

## Paclitaxel and Pegylated Liposomal Doxorubicin in Recurrent Head and Neck Cancer: Clinical and Unexpected Pharmacokinetic Interactions

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**Abstract.** *Background:* The combination of paclitaxel (PTX) with pegylated liposomal doxorubicin (PLD) is an interesting treatment for recurrent head and neck cancer. The pharmacokinetic behavior may depend on the interval between the intravenous administration of the two drugs. This study evaluates the clinical efficacy, toxicity and any possible interval-dependent pharmacokinetic interactions. *Patients and Methods:* Thirty patients were randomized to receive 80 mg/m<sup>2</sup> PTX weekly and 12.5 mg/m<sup>2</sup> PLD every two weeks at administration intervals of 0, 1, 3, 12 or 24 hours. Blood sampling was performed at day 1 and 15 and pharmacokinetics of PTX, PLD and Cremophor EL were evaluated by non-compartmental analysis. *Results:* Neutropenia was the most frequent side-effect (100% of patients; 30% grade 3-4). Hand-foot syndrome was severe in only 3% of patients. Overall response rate was 30%, with 3% complete responses and 27% partial responses. Stable disease and progression were 43% and 27%, respectively. Median response duration and overall median survival were 5.5 and 10 months respectively. Co-administration of PLD markedly reduced C<sub>max</sub> and the area under the curve (AUC), and increased PTX clearance. The differences in the PTX AUC and clearance between the 0 h and the 24 h experimental arms were statistically significant. *Conclusion:* The PTX/PLD combination plays a palliative role (clinical benefit in 73% of patients) and has good tolerability. The PTX pharmacokinetic profile was unexpectedly affected by different administration time intervals; in the 0 h arm the

AUC was reduced to one fourth, therefore a schedule with PTX on day one, PLD on day two may be preferred.

The prognosis of patients with recurrent or metastatic head and neck cancer (HNC) receiving conventional chemotherapeutic treatment is poor, with a 6-month median survival time in most studies conducted over the last 20 years. The combination of cisplatin with 5-fluorouracil emerged as a favorable treatment regimen in the 1980s and '90s; although it can induce response rates of 20% to 40%, it has little effect on overall survival in recurrent disease (1-2).

Treatment of recurrent cancer should also consider that many patients now receive cisplatin alone or in combination as first-line treatment for locally advanced disease. In view of the fact that palliation rather than cure is the mostly likely result of the therapeutic intervention in patients with recurrent head and neck cancer, new combinations should satisfy the goal of being active and easily tolerable.

Paclitaxel and docetaxel have shown significant activity against advanced HNC (3-4), whereas gemcitabine has only modest activity as a single agent (5). Paclitaxel (PTX) (Taxol; Bristol-Myers Squibb, Rome, Italy) is a very active drug; a weekly schedule may be both better tolerated and more efficacious than administration every 21 days (6).

A number of phase I and II trials (7-9) have shown that pegylated liposomal doxorubicin (PLD) (Caelyx; Schering-Plough, Milan, Italy) is highly active in chemotherapy-naive patients (overall response rate (ORR)=44-50%) and also in those pre-treated with chemotherapy (ORR=17-33%). Clinical studies with PLD (7-9) have reported low-grade 3-4 hematological, mucosal or cardiac toxicity and a particular pattern of mucositis and skin toxicity similar to that reported with protracted infusions of doxorubicin. The efficacy of PTX and PLD in locally advanced or recurrent HNC, as well as the complementary mechanisms of action and different toxicity profiles of the two drugs, make their combination an interesting regimen for trials, and the association has been

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explored in HNC as well as in other types of solid tumors (10-13). The pharmacokinetic (pk) behavior of both drugs could be influenced by their association, due to the excipient of PTX, polyethoxylated castor oil – Cremophor EL: the excipient could destabilize the liposomes of PLD, leading to the premature release both of doxorubicin from the vesicles and of phospholipidic components of PLD. For these reasons, it appears important not only to investigate the effect of the drug administration sequence, but also the timing interval between administration of the two drugs.

The pharmacokinetic interaction between PTX and PLD has been evaluated; in most of these studies the drug with the longer half-life (PLD) was administered first, followed by the drug with the shorter half-life (PTX). Briasoulis *et al.* (14-15) reported that administration of PLD followed by PTX, *versus* administration of PLD as a single agent, led to an increased area under the curve (AUC) and a decreased clearance of PLD; however, the study did not evaluate the pharmacokinetics of PTX. Campos *et al.* studied the effect of the sequence PLD/PTX (16) and showed that PLD does not alter the pharmacokinetics of PTX. Janinis *et al.* (17) examined the effects of inverting the sequence of administration, PLD/PTX or PTX/PLD, and showed that prior administration of PTX did not appear to affect the pharmacokinetics of PLD, whereas prior administration of PLD slightly increased the plasma concentration and AUC of PTX. However, all these studies are limited by the small series considered and the administration sequence PTX followed by PLD was poorly studied from a clinical and pharmacokinetic point of view.

The goal of the present study was to assay the clinical response and toxicity of the PTX/PLD association in HNC and evaluate the pharmacokinetics of PLD, PTX and Cremophor EL, when PLD administration is simultaneous or 24 h after the end of PTX infusion, in order to investigate the possible pharmacokinetic interactions when the drug with shorter half-life was administered first.

## Patients and Methods

**Patient population.** A non-randomized trial was conducted to investigate the feasibility, antitumor activity and pharmacokinetic data of a combination of PTX and PLD in 30 patients with recurrent, histologically confirmed and measurable loco-regional squamous cell carcinoma of the head and neck, not amenable to conventional surgical or radiotherapeutic management. Patients with measurable distant metastases were eligible. Prior radiation therapy was allowed but must have been completed at least 4 weeks before enrollment in the study. If the only site of measurable disease was within the radiation field, disease was required to be progressing in spite of radiation therapy. Patients who had received induction chemotherapy as part of their initial treatment, or synchronous chemotherapy with radiation therapy, were eligible as long as such therapy had been completed at least 6 months before enrollment. Prior palliative chemotherapy for recurrent disease was allowed but must have been completed at least 4 weeks before enrollment, and the measurable lesion should be in progression.

All patients were required to have an Eastern Cooperative Oncology Group (ECOG) PS of  $\leq 2$ , complete recovery from previous diagnostic and therapeutic procedures, life expectancy  $> 3$  months, and adequate enteral intake ( $\geq 2,000$  kcal/day). There was no age restriction. Patients were required to be free from detectable infection and have adequate hematological (WBC count  $> 4,000/\mu\text{l}$  or a normal absolute granulocyte count, platelet count  $> 100,000/\mu\text{l}$ , hemoglobin  $> 10$  g/dl), renal (creatinine  $< 1.5$  mg/dl), hepatic (total bilirubin  $< 1.8$  mg/dl; aspartate aminotransferase (AST)  $<$  twice normal), and neurological (peripheral neuropathy from previous therapy or unrelated disease  $<$  grade 2) functions. Patients with known brain or leptomeningeal metastases were excluded, as were those with previous or concurrent malignancy (except curatively treated basal or squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix), bone involvement only was allowed. All patients signed a written informed consent. The study was conducted in accordance with the Tokyo-Helsinki Declaration and Good Clinical Practices, and was approved by the local Ethics Committee.

**Pre-treatment and follow-up evaluations.** General assessment prior to treatment included medical history and physical examination, including a detailed neurological and hematological examination, blood chemistry (creatinine, urea, glucose, transaminases, gamma-glutamyl transferase (GGT), alkaline phosphatase, ionogram, bilirubin, protidogram and electrophoresis), urine, ECG and chest X-ray. Blood tests were weekly throughout the course of therapy. Tumor status was determined by computerized axial tomography. Tumor size was expressed as the product of the two major perpendicular diameters, or as the sum of the products in the case of multiple lesions. Neurological examination was repeated every two cycles or if the patient showed symptoms. Standard antiemetic treatment was given to all patients. Palmar-plantar erythrodysesthesia (PPE) was prevented by oral administration of corticosteroids (during the first week after infusion) and pyridoxine (100 mg/day).

**Treatment plan.** Treatment consisted of infusion of  $80 \text{ mg/m}^2$  PTX weekly over 1 hour, followed by  $12.5 \text{ mg/m}^2$  PLD dissolved in 250 ml 5% dextrose over 1 hour, every 2 weeks with standard premedication. Treatment was continued for 6 weeks followed by 2 weeks' rest. When a dose reduction was required, no dose re-escalation was allowed. The absolute neutrophil count (ANC) was required to be  $> 1,500/\mu\text{l}$  and the platelet count  $> 100,000/\mu\text{l}$  before any drug administration. If the ANC was between 1,000 and 1,500/ $\mu\text{l}$ , the drugs were administered with granulocyte colony-stimulating factor (G-CSF) to maintain dose intensity. If the ANC was  $< 1,000/\mu\text{l}$ , administration was postponed for 1 week and G-CSF treatment was initiated. A 25% dose reduction was applied when, despite the use of G-CSF, neutropenia and thrombocytopenia had been present  $> 7$  days or in the case of neutrophil count of  $500-1,000/\mu\text{l}$  and/or platelet count of  $50,000-99,000/\mu\text{l}$ . In cases of grade 3 peripheral neurotoxicity or any grade 4 toxicity, except alopecia, the patient was taken out of the study. Toxicity criteria were those adopted by the WHO (18).

For the pharmacokinetic study, 30 patients were randomized to receive the drug in one of the following administration intervals between drugs: 0 (9 patients), 1 (4 patients), 3 (5 patients), 12 (3 patients) or 24 hours (9 patients). Pk parameters were evaluated during the first cycle.

**Response evaluation.** Patients were evaluated at the end of each cycle and the response to therapy was recorded. Complete response (CR) was defined as the disappearance of all evidence of tumor, including

normalization of X-rays and biochemical tests for a minimum of 4 weeks. Partial response (PR) was defined as a 50% or greater decrease in the sum of the products of the greatest perpendicular diameters of all lesions for a minimum of 4 weeks without the appearance of any new lesions. Stable disease (SD) was defined as a measurable response less than that required for a PR or <25% increase in the sum of the products for a minimum of 4 weeks, and progressive disease (PD) as an unequivocal increase of at least 25% in the size of any measurable lesion or the appearance of new lesions.

Patients with no change, PR or CR were eligible for continuation of therapy. Patients discontinued treatment if they had PD after the first two cycles or at any time thereafter, or if side-effects were deemed unacceptable by the patient or the principal investigator. A maximum of six cycles was allowed. The duration of the response was measured from the date of its documentation to the date of relapse or death. Survival was calculated from the start of chemotherapy until the last checkup or death. Progression, duration of response, and survival times were calculated using the Kaplan and Meier method (BMDP Statistical Software, University of California, 1990). Category data were obtained by multivariate analysis using polychotomous stepwise logistic regression. The data on time to progression, duration of response and survival were updated on August 1, 2007.

**Blood samples.** Blood samples were collected in tubes from a large vein in the arm not receiving the drug infusion and immediately centrifuged at 2500  $\times g$  for 10 min at 4°C. The plasma was separated, frozen and stored at -70°C until analysis.

For PTX and Cremophor EL, 7 blood samples were drawn: immediately before PTX infusion, at the end of PTX infusion, 1, 3, 5, 24 and 48 hours after the end of PTX infusion. For PLD, 7 blood samples were drawn: immediately before PLD infusion, at the end of PLD infusion, and 2, 4, 24, 48, and 168 hours after the end of PLD infusion.

#### *Analytical procedures.*

**Paclitaxel:** PTX plasma concentrations were measured with a modification of a known procedure (19). A volume of 200  $\mu l$  of acetonitrile, 300  $\mu l$  of deionized water and 5 ml of *tert*-butylmethylether were added to 100  $\mu l$  of plasma samples in borosilicate glass tubes. The mixture was vortexed for 30 s and centrifuged at 2500  $\times g$  for 15 min. Upon centrifugation, 3 ml of the organic layer were transferred to a clean glass tube and evaporated to dryness under nitrogen at 40°C. The residue was then reconstituted with 200  $\mu l$  of 60% acetonitrile in deionized water and mixed on a vortex mixer for 90 s. A portion (100  $\mu l$ ) of the reconstituted sample was injected into the chromatograph. Chromatographic separations were achieved using a Symmetry C18 column (250 $\times$ 4.6 mm i.d., particle size 5 mm) and a Symmetry C18 guard column supplied by Waters (Vimodrone, Milan, Italy). The mobile phase, consisting of acetonitrile-0.1% phosphoric acid in deionized water (60:40, v/v) (Milli-Q Plus System, Millipore, Milford, MA, USA) was passed through a 0.22- $\mu m$  membrane filter and degassed by ultra sonication under vacuum before use. The flow-rate of the mobile phase was maintained at 1.0 ml/min. Chromatography was performed at ambient temperature (20 $\pm$ 2°C) and the UV detection wavelength was 227 nm; paclitaxel retention time was about 10 min. Drug concentrations were determined from the peak area ratios *versus* a standard curve obtained with the same procedure. The limit of quantitation for this method was 5 ng/ml.

**Pegylated liposomal doxorubicin.** A modified plasma extraction

procedure for total doxorubicin from PLD was used (20). A 500  $\mu l$  plasma aliquot was fortified with 50  $\mu l$  CH<sub>3</sub>CH<sub>2</sub>OH and vortex mixed. The further addition of 50  $\mu l$  Triton® X-100 3% was required to break the liposome vesicles. After a second vortex mixing procedure, 50  $\mu l$  of 65% 5-sulphosalicylic acid were added to precipitate both liposomal components and plasma proteins, which were removed by 10 min 20,000  $\times g$  centrifugation. After discarding the pellet, the resulting solution was fortified with 15  $\mu l$  of 1.8 M PBS, diluted 1:20 with 25 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>/0.03 M H<sub>3</sub>PO<sub>4</sub>-CH<sub>3</sub>CN (85:15 v/v) and filtered through Millex SLCR filters; 200  $\mu l$  were injected into the HPLC system as described in (21) with minimal modification. HPLC analysis was performed with a 15 min linear gradient (flow rate 1 ml/min) from 25 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>/0.03 M H<sub>3</sub>PO<sub>4</sub>-CH<sub>3</sub>CN 85:15v/v, to 25 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>/0.03 M H<sub>3</sub>PO<sub>4</sub>-CH<sub>3</sub>CN 50:50 v/v; the column was equilibrated at the initial conditions for 15 min between analyses. Chromatography was performed at ambient temperature (20 $\pm$ 2°C) and the column effluent was monitored at an excitation wavelength of 475 nm and an emission wavelength of 580 nm; doxorubicin retention time was about 11 min. The limit of quantitation for this method was 5 ng/ml.

**Cremophor EL.** Extraction of plasma samples for the analysis of Cremophor EL was performed as described by Sparreboom *et al.* (22). Briefly, 500  $\mu l$  of acetonitrile were added to 50  $\mu l$  of plasma samples and mixed by vortex for 1 min. Subsequently, 2 ml of *n*-butyl chloride were added, followed by vigorous mixing for 5 min. The organic layer was separated by centrifugation (4000  $\times g$ ; 5 min), transferred to a clean glass tube, and dried under nitrogen at 60°C for 30 min. The residue was reconstituted in 50  $\mu l$  of water by vortex mixing, and a 25- $\mu l$  volume was pipetted into a 96-well flat-bottom cluster. Finally, 250  $\mu l$  of water-diluted (1:4, v/v) Coomassie brilliant blue G-250 reagent was added and the absorbance maximum of the dye at 595 nm after binding to Cremophor EL and the simultaneous decrease in absorbance at 450 nm were measured within 24 h against a reagent blank using a Titertek Multiskan Plus MKII microplate reader. The limit of quantitation for this method was 0.5  $\mu l/ml$ .

#### *In vitro study of PTX-PLD interaction.*

The interaction between PTX and PLD was evaluated in 0.9% NaCl, 45 mg/ml human albumin in 0.9% NaCl, or human plasma. For each condition, the paclitaxel (final concentration 1  $\mu g/ml$ ) was incubated in a glass centrifuge tube without or with PLD (final concentration 20  $\mu g/ml$ ) already present in the incubation mixture or added 10 min after the addition of PTX.

**Incubation of PTX alone.** Ten  $\mu l$  of 100  $\mu g/ml$  PTX in 0.9% NaCl were added to 990  $\mu l$  of 0.9% NaCl, 990  $\mu l$  of 45 mg/ml human albumin or 990  $\mu l$  of human plasma and incubated for 20 min at 37°C.

**Incubation of PTX in the presence of PLD.** Ten  $\mu l$  of 100  $\mu g/ml$  paclitaxel in 0.9% NaCl were added to 10  $\mu l$  of 2 mg/ml PLD in 980  $\mu l$  of 0.9% NaCl, 980  $\mu l$  of 45 mg/ml human albumin or 980  $\mu l$  of human plasma and incubated for 20 min at 37°C.

**Incubation of PTX followed by the addition of PLD.** Ten  $\mu l$  of 100  $\mu g/ml$  PTX in 0.9% NaCl were added to 980  $\mu l$  of 0.9% NaCl, 980  $\mu l$  of 45 mg/ml human albumin or 980  $\mu l$  of human plasma and incubated for 10 min at 37°C. Ten  $\mu l$  of 2 mg/ml Caelyx were then added and the mixture incubated at 37 °C for a further 10 min.

At the end of each incubation, the sample was centrifuged at 5000  $\times g$  for 30 min to precipitate unbound PTX as insoluble aggregates and 500  $\mu l$  of the supernatant were transferred to a clean glass tube. The residual PTX was extracted and quantified by HPLC as described above.

**Pharmacokinetic and statistical evaluation.** Non-compartmental pk analysis (NCA) was performed during the first course using Kinetica 2000 4.1.1 software (InnaPhase Corp., Philadelphia USA); the single NCA assumption was that the terminal elimination phase can be approximated by an exponential equation, so that a straight line can approximate the logarithmic transformation of data belonging to the terminal removal process. The main pk parameters determined in this study were  $C_{max}$ , AUC,  $K_{el}$ ,  $t_{1/2}$ , Cl and  $V_{ss}$ .  $C_{max}$  is the maximum observed plasma concentration; AUC is the area under the plasma concentration–time curve from  $t=0$  to  $t=\infty$  extrapolated by the software; the elimination rate constant  $K_{el}$  was estimated from the terminal portion of the log-transformed plasma concentration–time curve. The elimination half-life  $t_{1/2}$  was calculated as  $\ln 2/K_{el}$ ; total plasma clearance Cl was calculated as  $Cl=Dose/AUC$ ; the apparent steady-state volume of distribution  $V_{ss}$  was calculated using a non compartmental first-moment method, as  $V_{ss}=AUMC \times D/AUC$ .

Statistical evaluation was performed using Instat 3.05 software (Graphpad, San Diego USA). The Mann-Whitney non parametric test was used to compare pharmacokinetic values for the 0 hour and 24 hour arms.

## Results

**Patient characteristics.** Between January 2004 and August 2006, 30 patients were enrolled in the study. The characteristics of the patients are listed in Table I. The median follow-up was 25.4 months (range 12-44 months). Twenty-four patients had recurrent local-regional disease, 6 had metastatic disease. All patients had received prior radiation therapy, 14 had undergone previous surgical treatment; 18 had received platinum-based chemotherapy concomitant with radiation therapy. Ten patients (30%) had received prior palliative chemotherapy for recurrent disease; 4 patients (13%) had received 2 or more regimens. The commonest sites of primary disease were the oropharynx (50%) followed by the larynx (20%) and hypopharynx (20%). A total of 127 cycles were administered; patients received 2 to 6 cycles, with a median of 4 cycles per patient.

**Response.** All 30 patients enrolled and treated in the study were included in the calculation for response. One patient (3%; 95% confidence interval (CI) 0% to 12%) achieved CR, 8 (27%; 95% CI, 12% to 35%) achieved PR, 13 (43%) had SD, and 8 (27%) had PD. The overall response rate (CR+PR) was 33% (95% CI 12-41%).

Table II shows responses obtained according to patient characteristics. There were no significant differences in response rate as related to site of primary lesion, site of recurrence or prior therapy. Patients with a PS=0 had a significantly better response than patients with PS 1-2

( $p<0.0002$ ). Patients with disease-free survival >9 months had a statistically better response rate ( $p<0.0117$ ) than those with <9 months. Only one patient previously treated with palliative cisplatin-based chemotherapy responded (10%). The median response duration was 5.5 months (2-16 months). Overall median survival was 10 months (2-24 months). Median survival of patients who achieved CR or PR was 13 months (9-24 months).

**Toxicity.** Toxicity, assessed in all patients, is reported in Table III. No drug-related deaths occurred. The major dose-limiting toxicity was hematological. Six patients (20%) required 25% dose reduction; G-CSF administration was required in 9 patients (30%). Granulocytopenia occurred in all patients, grade 3-4 in 9 (30%). No patient developed severe infection. Grade 1-2 thrombocytopenia occurred in 8 patients (27%). Anemia was frequent (57%) but never severe. The commonest nonhematological side-effects were hand–foot syndrome (80%; grade 3 in 3%), mucositis (77%; grade 3 in 10%), nausea/vomiting (44%), constipation (47%) and peripheral neuropathy (47%).

**Pharmacokinetic results.** Plasma levels of total doxorubicin, PTX and Cremophor EL were evaluated in all 30 patients who received PTX followed by PLD after intervals ranging from 0 to 24 hours. Patients were randomized to the different intervals between administration, as follows: 0 hours for 9 patients, 1 hour for 4 patients, 3 hours for 5 patients, 12 hours for 3 patients and 24 hours for 9 patients.

**Pegylated liposomal doxorubicin.** Figure 1 shows the median plasmatic profile of total doxorubicin for each interval of administration between PTX and PLD. Doxorubicin  $C_{max}$  and the plasma concentrations during the distribution phase were slightly lower when PLD was administered immediately after PTX, versus an interval of 1 hour or more between the two drugs, whereas the slope of the elimination phase was comparable for all time intervals. There were no other differences in the principal doxorubicin pharmacokinetic parameter among all experimental arms (Table IV), as also indicated by Mann-Whitney non parametric analysis, comparing the 0 hour and 24 hour arms.

**Paclitaxel.** Figure 2 shows the median plasma profile of PTX for each interval of administration between PTX and PLD. Co-administration of PLD had a substantial effect on the  $C_{max}$  and the plasma concentrations profiles of PTX: the comparison of the main pharmacokinetic parameters (Table V) shows a marked decrease of AUC, with a marked increase of clearance when the interval of administration is 0 or 1 hour. The differences in the pharmacokinetic parameters between the 0 hour and the 24 hour experimental arms are statistically highly significant, as indicated by Mann-Whitney non-parametric analysis.

Table I. Patient and tumor characteristics.

Characteristic	No. of patients	%
No. entered	30	100
No. assessable for response/toxicity	30	100
Gender		
Male	24	80
Female	6	20
Age (years), median (range)	58 (37-70)	
Performance status (ECOG)		
0	8	26
1	17	58
2	5	16
Site of primary lesion		
Oral cavity	3	10
Oropharynx	15	50
Larynx	6	20
Hypopharynx	6	20
Site of recurrence		
Local	6	20
Nodal	6	20
Local + nodal	12	40
Metastasis only	6	20
Site of metastasis		
Lung	3	
Skin	2	
Lung + liver	1	
Prior therapy		
Surgery + radiotherapy	12	40
Concomitant chemoradiotherapy	16	53
Surgery + concomitant CT-RT	2	7
Palliative chemotherapy	10	30
1 scheme	6	
2 or more schemes	4	
Disease-free interval		
<9 months	12	40
>9 months	18	60

CT, chemotherapy; RT, radiotherapy.

*Cremophor EL*. Co-administration of PLD had no effect on the main pharmacokinetic parameters of Cremophor EL at any interval of administration, as confirmed by Mann-Whitney non-parametric analysis comparing the 0 hour and the 24 hour experimental arms.

*In vitro interaction between PTX and PLD*. In order to elucidate the interaction *in vitro* between PTX and PLD, PTX was incubated for 20 min at 37°C in 0.9% NaCl, human albumin in 0.9% NaCl, or human plasma in the absence of PLD or with PLD already present in the incubation mixture or added 10 min after the addition of PTX. The results demonstrated the ability of PLD to absorb PTX: the concentration of PTX extracted from the supernatant of the mixture incubated in the presence of PLD was significantly higher than that in the mixture incubated without PLD ( $p=0.0180$ ) for all incubation media (0.9% NaCl, human albumin in 0.9% NaCl, human plasma).

Table II. Response versus patient characteristics.

Characteristic	No. of patients	%	P
Performance status			
0	7/8	87	0.0002
1-2	2/22	9	
Site of primary lesion			
Oral cavity	1/3	33	NS
Oropharynx	4/15	27	
Larynx	2/6	33	
Hypopharynx	2/6	33	
Site of recurrence			
Local or nodal	7/12	58	NS
Local+nodal or mets	2/18	11	
Prior therapy			
Surgery + radiotherapy	5/12	42	NS
Concomitant CT+RT	3/16	19	
Surgery + CT+RT	0/2	0	
Palliative CT	1/10	10	
Disease-free interval			
<9 months	0/12	0	0.0117
>9 months	9/18	50	

NS, not significant; T, tumor; N, nodal; CT, chemotherapy; RT, radiotherapy.

Table III. Toxicity.

Characteristic	Grade			
	1 No. (%)	2 No. (%)	3 No. (%)	4 No. (%)
Hematological				
Hemoglobin	12 (40)	5 (17)	0	0
Granulocytes	3 (10)	18 (60)	6 (20)	3(10)
Platelets	5 (17)	3 (10)	0	0
Infection	5 (17)	3 (10)	0	0
Neurotoxicity				
Peripheral	8 (27)	6 (20)	0	0
Constipation	8 (27)	6 (20)	0	0
Asthenia	8 (27)	5 (17)	3 (10)	0
Gastrointestinal				
Nausea/vomiting	8 (27)	5 (17)	0	0
Diarrhea	3 (10)	0	0	0
Mucositis	12 (40)	8 (27)	3 (10)	0
Hand-foot syndrome	15 (50)	8 (27)	1 (3)	0
Fluid retention	8 (27)	3 (10)	0	0

## Discussion

This study evaluated the activity and pharmacokinetics of the drug combination PTX/PLD in 30 patients affected by recurrent squamous cell carcinoma of the head and neck.

To determine the best schedule, characterized by minimal toxicity and high efficacy, we carried out a pharmacokinetic

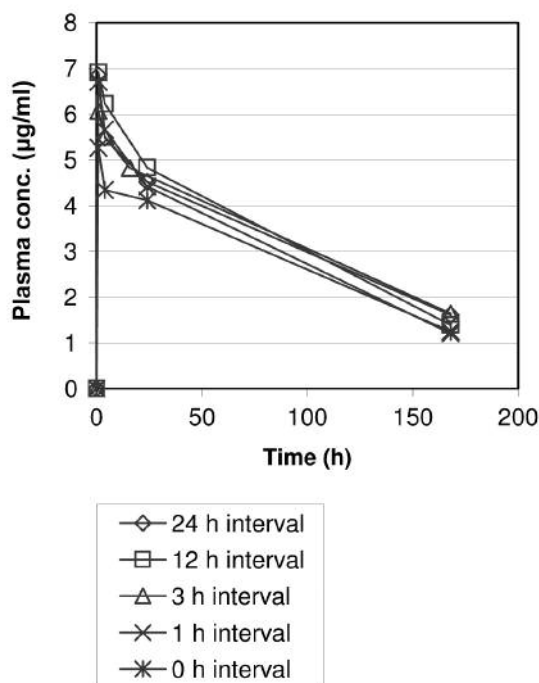


Figure 1. Median plasma concentration of pegylated liposomal doxorubicin for the different administration intervals.

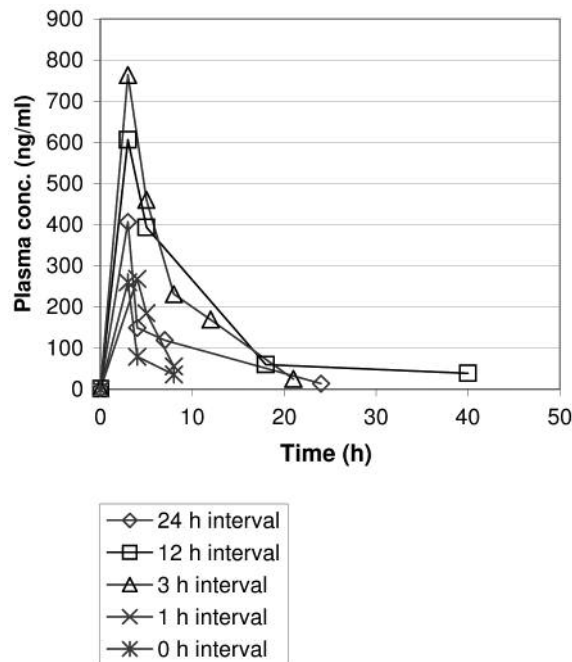


Figure 2. Median plasma concentration of paclitaxel for the different administration intervals.

study, concomitant to the clinical study, to establish any possible drug interactions. In particular, we wanted to determine whether the use of different time intervals between administration of the two drugs, PTX and PLD, used in the treatment of recurrent head and neck cancer, could influence their pk parameters or toxicity. Most previous studies (14-16) have shown that administration of PTX immediately after PLD significantly changes the AUC and clearance of PLD. The reverse therapeutic protocol (PTX followed by PLD) has rarely been employed in clinical trials. One exception is the study by Janinis *et al.* (17) that showed that prior administration of PTX did not appear to affect the pharmacokinetics of PLD, whereas prior administration of PLD, in agreement with other studies, resulted in slightly higher plasma concentration and AUC of PTX. In our study, we chose to administer PTX followed by PLD since, from the pk standpoint, it is preferable to administer the drug with the shorter half-life first, in this instance PTX, followed by the drug with the longer half-life, *i.e.* PLD (more than 15 days) (23). Following the PTX/PLD schedule, we were able to compare the pk data obtained by administering PLD 24 h after PTX with those obtained by different schedules (PLD concomitant to PTX, or PLD administered 1 h, 3 h or 12 h after PTX). In our opinion, the PTX/24 h PLD schedule may be very similar to the administration of PLD or PTX in monotherapy, since after 24 h, the very low residue of PTX in the plasma should not interfere with PLD pharmacokinetics.

To explain the results reported in the literature, we first hypothesized that the PTX excipient (Cremophor EL), a surface-active agent, might favor the early release of doxorubicin from the PLD vesicles, causing a substantial increase in plasma doxorubicin. Comparing pk data obtained after co-administration of PTX/PLD with those relating to different time intervals (1 h to 24 h), we found no substantial pk interference of PTX *vs.* PLD. On the contrary, other studies (15) following similar but not identical conditions (PTX immediately after PLD), report a clear increase of the PLD AUC, leading to higher systemic exposure to PLD. Moreover, by monitoring Cremophor EL in the different schedules, we showed that the plasma concentration of this excipient was not influenced by PLD or PTX plasma concentrations, reaching a maximum plasma concentration following the co-administration schedule.

The influence of PLD on the pk parameters of PTX appeared evident when the therapeutic regimen characterized by the concomitant administration of the two drugs was compared with those employing different time intervals. A dramatic and statistically significant reduction of AUC of PTX, corresponding to an increased clearance, were observed when the two drugs were administered simultaneously. Various hypotheses may explain these experimental data. In theory, a drug such as PTX may be rapidly eliminated from the plasma to the liver due to the simultaneous presence of an associated drug that stimulates liver metabolism. To our knowledge, it is

Table IV. Median values of the main pharmacokinetic parameters for pegylated liposomal doxorubicin.

Admin. interval	0 h 9 cycles (range)	1 h 4 cycles (range)	3 h 5 cycles (range)	12 h 3 cycles (range)	24 h 9 cycles (range)	<i>P</i> Mann-Whitney*
$C_{max}$ (ng/ml)	5110.6 (4100.0-8098.4)	6706.8 (4996.8-7227.0)	6076.4 (5467.3-11260.0)	6917.5 (6260.5-7046.1)	6853.9 (5033.1-7828.0)	0.0360
AUC (ng/ml)*(h)	676350 (253220-1073450)	606830 (540596-990028)	749600 (723569-1428010)	723830 (688283-734389)	739620 (410960-1053160)	0.3704
$K_{el}$ (1/h)	0.0073 (0.0055-0.0138)	0.0085 (0.0068-0.0090)	0.0075 (0.0041-0.0088)	0.0084 (0.0079-0.0090)	0.0075 (0.0050-0.0147)	0.7430
$t_{1/2}$ (h)	94.282 (50.040-126.005)	82.179 (87.485-101.693)	92.131 (78.798-168.709)	82.358 (77.150-87.919)	92.637 (47.047-138.838)	0.7430
MRT (h)	135.05 (71.16-183.56)	119.20 (107.91-148.16)	132.86 (115.12-245.34)	119.34 (111.87-126.36)	133.34 (67.65-138.77)	0.8148
CI (ml/h)	30.697 (19.563-82.932)	36.342 (22.222-40.468)	29.023 (15.406-41.361)	29.786 (29.358-31.964)	28.731 (20.472-51.708)	0.4234
$V_{ss}$ (ml)	4374.7 (2483.5-5901.5)	4085.5 (3292.5-4922.8)	4239.9 (2198.9-4761.2)	3575.7 (3554.6-3709.6)	3739.7 (3439.0-4111.8)	0.2359

\*The Mann-Whitney non-parametric test was used to compare pharmacokinetic values for the 0 h and 24 h arms;  $p>0.05$  non-significant difference,  $p<0.05$  significant difference.

Table V. Median values of the main pharmacokinetic parameters for paclitaxel.

Admin. interval	0 h 9 cycles (range)	1 h 4 cycles (range)	3 h 5 cycles (range)	12 h 3 cycles (range)	24 h 9 cycles (range)	<i>P</i> Mann-Whitney*
$C_{max}$ (ng/ml)	260.50 (219.32-531.17)	403.29 (296.95-754.97)	764.09 (380.66-1083.92)	606.56 (399.77-695.60)	459.31 (250.33-732.52)	0.0745
AUC (ng/ml)*(h)	924.6 (737.5-1784.8)	1620.2 (1208.4-4524.3)	4675.3 (2317.8-6769.0)	5273.1 (3384.3-7696.1)	1968.7 (945.8-6127.4)	0.0037
$K_{el}$ (1/h)	0.2517 (0.0853-0.3904)	0.2312 (0.1121-0.7411)	0.2077 (0.1631-0.2516)	0.0709 (0.0483-0.2426)	0.1809 (0.0250-0.3722)	0.1388
$t_{1/2}$ (h)	2.773 (1.830-8.126)	2.998 (0.635-6.184)	3.338 (2.754-4.251)	9.773 (2.857-14.350)	3.832 (1.862-27.676)	0.1388
MRT (h)	3.580 (2.295-8.106)	5.351 (2.073-6.872)	6.182 (4.571-6.760)	13.036 (5.158-20.324)	4.116 (2.523-35.933)	0.1996
CI (ml/h)	142764 (76201-184401)	87898 (33155-133374)	28662 (20831-59540)	26171 (18451-40776)	67454 (19421-143793)	0.0055
$V_{ss}$ (ml)	579219 (217190-671905)	308006 (178709-715195)	174222 (128628-402505)	240521 (210306-531891)	546092 (181588-697848)	0.8148

\*The Mann-Whitney non-parametric test was used to compare pharmacokinetic values for the 0 h and 24 h arms;  $p>0.05$  non-significant difference,  $p<0.05$  significant difference.

very improbable that PLD may induce the liver monooxygenases CYP2C8 or CYP3A4, which are usually responsible for PTX hydroxylation. We have already demonstrated that PLD associated with a drug characterized by high hepato-biliary clearance, vinorelbine, was able to lower the plasma elimination rate, causing a rise in plasma concentration (24). An alternative explanation is that PTX, a drug that is closely bound to plasma proteins, may be displaced from the binding sites by PLD, with a sequential decrease in AUC. We know that PLD is a drug not bound to plasma protein and characterized by a low distribution volume; consequently competition for protein binding between PLD and PTX is not feasible.

It is very probable that PLD may affect pk of PTX through physicochemical interaction, as suggested by a study by Fahr *et al.* concerning the interaction of lipophilic drugs with liposomal membranes (25). A very lipophilic drug such as PTX could be absorbed on the surface of the lipid vesicles of PLD, causing a lowering of plasma PTX concentration. We aimed to verify this suggestion by incubating *in vitro* PTX at concentrations similar to that used in the clinical experiments with PLD, in the presence or absence of albumin. We found that in both cases, PLD was effectively able to capture PTX, lowering its concentration. This might explain the anomaly of a drug such PLD that “induces” the elimination from plasma of paclitaxel, lowering its plasma concentration.

Our heavily pretreated patients (all patients had received radiotherapeutic treatment, more than 50% had been submitted to concomitant cisplatin administration, and 10 patients, 30%, had received a cisplatin based palliative chemotherapy for recurrent disease) achieved a good overall response rate (30%), with CR in 3% of patients; these results are comparable to those reported with another PLD/PTX schedule (10)). In our experience, a lower responsiveness was observed in patients previously treated with concomitant cisplatin and radiotherapy (overall response rate=15%) than in chemotherapy-naive patients (42%); we had a 10% response rate in patients previously treated with one or more palliative cisplatin-based schemes.

Prognostic factors for response were PS and disease-free interval; the prognostic role of PS has been reported in many series (26-28), whereas the role of the disease-free interval has not frequently been evaluated. We chose a 9-month cut-off because recent evidence shows its prognostic value in a large number of cases (29).

The impact of our combination on survival can be acceptable within a palliative framework (median overall survival, 10 months). As expected, the main toxicity was neutropenia, characterized by rapid recovery. Severe infection occurred in no case. Thrombocytopenia and anemia were never severe. Only 10% of patients had grade 3-4 mucositis, although all patients had previously undergone radiotherapy (60% received a radiation dose >65 Gy). The

severity and extent of all other toxicities was within the limits of a well-tolerated combination. Hand-foot syndrome was frequent but rarely severe.

In conclusion, taking together clinical, toxicological and pharmacokinetic results, we may conclude that the PTX-PLD combination can play a palliative role (clinical benefit in 73% of patients) with acceptable toxicity. From the pharmacokinetic data, to obtain a minimal drug interaction, the best administration sequence would appear to be PTX followed after 24 h by PLD.

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