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ORIGINAL ARTICLE

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Comparative pharmacokinetics, tissue distribution, and therapeutic effectiveness of cisplatin encapsulated in long-circulating, pegylated liposomes (SPI-077) in tumor-bearing mice

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Abstract *Purpose*: The pharmacokinetics (PK), biodistribution and therapeutic efficacy of cisplatin encapsulated in long-circulating pegylated (Stealth®) liposomes (SPI-077) were compared with those of nonliposomal cisplatin in two murine (C26 colon carcinoma and Lewis lung) tumor models. *Methods*: In therapeutic effectiveness studies, mice bearing murine C26 or Lewis lung tumors received multiple intravenous doses of SPI-077 or cisplatin in a variety of treatment schedules and cumulative doses. In the PK and biodistribution study, mice received a single intravenous bolus injection of 3 mg/kg of either SPI-077 or cisplatin 14 days after inoculation with 10^6 C26 tumor cells. Plasma and tissues were analyzed for total platinum (Pt) content by graphite furnace (flameless) atomic absorption spectrophotometery (GF-AAS). *Results*: Efficacy studies showed that SPI-077 had superior antitumor activity compared to the same cumulative dose of cisplatin. When lower doses of SPI-077 were compared to cisplatin at its maximally tolerated dose in Lewis lung tumors, equivalent SPI-077 antitumor activity was seen at only half the cisplatin dose. Higher cumulative doses of SPI-077 were well tolerated and had increased antitumor effect. SPI-077 PK were characterized by a one-compartment model with nonlinear (saturable) elimination, whereas cisplatin PK were described by a two-compartment model with linear elimination. SPI-077 had a 55-fold higher volume of distribution, 3-fold higher peak plasma levels, and a 60-fold larger plasma AUC compared with cisplatin. In addition, SPI-077-treated animals displayed a 4-fold reduction in Pt delivered to the kidneys (primary target organ of toxicity) relative to cisplatin, but a 28-fold higher tumor AUC than cisplatin. *Conclusions*: Based on the results of our studies, encapsulation of cisplatin in long-circulating pegylated liposomes has overcome limitations experienced with other liposomal cisplatin formulations. SPI-077 has a prolonged circulation time and increased tumor Pt disposition, and its antitumor effect is significantly improved compared to cisplatin in murine colon and lung cancer models.

Key words Cisplatin · Stealth® liposomes · SPI-077® · C26 colon · Lewis lung

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Introduction

Cisplatin (cis-dichlorodiammineplatinum (II); CDDP) is one of the most widely used agents in the treatment of a variety of solid tumors, particularly genitourinary, head and neck and lung tumors [3]. However, expansion of the clinical utility of cisplatin has been limited by its toxicity, as well as the emergence of intrinsic and acquired resistance in many common tumor types. A variety of strategies have been implemented to circumvent cisplatin-related toxicity although no strategy has been successful in preventing the nephrotoxicity and cumulative neurotoxicity that emerge with continued cisplatin treatment [7, 15]. Extensive efforts have been devoted to developing platinum analogues to which tumors do not display cross-resistance [8, 11, 12, 18], focusing on the development of platinum derivatives that are less toxic and more active than the parent compound [12, 17].

The development of liposomal formulations that contain cisplatin has been hampered by poor water solubility and low lipophilicity of the free drug, which result in unstable liposome formulations that leak their contents rapidly during storage or within the bloodstream. SPI-077 (Stealth® liposomal cisplatin) is a formulation of cisplatin encapsulated in sterically stabilized, long-circulating liposomes containing methoxypolyethylene glycol (MPEG). With sterically stabilized liposomes, the plasma pharmacokinetics and tissue distribution of the encapsulated material is characteristic of the liposome, not the

internalized drug, i.e. the behavior of the liposomes is similar regardless of their encapsulated contents [20]. Because of their small size, long circulation time and reduced interaction with formed elements of the blood, stable formulations of sterically stabilized liposomes tend to accumulate in tumors, presumably due to leakage through the often compromised tumor vasculature [5, 22]. Often, much higher doses of liposome-encapsulated material can be administered without toxicity, but with increased efficacy leading to changes in the safety and therapeutic effectiveness profile of an established chemotherapeutic agent [6, 21]

In this report we describe SPI-077, a new sterically stabilized liposome containing cisplatin. The in vitro plasma release rate, the in vivo pharmacokinetics and biodistribution, as well as the therapeutic effectiveness of SPI-077 relative to cisplatin in mice bearing C-26 colon or Lewis lung tumors were studied.

Materials and methods

Test articles

SPI-077 (Stealth® liposomal cisplatin) liposomes are comprised of hydrogenated soy phosphatidylcholine, methoxypolyethyleneglycol-distearoyl phosphatidyl-ethanolamine and cholesterol at an approximate 51:5:44 molar ratio. Briefly, preparation of the liposomes begins by dissolving the lipid components in ethanol followed by addition to an aqueous cisplatin solution. The resulting liposomes are then sized by extrusion through polycarbonate membranes and diafiltered to removed unencapsulated cisplatin. The process produces liposomes with an average particle size of 110 nm, no unencapsulated drug in the stored preparation, a final drug concentration of 1 mg/ml and a drug to lipid ratio of 0.014.

Cisplatin (Platinol AQ®; Bristol Laboratories, Princeton, N.J.) is commercially available as a 1.0 mg/ml liquid solution.

In vitro leakage studies of SPI-077 liposomes

A 1 mg/ml stock solution of SPI-077 was used to spike EDTA-treated plasma at four different concentrations (70, 100, 200, and 500 μg/ml) which was then incubated at 37 °C for 24 h. The separation and quantitation of unencapsulated platinum (Pt) from liposome-encapsulated Pt was accomplished using a Sepharose CL-4B column followed by graphite furnace atomic absorption spectrometery (GF-AAS). Analytical determinations were done prior to incubation and 6 and 24 h thereafter.

C-26 and Lewis lung tumor models

Tumors were harvested from donor mice and minced finely in RPMI medium (Roswell Park Memorial Institute) with 10% fetal calf serum (FCS). Tumors were then digested with enzyme mix (10 ml 0.25% protease type IX and 0.25% collagenase type IV in Hank's balanced salt solution (HBSS) and 0.2 ml 0.02% DNase in HBSS) for 30–60 min at 37 °C. A single cell suspension of tumor cells was washed twice in RPMI medium with FCS, concentrated by centrifugation and resuspended in RPMI medium with FCS for inoculation. For C-26 tumors, 10^6 tumor cells (0.10 ml) were inoculated into the left flank of 5–6-week-old 20–25-g male Balb/c mice (Simonsen Laboratories, Gilroy, Calif.). Tumors were allowed to grow until all inoculated mice had palpable tumors (10–14 days postimplantation).

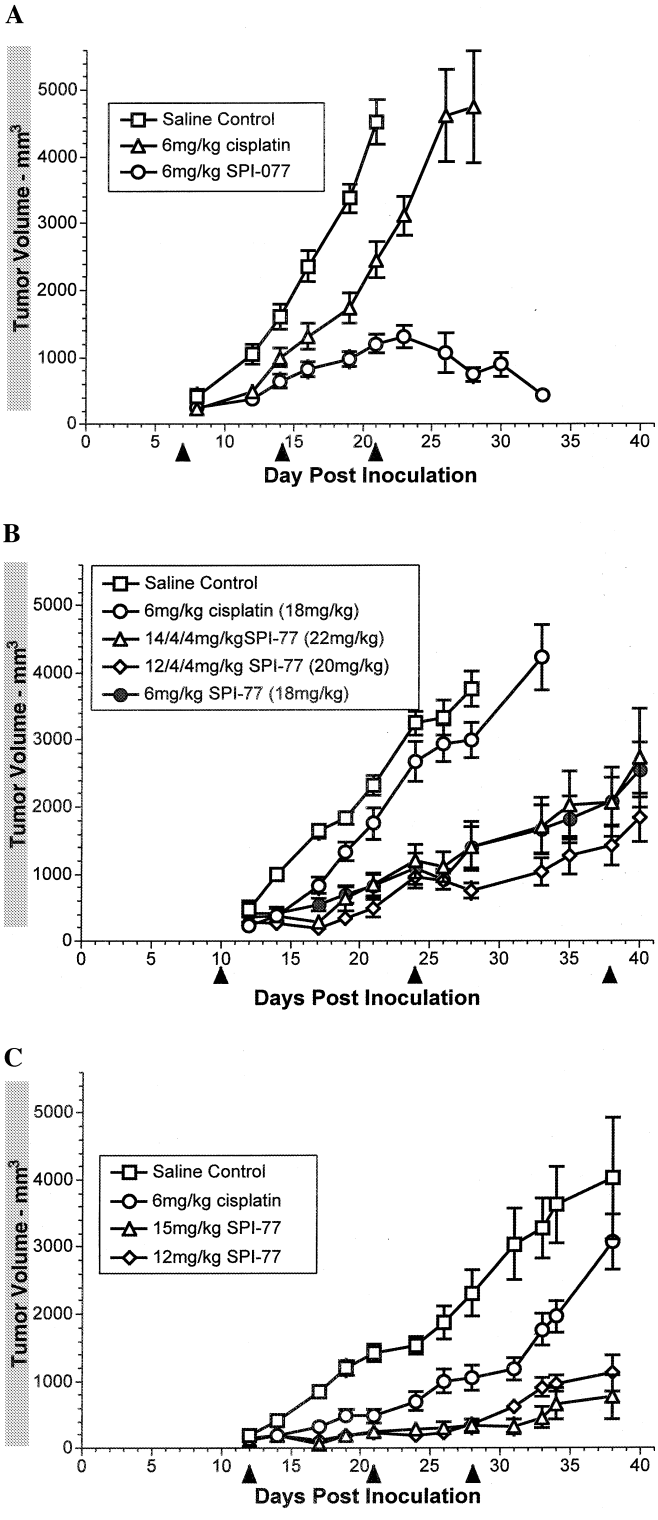


Fig. 1A–C Antitumor efficacy of SPI-077 and nonliposomal cisplatin against C26 colon carcinomas in Balb/c mice. All treatments administered intravenously at arrowheads. Data presented are means ± standard error. **A** C26-1 ($n = 16$ mice/group); **B** C26-2 ($n = 10$ mice/group); **C** C26-3 ($n = 10$ mice/group)

The Lewis lung tumor (LL/2, CRL-1642, ATCC, Rockville, Md.) model was grown in B6C3-F1 male mice (Simonsen Laboratories, Gilroy, Calif., or Taconic Farms, Germantown, N.Y.).

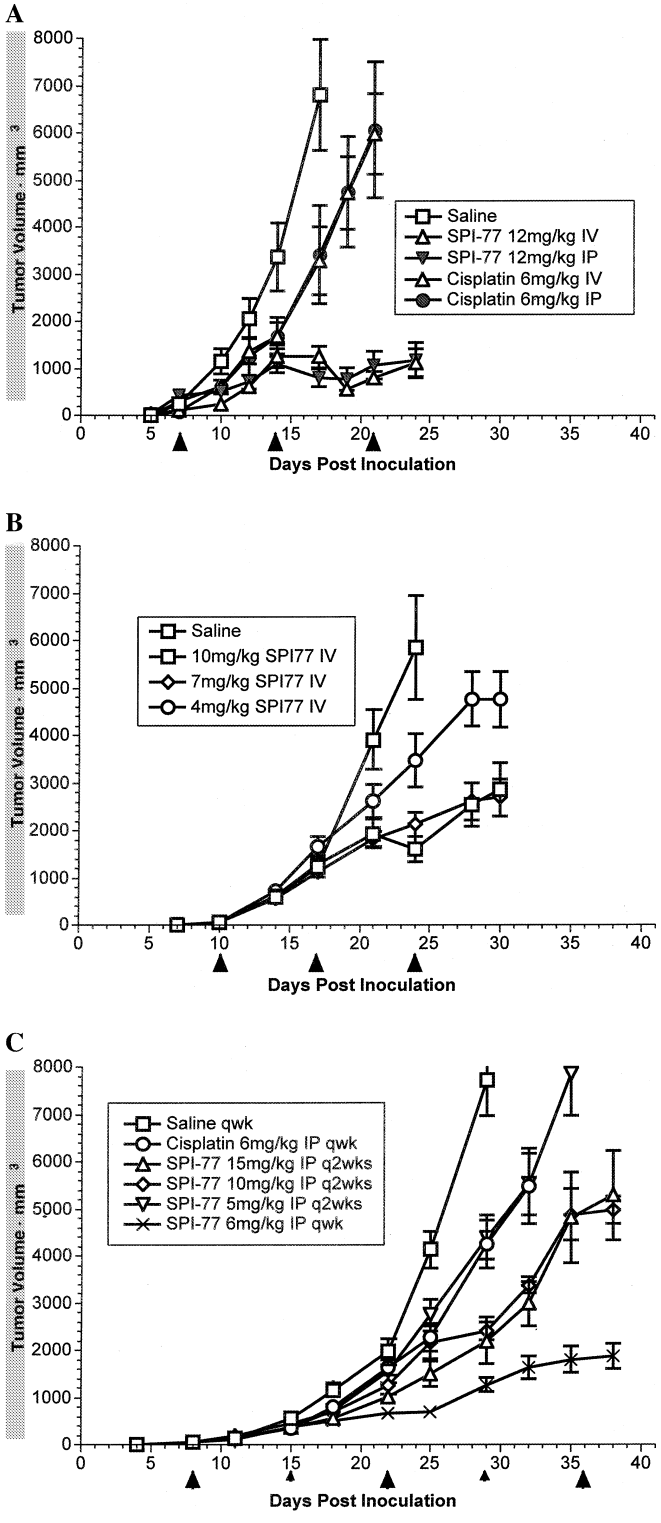


Fig. 2A–C Antitumor efficacy of SPI-077 and nonliposomal cisplatin against Lewis lung carcinomas in B6C3-F1 mice. Treatments administered intravenously or intraperitoneally (as indicated in the figure) at arrowheads. Data presented are means ± standard error of 10 mice/group. **A** LL-1; **B** LL-2; **C** LL-3

Tumors were harvested from donor mice and processed as above. Tumor cells (10^6, 0.1 ml) were inoculated into the left flank of 5–6-week-old male B6C3-F1 mice in each experiment.

In vivo antitumor activity

In all studies, animals were maintained in 12-h light 12-h dark cycles, had access to rodent chow and water *ad libitum*, and housed according to the guidelines of the Institute of Laboratory Animal Research (ILAR) Guide (1996) and the SEQUUS Institutional Animal Care and Use Committee. Animals were euthanized using the guidelines of the American Veterinary Medical Association (AVMA) Panel on Euthanasia of Laboratory Animals at the conclusion of each experiment or if the animal had greater than 15% loss of body weight from experiment initiation or tumor volume greater than 6000 mm^3.

Animals were observed daily for general well being. Tumor volume and body weights were measured for individual animals three times weekly in all experiments. Tumors were measured in three dimensions and tumor volume (mm^3) calculated as the product of these dimensions. Treatment was initiated as soon as a majority of experimental animals had palpable tumor masses ($\sim$100 mm^3). Treatment regimens for all studies are listed in Figs. 1 and 2.

Pharmacokinetics and biodistribution studies

On the day of dosing, mice received a 3 mg/kg dose of SPI-077 or cisplatin by tail vein injection (6–10 ml/kg). Doses were based on individual animal body weight. Animals were euthanized (two to four per time-point) by inhalation of isoflurane anesthetic at 0.05, 1, 4, 8, 24, 96, and 168 h after dosing. Blood was collected immediately from the aorta into heparinized tubes and centrifuged to obtain plasma. Liver, spleen, kidneys and tumors were removed, washed with saline, blotted dry and weighed prior to freezing. Plasma and tissues were stored frozen (−80 °C) until analysis.

Plasma and tissue analysis

Sample preparation

Plasma samples were diluted with acidified (0.05% nitric and 0.05% hydrochloric acid) water and analyzed directly. Tissue samples were digested in concentrated hydrochloric and nitric acid prior to analysis. After cold, overnight incubation in the acid mixture, the tissue slurry was gently heated to achieve partial digestion. Samples were cooled and hydrogen peroxide was added followed by heating to complete the digestion.

Assay methodology

Total Pt concentrations were determined in plasma or tissue using a sensitive and specific GF-AAS assay. The assay did not differentiate between liposomal and nonliposomal Pt or between free and protein-bound Pt. The linear range of the assay in plasma and tissues was 0.05 to 0.40 μg/ml, and the sensitivity was 0.05 μg/ml. The interday and intraday assay variability for plasma and tissues did not exceed 8.3% and 11%, respectively.

Pharmacokinetic analysis:

Pharmacokinetic parameter values were obtained by modeling the pooled plasma concentration versus time data using iterative nonlinear weighted regression with maximum likelihood estimation. The Pt concentrations were weighted by assuming that the residual ('error') standard deviations of the concentrations (σ) were linearly related to the true values (Y): $\sigma = \upsilon_1 Y + \upsilon_2$, in which υ_1 and υ_2 are the variance parameters [4]. In this analysis, candidate structural population models and a model for the residual variance of the observations, (i.e. plasma concentrations), were fitted to the experimental data. Model discrimination was accomplished using the Rule of Parsimony and Akaike's Information Criterion (AIC). Numerical integration was used to calculate the plasma area-under-

the-curve (AUC) and the trapezoidal rule was employed to determine tumor AUC.

Statistical analysis

SAS procedure PROC MIXED with unspecified covariance structure was used to evaluate treatment and time effects on tumor growth (repeated measurement analysis). The log growth rate was estimated for each treatment group and used in the statistical analysis. Differences with P-values <0.05 were considered significant with adjustments for type I error.

Results

In vitro studies

No detectable leakage of Pt occurred at any of the SPI-077 concentrations evaluated at any time-point. These findings suggest that the SPI-077 liposomes are stable in plasma and that the Pt measured in the circulation during the pharmacokinetic studies is liposome-encapsulated. A large amount of Pt in the plasma would indicate a high rate of leakage from the liposomes.

In vivo antitumor activity

C26 colon carcinoma

Initial experiments compared the antitumor activity of nonliposomal cisplatin at its maximally tolerated dose (MTD; 6 mg/kg weekly $\times$ 3) to the same dose of SPI-077. SPI-077 treatment resulted in a log growth rate of 0.03 which was significantly less than cisplatin-treated tumors, which had a log growth rate of 0.07 ($P < 0.0001$). Tumors in the saline-treated control mice had a log growth rate of 0.09, which was not significantly different from that in cisplatin-treated mice ($P = 0.0951$) but was significantly greater than in SPI-077-treated mice ($P < 0.0001$; Fig. 1A).

The dose schedule and total dose of SPI-077 was altered in subsequent experiments to characterize further its MTD and optimal dosing schedule. Administering a large loading dose (12 mg/kg) followed by two lower doses (4 mg/kg each) given at 2-week intervals resulted in similar antitumor activity (log growth rate of 0.030) to treatment with 6 mg/kg SPI-077 every other week (log rate of 0.033; $P = 0.812$), perhaps owing to the similar cumulative dose (Fig. 1B). In another study, weekly treatment with SPI-077 at its MTD (15 mg/kg $\times$ 3) had significantly greater antitumor activity (log growth rate of 0.031) compared to cisplatin at its MTD (log growth rate of 0.055; $P = 0.0033$; Fig. 1C). The antitumor efficacy of a moderate dose of SPI-077 (12 mg/kg weekly $\times$ 3; log growth rate of 0.040) was slightly but not significantly ($P = 0.3163$) less than that of the 15 mg/kg dose.

Lewis lung carcinoma

At its MTD (15 mg/kg weekly $\times$ 3), SPI-077 produced a log growth rate of 0.096 by intravenous administration and 0.036 by intraperitoneal administration. SPI-077 given by both routes had significantly greater antitumor efficacy compared to cisplatin at its MTD (6 mg/kg weekly $\times$ 3), which produced log growth rates of 0.171 by intravenous administration and 0.210 by intraperitoneal administration ($P < 0.0001$; Fig. 2A). In this study, SPI-077 and cisplatin were administered by intravenous or intraperitoneal injection to evaluate infusion rate-related toxicity of SPI-077. Both routes of administration resulted in similar antitumor efficacy for both agents. The toxicity of SPI-077 was somewhat less when administered by intraperitoneal injection, with reduced weight loss over the course of the experiment (data not shown) while cisplatin toxicity was not affected by the route of administration.

Subsequent studies in Lewis lung tumor-bearing mice demonstrated a dose response for SPI-077, with 10 mg/kg SPI-077 (log growth rate of 0.111) having more antitumor activity than 7 mg/kg SPI-077 (log growth rate of 0.113) and 4 mg/kg SPI-077 (log growth rate of 0.124) when each was given weekly for 3 weeks. All SPI-077 treatments were significantly more active than saline (log growth rate of 0.174; $P < 0.0009$), but no SPI-077 dose level was significantly different from another (Fig. 2B). SPI-077 at 6 mg/kg given weekly for 5 weeks (log growth rate of 0.07) had superior antitumor activity to cisplatin given at 6 mg/kg on the same schedule (log growth rate of 0.12; $P < 0.0001$; Fig. 2C). SPI-077 at 15 mg/kg given every other week for three treatments had significant antitumor activity (log growth rate of 0.09), but was not as effective as 6 mg/kg SPI-077 given weekly, despite the higher cumulative dose ($P = 0.0065$). SPI-077 at 5 mg/kg given every other week for three treatments (log growth rate of 0.11) had similar antitumor activity to cisplatin at 6 mg/kg given weekly for five treatments ($P < 0.092$).

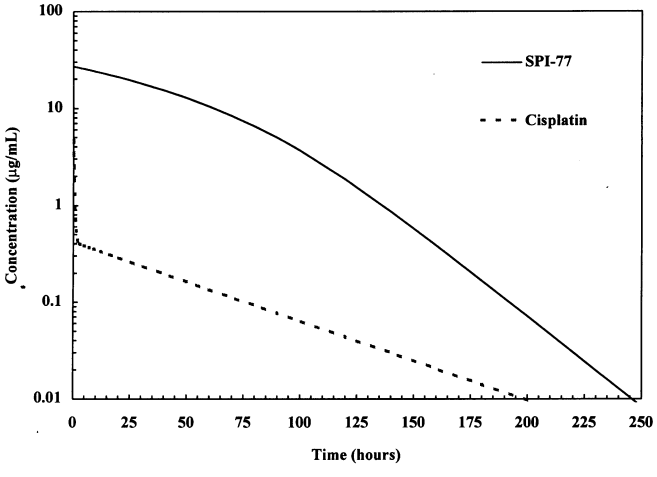


Fig. 3 Predicted concentration-time profile of Pt in plasma following SPI-077 or cisplatin administration

Table 1 Pharmacokinetic parameter estimates for SPI-077 and cisplatin (CisPt) in C26 colon tumor bearing mice (see text for definitions of parameters). *NA* not applicable

Drug	Vc (l/kg)	Vss	CLd	CLt (ml/h/kg)	CLi	Km (μg/ml)	AUC (μg/ml · h)	$\alpha t_{1/2}$ (h)	$\beta t_{1/2}$ (h)
SPI-077	NA	0.11	NA	NA	4.8	10	1461	NA	16[a]
CisPt	0.56	6.2	1347	125	NA	NA	24	0.24	37

[a] Apparent half-life

Pharmacokinetics

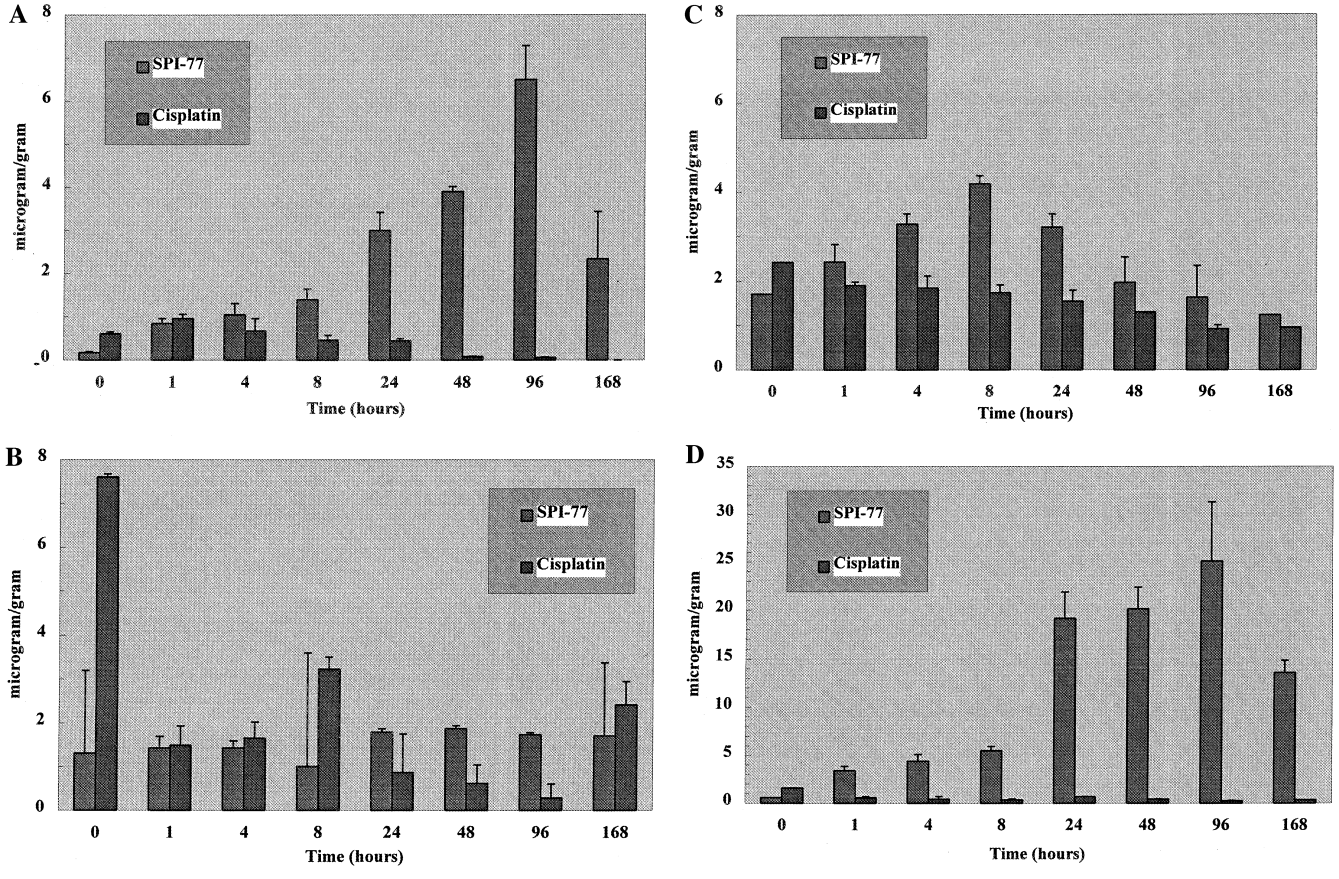
SPI-077 was cleared from the circulation much more slowly than cisplatin (Fig. 3). The SPI-077 plasma Pt concentration (Cp) versus time data were characterized best by a one-compartment model with nonlinear (saturable) elimination. The parameters used to describe the nonlinear pharmacokinetic model were the volume of distribution at steady-state (Vss), intrinsic clearance (CLi), and the Michaelis-Menten constant (Km), where the product of CLi and Km equals the maximum velocity or rate of elimination (Vmax) of drug. Km is the plasma concentration of Pt at which the rate of elimination is 50% of Vmax. The following equation describes the rate of elimination of platinum from the plasma following administration of SPI-077:

$$\text{Rate of elimination (mg/h)} = \frac{\text{CLi} \cdot \text{Km} \cdot \text{Cp}}{\text{Km} + \text{Cp}}$$

At plasma concentrations far below the Km value the rate of elimination approximates linear pharmacokinetics (i.e. a twofold increase in dose results in a proportionate increase in concentrations), whereas, at concentrations exceeding Km, the rate of elimination approaches Vmax, thus becoming saturable. Because SPI-077 exhibited nonlinear pharmacokinetics, the half-life could not be determined in SPI-077-treated animals; however, at plasma concentrations far below the Km value, an "apparent" half-life could be estimated by determining the slope of the line between the two final time-points.

The cisplatin plasma data were characterized best by a two-compartment model with linear elimination. The

Fig. 4A–D Concentration-time profile of Pt in tumor (**A** C26 murine colon carcinoma) and various tissues (**B** kidney, **C** liver, **D** spleen) following a single i.v. dose of SPI-077 or cisplatin. Values indicated are means + SD



parameters used to describe the model were the following: Vc – volume of distribution of the central compartment, Vp – volume of distribution of the peripheral compartment (Vss = Vc + Vp), CLd – distributional clearance between the central and peripheral compartments, and CLt – total plasma clearance.

The maximum concentration observed (Cmax) was higher in SPI-077-treated mice (30 μg/ml) than in mice treated with the same dose of cisplatin (8.6 μg/ml), and the AUC was more than 60-fold greater in the SPI-077-treated animals than in the cisplatin-treated animals (Table 1). The elimination rate of SPI-077 and volume of distribution were smaller in SPI-077-treated mice, and the plasma half-life was similar in SPI-077- and cisplatin-treated mice. However, the terminal half-life in the cisplatin-treated mice represented less than 5% of the AUC, and in SPI-077-treated mice represented more than 90% of the AUC. This difference in half-life and associated AUC between formulations was due to the persistence of protein-bound Pt following cisplatin administration, whereas the plasma Pt measured following SPI-077 represented liposome encapsulated drug.

Tissue distribution

Peak Pt levels were measured in most tissues within 1 hour of cisplatin treatment and, with the exception of the kidney, Pt concentrations were generally lower than those following treatment with the same dose of SPI-077 (Fig. 4). Peak tissue Pt levels were measured much later following SPI-077 treatment (48–96 h after treatment) and remained elevated at the end of the study. Organs responsible for removing SPI-077 liposomes from the bloodstream, namely the liver and spleen, had elevated Pt levels (1.7- to 16.2-fold higher than in cisplatin-treated mice), while the kidney, the primary target organ of cisplatin toxicity, had more than fourfold lower Pt levels than in cisplatin-treated mice.

Tumor Pt levels following SPI-077 treatment, which peaked at 96 h after treatment, were more than 6.7-fold higher than following cisplatin treatment, which peaked at 1 h after treatment (Fig. 4). Tumor levels remained high in SPI-077-treated animals, with 2.3 μg Pt/g of tumor measured at 168 h after treatment. Tumor exposure as measured by the tumor AUC was 28-fold higher following SPI-077 administration (694 and 25 μg/g · h in SPI-077- and cisplatin-treated animals, respectively).

Discussion

Our studies showed that SPI-077 is a stable, long-circulating liposomal formulation of cisplatin with significantly improved antitumor activity relative to nonliposomal cisplatin. These results are consistent with other reports on the activity of sterically stabilized liposome-encapsulated anticancer agents, such as Doxil® and vincristine and represent an improvement over other liposomal cisplatin formulations that have been hampered by poor stability and encapsulation difficulties [1, 10, 18, 19]. Treatment with SPI-077 dose-dependently inhibited tumor growth, with apparently equivalent antitumor activity at half the administered cisplatin dose, an apparent twofold increase in antitumor effectiveness. At equivalent cumulative doses of SPI-077 and cisplatin, SPI-077 demonstrated superior antitumor efficacy in all cases. Increasing the cumulative dose of SPI-077 could further improve the antitumor effects.

SPI-077 remained in the bloodstream significantly longer than nonliposomal cisplatin, as reflected by differences in plasma AUC. After dosing, the majority of the administered SPI-077 dose was found in the plasma, and secondarily in the organs of the mononuclear phagocytic system (MPS; liver and spleen). SPI-077 pharmacokinetics appeared similar to those of drugs encapsulated in sterically stabilized liposomes, with a high AUC and slow clearance rate. However, SPI-077 pharmacokinetics were best characterized by a nonlinear (saturable) model. Previous studies on Doxil have shown linear pharmacokinetics at low Doxil doses (10, 20 or 40 mg/m^2) [14], although more recent studies indicate that Doxil exhibits nonlinear or saturable pharmacokinetics at higher doses [2]. Saturable kinetics may be affected by a lower drug-to-lipid ratio in the SPI-077 formulation relative to Doxil. The overall impact of saturable kinetics remains to be determined, although no obvious impact has been observed in phase I clinical trials with SPI-077 thus far [16].

Although high amounts of Pt were measured in plasma and tissues with SPI-077 treatment, cisplatin-associated toxicity did not appear to increase. In fact, preliminary studies with SPI-077 have shown that mice tolerate a single intravenous SPI-077 dose up to 100 mg/kg without mortality (data not shown). This compares to the single dose LD_{50} for cisplatin in mice of 12 mg/kg. While the antitumor activity of cisplatin depends on its total administered dose and cumulative AUC [9], its toxicity appears related to peak plasma Pt concentration, more specifically, peak filterable Pt concentration [13]. While a high concentration of Pt in plasma ultrafiltrate (PUF) positively correlates with antitumor effect, lower levels correlate with reduced cisplatin toxicity. SPI-077 presumably avoids a high peak plasma concentration of free, filterable cisplatin, since most of the drug is retained in the liposome during circulation gradually releasing with time. Assuming that the release of cisplatin from SPI-077 liposomes remains constant, the tumor and other tissues, as well as plasma, are continuously exposed to a low-dose cisplatin environment, and the amount of filterable Pt remains proportionately low. Based on the superior antitumor activity of SPI-077 in the tumor models, it seems reasonable to suggest that levels of Pt in the PUF following SPI-077 administration are sustained, if not increased, relative to the same dose of cisplatin. Further pharmacokinetic studies will be required to confirm this supposition.

By delivering more cisplatin to tumor tissue with SPI-077, the antitumor effect of the encapsulated cisplatin can be directed locally at the tumor site. Toxicity, in contrast, can be ameliorated by lowering the peak exposure of target organs to unencapsulated cisplatin. Platinum levels in the kidney, a target organ of cisplatin toxicity, were significantly lower in animals treated with SPI-077 than in those that received the same dose of cisplatin. Tumor Pt levels, however, were significantly higher in animals treated with SPI-077 most likely because of its prolonged circulation and the tendency of the small liposomes to extravasate in tumors [5]. While Pt concentration in the liver and spleen were both high in animals treated with SPI-077, there were no associated increases in hepatic or splenic toxicity.

In summary, encapsulation of cisplatin in sterically stabilized liposomes has overcome many of the limitations experienced with other liposomal formulations. Circulation time is lengthened, tumor disposition of Pt is increased and the antitumor effect of SPI-077 is enhanced compared to nonliposomal cisplatin. Based on the results of these studies, Stealth® liposomal cisplatin, SPI-077, is more active than cisplatin in murine colon and lung cancer models. Owing to its reduced toxicity, higher cumulative doses of SPI-077 can be administered without significant detrimental effect, potentially greatly enhancing the clinical utility of SPI-077 for the treatment of a variety of cancers.

References

1. Allen TM, Newman MS, Woodle MC, Mayhew E, et al (1995) Pharmacokinetics and anti-tumor activity of vincristine encapsulated in sterically stabilized liposomes. Int J Cancer 62: 199
2. Amantea MA, Forrest A, Northfelt DW, Mamelok R (1997) Population pharmacokinetics and pharmacodynamics of pegylated liposomal doxorubicin in pateints with AIDS-related Kaposi's sarcoma. Clin Pharmacol Ther 61: 301
3. Comis RL (1994) Cisplatin: the future. Semin Oncol 21: 109
4. D'Argenio, Schumitzky A (1979) A program package for simulaiton and parameter estimation in pharmacokinetic systems. Comput Programs Biomed 9: 115
5. Dvorak HF, Nagy JA, Dvorak JT, Dvorak AM (1988) Identification and characterization of the blood vessels of solid tumors that are leaky to circulating macromolecules. Am J Pathol 133: 95
6. Gabizon A, Catane R, Uziely B (1994) Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res 54: 987
7. Hacker MP (1991) Toxicity of platinum-based anticancer drugs. In: Powis G, and Hacker MP (ed) The toxicity of anticancer drugs. McGraw-Hill, New York, p 82
8. Kelland RL, McKeage MJ (1994) New platinum agents. A comparison in ovarian cancer. Drugs Aging 5: 85
9. Kurihara N, Kubota T, Hoshiya Y, Otani Y, et al (1996) Antitumor activity of cis-diamminedichloroplatinum (II) against human tumor xenografts depends on its area under the curve in nude mice. Clin Res 26: 436A
10. Lasic DD (1996) Doxorubicin in sterically stabilized liposomes. Nature 380(6574): 561
11. McKeage MJ, Boxall F,Jones M (1994) Lack of neurotoxicity or oral *bis*-acetat-ammine-dichloro-cyclohexylamine-platinum(IV) (JM216) in comparison to cisplatin and tetraplatin. Cancer Res 54: 629
12. McKeage MJ, Mistry P, Ward J, Boxall FE, et al (1995) A phase I and pharmacological study of an oral platinum complex (JM216): dose-dependent pharmacokinetics with single dose administration. Cancer Chemother Pharmacol 36: 451
13. Nagai N, Ogata H (1997) Quantitative relationship between pharmacokinetics of unchanged cisplatin and nephrotoxicity in rats: importance of area under the concentration-time curve (AUC) as the major toxicodynamic determinant in vivo. Cancer Chemother Pharmacol 40: 11
14. Northfelt DW, Martin FJ, Working PK, Volberding PA, et al (1996) Doxorubicin encapsulated in liposomes containing surface-bound polyethylene glycol: pharmacokinetics, tumor localization and safety in patients with AIDS-related Kaposi's sarcoma. J Clin Pharmacol 36: 55
15. Ogilvie GK, Fettman MJ, Jameson VJ, Walters LM, et al (1992) Evaluation of a one-hour saline diuresis protocol for administration of cisplatin to dogs. Am J Vet Res 53: 1666
16. Schellens JHM, Meerum T, Groenewegen G, et al (1998) Phase I and pharmacologic study of SPI-77, a novel Stealth® liposomal encapsulated formulation of cisplatin (CDDP). Eighty-ninth Annual Meeting, Proceedings of American Association for Cancer Research, New Orleans
17. Schilder RJ, LaCreta FP, Perez RP (1994) Phase I and pharmacokinetic study of ormaplatin (tetraplatin, NSC363812) administered on a day 1 and 8 schedule. Cancer Res 54: 709
18. Steerenberg PA, Storm G, deGroot G, Claessen A, et al (1988) Liposomes as a drug carrier system for cis-diamminedichloroplatinum(II). II. Antitumor resistance in vivo, induction of drug resistance, nephrotoxicity and Pt distribution. Cancer Chemother Pharmacol 21: 299
19. Vaage J, Donovan D, Uster P, Working P (1997) Tumour uptake of doxorubicin in polyethylene glycol-coated liposomes and therapeutic effect against a xenografted human pancreatic carcinoma. Br J Cancer 75: 482
20. Woodle MC, Lasic DD (1992) Sterically stabilized liposomes. Biochem Biophys Acta 1113: 171
21. Woodle MC, Newman MS, Working PK (1995) Biological properties of sterically stabilized liposomes. In: Lasic DD, Martin FM (ed) Stealth liposomes. CRC Press, Boca Raton, p 103
22. Wu NZ, Dewhirst MW (1993) Measure of tissue uptake of intravenously injected macromolecules using fluorescence video-microscopy. Microvasc Res 46: 231