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ORIGINAL ARTICLE

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Pharmacokinetics of oxaliplatin (NSC 266046) alone and in combination with paclitaxel in cancer patients

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Abstract Oxaliplatin (OPT), a third-generation platinating agent, is currently being evaluated in a phase II clinical trial in head and neck cancer patients and in a phase I clinical trial in combination with paclitaxel (TXL). *Purpose:* The aim of this study was to investigate the pharmacokinetics and biological correlates of OPT alone as well as the potential pharmacokinetic interaction between OPT and TXL. *Methods:* In the phase II study, OPT was given alone as a 2-h i.v. infusion at 60 mg/m^2 weekly for 4 weeks with the cycle repeated after a 2-week rest. In the concurrent phase I combination trial OPT was also given as a 2-h i.v. infusion, but followed by a 1-h i.v. infusion of TXL, weekly for 4 weeks with the cycle repeated after a 2-week rest. The clinical pharmacokinetics of OPT alone and in combination with TXL were investigated in the first cycle of each treatment protocol. The platinum levels in plasma, plasma ultrafiltrate (PUF) and urine were measured by a fully validated inductively coupled plasma mass spectrometry (ICPMS) method. *Results:* In the ten patients receiving OPT alone, the concentration-time profiles of total platinum exhibited a biexponential decline both in plasma and in PUF. The peak levels of platinum were 2.72 ± 0.41 μg/ml in plasma and 1.36 ± 0.42 μg/ml in PUF at the end of the OPT infusion, and the platinum levels were still detectable at >10 ng/ml 94 h after the OPT infusion. The mean terminal $t_{1/2}$ values of total platinum in plasma and in PUF were 58.9 h and 22.8 h, respectively. The AUC of ultrafilterable platinum represented about 10% of that of the total plasma platinum. The platinum levels in the DNA fraction of peripheral white blood cells (WBC) correlated with the platinum levels in PUF ($r = 0.77$, $P < 0.01$). In the phase I combination study, the dose level of OPT was escalated from 35 mg/m^2 to 60 mg/m^2. The concentration-time profiles of platinum in the combination trial also showed biexponential decay in plasma and in PUF as exhibited by OPT alone. However, the terminal elimination rate constant (β) of total plasma platinum increased at all dose levels of OPT when combined with TXL at 45 mg/m^2 ($P < 0.05$). A concomitant increase in clearance (CL) of total plasma platinum was observed at the OPT dose level of 45 mg/m^2 in combination with TXL at 45 mg/m^2. No statistically significant difference in the 24-h urinary elimination of total platinum was detected between the combination groups and the single-agent group. The AUC values of total platinum in PUF were proportional to OPT doses ranging from 35 to 60 mg/m^2, whether OPT was given alone or in combination with TXL. *Conclusions:* OPT clearance may be enhanced by TXL when the two agents are used in combination in patients. The Pt-DNA adduct level in peripheral WBC was found to be a good indicator for oxaliplatin exposure in patients, and should be further exploited for potential tumor drug exposure.

Keywords Platinum · Taxol · Cancers · Phase I and phase II clinical trials · Pharmacokinetics

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Introduction

Oxaliplatin (*trans-l*-1,2-diaminocyclohexane oxalatoplatinum), a third-generation anticancer platinum derivative, has shown a wide spectrum of antitumor activity both in preclinical models [10, 17] and in human malignancies [6, 16, 19] including tumors resistant to cisplatin [3, 6, 18, 19]. Oxaliplatin lacks the nephrotoxicity of cisplatin and myelosuppressive action of carboplatin [4], but it produces reversible transient peripheral neuropathy, which can be enhanced by exposure to cold [4]. The mechanism of action of

oxaliplatin is similar to that of other platinum drugs and is mainly through the formation of platinum-DNA adducts [21, 25]. The diaminocyclohexane (DACH) platinum-DNA adducts formed by oxaliplatin are more effective in inhibiting DNA synthesis [13, 25], and therefore are more cytotoxic, than DNA adducts formed from cisplatin and carboplatin [19, 23, 25]. The difference in cytotoxicity has been attributed to the decreased repair of DACH platinum-DNA adducts due to steric hindrance of the DACH ring [22].

Oxaliplatin has been evaluated in clinical trials in a variety of malignancies and has shown activity in non-Hodgkin's lymphoma [8], endometrial cancer [24], non-small-cell lung cancer [15], and colon cancer [1, 2], when administered at 130 mg/m^2 as a 2-h i.v. infusion every 3 weeks. Currently, a phase II clinical study of oxaliplatin is being conducted at the Ohio State University Medical Center to explore the activity and toxicity of a weekly dose of 60 mg/m^2 in the treatment of head and neck cancers. Based on the synergism of paclitaxel with other platinum anticancer drugs, such as cisplatin and carboplatin [5, 20], a phase I trial of the combination of weekly oxaliplatin and paclitaxel is also being studied concurrently. We report the pharmacokinetics of oxaliplatin in both clinical trials, with a particular interest in the potential pharmacokinetic interaction between oxaliplatin and paclitaxel, as well as the biological correlates of oxaliplatin in patients.

Material and methods

Drugs

Oxaliplatin was supplied as freeze-dried powder by the National Cancer Institute. The powder was reconstituted by adding 5% dextrose (D5W) to yield a 5-mg/ml solution. This solution was then diluted in an infusion solution of 500 ml D5W. Paclitaxel was obtained commercially as a sterile concentrated solution of 6 mg/ml in 50% Cremophor-EL and 50% USP alcohol (Taxol, Bristol Myers Squibb). Paclitaxel at the appropriate dose was diluted with 250 ml D5W.

Patients

A phase II study with oxaliplatin alone in head and neck cancers and a phase I combination study with oxaliplatin and paclitaxel were implemented at the Ohio State University Medical Center. These studies were approved by both the Cancer Therapy Evaluation Program (CTEP) at the National Cancer Institute, and by the Institutional Review Boards of Ohio State University. Patients who entered on either protocol had histologically confirmed malignancy, and had adequate renal, hepatic and marrow function defined as follows: creatinine clearance ≥60 ml/min per 1.73 m^2, total bilirubin within normal institutional limits, ASG (SGOT)/ALT (SGTP) no more than 2.5 times the institutional upper limit of normal, leukocytes ≥3000/μl, absolute neutrophils ≥1500/μl, and platelets ≥100,000/μl. All patients provided written consent.

Drug administration

For the phase II study, oxaliplatin was given alone as a 2-h i.v. infusion at 60 mg/m^2 weekly for 4 weeks, with the cycle repeated after a 2-week rest. The phase I protocol called for a 2-h i.v. infusion of oxaliplatin followed by a 1-h i.v. infusion of paclitaxel, with the regimen repeated weekly for 3 weeks to complete one cycle of treatment, followed by a 2-week rest period prior to the next cycle of treatment. The doses of oxaliplatin were escalated according to the scheme 35, 45, 60, 60, 60, and 75 mg/m^2, with the corresponding paclitaxel doses of 45, 45, 45, 60, 80, and 80 mg/m^2, respectively, in the phase I study.

Blood sampling

Blood samples were collected at 0, 0.5, 1.5, 2, 2.25, 3, 6, 24, 48, 80, and 96 h from the beginning of the oxaliplatin infusion (BOI) in the phase II study and at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7, 9, 13, 24, 27, 39, 51, and 74 h from BOI in the phase I combination study. All blood samples were collected into heparinized tubes and placed on ice for no longer than 1 h. Each blood sample was then centrifuged at 2000 *g* and 4°C for 10 min to separate the plasma from the blood cells. An aliquot of the resultant plasma was immediately loaded onto an Amicon Centrifree micropartition device (30,000 Da cutoff; Millipore Corporation, Bedford, Mass.) and centrifuged at 1800 *g* and 4°C for 30 min to obtain plasma ultrafiltrate (PUF). Urine was collected from each patient during the 24 h from BOI in both settings. All plasma, PUF and urine samples were stored at –80°C until analysis.

Platinum assay

Total platinum in patient plasma, PUF and urine was measured by inductively coupled plasma mass spectrometry (ICPMS). A Perkin-Elmer ICPMS Elan 6000 system (Perkin-Elmer, Norwalk, Ct.) was used to determine total platinum levels. The instrument was fitted with a cross-flow nebulizer and a Scott spray chamber, a Fassel-type torch and an Elan 6000 data system. Before each batch of samples, the instrument was optimized with the standard tuning solution containing 10 ppb Mg, Rh, Pb, Ba, and Ce to obtain signals of Mg $>$20,000 cps, Rh $>$150,000 cps, Pb $>$100,000 cps, $Ba^{++}/Ba \leq 0.03$, $CeO/Ce \leq 0.03$, and background $<$30 cps. For some assays, an autosampler (AS 90, Perkin Elmer) was used. The peak jumping mode for spectral scanning was selected to monitor ^{194}Pt, ^{195}Pt and ^{196}Pt ions.

Plasma and PUF in 50-μl aliquots were diluted to 5 ml and 1.5 ml, respectively, with deionized water. The diluted samples were delivered to the nebulization chamber via a peristaltic pump. The liquid was converted to aerosol by the nebulizer to enter the ICP torch where an ion plasma was formed. The platinum ions generated were detected by a quadrupole mass analyzer. Prior to sample analysis, the method was validated using human plasma and PUF. Calibration curves were constructed from 5 to 10,000 ng/ml and 2 to 4000 ng/ml for human plasma and PUF, respectively. Within-day and between-day precisions were evaluated for at least three different concentrations and six replicates at each concentration level. Total platinum levels in urine were also analyzed by ICPMS after diluting 25-μl aliquots of the urine samples to 5 ml with deionized water.

Pharmacokinetic and statistical analysis

The concentration-time profiles of platinum derived from oxaliplatin in plasma and PUF from each patient were fitted to a two-compartment model with a zero-order input using WinNonlin software (Version 3.0, Pharsight Corporation, Mountain View, Calif.). The estimated pharmacokinetic parameters of platinum obtained from the phase I combination study were compared with those obtained from oxaliplatin single-drug therapy in the phase II study. The significances of the differences were evaluated using an unpaired *t*-test, except for the percent urinary excretion for which nonparametric tests were used to evaluate potential pharmacokinetic interaction between oxaliplatin and paclitaxel.

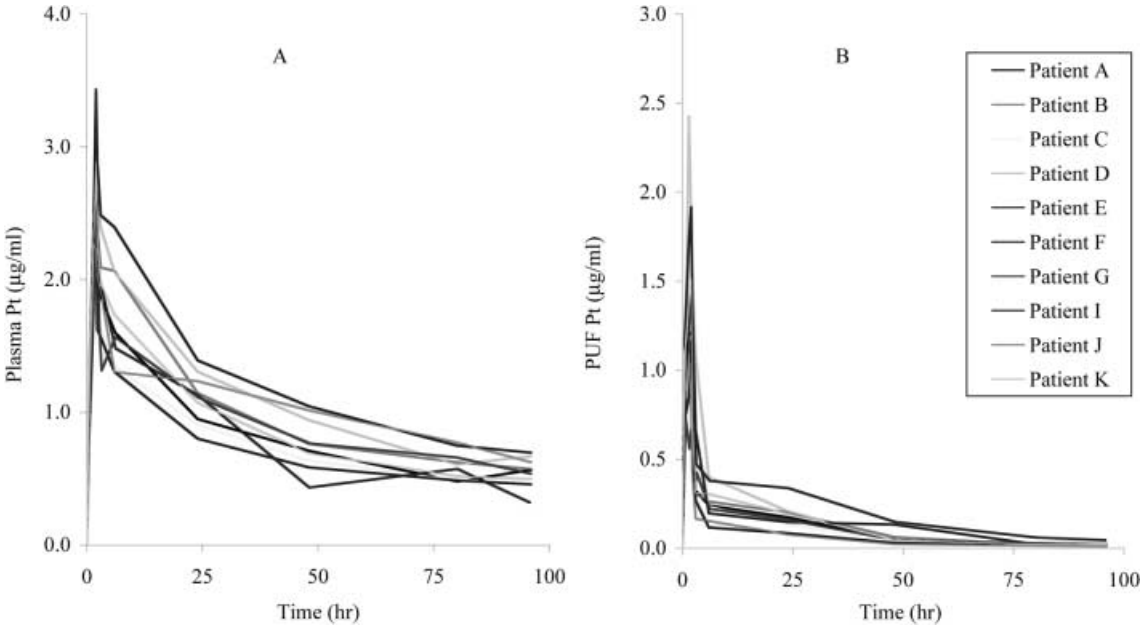


Fig. 1A, B. Concentration-time profiles of total platinum in plasma (**A**) and PUF (**B**) in head and neck cancer patients receiving oxaliplatin alone at 60 mg/m^2 as a 2-h i.v. infusion

Platinum-DNA adduct analysis in peripheral WBC

Whole blood (10 ml) was collected from each patient at 2 and 72 h from BOI in the phase II study. These specimens were collected using Vacutainer mononuclear cell preparation tubes (CPT; Becton Dickinson, Franklin Lakes, N.J.) which contain lithium heparin, polyester gel and Ficoll Hypaque gradient solution for separation of mononuclear cells. WBC were isolated following centrifugation at 1500 *g* for 15 min at room temperature. The WBC were then pelleted in a microcentrifuge tube. The DNA was isolated using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minn.). In this procedure, the WBC were lysed first using the cell lysis solution. The protein was then precipitated with protein precipitation solution, and the DNA in the supernatant was then precipitated with 100% isopropanol. The DNA was resolubilized in 100 μl TE buffer (1 m*M* Tris, pH 8.0, 0.1 m*M* EDTA) for DNA quantitation and platinum analysis. Since DNAs show maximum UV absorbance at 260 nm, while the proteins exhibit maximum absorbance at 280 nm, the purity of the DNA fraction was evaluated in terms of the ratio of the UV absorbance at 260 nm to that at 280 nm (A_{260}/A_{280}). The amount of DNA isolated from the WBC was quantitated using a specific fluorochrome, picogreen (a molecular probe). The platinum contents in the DNA fraction were determined by ICPMS after appropriate dilution. Calf thymus DNA was used to construct the standard curve. DNA obtained from normal (untreated) individuals was used as a negative control.

Results

Validation of ICPMS assay of oxaliplatin in human plasma and PUF

The limit of detection (LOD) of oxaliplatin by ICPMS was found to be 1 ng/ml in both plasma and PUF using the criterion of a 3:1 signal to noise ratio. The assay was linear in the ranges 5–10000 ng/ml in human plasma and 2–4000 ng/ml in PUF. The within-run coefficients of variation (CV) were 5.5%, 3.2%, and 2.0% at 5, 500, and 5000 ng/ml, respectively, in human plasma ($n=6$). In PUF, the within-run CVs were 3.1%, 2.5% and 1.6% at 2, 100, and 2000 ng/ml ($n=6$), respectively. The respective between-run CVs in human plasma were 6.4%, 2.8%, and 2.0% at 5, 500, and 5000 ng/ml, and for human PUF were 4.5%, 3.1%, and 2.6% at 2, 100, and 2000 ng/ml (all $n=3$).

Pharmacokinetics of oxaliplatin alone

In the phase II single-agent study, a total of ten patients received oxaliplatin alone at 60 mg/m^2 as a 2-h i.v. weekly infusion. The concentration-time profiles of total platinum derived from oxaliplatin in plasma and PUF showed biexponential decay in these patients (Fig. 1). All these concentration-time data were fitted to a two-compartment model with a zero-order input, and the relevant pharmacokinetic parameters thus obtained are summarized in Table 1. The total platinum concentration at the end of the oxaliplatin infusion (C_{end}) was 2.72 ± 0.41 μg/ml in plasma and 1.36 ± 0.42 μg/ml in PUF. The mean initial elimination half-lives ($t_{1/2\alpha}$) of total platinum in plasma and PUF were 0.47 h and 0.25 h, respectively, and the corresponding mean terminal elimination half-lives ($t_{1/2\beta}$) were 58.9 h and 22.8 h, respectively. The steady-state volume of distribution (V_{ss}) of total plasma platinum and PUF platinum were 34.8 l and 136.9 l, respectively. The mean areas under the concentration-time curves (AUC) of plasma platinum and PUF platinum were 130.4 μg·h/ml and 11.4 μg·h/ml, respectively. The total body clearance (CL) of platinum was 0.42 l/h in plasma and 4.8 l/h in PUF. Thus,

Table 1. Relevant pharmacokinetic parameters of total platinum in plasma and PUF and urinary elimination of platinum in patients receiving a 2-h i.v. infusion of oxaliplatin at 60 mg/m^2. Data presented are mean ± SD ($n = 10$)

Parameter	Plasma Pt	PUF Pt
C_{end} (μg/ml)[a]	2.72 ± 0.41	1.36 ± 0.42
C_{max} (μg/ml)[b]	2.69 ± 0.34	1.38 ± 0.41
$AUC_{0-\infty}$ (μg·h/ml)	130.4 ± 27.2	11.43 ± 3.97
α (1/h)	1.47 ± 0.53	2.73 ± 1.17
$t_{1/2\alpha}$ (h)	0.47 (0.40–1.85)	0.25 (0.16–1.30)
β (1/h)	0.012 ± 0.002	0.029 ± 0.009
$t_{1/2\beta}$ (h)	58.92 (52.01–82.93)	22.82 (14.33–24.25)
V_{ss} (l)	34.78 ± 8.04	136.93 ± 52.67
CL (l/h)	0.42 ± 0.09	4.81 ± 1.93
24-h urinary elimination (%)	38.9 ± 7.2	

[a]Experimental values
[b]From curve fitting

the free drug showed a shorter elimination half-life and a more rapid clearance than the total drug, as expected. The AUC of free platinum was 8.8% of the total plasma platinum. The 24-h urinary elimination of platinum was 38.9 ± 7.2%.

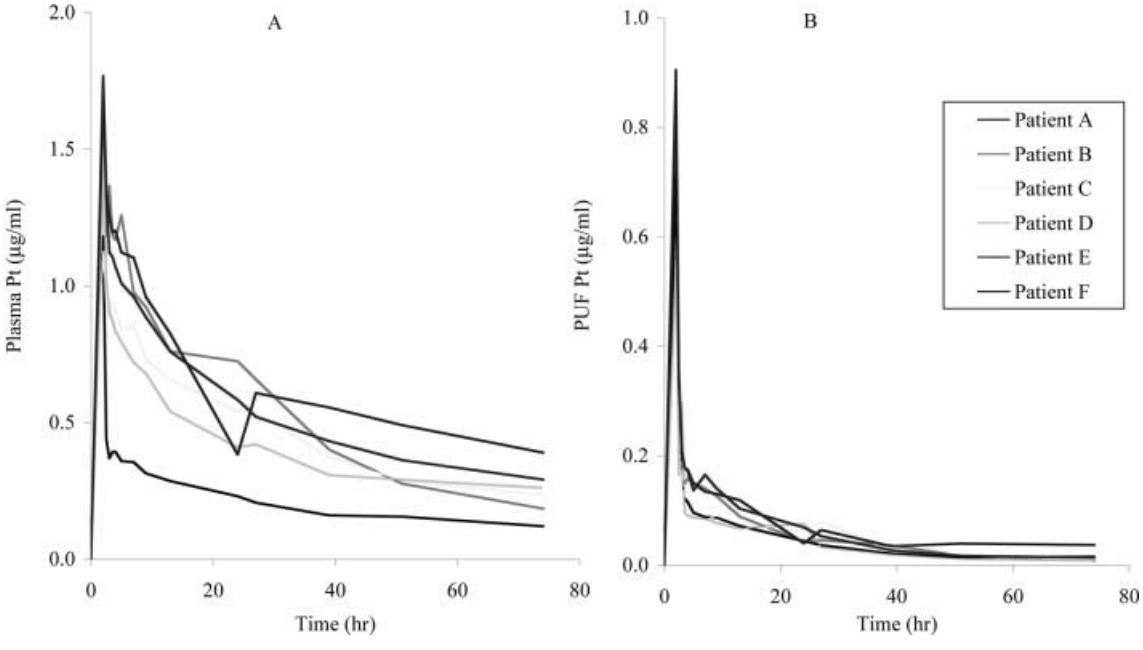
Pharmacokinetics of oxaliplatin in combination with paclitaxel

In the phase I combination study, three dose levels of oxaliplatin were investigated in combination with paclitaxel (45 mg/m^2). The concentration-time profiles of total platinum in plasma and in PUF in the six patients receiving the first dose level of oxaliplatin (35 mg/m^2) are shown in Fig. 2. The concentration-time profiles in three patients receiving oxaliplatin at 45 mg/m^2 are shown in Fig. 3, and those in three patients receiving 60 mg/m^2 are shown in Fig. 4. The concentration-time profiles of platinum both in human plasma and in PUF also followed biexponential decay when oxaliplatin was given in combination with paclitaxel. The total plasma platinum profiles appeared to be more variable in the combination groups as compared to those in the single agent group.

The concentration-time data were fitted to a two-compartment model with a zero-order input, and the relevant pharmacokinetic parameters estimated are summarized in Table 2. The AUC of total platinum in PUF was essentially proportional to the doses of oxaliplatin, regardless of whether it was given as a single agent or in combination with paclitaxel (Fig. 5). This result indicates that the pharmacokinetics of oxaliplatin were linear in the dose range 35–60 mg/m^2, a finding consistent with the data reported by Graham et al. [9].

Consistent increases in the mean terminal elimination rate constant (β) of total plasma platinum were observed in all the combination groups when compared with that in the single-drug group ($P < 0.05$). A concomitant increase in the CL of total plasma platinum was found only at 45 mg/m^2 of oxaliplatin in combination with paclitaxel, when compared with that of the single drug (60 mg/m^2) ($P < 0.01$). A similar trend was also observed in the combination groups at the lower dose (35 mg/m^2), but the difference was not statistically significant. No difference in the CL value was observed between the combination group receiving oxaliplatin at 60 mg/m^2 and the single-agent group. The V_{ss} values of total

Fig. 2A, B. Concentration-time profiles of total platinum in plasma (**A**) and PUF (**B**) in patients receiving oxaliplatin at 35 mg/m^2 as a 2-h i.v. infusion combined with paclitaxel at 45 mg/m^2 as a 2-h i.v. infusion



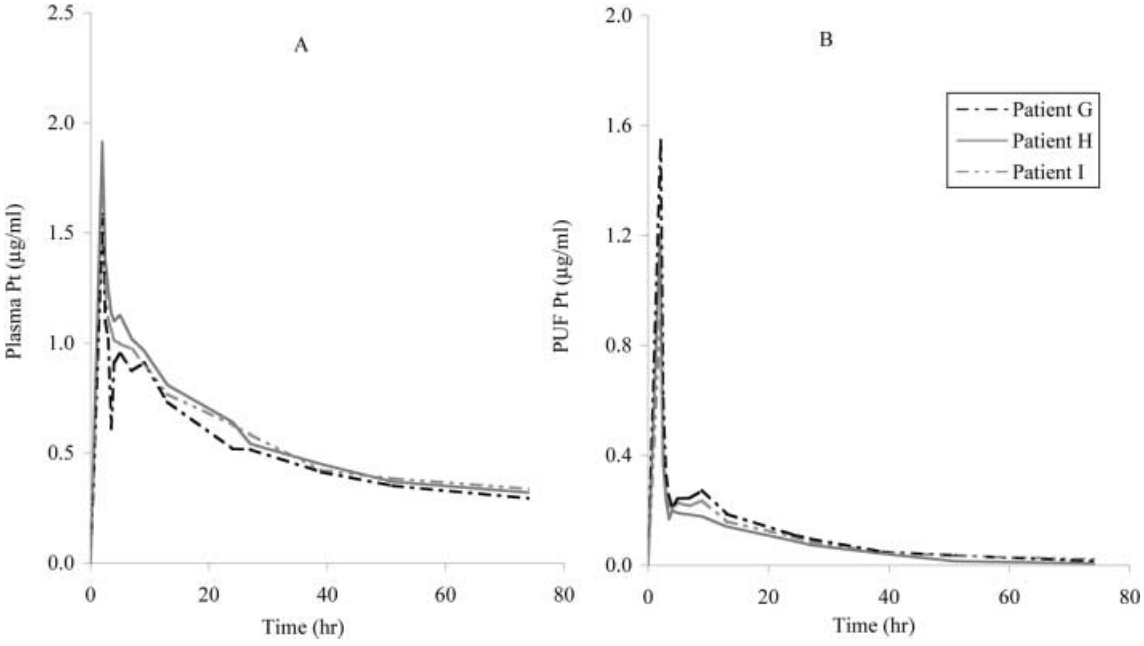


Fig. 3A, B. Concentration-time profiles of total platinum in plasma (**A**) and PUF (**B**) in patients receiving oxaliplatin at 45 mg/m^2 as a 2-h i.v. infusion combined with paclitaxel at 45 mg/m^2 as a 2-h i.v. infusion

plasma platinum in all groups were essentially the same. The increase in β of total platinum, however, was not observed in PUF, except that in the combination group receiving oxaliplatin at 45 mg/m^2. No difference in 24-h urinary elimination of total platinum was observed between the combination groups and the single-agent group.

Platinum levels in the DNA of peripheral WBC

The platinum levels in the DNA fraction of peripheral WBC, isolated from six head and neck cancer patients treated with oxaliplatin alone, are shown in Table 3. The total platinum levels in the DNA fractions were higher at earlier time-points (i.e. 2 h after BOI) in most patients and had decreased by later time-points (i.e. 72 h after BOI), consistent with the change in plasma platinum

Fig. 4A, B. Concentration-time profiles of total platinum in plasma (**A**) and PUF (**B**) in patients receiving oxaliplatin at 60 mg/m^2 as a 2-h i.v. infusion combined with paclitaxel at 45 mg/m^2 as a 2-h i.v. infusion

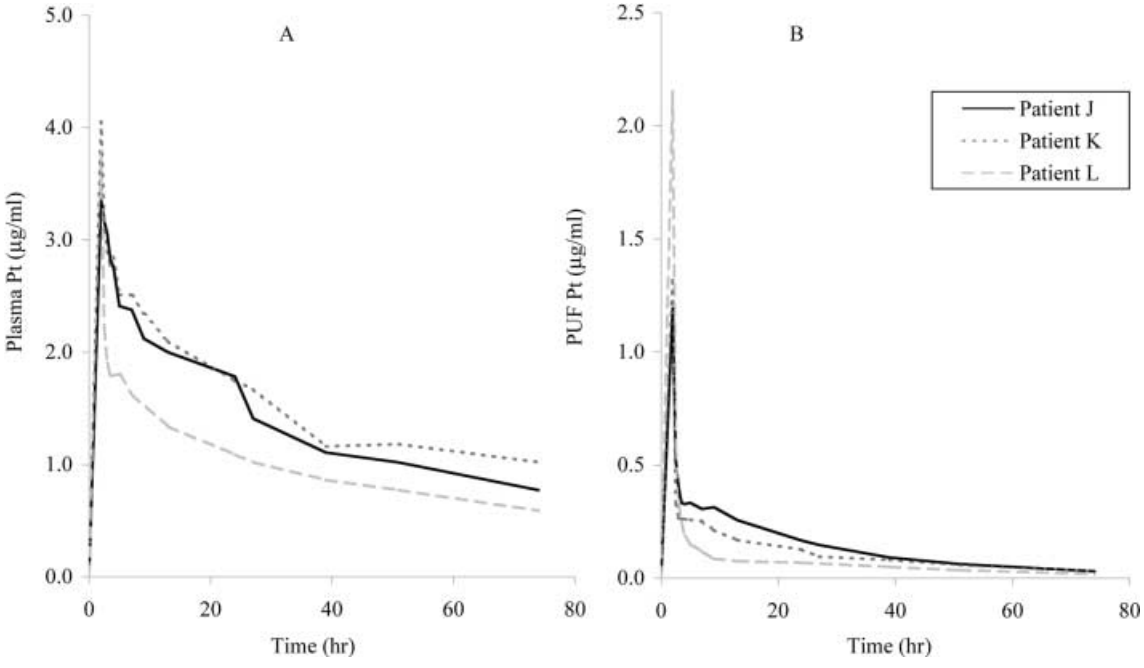


Table 2. Relevant pharmacokinetic parameters of total platinum in plasma and PUF as well as in urine obtained from patients receiving a 2-h i.v. infusion of oxaliplatin at 35, 45 and 60 mg/m^2 followed by a 1-h i.v. infusion of paclitaxel at 45 mg/m^2. Data presented are means ± SD

Parameter	35 mg/m^2 ($n = 6$)		45 mg/m^2 ($n = 3$)		60 mg/m^2 ($n = 3$)	
	Plasma Pt	PUF Pt	Plasma Pt	PUF Pt	Plasma Pt	PUF Pt
C_{end} (μg/ml)[a]	1.49 ± 0.28	0.694 ± 0.146	1.72 ± 0.17	1.21 ± 0.33	3.73 ± 0.35	1.55 ± 0.52
C_{max} (μg/ml)[b]	1.32 ± 0.24	0.692 ± 0.107	1.69 ± 0.11	1.22 ± 0.32	3.66 ± 0.30	1.79 ± 0.79
$AUC_{0-\infty}$ (μg·h/ml)	43.7 ± 14.4	4.65 ± 0.81	54.8 ± 1.8	8.02 ± 1.47	144.0 ± 31.9	13.9 ± 7.2
α (1/h)	1.63 ± 2.25	3.23 ± 1.11	1.81 ± 0.94	3.26 ± 0.06	1.26 ± 1.22	3.46 ± 1.88
$t_{1/2\alpha}$ (h)	0.36 (0.12–5.16)	0.22 (0.15–0.37)	0.38 (0.24–0.64)	0.21 (0.18–0.26)	0.55 (0.26–2.37)	0.20 (0.13–0.40)
β (1/h)	0.019 ± 0.007**	0.038 ± 0.006	0.018 ± 0.001**	0.042 ± 0.006**	0.016 ± 0.001*	0.031 ± 0.005
$t_{1/2\beta}$ (h)	42.7 (26.8–84.3)	18.4 (15.9–23.6)	38.7 (35.9–42.2)	16.3 (14.0 –18.4)	44.7 (42.0–47.2)	22.7 (19.6–27.6)
V_{ss} (l)	44.4 ± 20.4	135.0 ± 34.6	42.9 ± 2.2	105.1 ± 25.5	26.0 ± 5.1	146.0 ± 16.0
CL (l/h)	0.703 ± 0.497	6.62 ± 1.52	0.837 ± 0.006**	5.87 ± 1.21	0.417 ± 0.093	5.81 ± 0.70
24-h urinary elimination (%)	41.8 ± 8.6		49.5 ± 5.4		46.2 ± 20.6	

* $P < 0.05$, ** $P < 0.01$, vs corresponding parameter in the single-drug group (Table 1)
[a]Experimental values
[b]From curve fitting

concentration. The A_{260}/A_{280} ratios of these DNA fractions were consistently less than 1.4, although the proteins had been removed by precipitation twice from the DNA. Theoretically, the average A_{260}/A_{280} ratio for DNA is about 2 [11]. These findings suggest that a complex or strong association between protein and DNA forms in the WBC in oxaliplatin-treated patients. Woynarowski et al. [26] have recently reported similar findings of an in vitro cell study. The platinum levels in the DNA fraction of peripheral WBC of the patients were about 100-fold higher than those found in another in vitro study by Pendyala et al. [17], in which 60 μ*M* oxaliplatin was incubated with L1210 cells. The data also showed that the platinum levels in the DNA fraction correlated with the platinum levels in PUF (Fig. 6), with a correlation coefficient of 0.77 ($P < 0.01$).

Discussion

Because of the lability of most of the platinating agents and the formation of complex transformation products in biological fluids, most of the assay methods for platinating agents measure total platinum by flameless atomic absorption spectroscopy (FAAS) and by the more sensitive method of ICPMS [9]. Furthermore, because of the irreversible plasma protein binding of many platinum species, platinum levels in PUF have been used in most of the pharmacokinetic studies of the platinating agents. In general, this approach is adequate for pharmacokinetic studies, since a number of free platinum species are considered to be pharmacologically active. Since the previously reported ICPMS method lacks assay validation [7], we have provided a full validation of the ICPMS assay in plasma and PUF, and in urine in the present study.

Pharmacokinetic profiles of oxaliplatin alone in patients in the present study showed biexponential decay, similar to those reported by Massari et al. [14]. Our data, however, are different from those reported by Graham et al. [9], whose data demonstrate a triexponential decay of free platinum derived from oxaliplatin with a mean terminal half-life of 273 h, much longer than the 22.8 h obtained in the present phase II study. This discrepancy in terminal half-life may have been due to the difference in duration of drug level monitoring, as Graham et al. collected blood samples for 22 days, compared to the 96 h in our study. This difference may also have been due to a difference in dose, as Graham et al. employed a 2-h i.v. infusion of oxaliplatin at 130 mg/m^2, twice the level used in our study.

Evidence of increased CL and β (decreased $t_{1/2\beta}$) in plasma platinum was observed in the combination groups compared to those in the single-agent group. However, no corresponding increase in the 24-h urinary elimination was found, although urinary excretion is the major route of platinum elimination. No change in V_{ss} was observed in the combination groups. These findings indicate that paclitaxel may increase the elimination of platinum via pathways other than urinary elimination.

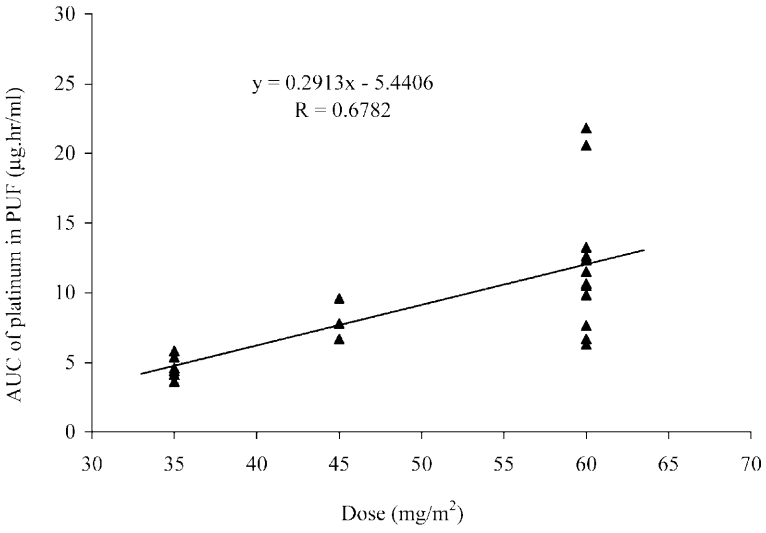


Fig. 5. Correlation of the mean AUCs of total platinum in PUF with the doses of oxaliplatin in patients receiving a 2-h i.v. infusion of oxaliplatin either alone (60 mg/m^2) and in combination with paclitaxel (oxaliplatin doses of 35, 45 and 60 mg/m^2) ($P < 0.05$)

Table 3. Platinum levels in the DNA fraction of peripheral WBC from head and neck cancer patients treated with oxaliplatin at 60 mg/m^2. The corresponding A_{260}/A_{280} ratios of the DNA fraction are as indicated as an index of relative properties of DNA to protein (*BOI* beginning of oxaliplatin infusion)

Patient no.	2 h after BOI		72 h after BOI	
	ng Pt/μg DNA	A_{260}/A_{280}	ng Pt/μg DNA	A_{260}/A_{280}
1	3.227	0.97	0.068	1.45
2	3.090	0.96	0.137	1.42
3	3.188	0.91	2.467	0.82
4	6.699	0.86	0.319	1.28
5	1.806	1.10	0.630	1.14
6	1.140	0.94	2.074	0.77

It has been reported that platinum generated from oxaliplatin may be irreversibly bound to plasma protein, taken up by red blood cells, and bound to tissues [12, 16]. An increase in irreversible plasma protein binding, red blood cell uptake, and tissue binding may all contribute to the increased CL and decreased $t_{1/2\beta}$ of platinum in the combination study. The increase in CL was not consistent among the combination groups at different dose levels of oxaliplatin. This inconsistency may have been due to the different oxaliplatin doses, and therefore, resulted in concentration-dependency in the increases in CL caused by paclitaxel. The exact mechanism of the alteration in CL by paclitaxel remains unclear at this time, and needs to be further investigated using both animal and in vitro experiments.

Since oxaliplatin is thought to exhibit its anticancer activity by forming DACH-platinum-DNA adducts, the platinum-DNA adduct level in the peripheral WBC of patients may be a useful pharmacodynamic biomarker for oxaliplatin exposure. The platinum levels in the DNA fraction correlated with the free platinum levels in plasma in patients treated with oxaliplatin. The platinum-DNA adduct levels may also be a useful marker of drug exposure in tumors. The association of protein with

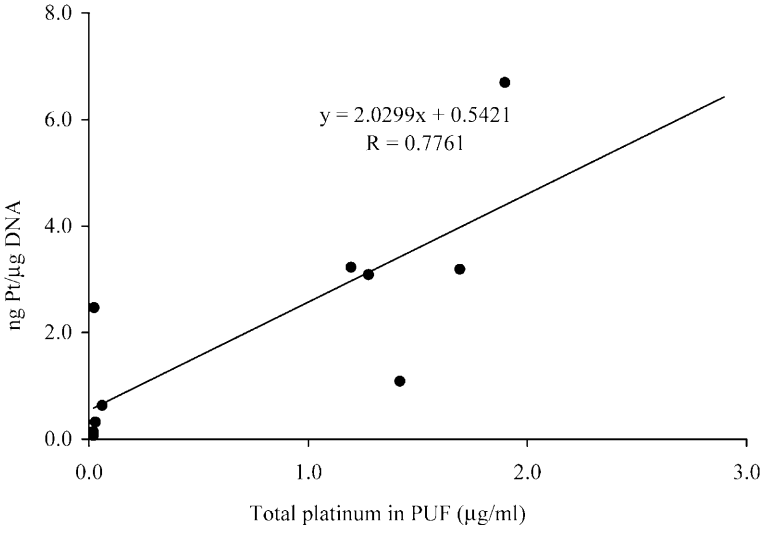


Fig. 6. Correlation of the platinum levels in the DNA fraction of peripheral WBC with the platinum concentration in PUF in patients treated with oxaliplatin at 60 mg/m^2. The correlation coefficient is 0.7761 ($P < 0.01$)

extracted DNA suggests the formation of protein-platinum-DNA crosslinks. This possibility will be further investigated by other methods.

In summary, an apparent alteration of pharmacokinetics of oxaliplatin by paclitaxel was observed in patients. Platinum levels in the DNA of peripheral WBC correlated with free platinum plasma levels in patients treated with oxaliplatin. The platinum-DNA adduct level in peripheral WBC has been shown to be a good indicator of oxaliplatin exposure in patients, and should be further exploited as a potential indicator of tumor drug exposure.

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