

## Pharmacokinetics of Liposomal Cisplatin (Lipoplatin) in Combination with 5-FU in Patients with Advanced Head and Neck Cancer: First Results of a Phase III Study

C.F. JEHN<sup>1</sup>, T. BOULIKAS<sup>2</sup>, A. KOURVETARIS<sup>2</sup>, K. POSSINGER<sup>1</sup> and D. LÜFTNER<sup>1</sup>

<sup>1</sup>Medizinische Klinik und Poliklinik mit Schwerpunkt Onkologie und Hämatologie Charité, Campus Mitte, Universitätsmedizin Berlin, Humboldt-Universität zu Berlin Charitéplatz 1, 10117 Berlin, Germany;

<sup>2</sup>Regulon, Inc., 715 N. Shoreline Blvd., Mt View, CA 94043, U.S.A.

**Abstract.** *Background:* Lipoplatin, a novel liposomal formulation of cisplatin, is composed of cisplatin and liposomes based on dipalmityl phosphatidyl glycerol (DPPG), soy phosphatidyl choline (SPC-3), cholesterol and methoxypolyethylene glycol-distearoyl phosphatidylethanolamine (mPEG2000-DSPE). Liposomal encapsulation of cisplatin is designed to increase safety and tolerability by decreasing, e.g., nephrotoxicity through decreased exposure of organs to cisplatin, while effectively delivering the drug to the tumor. In an ongoing phase III trial comparing cisplatin to lipoplatin (both in combination with infusional high-dose 5-Fluorouracil) in advanced head and neck cancer (HNC), a sub-study to determine the pharmacokinetic profile of lipoplatin in comparison to conventional cisplatin was undertaken. *Materials and Methods:* In total, twelve patients with advanced HNC received a combination chemotherapy with either lipoplatin/5-FU or cisplatin/5-FU. Plasma samples were analyzed for concentration of total platinum in patients from both arms. *Results:* All twelve patients from the pharmacokinetic sub-study were male Caucasians at a mean age of 60 years. There was no difference in age or kidney function between the two treatment groups. The total body clearance for cisplatin was 1.25 L/(hxm<sup>2</sup>) for the liposomal formulation, compared to 0.62 L/(hxm<sup>2</sup>) for conventional cisplatin. The terminal half life was half as long for lipoplatin (10.98 h) as compared to cisplatin (24.5h). Even though the maximum observed concentration in the plasma (C<sub>max</sub>) was greater for lipoplatin than for cisplatin, the area under

the concentration time-curve (AUC) was less (6.5 µg/ml vs. 4.07 µg/ml and 66.85 µg/h/ml vs. 130.33 µg/h/ml, respectively). *Conclusion:* The pharmacokinetic profile of lipoplatin (in combination with 5-FU) suggests that the liposomal formulation results in a greater body clearance and shorter half life than conventional cisplatin, which confirms the clinical observation of decreased toxicity, especially renal deterioration.

Cisplatin is one of the most active chemotherapeutic agents used in the treatment of advanced squamous cell carcinoma of the head and neck (SCCHN). However, its clinical efficacy is contrasted by its toxicity profile. In particular renal toxicity, peripheral neuropathy and hematotoxicity limit its clinical use and a cumulative effect of successive doses is apparent (1).

The application of liposomes as drug carriers offers the possibility to manipulate the pharmacokinetics of drugs and to improve their efficacy and reduce toxicity (2). Lipoplatin is a liposomal formulation of cisplatin. The anionic lipid dipalmityl phosphatidyl glycerol (DPPG) gives lipoplatin its fusogenic properties in the liposomal formulation. These nanoparticles (110 nm) extravasate preferentially into solid tumors and their metastases through the altered, leaky vasculature. The entrance of lipoplatin particles into the kidney tubule cells is limited as a fusion of the lipid capsule with the cell membrane is needed for reactivity (3). Through this mechanism the release of cisplatin into the system is reduced and the toxic side-effects are decreased. Animal studies show five times lower levels of platinum in kidneys after a lipoplatin infusion compared to cisplatin treatments at equal dosing (4).

To test the efficacy and toxicity profile in a clinical setting a subprotocol to a randomized, multicenter phase III trial of SCCHN was designed, in which conventional cisplatin or the liposomal formulation of cisplatin (lipoplatin) was used in combination with 5-FU. The total platinum concentration was measured in both arms of the study following administration of either cisplatin (and 5-FU) or lipoplatin (and 5-FU).

*Correspondence to:* PD Dr. med. Diana Lüftner, Medizinische Klinik und Poliklinik mit Schwerpunkt Onkologie und Hämatologie Charité, Campus Mitte, Universitätsmedizin Berlin, Humboldt-Universität zu Berlin Charitéplatz 1, 10117 Berlin, Germany. Tel: +49 30 450 55 32 59, Fax: +49 30 450 51 39 19, e-mail: diana.lueftner@rz.hu-berlin.de / diana.lueftner@charite.de

*Key Words:* Liposomal cisplatin, lipoplatin, 5-fluorouracil, head and neck cancer.

Table I. Patient characteristics relevant for the pharmacokinetic and pharmacodynamic evaluations.

Parameter	Lipoplatin	Cisplatin	T-test
Dose (mg/m <sup>2</sup> )	100	100	
Dosing interval	day 1, 8, 15 q3w	day 1 q3w	
Male/female	6/0	6/0	
Age (years)	59.2±5.0	61.2±7.2	N.S.
Height (cm)	178.5±3.8	174.7±5.4	
Weight (Kg)	71.6±13.4	62.9±9.8	
CrCl (ml/min)	93.73±16.5	91.1±27.4	N.S.

N.S.=not significant; CrCl: Creatinin clearance.

**Materials and Methods**

Plasma samples for pharmacokinetic evaluation were collected during the first and second cycles of treatment only. This study was approved by the institutional review board and all patients signed informed consent forms.

Only patients with histologically confirmed SCCHN (primary metastatic or patients with relapsed/progressive disease) between the age of 18-75 years with sufficient renal function defined as creatinine clearance >50 ml/min where included in the study. After stratification (criteria: primary metastatic disease, recurrent or progressive SCCHN, prior chemotherapy/no prior chemotherapy, prior cisplatin-based chemotherapy/prior non-cisplatin based chemotherapy and center), patients were randomized between the following arms: Arm A: patients received 100 mg/m<sup>2</sup>/d lipoplatin (d 1,8,15) plus 1000 mg/m<sup>2</sup>/d 5-FU (d 1-5 continuous infusion) every three weeks for six cycles; arm B: patients received 100 mg/m<sup>2</sup>/d cisplatin (d 1) plus 1000 mg/m<sup>2</sup>/d 5-FU (day 1-5 continuous infusion) every three weeks for six cycles. The main endpoints for this pharmacokinetic analysis were the maximum observed concentration in the plasma (C<sub>max</sub>), the terminal half-life (t<sub>1/2</sub>), the area under the concentration-time curve (AUC), the total body clearance (CL) and the volume of distribution at steady state (V<sub>ss</sub>). Each variable was recorded for total platinum.

**Pharmacokinetic sampling.** For drug concentration assays, 10 ml of blood was collected in tubes containing EDTA. Samples were collected at 0, 2, 4, 8, 12, 24, 48 and 120 h after the start of the IV infusion of lipoplatin (infusion duration of 4h) and cisplatin (infusion duration of 1h). The 5-FU continuous infusion (day 1-5) was initiated directly after these infusions were completed. Plasma samples were diluted with water before the analytic procedures.

**GF-AAS (Graphite Furnace Atomic Absorption Spectrometry) assay.** Total platinum concentrations were determined in plasma using a GF-AAS (Graphite Furnace Atomic Absorption Spectrometry) assay. Standard solutions were prepared by the dilution of cisplatin<sub>(5)</sub> with water. A solution of Triton X-100 (1 ml/L) and nitric acid (0.02 g/L) was used as matrix modifier (5). The linear range of the assay in plasma was 0.03-0.35 µg Pt/ml, and the limit of detection did not exceed 14 ng Pt/ml. Further details of the furnace program were as

Table II. Mean and standard deviation of the pharmacokinetic parameters of lipoplatin vs. conventional cisplatin.

Parameter (unit)	Lipoplatin	CV%	Cisplatin	CV%	T-test
AUC (µg/h/ml)	66.9±39.5	59	130.3±69.4	53	NS
C <sub>max</sub> (µg/ml)	6.5±4.5	67	4.1±2.1	52	N.S.
MRT (h)	15.9±7.7	49	35.4±14.5	41	p<0.05
kel (h <sup>-1</sup> )	0.1±0.07	29	0.03±0.01	33	p<0.01
t <sub>1/2</sub> (h)	11.0±5.4	49	24.5±10.1	41	p<0.05
CL L/(h x m <sup>2</sup> )	1.3±0.6	46	0.6±0.3	47	p<0.05
V <sub>ss</sub> (L/m <sup>2</sup> )	15.2±5.4	35	18.3±3.1	17	N.S.

AUC=area under the curve; C<sub>max</sub>=maximum concentration; k<sub>el</sub>=elimination constant; t<sub>1/2</sub>=half life; CL=clearance; V<sub>ss</sub>=steady state; CV=coefficient of variation. MRT=mean residence time

follows: wavelength 265.9 nm; slit width (nm) 0.2 L; read time 4 sec; injected sample volume 20 µl; furnace temperature escalation program 6 steps -90°C, 100°C, 300°C, 450°C, 1400°C and 2650°C.

**Statistical analysis.** Estimates of pharmacokinetic parameters were obtained by non-compartmental analysis, based on statistical moment theory (6, 7). These parameters are determined by a numerical integration procedure such as the trapezoidal rule which was employed to determine the plasma AUC. The mean residence time (MRT) was calculated from the equation MRT=AUMC/AUC, where AUMC is the area under the first moment curve, and MRT represents the time for 63.2% of the administered dose to be eliminated. 1/K<sub>elim</sub> (elimination rate constant) is the MRT, the statistical moment analogue to half-life t<sub>1/2</sub>. The t<sub>1/2</sub> (elimination half-life) was calculated form the formula t<sub>1/2</sub>=0.693 x (1/K<sub>elim</sub>). The CL value was calculated from CL=Di.v./AUC, where Di.v. is the intravenous dose of lipoplatin/cisplatin and AUC the relative area under the curve. The V<sub>ss</sub> was calculated form the formula V<sub>ss</sub>=(infused dose x AUMC/AUC<sup>2</sup>)-(infused dose x T/(2xAUC)), where T is the infusion time. Creatinin clearance (CrCl) was estimated by the method of Trollfors *et al.* (8).

The statistical significance of differences between the pharmacokinetic parameters of lipoplatin and cisplatin were evaluated by using the unpaired 2-sample T-test with a significance level of 0.05 (2-tailed). The T-test for unequal variances was used in cases where the F-test for variances showed group variances to be unequal. All results were reported as mean±standard deviation (SD). As statistical software SPSS 12.00 was used.

**Results**

Pharmacokinetic measurements were performed on twelve patients (six patients from the lipoplatin arm and six patients from the cisplatin arm). As shown in Table I, all subjects were male and showed no significant difference in age or renal function as measured by the CrCl.

Both, lipoplatin and cisplatin, were infused at the same dose (100 mg cisplatin/m<sup>2</sup>) and showed similar plasma concentration profiles (Figure 1). However, the pharmacokinetics of lipoplatin differed significantly from those of cisplatin. Figures 2 and 3 show the kinetics of total

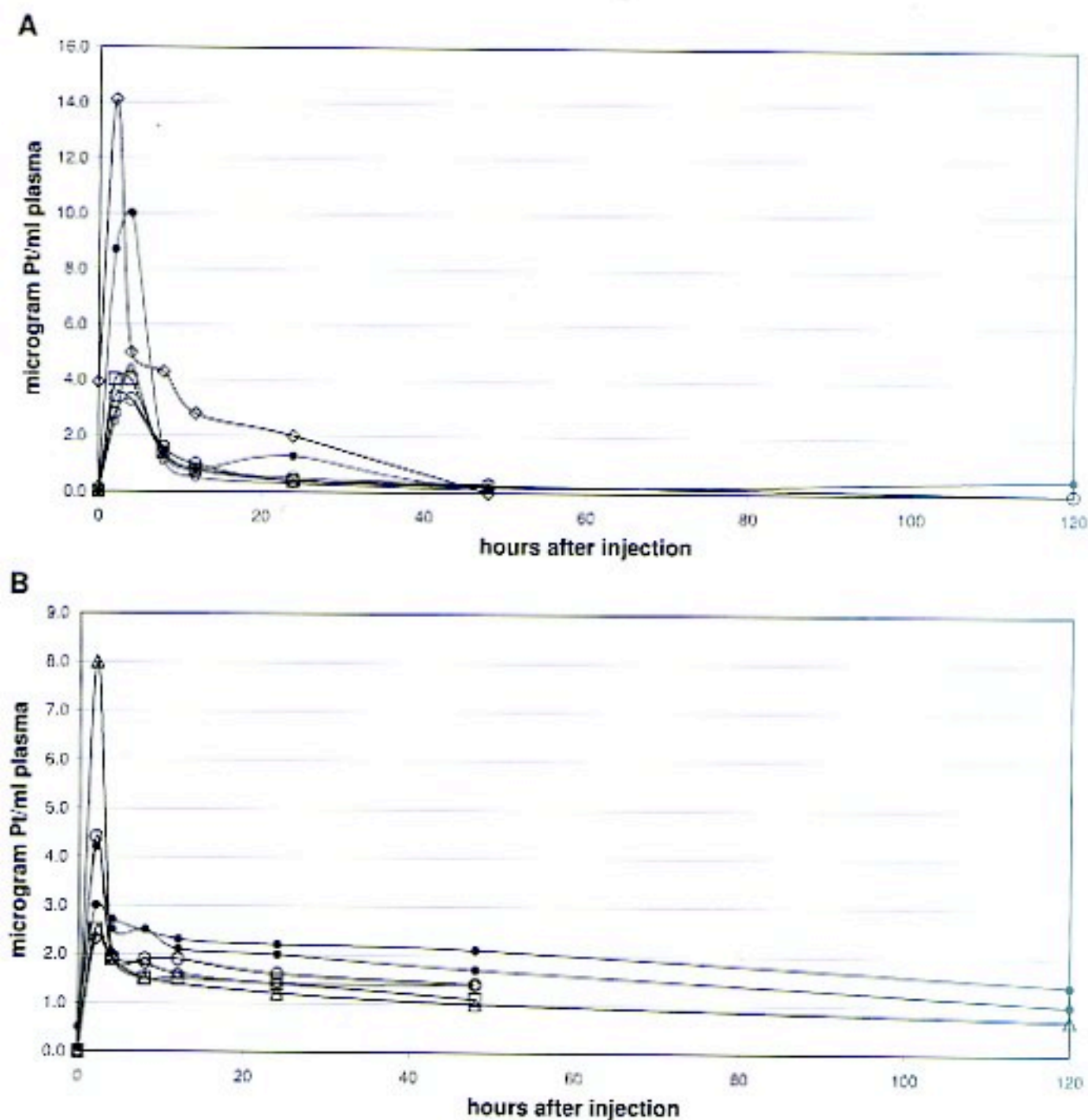


Figure 1. Concentration-time profile of the total plasma concentration of platinum after the lipoplatin infusion in liposomal formulation (A) and of platinum after the infusion of the unformulated native drug (B). Concentration-time profiles of all 12 patients depicted individually (6 patients lipoplatin group; 6 patients cisplatin group).

platinum for each patient. Mean concentrations are shown in Figure 3. Table II presents the mean observed and calculated pharmacokinetic variables for all patients. Patients treated with lipoplatin showed approximately 60% higher  $C_{max}$  compared to the cisplatin-treated group (statistically not significant). In addition,  $C_{max}$  was observed later in patients treated with lipoplatin, after which the concentration declined rapidly, resulting in an increased AUC for the cisplatin group (statistically not significant).

The platinum plasma concentration in the lipoplatin group reached the level of the conventional cisplatin group eight hours after infusion, while falling below the detection limit of 14 ng/ml by 48-120 h. In the cisplatin-treated group, the plasma level remained above the limit of detection for the

entire observation time. The  $K_{el}$  was significantly higher in patients treated with lipoplatin ( $p < 0.01$ ), resulting in a reduced half-life  $t_{1/2}$  and MRT ( $p < 0.05$ ). Lipoplatin exhibited a smaller volume of distribution compared to cisplatin (statistically not significant). The total clearance of lipoplatin was 2-fold higher than the clearance of cisplatin ( $p < 0.05$ ).

## Discussion

This study characterized the multiphasic plasma concentration profile of lipoplatin compared to cisplatin in patients treated with a combination chemotherapy with 5-FU for SCCHN. Liposomal formulations are known to alter the pharmacokinetics of drugs which it is proposed accounts for the

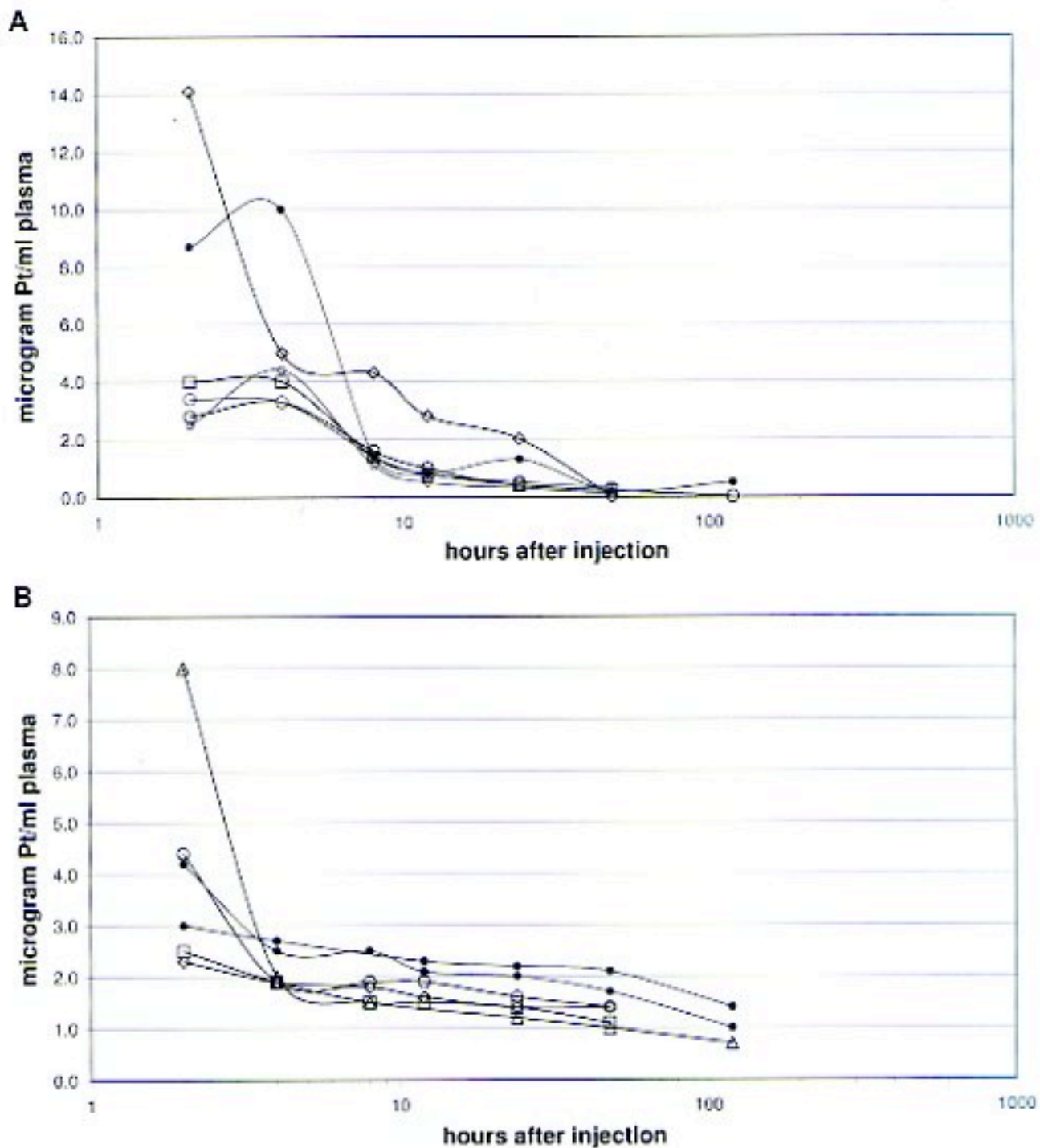


Figure 2. Concentration-time (logarithmic time scale) profile of the total plasma concentration of platinum after lipoplatin infusion in liposomal formulation (A) and of platinum after the infusion of the unformulated native drug (B). Concentration-time profiles of all 12 patients depicted individually (6 patients lipoplatin group; 6 patients cisplatin group).

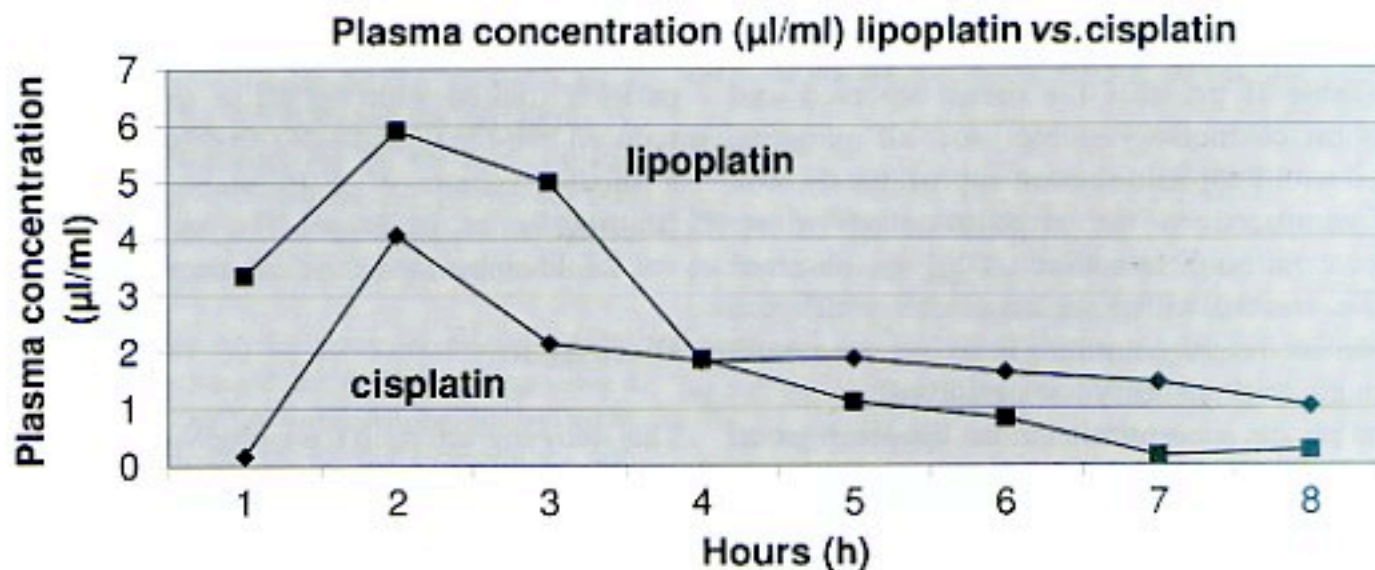


Figure 3. Comparative median plasma concentration profiles of total platinum after infusion of lipoplatin vs. conventional cisplatin.

different toxicity profiles of liposomal drugs (9). Of special interest in this regard is nephrotoxicity which is reduced with lipoplatin to a clinically relevant extent (10). Conventional cisplatin is rapidly and irreversibly bound to plasma proteins and only 25% of cisplatin is excreted renally during the first 24 h (1). This slow elimination is also due to the high tubular reabsorption in the kidneys. There is practically no elimination through hepatic metabolism (1). Since the groups compared showed no difference in kidney function, the observed marked differences in pharmacokinetics may be intrinsic to the liposomal formulation. The 2-fold higher clearance of lipoplatin could be explained by a reduced or missing renotubular reabsorption of cisplatin in liposomal formulation. In consequence, the nephrotoxicity would be reduced as demonstrated by clinical data and the preliminary clinical outcome of this study to be reported elsewhere. In addition, the terminal half-life for lipoplatin is 10.9 h and is roughly 50% shorter than conventional cisplatin (24.5 h). Interestingly, the total platinum plasma concentration reached higher values with the liposomal formulation than with conventional cisplatin even though the same dose was administered. The resulting lower AUC of total platinum for the liposomal formulation can be explained by the shorter half-life and the higher clearance.

We observed a fairly large inter-patient variability in pharmacokinetic parameters for total cisplatin with a coefficient of variation (CV) for clearance of 47%, for AUC of 55% and for  $C_{max}$  of 60%, respectively, in both treatment groups. Such variability is not surprising in cancer chemotherapy (11). Although the reasons for these differences are unclear, confounding factors such as small sample size, the presence of hepatic and renal dysfunction in patients receiving chemotherapy and the fact that some studies were conducted with different combination regimens are likely to account for this variation (12).

In summary, the pharmacokinetics of lipoplatin appear to be characterized by a reduced volume of distribution, a shorter half-life and higher plasma clearance compared with what would be expected in patients receiving conventional cisplatin (13). Furthermore, lipoplatin differs from cisplatin in that it reaches a higher and later maximum plasma concentration. However, lipoplatin exhibits a lower AUC than cisplatin, presumably due to reduced or missing renal tubular reabsorption of the liposomal formulation.

## Conclusion

The pharmacokinetic profile of lipoplatin suggests that the liposomal formulation results in a greater body clearance and shorter half life than conventional cisplatin, which confirms the clinical observation of decreased toxicity, especially renal deterioration.

## References

- Schellens JHM, Ma J, Planting AS, van der Burg ME, van Meerten E, de Boer-Dennert M, Schmitz PI, Stoter G and Verweij J: Relationship between the exposure to cisplatin, DNA-adduct formation in leucocytes and tumor response in patients with solid tumors. *Br J Cancer* 74: 323-326, 1996.
- Massing U and Fuxius S: Liposomal formulations of anticancer drugs: selectivity and effectiveness. *Drug Resist Update* 3: 171-177, 2000.
- Boulikas T, Stathopoulos GP, Volakakis N and Vougiouka M: Systemic Lipoplatin infusion results in preferential tumor uptake in human studies. *Anticancer Res* 25(4): 3031-3040, 2005.
- Devarajan P, Tarabishi R, Mishra J, Ma Q, Kourvetaris A, Vougiouka M and Boulikas T: Low renal toxicity of lipoplatin compared to cisplatin in animals. *Anticancer Res* 24(4): 2193-2200, 2004.
- Jetske M, Terwogt M, Tibben M, Welbank H, Schellens JHM and Beijnen JH: Validated method for the determination of platinum from a liposomal source (SPI-77) in human plasma using graphite furnace Zeeman atomic absorption spectrometry. *Fresenius J Anal Chem* 366: 298-302, 2000.
- Chan KKH and Gibaldi M: Estimation of statistical moments and steady-state volume of distribution for a drug given by intravenous infusion. *J Pharmacokinetic Biopharm* 10: 551-558, 1982.
- Gibaldi M and Perrier D: *Pharmacokinetics*, 2nd Edn., Dekker, USA, 1982.
- Trollfors B, Alestig K and Jagenburg R: Prediction of glomerular filtration rate from serum creatinine, age, sex and body weight. *Acta Med Scand* 221(5): 495-498, 1987.
- Fielding RM: Relationship of pharmacokinetically calculated volumes of distribution to the physiologic distribution of liposomal drugs in tissue: implications for the characterisation of liposomal formulations. *Pharm Res* 18: 238-242, 2001.
- Stathopoulos GP, Boulikas T, Vougiouka M, Delicostantinou G, Rigatos S, Darli E, Viliotou V and Stathopoulos JG: Pharmacokinetics and adverse reactions of a new liposomal cisplatin (Lipoplatin): phase I study. *Oncol Rep* 13(4): 589-595, 2005.
- Fielding RM, Mukwaya G and Sandhaus RA: Clinical and preclinical studies with low-clearance liposomal amikacin (MiKasome). *In: Long-circulating Liposomes: Old Drugs, New Therapeutics*. Woodle MC and Storm G (eds.), Landes Bioscience, Georgetown, Tex, pp. 213-225, 1998.
- Stathopoulos GP, Boulikas T, Vougiouka M, Rigatos SK and Stathopoulos JG: Liposomal cisplatin combined with gemcitabine in pretreated advanced pancreatic cancer patients: a phase I-II study. *Oncol Rep* 15(5): 1201-1204, 2006.
- Kurata T, Tamura T, Shinkai T, Ohe Y, Kunitch H, Kodama T, Kakinuma R, Matsumoto T, Kubota K, Omatsu H, Nishiwaki Y and Saiio N: Phase I and pharmacological study of paclitaxel given over 3h with cisplatin for advanced non-small cell lung cancer. *Jpn J Clin Oncol* 31(3): 93-99, 2001.

Received October 13, 2006

Revised December 13, 2006

Accepted December 18, 2006