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Interaction of cisplatin with other cytotoxics and non-steroidal anti-inflammatory drugs

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This study investigated a possible interaction between cisplatin, other cytotoxics and non-steroidal anti-inflammatory drugs. Experiments were performed in quadruplicate. Plasma was spiked with cisplatin with or without another cytotoxic or non-steroidal anti-inflammatory drug. The results were analysed by Student's *t*-test and a p value of less than 0.05 was accepted as statistically significant. No interaction between cisplatin and the other cytotoxics was demonstrated. However, an increase in free cisplatin was noted when mixed with indomethacin ($p = 0.019$). No interaction with the other non-steroidal anti-inflammatory drugs was demonstrated.

Key words: Cisplatin, cytotoxics, non-steroidal anti-inflammatory drugs, cytotoxic interactions.

Introduction

Over the last decade, cisplatin (CDDP) has become a useful drug in the treatment of a number of tumors. With the increasing use of combination chemotherapy, CDDP is commonly prescribed with other cytotoxics. There is a large variation in free CDDP levels noted in the plasma of patients receiving platinum-containing regimens. Despite the number of regimens using CDDP, few pharmacological studies[1,2] have addressed the question of whether interactions with other cytotoxics or analgesics occur, and how, if at all, these might contribute to the inter-patient variation in free CDDP levels described.[3]

Alterations in the pharmacokinetics of CDDP have been attributed to variations in renal function and serum protein levels. However, the possibility that interactions with other drugs may be responsible for this variation has not been addressed. Within the cytoplasm, CDDP is aquated to become a reactive species capable of complexing a number of molecules.[4] Sauter *et al.*[5] studied the interaction of CDDP with a number of other cytotoxics in an *in vitro* rapid cell culture system and found that thiols and cyclophosphamide reduced the cytopathic effect of CDDP. This suggests that either a direct biochemical interaction was occurring or that the drugs were interfering with the other's biological action.

This study reports the pharmacokinetics of five patients receiving CDDP. There was considerable variation in pharmacokinetic parameters. The possibility that this variation was the result of drug interactions between CDDP and other agents administered concurrently was investigated *in vitro* by mixing experiments involving the addition of known quantities of CDDP to other cytotoxics and several analgesics commonly used in this patient population.

Materials and methods

Patients

Five patients treated with CDDP for carcinoma of the ovary, testicular carcinoma and transitional cell carcinoma of the bladder had pharmacokinetic studies performed. After obtaining informed consent, the patients' serum albumin and protein concentrations, renal and hemopoietic function were recorded. All drugs administered concurrently were documented. A 21 gauge butterfly needle was inserted and a 60–90 min infusion of CDDP (50–100 mg/m^2) commenced. Blood was taken at 0, 30, 60, 90, 120, 240, 300 and 1200 min following

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the conclusion of the CDDP infusion. Samples were processed immediately as free CDDP deteriorates rapidly (see below).

High performance liquid chromatography (HPLC) assay for CDDP

CDDP was analysed according to the technique of Drummer *et al.*[6] The blood samples were collected and centrifuged at 2000 rev/min for 20 min using Amicon ultrafiltrate cones. The 500 μl of ultrafiltrate, 40 μl of NiCl (internal Standard) and 100 μl of diethylthiolcarbamate (DDTC) was added. The stabilizing agent, DDTC, was added within 30 min of collection of the blood. Specimens were incubated for 1 h at 37°C and extracted with 200 μl of chloroform. Ten μl was injected into the HPLC system which consisted of a Waters 510 dual piston pump, a Waters Variable Wavelength Lambda Max 410 Spectrophotometer and a Rheodyne 7125 injector. A 5 μm cyano Activon with a pre-column was used. The mobile phase consisted of heptane (88%) and propanol-2 (12%) and the effluent was monitored at 254 nm. Pump speed was 2.6 ml/min.

To determine the stability of free CDDP, normal plasma obtained from volunteers was spiked with CDDP (2 μg/ml final concentration), protected from light and incubated at 37°C for 0, 0.5, 1, 2 and 4 h. At each time period the samples were processed in quintuplicate. Following incubation, the samples were ultra-filtered and the free CDDP concentration measured.

The stability of the ultra-filtrate following derivitization with DDTC was also determined. The plasma ultra-filtrate (500 μl) was spiked with CDDP (2 μg/ml final concentration), derivitized with DDTC (see above) and then stored at 4°C for 0, 1, 2, 3 and 7 days. After this period had elapsed, free CDDP concentrations were determined. The estimates at each time frame were performed in quintuplicate.

In vitro studies

Normal plasma and/or saline (1.8 ml) was spiked with CDDP to yield a concentration of 1 μg/ml, following which 100 μl of the drugs detailed below were added to achieve the quoted concentrations (pH = 7.4). Experiments were performed in quadruplicate. Samples were incubated for 1 h at 37°C and the tubes were covered with aluminum foil as CDDP is light-sensitive. On completion of incubation, the free CDDP concentration was determined.

Non-steroidal anti-inflammatory drugs (NSAID). Aspirin (15 μg/ml), flufenamic acid (5 μg/ml), indomethacin (12.5 μg/ml), piroxicam (10 μg/ml) and sulindac (5 μg/ml) were studied. The concentrations used approximated the peak concentrations previously reported in plasma following oral administration. Aspirin was dissolved in distilled water and the other NSAIDs were dissolved in 1 M NaOH.

Cytotoxics. The following cytotoxics were studied (μg/ml): adriamycin, 250; cyclophosphamide, 100; vinblastine, 100; vincristine, 100; etoposide, 200; 5-fluorouracil, 120; methotrexate, 250. The concentrations chosen for investigation *in vitro* are pharmacologically active.

Statistical analysis

The α and β half-life, peak plasma concentration and area under the curve (AUC) were calculated using a non-linear least-squares program[7]. The pharmacokinetic parameters were correlated with patients' serum protein, albumin, creatinine, hemoglobin and white cell count using correlation coefficients. Multivariate analysis was performed by stepwise regression.[8]

For the *in vitro* studies, the mean ± standard deviation of the ratio of CDDP to the internal standard were compared for CDDP alone and CDDP mixed with the NSAIDs, or cytotoxics. Results were compared using an unpaired Student's *t*-test.

Results

Pharmacokinetic studies

The mean age of the patients was 56 years (Table 1). The mean hemoglobin was 12.4 ± 0.6 g/l, white cell count $6.6 \pm 0.9 \times 10^3/mm^3$ and platelets $267 \pm 41 \times 10^3/mm^3$. No patient had a third space collection, e.g. pleural effusion or ascites, at the time of the study.

The results of the pharmacokinetic studies are demonstrated in Table 1 and Figure 1. There was a large inter-patient variation in the AUC partly explained by the different doses used. Factors such as dose, infusion time and albumin predicted for

Table 1. Pharmacokinetic characteristics in patients receiving CDDP chemotherapy. Patients received variable doses of CDDP according to their body surface area and underlying tumor

Patient	Dose (mg/m^2)	Infusion time (h)	Half-lives (min)		AUC[a]	Creatinine mmol/l	Albumin (g/l)	Peak conc. (ng/ml)	Age (years)
			α	β					
1	60	2.0	54	559	1846455	0.08	41	160	58
2	70	1.0	70	802	13563737	0.09	43	549	71
3	100	1.0	82	829	26508989	0.10	46	1473	65
4	33	1.5	65	1212	260111	0.08	43	210	26
5	70	1.0	82	1198	481655	0.11	43	190	61

[a] Area under the curve (ng/ml/h).

the α half-life and AUC. The other drugs that patients were receiving concomitantly are documented in Table 2. There was suggestion of a correlation between cyclophosphamide and the α half-life of free CDDP although this did not reach statistical significance ($r = 0.779$; $p = 0.12$). Furthermore, on multivariate analysis using stepwise regression cyclophosphamide was not an independent predictor for the α half-life. The other cytotoxics listed in Table 2 did not correlate with the pharmacokinetic parameters.

CDDP assay

Stability of free CDDP. In normal plasma samples spiked with CDDP and incubated at 37°C the free

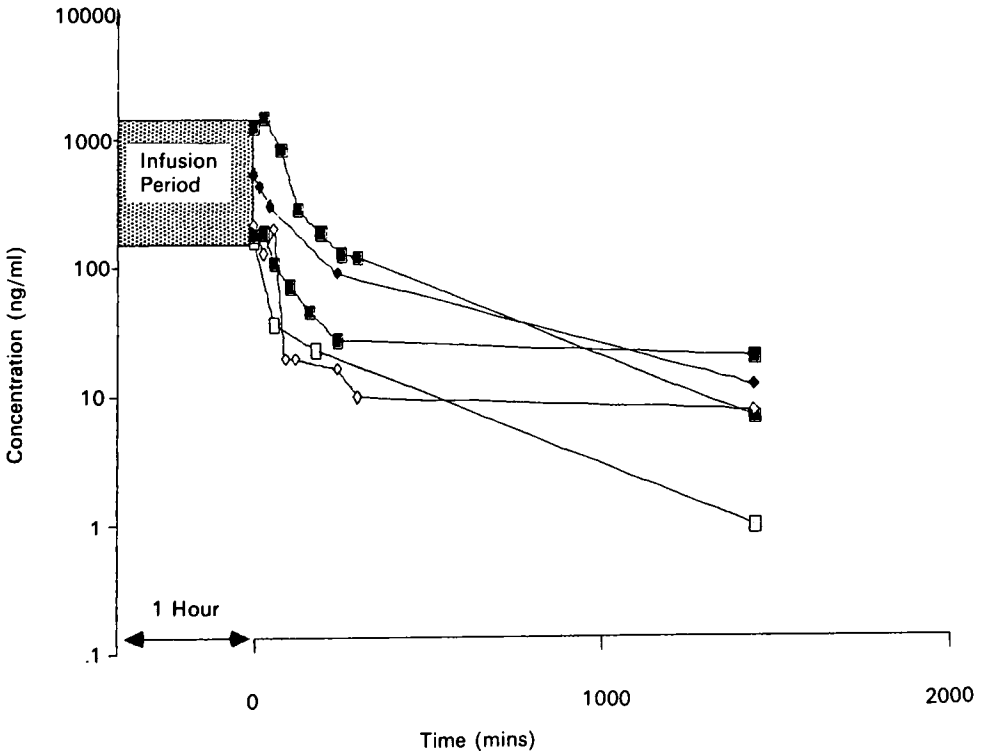

Figure 1 The pharmacokinetics of five patients (1–5) receiving CDDP. (□) 1; (◆) 2; (■) 3; (◇) 4; (■) 5.

Table 2. NSIADs and other cytotoxics taken by the five patients studied at the time of CDDP infusion. Mannitol, prochloperazine and lorazepam, although not tested in this study, did not interact with CDDP (data not shown)

Patient	Cytotoxic	NSAID
1	Cyclophosphamide	Naproxen
	Adriamycin	Mannitol
2	Methotrexate	Mannitol
		Lorazepam
	Adriamycin	Naproxen
	Vinblastine	
3		Mannitol
		Prochloperazine
4	VP-16	Lorazepam
		Mannitol
5	Methotrexate	Mannitol
	Adriamycin	Sulindac
	Vinblastine	

CDDP concentration in plasma decreased with increasing time (Figure 2). Plasma incubated for 30 min had a 10% reduction in free CDDP compared with that analysed immediately (unpaired t-test; $p = 0.73$). In the samples incubated for 4 h the free CDDP concentration had fallen by approximately 60% (unpaired t-test; $p < 0.0001$).

The derivitized samples stored at 4°C were stable at 7 days (unpaired t-test; $p = 0.110$) (Figure 3).

In vitro studies

The ratio of the height of the CDDP peak to the internal standard did not alter significantly for each of the NSAIDs used except in the case of indomethacin. However, when CDDP was admixed with a number of other NSAIDs, including aspirin, flufenamic acid, piroxicam (10 μg/ml) and sulindac, no statistically significant interaction was observed (Table 3). In the case of indomethacin there was an increase of 16% in the free CDDP concentration compared to the control ($p < 0.05$, Table 3).

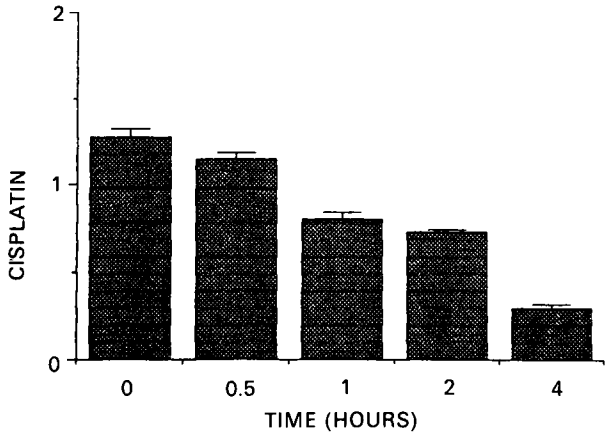

Figure 2. Concentration of CDDP in normal plasma spiked with CDDP vs time; samples were incubated at 37°C. After incubation for 4 h, the free CDDP has fallen by approximately 60% (unpaired t-test: $p < 0.0001$).

Table 3. The ratio of CDDP to nickel chloride (NiCl) in normal plasma spiked with CDDP alone or in combination with NSAIDS. Data points were collected in quadruplicate. The p value was obtained using an unpaired Student's t-test in each case

Drugs	CDDP/NiCl (mean ± SEM)	p Value (NSAID and CDDP)
CDDP alone	0.5935 ± 0.0444	
CDDP + flufenamic acid	0.5360 ± 0.0304	0.176
CDDP + indomethacin	0.7040 ± 0.0052	0.019
CDDP + piroxicam	0.6400 ± 0.1021	0.600
CDDP + aspirin	0.5245 ± 0.0389	0.241
CDDP + sulindac	0.6133 ± 0.0444	0.659

In samples of plasma or saline (data not shown) incubated with CDDP in the presence of other cytotoxics there was no statistically significant change in the free CDDP concentration (Table 4) as determined by HPLC.

Discussion

Wide variations in the pharmacokinetics of CDDP have been documented in a number of studies.[9] Postulated mechanisms include differences in serum protein concentrations and/or variability in renal

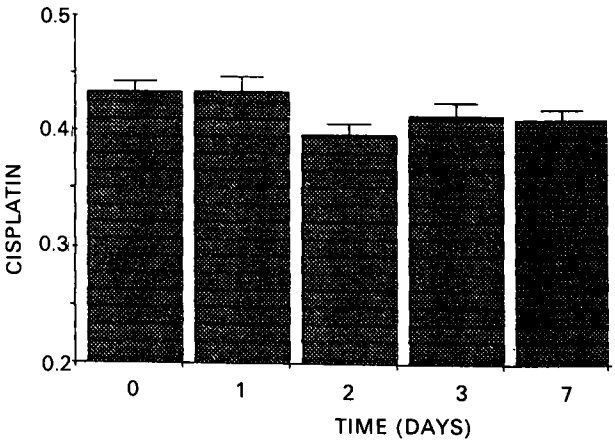

Figure 3. The concentration of free CDDP measured in plasma ultrafiltrate which has been derivitized with NiCl and stored at 4°C for varying periods of time. The derivitized ultrafiltrate was stable for 7 days with no significant change in the estimation of free CDDP.

Table 4. The ratio of CDDP/NiCl in plasma spiked with CDDP alone or in combination with other cytotoxics. The *p* value was obtained by using an unpaired Student's *t*-test

Drug	CDDP/NiCl (mean ± SEM)	*p* Value (cytotoxic and CDDP)	No. of samples
CDDP alone	0.7400 ± 0.0177		
CDDP + adriamycin	0.7114 ± 0.0248	0.366	8
CDDP + etoposide	0.7058 ± 0.0131	0.296	4
CDDP + cyclophosphamide	0.7350 ± 0.0118	0.842	5
CDDP + methotrexate	0.7020 + 0.0166	0.212	8
CDDP + vinblastine	0.7164 ± 0.0211	0.416	6
CDDP + vincristine	0.7512 ± 0.0104	0.721	4
CDDP + 5-FU	0.7100 ± 0.0231	0.342	4

excretion. However, Reece *et al.*[9] recently demonstrated that the patient-to-patient differences in the pharmacokinetics of CDDP could not be completely accounted for by changes in creatinine clearance.

Assay methodology may represent one possible technical factor that might explain this patient-to-patient variability but the stability and reproducibility of CDDP analysis in this assay was determined and found to be satisfactory. In our hands there was less than a 10% reduction in free CDDP provided the derivitizing agent was added to the blood specimen within 30 min of venesection. Furthermore, the derivitized CDDP was stable for at least 7 days at 4°C. If this factor were to be ignored, then significant variations in pharmacokinetics may occur.

This pilot study of five patients confirmed the large inter-patient variability in CDDP kinetics. Were drug interactions with other cytotoxics contributing to the changes in pharmacokinetics seen in this patient group, then predominantly a change in the α half-life would be expected. The α half-life of CDDP was reduced in patients receiving cyclophosphamide. However, this was not statistically significant and was not an independent predictor on stepwise regression. No correlation between the pharmacokinetic parameters of CDDP and the other cytotoxic drugs investigated could be demonstrated (data not shown).

To confirm the suggestion that cytotoxics or NSAIDs do not contribute to the inter-patient variability in CDDP kinetics, *in vitro* studies were performed. The results of these experiments did not demonstrate a significant interaction between CDDP and any of the other cytotoxics studied. These data do not exclude an interaction at the cellular level as this would not necessarily alter CDDP levels *in vivo* or *in vitro*.

In view of the number of patients on chemotherapy also receiving NSAIDs, a potential interaction with CDDP was also investigated *in vitro*. Indomethacin was the only example of an NSAID tested to demonstrate a change in free CDDP concentrations when the two were mixed. As both molecules are highly protein-bound this may account for this interaction. The clinical significance of this will require further investigation.

This study did not demonstrate any significant biochemical interaction between CDDP and the other commonly used cytotoxics or NSAIDs except for indomethacin. However, this study does not exclude *in vivo* pharmacological interactions as the basis of the inter-patient variability in CDDP kinetics.

Conclusion

In summary, this paper investigates interaction between cisplatin and other cytotoxics and non-steroidal anti-inflammatory drugs. The small interaction between cisplatin and indomethacin was statistically significant ($p = 0.019$). No other interaction could be demonstrated.

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