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Influence of glutathione administration on the disposition of free and total platinum in patients after administration of cisplatin

R. Leone[1], M. E. Fracasso[1], E. Soresi[2], G. Cimino[2], M. Tedeschi[3], D. Castoldi[3], V. Monzani[3], L. Colombi[3], T. Usari[3], and A. Bernareggi[3]

[1] Institute of Pharmacology, Policlinico Borgo Roma, Verona, Italy
[2] Divisione pneumologica "A. Piazza", Ospedale Niguarda, Milano, Italy
[3] Boehringer Mannheim Italia SpA, Research Center, Monza, Italy

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Summary. The kinetics of platinum (Pt) was studied in 12 patients suffering from non-small-cell lung cancer or pleural mesothelioma. Each subject received an infusion of cisplatin (CDDP, 80 mg/m^2), and six patients were pretreated with glutathione (GSH, 2.5 g given i.v.) at 15 min prior to the cisplatin infusion. After a 3- to 4-week interval, all patients were given a second course of treatment on the same schedule. A biexponential model was fitted to plasma concentrations of total and ultrafilterable Pt. The excretion of Pt in urine was evaluated during the first 48 h after the CDDP infusion. Following the administration of CDDP alone or with GSH pretreatment, the pharmacokinetic parameters of Pt did not significantly differ between the treatments. Also, the unbound fraction determined at each sampling time did not vary significantly between the treatments. However, it is noteworthy that the mean values obtained for the terminal half-life, the volume of distribution, the renal clearance, the percentage of the dose excreted in the urine, and the mean residence time of total Pt were higher in patients who had been pretreated with GSH, suggesting that GSH might increase both the rate of Pt elimination and the extent of Pt distribution and, as a consequence of the latter, might prolong the residence time of Pt in the body. In addition, the unbound fraction of Pt from the 4th to the 48th h was higher following the first dose of CDDP+GSH than after treatment with CDDP alone. Because of the rather high variability in the values of the parameters obtained, further work is planned using a larger number of patients.

Introduction

Cisplatin (CDDP) is a widely used antitumor agent that is effective in the treatment of some solid tumors, including lung cancer. The pharmacokinetics of Pt has been studied in cancer patients who have received CDDP treatment [4, 5, 10, 11]. Evidence of a dose-response effect for CDDP has led to the use of high doses of this drug [7]. Since nephro- and neurotoxicity are the major dose-limiting side effects, a variety of strategies have been proposed to protect the kidney and neurological functions following CDDP treatment. It has recently been shown that the administration of glutathione (GSH) provides protection against CDDP-induced nephrotoxicity without reducing the antitumor activity of the cytotoxic agent [3, 12, 13]. The aim of the present study was to investigate the effect of GSH pretreatment on the kinetics of total and ultrafilterable (free) platinum (Pt) in patients receiving CDDP for the treatment of non-small-cell lung cancer or pleural mesothelioma.

Offprint requests to: A. Bernareggi, Boehringer Mannheim Italie, Research Center, Viale delle Libertà Km 0,750, 20052 Monza (ITALY)

Patients and methods

Experimental design. The disposition of free and total Pt after the administration of CDDP was studied in two randomized groups of six patients each. Eligibility criteria included histologically documented, inoperable non-small-cell lung carcinoma (ten patients) or pleural mesothelioma (two cases), an age of <70 years, no prior chemotherapy, a leukocyte count of >3500 cells/mm^3, a hemoglobin value of >11 g/dl, and a serum creatinine level of <1.5 mg/dl. All subjects were required to show an Eastern Cooperative Oncology Group performance status of <3 and a life expectancy of ≥4 months. Individuals exhibiting severe metabolic disease or cardiopathy were excluded. Informed consent was obtained from each patient.

The treatment regimen consisted of 120 mg/m^2 etoposide given on days 1, 8, 15, and 22 as a 30-min i.v. infusion in 100 ml normal saline and 80 mg/m^2 CDDP given on day 2 and between days 23 and 30 as a 15-min i.v. infusion in 100 ml normal saline. On a random basis, one of the two groups was also given a 15-min i.v. infusion of 2.5 g GSH in 100 ml normal saline just before the administration of CDDP. Uniform i.v. hydration (1250 ml fluid) without diuretics was used; at 1 h prior to the initiation of the CDDP infusion, patients were hydrated with 250 ml normal saline. Posthydration with 1000 ml normal saline was continued for 4 h. Subjects received promazine (25 mg given i.m.) and hydrocortisone (200 mg given i.v.) immediately before CDDP administration and dexamethasone (8 mg given i.v.) and alizapride (350 mg given i.v.) after the CDDP infusion as standard antiemetic treatment.

Table 1. Patients' characteristics

Initials	Sex	Age (years)	Weight (kg)	Height (m)	Total dose of CDDP (mg)
CDDP group:					
M.D.	M	60	72	1.7	144
V.C.	M	68	68	1.67	140
E.O.	M	56	83	1.78	160
L.C.	M	49	82	1.68	152
G.M.	M	47	67	1.69	140
E.P.	M	60	84	1.74	158
CDDP+GSH group:					
G.M.	F	65	69	1.64	140
A.B.	M	53	69	1.7	144
N.Q.	M	52	84	1.8	160
S.C.	M	68	50	1.6	120
A.C.	F	63	53	1.68	124
R.E.	M	51	56	1.7	128

The demographic characteristics of our patients are reported in Table 1. Complete blood cell counts, hepatic and renal functions, fasting blood glucose, plasma total protein, albumin, and electrolytes were evaluated before each CDDP infusion.

Sample collection. Blood samples were drawn into heparinized tubes prior to the administration of CDDP, at the end of the CDDP infusion, and at 5, 15, and 30 min and 1, 2, 4, 7, 24, and 48 h after the infusion. Blood samples were immediately centrifuged at 12,000 *g* for 2 min at room temperature, and the plasma was separated and divided into two aliquots. The first was frozen and stored at –20° C until the analysis of total Pt. The second aliquot was immediately ultrafiltered through Centriflo CF50A cones (Amicon; cut-off, 50,000 Da) by centrifugation at 1,000 *g* for 10 min at 4° C. The ultrafiltrate was frozen and stored at –20° C until the analysis of free Pt.

Total and free Pt assay. The total Pt concentration in plasma samples was determined after a 1 : 10 dilution of the latter with 0.05% Triton solution. The free Pt concentration was measured in most ultrafiltrates without any dilution; when necessary, the ultrafiltrates were diluted with 0.1% Triton solution. Urine samples were diluted 1 : 2 with distilled water. Aliquots of 10 µl were analyzed by flameless atomic absorption spectrometry (Varian model 1475-GTA 95). The limit of quantitation was 5 ng/ml for plasma samples and 0.5 ng/ml for urine.

Pharmacokinetic analysis. A biexponential model for i.v. infusion (Eq. 1) was fitted to total and free Pt concentrations using $1/\hat{C}^2$ as a weighting factor (Siphar program; R. Gomeni, Simed, Créteil, France) according to the formula:

$$c = \sum_{i=1}^{2} ci\,(1-e^{-\lambda i\,T})\,e^{-\lambda i\,(t-T)}/\lambda i T, \quad (1)$$

where c is the concentration at any time, ci and λi represent the hybrid coefficients and the exponents of each exponential term (had an i.v. bolus dose been given), respectively, and T indicates the infusion time. The platinum concentration at the end of the infusion (c_{max}) was obtained from the experimental data. Other pharmacokinetic parameters were calculated according to standard relationships [6]. The half-lives in the distribution phase ($t_{1/2}\alpha$) and in the elimination phase ($t_{1/2}\beta$) were calculated as:

$$t_{1/2}\,i = \ln(2)/\lambda i. \quad (2)$$

The area under the concentration-time curves from time zero to 48 h (AUC_{0-48}) and extrapolated to infinity (AUC) were obtained using the following equations in which t′ is the post-infusion time:

$$AUC_{0-48} = \sum_{i=1}^{2} ci/\lambda i + ci(e^{-\lambda i T}-1)e^{-\lambda i\,t'}/\lambda i^2 T \quad (3)$$

$$AUC = \sum_{i=1}^{2} ci/\lambda i. \quad (4)$$

The plasma clearance of total platinum (C and unbound platinum (C_u) were calculated as the ratio of the delivered dose (D) expressed as elemental platinum (D = 52.4 mg/m^2) to the respective AUC value for total and unbound Pt. The renal clearance (C_R) was calculated as the ratio of the amount of Pt excreted in the 0- to 48-h urine specimen Ae_{0-48}) to the AUC_{0-48} value for total Pt. The mean residence time (MRT) was calculated as the ratio of the area under the first moment curve (AUMC) to the AUC. The initial volume of distribution (V_1) and the steady-state volume of distribution (V_{ss}) were calculated according to the following equations:

$$V_1 = D/(c_{1+2}) \text{ and} \quad (5)$$

$$V_{ss} = C \times MRT. \quad (6)$$

The unbound volume of distribution (V_u) was calculated as the ratio of C_u to λ2 for the unbound Pt. The unbound fraction (fu) was obtained as the ratio of the unbound Pt concentration to the total Pt concentration at each sampling time.

Statistical analysis. Statistical analysis was done by one-way analysis of variance for repeated measurements, with treatments, times (first and second administration), and times × treatments (interaction) as sources of variation.

Results

The temporal profiles of total and ultrafilterable Pt concentrations in plasma following the first i.v. dose of CDDP in the presence and absence of GSH pretreatment are shown in Fig. 1 and 2, respectively. The profiles observed following the second administration were very similar. The mean pharmacokinetic parameters calculated from plasma and urinary data are listed in Tables 2 and 3. Figure 3 illustrates the excretion of Pt during the first 24 and 48 h following the CDDP infusion.

Modest interpatient variability was observed in the plasma concentration values at the different sampling times. The concentration measured in plasma at the end of the infusion (c_{max}) was similar in all subjects and did not depend on the treatment, the mean values ranging from 4.71 (CDDP, first dose) to 5.44 mg/l (CDDP, second dose). Similarly, the mean values for AUC_{0-48} ranged from 72.1 (CDDP+GSH, first dose) to 92.4 mg h l^{-1} (CDDP+GSH, second dose) and did not differ significantly

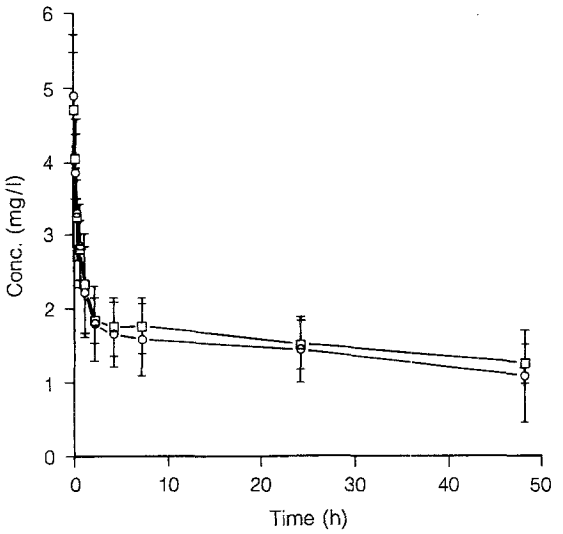

Fig. 1. Temporal profiles of total Pt concentrations (*Conc*) following the administration of the first dose of CDDP alone (□) or with GSH pretreatment (○)

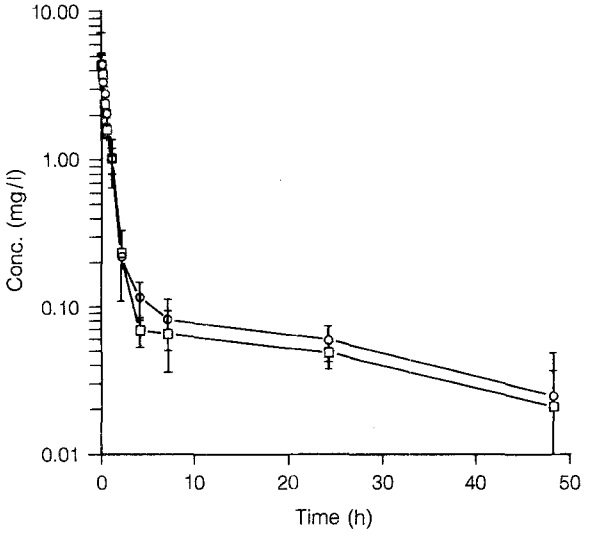

Fig. 2. Temporal profiles of ultrafilterable Pt concentrations (*Conc*) following the administration of the first dose of CDDP alone (□) or with GSH pretreatment (○)

between the treatments. Higher interpatient variability was associated with the other estimated parameters, whose values were dependent on the estimate of the terminal slope of the plasma curves (e. g., AUC, C, and $t_{1/2}\beta$).

Following all treatments, the concentration of total Pt subsided after the peak in a biexponential manner; the initial rapid decay ($t_{1/2}\alpha$, from 0.36 to 0.45 h), which was mainly related to the distribution process, was followed by a prolonged and slow apparent terminal phase ($t_{1/2}\beta$, from 85.6 to 143.2 h). Values of ca. 2 mg/l were attained at 4 h after CDDP administration, and at the 48th h the concentration remained as high as >1 mg/l. The c_{max} value for free Pt at the end of the infusion could be superimposed over the concentration of total Pt, indicating that all of the drug in plasma was unbound immediately after its administration. The concentration of unbound Pt decreased in a biexponential fashion. The half-life associated with the distribution phase ($t_{1/2}\alpha$, from 0.43 to 0.46 h) was similar to that of total Pt, whereas the apparent terminal half-life ($t_{1/2}\beta$, from 39.4 to 87.7 h) was much shorter than that of total Pt due to the time dependency of the binding in plasma. The extent of Pt protein binding in plasma increased with time: during the first 4 h, the unbound fraction (fu) decreased from approx. 0.95 to 0.05, and it dropped to 0.03 at the 48th h. Figure 4 shows the time dependency of the fu fraction of Pt. This phenomenon has previously been reported for Pt pharmacokinetics [4].

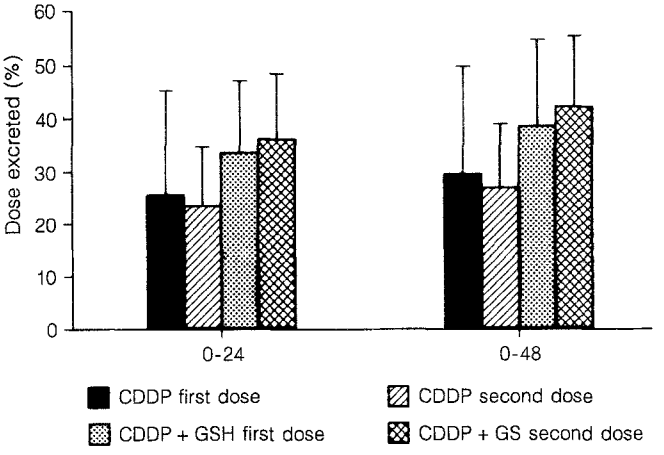

Fig. 3. Excretion of Pt in urine at 24 and 48 h after the CDDP infusion

Table 2. Mean pharmacokinetic parameters of total platinum following the administration of CDDP or CDDP+GSH

	CDDP		CDDP+GSH	
	1st dose	2nd dose	1st dose	2nd dose
c_{max} (mg/l)	4.71 (0.77)	5.44 (1.11)	4.9 (0.82)	5.39 (1.25)
$t_{1/2}\alpha$ (h)	0.36 (0.15)	0.37 (0.15)	0.43 (0.18)	0.45 (0.19)
$t_{1/2}\beta$ (h)	85.6 (16.5)	88.1 (36)	120.7 (57.1)	143.2 (64.4)
AUC (mg h l^{-1})	230.1 (64.7)	262.1 (121.3)	300.8 (164.4)	450.1 (239.3)
AUC_{0-48} (mg h l^{-1})	75.1 (14.5)	82.4 (14.1)	72.1 (19.3)	92.4 (19.9)
MRT (h)	122.6 (24)	126.1 (51.8)	172.9 (82.2)	205.5 (92.8)
C (ml h^{-1} kg^{-1})	5.92 (1.4)	5.75 (2.48)	6.17 (3.54)	4.01 (2.05)
C_R (ml h^{-1} kg^{-1})	5.31 (3.67)	4.26 (1.93)	7.51 (2.25)	6.37 (0.68)
Ae_{0-48} (%)	29.7 (19.7)	26.8 (11.9)	38.5 (15.6)	42.1 (12.9)
V_1 (l/kg)	0.25 (0.04)	0.22 (0.04)	0.28 (0.06)	0.26 (0.09)
V_{ss} (l/kg)	0.7 (0.1)	0.63 (0.09)	0.89 (0.33)	0.69 (0.23)

SD values are shown in parentheses

Table 3. Mean pharmacokinetic parameters of unbound platinum following the administration of CDDP or CDDP+GSH

	CDDP		CDDP+GSH	
	1st dose	2nd dose	1st dose	2nd dose
c_{max} (mg/l)	4.76 (0.57)	5.02 (1.4)	5.07 (1.58)	4.5 (1.33)
$t_{1/2}\alpha$ (h)	0.43 (0.03)	0.46 (0.06)	0.43 (0.04)	0.43 (0.1)
$t_{1/2}\beta$ (h)	39.4 (11.4)	87.7 (52.8)	42.7 (33.8)	50.1 (42.8)
AUC (mg h l^{-1})	7.02 (0.56)	11.84 (2.07)	9.04 (2.45)	10.87 (7.67)
AUC_{0-48} (mg h l^{-1})	5.4 (0.55)	6.26 (1.12)	6.57 (0.93)	6.58 (1.72)
C_u (ml h^{-1} kg^{-1})	181 (16.3)	108.9 (16.1)	181.6 (33.5)	182.9 (90.2)
V_u (l/kg)	10.37 (3.74)	12.9 (6.31)	7.27 (1.52)	9.17 (3.02)

SD values are shown in parentheses

Platinum proved to be widely distributed in the body: the values for the volume of distribution ranged from 0.63 (CDDP, second dose) to 0.89 l/kg (CDDP+GSH, first dose), indicating that Pt is distributed to both the extracellular and the intracellular fluids. Platinum was slowly cleared from the body, the total clearance ranging from 4.01 (CDDP+GSH, second dose) to 6.17 ml h^{-1} kg^{-1} (CDDP+GSH, first dose).

The occurrence of a high volume of distribution and a low clearance resulted in a long terminal half-life and a high mean residence time (MRT, 122.6–205.5 h) for Pt. In 48 h, the urinary recovery of the administered dose ranged from 26.8% (CDDP, second dose) to 42.1% (CDDP+GSH, second dose), confirming the slow elimination of Pt from the body. The renal clearance values ranged from 4.26 (CDDP, second dose) to 7.51 ml h^{-1} kg^{-1} (CDDP+GSH, first dose) and were similar to those found for total clearance. This indicates that the elimination of Pt from the body occurs mainly by renal excretion. The Pt pharmacokinetic parameters obtained in the present study are consistent with previously published data [5, 10, 11].

Discussion

The effect of GSH pretreatment on the pharmacokinetics of total and free platinum was investigated in 12 cancer patients following i. v. infusions of 80 mg/m^2 CDDP in the presence or absence of GSH pretreatment. For ethical reasons, the last sampling time was at 48 h after the administration, an early cutoff point for a drug that is characterized by a long elimination half-life, and the terminal phase of the plasma curve was defined by a few experimental points. Therefore, some degree of uncertainty might be associated with the estimate of the terminal rate constant and, consequently, with the pharmacokinetic parameters derived. The AUC value calculated using the trapezoidal

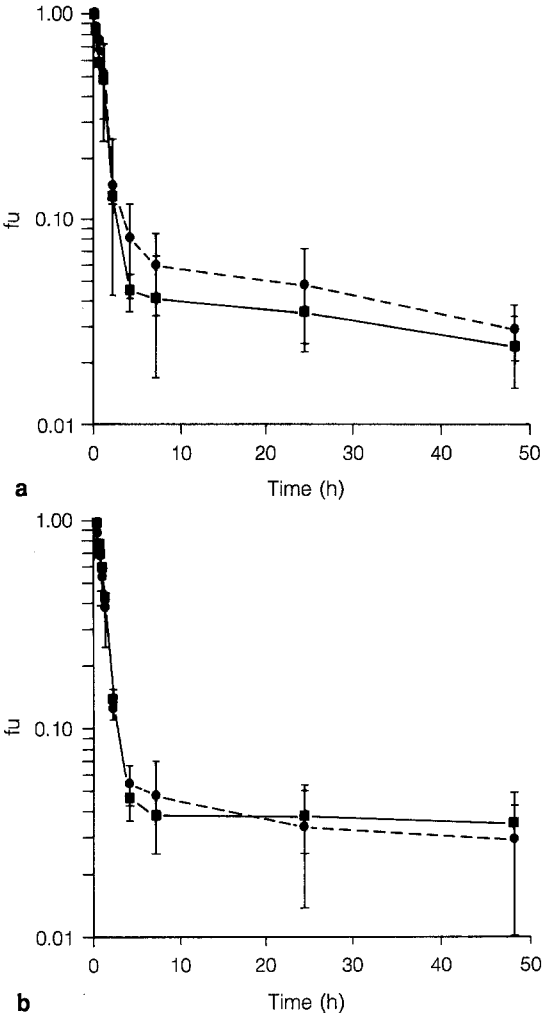

Fig. 4 a, b. Time dependency of the unbound fraction of Pt after the **a** first and **b** second administration of CDDP (■) and CDDP+GSH (●)

rule from time zero to the last measured concentration accounted for only about 20%–30% of the total AUC, indicating that most of the curve was not experimentally inspected but rather predicted by the fitting model used (Eq. 1).

Influence of GSH pretreatment

Following the administration of CDDP alone or with GSH pretreatment, most of the pharmacokinetic parameters of CDDP were not significantly different between the treatments. Neither the parameters defining the rate ($t_{1/2}$) and the extent (V_1, V_{ss}) of distribution nor those quantifying the rate (C, C_R) and the extent (Ae_{0-48}) of elimination were significantly different following the administration of CDDP in the presence vs the absence of GSH pretreatment. Moreover, the unbound fraction determined at each sampling time did not vary significantly between the treatments.

The relatively high variability in the values of the parameters and the small number of patients treated may explain the lack of statistically significant differences between the treatments. In comparing the mean values ob-

tained for the pharmacokinetic parameters following the administration of CDDP vs CDDP+GSH, some considerations seem noteworthy. The GSH pretreatment appeared to increase both the rate of total Pt elimination and the extent of total Pt distribution and, as a result of the latter, seemed to increase the residence time of Pt in the body. In fact, after the first administration of both medications, the volume of distribution increased from 0.7 to 0.89 l/kg; the renal clearance, from 5.31 to 7.51 ml h^{-1} kg^{-1}; the percentage of the administered dose excreted in the 0- to 48-h urine sample, from 29.7% to 38.5%; the AUC, from 230 to 301 mg h l^{-1}; the MRT, from 122.6 to 172.9 h; and the terminal half-life, from 85.6 to 120.7 h.

The possible influence of GSH on the distribution and elimination of Pt could be the result of the effect of GSH on Pt binding to plasma protein. It is generally recognized that only the free drug is available for tissue distribution and elimination [8]; an increase in the free fraction of Pt results in an increased volume of distribution and clearance, the drug having a low extraction ratio. We actually observed that the unbound fraction of Pt from the 4th to the 48th h was higher following the first dose of CDDP+GSH than after treatment with CDDP alone (Fig. 4). Accordingly, the clearance value for unbound platinum (C_u) did not vary after the first infusion of CDDP and CDDP+GSH (Table 3).

Following i.v. administration, exogenous GSH rapidly disappears from the plasma compartment [1] and is removed by the kidney, in which high concentrations of the thiol compound are achieved [13]. In fact, GSH is not taken up by most of the cells, except for those tissues showing substantial expression of γ-glutamyl-transpeptidase (γ-GT) on the cell membrane surface, mainly in the kidney. Since the nephrotoxicity of cisplatin has been ascribed to its reaction with the thiol groups of membrane proteins of renal tubules [2], GSH may reduce this toxicity by competing with protein sites for reactive platinum intermediates.

Therefore, the observed reduction in the nephrotoxicity of CDDP given with GSH pretreatment might be interpreted as the consequence of both an increase in the renal clearance of the former (due to the increased unbound fraction of CDDP in plasma) and a reduction in CDDP's interaction with the thiol groups of membrane proteins of renal tubules. Extracellular GSH does not interfere with the cytotoxic activity of cisplatin [9] and as the most common tumor histotypes express relatively low levels of γ-GT, GSH uptake by the tumor cells is unlikely. Therefore, the extracellular or intracellular inactivation of toxic platinum species by GSH in the tumor is not expected.

Time dependency of Pt kinetics

In a comparison of the pharmacokinetic parameters obtained following the first and the second dose, no time dependency of Pt pharmacokinetics was found after the administration of CDDP alone or with GSH pretreatment. The parameters obtained after the first administration did not significantly change following the second dose.

Tolerability

The tolerability of the present regimen was evaluated by determinations of blood cell counts, blood urea nitrogen values, and levels of serum creatinine, serum electrolytes, SGOT, SGPT, alkaline phosphatase, and serum total bilirubin. No major side effect was observed following treatment in either of the groups. Nephrotoxicity did not occur in the two groups, as expected due to the low CDDP dose given, to the short duration of treatment, and to the limited number of patients.

Conclusions

In the present study, the pharmacokinetics of cisplatin was studied in cancer patients following the administration of cisplatin in the presence or absence of GSH pretreatment. Due to high variability of the estimates of the parameters and to the limited number of patients, the pharmacokinetic parameters of free and total platinum did not differ significantly between the treatments. However, a comparison of the mean values obtained for most pharmacokinetic parameters calculated for total Pt after both administrations suggested that GSH pretreatment may increase both the extent of CDDP tissue distribution and the rate of the drug's elimination. This may explain some recent observations indicating that GSH pretreatment diminishes cisplatin's nephrotoxicity without reducing its antitumor activity. Further work using a larger number of patients is planned.

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