Cancer Chemother Pharmacol (2002) 50: 445–453
DOI 10.1007/s00280-002-0531-6

ORIGINAL ARTICLE

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Dosing sequence-dependent pharmacokinetic interaction of oxaliplatin with paclitaxel in the rat

Received: 6 May 2002 / Accepted: 29 August 2002 / Published online: 16 October 2002

Abstract *Background*: In a phase I clinical trial of oxaliplatin (OPT) in combination with paclitaxel (PXL), a pharmacokinetic interaction was observed when OPT was given as a 2-h i.v. infusion followed by a 1-h i.v. infusion of PXL. The purpose of this study was to use a rat model to evaluate whether the pharmacokinetic interaction between OPT and PXL is dosing sequence-dependent. *Methods*: One group of rats was given OPT as a 2-h i.v. infusion followed by a 1-h i.v. infusion of PXL formulated in 50% Cremophor EL (CrEL)/50% ethanol (OPT→fPXL), similar to the current phase I clinical protocol. In a second group of rats, the fPXL was infused first to reach a quasi-steady-state plasma level of PXL, followed by an i.v. bolus dose of OPT (CIfPXL→OPT). In a third group of rats, fPXL was replaced with the formulation vehicle, CrEL, which was infused in the same manner as in the second group. Each combination was accompanied with a control of either OPT alone or with replacement of PXL with dextrose 5% in water (CID5W→OPT). The total platinum (Pt) levels in plasma and plasma ultrafiltrate were measured by a validated inductively coupled plasma mass spectrometry (ICPMS) method. The protein binding, red blood cell (RBC) uptake and urinary elimination of Pt were also examined in each group of rats. *Results*: The concentration-time profiles of plasma Pt and ultrafiltrable Pt followed triexponential decays in all groups of rats. In the rat receiving OPT→fPXL, the terminal elimination rate constant (γ) of plasma Pt increased, with essentially no change in the total body clearance (CL) and the AUC value, when compared to those without PXL infusion (CID5W→OPT). The steady-state volume of distribution (V_{ss}) of the ultrafiltrable Pt also showed an increase in the combination group receiving OPT→fPXL ($P < 0.01$). These results were similar to those from the clinical trial, although the magnitude of change was less. However, in the CIf-PXL→OPT group, both CL and V_{ss} of Pt in plasma and plasma ultrafiltrate decreased, with corresponding increases in AUCs ($P < 0.01$). The 24-h urinary elimination of total Pt increased in both combination groups, irrespective of the dosing sequence. No difference in protein binding of Pt was observed among the groups. There was a decrease in RBC uptake in the presence of steady-state level of fPXL, but the same was not observed in the OPT→fPXL group. Additionally, similar results were observed with OPT in combination with CrEL alone. *Conclusions*: These results suggest that alterations in the pharmacokinetics of OPT by fPXL are dosing sequence-dependent and mainly caused by the formulation vehicle CrEL. It is suggested that the dosing sequence of fPXL followed by OPT would be more clinically favorable because it would prolong the residence of OPT in systemic circulation. It is further recommended that the use of other formulations of PXL without CrEL or docetaxel would avoid the complication effect of CrEL.

Keywords Platinum · Paclitaxel · Drug interaction · Pharmacokinetics · Cremophor EL

This work was supported by PHS grants UO1 CA 76576 and UO1 CA 63185, both from the National Cancer Institute.

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Introduction

Oxaliplatin (*trans*-L-1,2-diaminocyclohexane oxalatoplatinum, OPT) is a third-generation anticancer platinum compound. In comparison to other platinum

compounds, OPT lacks the nephrotoxicity of cisplatin and myelosuppression of carboplatin, but it produces a reversible cold-sensitive peripheral neuropathy [8]. Anticancer platinum compounds are generally thought to exhibit their antitumor activity though the formation of platinum-DNA adducts that block DNA replication and transcription, resulting in cell death in actively dividing cells as well as the induction of apoptosis [17]. OPT exhibits a wide spectrum of antitumor activity, including in tumors resistant to cisplatin and carboplatin [6, 10, 18, 19]. OPT is more cytotoxic than cisplatin and carboplatin, in that it requires fewer DNA adducts to achieve an equal level of cytotoxicity [5].

Paclitaxel (PXL) is a unique mitotic poison, which promotes the assembly of microtubules and stabilizes them against depolymerization [15]. PXL also causes apoptosis of tumor cells [2]. PXL has shown significant clinical activity in several human malignancies including ovarian cancer, non-small-cell lung cancer and breast cancer [20]. The major toxicity found after PXL treatment is the inhibition of rapidly proliferating cells with resultant myelosuppression, alopecia and mucositis, and peripheral neuropathy.

Anticancer agents are rarely used alone in cancer chemotherapy. Effective cancer treatment usually depends on the identification of a suitable combination of two or more chemotherapy agents to increase the efficacy and to decrease the toxicity of the chemotherapy [9]. OPT and PXL show different mechanisms in their antitumor activities and do not share common major toxicities, except neuropathy. Moreover, PXL has shown synergism with cisplatin and carboplatin both in vitro and in vivo [20]. Based on the above aspects, an empirically designed combination of OPT and PXL was evaluated in a phase I clinical trial at The Ohio State University Comprehensive Cancer Center.

When drugs are used in combination, the possibility of drug-drug interaction at the pharmacokinetic level and/or the pharmacodynamic level exists. In the phase I clinical study, we have found that the terminal elimination rate constant (β) and the total body clearance (CL) of plasma platinum increased when OPT was administered as a 2-h i.v. infusion followed by a 1-h i.v. infusion of PXL, as compared to those when OPT was given alone in a separate phase II clinical trial [13]. This comparison was based on different patient populations and the interaction may not have been assessed under optimal conditions. For example, in the clinical combination study, both investigated agents were given by a sequential short-term infusion. Both OPT and PXL plasma concentrations decayed simultaneously according to their own pharmacokinetic properties. Under this situation, one agent would not experience the full presence of the other.

To circumvent these problems, an animal model was adopted and several experimental designs were used. First, the pharmacokinetics of OPT in the presence of a steady-state plasma level of PXL was determined and compared to those obtained from OPT alone. Since Cremophor EL (CrEL), the formulation vehicle of PXL has been reported to alter the distribution and disposition of many drugs [7, 11, 24, 25], the effect of CrEL on the pharmacokinetic behavior of OPT was examined in the rat. Secondly, another group of rats was used to simulate the exact clinical dosing regimen, which was a 2-h i.v. infusion of OPT followed by a 1-h i.v. infusion of formulated PXL (fPXL). When compared to the corresponding control group, namely a 2-h i.v. infusion of OPT followed by a 1-h i.v. infusion of dextrose 5% in water (D5W), these results allowed the assessment of the sequence-dependency of the drug-drug interaction between OPT and PXL. In addition to the in vivo studies, the in vitro plasma protein binding and red blood cell (RBC) uptake of OPT in the absence and presence of PXL or CrEL were determined to aid the assessment of the mechanism of the pharmacokinetic interaction.

Material and methods

Drugs and chemicals

OPT, as a white crystalline powder, was a generous gift from Desynth (Buenos Aires, Argentina). PXL injection solution (Taxol, 6 mg/ml in 50% CrEL and 50% ethanol; Bristol-Myers Squibb, Princeton, N.J.) was purchased from The Ohio State University Hospital Pharmacy. CrEL was purchased from Sigma (St Louis, Mo.). Upon drug administration, an appropriate amount of each drug was diluted with D5W. All of these agents were used without further purification.

Animal protocols

A total of 30 male Sprague-Dawley rats were used in the study. The average body weight was 280 ± 29 g (mean $\pm$ SD). The animal protocols were written according to the Institutional Laboratory Animal Care and Use Committee (ILACUC) of the Ohio State University Laboratory Animal Resources (ULAR), and in accordance with the "Principles of Laboratory Animal Care" of the NIH. All rats received both jugular vein and tail vein cannulation. The rats were anesthetized with ketamine (Bedford Laboratory, Bedford, Ohio) at a dose of 100 mg/kg body weight. The right jugular vein of each rat was catheterized with a PE-50 cannula [1]. The fur around the ventral right side of the neck was shaved and the skin was sterilized with 70% alcohol. A 2-cm incision was made, and the neck muscle was gently separated to expose the right jugular vein. A small nick was made, and a 15-cm long beveled piece of PE-50 tubing (ID 0.58 mm, OD 0.965 mm; Clay Adams brand, Becton Dickinson, Sparks, Md.) was inserted and pushed into the right atrium. The muscle and skin were then closed with stitches and the cannula was exteriorized under the skin at the back of the neck. The blood flow was examined and the tubing was filled with heparinized (25 IU/ml) D5W.

A second catheter was inserted into a lateral tail vein. The tail vein was dilated by applying a tourniquet around the base of the tail. The vein was cannulated by first piercing it with a 20G needle (Angiocath; Becton Dickinson) and a PE-10 tube (ID 0.28 mm, OD 0.61 mm, Clay Adams brand; Becton Dickinson) was inserted into the barrel of the needle. The needle was then withdrawn, leaving the catheter in the vein. The catheter was filled with heparinized D5W solution prior to insertion. A small volume of D5W solution was injected and withdrawn to examine the success of the i.v. cannulation. To anchor the catheter, a piece of adhesive tape was wrapped around the catheter and the tail. Then a flexible sheath was wrapped around the tail to protect the insertion point

and a 30-cm section of compression spring was threaded over the catheter and taped to the sheath to prevent the rat from chewing the catheter. The rat was then put into a metabolic cage with access to food and water ad libitum. The spring and the catheter were passed though the top of the cage. The catheter was then connected to a swivel (Harvard Apparatus, Holliston, Mass.), which was also connected to an infusion pump (model KDS200, Fisher, Pittsburgh, Pa.) and the spring was fixed to a cross rod on the top of the cage to prevent the catheter from pulling back into the cage and also to allow the animal 360° mobility within the cage. The jugular vein catheter was used for blood sampling, and the tail vein catheter was used for drug infusion.

Dosing regimen

Thirty rats were divided into two groups of 18 and 12 rats. The first group was further divided into three subgroups of six each. These subgroups of rats were designated as combination 1 (CIfPXL→OPT), control 1 (CID5W→OPT), and CrEL control (CICrEL→OPT). In the CIfPXL→OPT group, each rat was given 16 mg/kg of fPXL as an 8-h i.v. infusion via the tail vein cannula at the rate of 1 ml/min. At the end of the 4th hour of fPXL infusion, the achievement of a quasi-steady-state level of PXL was expected. An i.v. bolus dose of OPT at 12 mg/kg (equivalent to 130 mg/m^2) was then given via the jugular vein cannula, while the infusion was continued for an additional 4 h. In the control CID5W→OPT group, D5W was infused to each rat at 1 ml/min instead of fPXL for 8 h. In the control CICrEL→OPT group, the amount of CrEL equivalent to that present in the fPXL formulation was estimated and was infused with D5W into each rat at 1 ml/min (1.3 ml/kg) for 8 h. At the end of the 4th hour of infusion, the rats were given an i.v. bolus injection of OPT as in the combination group. The doses of OPT and PXL were chosen based on the maximum tolerated dose (MTD), or in the clinically relevant dose range when they were used clinically as the single drug [8, 20]. To avoid the complication due to potential circadian pharmacokinetics of OPT [3], the drug was given to the rats at the same time ($\pm$0.5 h) on each day of the experiment.

The second group of 12 rats was divided into two subgroups, with six per subgroup, denoted as combination 2 or OPT→fPXL and control 2 or OPT→D5W. In the OPT→fPXL group, the rats were given OPT at 7 mg/kg (equivalent to 75 mg/m^2) as a 2-h i.v. infusion followed by a 1-h i.v. infusion of fPXL (80 mg/m^2), the exact schedule that was used in the clinical combination study. The dose of OPT was slightly lower than that used in the CIfPXL→OPT group, in order to mimic that which was used in the clinical protocol. The pharmacokinetics of OPT were found to be linear between 75 and 130 mg/m^2. The OPT→D5W group was given 7 mg/kg of OPT as a 2-h i.v. infusion followed by a 1-h i.v. infusion of D5W. The animal grouping designation is summarized in Table 1.

Table 1 Summary of animal study designations (see text for dose details)

Group designation	Dosing sequence	Function	No. of rats
CIfPXL→OPT	Formulated PXL i.v. infusion + OPT i.v. bolus	Combination 1	6
CICrEL→OPT	CrEL i.v. infusion + OPT i.v. bolus	Formulation control	6
CID5W→OPT	D5W i.v. infusion + OPT i.v. bolus	D5W control 1	6
OPT→fPXL	OPT 2-h i.v. infusion + fPXL 1-h i.v. infusion	Combination 2	6
OPT→D5W	OPT 2-h i.v. infusion + D5W 1-h i.v. infusion	D5W control 2	6

Blood, urine and tissue sampling

Serial blood samples (250 μl each) were drawn from the rats via the jugular vein cannula according to a predetermined schedule. Heparinized (25 IU/ml) D5W at a volume equal to the drawn blood volume was injected into the rats to replace the lost blood. Plasma was obtained from each blood sample by centrifugation at 2000 *g* and 4°C for 5 min. After separation, the RBCs were washed with 1 ml cold D5W immediately, and collected after centrifugation. The plasma (50 μl) was quickly mixed with 250 μl of blank rat plasma and immediately centrifuged in an Amicon Centrifree micropartition system (30,000 Da cutoff; Millipore Corporation, Bedford, Mass.) at 1800 *g* and 4°C for 10 min to obtain plasma ultrafiltrate (PUF). The purpose of adding blank plasma was to fulfill the minimum volume requirement of the micropartition device. The time frame of the dilution did not alter the protein-binding status, as the process of protein binding of OPT is rather slow. The rest of the plasma was collected into a microcentrifuge tube. Urine samples were collected from each rat over a 24-h period after OPT dosing. At the end of the blood sampling, the rats were killed. Liver, kidneys, heart and lungs were dissected from each rat, and rinsed with cold sodium phosphate buffer (PBS). The plasma, PUF, RBCs, urine and tissue samples were stored at –80°C until analysis.

Platinum assay

The total platinum in each biological sample was analyzed by inductively coupled plasma mass spectrometry (ICPMS). A Perkin-Elmer ICPMS Elan 6000 (Perkin-Elmer, Norwalk, Ct.) was used in the sample analysis. The total platinum in rat plasma, PUF and urine was measured after the direct dilution of the samples with deionized water. Briefly, 20 μl plasma, 50 μl PUF and 25 μl urine were diluted with deionized water to 5 ml, 3 ml and 10 ml, respectively. RBCs and tissues were digested with concentrated HNO_3 and then reconstituted with 2% HNO_3 before ICPMS analysis. Iridium was used as the internal standard. Briefly, 100 ng of iridium was added to 25 μl RBCs or 0.1 g tissue sample. Then 0.5 ml of optically pure HNO_3 (Fisher, Pittsburgh, Pa.) was added to each sample. The samples were then heated at 90°C overnight. The residue was redissolved in 5 ml 2% HNO_3. If the platinum level was found to be too high in the analysis, the samples were further diluted for repeat analysis. The calibration curves of OPT were constructed using the corresponding blank matrix. The within-day and between-day validations were performed at three different concentrations with six replicates at each concentration level.

PXL analysis by HPLC

Plasma concentrations of PXL in the combination CIfPXL→OPT group were determined to ascertain the attainment of the quasi-steady-state level at the time when OPT was injected. The HPLC-UV method adapted from that of Schiller et al. [21] with modification was used. Briefly, to 25 μl rat plasma was added 250 ng 2′-benzyl-2,2-dimethylpropionanilide (BDPA; Aldrich, Milwaukee, Wis.) as the internal standard. After a brief mixing by vortex, 500 μl acetonitrile was added to precipitate the plasma proteins. The protein precipitates were separated by centrifugation at 2000 *g* for 5 min at 4°C. The supernatant was collected and dried under a stream of nitrogen. The residue was reconstituted in 100 μl of HPLC mobile phase. A 50-μl aliquot of the resultant solution was used for HPLC analysis. Serial standard solutions of PXL in rat plasma ranging from 1 to 10 μg/ml were prepared as the calibration curve.

A Shimadzu LC-10AT VP pump, coupled with a Shimadzu SIL-10AD VP autosampler and a Shimadzu SCL-10A VP system controller, was used for the analysis. The compounds were separated on a Beckman Ultrasphere ODS column (4.6×150 mm, 5 μm) coupled with a precolumn (Beckman Ultrasphere, ODS, 5 μm, 4.6 (ID)×7.5 mm). The mobile phase consisted of acetonitrile and 35 m*M* acetic acid in the volume ratio of 46:54, and the flow rate was 1 ml/min with a run time of 20 min. After each run, a washing

step was added to eliminate interference before the next injection. The washing step consisted of a 2-min gradient run from 100% of the mobile phase to 90% acetonitrile, which was maintained for 6 min, followed by a 2-min gradient return to the mobile phase. A 6-min equilibration period with the mobile phase was required prior to the next run. A Spectroflow 757 (ABI Analytical, Krabs Division) UV detector was used for detection, and the detection wavelength was 230 nm. The chromatogram was recorded and integrated by a Shimadzu C-R5A Chromatopac recorder.

Pharmacokinetic and statistical analysis

The concentration-time data were analyzed by WinNonlin (version 3.0; Pharsight Corporation, Mountain View, Calif.) to obtain the pharmacokinetic model and parameters of OPT. The mean value and standard deviation of each parameter in each study group were calculated using Microsoft Excel. Data from each group were compared by the unpaired Student's *t*-test, except in the percentage of urinary elimination, which was tested by a non-parametric test.

Stability studies

The stability of OPT was examined at 2.5 μg/ml in water in the absence and presence of PXL for 24 h. According to a predetermined time schedule, an aliquot of each solution was taken and carboplatin was added as the internal standard. The solution was immediately extracted with ethyl acetate to remove PXL. A 5-μl aliquot of the aqueous phase was analyzed immediately for detection of unchanged OPT on a Finnigan LCQ LC/MS system (Thermoquest, San Jose, Calif.).

In vitro plasma protein binding

The plasma protein binding study of OPT was carried out in rat plasma alone and in the presence of PXL or CrEL by an ultrafiltration method. OPT at 10 μg/ml was used in all of the protein binding experiments. For OPT alone, OPT was incubated in rat plasma at 37°C for different times. For the combination of OPT with PXL, PXL at 5 μg/ml was added separately to the rat plasma 1 h before, simultaneously, and 1 h after OPT exposure. For the combination of OPT with CrEL, CrEL at 1 μl/ml was added separately to the rat plasma in the same manner as before. A 400-μl aliquot was removed from each drug-containing plasma sample 10, 20 and 40 min, and 1, 2, 4, 6, 8 and 12 h after OPT exposure, and processed though an Amicon Centrifree micropartition device (Millipore Corporation, Bedford, Mass.) to obtain the PUF. Another 100-μl aliquot of each plasma sample was collected for the assay of total plasma platinum. The platinum levels in the PUF and the total plasma were measured by ICPMS after an appropriate dilution by deionized water. The percentage of unbound drug at each time-point was obtained by dividing the platinum concentrations in the PUF by those in the corresponding plasma samples.

In vitro RBC uptake of OPT

RBC uptake of OPT was carried out in freshly prepared rat RBCs alone and in the presence of PXL or CrEL. Rat RBCs were freshly collected from a Sprague-Dawley rat and washed with D5W to remove plasma proteins. The RBCs were then resuspended in an equal volume of PBS. OPT at 10 μg/ml was used in all of the study. For the control study, OPT was incubated with the freshly prepared rat RBCs alone for different times. For the combination of OPT and PXL, PXL at 5 μg/ml was added separately to the fresh rat RBCs 1 h before, simultaneously, and 1 h after OPT exposure. For the combination of OPT and CrEL, CrEL at 1 μl/ml was added to the fresh rat RBCs in the same manner as before. A 100-μl aliquot of the RBC suspension was removed from each sample 5, 10, 20, 40, 60, 90, 120, 240 and 360 min following OPT exposure, and centrifuged at 1000 *g* for 1 min. The supernatant was collected for determination of the platinum concentration in the incubation medium. The RBCs were washed with cold PBS twice and then collected for the determination of platinum levels. Briefly, 0.2 ml of optically pure HNO_3 and 100 ng iridium were added to each RBC pellet. The mixture was heated at 90°C overnight. The residue was resuspended in 5 ml 2% HNO_3, and analyzed by ICPMS.

Results

Pharmacokinetics of OPT alone following an i.v. bolus injection and in the presence of steady-state level of fPXL or CrEL

In rats receiving 12 mg/kg of OPT alone (CID5W→OPT group), the concentration-time profiles of total platinum showed triexponential declines in both plasma and PUF (Fig. 1). Thus, the concentration-time data of the six rats were fitted to a thee-compartment model. The major pharmacokinetic parameters as estimated by WinNonlin software are summarized in Table 2. As shown, the mean initial half-lives ($t_{1/2\alpha}$) were 4.4 min and 4.1 min, the intermediate half-lives ($t_{1/2\beta}$) were 1.62 h and 0.76 h, and the terminal half-lives ($t_{1/2\gamma}$) were 26.7 h and 18.6 h for plasma platinum and PUF platinum, respectively. The

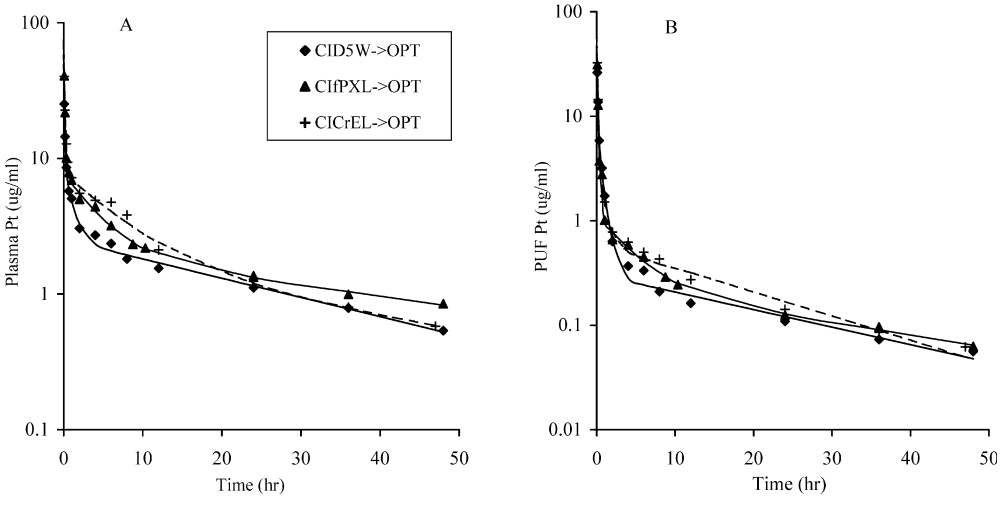

Fig. 1A, B Representative concentration-time profiles of total platinum in rat plasma (**A**) and plasma ultrafiltrate (**B**) with 12 mg/kg of OPT given alone as an i.v. bolus injection, and in the presence of fPXL and CrEL

area under the concentration-time curve ($AUC_{0-\infty}$) of total plasma platinum was 49.8 μg·h/ml, 14.8% of which was in the PUF. The total body clearance (CL) values of platinum in plasma and in PUF were 2.05 ml/min per kg and 13.5 ml/min per kg, respectively. The steady-state volumes of distribution (V_{ss}) of total platinum in plasma and in PUF were 1.27 l and 3.78 l, respectively. The platinum levels in the RBCs of the rats receiving 12 mg/kg of OPT reached a plateau of 30 μg/ml in 10 min, and this level remained unchanged during the rest of sampling time up to 48 h (Fig. 2).

The 24-h urinary elimination of total platinum was 33.6%. A significant amount of platinum was detected in the selected tissues 48 h after OPT administration, with the highest platinum levels in the kidneys, followed by liver, lungs, muscles and heart (Table 3). In the combination CIfPXL→OPT group, 4 h into the fPXL infusion, PXL levels reached a mean quasi-steady-state plasma level (C_{ss}) of 4.6 μg/ml as analyzed by HPLC. In the presence of a steady-state level of fPXL, the total plasma platinum and PUF platinum concentration-time profiles also showed triexponential decays (Fig. 1). The computed pharmacokinetic parameters of plasma platinum and free platinum are summarized in Table 2. Compared to those of the control CID5W→OPT group, platinum concentrations 5 min after OPT injection (C_{5min}, measured maximum drug concentration), C_{max} (curve-fitted maximum concentration) and $AUC_{0-\infty}$ of total plasma platinum increased, while CL of total plasma platinum decreased ($P < 0.01$). PUF platinum showed a similar increase in C_{5min} and $AUC_{0-\infty}$, and a decrease in CL ($P < 0.01$); however, the increase in C_{max} was not statistically significant.

There was a slight decrease in V_{ss}, in the combination CIfPXL→OPT group, but the difference was not significantly different. No significant difference in the elimination $t_{1/2}$ values both in total plasma platinum and in free platinum between these two groups was detected. The platinum levels in the RBCs at the individual time-points were not significantly different from those of the CID5W→OPT group due to the large variation in the data (Fig. 2), but the AUC_{0-48h} of the RBC platinum was lower than that of the CID5W→OPT group ($P < 0.05$). The platinum levels in different tissues at 48 h showed no difference between the combination CIfPXL→OPT group and the control CID5W→OPT group (Table 3), both given i.v. bolus OPT at 12 mg/kg. Since the other control group (OPT→D5W) was given a lower dose of OPT (7 mg/kg) than the CID5W→OPT group, the tissue platinum levels of these two control groups were approximately proportional to the dose.

Since the clinically used formulation of PXL contains a pharmacologically active vehicle CrEL [24, 25], the changes in pharmacokinetic parameters of platinum generated from OPT may be caused by CrEL. To investigate this possibility, another control experiment with CrEL infusion in combination with OPT was conducted. Representative concentration-time profiles

Table 2 Pharmacokinetic parameters of plasma platinum and PUF platinum when 12 mg/kg of OPT was given as an i.v. bolus injection in the absence and presence of steady state levels of fPXL or CrEL. Data are presented as mean ± SD (n = 6)

Parameter	CID5W→OPT (control 1)		CIfPXL→OPT (combination 1)		CICrEL→OPT (CrEL control)	
	Plasma Pt	PUF Pt	Plasma Pt	PUF Pt	Plasma Pt	PUF Pt
α (1/h)	9.55 ± 4.92	9.43 ± 0.67	10.55 ± 2.75	10.20 ± 1.54	10.20 ± 1.54	10.81 ± 1.95
β (1/h)	0.91 ± 0.69	0.21 ± 0.10	1.34 ± 1.20	0.48 ± 0.64	1.25 ± 0.48	0.25 ± 0.09
γ (1/h)	0.037 ± 0.012	0.026 ± 0.009	0.036 ± 0.012	0.024 ± 0.01	0.044 ± 0.005	0.024 ± 0.006
C_{max} (μg/ml)	24.45 ± 4.77	24.10 ± 10.90	40.74 ± 9.24*	36.54 ± 14.24	46.68 ± 11.33*	42.56 ± 10.79**
C_{5min} (μg/ml)	12.70 ± 1.65	10.91 ± 1.52	20.07 ± 4.11*	16.97 ± 4.11*	21.28 ± 2.36*	19.44 ± 4.56*
$AUC_{0-\infty}$ (μg·h/ml)	49.85 ± 12.10	7.40 ± 1.12	70.12 ± 8.57*	10.14 ± 1.56*	79.28 ± 14.02*	10.92 ± 1.95*
$t_{1/2\alpha}$ (h)[a]	0.074 (0.066–0.080)	0.069 (0.036–0.102)	0.069 (0.054–0.075)	0.066 (0.046–0.081)	0.064 (0.053–0.097)	0.065 (0.053–0.075)
$t_{1/2\beta}$ (h)[a]	1.62 (0.72–6.01)	0.762 (0.43–4.57)	1.44 (0.36–4.18)	0.531 (0.18–2.74)	2.78 (1.98–5.22)	0.553 (0.35–1.465)
$t_{1/2\gamma}$ (h)[a]	26.73 [19.55–48.67]	18.58 [13.91–38.34]	29.06 [19.32–60.63]	19.14 [14.47–45.01]	28.29 [20.52–56.06]	15.91 [13.04–18.24]
V_{ss} (l)	1.27 ± 0.42	3.78 ± 2.53	0.962 ± 0.26	3.04 ± 1.78	0.739 ± 0.213**	1.84 ± 0.31*
CL (ml/min/kg)	2.05 ± 0.38	13.48 ± 1.85	1.38 ± 0.15*	10.15 ± 1.55*	1.42 ± 0.43**	9.08 ± 1.70*
24-h urinary excretion (%)	33.61 ± 6.42		38.92 ± 9.64		51.92 ± 5.24*	

* $P < 0.01$, ** $P < 0.05$, vs control CID5W→OPT group
[a]Harmonic mean values with ranges

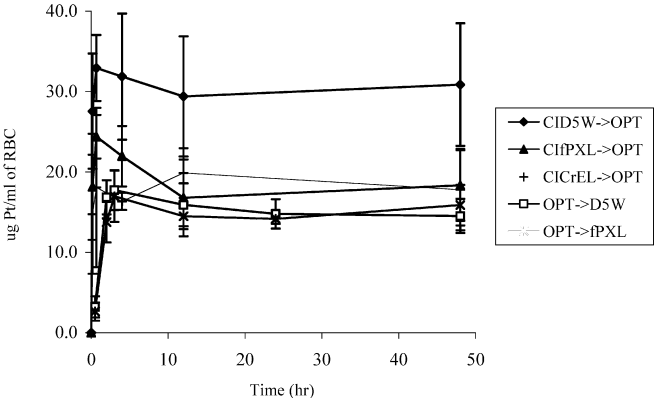

Fig. 2 Platinum levels in rat RBCs after the rats received OPT alone and in combination with fPXL and CrEL. Data are presented as means ± SD ($n = 6$). OPT was administered at 12 mg/kg (*solid diamonds*, *solid triangles*, *crosses*) and 7 mg/kg (*open squares*, *asterisks*)

of total platinum in rat plasma and PUF also showed triexponential decays in the presence of CrEL (Fig. 1). The pharmacokinetic parameters are also shown in Table 1. As shown, infusion of CrEL resulted in a similar increase in C_{5min}, C_{max} and $AUC_{0-\infty}$, and decrease in CL to those obtained with fPXL infusion, in both total plasma and free platinum. There was a significant decrease in V_{ss} of total plasma platinum ($P < 0.05$) and an increase in 24-h urinary excretion of total platinum ($P < 0.01$) in the CrEL control CICrEL→OPT group, when compared to the control CID5W→OPT group. The platinum levels in the rat RBCs were similar to those of the combination CIfPXL→OPT group, and also lower than those of the control CID5W→OPT group (Fig. 2). The tissue distribution of platinum was not significantly different from those of the CID5W→OPT group and the CIfPXL→OPT group (Table 3). Therefore, the alteration in OPT pharmacokinetics in the combination with fPXL was also seen with CrEL, suggesting that the effect may have been due to CrEL itself.

Pharmacokinetics of OPT with a 2-h i.v. infusion alone and followed by a 1-h i.v. infusion of fPXL

In the control OPT→D5W group (control 2), the rats were given OPT alone at 7 mg/kg as a 2-h i.v. infusion. The post-infusion concentration-time profiles of total plasma platinum and PUF platinum showed triexponential decays, similar to that in the clinical situation (Fig. 3). The platinum levels in the RBCs increased, while the OPT infusion was continued, and reached a plateau at the end of OPT infusion (Fig. 2). The plateau levels of platinum in RBCs and in the selected tissues in this control group were lower than those of the control CID5W→OPT group (Table 3), as expected with a lower dose. In the combination OPT→fPXL group, the concentration-time profiles of platinum also declined triexponentially. Comparison of the pharmacokinetic parameters of the two groups showed that there was a significant increase in the terminal decay rate constant of total plasma platinum ($P < 0.05$) and in V_{ss} of free platinum in the combination OPT→fPXL group ($P < 0.05$; Table 4). The data also showed a decrease in C_{max} and C_{end} of free platinum in the combination OPT→fPXL group, which cannot be explained at this time. No significant difference in 24-h urinary elimination was detected (Table 4), nor in RBC platinum (Fig. 2). It appeared that fPXL administered after OPT increased the platinum levels in the rat liver, heart, kidneys ($P < 0.01$) and lungs ($P < 0.05$; Table 3), which may have contributed to the increased V_{ss} of free platinum, although the increase in the first two tissues was not statistically significant.

Stability of OPT in aqueous buffer

The stability of OPT aqueous buffer solution alone and in the presence of PXL at 37°C was monitored by electrospray LC/MS. The result showed that intact OPT was stable for over 24 h and its stability was not influenced by the presence of PXL.

In vitro plasma protein binding of OPT

When incubated in rat plasma, OPT gradually bound to plasma protein with a half-maximal binding time of about 80 min (Fig. 4). The binding had reached a plateau by 6 h following OPT incubation, similar to data reported previously [16]. The extent of OPT bound to rat plasma protein was about 90% and was not dependent on OPT concentration (data not shown). In the presence of either PXL or CrEL, no significant change in the rate or extent of OPT plasma protein binding was observed.

Table 3 Platinum levels in different tissues of rats treated with OPT. The data are means ± SD ($n = 6$) in units of micrograms platinum per gram tissue

	Liver	Kidney	Lung	Heart	Muscle
CID5W→OPT	4.63 ± 0.71	9.35 ± 0.99	2.31 ± 0.43	0.87 ± 0.04	3.13 ± 0.52
CIfPXL→OPT	4.74 ± 0.43	9.21 ± 0.78	2.67 ± 0.64	1.14 ± 0.46	
CICrEL→OPT	3.49 ± 0.30	8.00 ± 0.54	2.17 ± 0.33	0.91 ± 0.35	
OPT→D5 W	1.85 ± 0.26	4.48 ± 0.44	1.15 ± 0.13	0.53 ± 0.10	
OPT→fPXL	2.11 ± 0.48	7.19 ± 0.54*	1.96 ± 0.48**	0.60 ± 0.10	

*$P < 0.01$, **$P < 0.05$, vs the corresponding control group

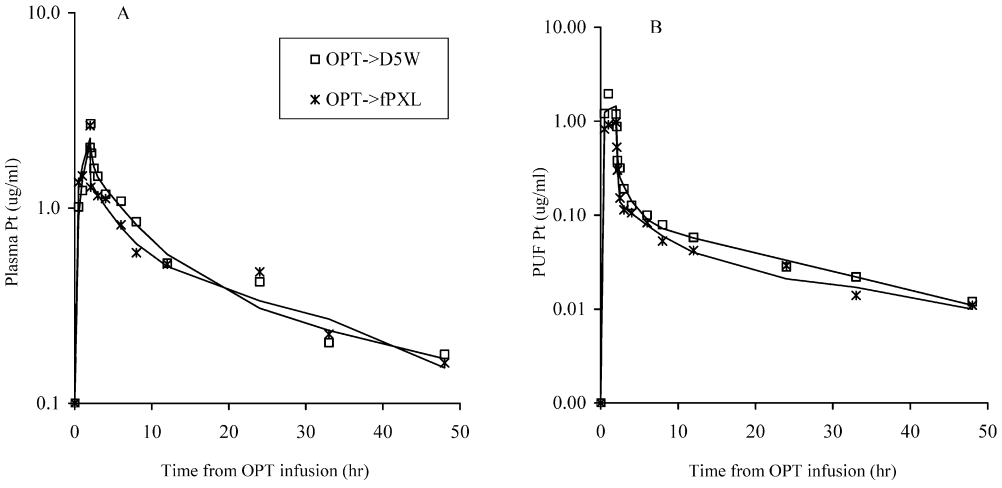

Fig. 3A, B Representative concentration-time profiles of total platinum in rat plasma (**A**) and PUF (**B**) when OPT was given alone as a 2-h i.v. infusion at 7 mg/kg and followed by 1 h of fPXL

Table 4 Pharmacokinetic parameters of plasma platinum and PUF platinum when 7 mg/kg of OPT was given as an 2-h i.v. infusion followed by a 1-h i.v. infusion of fPXL or D5W. The data are means ± SD ($n = 6$)

Parameter	OPT→D5W		OPT→fPXL	
	Plasma Pt	PUF Pt	Plasma Pt	PUF Pt
α (1/h)	9.23 ± 6.34	9.42 ± 4.32	11.49 ± 4.30	13.10 ± 5.65
β (1/h)	0.353 ± 0.397	0.67 ± 0.54	0.35 ± 0.29	0.78 ± 0.63
γ (1/h)	0.023 ± 0.006	0.049 ± 0.012	0.032 ± 0.01*	0.038 ± 0.028
C_{max}(μg/ml)	2.40 ± 0.47	1.37 ± 0.14	2.52 ± 0.33	0.90 ± 0.14*
C_{end} (μg/ml)	2.76 ± 0.76	1.32 ± 0.10	2.63 ± 0.13	0.87 ± 0.16*
$AUC_{0-\infty}$ (μg·h/ml)	38.84 ± 7.61	5.86 ± 1.94	36.20 ± 6.72	4.96 ± 1.71
CL (ml/min/kg)	3.08 ± 0.61	21.22 ± 4.99	3.34 ± 0.58	25.58 ± 7.55
V_{ss} (l)	1.94 ± 0.50	3.33 ± 1.39	1.59 ± 0.41	7.98 ± 3.57*
24-h urinary excretion (%)		45.42 ± 6.93		53.10 ± 2.47

*$P < 0.05$ vs the corresponding parameter in the OPT→D5W group

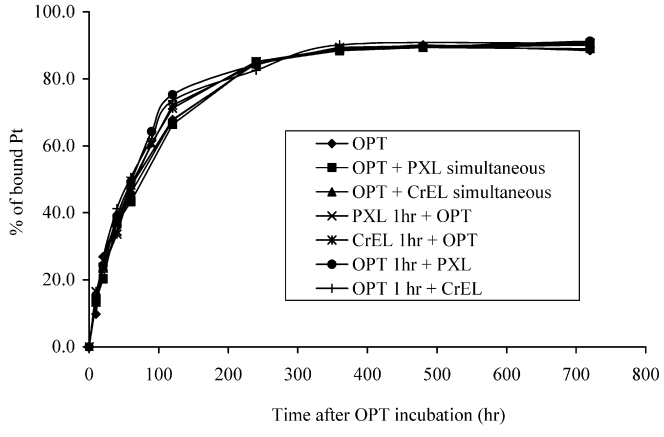

Fig. 4 In vitro protein binding of OPT in rat plasma in the absence and presence of PXL or CrEL. The data presented as the mean values of duplicate determinations

In vitro RBC uptake of OPT in the absence and presence of PXL or CrEL

In vitro, OPT alone was rapidly taken up by RBCs, with a half-maximal time of 50 min, and reached a plateau by 2 h of OPT incubation (Fig. 5). Preincubation of RBCs with PXL for 1 h slightly decreased the rate and extent of RBC uptake of platinum (about 10%). However, with the preincubation of RBCs with CrEL, the rate and extent of OPT uptake by RBCs were significantly decreased. The extent of the RBC uptake of platinum was only about 20% of that of OPT alone.

Discussion

It has been reported that OPT is irreversibly bound to rat plasma proteins and taken up by RBCs both in vitro [16] and in vivo [14]. Our results are consistent with the reported data. In both control groups, where OPT was given alone, approximately 85% of total plasma platinum was bound to the plasma proteins, as evidenced by comparing the $AUC_{0-\infty}$ of PUF platinum to that of total plasma platinum. The total plasma platinum also showed higher values of $t_{1/2\beta}$, $t_{1/2\gamma}$ and AUC, and lower CL than free platinum. This indicates that total plasma platinum was eliminated much more slowly than free platinum. The V_{ss} of total plasma platinum was found to be lower than that of free platinum, suggesting that the

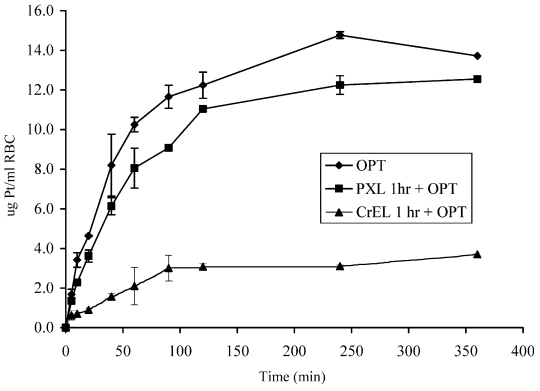

Fig. 5 In vitro RBC uptake of OPT in the absence and presence of PXL and CrEL The data are presented as means ± SD ($n = 3$) (see text for details)

extensive plasma protein binding of OPT reduced the distribution of platinum. Free platinum showed a larger value of V_{ss} (3.78 l) probably due to the extensive tissue distribution of free platinum. The tissue platinum levels were highest in the kidneys, followed by the liver, muscles, lungs and heart, in the same rank order as reported in mice after an i.v. injection of 7 mg/kg of OPT [3]. Once achieved, the plateau platinum levels in the rat RBCs remained unchanged during the 48-h period of study, which confirmed the irreversibility of RBC uptake of OPT.

To investigate the potential pharmacokinetic interaction, a rat model was used with an initial design of maintaining fPXL at steady-state plasma levels to reduce one of the variables, while studying the pharmacokinetics of OPT. In the presence of a steady-state level of fPXL, C_{end}, C_{max}, and AUC of total plasma platinum and of free plasma platinum were found to increase, while CL decreased, when compared to those of OPT alone. Similar results were found in the CrEL control group. This result suggests that the alteration in pharmacokinetics of OPT by fPXL in the rat might be due to the formulation vehicle of PXL, CrEL alone. In contrast to the decrease in CL of total platinum, an increase in 24-h urinary elimination was observed in the CrEL control group. The opposing results in CL and urinary elimination suggests a possible decrease in other elimination pathways of platinum from plasma.

The above pharmacokinetic alteration by fPXL in the rat differed from recent data on pharmacokinetic interaction between fPXL and OPT observed clinically [13]. It was found that CL increased with a decrease in the terminal half-life of plasma platinum, when OPT was given first as a 2-h i.v. infusion followed by a 1-h i.v. infusion of PXL in patients. The reason for this opposite trend of pharmacokinetic interaction could be due to either the different dosing sequence of the two agents, or the species difference between the two studies. In order to assess the potential sequence dependency of the pharmacokinetic interaction, a second group of rats was used to simulate exactly the same dosing regimen that was used in the patients (combination 2, OPT→fPXL). Interestingly, in this group, an increase in CL of total plasma platinum was found in the rat, similar to the result of the clinical combination study. There was also an increase in V_{ss} when OPT was given before fPXL, although the magnitude of the changes was smaller than those in the clinical studies. The result from both combination studies suggests that the alteration in the pharmacokinetics of OPT by fPXL may be dosing sequence-dependent.

In order to sort out the possible mechanism of the pharmacokinetic interaction, in vitro drug stability, plasma protein binding and RBC uptake were investigated. Clinically achievable concentrations of OPT, PXL, and CrEL were chosen for the in vitro studies. The in vitro study showed that PXL and CrEL had little influence on the stability and plasma protein binding of OPT. Therefore, the alteration in the pharmacokinetics of OPT by fPXL and CrEL was not due to the change in plasma protein binding. This was further confirmed by the similar unchanged free fractions of platinum in rat plasma in the combination groups. However, there was a significant decrease in the rate and extent of RBC uptake of OPT in vitro when fresh rat RBCs were preincubated with CrEL. Free platinum may be cleared from rat plasma by several pathways, such as urinary elimination, irreversible plasma protein binding, RBC uptake, and tissue binding. The decrease in RBC uptake may partially explain the increase in plasma concentration and AUC, as well as decreases in CL and V_{ss} of total and free plasma, when fPXL and CrEL were given prior to OPT. The mechanism by which CrEL decreases the RBC uptake when CrEL is given prior to OPT might be due to either (1) the entrapment of OPT into micelles [12, 23], or (2) alteration in cell membrane permeability by an increase cell membrane fluidity [4, 22, 26].

The increase in AUC and decrease in CL of total platinum when fPXL and CrEL preceded OPT may also have been caused by a decrease in tissue binding. This hypothesis was partially reflected by the decrease in V_{ss} of total platinum in both the combination groups (CIfPXL→OPT and OPT→fPXL) and the CICrEL→OPT group. However, there was no change in platinum concentrations in several small organ tissues examined between the CIfPXL→OPT group and its control group (CID5W→OPT), or between the CrEL control group (CICrEL→OPT) and its control group (CID5W→OPT). Unfortunately, the more massive tissues, such as skeletal muscles, skin, and bones, were not assayed for platinum content; therefore, a thorough examination of the changes in tissue distribution of platinum could not be made.

When OPT was given prior to fPXL and CrEL, there was a small increase in the terminal elimination constant of total plasma platinum and V_{ss} of free platinum in the combination OPT→fPXL group relative to the control OPT→D5W group, although the corresponding AUC values did not show changes. The increase in V_{ss} may have been due to the prevention of redistribution of free

platinum in the tissue space to the blood stream by fPXL. As mentioned above, the incomplete organ tissue measurement did not permit direct confirmation by organ tissue distribution values. The RBC platinum levels were also similar between these two subgroups. The latter is reasonable since the majority of RBC binding of OPT took place before fPXL was infused. There was no difference in the 24-h urinary elimination between the control OPT→D5W and combination OPT→fPXL groups.

In rats given the same dosing schedule as the phase I clinical trial, a similar trend of alteration in the pharmacokinetic parameters to that in humans was observed, albeit to a lesser extent. Therefore, this indicates that the pharmacokinetic interaction is not species dependent.

In conclusion, the alteration in pharmacokinetics of OPT by fPXL was dosing sequence-dependent, and the effect was mainly due to the formulation vehicle of PXL, CrEL. The mechanism of the pharmacokinetic changes remains obscure, but may be in part due to alteration in RBC uptake and tissue distribution of OPT, when OPT is combined with fPXL. Based on the pharmacokinetic results, it would seem that the dosing sequence of fPXL followed by oxaliplatin would be more clinically favorable because this sequence will prolong the residence of OPT in the systemic circulation and decrease the loss of OPT due to RBC uptake and binding. However, other factors such as the pharmacodynamics need to be considered, as CrEL could decrease platinum-DNA adduct formation (data not shown), although this effect in vivo may be considerably slower. Perhaps, it would be best to avoid using CrEL in the formulation, by using alternate formulations of PXL such as nanoparticles or other effective congeners such as docetaxel.

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