonic acid (DMPS) and meso-2,3-dimercaptosuccinic acid (DMSA), on the antitumor activity of cisplatin (CDDP). In the *in vivo* experiments, DMPS showed a clear synergistic effect and significantly enhanced the antitumor activity of CDDP in terms of survival and life span in mice transplanted with ascites sarcoma 180 cells (S180 cells) at a dose of <100  $\mu\text{mol/kg}$ , *s.c.*, but not at a dose of >500  $\mu\text{mol/kg}$ . On the other hand, DMSA did not enhance the antitumor activity of CDDP. DMPS (50  $\mu\text{mol/kg}$ , *s.c.*) combined with CDDP also potently suppressed [<sup>3</sup>H]thymidine uptake in S180 cells implanted in mice, whereas DMSA did not. In the *in vitro* experiments, DMPS ( $10^{-6}$  to  $10^{-5}$  M) produced a time- and dose-dependent decrease in intracellular  $\text{Ca}^{2+}$  concentrations ( $[\text{Ca}^{2+}]_i$ ) in S180 cells and, in combination with CDDP, yielded a significant increase in intracellular platinum accumulation compared to that in cells treated with CDDP alone. These results indicate that DMPS used in combination with CDDP may be of considerable benefit in enhancing the cytotoxicity of CDDP in tumor cells, especially at a low dose. The results also suggest that the enhancing effect of DMPS is closely related to a decrease in  $[\text{Ca}^{2+}]_i$  and that the suitable dose and adequate administrative time of DMPS are important for its effective action.

**Keywords:** 2,3-dimercapto-1-propanesulfonic acid (DMPS), meso-2,3-dimercaptosuccinic acid (DMSA), cisplatin, sarcoma 180 cell, antitumor activity

**Introduction**

2,3-Dimercapto-1-propanesulfonic acid (DMPS) and meso-2,3-dimercaptosuccinic acid (DMSA) are dithiol metal chelators generally considered suitable for treating cases of heavy metal poisoning, including those caused by lead (1–3), mercury (4–7), cadmium (8, 9), and copper (10). These two dimercapto-compounds are highly water soluble, can be taken orally, and are relatively nontoxic, all of which contributes to their excellence for use in detoxication (11).

Many transporters such as organic anion transporters

and organic cation transporters are recognized as key determinants of drug disposition in tissues. Specific transporters in cell membranes allow efficient directional movement of drugs. There is evidence that DMPS and DMSA access the intracellular compartment via the organic anion transporter OAT1 and a sodium-dependent dicarboxylate transporter, respectively, and must do so in order to exert an antidotal effect (12, 13).

Cisplatin (CDDP), a platinum metal complex, is one of the most effective antitumor agents for the treatment of genitourinary cancers, particularly testicular, ovarian, and bladder cancers. However, it can induce significant renal dysfunction and occasional acoustic nerve dysfunction (14). The antidotal effects of a number of chelating agents, including 2,3-dimercaptopropanol, deferoxamine, DMPS, and DMSA, on CDDP-induced nephrotoxicity have been studied; and the results suggest that they mini-

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mize the renal toxicity of CDDP (15 – 17). However, to the authors' knowledge, no studies have investigated the synergistic effects of dimercapto-compounds on the cytotoxicity of CDDP in tumor cells.

An earlier study indicated that DMPS enhanced the antitumor activity of arsenic trioxide in mice transplanted with ascites sarcoma 180 cells (S180 cells), especially at a low dose (18). This suggested that DMPS might also enhance the cytotoxicity of other antitumor agents on tumor cells. To further elucidate the effect of dimercapto-compounds on the cytotoxicity of antitumor agents, we investigated the interactions between dimercapto-compounds and CDDP.

In the present study, we investigated the effect of dimercapto-compounds on the antitumor activity of CDDP in mice transplanted with 180 cells in terms of survival and life span. We further measured intracellular platinum and  $\text{Ca}^{2+}$  concentrations ( $[\text{Ca}^{2+}]_i$ ) to elucidate the cellular mechanisms involved in the process.

## Materials and Methods

### Animals

Four-week-old female SPF ddy mice were purchased from Japan SLC, Inc. (Hamamatsu). The animals were kept in cages with wood chips in an air-conditioned room (temperature  $23 \pm 2^\circ\text{C}$  and lights on between 6:00 am and 6:00 pm) and maintained on commercial laboratory chow and tap water ad libitum for at least one week before they were used in the experiments. All experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society and Tokyo Dental College. All materials used for the animals were sterilized by autoclaving or radiation.

### Chemicals

The chemicals used in this study were purchased from the indicated sources: CDDP, DMPS, DMSA, HEPES, bovine serum albumin, and calf thymus DNA (type 1) from Sigma-Aldrich (St. Louis, MO, USA); Dulbecco's Modified Eagle's Medium (DMEM), Hanks' balanced salt solution (HBSS), fetal calf serum, and antibiotics from Gibco BRL (Grand Island, NY, USA);  $[\text{}^3\text{H}]$ thymidine from Daiichi Chemical Company (Tokyo); and fura-2 acetoxymethyl ester (fura-2/AM) from Molecular Probes (Eugene, OR, USA). HBSS-H was composed of HBSS containing 1.27 mM  $\text{CaCl}_2$ , 0.81 mM  $\text{MgSO}_4$ , and 30 mM HEPES (pH 7.4). All other reagents used were of the highest grade commercially available.

### Preparation and administration of drug

DMPS, DMSA, and CDDP were dissolved in physio-

logical saline, filtered through a  $0.32\text{-}\mu\text{m}$  filter (Millipore Co., Bedford, MA, USA), and prepared immediately before use. A volume of 0.1 ml per 10 g body weight was administered in each mouse. Control mice were given an equal volume of physiological saline.

### Experimental procedures for in vivo tests

S180 cells were a gift from Amano Enzyme, Inc. (Nagoya). A total of  $10^6$  cells suspended in 0.2 ml phosphate-buffered saline (PBS) were injected into the mice via the abdominal cavity. Five mice weighing 23 – 25 g each were prepared in each group. Administration of CDDP with or without dimercapto-compounds was initiated at 5 days after transplantation of the S180 cells and continued for 4 days, once a day. When CDDP was administered at  $5.7\text{ }\mu\text{mol/kg}$ , s.c. or  $17\text{ }\mu\text{mol/kg}$ , p.o., dimercapto-compounds were subcutaneously administered at the same time or at 1 h before CDDP administration, respectively. The antitumor effect was evaluated by increase in life span using the formula  $\text{ILS (\%)} = [(aD/aN) - 1] \times 100$ , where aD is the average number of survival days in the drug-treated group and aN is the average number of survival days in the drug-untreated group. Life span changes were compared between the drug-untreated and CDDP-treated mice transplanted with tumor cells.

### Cell culture

In the in vivo experiment, S180 cells ( $10^6$  cells/0.2 ml PBS) were transplanted into the abdominal cavity of the mice at 7-day intervals. In the in vitro experiment, cells were suspended in DMEM containing 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100  $\mu\text{g/ml}$ ) and then cultured in the incubator at  $37^\circ\text{C}$  under a humidified atmosphere of 5%  $\text{CO}_2$  and 95%  $\text{O}_2$ .

### Measurement of platinum and DNA in S180 cells

Approximately 1 ml S180 cells was collected from the abdominal cavity of the mice at 1 to 5 h after the final administration of CDDP with or without DMPS. Collected cells were lightly washed twice with cold PBS and collected by centrifugation at 1,000 rpm. The pellets obtained were re-suspended in 1 ml PBS and used for measurement of platinum and DNA contents. Measurement of platinum was carried out according to the method described previously (19, 20). Briefly, 1 ml 50%  $\text{Mg}(\text{NO}_3)_2$  was added to the pellet followed by heating for 30 min at  $70^\circ\text{C}$ . A 0.5-ml aliquot of 10 N  $\text{HNO}_3$  was then added to the pellet, after which it was heated for 90 min at  $120^\circ\text{C}$  and then maintained at a temperature of  $140^\circ\text{C}$  –  $160^\circ\text{C}$  until it turned to ash. The product obtained was dissolved in 3 ml 10 N HCl, followed by addition of 0.3 ml 40% KI. The solution was mixed with 3

ml  $\text{CHCl}_3$ . The organic layer containing platinum was transferred to a tube containing 0.26%  $\text{Mg}(\text{NO}_3)_2$ . Finally, the platinum was re-collected from the aqueous layer and its concentration determined with a flameless atomic absorption spectroscope (model AA-640-13; Shimadzu Corporation, Kyoto) equipped with a graphite furnace atomizer (model GFA-3, Shimadzu Corporation). CDDP was used as the standard of platinum. The amount of platinum in the cell was expressed as nmol/ $\mu\text{g}$  DNA. Measurement of DNA was carried out by the diphenylamine method using calf thymus DNA as the standard (21).

#### $[^3\text{H}]$ Thymidine uptake in S180 cells

**Experiment 1:** S180 cells (approximately  $10^5$  cells) collected from mice treated with CDDP and dimercapto-compounds were washed with cold PBS, seeded onto a 96-well plate, and incubated with  $[^3\text{H}]$ thymidine (37 kBq/ml) for 2–24 h under the conditions described above. Cells were collected with a cell-harvester and radioactivity measured with a liquid scintillation counter.

**Experiment 2:** S180 cells (approximately  $10^5$  cells) collected from the drug-untreated mice were washed with cold PBS and cultivated with CDDP in 2 ml culture medium in a 35-mm well plate for 24 h under the conditions described above. Cells were further incubated with  $[^3\text{H}]$ thymidine (37 kBq/ml) for 6 h, collected with a cell-harvester, and radioactivity measured with a liquid scintillation counter.

#### Measurement of $[\text{Ca}^{2+}]_i$

S180 cells collected from the drug-untreated mice were suspended in PBS and seeded onto a non-fluorescent glass plate attached to a silicon rubber well (15-mm diameter, flexiPERM; Heraeus Instruments, Hanau, Germany). Cells were loaded with 2  $\mu\text{M}$  fura-2/AM in DMEM and further incubated for 1 h at  $37^\circ\text{C}$  in the  $\text{CO}_2$  incubator. After incubation, cells were rinsed twice with HBSS-H, re-suspended in the same medium, and incubated at room temperature for 15 min for dye equilibration. For the calcium-deficient experiments,  $\text{CaCl}_2$  was omitted from the HBSS-H. Fluorescent imaging and analysis were performed using the ARGUS 50 (Hamamatsu Photonics K.K., Hamamatsu). Fluorescence was monitored in 'ratio mode' by alternating the excitation wavelength between 340 and 380 nm and measuring fluorescence emitted at 510 nm (22).  $[\text{Ca}^{2+}]_i$  was calculated from the ratio of fluorescence using the calibration curve.

#### Statistical analyses

Statistical analyses were performed by Student's *t*-test and a one-way analysis of variance (ANOVA) followed

by the Dunnett's test. The results obtained were expressed as the mean  $\pm$  S.D. A *P*-value of less than 0.05 was considered to be statistically significant.

## Results

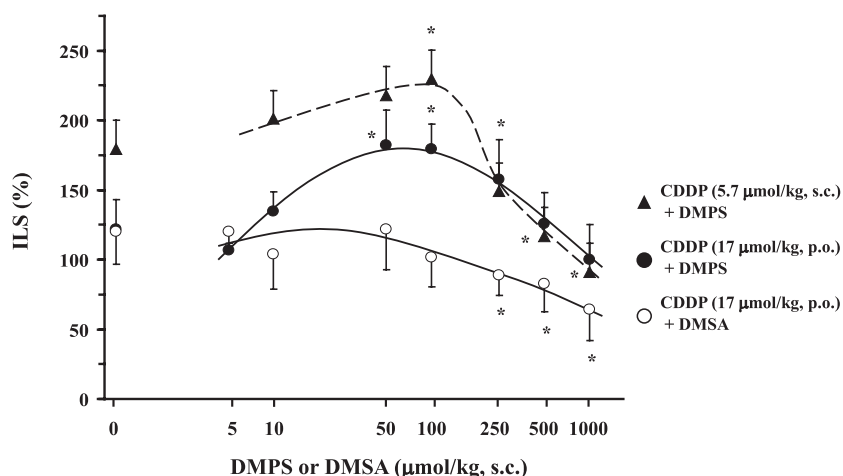
### *Antitumor activity of CDDP with dimercapto-compounds in vivo*

In mice transplanted with S180 cells, CDDP at a dose of 17  $\mu\text{mol/kg}$ , p.o. yielded 119% for ILS and 17.7 days for survival, which was approximately 2-fold the survival in the non-treated mice (Table 1). This CDDP activity was significantly enhanced by simultaneous treatment with DMPS (Fig. 1). At 50, 100, and 250  $\mu\text{mol/kg}$ , s.c. DMPS, ILS values increased to 179%, 174%, and 155%, respectively. No effect was observed at the high dose of 500 or 1,000  $\mu\text{mol/kg}$ . On the other hand, DMSA showed no clear synergistic effect on the antitumor activity of CDDP (Fig. 1). DMSA at a dose of  $>100$   $\mu\text{mol/kg}$ , s.c. yielded a decrease in ILS values. We further examined the synergistic effect of DMPS in terms of the administration route of CDDP. Subcutaneous administration of CDDP at a dose of 5.7  $\mu\text{mol/kg}$  significantly increased the ILS value to 184%, compared with 119% by p.o. administration (Table 1). Number of survival days was 19.9 days, also being slightly higher than the 17.7 days yielded by p.o. administration. This ILS value obtained with CDDP s.c. administration was further enhanced by simultaneous treatment with DMPS, which produced the maximum effect at a dose of 100  $\mu\text{mol/kg}$ , s.c. (Fig. 1). DMPS at doses of 250 and 500  $\mu\text{mol/kg}$  decreased ILS values to 80% and 64% of those with CDDP alone, respectively. These results indicate that specific doses are required to optimize the synergistic effect of DMPS with CDDP and that s.c. administration of CDDP yields more favorable results in vivo.

**Table 1.** Effects of administration route on antitumor activity of CDDP

	Survival days	ILS (%)
Vehicle (p.o.)	8.1 $\pm$ 0.9	—
CDDP (17 $\mu\text{mol/kg}$ , p.o.)	17.7 $\pm$ 1.2	119 $\pm$ 24
Vehicle (s.c.)	7.0 $\pm$ 0.5	—
CDDP (5.7 $\mu\text{mol/kg}$ , s.c.)	19.9 $\pm$ 1.2	184 $\pm$ 15*

S180 cells ( $10^6$  cells) were transplanted into the abdominal cavity of each mouse; and starting at 5 days after transplantation, CDDP was p.o. and s.c. administered once a day for 4 days. Survival days and ILS values were estimated in each group. Each value represents a mean  $\pm$  S.D. (n = 5). \**P* < 0.05, significantly different from the value obtained with p.o. administration of CDDP.



**Fig. 1.** Dose-response effect of dimercapto-compounds on antitumor activity of CDDP. Procedures for the transplantation of S180 cells into mice and administrations of CDDP and dimercapto-compounds at the indicated doses were the same as those described in Table 1. DMPS and DMSA were s.c. administered 1 h before p.o. administration of CDDP or at the same time as s.c. administration of CDDP. The ILS value was estimated in each group. Each value represents a mean  $\pm$  S.D. ( $n = 5$ ). \* $P < 0.05$ , significantly different from value obtained with CDDP alone.

### *[<sup>3</sup>H]Thymidine uptake in S180 cells*

To examine the bioactivity of S180 cells in mice treated with CDDP and dimercapto-compounds, we measured thymidine uptake (Fig. 2). In S180 cells collected from the control mice, uptake showed a linear increase for up to 6 h after incubation, with a slow increase until 24 h after that. CDDP at a dose of 5.7  $\mu\text{mol/kg}$ , s.c. remarkably suppressed uptake in the cells in vivo. Uptake suppressed by CDDP was further inhibited by simultaneous treatment with DMPS at 5 and 50  $\mu\text{mol/kg}$ , s.c. However, at a dose of 1,000  $\mu\text{mol/kg}$ , uptake was increased to a level greater than that with CDDP alone (Fig. 2A). DMSA at 5 and 50  $\mu\text{mol/kg}$ , s.c. had no effect on the thymidine uptake obtained with CDDP. At 1,000  $\mu\text{mol/kg}$ , DMSA increased uptake to a level greater than that with CDDP alone (Fig. 2B). These results showed that DMPS, but not DMSA, enhanced the sensitivity of S180 cells to CDDP and strongly suggest an enhancing effect for DMPS on the antitumor activity of CDDP in vivo.

In the in vitro experiments, we further examined the sensitivity of S180 cells to CDDP (Fig. 3). CDDP at a dose of  $>10$  nM showed a dose-dependent inhibitory curve on thymidine uptake in cultured S180 cells. CDDP at doses of 50 and 1,000 nM suppressed uptake to approximately 54% and 14% of control levels, respectively. This indicates that S180 cells have a high sensitivity to CDDP at doses of  $>10$  nM.

### *Effect of DMPS on CDDP content in S180 cells*

To investigate the pharmacokinetics of CDDP in S180 cells in vivo, we measured platinum content in the cells treated with CDDP at 5.7  $\mu\text{mol/kg}$ , s.c. and DMPS at 50  $\mu\text{mol/kg}$ , s.c., which was the effective combination in terms of antitumor activity in vivo (Fig. 4). In the CDDP-treated cells, intracellular platinum concentrations de-

creased to 67% and 33% of the initial levels at 4 and 5 h after treatment, respectively. In the CDDP plus DMPS-treated cells, intracellular platinum concentrations remained high for a long time. They were the same as the initial levels at 4 h after both drug treatments and were 77% of the initial levels at 5 h after treatment.

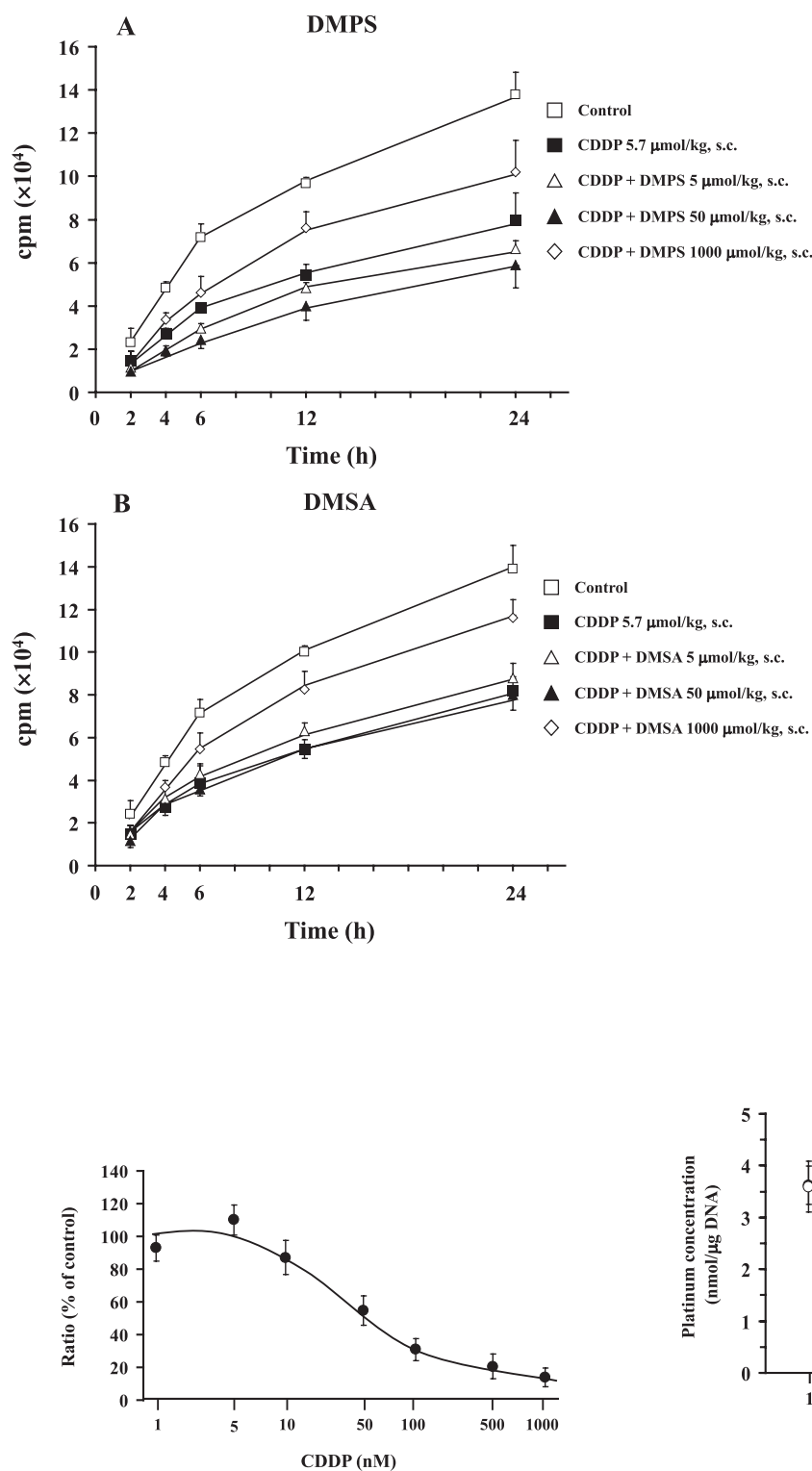
### *Kinetics of $[\text{Ca}^{2+}]_i$ in S180 cells*

Maintenance of intracellular  $\text{Ca}^{2+}$  homeostasis is critical to many cellular functions that rely on  $\text{Ca}^{2+}$  as a messenger. We measured  $[\text{Ca}^{2+}]_i$  in the S180 cells after treatment with DMPS. In the absence of extracellular  $\text{Ca}^{2+}$ , DMPS evoked a time- and dose-dependent inhibitory response in fluorescent intensity of  $\text{Ca}^{2+}$ /fura-2 complexes in the cells (Fig. 5A). The  $[\text{Ca}^{2+}]_i$  significantly decreased to 74% and 62% of basal levels within 2 min after treatment with DMPS at  $10^{-6}$  and  $10^{-5}$  M, respectively (Fig. 5B). In the DMPS-untreated cells, no change was observed during the period monitored.

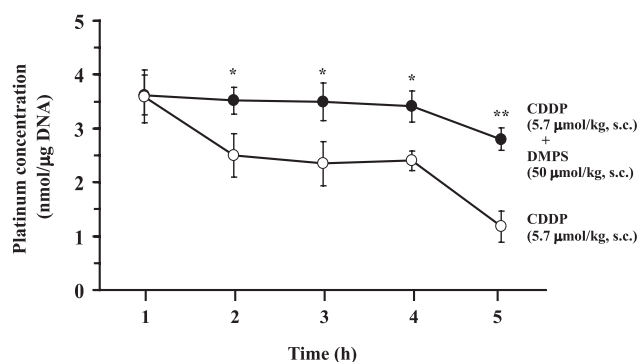
## **Discussion**

This is the first study to show that DMPS, which is the preferred antidote for heavy metal poisoning, effectively enhanced the cytotoxicity of CDDP in tumor cells, whereas DMSA did not.

When either of the two dimercapto-compounds and CDDP were simultaneously administered, DMPS at low doses of  $<250$   $\mu\text{mol/kg}$ , s.c. significantly enhanced the antitumor activity of CDDP on ILS (%), whereas high doses of  $>500$   $\mu\text{mol/kg}$  yielded a decline in the active effects of CDDP. It is especially noteworthy that a dose of 50 to 100  $\mu\text{mol/kg}$ , s.c. resulted in maximum enhancement. On the other hand, DMSA showed no enhancement of the active effect of CDDP in vivo. These results indicate that specific doses of DMPS are required to optimize

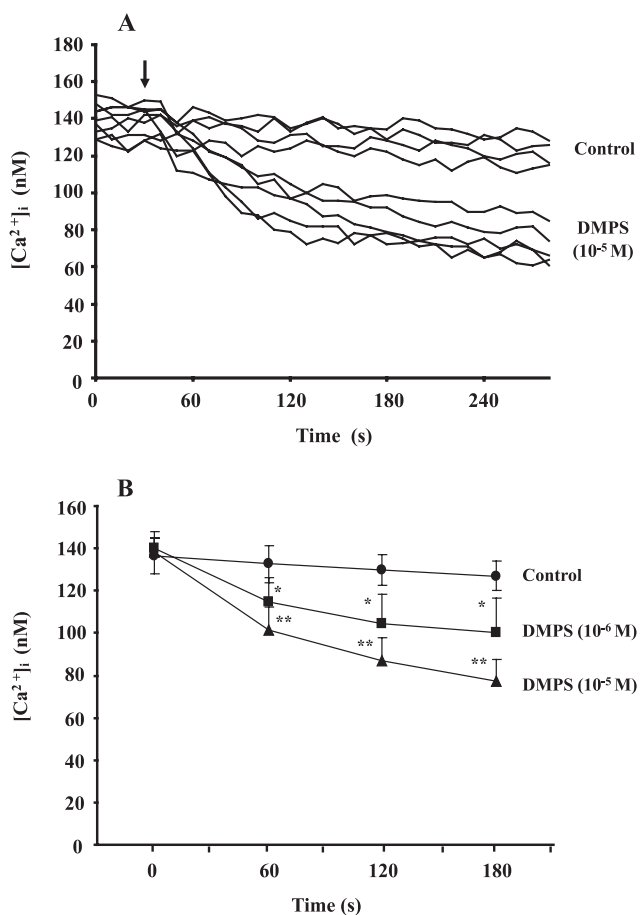


**Fig. 2.**  $[^3\text{H}]$ Thymidine uptake in S180 cells collected from mice treated with CDDP and dimercapto-compounds. S180 cells ( $10^6$  cells) were transplanted into mice; and starting at 5 days after transplantation, CDDP and dimercapto-compounds were administered once a day for 4 days. Cells were collected 1 h after the final administration of each drug, washed with PBS, seeded in a 96-well plate, and further incubated with  $[^3\text{H}]$ thymidine (37 kBq/ml) for the indicated times. Radioactivity was measured as described in Materials and Methods. DMPS (A) and DMSA (B) were s.c. administered at the indicated doses with CDDP (5.7  $\mu\text{mol/kg}$ , s.c.) Each value represents a mean  $\pm$  S.D. (n = 5).



**Fig. 4.** Platinum concentration in S180 cells collected from mice treated with CDDP and DMPS. S180 cells ( $10^6$  cells) were transplanted into mice and CDDP (5.7  $\mu\text{mol/kg}$ , s.c.) and DMPS (50  $\mu\text{mol/kg}$ , s.c.) were administered at 5 days after transplantation. Cells were collected at the indicated times after administration and platinum concentrations in cells measured as described in Materials and Methods. Each value represents a mean  $\pm$  S.D. (n = 5). \* $P < 0.05$ , \*\* $P < 0.01$ , significantly different from the value obtained with CDDP alone.





**Fig. 5.** Effect of DMPS on  $[Ca^{2+}]_i$  in S180 cells. S180 cells collected from drug-untreated mice were seeded onto a non-fluorescent glass plate, cultivated in DMEM, and added with 2  $\mu$ M fura-2/AM as described in Materials and Methods. Before measurement, the medium was replaced with  $Ca^{2+}$ -free HBSS-H. A) Trace of fluorescence intensity after treatment with DMPS. This figure shows data obtained from 4 to 5 independent experiments. DMPS ( $10^{-5}$  M) and vehicle were added at the point indicated by the arrow. B)  $[Ca^{2+}]_i$  in S180 cells after treatment with DMPS. This figure shows data at the times obtained from panel A. Each value represents a mean  $\pm$  S.D. ( $n = 4 - 6$ ). \* $P < 0.05$ , \*\* $P < 0.01$ , significantly different from control values.

its synergistic effect. However, DMPS and DMSA at high doses decreased ILS values of CDDP. Many investigators have demonstrated that a dose of dimercapto-compound of more than 1,000  $\mu$ mol/kg is effective as an antidote to heavy metal poisoning (7, 19, 23–25). Therefore, it is possible that they reacted with CDDP as a metal chelator for heavy metal poisoning, excreting it from the body.

It appears that both appropriate dosage and administration time of DMPS are necessary to achieve the effective synergism with CDDP. Oral and subcutaneous administration results in a time-lag in blood levels between the two drugs. We believe that DMPS exerts a cytotoxic ef-

fect on the S180 cell itself. We empirically confirmed that the time-lag between p.o and s.c. was 30 to 60 min (data not shown). The synergistic effects of DMPS and CDDP were observed with both s.c. administration and time-lag administration. It seems likely that a time-difference in the blood levels of DMPS and CDDP is an important factor in maximizing their synergistic effects.

Contrary to expectations that DMPS would lower the cytotoxicity of CDDP to S180 cells, a low dose of DMPS produced the reverse effect. To confirm this unexpected finding *in vivo*, we measured [ $^3$ H]thymidine uptake in S180 cells collected from mice treated with CDDP and DMPS. When the mice were treated with CDDP at a dose of 5.7  $\mu$ mol/kg, s.c., thymidine uptake was further potently suppressed by simultaneous treatment with DMPS at doses of 5 and 50  $\mu$ mol/kg, s.c. No similar effect was observed in mice treated with DMSA. In terms of *in vitro* thymidine uptake in cultured S180 cells, the cells showed sensitivity to CDDP at dose of  $>10$  nM. These results indicate that S180 cells are drastically damaged by CDDP, and they support the enhancing effects of DMPS, but not DMSA, on the antitumor activity of CDDP *in vivo*. The results also suggest that DMPS may act on the S180 cell itself, together with CDDP, but not on detoxification of the host.

It has been shown that DMPS is transported into cells via the organic anion transporter hOAT1 in antidotal activity in the kidney and red blood cells (12, 22, 26). The tumor cell membrane transport system is comprised of a permeable glycoprotein (P-gp) family, an organic anion transporter (OAT1, 2, and 3 and OAT-K1), an organic anion transporter polypeptide (OATP1), and an organic cation transporter (OCT2) (27–30). Apart from the P-gp family, tumor cell membrane transporters were shown to function in the influx of drugs into cells. It was pointed out that these transporters decreased in cells resistant to chemotherapeutic agents (31). P-gp, an ATP-binding cassette drug efflux pump, functions in the efflux of various toxic compounds and chemotherapeutic agents from cells. Multidrug-resistant tumor cells accelerate outward transport via P-gp and reduce cellular drug accumulation. P-gp has been found to be associated with multiple molecules, and alteration of cellular properties has been attributed to this interaction (32, 33). P-gp function can be blocked with either calcium-channel blockers or inhibitors of calmodulin and cyclosporine A, which bind to the inner binding site of P-gp, competing with P-gp substrates, thus blocking drug efflux from cells (34, 35). These results indicate that intracellular  $Ca^{2+}$  homeostasis is intimately related to the mechanism of P-gp function, and a lowering of  $[Ca^{2+}]_i$  in cells presumably depresses P-gp function. In this study, DMPS treatment produced a time- and dose-dependent decrease in the

intensity of  $\text{Ca}^{2+}$ /fura-2 in S180 cells in the absence of extracellular  $\text{Ca}^{2+}$ . This suggests that DMPS decreases  $[\text{Ca}^{2+}]_i$  in cells, affecting cellular function and transduction. Consequently, we hypothesize that, after being taken up in S180 cells via the organic anion transporter, DMPS 1) binds to intracellular  $\text{Ca}^{2+}$ , decreasing  $\text{Ca}^{2+}$  levels, inhibiting the efflux function of P-gp, and increasing intracellular accumulation of drugs; 2) binds to the binding site of P-gp, competing with P-gp substrates, and inhibiting P-gp function; or 3) accelerates the transport function-related cellular influx of CDDP. Our hypothesis noted in 1) above is strongly supported by the observation that high concentrations of CDDP persisted for a long time in the DMPS-treated S180 cells.

In contrast to the effective action of DMPS, DMSA had no enhancing effect on the cytotoxicity of CDDP on S180 cells or thymidine uptake. The clear distinction between DMPS and DMSA in terms of their interaction with CDDP *in vivo* may be explained by differences in their respective transport systems. DMSA appears to be taken up by a sodium-dependent dicarboxylate transporter (13), but the localization of this transporter remains to be identified in tumor cells. We believe that DMSA can not permeate the membrane of a S180 cell due to its transport system, which thus negates its function against such cells. Further investigation will be necessary to elucidate the mechanism of its transporter.

In conclusion, the present results demonstrate that while DMPS enhances the cytotoxicity of CDDP to S180 cells, DMSA does not, and that its enhancing effect is induced at a low dose and is closely related to a decrease in intracellular  $\text{Ca}^{2+}$  concentrations.

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