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Factors affecting human autopsy kidney-cortex and kidney-medulla platinum concentrations after cisplatin administration

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Abstract. The objective of this study was to determine factors that affect cisplatin concentrations in human kidney cortex. We used flameless atomic absorption spectrophotometry to assay platinum in autopsy specimens of kidney cortex obtained from 83 cisplatin-treated patients. Concentrations were correlated with pretreatment factors and treatment conditions using univariate nonparametric statistics. Hierarchical stepwise multiple regression analyses of transformed (to normalize) data were then used to assess which factors were most important, controlling for other factors. Kidney-cortex platinum concentrations varied from 0 to 14.8 μg/g (median, 2.04 μg/g). The cumulative lifetime dose of cisplatin ranged from 10 to 1120 mg/m^2 (median, 112 mg/m^2). The time from the last cisplatin dose to death was <1–609 days (median, 38 days). According to univariate statistics, factors that correlated ($P<0.05$) with kidney-cortex platinum concentrations were the cisplatin dose per course, the pretreatment serum urea level, metoclopramide use (positive correlations), the time from the last cisplatin treatment to death, and the pretreatment serum albumin value (negative correlations). Factors that approached significance ($0.05 \leq P \leq 0.10$) were a history of hypertension, hyperbilirubinemia (positive), the serum calcium level, and phenytoin use (negative). In the multiple regression analysis, after controlling for the cisplatin dose per course and the time from the last treatment to death, only concurrent metoclopramide and phenytoin use entered the model. The hydration volume did not affect corrected kidney-cortex or kidney-medulla platinum concentrations. The following conclusions were reached: (1) it may be feasible to use lower hydration volumes than those used routinely, (2) any effect of hydration volume on cisplatin nephrotoxicity may not be mediated via a reduction in kidney-cortex platinum concentrations, (3) higher cisplatin doses might be tolerated with new 5-hydroxytryptamine-3 (5HT-3) antiemetics than were tolerated with metoclopramide, and (4) phenytoin should be tested for its ability to reduce cisplatin nephrotoxicity.

Introduction

Cisplatin is one of the most active solid-tumor chemotherapy drugs available. Although severe nephrotoxicity was a problem in early clinical studies of cisplatin, vigorous hydration and mannitol administration reduced nephrotoxicity [1]. There are few data available on exactly how much hydration is required to protect the kidneys. Some studies suggest that nephrotoxicity decreases with increased hydration volume [2], whereas others have shown little impact of total hydration volume on nephrotoxicity as long as some degree of hydration is given [3].

Concurrent use of the diuretic mannitol reduces cisplatin nephrotoxicity in both animals [4, 5] and humans [10]. Mannitol did not alter kidney platinum concentrations in animals [5], and there are no data on its effect on kidney platinum concentrations in humans. Other diuretics have had variable effects on cisplatin nephrotoxicity [4–8] and kidney platinum concentrations in animals [4, 6, 9].

In different clinical and preclinical studies, various other factors have also been found to affect the degree of cisplatin nephrotoxicity. For example, high peak plasma platinum concentrations may augment cisplatin nephrotoxicity [11–13], and steps taken to reduce peak plasma platinum concentrations (such as slow as opposed to rapid administration [14–16] or multiple-day as opposed to single-day fractionation of a given total dose [17]) may also reduce cisplatin nephrotoxicity [14–16] and decrease kidney pla-

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tinum concentrations [17]. Several medications have also been reported to reduce [18–46] or augment [47–49] cisplatin nephrotoxicity with reduction in [22, 27, 44, 50], augmentation of [19], or no change in [19, 30] kidney platinum concentrations.

Various physiologic factors have also been found to correlate with nephrotoxicity. For example low hemoglobin, serum albumin, calcium and chloride levels as well as high serum bilirubin and uric acid values may correlate with cisplatin nephrotoxicity in humans [3, 51]. Cisplatin-DNA adduct concentrations in rats may be affected by the route of drug administration (intravenous versus intraperitoneal), diet, gender, and hormonal status [52].

In animals, the highest kidney platinum concentrations after cisplatin administration have been found in the renal cortex [53] and corticomedullary junction [53, 54], and the major nephrotoxic effects of cisplatin appear to be on renal tubules in this region [5, 53, 55, 56]. However, it is unclear whether cisplatin nephrotoxicity is a concentration-dependent phenomenon, since platinum-based drugs with differing degrees of nephrotoxicity achieve comparable concentrations in animal kidneys [55] and since the concentration of platinum is at least as high in liver as it is in kidney [57, 58], yet cisplatin hepatic toxicity is very uncommon.

In a previous study of platinum concentrations in kidneys of 30 patients who had received cisplatin 0–240 days antemortem, we found that kidney-cortex platinum concentrations correlated with nephrotoxicity [58]. In this paper, we report results obtained in an additional 53 patients. In an effort to understand better the effect of various factors on cisplatin nephrotoxicity, we also assessed which of these factors correlated with platinum concentrations in autopsy specimens of kidney-cortex in the entire population of 83 patients.

Materials and methods

Between 1982 and 1990, unfixed autopsy samples of kidney were collected from 83 patients who had received cisplatin at some time antemortem as treatment for a malignancy. Renal cortex was separated from renal medulla by sharp dissection and tissues were stored frozen (−50° C) until assayed for platinum using a variation of a method employed in our earlier studies [58].

Samples were assayed for platinum by flameless atomic absorption spectrophotometry using an electrothermal atomization atomic absorption spectrometry system consisting of a Varion Techtron AA-1745 spectrophotometer, a GTA-95 graphite tube atomizer with an autosampler and an Epson RX-80 printer. The instrument conditions for the measurement of platinum were: wavelength, 265.9 nm; slit width, 0.2 nm; and lamp current, 10 mA. Argon was used as the sheath gas. The graphite tubes were pyrolitically coated. During the drying step, the furnace temperature was 95° C (ramp, 15 s; hold, 25 s). During the ashing step, the furnace temperature was 1300° C (ramp, 25 s; hold, 20 s). During the atomization step, the furnace temperature was 2700° C (ramp, 1 s; hold, 2 s; internal flow rate, 0 ml/min). Matrix-matched standards were prepared from a 1-g/l solution of cisplatin (Platinol, Bristol-Myers Squibb Pharmaceuticals, Montreal). Nitric acid (BDH, Toronto) was of Analar grade, and water was distilled and then deionized.

Samples (0.2 g wet weight) were digested with 2.5 ml 70% nitric acid for 90 min at 135° C in Teflon pressure-decomposition vessels with stainless steel casings [59]. After cooling, the digests were washed quantitatively into 20-ml glass beakers with distilled deionized water, and the contents were evaporated to dryness on a hot plate. The residues were allowed to dry and were then dissolved in exactly 1–2 or 5 ml 0.5% (v/v) nitric acid, depending on the sample weight. Undissolved material was separated by means of acrodisc filters (0.45 μM) attached to a syringe. An intermediate platinum stock solution was used for calibrating the instrument. It was prepared by dissolving a control tissue sample in nitric acid as described above, then spiking the dissolved control sample with the cisplatin injection solution to give a concentration of 325 μg/l.

The instrument was calibrated with three working standards of 32.5, 65.0, and 130 μg/l by dispensing into the graphite furnace 2, 4, and 8 μl intermediate stock solution made up in each case to a volume of 20 μl with 0.5% (v/v) nitric acid. Sample solutions of 15 μl were also made up to a total volume of 20 μl and then analyzed for platinum using the concentration mode of the spectrophotometer. Sample solutions with concentration readings outside the calibration range were diluted accordingly, then reanalyzed. All measurements were done in duplicate. After the analysis of every two samples, the instrument was recalibrated with the intermediate working standard by a resloping procedure. The lower limit of quantitation for this method was 0.05 μg/g as compared with the lower limit of quantitation of 0.08 μg/g for our earlier assay methodology used in the first 30 of our 83 patients [58].

Patients' charts were reviewed for patients' characteristics and treatment details. Kidney tissue variables included kidney-cortex and kidney-medulla platinum concentrations.

All statistical analyses were performed using the computer programs SPSS-PC (version 4.01) and BMDP PC-90. Since many of the continuous variables were not normally distributed, only the following non-parametric tests were used for initial analyses: Spearman rank-order correlation, to examine associations between kidney tissue variables and all continuous variables, and Kruskal-Wallis one-way analysis of variance (K-W ANOVA), to examine differences in kidney tissue variables between (among) groups defined by the nominal and ordinal factors.

Based in part on these analyses, variables were selected for inclusion in hierarchical stepwise multiple regression. The first block of variables entered into the models included the dose per course and the cumulative dose. The second block included factors that were statistically significant ($P<0.05$) in the univariate analyses. The third block included biologically important but not statistically significant variables.

Prior to running the multiple regression, we tested for normality the distribution of each of the continuous variables to be included in the model using the Kolmogorov-Smirnov (K-S) test. Any variable with a P value of <0.10 was transformed. Initially, square root and log base-10 transformations were used. If these transformations were not successful in normalizing a variable, the z-scores of the raw scores were examined. Any individual data point with a z-score of >3.5 was reduced to the next lowest observed value with a z-score of <3.5. These "truncated" variables, along with their square-root and log-10 transforms, were then tested for normality using the K-S test. This procedure was repeated as necessary. These steps were sufficient for normalizing all variables except albumin.

The dependent measures also had to be transformed. The square roots of kidney-cortex and kidney-medulla platinum concentrations were used in the multiple regression analyses. Dummy coding (0 = no vs 1 = yes or 0 = normal vs 1 = abnormal) was used for the dichotomous variables, and the five-level ordinal factor, performance status, was treated as a continuous variable.

Multiple regression uses listwise deletion of missing cases. Among the total of 83 patients, 58 had nonmissing values for all variables to be included in the kidney-cortex platinum concentration analysis and 56, for the kidney-medulla platinum analysis. Since most patients (over 72%) had values missing for only one factor (maximum of three), missing values for each factor were replaced with means, and patients with missing information as to whether or not they had received a specific drug along with cisplatin were coded as if they had not received the drug. One factor to be included in block 3 of the cortex model, cisplatin infusion duration, was not considered for the analysis,

Table 1. Spearman rank-order correlation coefficients: kidney-cortex and kidney-medulla platinum concentrations versus continuous patient factors

Characteristic	Spearman rank-order coefficients	
	Kidney-cortex platinum concentration	Kidney-medulla platinum concentration
Cisplatin dose mg/m²:		
Per course	0.32*[c]	0.34*[c]
Cumulative	0.16[c]	0.26*[c]
Time[a]	−0.45*[d]	−0.36*[d]
Age	0.01	−0.11
Creatinine	0.05	0.04
Urea	0.26*[d]	0.20**[e]
Uric acid	−0.04	0.04
Hemoglobin	−0.16	−0.05
Albumin	−0.22*[d]	−0.11
Total protein	−0.14	−0.03
Calcium	−0.20**[e]	−0.10
Magnesium	0.10	0.03
Chloride	−0.05	−0.06
Sodium	0.02	0.05
Potassium	−0.08	−0.06
Carbon dioxide	0.05	0.10
Phosphorus	−0.06	0.03
Bilirubin	0.14[e]	0.16
AST	0.16	0.08
ALT	0.12	0.07
Lactate dehydrogenase	0.16	0.25*[d]
Glucose	−0.07	−0.03
Hydration volume[b]	−0.13[e]	−0.16[e]
Mannitol (20%) volume	0.02[e]	−0.05
Cisplatin infusion duration	0.02	0.04

* $P < 0.05$

** $0.05 < P < 0.10$

[a] Time in days from the last treatment with cisplatin to death

[b] Total volume of intravenous fluids on the day of cisplatin administration

[c] Included in block 1 in multiple regression analyses: cisplatin dose factors

[d] Included in block 2 in multiple regression analyses: factors correlating significantly with kidney platinum concentrations in univariate analyses

[e] Included in block 3 in multiple regression analyses: other factors of potential biological significance

as over 50% of the cases had missing values for this variable despite a thorough review of patients' medical records, nursing notes, and pharmacy records.

Table 2. Median human autopsy kidney-cortex and -medulla concentrations as a function of categorical patient factors

	Kidney-cortex platinum concentration [μg/g]		Kidney-medulla platinum concentration [μg/g]	
	Median	(*n*)	Median	(*n*)
Gender:				
F	2.10	(31)	1.37	(30)
M	1.98	(52)	2.00	(50)
History of diabetes:				
No	2.04	(77)	1.80	(75)
Yes	1.64	(6)	1.30	(5)
History of hypertension:				
No	1.75	(66)**[b]	1.59	(64)*[a]
Yes	2.27	(17)	2.79	(16)
History of atherosclerosis:				
No	2.12	(41)	1.71	(40)
Yes	1.98	(42)	2.00	(40)
Total volume i.v. hydration on day of cisplatin administration:				
<1 l	2.11	(28)	1.98	(28)
1–2 l	2.17	(42)	2.08	(40)
>2 l	1.39	(12)	1.23	(11)
Hyperbilirubinemia:				
No	1.91	(76)**[b]	1.75	(73)
Yes	4.00	(5)	3.40	(5)
ECOG performance status				
0	1.31	(8)[b]	1.30	(8)[b]
1	1.45	(15)	1.51	(14)
2	2.27	(21)	2.40	(19)
3	2.18	(25)	1.40	(25)
4	2.50	(12)	2.50	(12)
Route of cisplatin administration:				
Intravenous	2.17	(54)	2.10	(52)
Intracarotid	1.59	(15)	1.57	(15)
Intrahepatic	3.04	(8)	2.20	(7)
Other intraarterial	1.09	(6)	1.54	(6)
Number of days cisplatin was given on each course:				
1 day	1.91	(68)	1.67	(65)
2–5 days	2.18	(15)	1.78	(15)

* $P < 0.05$, K-W ANOVA

** $0.05 < P < 0.10$, K-W ANOVA

[a] Included in block 2 in multiple regression analyses: factors correlating significantly with kidney platinum concentrations

[b] Included in block 3 in multiple regression analyses: other factors of potential biological importance

Results

The 83 patients included in this study included 52 men and 31 women. They had received lifetime cumulative cisplatin doses of 10–1120 (median, 112) mg/m² and had died at <1–609 (median, 38) days after their last treatment with cisplatin. Kidney-cortex platinum concentrations ranged from 0 (i.e., <0.05 μg/g) to 14.8 (median, 2.04) μg/g, whereas kidney-medulla concentrations varied from 0 to 15.5 (median, 1.78) μg/g.

Table 1 summarizes the Spearman rank-order correlation coefficients of kidney-cortex and kidney-medulla platinum concentrations versus various continuous independent variables, and Tables 2 and 3 summarize human autopsy kidney-cortex and -medulla concentrations as a function of categorical patient demographic/history factors and concurrent drug use. These univariate statistics must be interpreted cautiously, since the large number of statistical tests increases the possibility of false positives.

Table 3. Median human autopsy kidney-cortex and -medulla concentrations as a function of concurrent drug use

	Kidney-cortex platinum concentration [μg/g]		Kidney-medulla platinum concentration [μg/g]	
	Median	(n)	Median	(n)
Corticosteroids:				
No	1.92	(7)	1.24	(7)
Yes	2.04	(75)	1.88	(72)
Prochlorperazine:				
No	2.15	(30)	2.10	(30)
Yes	1.98	(52)	1.67	(49)
Metoclopramide:				
No	1.00	(33)*[a]	1.22	(32)*[a]
Yes	2.45	(50)	2.30	(48)
Phenytoin:				
No	2.19	(60)**[b]	2.30	(57)*[a]
Yes	1.60	(23)	1.44	(23)
Mannitol:				
No	2.04	(13)[b]	2.40	(13)[b]
Yes	1.83	(60)	1.71	(58)
Doxorubicin:				
No	2.13	(68)	2.00	(67)
Yes	1.05	(15)	1.57	(13)
Cyclophosphamide:				
No	2.07	(74)	1.78	(72)
Yes	1.45	(9)	2.15	(8)
Etoposide:				
No	1.98	(60)	1.59	(57)
Yes	2.12	(23)	2.00	(23)

* $P < 0.05$, K-W ANOVA
** $0.05 \leq P \leq 0.10$, K-W ANOVA
[a] Included in block 2 in multiple regression analyses: factors correlating significantly with kidney platinum concentrations
[b] Included in block 3 in multiple regression analyses: other factors of potential biological importance

Kidney-cortex platinum concentrations

In the univariate analyses, factors that were associated ($P \leq 0.05$) with kidney-cortex platinum concentrations were the cisplatin dose per course, the pretreatment serum urea level, metoclopramide use (positive correlations), the time from the last cisplatin treatment to death, and the pretreatment serum albumin value (negative correlations). Factors that approached significance ($0.05 \leq P \leq 0.10$) were a history of hypertension, hyperbilirubinemia (positive correlations), the serum calcium level, and phenytoin use (negative correlations).

Results of the multiple regression analysis (Table 4) indicated that after controlling for the cisplatin dose per course, the patient characteristics that were significantly associated with kidney-cortex platinum concentrations were concurrent metoclopramide use (positive coefficient), the time from the last treatment to death, and concurrent phenytoin use (negative coefficients).

All except 13 patients had received mannitol with their cisplatin. The major exceptions were patients who had received very low cisplatin doses. Mannitol use did not correlate significantly with kidney-cortex or -medulla platinum concentrations in univariate analysis or following correction for the cisplatin dose in multivariate analyses. Nevertheless, kidney platinum concentrations did appear to be somewhat lower in those patients who had received mannitol (Table 3). In addition, all except 28 of the patients received total intravenous hydration volumes of ≥1000 ml in the 24-h period around the time of cisplatin administration. In this patient population, the hydration volume did not correlate significantly with the cisplatin dose per course ($r = -0.02$). As noted in Table 2, kidney-cortex platinum concentrations were comparable in patients who had received total intravenous hydration of < 1 l and in those who had received 1–2 l. Patients who had received > 2 l intravenous fluids did have the lowest median kidney-cortex platinum concentrations, but there were only 12 patients in this group, and the differences did not achieve statistical significance in either univariate or multivariate analyses, despite correction for the effect of the cisplatin dose per course by multiple regression.

Since the time from the last treatment to death accounted for 16% of the total variability in kidney-cortex platinum concentrations (controlling for the dose per course), whereas the dose per course uniquely accounted for only 7% of the variability, we were concerned that our analyses might be sensitive only to factors that affected long-term retention of platinum in tissues and that they might be less sensitive to any additional factors that might affect only short-term cisplatin retention. Hence, we repeated the regression analysis on the 50% subpopulation of patients (41 patients) having the shortest intervals from the last treatment to death. After controlling for the time from the last treatment to death and the cisplatin dose per course, the only factor that entered the model was concurrent use of metoclopramide.

Kidney-medulla platinum concentrations

Factors that correlated ($P < 0.05$) with kidney-medulla cisplatin concentration in univariate analyses (Tables 1–3) were the cumulative lifetime cisplatin dose, the cisplatin dose per course, the lactate dehydrogenase level, a history of hypertension, metoclopramide use (positive correlations), the time from the last treatment to death, and phenytoin use (negative correlations). The serum urea concentration was of borderline importance (positive). Multiple regression analysis for kidney-medulla platinum concentrations (Table 5) gave results that were very similar to those obtained for kidney cortex.

Discussion

In our multivariate analysis, the time from the last treatment to death explained 16% of the variability in kidney-cortex platinum concentrations after controlling for the cisplatin dose per course, and kidney platinum concentrations deceased only slowly over several months. This

Table 4. Summary of results of hierarchical stepwise multiple regression model: the dependent variable is the square root of kidney-cortex platinum concentration

Block	Variables included in block for stepwise analysis	In final model	R	R^2	Change in R^2	Beta (final)
1	sqrt Dose/course cisplatin $\log_{10}$ Cumulative dose mg/m^2	+	0.27	0.07	0.07	0.111
2	$\log_{10}$ (Time + 10)[a] $\log_{10}$ Urea[b] $\log_{10}$ Albumin[b] Metoclopramide (no = 0, yes = 1)	+	0.48	0.23	0.16	–0.423* 0.338*
3	Calcium $\log_{10}$ Bilirubin[b] Hypertension (no/yes) Hyperbilirubinemia (no/yes) ECOG Performance status (0–4) Phenytoin (no/yes) Mannitol (no/yes) Volume of i.v. fluid in total	+	0.62	0.38	0.03	–0.189*

sqrt, Square root
* $P < 0.05$, t-test for coefficient, final model
[a] Time from last cisplatin treatment to death, in days
[b] Transformation included "truncated" extreme data values
[c] The change in R^2 as one goes down the column indicates the amount of variability in kidney-cortex platinum concentration uniquely explained by a variable, controlling for the prior factors included in the model. Hence, the dose per course explains 7% of the variability and the time from treatment to death explains 16%, controlling for dose per course and other factors

Table 5. Summary of results of hierarchical stepwise multiple regression model: the dependent variable is the square root of kidney-medulla platinum concentration

Block	Variables included in block for stepwise analysis	In final model	R	R^2	Change in R^2	Beta (final)
1	sqrt Dose/course cisplatin $\log_{10}$ Cumulative dose mg/m^2	+	0.34	0.12	0.12	0.151
2	Metoclopramide (no = 0, yes = 1) $\log_{10}$ (Time + 10)[a] $\log_{10}$ LDH[b] Hypertension (no/yes) Phenytoin (no/yes)	+ + +	0.51 0.60 0.67	0.26 0.36 0.44	0.14 0.10 0.09	0.371* –0.363* –0.305*
3	$\log_{10}$ Urea[b] Total volume i.v. fluids ECOG performance status (0–4) Mannitol (no/yes)					

sqrt, Square root
* $P < 0.05$, t-test for coefficient, final model
[a] Time from last cisplatin treatment to death, in days
[b] Transformation included "truncated" extreme data values
[c] The change in R^2 as one goes down the column indicates the amount of variability in kidney-cortex platinum concentration uniquely explained by a variable, controlling for the prior factors included in the model. Hence, dose per course explains 12% of the variability, time from treatment to death explains 10%, controlling for dose per course, etc

finding is similar to that obtained in our earlier study [58] on the first 30 of this series of 83 patients.

In our earlier study, we did not find a correlation between kidney-cortex platinum concentrations and the cumulative lifetime cisplatin dose [58]. In the present study, involving a larger number of patients, kidney-cortex platinum concentrations still did not correlate significantly with the cumulative cisplatin dose in univariate nonparametric analysis. In multivariate analyses, the cisplatin dose per course emerged as being more closely associated with kidney-cortex platinum concentrations than was the cumulative cisplatin dose. This could be explained if most drug washes out of the kidney after each treatment and only the last (most recent) treatment course is accounting for drug retained in the kidney. However, our ability to detect cisplatin in human kidney autopsy samples for many months after the last treatment argues strongly against this explanation. Alternatively, this observation raises the question as to whether most cisplatin uptake into the kidney occurs with the first course of treatment, with little uptake occurring during subsequent treatments. However, this would be difficult to explain pharmacologically, and it goes against animal data published by Litterst and Schweitzer [60] that suggest that tissue retention of drug may actually be greater with later versus earlier drug courses. Our observations are nevertheless in keeping with the findings by at least some other investigators that cisplatin nephrotoxicity [11, 61–64] and kidney platinum-DNA adduct concentrations [65] correlate poorly with the cumulative cisplatin dose.

We have recently found in studies in mice that cell membrane lipids in the mouse kidney undergo substantial change after treatment of the animal with cisplatin (J.M. Molepo and R. Goel, unpublished data). We have also found in studies in tumor cell lines that the cell-membrane lipid characteristics appear to correlate with cisplatin uptake [66, 67]. Hence, one might speculate that the first treatment with cisplatin induces cell membrane changes in the kidney that then limit the uptake of further cisplatin into the kidney.

Alternatively, it is possible that the potential effect of high kidney platinum concentrations on nephrotoxicity could help explain the relatively poor correlation of kidney-cortex platinum concentrations with the cumulative cisplatin dose; i.e., patients with particularly high kidney-cortex concentrations after an early course of therapy might also have experienced early clinical cisplatin nephrotoxicity and might therefore have had their retreatment limited. Hence, early high kidney-cortex platinum concentrations would result in the inability to deliver high cumulative cisplatin doses. The latter explanation is in keeping with our earlier studies of cisplatin nephrotoxicity in which we had found an inverse correlation between the cumulative cisplatin dose and clinical nephrotoxicity with the first cisplatin treatment [3].

We are unaware of any data linking the antiemetic drug metoclopramide to cisplatin nephrotoxicity in animals [68] or in the clinical setting. However, metoclopramide antagonizes renovascular dopamine receptors [68] and augments cisplatin antitumor efficacy in preclinical systems, possibly through direct or indirect inhibition of the DNA repair enzyme polyadenosine-diphosphoribosyl transferase [69]. One might speculate that if metoclopramide inhibited the removal of platinum adducts from kidney DNA, it could thereby increase kidney platinum concentrations by increasing the amount of platinum left bound to DNA. An argument against this explanation is the observation that only a relatively small fraction of total cellular cisplatin is bound to DNA [70, 71].

It is unclear whether this association of metoclopramide use with kidney-cortex platinum concentrations is of any clinical significance, as the association uniquely explained only 12% of the variability in kidney-cortex platinum concentrations in multivariate analysis. However, we have also previously found an association between metoclopramide use and the development of cisplatin peripheral neuropathy [72]. The antiemetic prochlorperazine *reduces* cisplatin nephrotoxicity in mice, but the mechanism of this nephroprotection is unknown [30]. It is possible that our results were due to a reduction in kidney platinum concentrations in those patients who had received prochlorperazine instead of metoclopramide as their major antiemetic rather than being due to augmentation of kidney platinum concentrations by metoclopramide. However, the mouse studies did not document any alteration in kidney platinum concentrations by prochlorperazine (despite the reduction in nephrotoxicity) [30], and the kidney-cortex platinum concentrations detected in our patients who had received prochlorperazine were only slightly lower than the concentrations measured in those who had not received this medication.

Since all of our autopsy specimens were collected in the era prior to availability of the 5-hydroxytryptamine-3 (5-HT3) antagonists, we have no data on the effect of 5-HT3 antagonists on kidney platinum concentrations, and we are not aware of any data indicating that there is less cisplatin nephrotoxicity with the administrations of 5-HT3 antagonists as compared with metoclopramide. Nevertheless, in light of our observations on the apparent effect of metoclopramide on kidney-cortex platinum concentrations, we feel that it would be worthwhile to test whether higher doses of cisplatin can be achieved safely with the 5-HT3 antagonists than is possible with metoclopramide.

Kidney-cortex platinum concentrations correlated inversely with phenytoin use in this study, and we had previously found that phenytoin use also correlated with reduced cisplatin nephrotoxicity [3]. The reasons for this association are unclear, although phenytoin is known to alter the flux of several cations across cell membranes [73]. However, a recent randomized study of phenytoin administration initiated immediately before the first course of cisplatin failed to indicate any protection from cisplatin nephrotoxicity (D. Stewart, unpublished data). Hence, any possible phenytoin protection from cisplatin nephrotoxicity may require prolonged phenytoin administration prior to the first dose of cisplatin.

Prehydration with or without mannitol has resulted in a substantial reduction in cisplatin nephrotoxicity [1, 2], but neither mannitol use nor the intravenous hydration volume correlated significantly with kidney platinum concentrations in our study. Since most patients were treated as outpatients, we did not have details of the amount of oral hydration patients had received, although it is our practice to encourage patients to drink six to eight glasses of fluid per day on the day of cisplatin administration and for the first several days after cisplatin treatment. In any event, within the limitations of this study, we could not detect any significant effect of the hydration volume on kidney-cortex platinum concentrations, and we have not found any significant effect of the hydration volume on the risk of cisplatin nephrotoxicity in our previous studies [3]. Interestingly, although there is general agreement that generous hydration is important for decreasing the risk of cisplatin nephrotoxicity, there is little information on the fluid volume that is optimal or on the minimal fluid volume that is required. The data from this study and from our previous studies of cisplatin nephrotoxicity [3] suggest that the minimally required fluid volume may be somewhat lower than that used routinely by many groups. Further study of this aspect is warranted, since it could potentially facilitate the administration of cisplatin on an outpatient basis.

In univariate analysis, associations of kidney-cortex platinum concentrations with hyperbilirubinemia and serum calcium levels approached statistical significance. Neither of these variables was significantly associated with kidney-cortex platinum concentrations after correction for associations with other factors, but both may merit further study. We previously found that hyperbilirubinemia was associated with increased cisplatin nephrotoxicity, whereas serum calcium levels correlated inversely with cisplatin nephrotoxicity [3]. On the other hand, we found that serum calcium values correlated directly and serum bilirubin le-

vels correlated inversely with human tumor platinum concentrations and plasma-tumor transfer constants after cisplatin administration [74] and that serum calcium levels correlated directly with the development of cisplatin-induced peripheral neuropathy [3, 72]. Hence, if serum bilirubin and calcium levels actually do affect tissue cisplatin uptake or retention, the nature of the effect may be complex and tissue-specific.

We are currently analyzing the effect of several different factors on cisplatin nephrotoxicity. As part of these studies, we plan to assess whether kidney-cortex platinum concentrations are associated with cisplatin nephrotoxicity after correction for associations with other factors. In our earlier study [58], cisplatin nephrotoxicity did correlate with kidney-cortex platinum concentrations after correction for the cisplatin dose and the time from the last cisplatin treatment to death, suggesting that cisplatin nephrotoxicity is tissue-concentration-dependent. If such an association were confirmed, it would support the concept that new methods designed to reduce kidney-cortex platinum concentrations could also reduce nephrotoxicity. We have previously found that the dorsal root ganglion is the part of the nervous system with the highest platinum concentrations after cisplatin administration, that it is the site of the most prominent and earliest nervous system damage, and that cisplatin peripheral neuropathy is proportional to platinum concentrations in the dorsal root ganglion [72, 75–79]. Moreover, in our previous studies, tumor platinum concentrations correlated with antitumor efficacy in humans [80]. Hence, cisplatin toxicity and efficacy may be concentration-dependent in humans. This possibility is in keeping with in vitro data and is also consistent with our observations on several other antineoplastic agents [81–85]. However, there must also be a substantial element of individual tissue susceptibility, since some organs that attain high concentrations of antineoplastic agents have only a low propensity to develop toxicity [57, 81–85].

In this study, we found that the factors that correlated most closely with kidney-medulla platinum concentrations in multivariate analyses were identical to those that correlated most closely with kidney-cortex platinum concentrations. Although it is possible that the kidney medulla could be an important site of cisplatin toxicity, both we [86, 87] and other investigators [53–56] have found that the major histopathologic evidence of cisplatin nephrotoxicity occurs in the kidney cortex and in the corticomedullary junction.

In summary, platinum concentrations in human autopsy specimens of kidney cortex correlated inversely with the time from the last treatment to death and directly with the cisplatin dose per course. Metoclopramide use was associated with increased kidney-cortex platinum concentrations, suggesting that higher cisplatin doses may be achievable with new 5-HT3 antagonists than with metoclopramide. Furthermore, patients who had received phenytoin showed reduced kidney-cortex platinum concentrations, suggesting that phenytoin should perhaps be studied further as a possible means of reducing nephrotoxicity and augmenting maximally achievable cisplatin doses. The intravenous hydration volume did not correlate significantly with kidney-cortex platinum concentrations, suggesting that it may be feasible to use hydration volumes lower than those used by some groups. This study and several previous studies we have conducted [57, 58, 75, 80–85, 88–93] indicate that despite the obvious problems involved in interpreting data on drug concentrations in human autopsy tissue, potentially useful information can be obtained.

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