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Selenium-induced Protection against *cis*-Diamminedichloroplatinum(II) Nephrotoxicity in Mice and Rats[1]

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ABSTRACT

The influence of selenium on *cis*-diamminedichloroplatinum(II) (c-DDP) nephrotoxicity in mice and rats was assessed, using single doses of both compounds. Sodium selenite, 2 mg of selenium per kg, given 1 h before c-DDP, greatly reduced blood urea nitrogen and creatinine levels and morphological kidney damage in both BALB/c mice and Wistar rats, while administration 1 h after c-DDP did not. Liver toxicity of selenium was evaluated by measuring serum glutamic pyruvate transaminase and serum glutamic oxalate transaminase and by routine histology. No liver damage was observed in animals treated with sodium selenite, 2 mg of selenium per kg, and physiological saline or c-DDP. Pretreatment with sodium selenite did not reduce the antitumor activity of c-DDP against MPC 11 plasmacytoma or Prima breast tumor in BALB/c mice.

The present results indicate that sodium selenite may provide protection against c-DDP nephrotoxicity, when it is given before c-DDP. Moreover, selenium has an antineoplastic activity against several tumors. The combination of these qualities may open new perspectives in cancer chemotherapy.

INTRODUCTION

c-DDP[3] is an important antineoplastic drug, widely used against various tumors (1). However, c-DDP has severe side effects, notably toxicity to the kidneys, the gastrointestinal tract, the peripheral nerves, and the bone marrow (1, 2). The pathology of c-DDP nephrotoxicity, the dose-limiting factor in clinical studies (3), as well as various methods to reduce c-DDP toxicity have been described (4), but its molecular mechanism is still unknown.

Several attempts have been made to improve the therapeutic index of c-DDP. (*a*) Various platinum analogues have been developed, which are less nephrotoxic than c-DDP. Carboplatin is indeed less nephrotoxic, but severe bone marrow depression and a different antitumor spectrum limit its usefulness (5). (*b*) Hydration and induction of chloruresis provide some protection against c-DDP nephrotoxicity (6). (*c*) Several agents have been tested for their ability to protect against c-DDP toxicity in animals (7, 8), but until now none of them has led to an improved therapeutic index of c-DDP in patients. The major problems encountered in this field are toxicity of the chemoprotectors investigated and reduction of the antitumor activity of c-DDP.

The present study focuses on the essential trace element selenium, which has been reported to reduce c-DDP lethality in mice, without reducing its antitumor activity against murine fibrosarcoma (9). Also in other respects, selenium is unique among chemoprotectors. There is growing evidence that selenium deficiency is an important predisposing factor in the development of cancer. Epidemiological reports of an inverse relationship between plasma selenium levels and incidence of cancer have been published (10, 11). The effect of selenium deficiency on tumorigenesis has been evaluated in two studies. In both studies selenium supplementation caused a decrease in tumorigenesis (12, 13). The cytotoxic activity of selenium against a variety of tumor cell lines has been well documented in several systems including Ehrlich ascites tumor-bearing mice (14–19), and selenocystine has already been used with some success in the treatment of human leukemias (20).

The experience with selenium as a chemoreceptor is limited to only one species, the mouse, and to two tumors (9, 21). Also, little is known about the influence of the sequence of administration of the two drugs nor about the time interval between administration of the two drugs. We have, therefore, examined the influence of sodium selenite on c-DDP-induced nephrotoxicity and antitumor activity in BALB/c mice. The tumors used were the Prima breast carcinoma as a solid tumor and the MPC 11 plasmacytoma, an ascitic tumor. We have also studied the influence of sodium selenite on c-DDP nephrotoxicity in Wistar rats, paying special attention to the influence of the time of injection of sodium selenite, relative to the time of administration of c-DDP.

MATERIALS AND METHODS

Laboratory Animals. Female BALB/c mice and male Wistar rats were obtained from the Central Institute for the Breeding of Laboratory Animals/Harlan Sprague-Dawley (CPB/HSD), Zeist, The Netherlands. The mice were 8 wk of age and weighed 18 to 20 g at the start of the experiments. The rats were used at an age of 8 to 9 wk and a weight of 240 to 260 g. All animals were provided with standard laboratory food (SRMA chow; Hope Farms, Woerden, The Netherlands) and water *ad libitum*.

Tumors. MPC 11 tumor cells were obtained from The Institute of Pathology, University of Utrecht, The Netherlands. The MPC 11 tumor originated as a plasmacytoma and was originally obtained from Dr. D. Catty, Birmingham, United Kingdom. The tumor cells were maintained by weekly passages in BALB/c mice. Freshly harvested ascitic cells were used in the experiments. Cells were counted with a hemocytometer. Transplantable Prima breast tumor cells were obtained from the Radiobiological Institute TNO, Rijswijk, The Netherlands. The Prima tumor originated as a breast carcinoma, induced by forced breeding in BALB/c mice bearing murine mammary tumor virus. The Prima tumor line was cultured *in vitro* in standard Dulbecco's modification of minimal essential medium (Gibco, Paisley, United Kingdom), supplemented with L-glutamine (500 mg/liter), 2-mercaptoethanol (60 μmol/liter), and 10% fetal calf serum (Sera-Lab, Ltd., Sussex, United Kingdom).

Chemicals. Sodium selenite, $Na_2SeO_3 \cdot 5H_2O$, was purchased from Merck, Darmstadt, Federal Republic of Germany. All doses of sodium selenite mentioned in this paper are expressed as mg of selenium per kg. c-DDP was synthesized in our laboratory, according to procedures for the synthesis and quality control described by Hoeschele *et al.* (22).

Kidney Function. Animals were divided at random into groups, which were treated with graded i.p. (mice) or i.v. (rats) injections of c-DDP in physiological saline. The influence of selenium on c-DDP toxicity was studied by injection of sodium selenite i.v. 1 h prior to or 1 h after c-DDP administration. Control groups were treated with injections of selenium or physiological saline. Each dose group consisted of 10 animals. Blood for measurement of BUN was obtained from the

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[3] The abbreviations used are: c-DDP, *cis*-diamminedichloroplatinum(II); BUN, blood urea nitrogen; MST, median survival time; sGPT, serum glutamic pyruvate transaminase; sGOT, serum glutamic oxalate transaminase.

retroorbital venous plexus in the mice or from a lateral tail vein in the rat. BUN and serum creatinine were measured daily in pilot studies (data not shown) and in more extensive studies at the time of maximally observed toxicity: Day 4 in BALB/c mice and Day 5 in Wistar rats. BUN and serum creatinine were measured spectrometrically using the Merckotest urea reagent kit and the Merckotest creatinine reagent kit from Merck, Darmstadt, Federal Republic of Germany.

Liver Function. sGPT and sGOT were determined on Days 1 and 4 in BALB/c mice and on Days 1 and 5 posttreatment in Wistar rats. sGPT and sGOT were measured with reagent kits from J. T. Baker, Deventer, The Netherlands.

Histology. Mice were sacrificed 4 days after the injection of c-DDP. Kidneys and livers were removed, weighed, and processed for light microscopy, by routine histology. Sections of 10 μm thickness were cut and stained in hematoxylin:eosin. All slides were examined without prior knowledge of the treatment given to the animal from which the specimen under investigation was taken.

Evaluation of Antitumor Activity. The influence of selenium on the antitumor activity of c-DDP against ascitic tumors was examined in BALB/c mice, i.p. inoculated with 10^6 MPC 11 tumor cells (Day 0). After 24 h, the mice were treated with a single i.p. dose of c-DDP. The influence of selenium was assessed by injecting sodium selenite i.p. 1 h prior to c-DDP. Control groups were treated with physiological saline instead of c-DDP. Mice were examined daily for occurrence of tumors. The experiments were terminated on Day 42 and MSTs were calculated.

BALB/c mice, inoculated with 0.5×10^6 Prima breast tumor cells s.c. in the left thigh (Day 0), were used to investigate the effect of selenium on the antitumor activity of c-DDP against solid tumors. One group of mice was treated with a single i.p. dose of c-DDP 24 h after inoculation of tumor cells. Another group was treated with a single i.p. injection of sodium selenite 1 h before c-DDP. Control groups were treated with physiological saline instead of c-DDP. The occurrence of tumors was examined daily by palpation. The experiments were terminated on Day 15: tumors were excised and weighed.

Statistics. Student's *t* test was used to evaluate the significance of differences between experimental groups.

RESULTS

Kidney Function. The results, summarized in Table 1, demonstrate a protective effect of sodium selenite against nephrotoxicity induced by various c-DDP doses in mice. Administration of c-DDP in a dose range of 11.5 to 16.0 mg/kg did increase BUN and creatinine levels at Day 4 posttreatment. Administration of sodium selenite 1 h before c-DDP did significantly decrease BUN and creatinine levels at all c-DDP doses tested with the exception of the highest dose. However, when sodium selenite was administered 1 h after c-DDP, no decrease in c-DDP-induced BUN and creatinine elevation was observed. Treatment of the animals with selenium, 2.0 mg/kg, alone did not cause a change in BUN or creatinine levels. In experiments with Wistar rats, similar results were obtained (Table 2). The data in Tables 1 and 2 demonstrate that selenium protects BALB/c mice and Wistar rats against c-DDP-induced nephrotoxicity, when it is administered 1 h prior to c-DDP, but not when it is given 1 h thereafter.

Liver Function. Selenium, 2.0 mg/kg, did not cause a significant increase in sGPT or sGOT levels in BALB/c mice at Day 4 posttreatment. Also administration of selenium followed by c-DDP injection 1 h later did not cause a significant increase in sGPT or sGOT levels. Similar results were obtained with measurements at Day 1 posttreatment and in experiments with Wistar rats (data not shown). Thus, selenium at doses up to 2.0 mg/kg did not cause liver damage.

Histology. The protective effect of selenium against c-DDP-induced kidney damage, as observed by BUN and creatinine measurements, was confirmed by routine histology. Fig. 1 demonstrates that tubules of the kidneys of mice treated with selenium, 2.0 mg/kg, and c-DDP, 13.0 mg/kg, 1 h thereafter, show less degeneration and less cell loss of the tubular epithelium at Day 4 posttreatment than those of mice treated with c-DDP, 13.0 mg/kg, alone.

No liver damage was observed by routine histology in BALB/c mice nor in Wistar rats, 4 days, respectively, 5 days after treatment with selenium, 2.0 mg/kg, with or without various c-DDP doses (light micrographs not shown).

The Influence of Selenium on the Antitumor Activity of c-DDP: MPC 11 Plasmacytoma. The antitumor activities of various c-DDP/selenium combinations in BALB/c mice, inoculated with MPC 11 tumor cells, are shown in Table 3. Selenium, 2.0 mg/kg, did not reduce the antitumor activity of c-DDP. At c-DDP doses as low as 6.5 mg/kg, the c-DDP/selenium combinations were equally effective against the tumor as c-DDP alone: there were no significant differences in MST of c-DDP-treated mice compared to mice treated with the corresponding c-DDP dose and selenium. Treatment with selenium, 2.0 mg/kg, alone, resulted in a MST identical to that of the control group treated with physiological saline.

The data in Table 3 also show that selenium protects BALB/c mice against c-DDP nephrotoxicity without reducing its antitumor activity against the MPC 11 tumor. When 8 BALB/c mice were inoculated with 10^6 MPC 11 tumor cells and treated with c-DDP, 13.0 mg/kg i.p., 24 h later, 6 animals had elevated BUN levels (mean, 155 ± 86 mg/100 ml for the whole group), 4 days after c-DDP treatment. The BUN levels of the remaining 2 animals were not elevated: these animals did not develop

Table 1 *The influence of selenium on the nephrotoxicity of c-DDP in BALB/c mice*

c-DDP (mg/kg)	Selenium[a] (mg/kg)	BUN (mg/100 ml)	Creatinine (mg/100 ml)
0[b]	0	21 ± 3[c]	0.54 ± 0.04
0	2.0[d]	20 ± 2	0.53 ± 0.02
10.0	0	20 ± 2	0.52 ± 0.05
10.0	2.0[d]	21 ± 3	0.55 ± 0.04
11.5	0	54 ± 45	1.2 ± 0.2
11.5	2.0[d]	21 ± 2[e]	0.57 ± 0.06[e]
13.0	0	99 ± 43	3.2 ± 0.4
13.0	2.0[d]	35 ± 30[e]	0.7 ± 0.1[e]
13.0	2.0[f]	110 ± 50	3.5 ± 0.8
14.5	0	159 ± 55	5.7 ± 1.5
14.5	2.0[d]	68 ± 62[e]	1.5 ± 1.0[e]
16.0	0	208 ± 26	6.5 ± 0.6
16.0	2.0[d]	196 ± 32	6.6 ± 0.7

[a] Selenium was administered as sodium selenite.
[b] Control animals were given injections of saline.
[c] Mean ± SD (n = 10).
[d] Selenium was given 1 h before c-DDP.
[e] $P < 0.05$ compared to the respective c-DDP alone-treated groups.
[f] Selenium was given 1 h after c-DDP.

Table 2 *The influence of selenium on the nephrotoxicity of c-DDP in Wistar rats*

c-DDP (mg/kg)	Selenium[a] (mg/kg)	BUN (mg/100 ml)	Creatinine (mg/100 ml)
0[b]	0	21 ± 2[c]	0.52 ± 0.02
0	2.0[d]	22 ± 2	0.53 ± 0.02
5.0	0	21 ± 2	0.54 ± 0.03
5.0	2.0[d]	21 ± 2	0.53 ± 0.04
6.0	0	36 ± 10	0.74 ± 0.07
6.0	2.0[d]	22 ± 2[e]	0.57 ± 0.06[e]
7.0	0	144 ± 43	5.1 ± 1.0
7.0	2.0[d]	31 ± 34[e]	0.7 ± 0.6[e]
8.0	0	172 ± 20	6.3 ± 0.5
8.0	2.0[d]	70 ± 63[e]	1.7 ± 1.5[e]
8.0	2.0[f]	156 ± 35	6.1 ± 0.7
9.0	0	208 ± 26	6.5 ± 0.6
9.0	2.0[d]	196 ± 32	6.6 ± 0.7

[a] Selenium was administered as sodium selenite.
[b] Control animals were given injections of saline.
[c] Mean ± SD (n = 10).
[d] Selenium was given 1 h before c-DDP.
[e] $P < 0.05$ compared to the respective c-DDP alone-treated groups.
[f] Selenium was given 1 h after c-DDP.

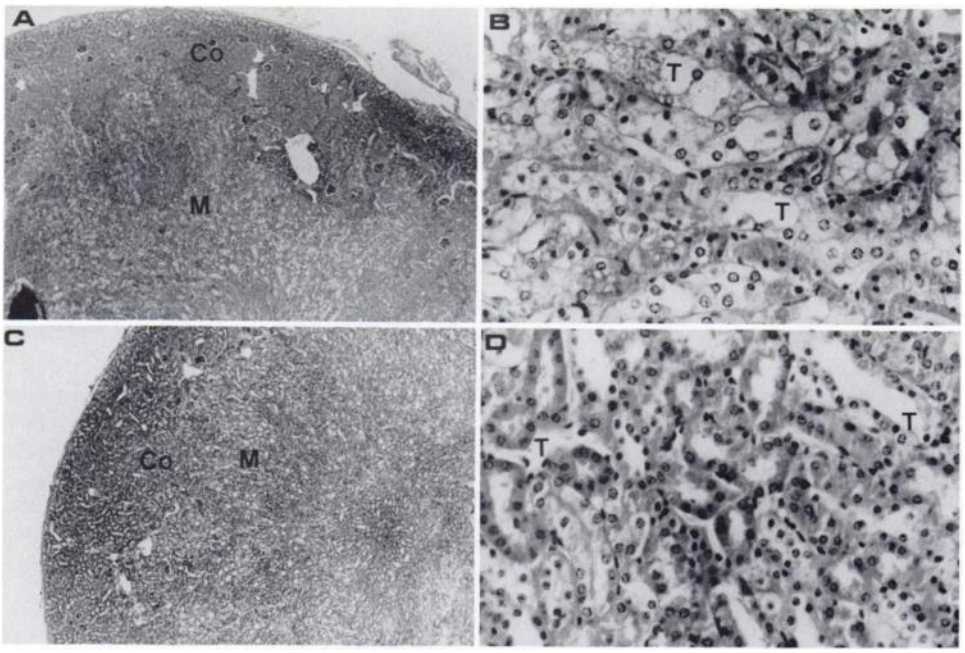

Fig. 1. Light micrographs of the kidneys from BALB/c mice 4 days after: *A*, i.p. injection of c-DDP, 13.0 mg/kg; *B*, magnification of *A* of a region in the medulla; *C*, injection of selenium, 2.0 mg/kg, as sodium selenite, and c-DDP, 13.0 mg/kg, 1 h thereafter; *D*, magnification of *C* of a region in the medulla. *Co*, cortex; *M*, medulla; *T*, tubules. H & E; *A* and *C*, × 40; *B* and *D*, × 400.

Table 3 *The influence of selenium on the nephrotoxicity and antitumor activity of c-DDP in BALB/c mice inoculated with MPC 11 tumor cells (n = 8)*

c-DDP (mg/kg)	Selenium (mg/kg)[a]	BUN (mg/100 ml)	Survival Day 7 (%)	Incidence Tumors Day 7 (%)	MST (days)	T/C[b] (%)
0	0	20 ± 2[c]	100	100	14	100
0	2.0	20 ± 2	100	100	14	100
4.0	0	21 ± 2	100	100	28 ± 2	200
4.0	2.0	21 ± 2	100	100	29 ± 2	207
6.5	0	20 ± 2	100	100	33 ± 3	236
6.5	2.0	21 ± 2	100	100	34 ± 3	243
9.0	0	21 ± 2	100	0	>42	>300
9.0	2.0	21 ± 2	100	0	>42	>300
11.5	0	21 ± 2	100	0	>42	>300
11.5	2.0	21 ± 2	100	0	>42	>300
13.0	0	155 ± 86	25[d,e]	0	>42	>300
13.0	2.0	21 ± 2[f]	100[d]	0	>42	>300
14.5	0	218 ± 43	0	0		
14.5	2.0	108 ± 120[f]	62.5[d,e]	0	>42	>300

[a] Selenium was given as sodium selenite 1 h before c-DDP.
[b] T/C, MST treated/MST control.
[c] Mean ± SD.
[d] Mice alive at Day 7 did not develop tumors as judged by daily examination and by autopsy on Day 42.
[e] Mice which were dead on Day 7 were presumed to have died from cisplatin toxicity.
[f] $P < 0.05$ compared to the respective c-DDP-treated groups.

Table 4 *The influence of selenium on the nephrotoxicity and antitumor activity of c-DDP in BALB/c mice inoculated with Prima breast tumor cells (n = 8)*

c-DDP (mg/kg)	Selenium (mg/kg)[a]	BUN Day 5 (mg/100 ml)	Survival Day 8 (%)	Incidence Tumors Day 8[c] (%)	Mean tumor wt Day 15 (g)	T/C[b] Day 15 (%)
0	0	21 ± 1[d]	100	100	2.0 ± 0.4	
0	2.0	22 ± 2	100	100	1.9 ± 0.3	95
6.5	0	21 ± 1	100	100	1.9 ± 0.4	95
6.5	2.0	21 ± 2	100	100	2.1 ± 0.4	105
9.0	0	21 ± 2	100	0	0.4 ± 0.2	20
9.0	2.0	22 ± 2	100	0	0.4 ± 0.1	20
11.5	0	22 ± 2	100	0	0.15 ± 0.06	7.5
11.5	2.0	22 ± 2	100	0	0.19 ± 0.07	9.5
13.0	0	127 ± 91	100	0	0.16 ± 0.05	8
13.0	2.0	23 ± 2[e]	100	0	0.12 ± 0.06	6
14.5	0	194 ± 37	0	0		
14.5	2.0	111 ± 98[e]	50	0	0.09 ± 0.05	4.5

[a] Selenium was given as sodium selenite 1 h before c-DDP.
[b] T/C, mean tumor weight treated mice/mean tumor weight control mice.
[c] Incidence of tumors was assessed by palpation.
[d] Mean ± SD.
[e] $P < 0.05$ compared to the respective c-DDP alone-treated groups.

tumors (MST > 42 days). MST of mice inoculated with 10^6 MPC 11 cells on Day 0 was 14 days. All mice ($n = 8$) treated with selenium, 2.0 mg/kg, and c-DDP, 13.0 mg/kg, survived: MST > 42 days. None of these animals had elevated BUN levels (mean, 21 ± 2 mg/100 ml) or developed tumors.

Prima Breast Tumor. As shown in Table 4, c-DDP in doses of 9.0 and 11.5 mg/kg was effective against the Prima breast tumor in BALB/c mice. Selenium, 2.0 mg/kg, was not effective against the Prima tumor and did not reduce the antitumor activity of c-DDP.

The data in Table 4 also show that selenium improves the therapeutic index of c-DDP in Prima tumor-bearing mice. Of 8 mice, treated with c-DDP alone, 13.0 mg/kg, 5 showed strongly elevated BUN levels (mean, 127 ± 91 mg/100 ml for the whole group) on Day 5, but all the animals survived. None of the mice of this group had tumors as assessed by palpation on Day 7. On the other hand, none of the mice ($n = 8$), treated with selenium, 2.0 mg/kg, and c-DDP, 13.0 mg/kg, showed kidney damage. All mice survived and none of them had tumors. BUN levels on Day 5 and mean tumor weight on Day 15 of mice treated with selenium, 2.0 mg/kg, alone, were not significantly different from those of the control group, treated with physiological saline.

DISCUSSION

The results presented demonstrate that pretreatment with sodium selenite renders mice and rats less susceptible to kidney damage due to subsequent c-DDP injection. Combinations of c-DDP and sodium selenite were equally effective against tumors as was c-DDP alone. A number of factors which could have an impact on these experiments will be discussed.

Choice of Tumor Models. There are several reports of antineoplastic activity of selenium (14, 15). Naganuma (21) has reported a protective effect of sodium selenite against c-DDP nephrotoxicity in Ehrlich ascites tumor-bearing mice. The antitumor activity of a combination of c-DDP and sodium selenite was equal to that of c-DDP alone. However, sodium selenite itself reduced the growth of Ehrlich ascitic tumors in those

experiments. Furthermore, the protective effect of selenium against c-DDP lethality, without alteration of its antitumor activity, in fibrosarcoma-bearing mice has been reported (9). However, in those studies the effect of the administered selenium doses on the liver was not investigated. The combination of selenium with c-DDP would be of no clinical value if it resulted in other limiting toxicities.

The results in Tables 1 to 4 show that selenium provides protection against c-DDP-induced nephrotoxicity without reducing its antitumor activity in BALB/c mice inoculated with MPC 11 plasmacytoma cells or Prima breast tumor cells. It is important to note that, in these studies, selenium also did not reduce the antitumor activity of much lower, nonnephrotoxic c-DDP doses. Also, selenium doses effective in protecting against c-DDP-induced nephrotoxicity were found not to cause liver damage.

Tumor inhibition of selenite is suggested to be preceded by the formation of selenodiglutathione (13), while the antitumor activity of c-DDP is based on formation of bifunctional adducts with DNA (23). Although selenite alone did not exert an antitumor activity, there could be a subclinical effect which is synergistic with c-DDP. Such a potentiation of the antitumor activity of c-DDP could mask a simultaneous reduction due to a reaction of c-DDP with selenium.

The Dose Schedule of c-DDP and Sodium Selenite. Selenium prevented death, when administered 4 h prior to an otherwise lethal single dose of c-DDP in mice (9). Reduction of c-DDP nephrotoxicity by sodium selenite, using a multiple dosage schedule of both drugs, has been reported (21). In these experiments, mice were treated for 5 days with a daily c-DDP dose of 10 μmol/kg i.p. and a simultaneous dose of sodium selenite, 10 μmol/kg s.c. In the present study a single dose of selenium, 2.0 mg/kg (= 25 μmol/kg), provided protection against kidney damage in rats if it was administered 1 h prior to a single dose of c-DDP, but not when it was given 1 h after c-DDP (Tables 1 and 2).

Since the distribution phase of c-DDP is 15 to 30 min (24), the above studies demonstrate that sodium selenite provides protection against cisplatin nephrotoxicity when it is administered before or simultaneously with c-DDP; that means before c-DDP has become bound to target sites in the tissues. c-DDP is supposed to exert its nephrotoxic activity by inactivation of thiol-containing enzymes in the kidneys (25, 26). A lack of protection of sodium selenite when administered in a single injection 1 h after c-DDP could be explained by assuming that c-DDP or one of its nephrotoxic metabolites (27) has already, irreversibly, inactivated these enzymes before selenium reaches the kidneys.

Since selenium has an antineoplastic activity against several tumors, the observed protection against c-DDP nephrotoxicity by selenium might have important implications for cancer chemotherapy: insight into the mechanism of the molecular interaction between selenium and c-DDP or its metabolites is required for optimizing clinical dosage schedules and modes of administration for c-DDP/selenium combinations. It is also an intriguing question whether nutritional selenium status and sensitivity of patients to c-DDP nephrotoxicity are related.

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