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Preclinical report

Pharmacological studies of cisplatin encapsulated in long-circulating liposomes in mouse tumor models

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We investigated the pharmacokinetics and therapeutic efficacy of cisplatin encapsulated in polyethyleneglycol-coated long-circulating liposomes in a formulation referred to as SPI-077, in three mouse tumor models (M-109 lung carcinoma inoculated s.c., J-6456 lymphoma inoculated i.p. and A-375 melanoma inoculated s.c.). Tumor-bearing mice were injected i.v. with single doses of SPI-077 and cisplatin. For pharmacokinetic experiments, mice were sacrificed at different timepoints post-treatment. Platinum levels were determined in plasma, spleen, liver, kidneys and tumors using flameless atomic absorption spectrophotometry. Survival times and/or tumor size were recorded for therapeutic studies. The pharmacokinetic studies revealed a prolonged circulation time and enhanced tumor uptake for SPI-077. In contrast to these results, no superior antitumor activity of SPI-077 over cisplatin could be observed in all tumor models. *In vitro* release experiments showed a negligible release (below 10%) of platinum from the liposomes. An *in vitro* cytotoxicity assay indicated a reduced cytotoxic activity of SPI-077 in comparison to cisplatin. We concluded that SPI-077 is being delivered to the tumor sites in a low bioavailability form, with extremely slow release kinetics. This explains the discrepant results of high platinum concentrations in tumors and reduced therapeutic activity after administration of SPI-077. [© 1999 Lippincott Williams & Wilkins.]

Key words: Cisplatin, liposomes, pharmacokinetics, SPI-077.

Introduction

Cisplatin [*cis*-dichlorodiamine platinum(II)] is a widely used anticancer agent. However, its antitumor activity is limited by undesirable side effects, e.g. nephrotoxicity, ototoxicity, neurotoxicity and to a lesser extent myelosuppression.[1,2] Among several attempts to reduce cisplatin-induced toxicities is the encapsulation of cisplatin in liposomes. Entrapment of drugs in liposomes modifies their pharmacokinetics, organ distribution and metabolism, leading in the case of doxorubicin, for example, to the prevention of some organ toxicities, such as doxorubicin cardiotoxicity and nephrotoxicity.[3] *In vitro* studies with a lipophilic cisplatin analog, *cis*-bis-neodecanoato-*trans*-*R*,*R*-1,2-diaminocyclohexane platinum(II) (NDDP) encapsulated in large multilamellar liposomes, showed a significantly higher cytotoxicity for the liposomal lipophilic cisplatin analog in comparison to free cisplatin against A-2780 ovarian carcinoma cells.[4] *In vivo* studies in mice bearing RIF-1 fibrosarcoma revealed that NDDP was effective in reducing tumor growth rate in non-reticulo-endothelial system (RES) organs only when incorporated into long-circulating, polyethylene glycol-coated liposomes.[5] By encapsulating cytotoxic drugs, such as the anthracyclines, into long-circulating liposomes, a high tumor accumulation of the anticancer agent at the tumor site is achieved.[6-8] Doxil® is a formulation of doxorubicin encapsulated in polyethyleneglycol-coated, long-circulating liposomes, referred to as Stealth® liposomes, with superior antitumor activity and a reduced toxicity profile in comparison to the free drug.[9] Stealth liposomes are sterically stabilized, long-circulating liposomes which are not recognized by the RES and have minimal interactions with the plasma components. In a formulation referred to as SPI-077, cisplatin is encapsulated into Stealth liposomes, with the aim of improving the drug localization in tumors, reducing toxicity and boosting antitumor activity. Previous studies on SPI-077 have reported a greater antitumor activity[10,11] and reduced toxicity[12] for SPI-077 compared to free cisplatin in mouse tumor models, using multiple dose schedules.

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In the present study we evaluate the pharmacokinetics, biodistribution and therapeutic efficacy of SPI-077 in comparison to cisplatin in A-375 human melanoma in nude mice, and in the murine tumors M-109 lung carcinoma and J-6456 lymphoma in BALB/c mice using a single injection schedule.

Materials and methods

Drugs

Cisplatin (Abiplatin®) was purchased from Teva-Abic (Netanya, Israel) in 1 mg/ml solution ready for use. Stealth liposomal CDDP, SPI-077, was kindly provided by Sequus Pharmaceuticals (Menlo Park, CA) as a liposome suspension in buffer with a cisplatin concentration of 1 mg/ml. The phospholipids used in the preparation of Stealth liposomal cisplatin are similar to those of Doxil® with a slight modification.[10] SPI-077 liposomes contain hydrogenated soy phosphatidylcholine (HSPC):*N*-(carbamoylmethoxypolyethylene glycol 2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine sodium salt (mPEG-DSPE):Chol at a molar ratio of 51:5:44. Cisplatin is passively encapsulated with a final drug:lipid ratio of 14 μg cisplatin/mg lipid. The mean vesicle diameter of SPI-077 is 110 nm.

Animals

Female BALB/c mice and nude mice, 8–9 weeks old, were obtained from the Animal House of the Hebrew University Hadassah-Medical School (Jerusalem, Israel) and housed in a specific pathogen-free facility of the Hebrew University-Hadassah Medical School.

Cells

Tumor cells (A-375 human melanoma, M-109 mouse lung carcinoma and J-6456 mouse lymphoma) obtained from frozen tumor stocks were passaged in *in vitro* culture and expanded to appropriate numbers.

Pharmacokinetic studies

J-6456 lymphoma. In this model 10^6 tumor cells were injected i.p. to each mouse. Nine days following tumor inoculation, mice were injected i.v. with either cisplatin or SPI-077 at a dose of 10 mg/kg. Each experimental group consisted of three or four mice. Following drug injection, animals were bled through retro-orbital puncture, and sacrificed at 4, 24, 48 and 96 h after treatment. The peritoneal cavity was washed with 3 ml phosphate-buffered saline (PBS). Ascitic cells, mostly tumor cells, were pelleted out through centrifugation, washed twice, weighed and stored at −20 C. The supernatant (ascitic fluid, cell-free) was also stored at −20 C until analysis. Platinum levels were measured in plasma, ascitic fluid and ascitic cells.

M-109 carcinoma. In this model 10^6 cells were injected s.c. to the right and left flank of each mouse. Fourteen days following tumor inoculation mice were injected i.v. with either cisplatin or SPI-077 at 10 mg/kg. Each experimental group consisted of three or four mice. Following drug injection animals were bled through retro-orbital puncture, and sacrificed at 4, 24, 48 and 96 h after treatment. Liver, kidneys, spleen and tumors were removed and weighed. Blood was collected in heparinized tubes and centrifuged to obtain the plasma. Plasma and tissues were stored at −20 C until analysis. Platinum levels were determined in plasma, liver, kidneys, spleen and tumors.

Antitumor activity studies

Treatment was always i.v. via the tail vein route. Because of preliminary data indicating that SPI-077 is less toxic than cisplatin as a single injection, we chose a higher dose range for the former (10 and 20 mg/kg). Cisplatin was used at 5 and 10 mg/kg dose range. The 10 mg/kg dose level can only be given with concomitant administration of saline (1 ml/mouse, i.p. or s.c.) to thwart renal toxicity.[14] Statistical significance of differences in survival and tumor weights were analyzed by the non-parametric ranking test (log-rank test) and Mann-Whitney test, respectively, and expressed as two-sided p values.

J-6456 lymphoma. In this model, 5 days after i.p. inoculation of 10^6 cells, mice were treated with cisplatin or SPI-077. Each experimental group consisted of 10 mice. To evaluate therapeutic activity, survival times were recorded for a period of 60 days post-treatment. Immediately after treatment all mice received a single injection of 1 ml of physiologic saline s.c.

M-109 lung carcinoma. In this model BALB/c mice were inoculated with 10^6 cells in both flanks. Fourteen days later, when tumors became palpable as small nodules, mice were treated with SPI-077 and cisplatin. Each group consisted of 10 mice. All mice received a single injection of 1 ml physiologic saline i.p. imme-

diately after treatment. Thirty-two days after treatment mice were sacrificed, tumors dissected and weighed to evaluate therapeutic efficacy.

In an additional therapeutic experiment with M-109 carcinoma, the activity of SPI-077 was examined under intensive saline treatment [1 ml i.p. injection of hypertonic saline (3%) twice daily for five consecutive days] and compared to the above protocol of a single i.p. injection of saline 0.9%. Each experimental group consisted of eight mice. Thirty-two days after treatment, tumors were dissected, weighed and platinum concentrations in the tumors determined.

A-375 melanoma. In this model 0.8×10^6 A-375 cells were inoculated s.c. into each of both flanks of nude mice. Two weeks later when tumors became palpable as tiny nodules mice were treated i.v. Each experimental group consisted of at least six mice. Within each group mice were individually labeled, weighed and the two largest perpendicular diameters (a,b) of tumors measured with the help of a caliper. Tumor volume was calculated according to the equation $a \cdot b^2/2$. Mice were weighed and tumors measured twice a week. On week 9 after treatment mice were sacrificed. Statistical significance of the changes in tumor volume was calculated by the paired t-test.

In vitro experiments

The *in vitro* cytotoxic effect of cisplatin and SPI-077 was assayed by a methylene blue staining method using the human melanoma cell line A-375 as described previously.[15]

To examine the *in vitro* release of cisplatin from SPI-077 liposomes, SPI-077 was incubated with human plasma and with malignant effusions of cancer patients at a concentration of 70 μg Pt/ml at 37°C. Samples were taken after 0, 3 and 7 days of incubation, and run on a Biogel A 5 m (BioRad, Hercules, CA) column to separate liposomal from protein-bound from free platinum. Elution of platinum was done with saline and fractions of 1 ml were collected. Platinum was determined in the fractions as described below.

Determination of platinum in plasma, tissue samples, ascitic fluid and ascitic cells

Plasma, tissue samples and cells were digested with concentrated. HNO_3 at 85°C for 1 h in a Techne Dri Block heater (Techne, Cambridge, UK) followed by centrifugation. Supernatant was diluted with double-distilled water to yield a final platinum concentration in the range of 50–250 ng/ml. Platinum was determined using a Varian-Zeeman (Model Spectra AA-300; Varian Techtron, Mulgrave, Australia) fully automatic flameless atomic absorption spectrometer (FAAS), equipped with a heated graphite atomizer on which the temperature control was modified to produce a gradual increase in temperature reaching a maximum of 2900°C. This method is for total platinum metal and does not differentiate the chemical or biological state of platinum.

To determine the recovery of platinum, blank plasma and tissue samples were spiked with 20 μg cisplatin or SPI-077 for 1 h, followed by digestion with nitric acid and dilution with double distilled water as described above.

A calibration curve with platinum concentrations in the range of 50–250 ng/ml was run before analysis of each sample type. Values reported were the average of two separate platinum determinations for each sample. The recovery of platinum after incubation of plasma and tissues (kidney, liver and spleen) with cisplatin or SPI-077 was 85–95%.

Results

Pharmacokinetic experiments

J-6456 lymphoma. In the J-6456 lymphoma model platinum concentrations were determined in the plasma, ascitic fluid and ascitic cells at different time points after treatment. Mean and standard deviations (in μg) of total platinum are presented in Figure 1. SPI-077 injection results in a very high plasma concentration of platinum. At 4 h, the platinum concentration in plasma in the SPI-077 group is approximately 50-fold greater than in the cisplatin group. Platinum clearance from plasma suggests that the distribution of SPI-077 is mono-exponential with a half-life exceeding 40 h. This is almost twice as long as the half-life of Doxil in the same mouse strain.[13] This difference is probably related to the higher lipid load of SPI-077 that causes RES saturation. Ninety-six hours after treatment with SPI-077 high amounts of platinum are still found in plasma (20.5 ± 3.8 compared to 0.44 ± 0.26 μg/ml after treatment with cisplatin). Peak platinum concentrations in the ascitic fluid are achieved between 48 and 96 h after SPI-077 injection, and are 10-fold greater than the peak concentration after cisplatin injection (5.6 μg/peritoneal wash for SPI-077 versus

0.6 μg/peritoneal wash for cisplatin). Platinum concentration in the ascitic cells is initially greater for mice receiving cisplatin, but gradually the concentration increases in mice receiving SPI-077, reaching a peak at around 96 h post-injection. This peak is about 5-fold greater than the cisplatin peak (2.07±0.58 μg/g for SPI-077 versus 0.407±0.05 μg/g for cisplatin).

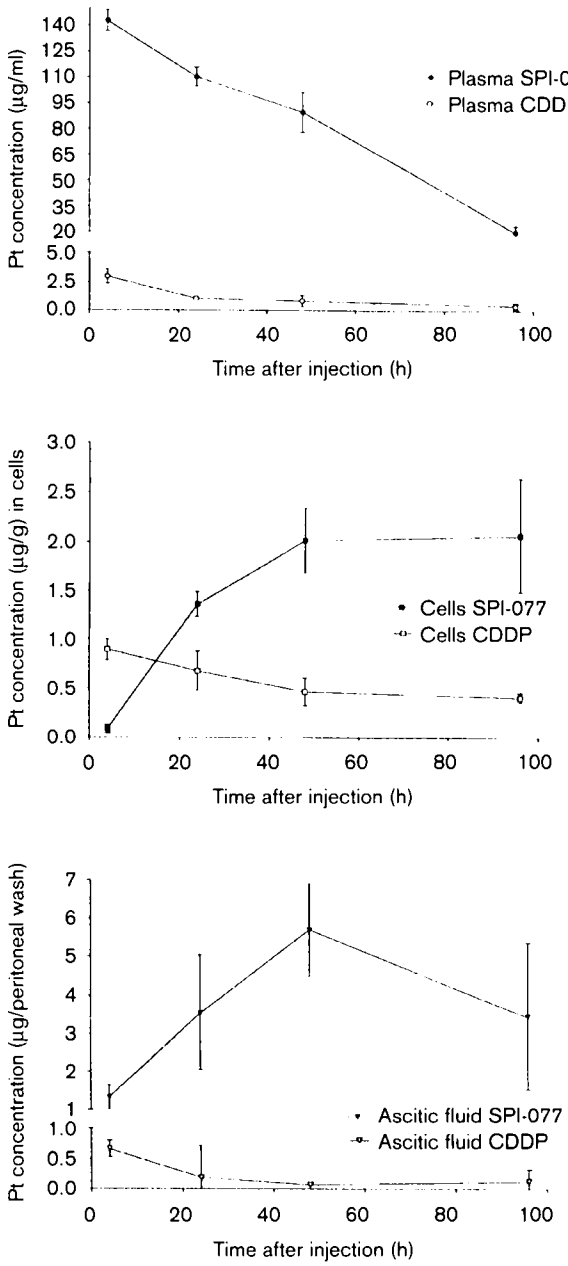

Figure 1. Pharmacokinetic profile of platinum in plasma, ascitic cells and ascitic fluid of female BALB/c mice bearing J-6456 lymphoma following treatment with either SPI-077 or cisplatin (CDDP) at 10 mg/kg.

To determine whether the platinum in the ascitic fluid is liposome-associated or free, we performed gel chromatography of some samples of the ascitic fluid to separate free from liposomal cisplatin on a Biogel A 5 m column. Platinum levels in all collected fractions were measured. Our results (data not shown) indicated that all the measurable platinum eluted in the liposome fractions, with no platinum detected in the free or protein-bound form. Since our calibration curve can detect 50 ng/ml platinum, the amount of platinum in the non-liposomal fraction must be extremely small, probably less than 5% of the total platinum in the ascitic fluid.

M-109 carcinoma. The plasma concentrations of platinum after injection of SPI-077 are similar to the platinum concentrations in the J-lymphoma experiment after injection of SPI-077 (see Figure 1). The half-life time of SPI-077 in plasma again exceeds 40 h. Figure 2 shows the tissue distribution of platinum in the liver, kidneys, spleen and tumors after administration of cisplatin or SPI-077.

The liver platinum values in the SPI-077 group show altogether minor changes between 4 and 96 h after injection, suggesting that the liver clearance becomes saturated within the first 4 h after injection. As expected, in the case of cisplatin the platinum peak values in the kidney, the excretory organ of cisplatin, are higher than those in the liver, spleen and tumor. In contrast, in the case of SPI-077, the platinum peak levels are highest in spleen, followed by tumor, liver and kidney in decreasing order. The spleen shows a continuous increase in platinum tissue concentration even by 96 h post-injection. This is again consistent with the saturation of liver and the concomitant ability of the spleen to clear liposomes in a non-saturable fashion by a filtration effect in the sinuses of the red pulp.[16]

The most striking effect in this biodistribution study is the increase in tumor concentration of platinum when SPI-077 is compared to cisplatin that reaches a 10-fold difference at 48 h. Even at 96 h post-injection high amounts of platinum (27.2±1.8 μg/g) are still found in tumors. Incidentally, the slight increase in platinum tumor concentration at 96 h after treatment with cisplatin is probably due to the relative shrinkage of the tumor mass due to the antitumor effect of cisplatin (see below). Our analytical methodology does not differentiate between encapsulated and free platinum, therefore we cannot decide in which state the platinum is present in the solid tumors. Double labeling experiments (lipid label and platinum label) may help clear this issue.

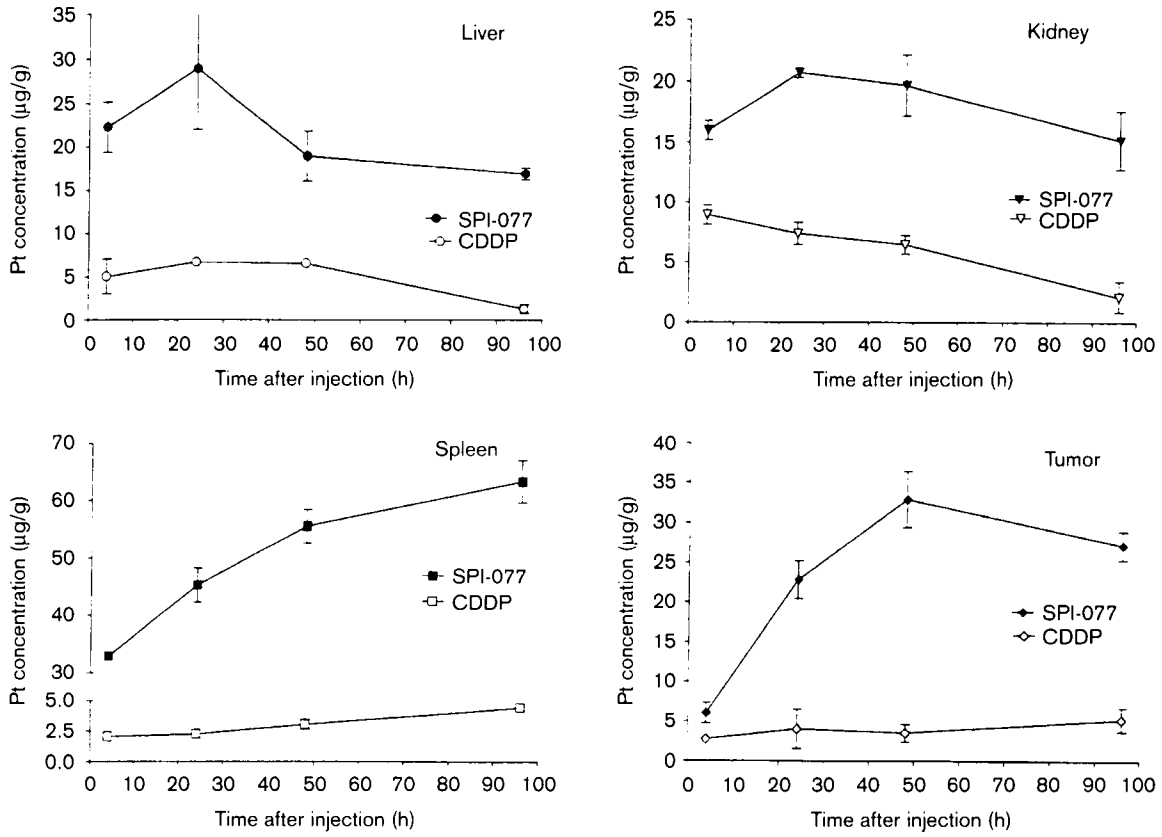

Figure 2. Pharmacokinetic profile of platinum in the liver, kidneys, spleen and tumors of female BALB/c mice bearing the s.c. M-109 tumor following treatment with either cisplatin (CDDP) or SPI-077 at 10 mg/kg.

Antitumor activity studies

J-6456 lymphoma. As can be seen in the survival curve in Figure 3, SPI-077 was ineffective in prolonging the survival at 10 and 20 mg/kg (17 versus 16 days for the control). Treatment with cisplatin produced a significant prolongation of the survival at the high dose, 10 mg/kg (50% increase in the median survival time, log rank test versus control; p=0.0003), while at 5 mg/ml cisplatin no significant increase in the survival time could be observed.

M-109 carcinoma. The greater efficacy of cisplatin over SPI-077 in this model is reflected in the number of the tumor-free (cured) animals and median tumor weight of mice receiving cisplatin (Figure 4). A dose-response effect is observed, with superior results for the high dose of cisplatin and SPI-077 over the respective lower doses. However, cisplatin 10 mg/kg was still more effective than SPI-077 20 mg/kg, despite the 2-fold difference in dose (p = 0.0175). In addition, platinum concentrations in the tumors were determined on day of sacrifice (day 32 post-treatment). For SPI-077 at 10 and 20 mg/kg the mean concentration of platinum was 2.2 ± 1.3 (n=18) and 3.9 ± 1.9 (n=11) µg/g tumor, respectively. For cisplatin at 5 and 10 mg/kg the mean concentrations were 0.15 ± 0.09 (n=6) and 0.27 ± 0.02 (n=3) µg/g tumor, respectively. When these results are compared to the 96 h values of platinum in tumors, we note that platinum levels have decreased by about 12-fold for SPI-077 (27.12 µg platinum after 96 h post-treatment versus 2.2 µg platinum after 32 days post-treatment) and by about 20-fold for cisplatin at 10 mg/kg (5.19 µg platinum after 96 h versus 0.27 µg platinum after 32 days).

Since cisplatin is known to be a highly reactive molecule, which degrades very easily in aqueous solution via nucleophilic replacement of the chloride ligands by water[17] and since the stabilizing effect of the chloride ion in the medium on the aquation reaction has proven to be effective in stabilizing cisplatin in solution,[13] we tested the effect of using intensive NaCl 3% treatment on the therapeutic efficacy of SPI-077 in the M-109 carcinoma model. Our results show that the intensive saline regime was

ineffective in augmenting the efficacy of SPI-077 when we examined the tumor weights of M-109 (Figure 5). The determination of platinum concentrations in the M-109 tumors 32 days after treatment showed that the mean platinum concentrations in the tumors of both groups receiving 10 mg/kg SPI-077 were similar (1.035 ± 0.59 μg/g for SPI-077+single saline injection and 1.31 ± 0.70 μg/g for SPI-077+multiple injections of NaCl 3%).

A-375 melanoma. Figure 6 compares the percent increase in tumor size in mice treated with SPI-077 and cisplatin. As can be seen, mice treated with SPI-077 at 10 and 20 mg/kg did not show any significant

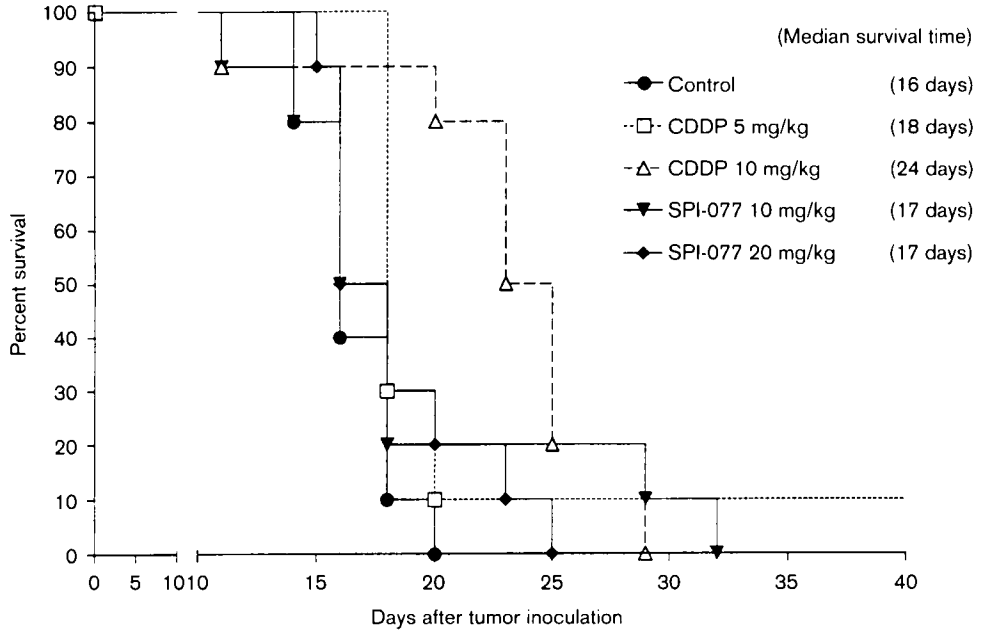

Figure 3. Survival curve of female BALB/c mice bearing the J-6456 lymphoma after i.v. treatment with different doses of either cisplatin (CDDP) or SPI-077.

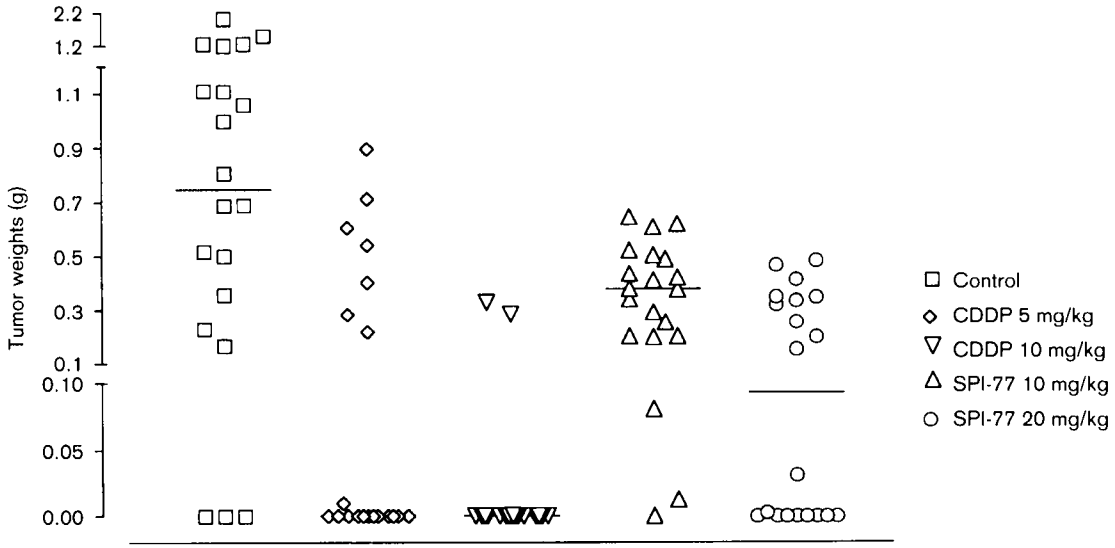

Figure 4. Distribution of M-109 tumor weights in female BALB/c mice, 32 days following treatment with either cisplatin (CDDP) or SPI-077 (horizontal lines represent median tumor weights).

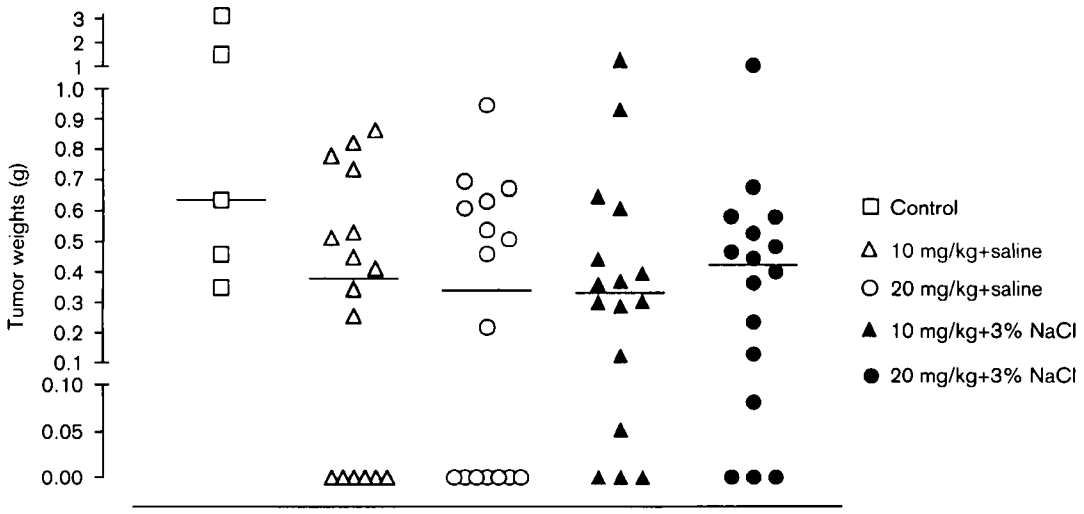

Figure 5. Distribution of tumor weights in female BALB/c mice inoculated with the M-109 tumor, 32 days post-treatment with SPI-077 under intensive hypertonic treatment.

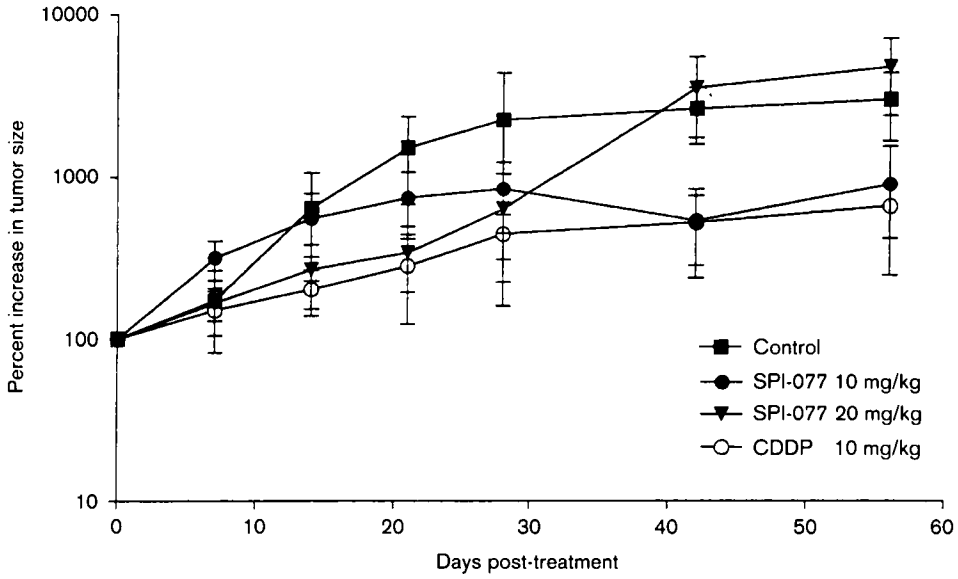

Figure 6. Percent increase in tumor size (volume) in nude mice inoculated with A-375 melanoma after receiving treatment with cisplatin (CDDP) and SPI-077.

reduction in tumor size. Cisplatin given as a single injection exerted moderate antitumor activity shown in the significant reduction of the tumor volumes in comparison to control animals ($p = 0.023$).

In vitro experiments

In the *in vitro* cytotoxic assay, in which the human melanoma cell line A-375 was exposed to various concentrations of cisplatin and SPI-077, we found that the IC_{50} for SPI-077 was greater than 3×10^{-4} M, whereas the IC_{50} for cisplatin was 2.5×10^{-6} M. Thus, at a concentration at which cisplatin exerted cytotoxic activity *in vitro*, SPI-077 proved to be inactive. The results of the *in vitro* release experiments of platinum from SPI-077 show a very slow to almost negligible release of platinum from the liposomes (below 10%) at the concentration studied, even after 1 week incubation in plasma and malignant effusion. These results

indicate that SPI-077 is highly stable in plasma and tissue fluids, and that most of the measured platinum in plasma and tissues during the pharmacokinetic studies is liposome-associated, thus suggesting reduced bioavailability of free platinum species.

Discussion

Long-circulating Stealth liposomes with encapsulated doxorubicin (Doxil) have been shown to increase drug deposition in tumors and antitumor activity.[8] In an attempt to extrapolate the results achieved with Doxil, other drugs such as cisplatin, vincristine and topoisomerase I inhibitors have been encapsulated in Stealth liposomes.[19,20] The antitumor activity of liposome-encapsulated vincristine and topoisomerase I inhibitors is increased after encapsulation into long-circulating liposomes when compared to the free drugs.

Previous pharmacokinetic and therapeutic studies with Stealth liposome-encapsulated cisplatin (SPI-077) point to a significantly improved antitumor activity relative to free cisplatin in mouse tumor models using multiple dose injections.[10,11] In addition, Working *et al.* compared the toxicity of cisplatin and SPI-077 in cynomolgus monkeys, and found that SPI-077 was less toxic and better tolerated than cisplatin.[12]

In the two mouse tumor models (M-109 and J-6456) investigated in this study cisplatin was clearly more effective than SPI-077. In the human A-375 melanoma model the therapeutic efficacy of SPI-077 and cisplatin was minimal in both cases. In contrast to the therapeutic observations with M-109 carcinoma, the pharmacokinetic study revealed enhanced accumulation of platinum in the M-109 s.c. tumors, reaching a peak concentration at 48 h post-injection (Figure 2).

Enhanced accumulation of platinum in the liver and spleen of mice receiving SPI-077 has not been associated with increases in the hepatic and/or splenic toxicity.[10] No significant impairment of bacterial clearance has been found in animals treated with Doxil,[21] indicating no damage to the RES in this case. Regarding SPI-077, bacterial clearance studies have not been reported. Therefore a subtle damage to the RES function cannot be discarded, especially given the large lipid doses used and the possibility of RES saturation.

In the case of J-6456 lymphoma increasing amounts of platinum were measured in the ascitic cells after injection of SPI-077, suggesting superior drug delivery to tumor cells with SPI-077 as compared to free cisplatin.

How can we reconcile the presence of high amounts of platinum in tumors and/or cells with the reduced therapeutic efficacy of SPI-077? The results of the *in vitro* cytotoxicity and leakage experiments indicate a very slow, almost negligible, release of platinum from the liposomes to the medium. The decreased *in vitro* cytotoxicity of SPI-077 in comparison to free cisplatin is consistent with this finding. Slow outflow of active platinum species from the liposomes under *in vivo* conditions and exposure of cisplatin to extracellular proteins and intracellular and extracellular quenchers may inactivate and detoxify the drug. An *in vivo* sustained release effect is suggested by the gradual increase of platinum concentrations in the ascitic cells, since Stealth liposomes are seldom taken up by tumor cells and interact minimally with cell membranes.[22] Cisplatin is prompt to inactivation in low Cl$^-$ environments due to an aquation reaction that removes the two Cl$^-$ atoms from the drug. The drug is then rapidly bound to proteins in an irreversible manner.[23] In addition, the susceptibility of cisplatin to nucleophilic substitution reactions and its readiness to react with biomolecules such as histidine and methionine have been reported.[24,25] In fact, reactivity of cisplatin with glutathione producing stable platinum complexes with no antitumor activity might also represent a route for loss of platinum released from the liposomes. In the case of free cisplatin bolus administration, the inactivation processes also take place, but they are probably less effective due to the fast movement of free cisplatin to the nucleus. The *in vivo* sustained release effect of liposomal platinum mimics to some extent the continuous route of administration. Several clinical studies report that cisplatin administered as continuous infusion does not exert a better therapeutic activity than cisplatin administered as bolus injection.[26,27] The extremely slow release of cisplatin from the liposomes and the fact that continuous exposure to cisplatin does not appear to improve antitumor activity are at least two of the factors that may account for the lack of efficacy improvement despite enhanced drug delivery to tumors.

The approach we carried out to circumvent one possible inactivation pathway of platinum released from liposomes was to create a high Cl$^-$ environment in the organism through administration of hypertonic NaCl (3%) for five consecutive days after treatment, since cisplatin is prompt to inactivation in a low Cl$^-$ environment. The single saline injection given in the studies described here may be optimal for cisplatin but sub-optimal in the case of SPI-077 which has a much more delayed tissue distribution, and therefore a delayed and a very slow release of platinum. The

hyperchloremia sustained during the critical days when SPI-077 is distributed to tumors may protect the drug from inactivation when it is released by the liposomes. Since peak tumor concentration is achieved between 2 and 4 days, this would mean that treatment with hypertonic NaCl and hyperchloremia has to be maintained for at least 5 days. Unfortunately, no better therapeutic efficacy of platinum after administration of SPI-077 could be achieved with the intensive Cl treatment.

Conclusions

In the tumor models investigated in this study, SPI-077 did not show superior antitumor activity in comparison to cisplatin, although high platinum amounts were found in the M-109 tumors and in the ascitic cells of J-6456 lymphoma after SPI-077 injection. Our observations are in contrast to the results of Newman *et al.*[10] and Vaage *et al.*,[11] who reported enhanced therapeutic efficacy for SPI-077. One possible explanation for these discrepant results is that the bioavailability of active platinum species from liposomes may be tumor dependent. For instance, phagocytic cells in some tumors may break down liposomes and release platinum at a higher rate before inactivation occurs. Alternatively the antitumor activity of cisplatin and SPI-077 may be affected differently by variations in dose schedule, whereby multiple dose administration of SPI-077, as used by Newman *et al.* and Vaage *et al.*, is pharmacodynamically more effective, possibly by reducing the RES saturation effect, in contrast to the single dose as used in this study. Further investigations examining various dose schedule regimens in different tumor models may help to clarify this issue.

At any rate, these experiments demonstrate that while Stealth liposomes are a powerful tool to deliver drugs to tumors, the bioavailability and pharmacology of the drug are still the critical determinants of the therapeutic outcome.

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