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# Sodium thiosulfate inhibits *cis*-diamminedichloroplatinum (II) activity

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***Summary.*** *To elucidate the mechanisms of inactivation of cis-diamminedichloroplatinum(II) (DDP) toxicity by its antidote sodium thiosulfate (STS), we studied the effects of STS on plasma concentrations of platinum (Pt) in vivo, binding of Pt to serum protein in vitro, and uptake of Pt by bacterial cells (E. coli, WP 2 uvrA strain) or cultured mouse tumor cells (FM3A) in vitro. STS did not significantly affect either plasma levels of total Pt or non-protein-bound Pt in vivo, but did inhibit binding of Pt to serum protein and cellular uptake of Pt in vitro. These results suggest that when DDP is given in combination with STS in vivo, the binding to macromolecules and entry of DDP into the cells are prevented due to formation of the Pt-thiosulfate complex in the extracellular fluid.*

## Introduction

*cis*-Diamminedichloroplatinum(II) (DDP), though a potent anticancer drug exhibiting a high activity against a variety of human cancers [4, 21, 22], is extremely toxic to the kidneys [3, 12]. Howell and Taetle [8] and Ishizawa et al. [11] reported that the toxicity of DDP can be reduced by sodium thiosulfate (STS). In our laboratory, 'two-route chemotherapy' with the combination of DDP and STS has been studied in intraperitoneal tumors [18], metastatic liver tumors [19, 20], and bladder tumors [16, 17] in rats, and excellent antitumor effects were obtained without systemic toxicity. In this therapy, the anticancer drug DDP was given locally into tumor tissues in high doses combined with systemic STS. The present study was undertaken to assess the mechanisms of inactivation of DDP by STS, which is the basis of our two-route chemotherapy.

## Materials and methods

DDP (Nippon Kayaku, Co., Ltd, Tokyo, Japan) and STS (purity 99.5%, Wako Pure Chemical Industries, Ltd, Osaka, Japan) were dissolved in 0.9% NaCl solution immediately before use. Female ddY mice (Kyudo, Co., Ltd, Kumamoto, Japan) weighing 22–27 g were used at 6–7 weeks of age. *E. coli* strain WP2 uvrA [6] was grown at 37° C in an L broth containing 10 g polypeptone, 5 g yeast extract, 5 g NaCl, and 1 g glucose in 1,000 ml distilled water. A cultured cell line, FM3A [14] derived from mouse mammary carcinoma was cultured at 37° C in Eagle's minimal essential medium (Nissui Seiyaku, Co., Ltd, Tokyo, Japan) containing 2% fetal bovine serum (M. A. Bioproducts, Walkersville, USA) and L-glutamine (0.3 mg/ml).

In pharmacokinetic studies of DDP, mice received DDP 10 mg/kg IV, immediately followed by STS 530 mg/kg (100-fold molar ratio to DDP) or saline IV, and killed 5 min to 96 h after the DDP injection. The plasma samples obtained from those mice were passed through a CF25 filter (Amicon Corp., Danvers, USA) by centrifugation (1,000 *g*, 10 min, 0–4° C) to obtain the plasma ultrafiltrate. Plasma and plasma ultrafiltrate were provided for the measurement of plasma total Pt and non-protein-bound Pt, respectively.

In the assay of Pt binding to serum protein in vitro, 1.8 ml newborn calf serum (NSC) (GIBCO Lab., New York, USA), 0.1 ml DDP solution, and 0.1 ml STS solution or saline were mixed and incubated in a test tube at 37° C, after which the mixture was passed through a CF25 filter by centrifugation (1,000 *g*, 10 min, 0–4° C) to obtain ultrafiltrate for the measurement of non-protein-bound Pt. Protein-bound Pt was determined by precipitation with cold 5% perchloric acid (PCA) as follows: an aliquot of 2 ml 10% PCA was added to 2 ml of the mixture after incubation. The acid-insoluble precipitate was washed three times with saline by centrifugation (2500 rpm, 30 min) and finally dissolved in 3 ml 1 *N* NaOH for the measurement of Pt content.

Cellular uptake of Pt by bacterial cells was determined as follows: bacterial cells were collected by centrifugation (2800 rpm, 15 min), washed three times, and suspended in saline to a final concentration of $5 \times 10^9$ cells/ml. Then 1 ml DDP solution and 1 ml STS solution or saline were added to test tubes containing 8 ml cell suspension and incubated at 37° C for 30 min. After incubation, the cells were collected by centrifugation (2800 rpm, 15 min) and washed three times with saline to remove extracellular DDP. To the washed cells, 1 ml conc. $HNO_3$ was added and the mixture was digested until $HNO_3$ was evaporated. Finally, digested cells were dissolved with 0.1 ml distilled water to measure Pt content.

The experiment relating to cellular uptake of Pt by cultured mouse tumor cells (FM3A) was performed in the same manner as that for bacterial cells, except that the final cell density was $1 \times 10^6$ cells/ml and centrifugation was performed at 800 rpm for 15 min. Incubation was carried out at 37° C for 30 min at various concentrations of DDP, with or without STS, at a molar ratio of 100 to DDP.

Pt concentrations were determined by flameless atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer, 180–70, Hitachi Co., Ltd, Tokyo, Japan). Under the assay conditions, the reliable detection limit was 0.1 μg/ml

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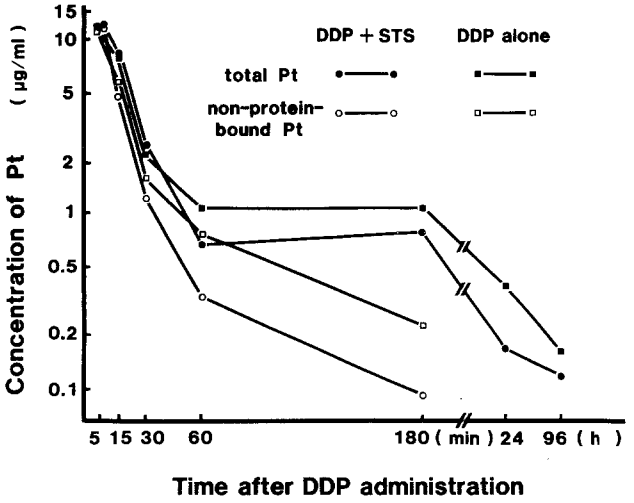


**Fig. 1.** Plasma levels of total and non-protein-bound Pt following IV administration of DDP with or without STS in mice. DDP was given IV in a dose of 10 mg/kg immediately followed by IV saline or STS (100-fold molar ratio to DDP). Each *point* is the mean level of three mice

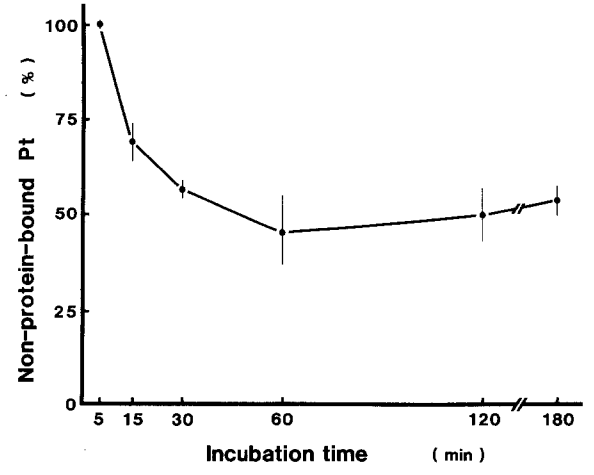


**Fig. 2.** Changes in non-protein-bound Pt in serum in vitro. DDP (2.0 μg/ml) was incubated at 37° C for the indicated times in newborn calf serum. % Non-protein-bound Pt = 100 times non-protein-bound Pt divided by added Pt. Each *point* is the mean of a triplicate sample; *bars* represent SD

for all samples. The recovery rate of Pt was > 90% in all experiments. Statistical analysis was performed by Student's *t*-test.

## Results

Figure 1 shows the time course of total Pt and non-protein-bound Pt levels in mouse plasma after administration of 10 mg/kg DDP with or without STS. There was no significant difference, either in total Pt or in non-protein-bound Pt levels, between the groups given DDP alone and those given DDP with STS (Student's *t*-test).

The effects of STS on binding of Pt to serum protein were also studied in vitro. The time course of the percent level of non-protein-bound Pt in serum is shown in Fig. 2. The concentration of non-protein-bound Pt decreased to 50% of the initial level at 30–60 min, and thereafter the decreased level was maintained for up to 180 min. The reaction of Pt with serum protein in this system reached an end-point within 30 min. Based on this result, the condition of 30 min incubation of DDP with STS in serum was used in the following study. Table 1 shows the effects of STS on the percent level of non-protein-bound Pt in serum, where DDP at a fixed concentration of 5.0 μg/ml was mixed with STS at 0 to 1,000-fold molar ratio to DDP. Although the percent level of non-protein-bound Pt increased depending on the increase of STS, there was no significant difference in the percent level of non-protein-bound Pt between the control group given DDP alone and groups given DDP and STS up to an STS dose of 500-fold molar ratio to DDP. Table 2 shows the effects of STS on the percent level of protein-bound Pt in serum where DDP 50 μg/ml was incubated with or without STS at a molar ratio of 100 in serum. The amount of protein-bound Pt was significantly decreased by STS. Particularly when DDP was preincubated with STS, less Pt was bound to serum protein than when DDP and STS were added to serum concurrently.

Figure 3 shows the uptake of Pt by bacterial cells exposed to DDP 50 μg/ml with STS at increasing molar ratios of 0–400 against DDP. The uptake of Pt was 244 ng (100%) per $10^{10}$ cells when bacterial cells were exposed to DDP without STS. According to the increase of STS the uptake of Pt decreased and the value was reduced to 47 ng (19%) when the molar ratio of STS to DDP was 100. Figure 4 indicates the uptake of Pt by bacterial cells exposed to various concentrations of DDP with or without STS at a molar ratio of 100 against DDP. A linear correlation was seen between DDP concentration and the

**Table 1.** Non-protein-bound Pt after incubation of DDP with STS at various molar ratios in serum in vitro

| Molar ratio (STS/DDP) | % Pt[a] (Mean ± SD) |
|---|---|
| 0 (control) | 60.0 ± 10.4 |
| 50 | 71.8 ± 3.2 |
| 100 | 73.4 ± 7.0 |
| 500 | 75.8 ± 5.6 |
| 1,000 | 84.6 ± 10.8[b] |

DDP (5.0 μg/ml) was incubated for 30 min at 37 °C with various doses of STS in newborn calf serum

[a] % Pt, 100 × non-protein-bound Pt/added Pt

[b] Significantly different from control ($P < 0.05$, Student's *t*-test)

**Table 2.** Protein-bound Pt after incubation of DDP with or without STS in serum in vitro

| Group | % Pt[b] (Mean ± SD) |
|---|---|
| DDP alone | 15.24 ± 1.36 (a) |
| DDP + STS | 6.48 ± 0.96 (b) |
| DDP + STS[a] | 2.74 ± 0.76 (c) |

DDP (50 μg/ml) was incubated at 37 °C for 30 min with or without STS at a molar ratio of 100 to DDP in newborn calf serum

[a] DDP and STS was preincubated at room temperature for 10 min before incubation in serum

[b] % Pt = 100 × protein-bound Pt/added Pt, *P* values for (a) vs (b), (a) vs (c), and (b) vs (c) are, respectively, < 0.001, < 0.001, < 0.01

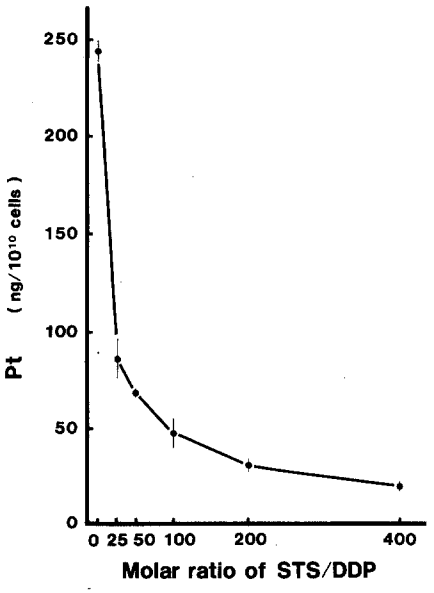


**Fig. 3.** Effects of STS on Pt uptake by bacterial cells as a function of molar ratios of STS to DDP. Cells were exposed for 30 min at 37° C to DDP 50 μg/ml with various doses of STS. Each *point* is the mean of a triplicate sample; *bars* represent SD

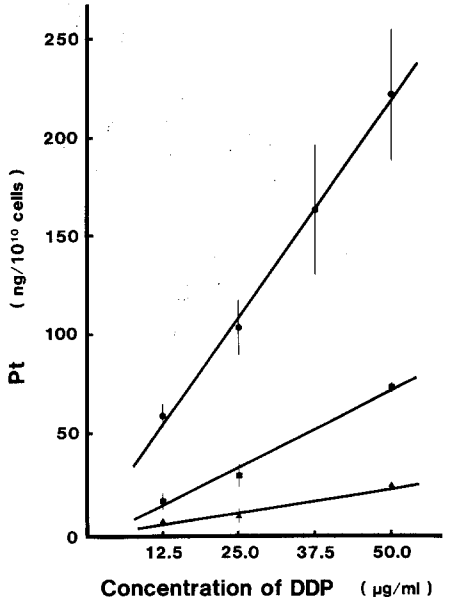


**Fig. 4.** Effect of STS on Pt uptake by bacterial cells as a function of DDP concentrations. Cells were exposed for 30 min at 37° C to various concentrations of DDP with (■, ▲) or without (●) STS (100-fold molar ratio to each DDP concentration). DDP, STS, and cells were mixed simultaneously and incubated (■). DDP and STS were preincubated for 10 min at room temperature before incubation with cells (▲). Each *point* is the mean of a triplicate sample; *bars* represent SD

uptake of Pt by bacterial cells, and the uptake was reduced to one-third of that with exposure to DDP alone by combining STS at a molar ratio of 100 with DDP. When DDP and STS were preincubated for 10 min before the exposure to bacterial cells the uptake of Pt was reduced to one-tenth of that seen with DDP alone.

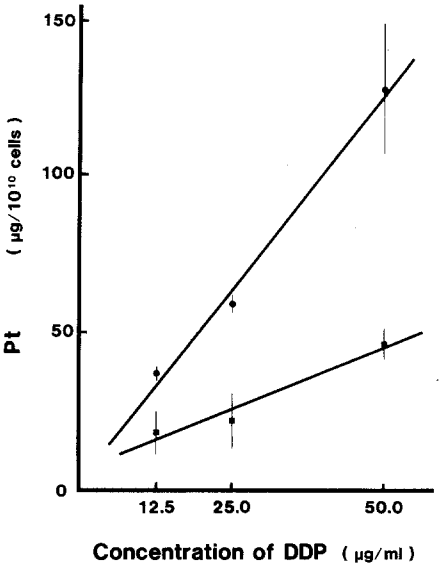


**Fig. 5.** Effects of STS on Pt uptake by FM3A cells as a function of DDP concentrations. Cells were exposed for 30 min at 37° C to various concentrations of DDP with (■) or without (●) STS (100-fold molar ratio to each DDP concentration). Each *point* is the mean of a triplicate sample; *bars* represent SD

Essentially similar results were obtained when cultured mouse tumor cells were used instead of bacterial cells (Fig. 5). The uptake of Pt by the tumor cells was also reduced to one-third of that with exposure to DDP alone by the combination of STS.

## Discussion

Cellular Pt determined in the present experiments using bacterial cells and tumor cells seemed to include not only intracellular Pt but also Pt adhering to the cell membrane. However, it is most likely that the Pt species weakly adhering to the cell membrane can be removed by extensive washing with saline after incubation with DDP. Manaka and Wolf reported that the uptake of Pt species by red blood cells occurs rapidly through cell membrane without significant binding of Pt to the membrane [13]. From these facts, it is conceivable that the Pt amount we estimated is almost the intracellular Pt.

DDP reacts with STS and form $Pt(S_2O_3)_4^{6-}$ according to platinum coordination chemistry [1], and the formation of this Pt-thiosulfate complex seems to be the main cause of inactivation of DDP by STS [11]. In the present study, we indicated that STS does not significantly affect either plasma levels of total Pt or non-protein-bound Pt in vivo, but does reduce the binding of Pt to serum protein and cellular uptake of Pt, in vitro, compared with findings recorded after DDP alone. Since Guarino et al. reported that the toxicity of DDP may be caused by inhibition of ATPase [7], the inhibition of binding of Pt to serum protein by STS which we observed suggests that STS can prevent DDP from attacking macromolecules such as ATPase. From these facts, we speculate that when DDP is given with STS in vivo the Pt-thiosulfate complex is formed in extracellular fluid and this complex is cleared from plasma without binding to macromolecules and cellular uptake.

The reduction of protein-bound Pt and cellular uptake of Pt by STS were enhanced by preincubation of DDP with STS for 10 min (Table 2, Fig. 4). This suggests that more Pt-thiosulfate complex was formed by preincubation than when DDP, STS, and serum or cell suspension were simultaneously mixed. However, the reaction of DDP with STS in extracellular fluid is considered to be rapid, because protein-bound Pt and cellular uptake of Pt were significantly reduced compared with the findings after DDP alone, even when STS was added to serum or cell suspension concurrently with DDP.

Active Pt species in vivo are thought to be present in the fraction of non-protein-bound Pt species, because protein-bound Pt species exhibit neither antitumor effects nor toxic effects [2, 5, 15]. Therefore, the non-protein-bound Pt species in plasma has been regarded as a biologically active Pt species. However, non-protein-bound Pt after DDP administration in combination with STS is probably not the same as that when DDP is administered alone, because STS converts DDP to an inactive species. The level of non-protein-bound Pt does not always reflect the biological activity of Pt species when DDP has been administered in combination with STS; our previous reports confirmed that STS completely reduced DDP toxicity [11, 16, 17, 19, 20], and in the present study STS did not affect the plasma level of non-protein-bound Pt in vivo. Howell et al. [9, 10] treated intraperitoneal tumors in humans using DDP combined with STS. They determined 'DDTC-reactive cisplatin' in the fraction of non-protein-bound Pt species using the chelating action of DDTC as the biologically active Pt species. The level of DDTC-reactive cisplatin they observed, however, does not always indicate the level of biologically active Pt species, because in our another study (data not shown), DDTC seemed to chelate Pt from the Pt-thiosulfate complex. As there is no appropriate method of determining the level of biologically active Pt species in vivo, particularly when DDP is given in combination with STS, we are now attempting to design a bioassay for DDP.

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## References

1. Basole F, Pearson RG (1958) A study of metal complexes; Mechanisms of inorganic reactions. Wiley, New York
2. Cole WC, Wolf W (1981) Renal toxicity studies of protein-bound platinum(*cis*). Chem Biol Interact 35: 341
3. Dentino M, Luft FC, Yum MN, Williams SD, Einhorn LH (1978) Long-term effect of *cis*-diamminedichloroplatinum (CDDP) on renal function and structure in man. Cancer 41: 1274
4. Einhorn LH, Donohue J (1977) *cis*-Diamminedichloroplatinum, vinblastin, and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 87: 293
5. Gormley PE, Bull JM, LeRoy AF, Cysyk R (1979) Kinetics of *cis*-dichlorodiammineplatinum. Clin Pharmacol Ther 25: 351
6. Green MH, Muriel WJ (1976) Mutagen testing using trp reversion in *Escherichia coli*. Mutat Res 38: 3
7. Guarino AM, Miller Ds, Arnold ST, Pritchard JB, Davis RD, Urbanek MA, Miller TJ, Litterst CL (1979) Platinate toxicity: past, present, and prospects. Cancer Treat Rep 63: 1475
8. Howell SB, Taetle R (1980) Effect of sodium thiosulfate on *cis*-dichlorodiammineplatinum(II) toxicity and antitumor activity in L1210 leukemia. Cancer Treat Rep 64: 611
9. Howell SB, Pfeifle CL, Wung WE, Olshen RA, Lucas WE, Yon JL, Green M (1982) Intraperitoneal cisplatin with systemic thiosulfate protection. Ann Intern Med 97: 845
10. Howell SB, Pfeifle CE, Wung WE, Olshen RA (1983) Intraperitoneal *cis*-diamminedichloroplatinum with systemic thiosulfate protection. Cancer Res 43: 1426
11. Ishizawa M, Taniguchi S, Baba T (1981) Protection by sodium-thiosulfate and thiourea against lethal toxicity of *cis*-diammine-dichloroplatinum(II) in bacteria and mice. Jpn J Pharmacol 31: 883
12. Madias NE, Harrington JT (1978) Platinum nephrotoxicity. Am J Med 65: 307
13. Manaka RC, Wolf W (1978) Distribution of *cis*-platin in blood. Chem Biol Interact 22: 353
14. Nakano N (1966) Establishment of cell lines in vitro from a mammary ascites tumor of mouse and biological properties of the established cell lines in a serum containing medium. Tohoku J Exp Med 88: 69
15. Patton TF, Himmelstein KJ, Belt R, Bannister SJ, Sternson LA, Repta AJ (1978) Plasma levels and urinary excretion of filterable platinum species following bolus injection and iv infusion of *cis*-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 62: 1359
16. Sagiyama K, Uozumi J, Baba T (1982) "Two route infusion chemotherapy" of *cis*-diamminedichloroplatinum(II) and its antidote, sodium thiosulfate, for rat bladder tumor [in Japanese]. Nippon Hinyokika Gakkai Zasshi (Jpn J Urol) 73: 287
17. Sagiyama K, Uozumi J, Aoki K, Baba T (1983) Efficacy of "two-route chemotherapy" using intra-arterial cisplatin and iv sodium thiosulfate, its antidote, in rat bladder tumor. Cancer Treat Rep 67: 567
18. Taniguchi S, Baba T (1982) "Two route chemotherapy" using *cis*-diamminedichloroplatinum(II) and its antidote, sodium thiosulfate, for peritoneally disseminated cancer in rats. Gann 73: 477
19. Uozumi J, Sagiyama K, Taniguchi S, Iwamoto Y, Aoki K, Baba T (1982) "Two route infusion chemotherapy" using *cis*-diammine-dichloroplatinum(II) and its antidote, sodium thiosulfate, for metastatic liver tumors in rats. Jpn J Surg 12: 456
20. Uozumi J, Sagiyama K, Aoki K, Iwamoto Y, Baba T (1983) Effectiveness of "two-route chemotherapy" using cisplatin and its antidote, sodium thiosulfate, on lifespan of rats bearing metastatic liver tumors. Cancer Treat Rep 67: 1067
21. Yagoda A (1979) Phase II trials with *cis*-diamminedichloroplatinum(II) in the treatment of urothelial cancer. Cancer Treat Rep 63: 1565
22. Young RC, Von Hoff DD, Gormley P, Makuch R, Cassidy J, Howser D, Bull JM (1979) *cis*-Dichlorodiammineplatinum(II) for the treatment of advanced ovarian cancer. Cancer Treat Rep 63: 1538

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