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Reduction of Cisplatin-Induced Nephrotoxicity in Vivo by Selenomethionine: The Effect on Cisplatin−DNA Adducts

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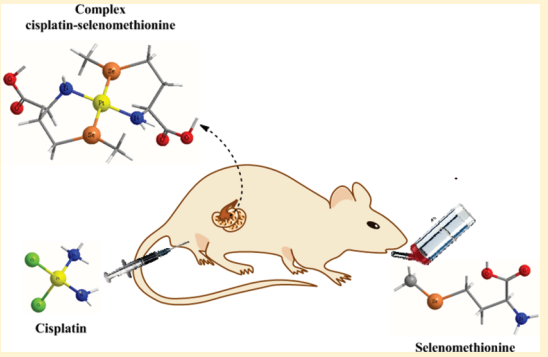
ABSTRACT: Cisplatin is one of the most effective chemotherapeutic agents, although its clinical use is limited by severe renal toxicity. This toxicity seems to be related to the accumulation of the drug in kidney tissues, leading to renal failure. For this reason, several compounds have been evaluated to ameliorate the nephrotoxicity induced by cisplatin. In the present investigation, we report the effect of the oral administration of selenomethionine before intraperitoneal cisplatin treatment. The preadministration of this Se species has been shown to have an important effect in reducing renal damage induced by cisplatin by increasing the excreted urea and improving creatinine clearance. Quantification of the level of DNA−cisplatin adducts in kidney and liver tissues was carried out by postcolumn isotope dilution analysis using liquid chromatography-inductively coupled plasma (LC-ICP-MS) as speciation set up. The level of DNA−cisplatin adducts in rats given Se-methionine in the drinking water before cisplatin administration was considerably lower in kidney tissues with respect to the animals drinking only water. Such effects were not observed in liver tissue. Initial speciation studies of Pt and Se conducted in kidney tissues of exposed animals by HPLC-ICP-MS have revealed the presence of cisplatin as part of a complex with Se-methionine, which can be eventually excreted into urine. This Pt−Se complex could explain the observed reduction of the kidney damage in Se-methionine-treated animals.



INTRODUCTION

Cisplatin [*cis*-diamminedichloroplatinum(II), CP] is one of the most effective antineoplastic drugs used in chemotherapy. The activity has been demonstrated against a variety of tumors, particularly, for head and neck, testicular, ovarian, bladder, and small cell lung cancers.[1−3]

However, as many antitumor agents, CP antitumoral properties are associated with undesirable physiological side effects such as nephrotoxicity, ototoxicity, neurotoxicity, and bone marrow suppression.[4] In particular, its renal toxicity is recognized as being dose-limiting.[5,6] The alterations in the kidney functions induced by CP are characterized by signs of injury, such as changes in urine volume, body weight, and glutathione status; increase of lipid peroxidation products in urine and kidney; and a significant elevation in serum creatinine and urea levels.[7,8]

The exact mechanism of CP-induced nephrotoxicity is not completely understood but is generally considered to be closely associated with oxidative damage.[9−12] In fact, this drug generates reactive oxygen species (ROS) such as superoxide anion and hydroxyl radicals, [11,13] stimulates renal lipid peroxidation, and decreases the activity of antioxidant enzymes in renal tissue.[7,14] CP is also able to conjugate with glutathion,[15] which is involved in the metabolism of ROS. Moreover, plasma concentrations of various antioxidants decreased significantly during CP-based chemotherapy in cancer patients, which may reflect a failure of the antioxidant defense mechanism against oxidative damage.[9,16] In this regard, the administration of antioxidants such as vitamin E, vitamin C, lycopene, melatonin, or tiopronin, before or after treatment with CP, has been used to diminish its nephrotoxicity in humans and animals.[7,17−19] Interestingly, these compounds may not necessarily destroy the therapeutic potential of CP since the mechanisms of CP nephrotoxicity are different from the mechanism of formation of bifunctional adducts with DNA, which produces the antitumor activity.[20−22]

Inorganic selenium as well as some of the organoselenium compounds also have been found to exhibit an effective chemoprotective role.[23−25] It has been hypothesized that this effect could be associated with the antioxidant effect of Se and its compounds that might diminish the oxidative stress produced by species such as CP in living organisms.[25] Thus, animal experiments have shown that selenium can reduce CP-induced nephrotoxicity and bone marrow depression without compromising the antitumor activity of this agent.[26] In this vein, two selenium compounds (sodium selenite and 2-phenyl-1,2-benzoselenazol-3-one known as ebselen) have demonstrated their capacity to reduce side effects of CP.[27−29] Similarly, selenomethionine[30,31] or selenosulfate,[32] administrated intraperitonally 1 h before CP, exhibited nephroprotective effects without interfering with the antitumor action of the drug. However, the mechanism of the

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Table 1. Instrumental Operating Conditions Used for Separation of Seleno-Species and CP−DNA Adducts by HPLC

	HPLC parameters in vitro studies	
reverse phase column	RP C_8 (250 mm × 2.1 mm i.d., 5 μm, Alltech, Perkin-Elmer Corp.)	
mobile phases	60 mM ammonium acetate (pH 5.8), 7.5% MeOH	
injection volume	20 μL	
flow rate	0.2 mL min^{-1}	
	HPLC speciation of kidney and urine	
SEC/ion exchange column	Shodex Asahipak GS-220 HQ (300 mm × 7.5 mm i.d., 6 μm, Showa Denko)	
mobile phases	10 mM ammonium acetate (pH 6)	
injection volume	20 μL	
flow rate	0.6 mL min^{-1}	
	HPLC parameters IDA analysis	
reverse phase column	RPZorbax XDB-C_{18} narrow-bore (150 mm × 2.1 mm i.d., 5 μm, Agilent)	
mobile phases	(A) 20 mM ammonium acetate (pH 6.5) in Milli-Q water	
	(B) 20 mM ammonium acetate (pH 6.5) in methanol	
gradient	time (min)	*B* (%)
	0	0
	5	0
	35	15
	39	15
	40	0
	60	0
injection volume	20 μL	
flow rate	0.2 mL min^{-1}	
spike postcolumn	10 ng g^{-1} ^{194}Pt in 1% nitric acid	
flow rate postcolumn	0.1 mL min^{-1}	

protective effect of Se species in the treatment of CP nephrotoxicity is still controversial. It is known that selenium can produce in vivo, among its metabolites, selenols (RSeH), which might be capable of scavenging CP, thereby preventing its nephrotoxicity. Baldew et al.[33] demonstrated that methyl-selenol is capable of reacting with CP in vitro, but they could not establish the identity of the reaction products.

Therefore, in the present work, we have tried to shed light on the mechanisms through which Se is active in reducing CP-induced nephrotoxicity in rats. For this purpose, we have evaluated the effect of Se-methionine administration on the formation of DNA−CP adducts in vivo in different organs, including kidney and liver. Because adducts formation is the final route of the therapeutic action of CP in tumor cells, it should be preserved in organs for which CP therapies are used. On the other hand, the collateral toxic effects observed in such therapies are also associated with the formation of adducts in organs that are not targets for the Pt chemotherapeutic treatment (e.g., kidney). Therefore, we try to establish if Se-methionine can be used to reduced CP adduct formation in kidneys but maintain it in other organs (e.g., liver). For this purpose, we have used a previously developed HPLC-ICP-MS setup and a quantification method based on isotope dilution.[34] Additionally, the levels of urea, creatinine, and creatinine clearance as well as the total Pt and Se accumulated in liver and other tissues have been evaluated in Wistar male rats treated with Se-methionine and CP. Initial speciation studies of Pt and Se also have been conducted in urine and kidney tissues.

EXPERIMENTAL PROCEDURES

Chemicals. Analytical reagent grade chemicals were used throughout unless otherwise stated. All solutions and dilutions were made with ultrapure deionized water (≥18.2 MΩ cm) from an Advance A10 Milli-Q system (Millipore, Bedford, MA). CP, sodium salt of the deoxyguanosine 5′-(mono)phosphate (dGMP), and selenomethionine (Se-MetH) were purchased from Sigma-Aldrich (St. Louis, MO). CP is carcinogenic and should be handled in a suitable fume hood, and appropriate personal protective equipment should be used. The mobile phases for HPLC separations were prepared by using methanol (HPLC grade, Merck, Darmstadt, Germany), ammonium acetate (Sigma-Aldrich), and acetic acid (Merck). Suprapure 30% hydrogen peroxide and analytical reagent-grade 65% nitric acid, additionally purified by subboiling distillation, both from Merck, were used for the microwave digestion of kidney and liver tissue samples. A 1000 mg L^{-1} amount of galium ICP standard was also obtained from Merck. All of the chemicals for the DNA isolation were purchased from Scharlau or Merck.

Enzymatic hydrolysis of DNA samples was conducted by incubation with Nuclease S1 (GE Healthcare, Barcelona, Spain). The activation buffer to dissolve the enzyme was provided by the manufacturer and contained 10 mM sodium acetate (pH 4.6), 150 mM sodium chloride, 0.05 mM zinc sulfate, and 50% glycerol. To remove the excess of enzyme, membrane ultracentrifugation devices were used (Centricon YM-10 centrifugal filter devices, Millipore).

Chromatographic Separation. All of the HPLC separations were carried out using a dual piston liquid chromatographic pump (Shimadzu LC-20AD, Shimadzu Corp., Kyoto, Japan) equipped with a sample injection valve by Rheodyne, model 7125 (Cotati, CA), fitted with a 20 μL injection loop. All of the connections were made of PEEK tubing (i.d. 0.25 mm). Three columns were used as follows: (A) Alltech RP C_8 (250 mm × 2.0 mm i.d., 5 μm): reverse phase column for in vitro studies of interaction between CP-dGMP adducts and selenomethionine; (B) Shodex Asahipak GS-220 HQ (300 mm × 7.5 mm i.d., 6 μm): size exclusion/ion exchange column for the speciation of Se−Pt species in kidney and urine from the rats; and (C) Agilent Zorbax RP XDB-C_{18}

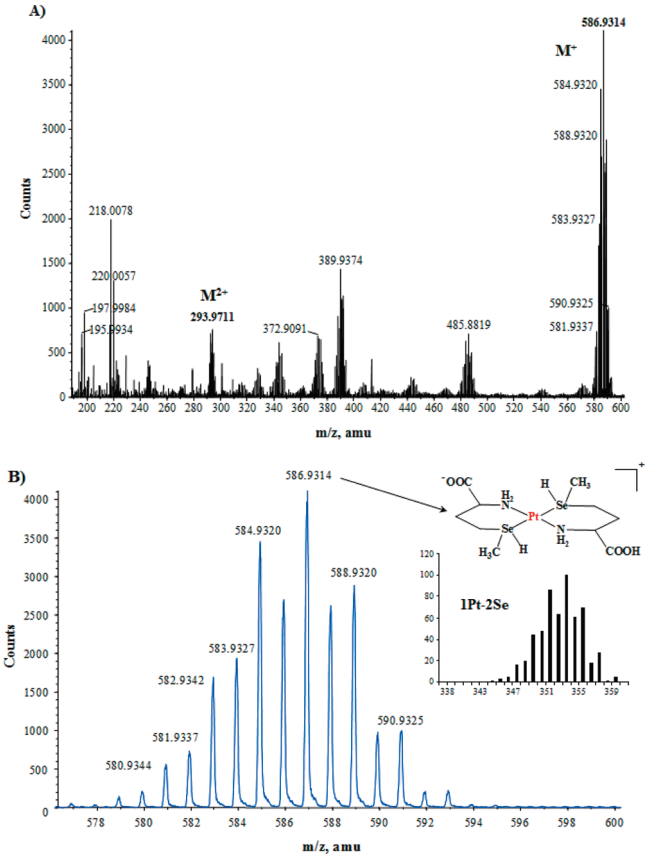

Figure 1. Mass spectra of the species obtained by incubation of Se-methionine:CP (2:1). (A) Full mass spectra and (B) magnification of the m/z 586.931, proposed structure, and molecular model for the complex with two Se atoms and one Pt atom.

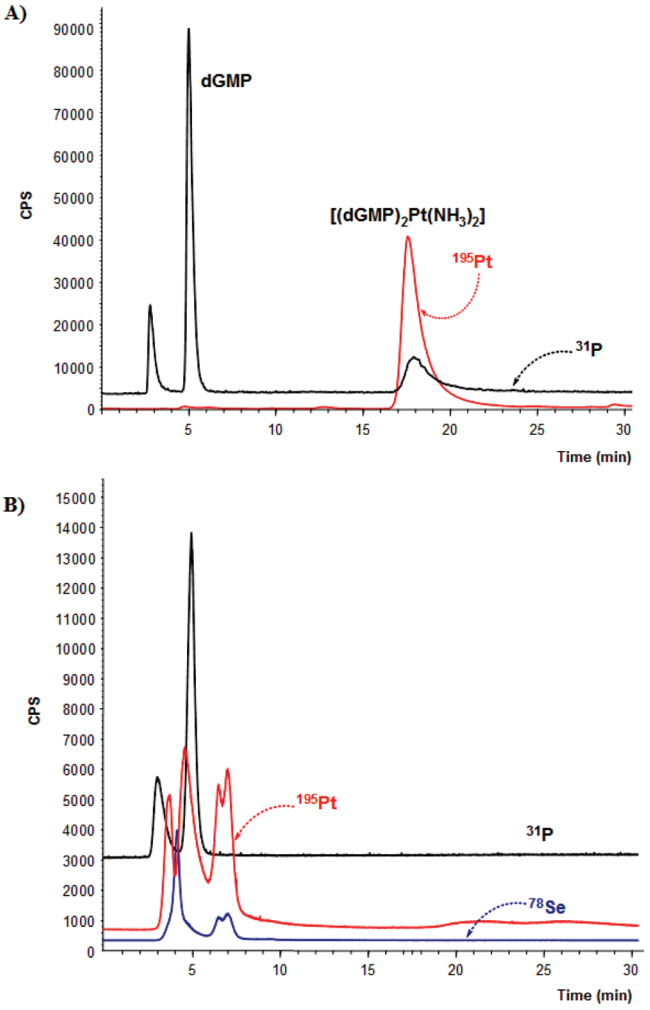

Figure 2. HPLC-ICP-MS chromatogram of adducts formed by (A) interaction between CP and dGMP, $[(dGMP)_2Pt(NH_3)_2]$ (Pt, red trace; and P, black trace), and (B) interaction between CP previously incubated with Se-methionine (1:2) and dGMP (Pt, red trace; P, black trace; and Se, blue trace).

narrow-bore (150 mm × 2.1 mm i.d., 5 μm): reverse phase column for quantitative analysis of kidney and liver CP− DNA adducts. All of the operating conditions are summarized in Table 1.

ICP-MS and ESI-MS Measurements. The ICP-MS instrument used in this study was an Agilent 7500ce (Agilent Technologies, Tokyo, Japan) equipped with a collision cell system. The Agilent system was fitted with a MicroMist concentric nebulizer (0.4 mL min^{-1}) with EzyFit, EzyLok arm, and EzyLok connector (Glass Expansion, Melbourne, Australia). The ESI quadrupole time-of-flight (ESI-q-TOF) instrument employed in this work was a QStar XL model (Applied Biosystems, Langen, Germany) equipped with the ion spray source and using N_2 as a nebulization gas. The scanned range goes from m/z 50 to m/z 1200, the instrument was daily calibrated, and the measurements were taken in positive mode.

Animals Enrolled in the Study. Treatment of the animals in this study followed ethical guidelines established by the Ethical Commission of the University of Oviedo. Approval of all animal procedures was obtained from the local ethics committee. Eleven male Wistar rats weighing 243−284 g were randomly distributed into three groups and housed individually in stainless steel cages in a pathogen-free University Laboratory Animal Research facility. They were kept under controlled temperature and illumination (24 °C; 12 h light/12 h dark cycles, respectively). Food and distilled water were provided ad libitum.

Experimental Design. Rats were acclimatized for 3 days. Groups of animals were, respectively: controls (C, $n = 3$), CP-treated ($n = 3$), and selenium−CP-treated (Se-CP, $n = 3$). The Se-CP group of animals was pretreated, before CP administration, with 100 mL of a solution of 100 μg mL^{-1} of Se-methionine in the drinking water. Because every rat weighted about 250 g and they drank about 20−25 mL per day, the total Se ingested was approximately 4 mg selenium per kg of body weight. The control and CP groups were treated only with distilled water in their drinking bottles. These treatments were extended for 11 consecutive days. The dose and time of treatment were defined in preliminary tests and on the basis of literature data.[30,31] On the eleventh day, the rats received intraperitoneal (ip) injection of 5 mg per kg of body weight of CP in 0.9% saline solution (groups CP and Se-CP) or only 0.9% saline solution (group C). From that moment on, distilled water was supplied to the entire group of animals. These treatments were repeated each 24 h for 7 successive days. The body weight of animals was recorded daily.

Prior to the animals being euthanized, isofluorane anesthesia at 5% vaporized in O_2 was used; the animals of each group were then sacrificed, and blood collection was obtained by cardiac puncture. Blood was sampled from ophthalmic veins to obtain serum after centrifugation at 3000 g for 15 min. Sera were stored at −20 °C before assay. The extracted organs (kidney and liver) were removed and protected against light. Then, organ tissue samples were frozen with liquid N_2 and rapidly lyophilized for 72 h in a Heto Lyolab 3000 freeze-driermodel from Heto-Holten (Allerød, Denmark). Finally, organs were individually stored at −80 °C until analysis.

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Table 2. Effects of Oral Administration of Se-Methionine before CP on CP-Induced Changes in Body Weight, Urinary Volume, Serum Creatinine, and Creatinine Clearance of Treated Rats[a]

Groups of animals	Change in Body weight (g)	Urinary volume ($mL \cdot 24h^{-1}$)	Serum creatinine ($mg \cdot dL^{-1}$)	Creatine clearance ($mL \cdot min^{-1}$)
C	34 ± 8	6.7 ± 0.8	0.34 ± 0.06	0.81 ± 0.09
CP	* -28 ± 7 ***	** 18.1 ± 1.9 ***	ns 4.3 ± 0.9 **	* 0.021 ± 0.004 ***
Se-CP	-13 ± 3 ***	11.3 ± 1.2 **	4.6 ± 1.2 **	0.036 ± 0.006 ***

[a] Values are means ± SDs for three animals in each group. Statistical significance (*$p < 0.01$, **$p < 0.05$, and ***$p < 0.001$; ns, not significant). Stars on the right hand side refer to differences with the control group, and stars on the left hand side refer to the differences among treated groups.

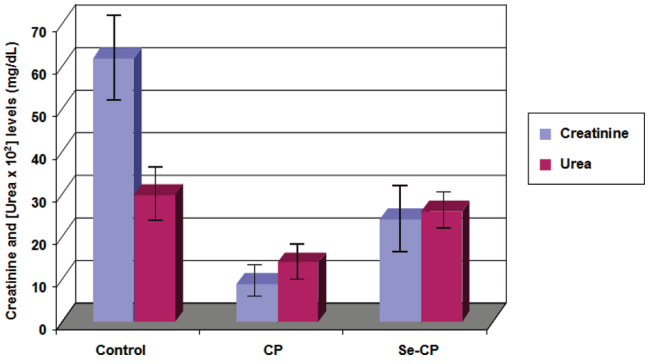

Figure 3. Levels of creatinine and urea in urine measured in the three groups of animals in the study: C, CP, and Se-CP (each experiment was conducted during the last week of treatment and corresponded to an average of 8 days and three animals in each group).

Urine was daily collected by using metabolic cages, and analysis of creatinine and urea was conducted using commercial kits. Creatinine clearance was calculated by use the formula: [urine creatinine] × [urinary volume h^{-1}]/[serum creatinine].

Kidney and Liver Pretreatment for Total Metal Determination. Total selenium and platinum contents in kidney and liver of the animals were measured by ICP-MS. For this purpose, after the tissues were freeze-dried, they were ground and homogenized in an agate mortar followed by a microwave digestion, using a microwave oven model Ethos I (Milestone, Socisole, Italy). Approximately 250 mg of tissue (either kidney or liver) was accurately weighed with a Precisa Instruments XB220A analytical balance (Dietikon, Switzerland) into microwave digestion vessels. Diluted sub-boiling HNO_3 (1:3 v/v, 8 mL) and 2 mL of H_2O_2 were added to the digestion vessels and digested by following the microwave program: (1) 3 min ramp to 95 °C, (2) 10 min ramp to 160 °C, (3) 3 min ramp to 180 °C, (4) 15 min hold at 180 °C, and (5) cooling.

It is worth stressing that microwave digestion vessels were thoroughly cleaned before all of the sample digestions making use of the same reagents and microwave program previously mentioned but in the absence of the sample. Moreover, at least one digestion blank was prepared for every 10 samples. Finally, the digests were stored at −20 °C until analysis.

Kidney and Urine Pretreatment for Metals Speciation Analysis. For speciation purposes, 200 mg of kidney rat was ground and homogenized in a special glass crush container with four volumes (w/v) of buffer (50 mM Tris/HCl, pH 7.4, Sigma). The homogenate was sonicated for 30 min and centrifuged at 15000*g* for 30 min. The supernatant liquid was filtered through 0.22 μm syringe filters. All preparative steps were performed at 4 °C. The supernatant was collected and stored at −20 °C until analysis. The urine samples were just filtered through 0.22 μm syringe filters and stored at −20 °C.

RESULTS AND DISCUSSION

Reaction between CP and Se-Methionine. To obtain maximum efficiency in the chemotherapeutic treatment with CP and maximum possible protection by selenium, it is necessary to investigate the way of administration of both compounds. One possibility is the coadministration of both compounds that has proved to be adequate in the case of other Se species such as sodium selenite.[28] We evaluated this possibility by incubating Se-methionine and CP and adding, afterward, deoxyguanosine monophosphate (dGMP) to the solution (since this is the target molecule for CP chemotherapeutic activity). The results obtained by mixing Se-methionine and CP at a 2:1 molar ratio after 24 h of incubation were evaluated by ESI-MS. The mass spectrum obtained, which is shown in Figure 1A, revealed the presence of a major species that correspond to $[Pt(Se\text{-}Met)(Se\text{-}MetH)]^+$ at m/z 586.931. It is also possible to observe the doubly charged species of this compound (M^{2+} m/z 293.971). The magnification of the mass spectrum (Figure 1B) shows the isotope distribution corresponding to two Se atoms and one Pt atom and exhibits quite good resolution. The structure of this species has been confirmed by MS/MS (data not shown).

Additionally, the second most abundant species corresponds to the ion at m/z 389.937 containing in this case, one Se and one Pt atom on the structure, which comes from the loss of one Se-methionine unit from the molecular ion (also proved by MS/MS, data not shown). Similar results have been found by other authors,[35] and such findings might explain the protection of Se-methionine against CP toxicity. However, unresolved questions remain regarding the chemotherapeutic effects of CP once such a complex with Se-methionine is formed. For this study, after the formation of the Se-methionine/CP complex (shown in Figure 1A), dGMP (DNA target for CP) was added to the incubation mixture in equimolar concentration with Se-methionine. This combination was left to react for 12 h, and the resulting mixture was injected in the chromatographic system. Our previous results[36] revealed that in the absence of Se-methionine, CP reacts with dGMP immediately after mixing by forming a complex that can be chromatographically separated from the excess of the nucleotide as can be seen in Figure 2A. However, if CP is previously complexed with Se-methionine, the chromatographic profile obtained is quite different, as shown in Figure 2B. In this latter case (Figure 2B), the Pt signals (red trace) are shown at about 3.5 and 5 min, respectively (maybe ascribed to the aqua complexes of the drug) and at approximately 7 min in a

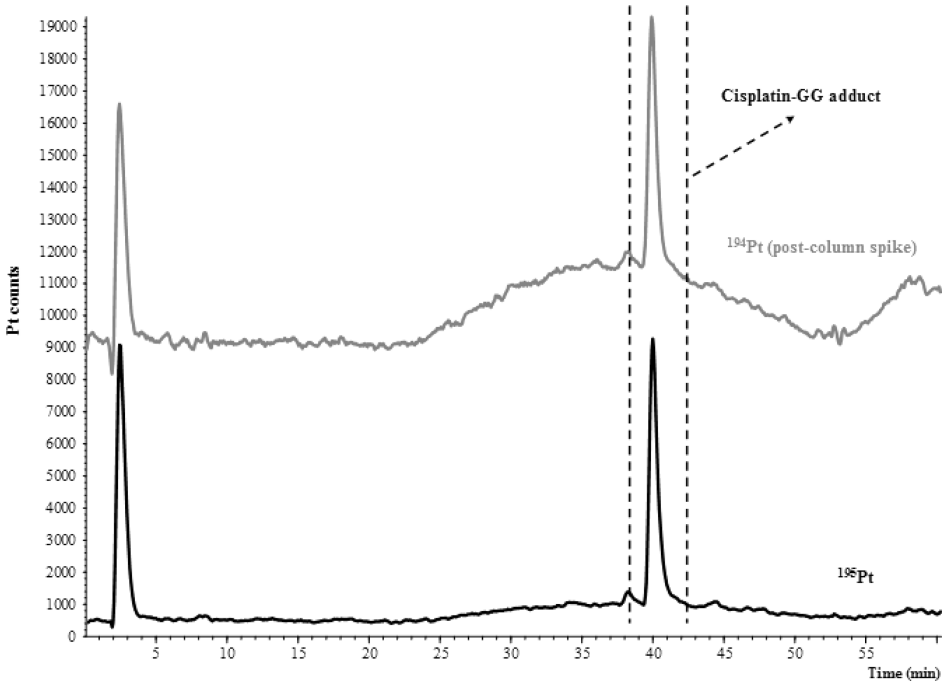

Figure 4. HPLC-ICP-MS chromatogram of DNA extracted from kidney of a rat from the Se-CP group followed by enzymatic hydrolysis. Postcolumn addition of the ^{194}Pt spike can be observed in the gray trace. Chromatographic conditions are reported in Table 1.

Table 3. Concentration of the Main CP Adducts (Shown in Figure 4) Found in Renal and Liver DNA from Rats That Were Administrated Different Treatments with Seleno-Methionine and CP[a]

	groups of animals	bisadduct (ng Pt/mg DNA)	total Pt (ng Pt/mg DNA)
rat kidney DNA	CP	89 ± 12	133 ± 35
	Se-CP	$31 \pm 5^{**}$	85 ± 24^{ns}
rat liver DNA	CP	54 ± 8	210 ± 43
	Se-CP	43 ± 7^{ns}	$116 \pm 16^{*}$

[a] Standard deviations were calculated for three different digestions of DNA samples from three animals in each group. Statistical significance of the mean values of the two groups is shown on the right hand side of the value ($^{*}p < 0.01$, $^{**}p < 0.05$, and $^{***}p < 0.001$; ns, not significant).

double peak where the Se signal (blue trace) can also be observed (ascribed to the CP−Se-methionine complex). However, the signal at 18 min corresponding to the $[(dGMP)_2Pt(NH_3)_2]$ complex shown in Figure 2A is negligible in Figure 2B. These results reveal that the efficacy of the CP treatment might be compromised when it is coadministrated with Se-methionine. Therefore, in vivo experiments with Se-methionine and CP were conducted by sequential administration of the compounds.

Furthermore, the studies on the protective effect of Se-methionine were also evaluated in the alternative order: (1) forming the adduct $[(dGMP)_2Pt(NH_3)_2]$ by direct interaction of CP with dGMP and (2) posterior addition of Se-methionine (in a stoichiometric ratio 1:1 with dGMP). In this case (data not shown), no reduction effect was observed on the peak corresponding to $[(dGMP)_2Pt(NH_3)_2]$ (at 18 min in Figure 2A). Therefore, Se-methionine competed with dGMP for interaction with CP, affecting the formation of the $[(dGMP)_2Pt(NH_3)_2]$ complex, but once the interaction of dGMP with CP has occurred, no effect of Se-methionine seems to take place.

In Vivo Experiments: Animal Weights, Excreted Urine, and Creatinine Levels in Urine and Plasma. Body weight variations are generally an important factor in toxicological studies. For this purpose, the three groups of animals in study (C, CP, and Se-CP) were weighed every day of the experiment, and Table 2 lists the obtained results. As can be seen, the control animals gained, on average, +34 g of body weight ($n = 3$) during the 21 days of the experiment. The body weight of animals that received intraperitoneal injections of CP (5 mg kg^{-1}) decreased by −28 g. However, when Se-methionine was administrated in drinking water 11 days before CP injection, the reduction in the body weight was less significant (−13 g). Because the weight loss of animals treated with CP can be, at least, partially due to the drug gastrointestinal toxicity, which accelerates the water elimination in urine, Se-methionine pretreatment seems to reduce such an effect.

In terms of the volume of excreted urine, the control group excreted relatively constant amounts of urine (6.7 mL per day), while the CP group excreted a significantly higher urinary volume (170% higher) 7 days after the drug injection as compared to the control group (Table 2). The animals previously treated with Se-methionine exhibited urinary levels in between the control and the CP groups, showing, once again, some effect on the Se-methionine pretreatment.

A third important parameter to take into account in the evaluation of the kidney damage produced by CP treatments is related to the plasma and urine creatinine levels and also the urea level.[37] Once the kidneys are severely injured, they reduce their capability to eliminate toxic substances, which results in the accumulation of undesired metabolic products (e.g., creatinine and urea) into the bloodstream. Results of such experiments and measurements can be observed in Figure 3, where the levels of

Table 4. Effect of Oral Administration of Seleno-Methionine (4 mg per kg of Body Weight for 11 Days) on Elevation of Selenium Levels in Kidney Rat and Liver[a]

Groups of animals	Conc. Se Kidney (μg · mL^{-1})	Conc. Se Liver (μg · mL^{-1})
C	2.1 ± 0.2	1.5 ± 0.2
CP	*** 2.2 ± 0.3 ns	** 1.6 ± 0.3 ns
Se-CP	*** 10.4 ± 1.2 ***	** 5.3 ± 0.9 **

[a] Values are means ± SDs for three animals in each group. Statistical significance (*p < 0.01, **p < 0.05, and ***p < 0.001; ns, not significant). Stars on the right hand side refer to differences with the control group, and stars on the left hand side refer to the differences among treated groups.

Table 5. Effect of Intraperitoneal Injection of CP (5 mg per kg of Body Weight ip for 7 Days) on Elevation of Platinum Levels in Kidney Rat and Liver[a]

Groups of animals	Conc. Pt Kidney (μg · mL^{-1})	Conc. Pt Liver (μg · mL^{-1})
C	0.0 ± 0.0	0.0 ± 0.0
CP	* 57.6 ± 4.1	ns 23.8 ± 3.4
Se-CP	* 41.2 ± 5.2	ns 22.3 ± 4.7

[a] Values are means ± SDs for three animals in each group. Statistical significance of the mean values of the two groups is shown on the left hand side of the value (*p < 0.01; ns, not significant).

creatinine and urea found in urine after the different treatments have been plotted. The CP injections reduced dramatically the renal function and produced an important decrease in the level of excreted creatinine and urea. However, the pretreatment with Se-methionine reduces the observed damage in both cases and to an almost normal (control) level for urea. Similar results can be observed when the creatinine clearance is evaluated. In this case, the level of urine creatinine (mg/mL) multiplied by the volume of excreted urine (in 24 h) is referred to the creatinine concentration in serum (mg/mL). These results reveal the amount of creatinine that is removed from blood over a given time interval and also has been calculated for the three groups of animals in study (see Table 2). Again, the elimination rate is approximately twice higher in the case of the Se-methionine pretreatment, which confirms a better renal function in Se-methionine-treated rats as well as the partial protection of the kidneys.

CP−DNA Adducts Measurement: Kidney versus Liver. The main mechanism through which CP exerts its cytotoxic action in tumor cells is the formation of covalent adducts with DNA molecules that finally yield to cell death. This is also the mechanism of CP cytotoxicity in nontumoral cells. However, to our knowledge, no information exists on the effect of Se-methionine preadministration in the formation of CP−DNA adducts in kidney samples (the main mechanisms of cytoxicity). By applying a previously developed methodology,[34] we evaluated here the level of adducts CP−DNA in the kidney and liver of the three groups of animals under scrutiny (C, CP, and Se-CP).

For this purpose, the kidney and liver tissues of the different animals were treated to isolate the DNA as we previously described.[34] After enzymatic digestion, the DNA samples are analyzed by HPLC-ICP-MS. Pt quantification has been done by postcolumn isotope dilution analysis (IDA), also called species-unspecific spiking mode, using a ^{194}Pt-enriched solution. All of the experimental conditions already have been optimized in previous publications.[34,36] Figure 4 shows the Pt chromatogram obtained with the proposed methodology and corresponding to the DNA extracted from rat kidney from the Se-CP group. In this figure, it is possible to observe a single species at 40 min that corresponds to the bifuncional adduct of CP with two adjacent guanine bases of the same strand of DNA (CP-GG) and some Pt in the void volume of the column as well as the continuous flow of the spike (gray trace). Using the well-established IDA equation, it is possible to quantify amounts of adducts in the kidney and liver samples. Thus, Table 3 shows the quantitative results of the bifuncional adduct and total eluted Pt in the different samples (corrected for column recovery, 68%).

The control group presented no adducts formation. Interestingly, the CP group presented an increase in the level of the formed CP−DNA adduct in rat kidney DNA when compared to the Se-CP group. In fact, kidney DNA adducts were significantly greater (about 3-fold) in CP-treated animals with respect to the Se-CP group. On the other hand, similar measurements were taken in the liver of exposed animals (see Table 3), and in this case, the two groups of animals (with and without Se-methionine pretreatment) did not exhibit significant differences in terms of formed CP−DNA adducts, even if total Pt was halved with the Se-methionine treatment. Such results support the role of Se-methionine as nephroprotective agent against CP, while the drug maintains its chemotherapeutic activity. This fact could be explained by considering the interaction between Se-methionine (previously accumulated in kidney) and CP (reported in the first section of the manuscript). As previously reported here, the reaction between both species resulted in a stable complex that could prevent the formation of higher level of CP−DNA adducts in this organ (Figure 2B).

Total Pt and Se Levels in Rat Kidney and Liver. Thus, it appears that the protective effect of Se-methionine against CP nephrotoxicty could be due to its tendency to bind directly to CP,

Table 6. Total Platinum and Selenium Urine Levels in the Treated Rats under Study[a]

Groups of animals	Conc. Se Urine (μg · mL^{-1})	Conc. Pt Urine (μg · mL^{-1})
C	2.0 ± 0.6	0.015 ± 0.002
CP	0.63 ± 0.05 * (***)	16.3 ± 1.5 *** (*)
Se-CP	5.7 ± 0.8 ** (***)	22.3 ± 2.7 *** (*)

[a] Values are means ± SDs for three animals in each group. Statistical significance (*$p < 0.01$, **$p < 0.05$, and ***$p < 0.001$; ns, not significant). Stars on the right hand side refer to differences with the control group, and stars on the left hand side refer to the differences among treated groups.

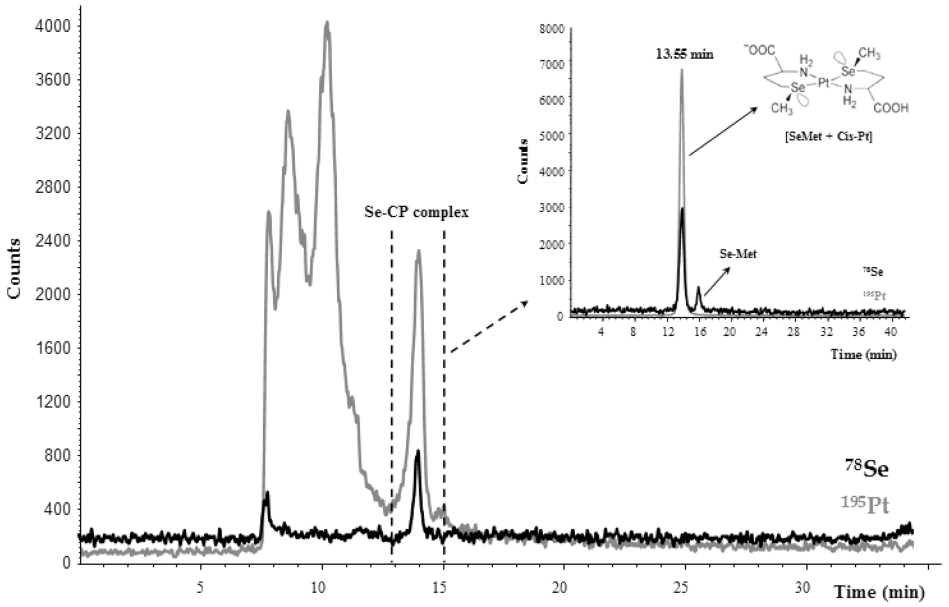

Figure 5. HPLC-ICP-MS chromatogram of kidney supernatant obtained from animals that were administrated Se-methionine before injection of CP. Chromatographic conditions are reported in Table 1.

forming a complex in the kidney that might protect this organ from the free form of CP (which causes cells damage). To confirm this hypothesis, we evaluated the levels of accumulated Pt and Se in the kidney and liver tissues of the exposed animals after acid mineralization of the samples.

Observed mean values for total Se and Pt concentrations in kidney and liver for the different situations are shown in Tables 4 and 5, respectively. No significant differences were observed in Se levels between control and CP treated animals. In the case of the Se-CP group, a statistically significant increment in concentration of the accumulated selenium is observed in kidney and liver tissues. It is noteworthy that the concentration of Se in kidney of this group with respect to the control group was about 5-fold, while in liver, it was only 3-fold. This finding supports the fact that more inhibition of CP−DNA adducts formation is observed in the tissue accumulating higher concentration of Se. Similarly, the levels of accumulated Pt in kidney and liver show a similar trend: In liver, there is no effect on the accumulated Pt when Se-methionine is given as pretreatment, but in kidney samples, the total Pt in the animals that did not received Se-methionine is significantly higher. Such results favor the hypothesis that in the kidney part of the CP present might form a complex with Se-methionine present to be excreted in urine, at a different rate than CP itself. This assumption was also confirmed by measuring total concentration of Se and Pt in the urine of the three groups of animals under study. The results obtained are shown in Table 6. As can be seen, an increment in the concentration of both Se and Pt is observed in urine of the Se-CP group with respect to the CP group. Such interaction reduces the damage in this organ (low level of adducts, higher urea in urine, and higher creatinine clearance). To further confirm this line of thinking, elemental speciation studies of Se and Pt were conducted in urine and kidney tissues.

Speciation of Se−Pt Compounds in Kidney and Urine. For speciation purposes, the crucial step is preserving the original species integrity in the sample during the whole analytical process. Therefore, the kidney samples were processed using extraction conditions that were different from those for DNA and, of course, different chromatographic conditions as well. After optimization of such conditions (see parameters in Table 1), it was possible to separate chromatographically the Pt- and Se-containing species in the kidney extract by HPLC-ICP-MS. The obtained results are shown in Figure 5 for the kidney extract of an animal from the Se-CP group (treated with Se-methionine before CP administration). The presence of a Se−Pt-containing species at 13.5 min is apparent and could be ascribed to the CP−Se-methionine complex previously observed by electrospray (see section 3.1). In fact, the CP−Se-methionine complex synthesized in vitro and chromatographically

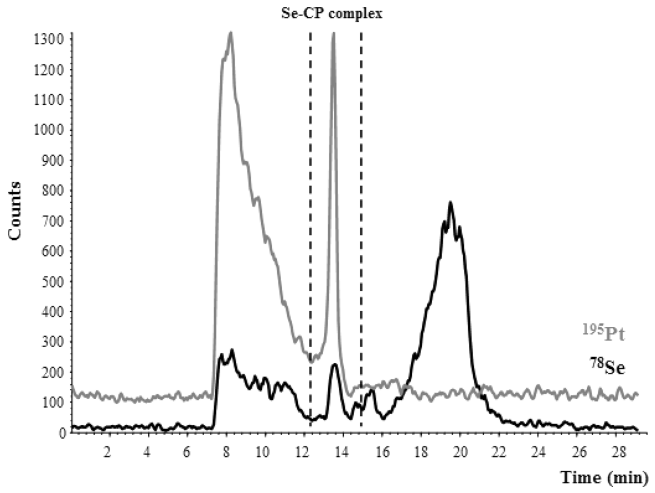

Figure 6. HPLC-ICP-MS chromatogram of urine sample obtained from animals that were administrated Se-methionine before injection of CP. Urine was collected on the 7th day treatment with CP. Chromatographic conditions are reported in Table 1.

separated, under the same chromatographic conditions, present a similar retention time to that of the Se−Pt-containing species (see the inset of Figure 5). This species was collected for further ESI-MS analysis, but unfortunately, the salt concentration was too high, while the species concentration was too low to obtain a reliable MS spectrum.

In spite of the limited intensity of the found Se−Pt species in the kidney extract; however, the coelution of Se and Pt suggests that Se-methionine could interact with CP to form a stable CP−Se-methionine complex (similar to the one formed in vitro), and so, it could be eventually excreted in urine. Therefore, similar speciation experiments were conducted in urine from Se-CP-treated animals, and the observed results are shown in Figure 6 for ^{195}Pt and ^{78}Se followed isotopes. As can be seen in this figure, it is possible to detect again a Se−Pt-containing compound at a similar retention time (13.5 min), apart from some other Pt and Se species, respectively. This species also could be confirmed (detected) using a different chromatographic setup consisting of a Superdex Peptide 10/300 GL column (data not shown).

CONCLUSIONS

In vitro experiments have revealed that chemical interaction between Se-methionine and CP reduces the capability of this platinum-based chemotherapeutic drug for reaction with DNA nucleobases. Because this is an undesired side effect, the Se-methionine nephroprotective role has been evaluated in vivo by sequential treatments of Se-methionine and then CP. In this regard, positive evidence on the protective role of Se-methionine on the CP-induced nephrotoxicity has been found. The evaluation of parameters such as creatinine and urea in urine and creatinine clearance revealed improvements in the renal function after oral administration of Se-methionine for 11 days before CP administration to rats. In addition, the quantitative evaluation of the DNA−CP adducts showed significant differences between groups. Using our ICP-MS new quantitative strategy, it has been possible to observe, for the first time, that Se-methionine treatment reduces the level of adducts in kidney tissues (where higher Se accumulation has been found). Importantly, the efficacy of the CP drug treatment turned out to be unaltered in the liver.

The speciation of kidney tissues and rat urine in the animals exposed to CP and Se-methionine revealed the presence, in both cases, of a species where Se and Pt coelute, and that corresponds to the retention time of the CP−Se-methionine complex previously synthesized.

All of the obtained evidence supports that using Se-methionine or Se-methionine containing nutritional supplements will provide a positive effect on the protection of the kidneys against CP-induced toxicity, when administrated in a sequential therapy. Such findings are of extraordinary interest to study and ameliorate CP side effects during chemotherapy, still one of the main limiting factors in the use of one of the most popular drug use against cancer today.

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