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# Sodium thiosulfate fails to increase the therapeutic index of intravenously administered *cis*-diamminedichloroplatinum (II) in mice bearing murine and human tumors*

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**Summary.** Intravenous (i.v.) administration of sodium thiosulfate reduces the toxicity of *cis*-diamminedichloroplatinum (II) (CDDP). This effect, which allows the use of increased CDDP doses, has been exploited clinically in the intraperitoneal (i.p.) treatment of intraabdominal tumors. Recently, attempts have been made to treat extraperitoneal tumors by concurrent i.v. administration of CDDP and sodium thiosulfate. We have tested this approach in mice bearing systemic L1210 leukemia, s.c. growing Lewis lung carcinoma, $C_3H$ mammary carcinoma, and a human sarcoma growing in athymic nude mice. In all cases the antitumor activity of CDDP was substantially reduced in a manner dependent on the thiosulfate dose. Increased doses of CDDP, permitted by reduced toxicity in the presence of thiosulfate, raised the antitumor activity. However, the latter did not exceed that obtained by much lower doses in the absence of thiosulfate. The present experiments in animal models thus fail to support the clinical use of CDDP given i.v. together with its antidote, sodium thiosulfate.

## Introduction

*cis*-Diamminedichloroplatinum (II), CDDP, is one of the most potent anticancer agents in current use, with activity against a wide range of human tumors [4, 6, 9, 18, 26–28]. However, CDDP also has strong side effects, particularly on the kidneys. In attempts to increase the therapeutic index of CDDP, a number of procedures have been introduced to reduce its dose-limiting nephrotoxicity. These include hydration [7] and the use of diuretics [11, 20, 25], as well as the administration of nucleophilic agents that react with the active species of CDDP [2, 5, 12–15, 17, 21–24, 29]. Hypertonic saline [8, 16, 19] has also been attempted. However, previous experiments on animal models have shown that the advantage gained in reduced toxicity by giving the drug in a high-NaCl vehicle was offset by a concurrent reduction in antitumor activity [1].

The use of sodium thiosulfate administration to reduce the toxicity of CDDP has been extensively studied by Howell et al. [12–14] and Uozomi et al. [23, 24]. Thiosulfate interacts with and neutralizes CDDP and has the advantage of being concentrated in the urine. It was shown, both in experimental animals [12, 23] and in patients [13, 14, 21], that when thiosulfate was concurrently given, much larger doses of CDDP could be tolerated. The data indicated that i.v. administration of thiosulfate will effectively inactivate CDDP reaching the systemic circulation after intracavitary CDDP administration [13, 14]. Partly on this basis, Howell and associates [13] later introduced i.v. administration of thiosulfate, concurrently with i.p. administration of CDDP, in the treatment of ovarian carcinoma and other tumors in the abdominal cavity.

The present study was prompted by recent attempts to treat patients with extraperitoneal tumors by giving both CDDP and thiosulfate concurrently by the i.v. route [21]. Since the validity of this procedure has not been adequately documented, we found it necessary to reexamine the approach in animal models. It was found that concurrent i.v. administration of thiosulfate markedly reduced the antitumor effect of CDDP in several murine and human tumor models in mice. However, the therapeutic index could not be increased by the use of the higher CDDP doses permitted due to the drug's decreased toxicity in the presence of thiosulfate. The data fail to support the clinical practice of giving both CDDP and thiosulfate by the i.v. route [21].

## Materials and methods

*Chemicals.* CDDP (clinical formulation, Platinol) was purchased from Bristol Laboratories, Syracuse, NY, USA, through the Pharmaceutical Department of the Norwegian Radium Hospital. The vials were stored at 4° C, and the drug was reconstituted immediately before use by the addition of distilled water. Sodium thiosulfate was obtained from E. Merck AG, Darmstadt, FRG, and was dissolved in 0.9% NaCl before use.

The CDDP and the sodium thiosulfate, dissolved in volumes of 0.2–0.3 ml, were injected separately and by separate punctures, into the tail veins of mice or intraperitoneally. The sodium thiosulfate was given shortly before the CCDP, and the injections were always performed at the same hour of the day (1 p.m. to 3 p.m.).

*Animals and tumors.* Male DBA/2, $B6D2F_1$ (C57BL/6xDBA/2)$F_1$ and $C_3D_2$ mice, 6–8 weeks old, were used. The animals were housed in plastic cages and were allowed free access to food pellets and tap water. There was a 12-h light-dark cycle.

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Nude, athymic mice (NMRI background) were bred in our own nude mouse facility from animals originating from the Laboratory Breeding and Research Center, Gl. Bomholt Gaard, Ry, Denmark. These animals were housed in isolators at a constant temperature (26° – 28° C) and humidity (60%–70%). The cages, bedding, and water were sterilized by autoclaving, and the food was sterilized by gamma irradiation.

The murine tumor lines, the L1210 mouse leukemia, the Lewis lung carcinoma, and the $C_3H$ mammary carcinoma were obtained originally from the National Cancer Institute, Bethesda, Md, USA. The leukemia was transplanted serially as ascites tumors in DBA/2 mice. The Lewis lung carcinoma and the $C_3H$ mammary carcinoma had been maintained in serial passage by i.m. injection of minced tumor tissue into the right hind legs of $B6D2F_1$ mice and $C_3D_2$ mice.

The human tumor line, MHMX, an undifferentiated sarcoma, had been established as subcutaneous xenografts in athymic, nude mice from biopsy specimens taken at the Norwegian Radium Hospital. The tumor was maintained by serial transplantation.

*Assay of antineoplastic activity.* In the case of the L1210 tumor, groups of 5–6 DBA/2 mice each were given an i.v. injection of $1 \times 10^6$ tumor cells. Twenty-four hours later, all animals except the mice in the control group were given sodium thiosulfate and CDDP, each in 0.2-ml doses. The anticancer activity of the different combinations was gauged by measuring the survival time of the treated, tumor-bearing mice. Antitumor activity was expressed as the mean percentage increase in the life span (ILS) compared to that of the untreated animals.

In the case of Lewis lung carcinoma and the $C_3H$ mammary carcinoma, minced tumor tissue was injected s.c. into $B6D2F_1$ and $C_3D_2$ mice (groups of six animals each). After the tumors had reached a mean diameter of 6–8 mm, the animals were randomized to the different groups. The treated animals were given a single injection of thiosulfate and of CDDP. The tumor growth was followed by measurements of two perpendicular diameters and was expressed as the mean tumor diameter difference from the start of treatment.

In the case of the human tumor line, MHMX, nude NMRI mice (groups of eight animals each) with subcutaneous tumors of about 5 mm were given the thiosulfate and CCDP doses i.v., three times with a weekly interval. The tumor growth was measured (see above) and expressed as the mean tumor diameter difference.

## Results

### *Effect of thiosulfate on the toxicity of CDDP*

The effect of thiosulfate administration on the toxicity of CDDP in mice is shown in Table 1. It can be seen that at the doses of 25 mg/kg and 30 mg/kg CDDP, concurrent administration of 800 mg/kg thiosulfate reduced the number of deaths and enhanced the survival time of the dying animals. The results confirm that thiosulfate is able to reduce the in vivo toxicity of CDDP [12–14, 22–23].

### *Reduction of antitumor activity of CDDP by thiosulfate*

*L1210 mouse leukemia.* The effect of increasing doses of thiosulfate on the antitumor activity of CDDP against L1210 mouse leukemia is shown in Fig. 1. Two doses were used, 5 mg/kg and 10 mg/kg. In our experience 10 mg/kg is the highest dose tolerated without unacceptable toxicity by these animals. It can be seen that, in the absence of thiosulfate, the doses of 5 mg/kg and 10 mg/kg gave increased ILSs of 35% and 70%, respectively. Administration of thiosulfate immediately before the CDDP resulted in a marked and dose-dependent reduction in the antitumor activity. For example, with 800 mg/kg thiosulfate, a dose which strongly reduced CDDP toxicity (Table 1) [12, 22], the ILS was reduced from 70% to about 35%, i.e., to the level obtained with only 5 mg/kg CDDP in the absence of thiosulfate.

The question arises as to whether an increased therapeutic index could be achieved by raising the CDDP dose over and above the maximum level tolerated in the absence of thiosulfate. The results in Fig. 2 show that this is not the case. When the CDDP dose was raised to 20 mg/kg in the presence of 800 mg/kg thiosulfate, the ILS was

**Table 1.** Ability of thiosulfate to reduce toxicity of CDDP in mice

| CDDP dose (mg/kg) | $S_2O_3$ | Survival time of dying animals (days ± SD[a]) | Survivors (no./total) |
|---|---|---|---|
| 20 | – | | 6/6 |
| 20 | + | | 6/6 |
| 25 | – | 5.9 ± 2.8 | 4/6 |
| 20 | + | | 6/6 |
| 30 | – | 4.7 ± 0.5 | 0/6 |
| 30 | + | 11.2 ± 4.4 | 3/6 |
| 35 | – | 3.4 ± 0.4 | 0/6 |
| 35 | + | 5.1 ± 0.4 | 0/6 |

Groups of six mice each were treated i.v. with the CDDP doses indicated in the presence (800 mg/kg) or absence of sodium thiosulfate. The animals were oberved for 4 weeks

[a] SD

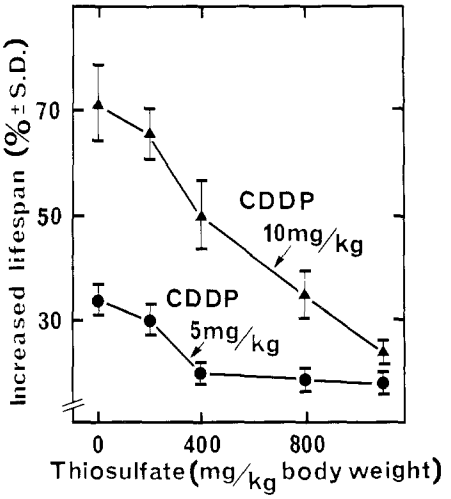


**Fig. 1.** Effect of thiosulfate on the antitumor activity of CDDP against L1210 leukemia. Groups of mice were injected with $1 \times 10^6$ L1210 tumor cells i.v., treated on the next day with either 5 mg or 10 mg CDDP/kg body weight and with increasing doses of thiosulfate i.v. as indicated, and the life span was recorded. Each data point represents the calculated ILS ± SD for 8–30 animals

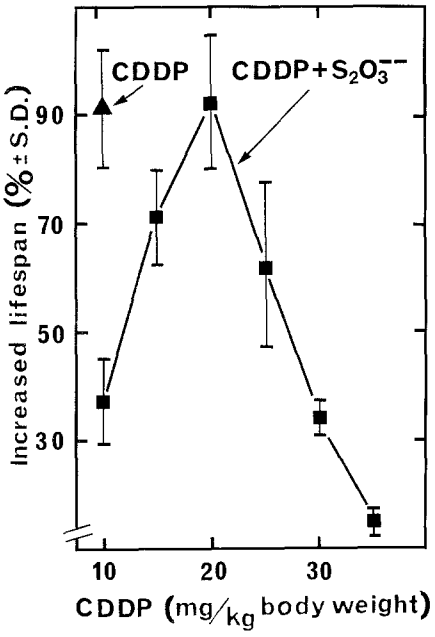


Fig. 2. Effect of increasing CDDP doses together with a fixed dose of thiosulfate on the ILS of L1210 leukemic mice. Groups of animals were treated with 800 mg/kg thiosulfate and immediately thereafter with CDDP doses as indicated (■). One group of animals was treated with 10 mg/kg CDDP in the absence of thiosulfate (▲). Each data point represents the average ILS ± SD of eight animals

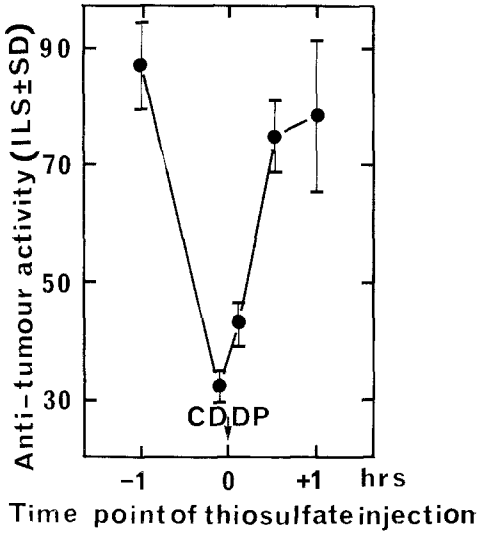


Fig. 3. Effect of time of injection of sodium thiosulfate, relative to administration of CDDP, on CDDP antitumor activity against L1210 leukemia. The thiosulfate dose was 800 mg/kg, and the CDDP dose 10 mg/kg. Each data point represents the mean ± SD of seven to eight animals

raised to the level observed with 10 mg/kg CDDP in the absence of thiosulfate. However, when the CDDP dose was raised further, the ILS decreased, apparently due to increasing toxicity.

Howell and Taetle [12] have shown that thiosulfate can reduce the toxicity of CDDP only when the thiosulfate is given within a short period of time, viz, between 1 h before and 30 min after CDDP administration. The results in Fig. 3 show that the ability of thiosulfate to counteract the

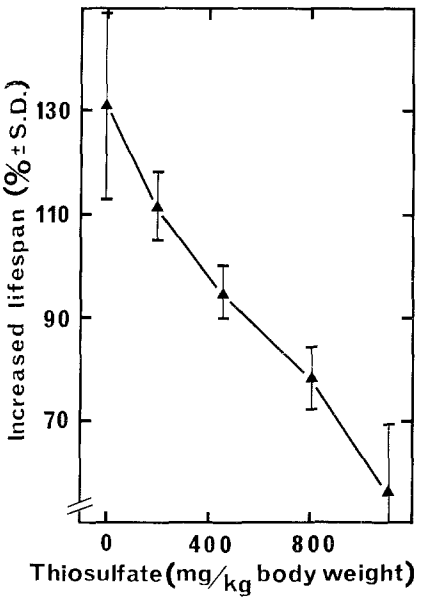


Fig. 4. Effect of thiosulfate on the antitumor activity of CDDP against L1210 leukemia. The CDDP (10 mg/kg) was given i.p., and the thiosulfate i.v. Each data point represents the mean ± SD of eight animals

antitumor activity of CDDP shows closely the same temporal relationships.

It has been reported that when CDDP was administered i.p. and the thiosulfate was given i.v., the plasma contained undiminished concentrations of active CDDP [13, 14]. The question was therefore asked as to whether the antitumor effect of CDDP exerted outside the peritoneal cavity would be unaffected by i.v. thiosulfate administration. The results in Fig. 4 do not support this view: in the experiment shown, mice with L1210 leukemia were treated i.p. with CDDP and i.v. with thiosulfate. It can be seen that thiosulfate administration produced a pronounced and dose-dependent reduction in the antitumor activity of the CDDP.

*Solid murine tumors.* Experiments with Lewis lung carcinoma are shown in Fig. 5. It can be seen that 10 mg CDDP given i.v. on day 6 had a definite although transient growth-inhibiting effect. When 800 mg/kg thiosulfate was given immediately prior to the CDDP, the growth curve was not significantly different from that of the untreated control animals.

Data from similar experiments with $C_3H$ mammary cancer are presented in Fig. 6. In this case, three levels of thiosulfate were used. It is apparent that CDDP alone had a strong growth-inhibiting effect. This was slightly reduced by 200 mg/kg thiosulfate, whereas 450 mg/kg and 800 mg/kg almost abolished the antitumor activity of CDDP.

*A human sarcoma.* The results obtained with a human sarcoma xenograft in athymic mice is shown in Fig. 7. The animals were treated three times, as indicated, with a dose of 6 mg/kg CDDP in the presence and absence of 400 mg/kg thiosulfate. Again, it is evident that the thiosulfate treatment clearly reduced the antitumor effect of CDDP.

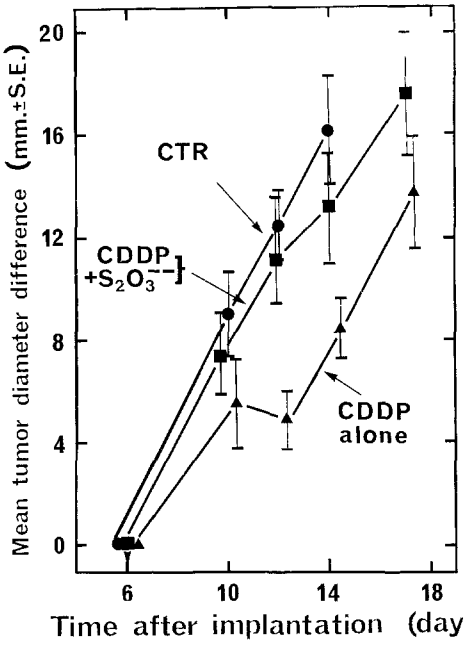


**Fig. 5.** Effect of thiosulfate on the antitumor activity of CDDP against s.c. growing Lewis lung carcinoma. The animals were treated on day 6 with CDDP (10 mg/kg) and with thiosulfate (800 mg/kg). Each data point represents the mean ± SE for five animals. ▲, CDDP alone; ■, CDDP ± $S_2O_3^{--}$; ●, untreated controls

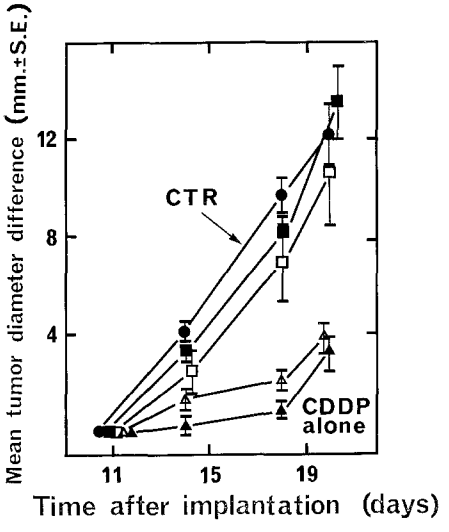


**Fig. 6.** Effect of increasing doses of thiosulfate on antitumor activity of CDDP against s.c. growing $C_3H$ mammary carcinoma in mice. The CDDP dose was 8 mg/kg. Each data point represents the mean ± SE for six animals. ▲, CDDP alone; △, CDDP + 200 mg/kg of $S_2O_3^{--}$; □, CDDP + 450 mg/kg of $S_2O_3^{--}$; ■, CDDP + 800 mg/kg of $S_2O_3^{--}$; ●, untreated controls

## Discussion

The clinical usefulness of CDDP is limited by its pronounced toxicity. Since the dose-effect curves for sensitive tumors are steep [10], treatment results might be significantly improved by the use of higher CDDP doses permitted by procedures reducing the dose-limiting toxicity of CDDP, provided that these do not concurrently reduce the

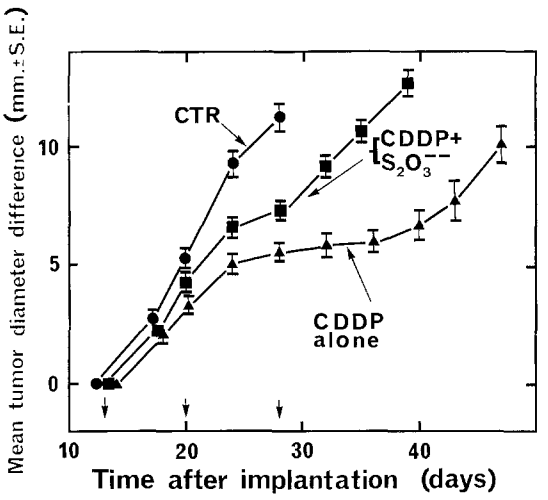


**Fig. 7.** Effect of thiosulfate on the growth-inhibiting effect of CDDP against an s.c. growing human sarcoma xenograft. The animals were treated as described in Materials and methods. The CDDP dose was 6 mg/kg and the thiosulfate dose was 400 mg/kg. Each data point represents the mean ± SE for eight animals. ▲, CDDP alone; ■, CDDP + $S_2O_3^{--}$; ●, untreated controls

antitumor activity of CDDP. Some procedures such as hydration [7] and the use of diuretics [11, 20, 25] have indeed increased the therapeutic index of CDDP.

The nucleophilic reagent thiosulfate interacts with CDDP to form covalently bonded complexes that are biologically inactive. Thus, when the two compounds are mixed together in the syringe, the CDDP becomes completely inactivated [12]. That thiosulfate counteracts the antiproliferative activity of CDDP under conditions where the two compounds are permitted to interact has been clearly demonstrated in several systems, both in vitro and in vivo [12–15, 23, 24]. However, the practice of giving thiosulfate i.v. in conjunction with i.p. administration of CDDP for the treatment of intraabdominal tumors has a rational basis. In this case, the antidote is administered into a different compartment than the CDDP, and this procedure permits the tumor to be exposed to increased concentrations of CDDP in the peritoneal fluid. On the other hand, the concurrent i.v. administration of thiosulfate and CDDP is a procedure which a priori should be viewed with caution for the reasons given above, and it should not be used without firm evidence of its validity.

In the present study on several leukemic and solid tumors, including a human tumor xenograft, the i.v. administration of thiosulfate consistently resulted in a strong reduction in the antitumor activity of CDDP when the latter was concurrently given by the same route. Significantly, the antitumor activity could not be increased above the level achieved with a much lower CDDP dose in the absence of thiosulfate. In view of the facts mentioned above, these findings did not come as a surprise.

The new strategy of Pfeifle and associates [21], involving concurrent administration of both CDDP and thiosulfate by the i.v. route, seems to be largely based on two premises. The first is that the i.v. use of thiosulfate in combination with CDDP blocks nephrotoxicity while retaining therapeutic activity [22]. However, what the data of Poore et al. [22] actually showed was that, in agreement with our findings, it was necessary to increase the CDDP dose to re-

tain antitumor activity when thiosulfate was concurrently given.

The second premise was that the amount of free, biologically active CDDP species in plasma is unchanged by thiosulfate administration [13, 14], a finding that seemed paradoxical [13, 14], since the thiosulfate might be expected to interact with and inactivate CDDP in plasma. Our own experiments, in which animals with systemic L1210 leukemia were treated with CDDP i.p. and thiosulfate i.v., provided evidence that the cytotoxic activity of CCDP in the blood was decreased. Thus, the data clearly showed that the antitumor effect decreased progressively with the thiosulfate dose. Moreover, Iwamoto et al. [15] have found that the plasma of rabbits treated with CDDP and thiosulfate i.v. had reduced antiproliferative capacity, and it was suggested [15] that the chemical assay used [3] by Howell and associates [13, 14] may not adequately measure the concentration of biologically active *cis*-platinum species in the presence of thiosulfate.

In conclusion, the present experiments fail to support the experimental basis for the concurrent i.v. use of CDDP and its antidote, sodium thiosulfate. Although in the animal models studied the thiosulfate protected against the toxicity of CDDP, permitting higher doses to be given, it markedly inhibited the antitumor activity of the drug. On these bases no increased clinical benefit is to be expected from this procedure.

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