0090-9556/93/2101-0030$02.00/0
DRUG METABOLISM AND DISPOSITION

Vol. 21, No. 1
Printed in U.S.A.

REDUCTION OF CISPLATIN NEPHROTOXICITY BY SODIUM SELENITE

Lack of Interaction at the Pharmacokinetic Level of Both Compounds

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(Received April 29, 1991; accepted August 20, 1992)

ABSTRACT:

Administration of sodium selenite (Na_2SeO_3) 1 hr before *cis*-diamminedichloroplatinum(II) (referred to herein as cisplatin) can protect against the nephrotoxicity of cisplatin. The pharmacokinetic aspects of this interaction were studied in rodents with radiolabeled selenite and cisplatin. Total [^{75}Se]selenium in plasma consisted of [^{75}Se]selenium in plasma proteins and [^{75}Se]selenite in plasma ultrafiltrate. After a short distribution phase, the elimination of [^{75}Se]selenite and total [^{75}Se]selenium proceeded biphasically in the rat, with an initial plasma elimination half-life of [^{75}Se]selenite of 22 ± 2 min. Coadministration of cisplatin had no effect on the initial nor on the much slower terminal elimination phase of [^{75}Se]selenite nor of total [^{75}Se]selenium. Sodium selenite, in doses protecting against the nephrotoxicity of cisplatin, did not significantly affect areas under the plasma concentration time curve from 0–6 hr nor the initial plasma half-lives of [^{195m}Pt]cisplatin ($t_{½}$, 28 ± 2 min) and total [^{195m}Pt]platinum ($t_{½}$, 30 ± 3 min) in plasma. The much slower terminal elimination phases in plasma and the cumulative urinary excretion of [^{195m}Pt]cisplatin and total [^{195m}Pt]platinum were neither influenced by sodium selenite. Sodium selenite does not react chemically with cisplatin *in vitro*. Apparently, bioactivation of selenite is required for its protective effect *in vivo*. Distribution studies in a mice tumor model indicated that [^{75}Se]selenium is concentrated strongly in the kidney and that the bioactivation of selenite also most likely occurs primarily in the kidneys. We conclude that sodium selenite protects rodents against cisplatin-induced nephrotoxicity without influencing the systemic availability of cisplatin and total platinum. Further studies are necessary to establish the clinical value of sodium selenite as chemoprotector against cisplatin-induced nephrotoxicity.

Cisplatin[1] is a chemotherapeutic drug that is effective against several human cancers, such as testis and ovarian cancer, head and neck cancer, and lung cancer (1, 2). However, the drug is also toxic to several tissues and organs such as the kidneys, gastrointestinal tract, peripheral nerves, and bone marrow (3). Nephrotoxicity, one of the most important side-effects of cisplatin, develops after several days, primarily in the S_3-segment of the proximal tubule (4). Events responsible for the toxicity of cisplatin, however, already occur shortly after administration of the drug (5). The severity of cisplatin nephrotoxicity is related to platinum concentrations in the kidneys (6). Administration of cisplatin with pre- and posthydration and mannitol-induced diuresis lowers the concentration of cisplatin in the kidneys: this reduces the nephrotoxicity of cisplatin, allowing higher doses with tolerable kidney toxicity. Higher doses of cisplatin are encouraged by a suggested dose-response relationship for treatment of cancers with cisplatin.

An alternative approach to protect against the side-effects of cisplatin is provided by chemoprotectors. Several sulfur-based nucleophilic agents, such as sodium thiosulfate (7), diethyldithiocarbamate (8), and S-2-(3-aminopropylamino)ethyl-phosphorothioic acid (WR-2721) (9), have been shown to reduce cisplatin-induced nephrotoxicity in animals. Major problems in this field are intrinsic toxicity of the chemoprotector [*e.g.* in the case of diethyldithiocarbamate (10)] or a lack of selectivity: sodium thiosulfate not only reduces the nephrotoxicity, but also the antitumor activity of cisplatin (11) by reacting chemically with cisplatin, forming a soluble, biologically inactive product (12). It has been shown that sodium thiosulfate thus causes reduced levels of active platinum in plasma (13).

Sodium selenite has recently also been shown to protect rodents against the nephrotoxicity of cisplatin, without reducing the antitumor activity of the drug (14). The mechanism by which sodium selenite protects the kidneys against the toxicity of cisplatin is not yet fully understood. It has been shown that sodium selenite does not react chemically with cisplatin. Reactions between cisplatin and nucleophilic metabolites of selenite are probably responsible for the protective effect of selenite against the nephrotoxicity of cisplatin (15, 16). For the design of optimal clinical dosage schedules, insight into the pharmacokinetics of selenite and its interaction at the level of pharmacokinetics of cisplatin is required.

The first aim of this study was to obtain some pharmacokinetic data of sodium selenite in rodents at dosage and treatment schedules that provide protection against cisplatin-induced nephrotoxicity. The second aim was to determine whether selenite has any effect on the early-phase pharmacokinetics and tissue distribution of cisplatin in rodents.

Materials and Methods

Chemicals. [^{75}Se]Selenite with a specific activity of 3 GBq/mg selenium was obtained from Dupont de Nemours (Dreieich, FRG). Sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) was obtained from Merck (Darmstadt, FRG).

This work was supported by a grant from the Dutch Cancer Society (Amsterdam, The Netherlands).

[1] Abbreviations used are: cisplatin, *cis*-diamminedichloroplatinum(II); SDS, sodium dodecyl sulfate; AUC, area under the plasma concentration time curve; $t_{½}$, elimination half-life.

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Chemicals for preparing the mobile phase of the chromatographic system were of HPLC grade. All other chemicals were of analytical grade.

Synthesis of Cisplatin. [^{195m}Pt]Cisplatin was synthesized as follows: platinum metal, isotopically enriched to 80% ^{194}Pt (Intersales Holland, Hengelo, The Netherlands), was irradiated in a thermal neutron flux of 2×10^{14} cm^{-2} sec^{-1} for 168 hr. The resulting ^{195m}Pt was converted to [^{195m}Pt]cisplatin, according to synthetic and test procedures described previously (17, 18).

Analytical Methods. HPLC analyses were performed on a reversed-phase HPLC system as described elsewhere (18). Briefly, 20 μl of a sample was injected on a 100 mm × 3.0 mm Spherisorb ODS column and eluted at a flow rate of 0.25 ml/min with a gradient from A to B, using the following two mobile phases: (A) 5 mM SDS and 10 mM sodium phosphate buffer (pH 2.6); (B) 5 mM SDS, 25% 2-propanol, and 60 mM sodium phosphate buffer (pH 2.6). Detection was performed with on-line ^{195m}Pt-radioactivity detection (18) or with on-line ^{75}Se-radioactivity detection (14).

Radioactivity measurements of tissue samples were performed on a γ-scintillation counter.

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 (Zeist, The Netherlands). Mice were 8 weeks of age and weighed 18–20 g at the start of the experiments. Rats were 8 to 9 weeks of age and weighed 240–260 g. All animals were provided with standard laboratory food (SRMA chow, Hope Farms, Woerden, The Netherlands) and water *ad libitum*.

Tumors. 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 cell-line was cultured *in vitro* in standard Dulbecco's modification of Minimal Essential Medium (Gibco, Paisley, UK), supplemented with L-glutamine (500 mg/liter), 2-mercaptoethanol (60 μmol/liter), and 10% fetal calf serum (Flow Laboratories (Zwanenburg, The Netherlands).

Pharmacokinetic Studies. Pharmacokinetic studies were performed in Wistar rats ($N = 5$), after cannulation of the carotid artery(19). Animals were treated with cisplatin and selenite. Both compounds were administered intravenously in a lateral tail vein in 1 ml physiological saline. Blood samples of 300 μl were taken from the cannula and immediately centrifuged to obtain plasma. Plasma samples were immediately ultrafiltered over YMT filters in an Amicon MPS-1 micropartition system (Amicon, Oosterhout, The Netherlands) for 20 min at 2000*g*. Ultrafiltrates were stored in liquid nitrogen and analyzed by HPLC within 24 hr.

AUCs were calculated by means of the trapezoidal rule. Elimination half-lives of the initial elimination phases ($t_{\frac{1}{2}}$) were calculated from the log-linear parts of the semilogarithmical curves of plasma concentrations versus time (20). No other pharmacokinetic parameters were calculated, because, on the one hand, this is known to be difficult in the case of the chemically very reactive and unstable cisplatin, and because, on the other hand, pharmacokinetic parameters of total radioactivity in the case of cisplatin are of limited value.

Rats used for urinary excretion experiments were not cannulated and were placed in metabolism cages 24 hr before the start of the experiments. Urine samples were collected at regular time intervals. At each time point the bladders were emptied by forced urination with diethylether anesthesia. Urine samples were filtered through 0.45 μm Millipore filters (Nihon Millipore, Kogyo Yonezawa, Japan) and stored in liquid nitrogen. Urine samples were analyzed within 24 hr after collection.

Pharmacokinetics of Selenite. Sodium [^{75}Se]selenite, 25 μmol/kg, was intravenously administered 1 hr before intravenous administration of 1 ml physiological saline. Blood samples were collected at 0, 5, 10, 20, 30, 60, 120, 240, and 360 min, and 24 hr after administration of [^{75}Se]selenite and urine samples at time points 1, 2, 4, 6, 12, and 24 hr. Filtered samples were analyzed for total ^{75}Se-radioactivity and for [^{75}Se]selenium compounds by HPLC with on-line ^{75}Se-radioactivity detection. The influence of cisplatin on the pharmacokinetics of [^{75}Se]selenite was studied in similar experiments in which cisplatin (17 μmol/kg) was intravenously administered instead of physiological saline.

Influence of Selenite on the Pharmacokinetics of Cisplatin. Sodium selenite (25 μmol/kg) was intravenously administered 1 hr before intravenous administration of [^{195m}Pt]cisplatin (17 μmol/kg). A control group was treated with 1 ml physiological saline instead of sodium selenite. Blood samples were collected at 0, 5, 10, 20, 30, 60, 120, 240, and 360 min, and 24 hr after administration of cisplatin and urine samples at time points 1, 2, 4, 6, 12, and 24 hr. Filtered samples were analyzed for total ^{195m}Pt-radioactivity and for [^{195m}Pt]platinum compounds by HPLC with on-line ^{195m}Pt-radioactivity detection.

Distribution Studies. BALB/c mice were inoculated subcutaneously with 0.5×10^6 Prima breast tumor cells in the left thigh (day 0). Mice with tumors greater than 0.5 g (day 8) were used for distribution studies. Mice were treated with [^{195m}Pt]cisplatin and [^{75}Se]selenite. At selected time points, groups of animals were killed, and tumors and kidneys were removed, weighed, and ^{195m}Pt- and ^{75}Se-radioactivity levels were determined. The following three groups were included in this study. Group I: sodium [^{75}Se]selenite (25 μmol/kg ip) was administered in 0.5 ml physiological saline, 1 hr later followed by 1 ml physiological saline. Group II: physiological saline (0.5 ml ip) was administered 1 hr later followed by [^{195m}Pt]cisplatin (45 μmol/kg ip) administered in 1 ml physiological saline. Group III: [^{75}Se]selenite (25 μmol Se/kg ip) was administered 1 hr later followed by [^{195m}Pt]cisplatin (45 μmol/kg ip) administered in 1 ml physiological saline. Time points of measurements were 30, 65, 75, 90, and 120 min after the first injection.

Statistics. Students' *t* test (unpaired) was used to evaluate the significance of differences between data from the experimental groups. The level of significance was set at $p < 0.05$.

Results

Pharmacokinetics of Selenite. The pharmacokinetics of selenite was studied in two groups of rats. One group was treated intravenously with sodium [^{75}Se]selenite and 1 hr later with physiological saline; the other group was treated with sodium [^{75}Se]selenite and 1 hr later with cisplatin. No significant differences were observed in HPLC radiochromatograms of plasma ultrafiltrate and urine samples from the two groups of rats at equal time points. In all plasma ultrafiltrate samples, only one ^{75}Se-containing compound was detected with HPLC (fig. 1*A*, *B*). This compound had a retention time of 2.5 min and was identified as [^{75}Se]selenite. It has previously been shown that the HPLC system used is suitable for the separation of selenite from a variety of organoselenium compounds, such as bis(glutathione)selenide (21). The detection limit of our HPLC system is 6.2×10^{-10} M Se for plasma ultrafiltrate and urine: the detector response is independent of both the matrix composition of the samples and the chemical structure of the selenium compounds (21).

In HPLC radiochromatograms of the urine samples, three ^{75}Se-containing peaks were observed (fig. 1*C*, *D*). The first peak was identified as [^{75}Se]selenite (retention time: 2.5 min). The identity of the other ^{75}Se-compounds remains to be established.

Plasma concentration of [^{75}Se]selenite and total [^{75}Se]selenium as a function of time are plotted semilogarithmically in fig. 2. Calculated pharmacokinetic parameters are summarized in table 1. Plasma [^{75}Se]selenium consisted of [^{75}Se]selenium in plasma proteins and [^{75}Se]selenite in plasma ultrafiltrate. The elimination of [^{75}Se]selenite and total [^{75}Se]selenium from plasma proceeded biphasically. The plasma elimination half-life, initial $t_{\frac{1}{2}}$, of [^{75}Se]selenite was 22 ± 2 min. A second elimination phase for both [^{75}Se]selenite and total [^{75}Se]selenium proceeded much slower (fig. 2). Because the experiments were terminated 24 hr after administration of sodium [^{75}Se]selenite, the kinetics of this

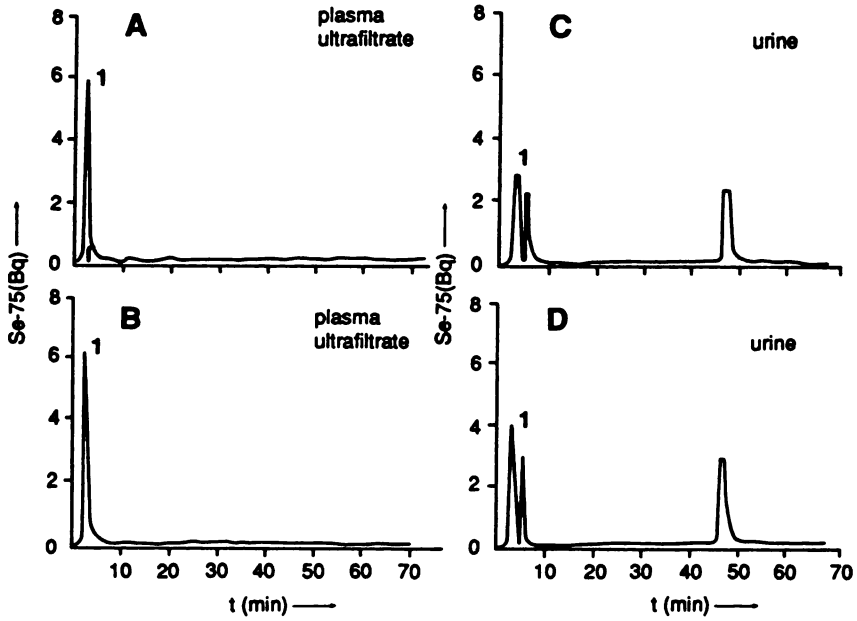

FIG. 1. *HPLC radiochromatograms of plasma ultrafiltrate (A, B) and urine samples (C, D) from Wistar rats, obtained 60 min after intravenous treatment with 25 μmol/kg sodium [^{75}Se]selenite (A, C) and 25 μmol/kg sodium [^{75}Se]selenite plus 17 μmol/kg cisplatin 1 hr later (B, D).*

Peak 1, with a retention time of 2.5 min, was identified as sodium [^{75}Se]selenite.

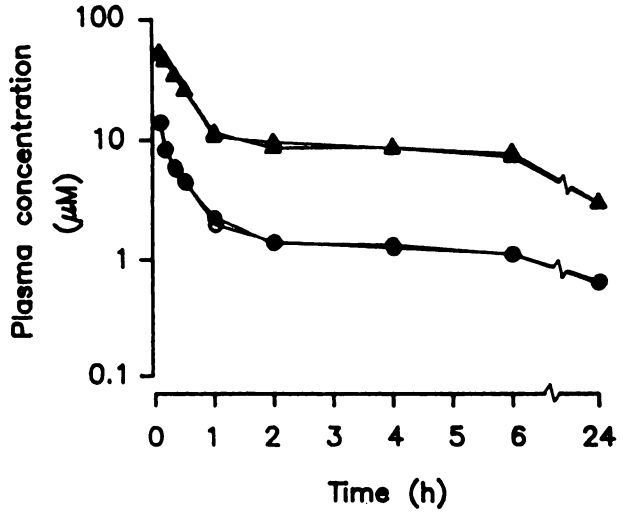

FIG. 2. *Semilogarithmic concentration vs. time plot of total [^{75}Se] selenium (Δ, ▲) and [^{75}Se]selenite (○, ●) in plasma of the Wistar rat after intravenous administration of 25 μmol/kg sodium [^{75}Se]selenite (○, Δ) and 25 μmol/kg sodium [^{75}Se]selenite plus 17 μmol/kg cisplatin 1 hr later (▲, ●).*

terminal phase could not be determined accurately. As shown in fig. 2 and table 1, coadministration of cisplatin did not influence the pharmacokinetics of [^{75}Se]selenite nor of total [^{75}Se]selenium.

Cumulative urinary excretion curves for [^{75}Se]selenite and total [^{75}Se]selenium are shown in fig. 3. Rats given sodium [^{75}Se] selenite alone excreted 30 ± 2.5% (N = 5) of the dose as total [^{75}Se]selenium in urine within 24 hr and 2.3 ± 0.2% of the dose in the form of unchanged [^{75}Se]selenite. Urinary excretion of [^{75}Se]selenite nor of total [^{75}Se]selenium was affected by coadministration of cisplatin (fig. 3).

Pharmacokinetics of Cisplatin. The pharmacokinetics of [^{195m}Pt]cisplatin was studied in two groups of rats: one treated intravenously with [^{195m}Pt]cisplatin and physiological saline, and one treated intravenously with [^{195m}Pt]cisplatin and sodium selenite. No significant differences were observed between the HPLC radiochromatograms of plasma ultrafiltrate and urine samples from the two groups of rats at equal time points (fig. 4). It has previously been shown that the HPLC system used is suitable for separation of cisplatin from various platinum complexes (18). In all plasma ultrafiltrate samples, two [^{195m}Pt] platinum compounds were detected, the first of which with a retention time of 2.1 min, was identified as [^{195m}Pt]cisplatin (18). The structure of the second compound (retention time 19.2 min) remains to be established: this compound was present in all samples as a fixed percentage (0.5%) of total [^{195m}Pt]platinum and is not considered to be a metabolite of [^{195m}Pt]cisplatin *in vivo*, because it was also formed when [^{195m}Pt]cisplatin was dissolved in plasma ultrafiltrate. De Waal *et al.* (22) have observed a similar platinum complex in plasma ultrafiltrate *in vitro* and have proposed that this compound is a hydrolysis product of cisplatin, probably *cis*-diammineaquachloroplatinum. The detection limit of our HPLC system is 3.3 × 10^{-8} M [^{195m}Pt] for plasma ultrafiltrate and urine; the detector response is independ-

TABLE 1

Pharmacokinetics of total [^{75}Se]selenium and [^{75}Se]selenite in plasma of rats treated intravenously with sodium [^{75}Se]selenite, either with or without cisplatin[a]

Compound Measured	Parameter	[^{75}Se]Selenite without Cisplatin	[^{75}Se]Selenite with Cisplatin
Total [^{75}Se]selenium	Initial $t_{\frac{1}{2}}$ (min)	25 ± 3	24 ± 2
	V (ml/kg)	139 ± 16	148 ± 15
	AUC (0–2 hr)	4.5 ± 0.4	4.1 ± 0.4
	AUC (0–6 hr)	7.1 ± 0.6	6.6 ± 0.6
[^{75}Se]Selenite	Initial $t_{\frac{1}{2}}$ (min)	22 ± 2	21 ± 2
	V (ml/kg)	312 ± 22	319 ± 17
	AUC (0–2 hr)	0.80 ± 0.05	0.78 ± 0.07
	AUC (0–6 hr)	1.1 ± 0.11	1.1 ± 0.11

[a] Mean ± SD (N = 5). AUCs are expressed in mmol·min^{-1}/liter.

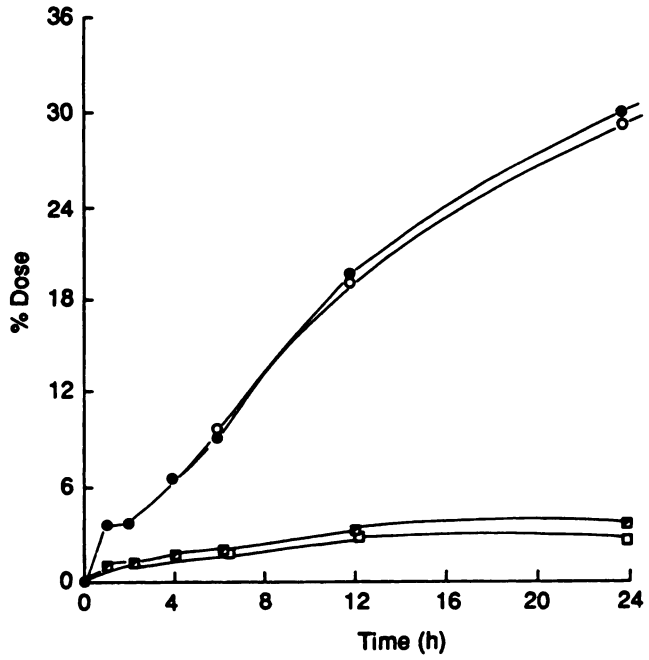

FIG. 3. *Cumulative urinary [^{75}Se]selenite (A) and total [^{75}Se]selenium (B) excretion curves as percentage of dose after intravenous treatment of the Wistar rat with 25 μmol/kg sodium [^{75}Se]selenite (●) and 25 μmol/kg sodium [^{75}Se]selenite plus 17 μmol/kg cisplatin 1 hr later (○).*

ent of both the matrix composition of the samples and the chemical structure of the platinum compounds (18).

In HPLC radiochromatograms of urine samples, several ^{195m}Pt-peaks were observed (fig. 4). The retention time of the first peak was 2.1 min, and this peak was identified as [^{195m}Pt]cisplatin (18). The identity of the other ^{195m}Pt-compounds remains to be established. These compounds, however, are not considered to be metabolites of [^{195m}Pt]cisplatin, because the same pattern of [^{195m}Pt]platinum peaks was observed in the HPLC chromatograms of control urine samples in which [^{195m}Pt]cisplatin was dissolved: apparently [^{195m}Pt]cisplatin decomposes in the urinary tract or the bladder by chemical reactions with constituents of urine, such as amino acids or thiols.

Influence of Selenite on the Pharmacokinetics of Cisplatin. Plasma concentrations of [^{195m}Pt]cisplatin and total [^{195m}Pt]platinum as a function of time are plotted semilogarithmically in fig. 5. After a short distribution phase, the curves proceeded biphasically. A second elimination phase for both [^{195m}Pt]cisplatin and total [^{195m}Pt]platinum proceeds much slower (fig. 5). This terminal elimination phase could not be determined accurately, however, because the experiments were terminated after 24 hr. Sodium selenite had neither effect on concentrations of [^{195m}Pt]cisplatin nor on total [^{195m}Pt]platinum concentrations in plasma. Calculated pharmacokinetic parameters are summarized in table 2. The initial plasma elimination-half-life for [^{195m}Pt]cisplatin, $t_{½}$, was 28 ± 2 min. The AUC, measured over the time interval 0–2 hr, was 2408 ± 293 μmol·min^{-1}/liter. Pharmacokinetic parameters of [^{195m}Pt]cisplatin and total [^{195m}Pt]platinum were not affected by sodium selenite (table 2).

Typical cumulative urinary excretion curves for total [^{195m}Pt]platinum are shown in fig. 6. Rats given [^{195m}Pt]cisplatin alone excreted 45 ± 5% of the dose in urine within 4 hr and 67 ± 6% (N = 5) within 24 hr. Urinary excretion of total [^{195m}Pt]platinum was not significantly affected by coadministration of sodium selenite (fig. 6).

Distribution Studies. As shown in table 3, ^{75}Se-levels in the kidneys of BALB/c mice, treated once intraperitoneally with sodium [^{75}Se]selenite were consistently higher (4 to 10 times) than those in the Prima tumors on all time points tested. Coadministration of [^{195m}Pt]cisplatin did not influence ^{75}Se-levels in tumor nor kidneys.

After intraperitoneal administration of [^{195m}Pt]cisplatin,

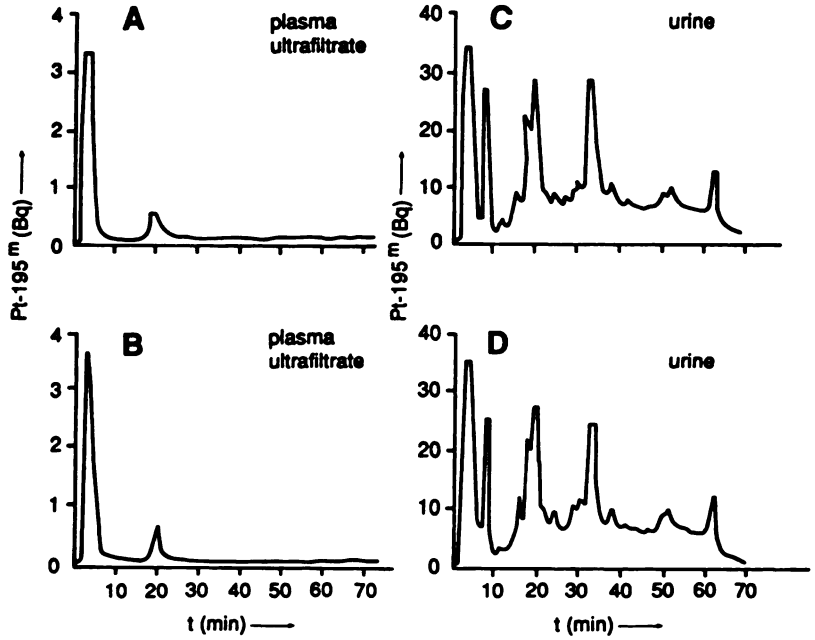

FIG. 4. *HPLC radiochromatograms of plasma ultrafiltrate (A, B) and urine samples (C, D) from the Wistar rat obtained 60 min after intravenous treatment with 17 μmol/kg [^{195m}Pt]cisplatin (A, C) and 25 μmol/kg sodium selenite plus 17 μmol/kg [^{195m}Pt]cisplatin 1 hr later (B, D).*

Peak 1, with a retention time of 2.1 min, was identified as [^{195m}Pt]cisplatin.

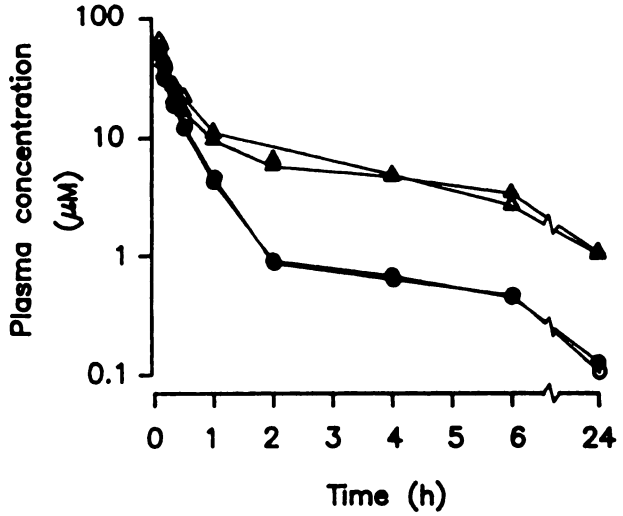

FIG. 5. *Semilogarithmic concentration vs. time plot of total [^{195m}Pt] platinum (△, ▲) and [^{195m}Pt]cisplatin (○, ●) in plasma of the Wistar rat after intravenous treatment with 17 μmol/kg [^{195m}Pt]cisplatin (△, ○) and 25 μmol/kg sodium selenite plus 17 μmol/kg [^{195m}Pt]cisplatin 1 hr later (▲, ●).*

TABLE 2

Pharmacokinetics of [^{195m}Pt]cisplatin and total [^{195m}Pt]platinum in plasma of rats treated intravenously with [^{195m}Pt]cisplatin, either with or without sodium selenite[a]

Compound Measured	Parameter	[^{195m}Pt]Cisplatin without Selenite	[^{195m}Pt]Cisplatin with Selenite
Total [^{195m}Pt]platinum	Initial $t_{½}$ (min)	30 ± 3	32 ± 2
	V (ml/kg)	372 ± 33	358 ± 28
	AUC (0–2 hr)	2.4 ± 0.3	2.7 ± 0.3
	AUC (0–6 hr)	2.7 ± 0.3	2.9 ± 0.3
[^{195m}Pt]Cisplatin	Initial $t_{½}$ (min)	28 ± 2	27 ± 2
	V (ml/kg)	504 ± 46	482 ± 34
	AUC (0–2 hr)	1.5 ± 0.2	1.6 ± 0.3
	AUC (0–6 hr)	1.6 ± 0.3	1.8 ± 0.2

[a] Mean ± SD (N = 5). AUCs were expressed in mmol·min^{-1}/liter.

^{195m}Pt-levels in the kidneys were also higher than those in the tumor: the kidney to tumor ratio was 2.3, 60 min after administration of [^{195m}Pt]cisplatin (corresponding to 120 min after administration of physiological saline), which is much lower than the ratio of 8 for ^{75}Se-levels observed 60 min after administration of a single dose of sodium [^{75}Se]selenite. Coadministration of sodium [^{75}Se]selenite 1 hr before [^{195m}Pt]cisplatin did not influence either ^{195m}Pt-levels in the tumor nor the kidneys (table 3).

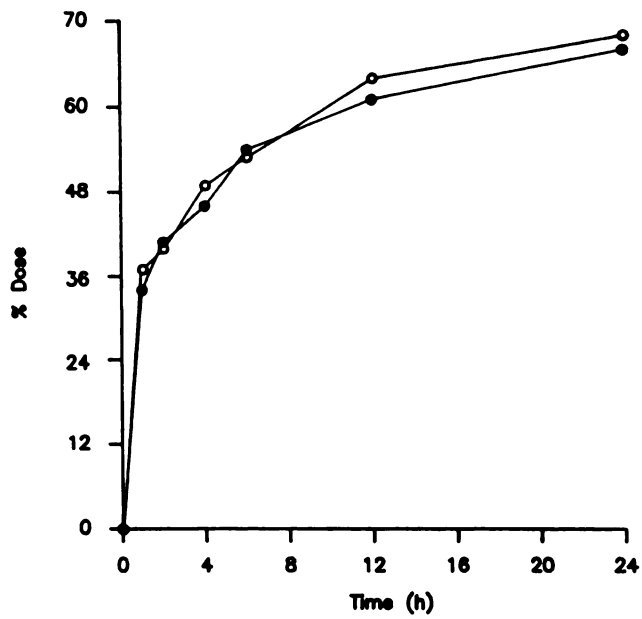

FIG. 6. *Cumulative urinary excretion curve of total [^{195m}Pt]platinum as percentage of the dose, after intravenous treatment of the Wistar rat with 17 μmol/kg [^{195m}Pt]cisplatin (●) or with 25 μmol/kg sodium selenite plus 17 μmol/kg [^{195m}Pt]cisplatin 1 hr later (○).*

TABLE 3

Levels of [^{195m}Pt]platinum and [^{75}Se]selenium in kidney and tumor of Prima breast tumor-bearing mice treated intraperitoneally with sodium [^{75}Se]selenite and/or [^{195m}Pt]cisplatin[a]

Selenite	Cisplatin	Time	Selenium		Platinum	
			Kidney	Tumor	Kidney	Tumor
μmol/kg	μmol/kg	min	nmol/g tissue		nmol/g tissue	
25	0	30	23 ± 14	6 ± 3	—	—
25	0	65	32 ± 1	4 ± 1	—	—
25	45	65	32 ± 3	4 ± 1	53 ± 10	14 ± 5
0	45	65	—	—	69 ± 8	14 ± 5
25	0	75	25 ± 8	6 ± 2	—	—
25	45	75	31 ± 8	5 ± 2	52 ± 20	21 ± 11
0	45	75	—	—	67 ± 5	20 ± 2
25	0	90	31 ± 2	5 ± 2	—	—
25	45	90	30 ± 3	5 ± 1	84 ± 20	18 ± 7
0	45	90	—	—	65 ± 13	20 ± 7
25	0	120	32 ± 6	4 ± 2	—	—
25	45	120	31 ± 9	3 ± 1	40 ± 20	11 ± 3
0	45	120	—	—	64 ± 10	28 ± 11

[a] Cisplatin was administered 1 hr after sodium selenite or physiological saline. Time was after administration of selenite or physiological saline. Mean ± SD (N = 3).

Discussion

Sodium selenite has been shown to protect Wistar rats against cisplatin-induced nephrotoxicity, when administered 1 hr prior to cisplatin (14). In this study, we have used recently developed sensitive and specific radioanalytical techniques for the analyses of [^{195m}Pt]cisplatin and metabolites (18). and [^{75}Se]selenite and metabolites (21) in order to study some pharmacokinetic aspects of a possible interaction between cisplatin and sodium selenite in rats and mice.

Pharmacokinetics of Selenite. Because no data on the kinetics of elimination of selenite after administration of a single dose of sodium selenite were available, we first studied the pharmacokinetics of sodium [^{75}Se]selenite in the rat. In the first 2 hr after single intravenous administration of sodium [^{75}Se]selenite, a rapid decline in plasma concentrations of [^{75}Se]selenite was observed (fig. 2). Only 2.3% of the dose was excreted in the urine within 24 hr as unchanged [^{75}Se]selenite. Most [^{75}Se]selenite appears to be eliminated from plasma by incorporation in proteins, such as glutathione peroxidase, and by metabolic conversion (23). Possibly irreversible binding of selenium to or incorporation in thiol-containing proteins probably explains the relatively slow terminal elimination phase for [^{75}Se]selenium from plasma. Selenite has been shown to be metabolized to hydrogen selenide (H_2Se), which is sequentially methylated to dimethylselenide ((CH_3SeCH_3)) and trimethylselenonium [$(CH_3)_3Se^+$] *via* methylselenol (CH_3SeH) (24). Dimethylselenide is excreted in exhaled air and trimethylselenonium $(CH_3)_3Se^+$ is excreted in

the urine (25). The major renal metabolite that we have observed (retention time: 48.4 min; fig. 1) is probably the trimethylselenonium ion. The plasma and urinary data presented in this study demonstrate that administration of cisplatin, 1 hr after sodium [^{75}Se]selenite administration, did not influence the elimination of [^{75}Se]selenite nor of total [^{75}Se]selenium from plasma (figs. 1–3 and table 1).

Pharmacokinetics of Cisplatin. The pharmacokinetics of the elimination of intravenous-administered cisplatin from plasma ultrafiltrate in Wistar rats, observed in this study (figs. 4–6), corresponded well with the pharmacokinetics of cisplatin in rodents, previously described by Van Hennik *et al.* (20) and Siddik *et al.* (26). After a short and rapid initial elimination phase of about 2 hr, both unchanged [^{195m}Pt]cisplatin and total [^{195m}Pt]platinum showed a second, much slower elimination phase, the elimination half-life of which could not be measured accurately. Because cisplatin is very reactive toward thiol- and amine-containing nucleophiles and because cisplatin decomposes rapidly to various reactive aquated hydrolysis products, more or less covalent binding to macromolecules in plasma probably contributes considerably to the slow terminal elimination phase of [^{195m}Pt]cisplatin and total [^{195m}Pt]platinum. The AUCs of [^{195m}Pt]cisplatin and total [^{195m}Pt]platinum were calculated accurately for the time period between 0–2 and 0–6 hr (tables 1 and 2), because the renal toxicity of cisplatin is generally thought to be related to peak level concentrations at a short time interval after dosing. Sodium selenite, administered at dose and time schedules providing protection against cisplatin-induced nephrotoxicity, did not significantly affect plasma AUCs nor the initial elimination half-lives nor the terminal elimination of [^{195m}Pt]cisplatin and total [^{195m}Pt]platinum (fig. 5, table 2). This means that coadministration of sodium selenite does not reduce the systemic availability of cisplatin in the first critical hours after administration of cisplatin. This is in contrast to the effects of other chemoprotectors. Goel *et al.* (12) have demonstrated that sodium thiosulfate, at doses providing protection against cisplatin-induced nephrotoxicity, reduced the plasma AUC of cisplatin and of diethyldithiocarbamate-reactive platinum species of cisplatin. It seems therefore obvious that application of sodium thiosulfate as chemoprotector against cisplatin-induced nephrotoxicity might suffer from a concomitant reduction of the antitumor activity of cisplatin as a result of reduced concentrations of cisplatin in plasma. Recently, Aamdal *et al.* (11) have demonstrated that sodium thiosulfate indeed fails to increase the therapeutic index of cisplatin in several tumor models. These results are in contrast to the experiments of Howell (27), however, who has shown that intraperitoneally administered sodium thiosulfate can increase the therapeutic index of intravenous-administered cisplatin.

Distribution of Selenium and Platinum. We have previously shown that sodium selenite protects rodents against cisplatin-induced nephrotoxicity when it is administered 1 hr before cisplatin, but not when administered 1 hr thereafter (14), indicating that the events responsible for the protective effect of selenite must occur before or shortly after administration of cisplatin. In this study we also measured ^{195m}Pt- and ^{75}Se-levels in both kidneys and tumors of Prima tumor-bearing BALB/c mice from 0.5 hr before up to 1 hr after administration of sodium [^{75}Se]selenite and [^{195m}Pt]cisplatin (table 3). Coadministration of sodium [^{75}Se]selenite did not influence ^{195m}Pt-levels in the kidneys nor in the tumor. Moreover, ^{75}Se-levels in the kidneys were much higher than those in the tumor, after administration of a single dose of sodium [^{75}Se]selenite. Apparently, [^{75}Se]selenium is concentrating strongly and selectively in the kidneys soon after administration of sodium [^{75}Se]selenite. A possible explanation for the protective effect of selenite might, therefore, be a selective inactivation of cisplatin by selenite in the kidneys. We have previously shown that selenite itself is not capable of reacting directly with cisplatin (15). The fact that, in this study, neither levels of [^{195m}Pt]cisplatin nor total [^{195m}Pt]platinum were decreased by coadministration of sodium selenite supports this suggestion. Although these results do not fully exclude the possibility that a direct reaction between cisplatin and selenite does occur in the kidneys, it seems most likely that bioactivation of selenite is required for its protective effect. Glutathione, present at much higher levels in the kidneys than in tumors (28), might well play a role in the bioactivation of selenite to protect species of selenite, such as nucleophilic thiols (15).

Conclusions

The results presented in this study demonstrate that sodium selenite does not protect BALB/c mice against cisplatin nephrotoxicity by reducing the systemic availability of cisplatin or total platinum. Apparently, concentration and bioactivation of selenite in the kidney are required for its protective effect. This bioactivation most probably occurs to a much higher extent in the kidney than in tumors. Further studies are necessary to establish the clinical value of selenite as chemoprotector against cisplatin-induced nephrotoxicity.

Acknowledgments. We are grateful to K. J. Volkers for synthesis of [^{195m}Pt]cisplatin and to Dr. R. G. M. Ten Berg and N. Bosnie for performing the cannulations.

References

1. A. W. Prestayko, S. T. Crooke, and S. K. Carter, Eds.: "Cisplatin: Current Status and New Developments." Academic Press, Inc., New York, 1980.
2. P. J. Loehrer and L. H. Einhorn: Cisplatin. *Ann. Intern. Med.* **100,** 704–713 (1984).
3. D. D. Von Hoff, R. Schilsky, C. M. Reichert, R. L. Reddick, M. Rozenweig, R. C. Young, and F. Muggia: Toxic effects of cisdichlorodiammine platinum in man. *Cancer Treat. Rep.* **63,** 1527–1531 (1979).
4. J. D. Blanchley and Hill, J. B.: Renal and electrolyte disturbances associated with cisplatin. *Ann. Intern. Med.* **95,** 628–632 (1981).
5. H. T. Heidemann, J. F. Gerkens, E. K. Jackson, and R. A. Branch: Attenuation of cisplatinum-induced nephrotoxicity in the rat by high salt diet, furosemide and acetazolamide. *Arch. Pharmacol.* **329,** 201–205 (1985).
6. C. F. J. Barnard: Platinum anti-cancer agents, twenty years of continuing development. *Platinum Metals Rev.* **33,** 162–167 (1989).
7. C. E. Pfeifle, S. B. Howell, R. D. Felthouse, T. B. S. Woliver, P. A. Andrews, M. Markman, and M. P. Murphy: High-dose cisplatin with sodium thiosulfate protection. *J. Clin. Oncol.* **3,** 237–244 (1987).
8. D. L. Bodenner, P. C. Dedon, P. C. Keng, J. C. Katz, and R. F. Borch: Selective protection against cis-diamminedichloroplatinum(II)-induced toxicity in kidney, gut and bone marrow by diethyldithiocarbamate. *Cancer Res.* **46,** 2751–2755 (1986).
9. J. M. Yuhas: Active versus passive absorption kinetics as the basis for selective protection of normal tissues by S-2-(3-aminopropylamino)-ethylphosphorothioic acid. *Cancer Res.* **40,** 1519–1524 (1980).
10. M. L. Rothenberg, Y. Ostchega, S. M. Steinberg, R. C. Young, S. Hummel, and R. F. Ozols: High-dose carboplatin with diethyldi-

thiocarbamate chemoprotection in treatment of women with relapsed ovarian cancer. *J. Natl. Cancer Inst.* **80**, 1488–1492 (1988).
11. S. Aamdal, O. Fodstad, and A. Pihl: Sodium thiosulfate fails to increase the therapeutic index of intravenously administered cis-diamminedichloroplatinum(II) in mice bearing murine and human tumors. *Cancer Chemother. Pharmacol.* **21**, 129–133 (1988).
12. R. Goel, S. M. Cleary, C. Horton, C., S. Kirmani, I. Abramson, C. Kelly, and S. B. Howell: Effect of sodium thiosulfate on the pharmacokinetics and toxicity of cisplatin. *J. Natl. Cancer Inst.* **81**, 1552–1560 (1989).
13. Y. Iwamoto, T. Kawano, M. Ishizawa, K. Aoki, T. Kuroiwa, and T. Baba: Inactivation of cis-diammine dichlorplatinum(II) in blood and protection of its toxicity by sodium thiosulfate in rabbits. *Cancer Chemother. Pharmacol.* **15**, 228–232 (1985).
14. G. S. Baldew, C. J. A. Van den Hamer, G. Los, N. P. E. Vermeulen, J. J. M. De Goeij, and J. G. McVie: Selenium-induced protection against cis-diamminedichloroplatinum(II) nephrotoxicity in mice and rats. *Cancer Res.* **49**, 3020–3023 (1989).
15. G. S. Baldew: Chemoprotection against cisplatin nephrotoxicity: a molecular approach with selenium compounds, Ph.D. thesis, Vrije Universiteit Amsterdam, The Netherlands, 1990.
16. G. S. Baldew, J. G. J. Mol, F. J. J. De Kanter, B. Van Baar, J. J. M. De Goeij, and N. P. E. Vermeulen: The mechanism of interaction between cisplatin and selenite. *Biochem. Pharmacol.* **41**, 1429–1437 (1991).
17. J. D. Hoeschele, T. A. Butler, J. A. Roberts, and C. E. Guyer: Analysis and refinement of the microscale synthesis of the [^{195m}Pt] -labeled antitumor drug, cis-dichlorodiammine-platinum(II), cis-DDP. *Radiochim. Acta* **31**, 27–36 (1982).
18. G. S. Baldew, K. J. Volkers, J. J. M. De Goeij, and N. P. E. Vermeulen: Determination of cisplatin and related platinum complexes in plasma ultrafiltrate and urine by high-performance liquid chromatography with on-line radioactivity detection. *J. Chrom. Biom. Appl.* **491**, 163–174 (1989).
19. G. Los, P. H. A. Mutsaers, W. J. F. Van der Vijgh, G. S. Baldew, P. W. De Graaf, and J. G. McVie: Direct diffusion of cis-diamminedichloroplatinum(II) in intraperitoneal tumors after intraperitoneal chemotherapy: a comparison with systemic chemotherapy. *Cancer Res.* **49**, 3380–3384 (1989).
20. M. B. Van Hennik, W. J. F. Van der Vijgh, I. Klein, F. Elferink, J. B. Vermorken, B. Winograd, and H. M. Pinedo: Comparative pharmacokinetics of cisplatin and three analogues in mice and humans. *Cancer Res.* **47**, 6297–6301 (1987).
21. G. S. Baldew, J. J. M. De Goeij, and N. P. E. Vermeulen: Determination of [^{75}Se]-labelled selenite and metabolites in plasma and urine by high-performance liquid chromatography with on-line radioactivity detection. *J. Chrom. Biom. Appl.* **496**, 111–120 (1989).
22. W. A. J. De Waal, F. J. M. J. Maessen, and J. C. Kraak: Analysis of platinum species originating from cis-diamminedichloroplatinum(II) (cisplatin) in human and rat plasma by HPLC-ICP-AES. *J. Chromatogr.* **273**, 301–327 (1987).
23. A. T. Diplock: Metabolic aspects of selenium action and toxicity. *CRC Crit. Rev. Toxicol.* **4**, 271–329 (1976).
24. H. E. Ganther: Pathways of selenium metabolism including respiratory excretory products. *J. Am. Coll. Toxicol.* **5**, 1–5 (1986).
25. R. J. Kraus, S. J. Foster, and H. E. Ganther: Analysis of trimethylselenonium ion in urine by high-performance liquid chromatography. *Anal. Biochem.* **147**, 432–436 (1985).
26. Z. H. Siddik, D. R. Newell, F. E. Boxall, and K. R. Harrap: The comparative pharmacokinetics of carboplatin and cisplatin in mice and rats. *Biochem. Pharmacol.* **36**, 1925–1932 (1987).
27. S. B. Howell: Intraperitoneal chemotherapy for ovarian carcinoma. *Contr. Oncol.* **29**, 72–83 (1988).
28. F. Y. F. Lee, M. J. Allalunis-Turner, D. W. Siemann: Depletion of tumour versus normal tissue glutathione by buthionine sulfoximine. *Br. J. Cancer* **56**, 33–38 (1987).
29. G. S. Baldew, K. J. Volkers, and C. J. A. Van den Hamer: Reduction of cisplatin nephrotoxicity by selenium: does metallothionein play a role? *Arch. Toxicol. Suppl.* **12**, 171–174 (1988).