Kinetics of Cisplatin and Its Monohydrated Complex with Sulfur-Containing Compounds Designed for Local Otoprotective Administration

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The anticancer drug cisplatin can cause permanent inner ear damage. We have determined the second-order degradation rate constant, k_{Nu}, of cisplatin and its more toxic monohydrated complex (MHC) in the presence of each of the sulfur-containing nucleophiles *N*-acetyl-L-cysteine, L-cysteine methyl ester, 1,3-dimethyl-2-thiourea, D-methionine, and thiosulfate, compounds that are under evaluation for local administration to prevent cisplatin-induced ototoxicity. MHC was isolated from a hydrolysis solution of cisplatin using liquid chromatography (LC). The degradations were evaluated by measuring the disappearance of MHC and cisplatin at 37°C and pH 7.4 in the presence of each of the nucleophiles using LC and photometric detection. The k_{Nu} of MHC and of cisplatin was 0.044 $M^{-1}sec^{-1}$ and 0.012 $M^{-1}sec^{-1}$ with *N*-acetyl-L-cysteine, 0.24 $M^{-1}sec^{-1}$ and 0.067 $M^{-1}sec^{-1}$ with L-cysteine methyl ester, 0.16 $M^{-1}sec^{-1}$ and 0.074 $M^{-1}sec^{-1}$ with 1,3-dimethyl-2-thiourea, 0.070 $M^{-1}sec^{-1}$ and 0.069 $M^{-1}sec^{-1}$ with D-methionine, and 3.9 $M^{-1}sec^{-1}$ and 0.091 $M^{-1}sec^{-1}$ with thiosulfate, respectively. Our results suggest that thiosulfate, as being the strongest nucleophile, is a promising candidate for local application in order to reduce the inner ear content of MHC and cisplatin. However, otoprotection is a multifactorial event, and it remains to be established how important nucleophilicity is for the effectiveness of the protecting agent. Exp Biol Med 231:1638–1645, 2006

Key words: antioxidants; *cis*-diamminedichloroplatinum; hydrated species; nucleophiles; ototoxicity

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Introduction

Cisplatin (Fig. 1a) holds a central position in the treatment of solid malignant tumors, such as testicular cancer, head and neck cancer, ovarian cancer, bladder cancer, and some pediatric malignancies. The monohydrated complex (MHC, Fig. 1b) is formed upon hydrolysis of cisplatin and is generally considered the key molecule in the antitumoral action of the drug (1). MHC is present in the blood from patients receiving cisplatin infusions, its area under the concentration-time curve (AUC) being approximately 15% of that of cisplatin (2). Inside the cell, the formation of MHC is more favored, since the concentration of chloride ions is lower (1).

Though cisplatin has been used clinically since the 1970s, the molecular basis for its cytotoxicity is still not fully elucidated. Treatment with cisplatin leads to the formation of platinum-DNA adducts, which are usually considered fundamental for the antitumoral effect (1). However, platinum adducts are formed with many other essential biomolecules, especially those containing sulfur (3). It has also been shown that cisplatin is cytotoxic to enucleated cells, inducing apoptotic signaling (4).

Cisplatin therapy is associated with gastrointestinal toxicity, nephrotoxicity, and ototoxicity. Of these, inner ear damage is the only side effect without any treatment available today. The high incidence of cisplatin-induced ototoxicity is a major problem, and it is more pronounced in children (5).

For many years, sulfur-containing compounds, due to their high affinity for platinum molecules, have been attractive candidates for development of protecting agents against cisplatin-induced nephrotoxicity and ototoxicity. Many of these agents are antioxidants and radical scavengers. Since it has been discovered that cisplatin toxicity may be related to oxidative stress (4, 6), the sulfur-containing compounds might offer cytoprotection through their nucleophilic as well as antioxidating properties.

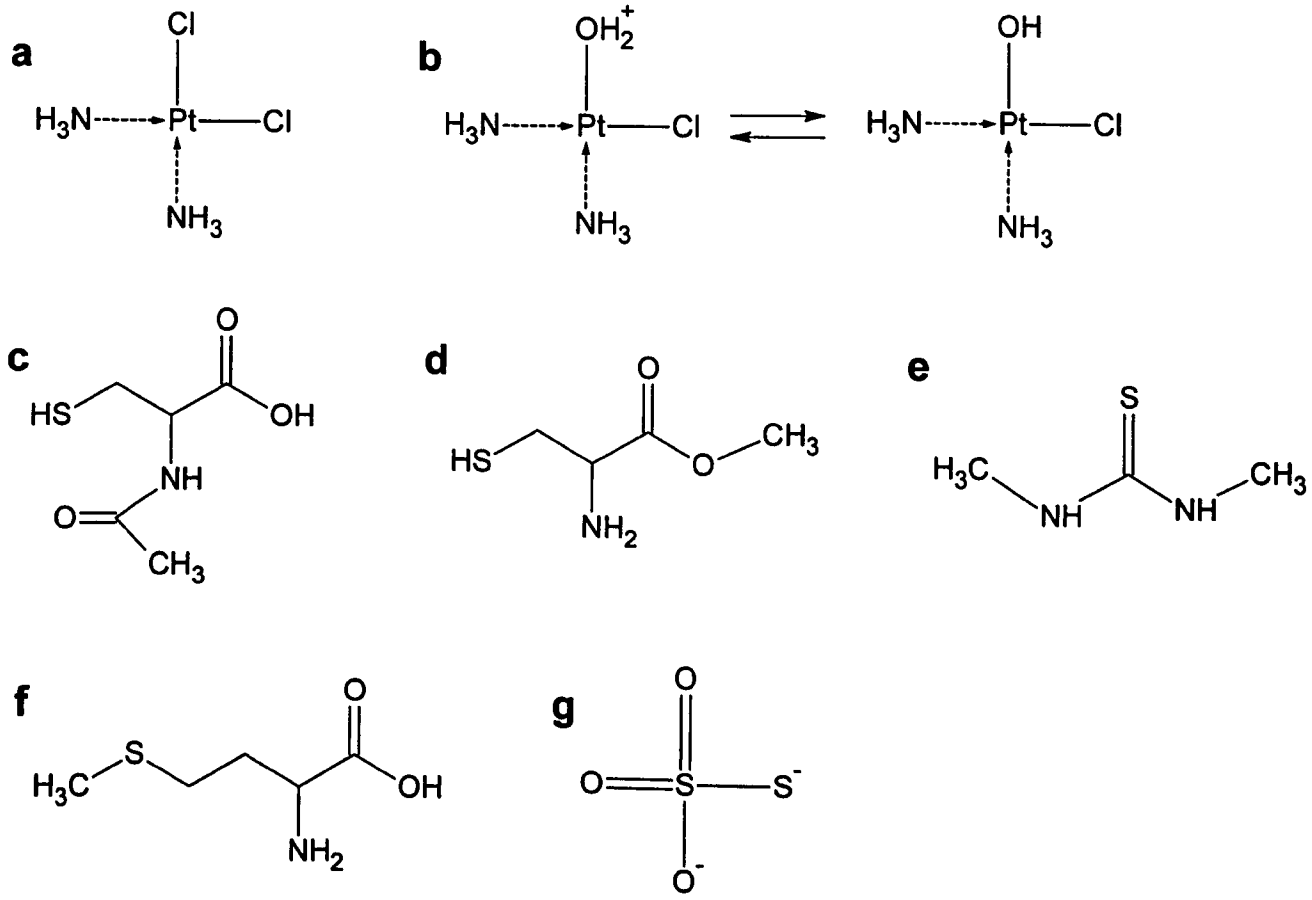

Figure 1. The molecular structures of the platinum and the sulfur-containing compounds used in the present study: cisplatin (a), the monohydrated cisplatin complex (MHC, b), *N*-acetyl-L-cysteine (c), L-cysteine methyl ester (d), 1,3-dimethyl-2-thiourea (e), D-methionine (f), and thiosulfate (g).

Several of the compounds, for example *N*-acetyl-L-cysteine (7, 8), 1,3-dimethyl-2-thiourea (9), methionine (10–12), and thiosulfate (8, 13, 14), have decreased the toxicity in experimental animals when administered systemically in conjunction with cisplatin. Thiosulfate has also shown nephroprotective potential in humans (15). Unfortunately, the coadministration may also reduce the antineoplastic activity (8, 11–13). The reductions in effects and side effects are most likely caused by a decrease in the AUC of cisplatin and/or MHC (15, 16). If the antitumoral properties of cisplatin are to be conserved, a protector with a high affinity for platinum compounds should probably not be distributed to the cancer tissue. *In vivo* studies have revealed that local administration of a sulfur-containing compound to the middle ear for uptake to the inner ear may be a possible clinical approach for otoprotection (12, 17–19).

Several properties may be important for the effectiveness of a locally administered otoprotectant. In addition to being a nucleophile and an antioxidant, the otoprotectant must be able to pass from the middle ear cavity to the inner ear, possibly primarily via the round window membrane. This thin membrane separates the basal part of the scala tympani in the cochlea from the middle ear cavity and can act as a semipermeable membrane. Cellular uptake into the target cells for cisplatin toxicity as well as clearance from the inner ear compartments must also be taken into consideration. In the present study, we have explored the nucleophilicity of five sulfur-containing compounds under evaluation for local otoprotective administration by determining the degradation rate constants of MHC and of cisplatin in the presence of these nucleophiles. The nucleophiles studied are *N*-acetyl-L-cysteine, L-cysteine methyl ester, 1,3-dimethyl-2-thiourea, D-methionine, and thiosulfate (Fig. 1c–g). To the best of our knowledge, this is the first paper presenting degradation rates of isolated MHC.

Materials and Methods

Chemicals. Cisplatin (Platinol 1 mg/ml, Bristol-Myers Squibb Company, New York, NY) was used in the degradation studies of cisplatin. Cisplatin (Sigma-Aldrich, St. Louis, MO) was used for preparation of MHC, similar to the method of Ehrsson *et al.* (20). In short, cisplatin was dissolved in water to a concentration of about 4 m*M* and was left to hydrolyze at room temperature for two days. Sodium hydroxide (1% v/v 1 *M*) was added to achieve the less reactive uncharged form of MHC (3). MHC was

isolated with a porous graphitic carbon column (Hypercarb, 100 × 4.6 mm i.d., particle size 5 μm, Thermo Electron Corporation, Runcorn, UK) and a mobile phase of sodium hydroxide (0.5 m*M*). Detection was performed photometrically at 283 nm. For quantification, comparison was made with a reference solution containing 1.1 m*M* MHC as determined with atomic absorption spectroscopy performed by Analytica AB (Täby, Sweden). To minimize the conversion of MHC to the dihydrated cisplatin complex and its possible polymerization (21), all alkaline solutions containing isolated MHC were protected from light and stored on ice or, if not used within one day, at −80°C. Sodium thiosulfate and *N*-acetyl-L-cysteine were purchased from Merck (Darmstadt, Germany). D-methionine, L-cysteine methyl ester, and 1,3-dimethyl-2-thiourea were derived from Sigma-Aldrich. The water used was purified with a Milli-Q Water Purification System from Millipore Corporate (Billerica, MA). All other chemicals were of analytical grade, were obtained from commercial suppliers, and were used without further purification.

Apparatus. The degradations of cisplatin and of MHC were evaluated by measuring their disappearance using an LC system consisting of a Valco Model C6W injector (Houston, TX) with a fixed loop volume of 20 μl for cisplatin and 5 μl for MHC, a LC-10ADvp pump, and a DGU-14A degasser, both devices from Shimadzu (Kyoto, Japan). MHC was analyzed with a Hypercarb column (150 × 3 mm i.d., particle size 3 μm, Thermo Electron Corporation) and quantified photometrically using microwave-assisted post-column derivatization with sodium *N,N*-diethyldithiocarbamate with an Initiator system from Biotage (Uppsala, Sweden), similar to the method of Ehrsson and Wallin (22), except that the mobile phase consisted of sodium hydroxide (1.0 m*M*). Cisplatin was analyzed with a self-packed strong anionic exchanger column (200 × 4.6 mm i.d.) of Nucleosil SB (particle size 5 μm, Macherey-Nagel, Düren, Germany) and a mobile phase of succinic acid (40% v/v 55 m*M* pH 5.0) and methanol (60% v/v), similar to the method described by Andersson and Ehrsson (23), except that no post-column derivatization was used. The UV absorbance was measured at 344 nm for MHC and 303 nm for cisplatin with a SPD-10AVvp UV-VIS detector (Shimadzu). The signal was plotted with a flatbed recorder (Kipp & Zonen, Delft, The Netherlands) and collected and processed with a Chromeleon integration system (version 6.20, Dionex Corporation, Sunnyvale, CA) for MHC and with a PC Integration Pack system (version 3.00, Kontron, München, Germany) for cisplatin.

Degradation of MHC. The degradation of MHC in the presence of *N*-acetyl-L-cysteine, L-cysteine methyl ester, 1,3-dimethyl-2-thiourea, D-methionine, and sodium thiosulfate, respectively, was performed at 37°C in HEPES (10 m*M*), pH 7.4. A fresh solution of the nucleophile to be studied was prepared immediately before the incubation. The initial concentration of MHC was 30 μ*M*, whereas that of the nucleophile ranged from 0.50 to 3.5 m*M*. All solutions were incubated in the dark in a thermostated water bath (Grant Instruments, Herts, UK) for at least four half-lives of the analyte. The pH was measured at the start and the end of the incubation. Samples were collected at appropriate intervals, quenched with ice-cold sodium hydroxide (25% v/v 0.1 *M*), and stored on ice for a maximum of 10 mins before freezing at −80°C. They were analyzed within 24 hrs.

Degradation of Cisplatin. The degradation of cisplatin in the presence of the same nucleophiles as in the MHC experiments was performed at 37°C in HEPES (0.10 *M*), pH 7.4. A fresh solution of the nucleophile to be studied was prepared immediately before the incubation. The initial concentration of cisplatin was 0.20 m*M*, whereas that of the nucleophile ranged from 5.2 to 29 m*M*. The concentration of chloride ions was 8.7 m*M* and derived from Platinol. Most probably, this had no effect on the degradation rate, since the sulfur-containing compounds are much stronger nucleophiles than the chloride ion (24, 25). Solutions were incubated as in the MHC experiments, and the pH was measured at the start and the end of the incubation. Samples were collected at appropriate intervals and quenched on ice until analysis, which was performed the same day.

Data Analysis. When the nucleophile is in large excess over the platinum compound, the substitution kinetics of square-planar Pt(II) compounds proceed by the general rate law for pseudo first-order kinetics

$$A = A_o \times e^{-k_{obs} \times t}, \quad (1)$$

where A is the concentration of the analyte at time t, A_o is the initial concentration of the analyte, and k_{obs} is the pseudo first-order rate constant of the analyte. The pseudo first-order rate constant can be expressed by

$$k_{obs} = k_1 + k_{Nu}[Nu] \quad (2)$$

and is thus dependent on k_1, which is the sum of the pseudo first-order rate constants for the reaction of the analyte with the solvent, for example HEPES and water; k_{Nu}, which is the second-order rate constant for the reaction of the analyte with a specific nucleophile; and $[Nu]$, which is the concentration of that nucleophile (26).

The concentrations of MHC and of cisplatin, represented by their LC-UV peak areas, were plotted *versus* time. The curves were fitted to the equation of one phase exponential decay according to Equation 1. By determining k_{obs} at a variety of different nucleophile concentrations, k_{obs} can be plotted versus $[Nu]$, and linear regression analysis will yield a straight line, with k_{Nu} being the slope and k_1 the y-intercept (Equation 2). Slopes were compared using regression analysis. All calculations were performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA).

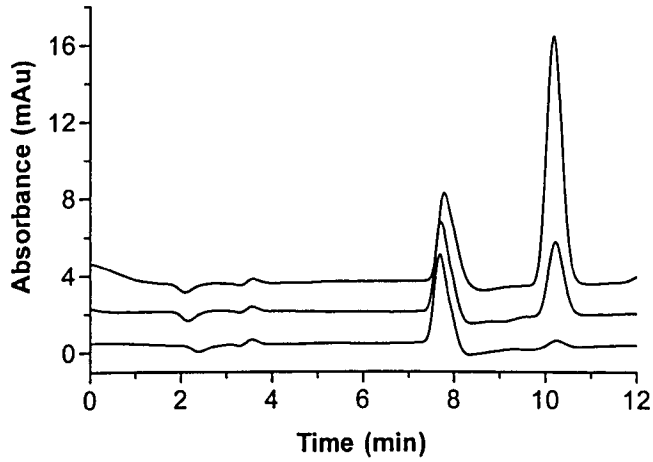

Figure 2. LC elution profiles of samples taken from a solution of MHC (initial concentration 30 μ*M*) incubated with thiosulfate (1.4 m*M*) at pH 7.4 and 37°C for 0.3 (top), 3.6 (middle), and 10.3 mins (bottom). The peaks at approximately 7.5 and 10.2 mins represent the front and MHC, respectively. The two top chromatograms have been shifted up along the *y* axis. Column: porous graphitic carbon column (Hypercarb 150 × 3 mm i.d., particle size 3 mm). Mobile phase: 1.0 m*M* sodium hydroxide. Flow rate: 0.2 ml/min. Detection: photometrically at 344 nm after on-line microwave-assisted post-column derivatization with *N,N*-diethyldithiocarbamate (2.7 m*M* in methanol, flow rate 0.2 ml/min) at 115°C.

Results

The disappearance of MHC and of cisplatin in the presence of the nucleophiles studied was followed using LC-UV, and representative chromatograms from different time points are given in Figures 2 and 3. As expected, the

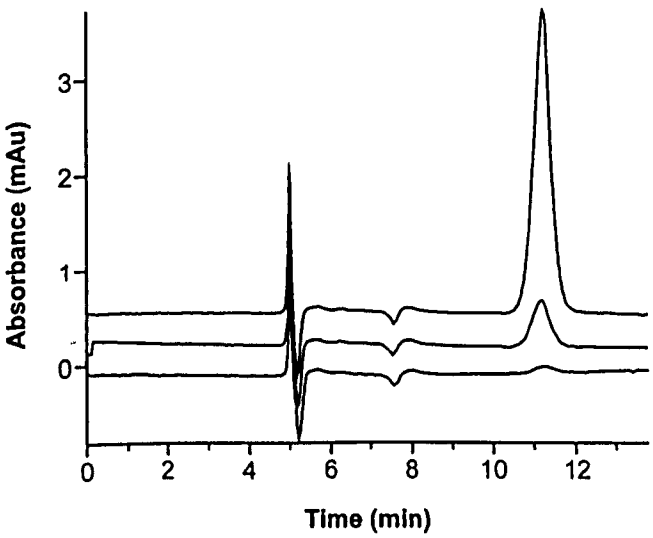

Figure 3. LC elution profiles of samples taken from a solution of cisplatin (initial concentration 0.20 m*M*) incubated with thiosulfate (11 m*M*) at pH 7.4 and 37°C for 0.3 (top), 45 (middle), and 105 mins (bottom). The peaks at approximately 5 and 11 mins represent the front and cisplatin, respectively. The two top chromatograms have been shifted up along the *y* axis. Column: self-packed strong anionic exchanger column (200 × 4.6 mm i.d.) of Nucleosil SB (particle size 5 μm). Mobile phase: succinic acid (40% v/v 55 m*M*, pH 5.0) and methanol (60% v/v). Flow rate: 0.5 ml/min. Detection: photometrically at 303 nm.

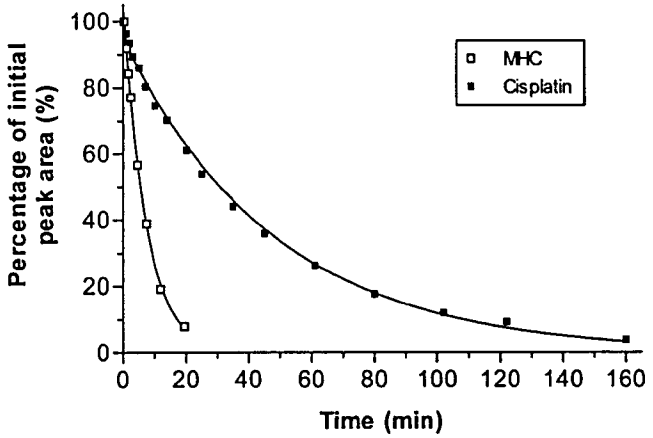

Figure 4. The time-dependent content of MHC (initial concentration 30 μ*M*) and of cisplatin (initial concentration 0.20 m*M*) of samples taken from solutions containing thiosulfate (0.50 m*M* for MHC and 5.3 m*M* for cisplatin) at 37°C and pH 7.4. Each symbol represents one sample. Quantification was performed with liquid chromatography and photometric detection.

decrease in analyte concentration proceeded according to Equation 1 and two representative plots are given in Figure 4.

In the presence of thiosulfate, the MHC content decreased significantly more quickly than that of cisplatin ($P < 0.0001$), as illustrated in Figure 4. The linear regression analyses of k_{obs} of MHC and of cisplatin versus the thiosulfate concentration can be seen in Figure 5. The k_{Nu} of MHC and of cisplatin with each of the nucleophiles studied is given in Table 1. The k_{Nu} of MHC was significantly larger than that of cisplatin in the presence of L-cysteine methyl ester ($P = 0.0035$) and 1,3-dimethyl-2-thiourea ($P = 0.041$), whereas it did not differ significantly in the presence of *N*-acetyl-L-cysteine ($P = 0.061$) or D-methionine ($P = 0.99$).

The MHC content decreased rapidly in solutions with L-

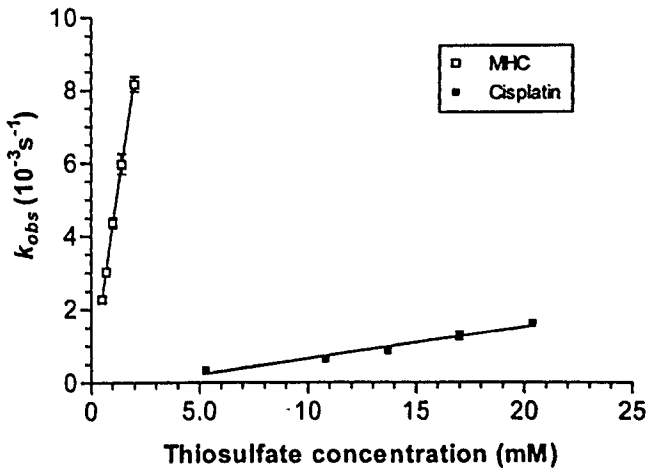

Figure 5. The observed degradation rate constant, k_{obs}, of MHC and of cisplatin versus the thiosulfate concentration at 37°C and pH 7.4. Each symbol represents one experiment. Bars represent 95% confidence interval.

Table 1. The Degradation Rate Constants of MHC and of Cisplatin in Solutions with Sulfur-Containing Compounds at 37°C and pH 7.4[a]

		N-Acetyl-L-cysteine	L-Cysteine methyl ester	1,3-Dimethyl-2-thiourea	D-Methionine	Sodium thiosulfate
MHC	k_{Nu} ± SEM (10^{-2} $M^{-1}sec^{-1}$)	4.4 ± 1.4	24 ± 1.8	16 ± 0.46	7.0 ± 0.39	390 ± 12
	95% CI (10^{-2} $M^{-1}sec^{-1}$)	0.01–8.8	19–30	15–18	5.7–8.2	360–430
	k_1 ± SEM ($10^{-5}sec^{-1}$)	11 ± 3.2	7.3 ± 2.2	6.5 ± 0.58	6.8 ± 0.49	37 ± 15
	95% CI ($10^{-5}sec^{-1}$)	1.4–22	0.2–14	4.7–8.4	5.2–8.3	−10 to 84
	r^2	0.77	0.98	1.0	0.99	1.0
	[*Nu*] (m*M*)	1.0–3.5	0.50–2.0	0.50–2.1	0.50–2.0	0.49–2.0
Cisplatin	k_{Nu} ± SEM (10^{-2} $M^{-1}sec^{-1}$)	1.2 ± 0.18	6.7 ± 0.51	7.4 ± 0.47	6.9 ± 0.83	9.1 ± 0.98
	95% CI (10^{-2} $M^{-1}sec^{-1}$)	0.06–1.8	5.1–8.3	6.0–8.9	4.3–9.6	6.0–12
	k_1 ± SEM ($10^{-5}sec^{-1}$)	6.3 ± 3.8	−10 ± 7.0	−12 ± 6.9	−7.2 ± 12	−20 ± 14
	95% CI ($10^{-5}sec^{-1}$)	−5.6 to 18	−32 to 12	−34 to 9.7	−44 to 30	−65 to 25
	r^2	0.94	0.98	0.99	0.96	0.97
	[*Nu*] (m*M*)	11–29	5.5–20	5.2–21	4.7–20	5.3–20

[a] The degradations of MHC and of cisplatin were evaluated by measuring their disappearance with liquid chromatography and photometric detection. The initial concentrations of the analytes were 30 μ*M* for MHC and 0.20 m*M* for cisplatin. One incubation per analyte was performed at five different concentrations for each nucleophile in HEPES buffer (10 m*M* for MHC and 0.10 *M* for cisplatin). The k_{Nu} is the second-order rate constant for the degradation of the analyte with a sulfur-containing nucleophile, k_1 is the sum of the pseudo first-order rate constants for the degradation of the analyte with the solvent, e.g., HEPES and water, and [*Nu*] is the concentration of the nucleophile. SEM represents standard error of the mean, r^2 the coefficient of determination, and CI the confidence interval.

cysteine methyl ester and, in particular, with thiosulfate compared to the other nucleophiles studied. All *P* values are given in Table 2.

Cisplatin was significantly more stable in solutions with *N*-acetyl-L-cysteine than with the other nucleophiles, as can be seen in Tables 1 and 2. The k_{Nu} of cisplatin in the presence of the other nucleophiles was in the same range (Table 1), although some of them differed significantly (Table 2).

Discussion

When cisplatin is dissolved in water, the chloride ions are displaced by water molecules in a stepwise manner, leading to the formation of primarily MHC and a dihydrated complex (27). The hydrated species of cisplatin have been shown to be more toxic *in vitro* (28–32) and more nephrotoxic *in vivo* (29, 32–35) compared to cisplatin. Furthermore, MHC has been shown to be more ototoxic than cisplatin *in vivo* (34). Under physiological conditions, MHC is probably the only hydrated species present in considerable amounts (27, 36, 37). However, few studies have been performed on isolated MHC (16, 31, 34), even though it is readily obtainable using the method of Ehrsson *et al.* (20). In this paper, we present the rate constants for the disappearance of isolated MHC and of cisplatin when incubated with five sulfur-containing nucleophiles, which

Table 2. Comparison of the Second-Order Rate Constants of MHC and of Cisplatin, Which Are Given in Table 1, Using Linear Regression Analysis

	Nucleophiles	*P* value
MHC	Thiosulfate versus all other nucleophiles	<0.0001
	L-Cysteine methyl ester versus *N*-acetyl-L-cysteine	0.00017
	L-Cysteine methyl ester versus 1,3-dimethyl-2-thiourea	0.0044
	L-Cysteine methyl ester versus D-methionine	<0.0001
	1,3-Dimethyl-2-thiourea versus *N*-acetyl-L-cysteine	0.00076
	1,3-Dimethyl-2-thiourea versus D-methionine	<0.0001
	D-Methionine versus *N*-acetyl-L-cysteine	0.23
Cisplatin	Thiosulfate versus *N*-acetyl-L-cysteine	0.00013
	Thiosulfate versus L-cysteine methyl ester	0.067
	Thiosulfate versus 1,3-dimethyl-2-thiourea	0.16
	Thiosulfate versus D-methionine	0.13
	L-Cysteine methyl ester versus *N*-acetyl-L-cysteine	<0.0001
	1,3-Dimethyl-2-thiourea versus *N*-acetyl-L-cysteine	<0.0001
	D-Methionine versus *N*-acetyl-L-cysteine	0.00036
	L-Cysteine methyl ester versus 1,3-dimethyl-2-thiourea	0.31
	L-Cysteine methyl ester versus D-methionine	0.82
	1,3-Dimethyl-2-thiourea versus D-methionine	0.59

are under evaluation for local otoprotective administration. As expected, our results suggest MHC as being more reactive than cisplatin. MHC disappeared extremely quickly in the presence of thiosulfate (Table 1). This preferred reactivity can be due to the electrostatic attraction between the partially positively charged MHC and the doubly negatively charged thiosulfate.

A few studies have been published on the reactivity of the hydrated complexes of cisplatin with different nucleophiles, predominantly those containing sulfur (38–43). Two of them include nucleophiles used in the present investigation, thiosulfate and methionine. In both studies, a mixture of cisplatin and its hydrated complexes was used. In the first paper, the reaction kinetics of a hydrolysis solution of cisplatin with the nucleophiles mesna, dimesna, and thiosulfate were examined (41). Thiosulfate was by far the most nucleophilic; the observed half-lives of MHC were less than 1 min at thiosulfate concentrations of 2.6 and 50 m*M* at pH 5.3 and 37°C (41). For comparison, a MHC half-life of 1.4 mins in a 2.0 m*M* thiosulfate solution was observed in the present study. At low pH, shorter half-lives are expected for MHC, since it has a pK_a of 6.56 (44) and its reactivity increases with a higher degree of protonation (3). In the second paper, the reaction of cisplatin and its hydrated species with L-methionine was explored at an unspecified pH (40). In contrast to the D-methionine results that are presented here, the degradation rate with the hydrolysis solution was faster than with cisplatin, with as well as without chloride ions present (40). A possible explanation for these diverging results is that the incubations may have been conducted at a lower pH than in the present investigation.

Local administration of an agent to prevent cisplatin-induced hearing damage is an attractive approach from several aspects. First, the risk of interaction with cisplatin at the tumor, leading to a reduced antitumoral effect, is minimized. Second, systemic toxic effects of the protector are avoided. Third, it is possible to achieve a higher local concentration of an otoprotectant in the inner ear compartments by a direct delivery via the round window membrane (45). Studies have confirmed that thiosulfate, methionine, and *N*-acetyl-L-cysteine may protect against cisplatin-associated hearing damage when applied locally to experimental animals (12, 17, 19, 46). 1,3-Dimethyl-2-thiourea has been shown to have otoprotective properties against ischemia-induced cochlear dysfunction when it has been administered locally to guinea pigs (47, 48). L-Cysteine methyl ester is a dark horse that was used in the present investigation because of its more lipophilic character (Fig. 1d), which might influence its uptake to the inner ear when administered to the middle ear. The data in Table 1 imply that L-cysteine methyl ester may effectively inactivate cisplatin and, to an even greater extent, MHC.

Thus, nucleophilicity is only one of several factors that might influence the otoprotective effects of an agent. *N*-acetyl-L-cysteine was by far the weakest nucleophile (Table 1), but *in vivo* experiments have revealed that it is able to reduce the ototoxic effects of cisplatin (17). The degradation of MHC with D-methionine was more than 50 times slower than with thiosulfate (Table 1). However, *in vivo*, round window application of D-methionine was shown to protect against hearing damage, whereas thiosulfate did not (19). Although the molar amount of D-methionine was about four times higher than that of thiosulfate and, as shown here, the degradation rate of cisplatin and MHC depends strongly on the nucleophile concentration, it is more likely that the different results were caused by the fact that D-methionine, as being more lipophilic, more easily passes the round window membrane than does thiosulfate.

The molecular structure of the reaction products between cisplatin/MHC and the sulfur-containing compounds depends on the time of incubation as well as on the concentration of the reactants (40, 49, 50). The fact that most of the nucleophiles have demonstrated otoprotective properties *in vitro* (51) and *in vivo* (7, 8, 10, 11, 17, 19, 46) strongly suggests that the molecules formed are less toxic than cisplatin and MHC. Nonetheless, a few renal studies have shown that *N*-acetyl-L-cysteine and methionine may, in some cases, exacerbate the nephrotoxicity of cisplatin (28, 50, 52).

The inner ear is divided into two extracellular compartments filled with either perilymph or endolymph. The composition of the perilymph resembles that of plasma ultrafiltrate, whereas the endolymph has a unique composition defined by high potassium, high chloride, and low sodium contents (53). After administration to the middle ear for uptake to the inner ear, the otoprotectant is expected first to be distributed to the perilymph (18, 54). Cisplatin has been detected in the perilymph of guinea pigs a few minutes to 1 hr after having been injected intraveneously (55). The existence of MHC in the inner ear fluids remains to be established. However, considering the composition of the inner ear fluids, their pH being similar to that of plasma (53), and the fact that MHC has been detected in blood of cisplatin-treated patients (2), it is rather likely that MHC is indeed present in the inner ear. Recently, it was shown that the ototoxicity of cisplatin may be influenced by local pH manipulation; otoprotection was achieved by application of alkaline PBS to the round window niche, whereas acidic PBS was found to be detrimental to the inner ear function (56). This indicates that MHC might be involved in the ototoxic side effects of cisplatin, since the reactivity of MHC is affected by the pH, whereas that of cisplatin is not (3).

Conclusion

Isolated MHC was shown to be significantly more reactive than cisplatin in the presence of the sulfur-containing nucleophiles L-cysteine methyl ester, 1,3-dimethyl-2-thiourea, and thiosulfate. Our results suggest that thiosulfate as being the strongest nucleophile is a promising

agent for local otoprotective administration. However, it remains to be established how important rapid complexation of MHC and cisplatin is for preventing inner ear damage.

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