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ORIGINAL ARTICLE

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# Phase I and pharmacokinetic study of SPI-77, a liposomal encapsulated dosage form of cisplatin

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**Abstract** *Purpose:* To investigate the safety and pharmacokinetics of a new liposomal formulation of cisplatin, SPI-77, in patients with advanced malignancies. *Patients and methods:* Patients with histologically proven malignancies not amenable to other treatment were eligible for this study. The starting dose of SPI-77 (cisplatin in Stealth liposomes) was 40 mg/m$^2$ administered every 4 weeks in a 2-h infusion, and doses were escalated up to 420 mg/m$^2$. Pharmacokinetic monitoring was performed in all patients and samples were analysed for platinum content by atomic absorption spectroscopy. Platinum-DNA (Pt-DNA) adduct levels in leucocytes (white blood cells, WBC) and tumour tissue were quantified using a sensitive $^{32}$P-postlabelling assay. *Results:* A total of 27 patients were accrued. The main toxicities observed were infusion-related reactions, which could be prevented by lowering the initial infusion rate, and anaemia. The pharmacokinetics of SPI-77-derived platinum were strikingly different from standard cisplatin. Free platinum levels in plasma ultrafiltrate samples were undetectable at the lowest dose levels (40 and 80 mg/m$^2$), and low but highly variable at higher doses of SPI-77. Plasma pharmacokinetics of total platinum were linear with small interpatient variability. The total body clearance of SPI-77 varied from 14 to 30 ml/h and was significantly lower than reported clearance values for cisplatin of 20 l/m$^2$ per h, due to the slow release of cisplatin from the liposomes. Pt-DNA adduct levels in WBC ranged from 0.02 to 4.13 fmol/μg DNA for intrastrand Pt-GG (guanine-guanine) adducts and from 0.02 to 1.27 fmol/μg DNA for intrastrand Pt-AG (adenosine-guanine) adducts, which is more than tenfold lower than after administration of a comparable dose of non-liposomal cisplatin. In tumour samples obtained from two patients treated at the highest dose-levels, relatively low levels of Pt-DNA adducts were observed. *Conclusions:* The results of this phase I trial show that the pharmacokinetic behaviour of cisplatin is significantly altered by its encapsulation in Stealth liposomes. The pharmacokinetics of SPI-77 are mainly dominated by the liposomal properties, resulting in high cholesterol concentrations and relatively low concentrations of (free) platinum in plasma, WBC and tumour tissue, which may explain the observed differences between the toxicity profiles of SPI-77 and cisplatin.

**Keywords** SPI-77 · Liposomal cisplatin · Stealth liposomes · Pharmacokinetics · Phase I study

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## Introduction

Cisplatin is a well-known and very potent anticancer agent frequently used against a variety of tumours, including ovarian, testicular, lung and head and neck cancer [3, 4, 7, 9, 10, 14]. The mechanism of action is based on a direct binding of cisplatin to DNA. The acute

dose-limiting toxicity associated with cisplatin therapy is nephrotoxicity, although this can be largely reduced with adequate hydration. Other commonly observed toxicities that hamper further dose increments are nausea and vomiting, peripheral neuropathies, ototoxicity, hypersensitivity reactions and myelosuppression. Research in the past decade has focused on the development of other platinum compounds with an improved therapeutic index, such as carboplatin [21, 25].

Liposomal encapsulation of cisplatin is an alternative approach to reducing systemic drug exposure and to delivering cisplatin to tumours with increased selectivity. SPI-77, a Stealth liposomal dosage form of cisplatin, was developed in order to increase the total dose of cisplatin that can be administered with no increase, and perhaps even a decrease, in systemic toxicity. Stealth liposomes are coated with methoxy-polyethylene glycol (MPEG) which makes them resistant to recognition by blood opsonins and removal from the blood stream. Due to their small size (approximately 110 nm), long circulation time and reduced interaction with blood components, Stealth liposomes tend to accumulate in tumours [28]. At these tumour sites, the liposomes can become extravasated through the abnormally permeable vessels characteristic of many tumours, thereby entering the interstitial space, where they gradually break down and release cisplatin to the surrounding tumour cells [5, 29]. If successful, the systemic exposure to free cisplatin is low, whereas high concentrations are achieved at the desired site.

SPI-77 has displayed less-acute toxicity in preclinical studies, as compared to cisplatin, and its antitumour effects are at least equivalent to those of cisplatin or carboplatin [12, 20]. In addition, antitumour effects are in general longer-lasting than after administration of cisplatin or carboplatin [20]. In vitro results have suggested that prolonged infusions of cisplatin may be favourable for therapeutic use [14]. The slow release of the drug from the liposomal carrier can mimic a continuous infusion with lower peak plasma levels and a prolonged drug exposure. A phase I dose-escalating study was performed in our institutes to evaluate the safety and the pharmacokinetics of SPI-77 in patients with cancer.

## Patients and methods

### Patient eligibility criteria

Patients were eligible if they had a histologically or cytologically proven malignancy not amenable to other treatment, and if they had an acceptable performance status (Karnofsky PS ≥70%). Other eligibility criteria included age ≥18 years, life expectancy ≥3 months, and adequate function of bone marrow function (absolute neutrophil count ≥2000/mm$^3$, platelet count ≥100,000/mm$^3$ and haemoglobin ≥9.0 g/dl), liver function (total bilirubin ≤ 2.0 mg/dl, ALT and AST not more than 1.5 times the upper limit of normal) and kidney function (creatinine clearance ≥50 ml/min). Previous anticancer chemotherapy, radiotherapy or immunotherapy had to be discontinued for at least 4 weeks before entry to the study (6 weeks in cases of treatment with nitrosourea, suramin or mitomycin C) and prior treatment with SPI-77 was not allowed. Patients were excluded if they had a history of abnormal cardiac function, hearing loss and/or an allergic reaction to cisplatin or other platinum-containing products. Further exclusion criteria were pregnancy, signs or symptoms of an acute infection requiring systemic therapy, neurological symptoms and psychiatric illness. Patients had to give written informed consent.

### Treatment plan

Three patients were enrolled per dose level (cohort). Each cohort received SPI-77 every 4 weeks to a total of six doses, or until disease progression, whichever occurred earlier. Initially, the infusion duration was set at 2 h. The three patients in the first cohort received SPI-77 at a dose of 40 mg/m$^2$ during each cycle and thereafter, dose escalation was scheduled as follows: 80, 120, 200, 320, 520 and 840 mg/m$^2$, provided that no dose-limiting toxicities were observed (for definition see Patient evaluation section). If a dose-limiting toxicity occurred in one of the three patients within one cohort, then three additional patients were treated at that level. The last patient at a dose level was observed for at least 4 weeks before the first patient at the subsequent dose level was treated. The maximum tolerated dose was defined as the dose below the dose at which two out of six patients experienced dose-limiting toxicity. No prophylactic antiemetic or hydration therapy was given.

### Chemicals

SPI-77 was provided by SEQUUS Pharmaceuticals (Brentford, UK) as an isotonic preservative-free suspension for parenteral administration containing 1.18 mg cisplatin/ml. The lipids in SPI-77 consist of fully hydrogenated soy phosphatidylcholine (HSPC) (40.0 mg/ml), cholesterol (17.1 mg/ml), and the polymer MPEG-DSPE (14.3 mg/ml), with a total lipid content of approximately 71 mg/ml. The mean liposome particle diameter is approximately 110 nm and the cisplatin encapsulation exceeds 90%. Prior to infusion, SPI-77 was further diluted with physiological saline (NaCl 0.9%) to a final concentration of 0.2 mg/ml. Nitric acid 65% for dilution of the samples was obtained from Merck, Darmstadt, Germany, and Triton X-100 was obtained from BDH, Poole, UK.

### Patient evaluation

Pretreatment evaluation included a complete medical history and a complete physical examination. In addition, an electrocardiogram, an audiogram and a neurological examination were performed. Prior to each administration, haematology and serum chemistry including lipid profile (cholesterol and triglyceride levels including the high density lipoprotein and low density lipoprotein subfractions) were checked. Urine analysis was performed and 24-h creatinine clearance determined. Haematology and serum chemistry were checked weekly. Urine analysis, audiogram and neurological examination were repeated at the end of every cycle. An electrocardiogram was repeated at the end of the treatment. Tumour evaluations were performed every other cycle. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria [15] and dose-limiting toxicities were defined as nephrotoxicity or non-reversible neurological toxicity grade 2 or higher in severity, or any other toxicity grade 3 or higher, with the exception of untreated nausea, vomiting, alopecia, weight change, fatigue, and allergic reactions, which were not considered dose-limiting. A decrease in creatinine clearance was used as an indicator for evaluation of nephrotoxicity. Creatinine clearances during treatment (between administrations) as well as at the end of the treatment (after the last administration) were determined, to detect acute and cumulative toxicities.

### Pharmacokinetic studies

Pharmacokinetic studies were performed in all except two patients. Blood samples were obtained from patients at 12 time-points up to 4 weeks after the first administration: preinfusion, at the end of the infusion, at 1, 3, 5, 24, 48, 72, 96 and 168 h after administration and

weekly thereafter for a total of three additional weeks. The samples (7 ml each) were collected in heparinized tubes, which were placed in an ice-bath (0–4°C) immediately after withdrawal. Whole blood was centrifuged at 4°C for 5 min (3000 *g*). Four 100-µl aliquots of the obtained plasma fraction were diluted tenfold (1:9) with 0.1% Triton X-100 plus 0.2% nitric acid solution. Of the remaining plasma, 1 ml was centrifuged for 10 min using Amicon Centrifee ultrafiltration devices to obtain approximately 0.2 ml plasma ultrafiltrate. All samples were stored at –20°C until analysis.

Urine samples were collected prior to infusion, and at 24, 48, 72 and 96 h after administration. Tumour samples were obtained from three patients: one patient with a melanoma, one patient with ascites from ovarian cancer and one patient with cervix cancer. The melanoma and the cervix cancer were biopsied on day 2 (the day after infusion of SPI-77) and ascites samples were taken on days 1, 5, 9, 15, 18, 26, 30, 36 and 41.

All platinum analyses were performed using a validated flameless atomic absorption spectroscopy method, with a lower limit of quantification of 0.125 µ*M*. [13]. Total platinum, i.e. free plus protein-bound plus liposomal encapsulated platinum, was measured in plasma. Total platinum concentrations were determined in diluted plasma samples and free platinum was determined in ultrafiltrate samples.

### Platinum-DNA adduct measurements

Prior to the infusion, at the end of the infusion and at 3, 24 and 48 h after the end of the infusion during course 1, whole blood samples of 14 ml were collected in heparinized tubes for platinum-DNA (Pt-DNA) adduct determination in leucocytes (white blood cells, WBC). Immediately after withdrawal, the samples were centrifuged at 4°C for 5 min (3000 *g*). Subsequently, the WBC fraction was isolated. WBC were purified by lysing contaminating red blood cells in the WBC fraction by incubation in 0.1% (w/v) ammonium chloride, 1 m*M* edetate disodium, and 1% (w/v) potassium bicarbonate for 20 min at 4°C. The WBC were washed twice with phosphate-buffered saline, and lysed in 0.01 *M* Tris-HCl, 2.32% (w/v) NaCl, and 2 m*M* edetate disodium, pH 7.30. The lysed WBC were directly used for DNA isolation or they were stored at –80°C until analysis. Pt-DNA adducts were quantified using a sensitive $^{32}$P-postlabelling assay, enabling the selective determination of intrastrand platinum and guanine-guanine (Pt-GG) and platinum and adenosine-guanine (Pt-AG) adducts down to a detection limit of 15 amol platinum/µg DNA [16].

### Pharmacokinetic parameters

Non-compartmental methods were applied to calculate the pharmacokinetic parameters of total platinum. The area under the plasma concentration-time curve (AUC) was determined using the linear trapezoidal method with extrapolation to infinity ($C_{last}/k$, in which $C_{last}$ is the last measured concentration). The AUC of Pt-DNA adducts was calculated up to $C_{last}$ without extrapolation to infinity. The elimination rate constant for total platinum (k) was calculated by linear regression analysis of the logarithmic plasma concentration-time curves. The total body clearance (Cl), the elimination half-life ($t_{1/2}$) and the volume of distribution at steady state ($V_{ss}$) were calculated using standard equations [6]. Pt-DNA adduct AUC was calculated using the trapezoidal method up to the last measured time-point at 48 h. Statistical analysis of the results was performed using SPSS/PC+ (SPSS/PC+ Advanced Statistics, version 6.1, 1994; Chicago, Ill.).

## Results

### Patients and treatment

A total of 27 patients were included in the trial. Patient characteristics are presented in Table 1. Six patients were treated at the starting dose of 40 mg/m$^2$, because of a grade 4 infusion-related reaction observed in one patient (for description see Toxicity section). Three patients were treated at a dose of 80 mg/m$^2$, three others at a dose of 120 mg/m$^2$ and three at a dose of 200 mg/m$^2$. Nine patients were treated at a dose of 320 mg/m$^2$, because two patients developed muscle weakness proximally in both legs, which was grade 2 to 3 in severity. Three patients were treated at a dose of 420 mg/m$^2$, after which the study was discontinued. A total of 69 courses of SPI-77 were administered with a median number of two courses per patient (range one to ten). One patient died after the second course due to disease progression. One patient went off-study after the second course due to the grade 4 infusion-related reaction. All other patients went off-study because of disease progression.

Because of the observed infusion-related reactions, the infusion duration was increased from 2 h to approximately 3 h at a dose of 120 mg/m$^2$ by lowering the infusion rate to 100 ml/h during the first 15 min, followed by an infusion rate of 700 ml/h for the remaining volume. Simultaneously, the final dilution of SPI-77 in 0.9% NaCl was decreased from 0.3 mg/ml to 0.2 mg/ml. As a result, the infusion duration at a dose of 320 mg/m$^2$ was approximately 5 h. In order to reduce the total infusion volume, the final dilution was reset at 0.3 mg/ml at a dose of 320 mg/m$^2$, which reduced the infusion duration to approximately 3 h. All patients were considered evaluable for toxicity and response.

**Table 1** Patient characteristics and responses. Values are number of patients, except age in years

| | |
|---|---|
| Number of patients | |
| Total | 27 |
| Male/female | 13/14 |
| Age (years) | |
| Median | 57 |
| Range | 34–75 |
| Karnofsky performance status (%) | |
| 70 | 2 |
| 80 | 2 |
| 90 | 11 |
| 100 | 12 |
| Prior therapy | |
| None | 1 |
| Surgery | 3 |
| Surgery + radiotherapy | 5 |
| Surgery + chemotherapy | 10 |
| Surgery + chemotherapy + hormonal therapy | 3 |
| Surgery + radiotherapy + hormonal therapy | 1 |
| Surgery + radiotherapy + chemotherapy | 4 |
| Tumour site | |
| Colon carcinoma | 5 |
| Renal cell carcinoma | 4 |
| Ovarian carcinoma | 5 |
| Cervix carcinoma | 1 |
| Mesothelioma | 3 |
| Sigmoid carcinoma | 1 |
| Pancreas carcinoma | 1 |
| Oesophagus carcinoma | 1 |
| Thyroid carcinoma | 2 |
| Melanoma | 2 |
| Prostate carcinoma | 1 |
| Adenocarcinoma with unknown primary | 1 |

## Toxicity

Haematological toxicity was mild. Anaemia grade 3 was the most severe haematological toxicity documented, which occurred in two patients (7%) during two courses (3%). Anaemia grade 2 occurred in nine patients (33%) during a total of 11 courses (16%). It should be noted, however, that anaemia grade 2 was pre-existent in three patients (11%) and anaemia grade 1 in ten patients (37%). One patient developed a leucocytopenia grade 1 during one course. Gastrointestinal toxicities were the most frequently observed non-haematological toxicities. One patient suffered from vomiting grade 3 during one course. Vomiting grade 1 or 2 occurred in ten patients (37%) during a total of 14 courses (20%). Nausea grade 1 or 2 was present in 13 patients (48%) during 22 courses (32%).

One patient experienced a grade 4 infusion-related reaction during the first few minutes of the second administration, which was the reason for study discontinuation in this patient. This patient developed hypotension, dizziness, sweating, back pain and paraesthesia within 10 min of the start of the second SPI-77 infusion. All symptoms resolved within 20 min of cessation of the infusion and administration of 10 mg dexamethasone plus 2 mg clemastine intravenously. Three other patients (11%) experienced minor reactions during the first administration. These reactions consisted mainly of flushing and a slight decrease in blood pressure. Typically, the reactions occurred during the first few minutes of administration. It was therefore decided to reduce the rate of infusion during the first 15 min to 100 ml/h (as described under patients and treatment), which prevented further infusion-related reactions. Ototoxicity manifested as transient tinnitus occurred in four patients (15%), during a total of five courses (7%).

Muscle weakness was observed in two patients (7%) during three courses (4%) at a dose of 320 mg/m$^2$, being severe (grade 2 or 3) in two courses (3%). The onset was after two courses. In both patients the toxicity manifested as a loss of strength of the proximal leg muscles. One patient died shortly thereafter due to disease progression and therefore the relationship between the observed toxicity and the study drug could not be properly assessed in this patient. The other patient underwent extensive neurological examination, but no objective abnormalities were observed on the electromyelogram. During the follow-up, the symptoms improved in this patient, indicating reversibility. It should be noted that the toxicity in both cases manifested as a loss of strength of the leg muscles, and both patients were already considerably weakened due to their disease. These toxicities were also the reason for the recruitment of six additional patients at a dose of 320 mg/m$^2$. These patients and the patients at a dose of 420 mg/m$^2$ underwent extensive neurological evaluation, consisting of an electromyelogram and vibration perception measurement, prior to study entry and every other course, or if symptoms developed. No weakness of muscle strength or abnormal test results for neurological side effects were observed in any of these six patients.

No significant decreases in creatinine clearance were observed at any dose administered, either during treatment or at the end of the treatment. Other toxicities consisted of skin toxicities, such as pruritus in five patients (19%) and stomatitis in three patients (11%), and 'flu-like symptoms, such as fever, in seven patients (26%) and fatigue in ten patients (37%). These toxicities were generally mild and never exceeded grade 1 or 2 in severity. Significant toxicities are shown in Table 2.

## Responses

Although response measurement was not a primary goal in the study, six patients (22%) had stable disease for at least two consecutive measurements. One of these patients with a renal cell carcinoma, who received four courses of SPI-77, showed a significant regression of tumour metastases, which lasted for 2 months. This patient was a 43-year-old chemonaive male. However, he did not reach a partial response, because no significant reduction was observed in the primary tumour. One patient with advanced pancreatic cancer with liver metastases showed stable disease during ten courses. Responses are further shown according to tumour site in Table 1.

## Pharmacokinetic studies

All except two patients underwent pharmacokinetic monitoring during the first course. Total platinum concentrations were measured in diluted plasma samples and free platinum concentrations were measured in plasma ultrafiltrate samples. Complete plasma concentration-time curves for total platinum were obtained in 25 patients during the first course. The mean plasma concentration-time curves per dose level are depicted in Fig. 1. Mean pharmacokinetic parameters for total platinum were derived from these curves and are presented in Table 3. A plot of the total platinum plasma AUC versus the administered dose of SPI-77 is shown in

**Table 2** Drug-related toxicity scored according to the CTC criteria

| Toxicity | Grade | No. of patients (%) | No. of courses (%) |
|---|---|---|---|
| Haematological | | | |
| Anaemia | 3 | 2 (7) | 2 (3) |
| Gastrointestinal | | | |
| Nausea | 3 | 1 (4) | 1 (1) |
| Vomiting | 3 | 1 (4) | 1 (1) |
| Infusion-related reactions | 4 | 1 (4) | 1 (1) |
| Neurological | | | |
| Sensory | 1 | 1 (4) | 1 (1) |
| Motor | 1 | 1 (4) | 1 (1) |
| Muscle weakness | 2 | 1 (4) | 1 (1) |
| | 3 | 1 (4) | 1 (1) |

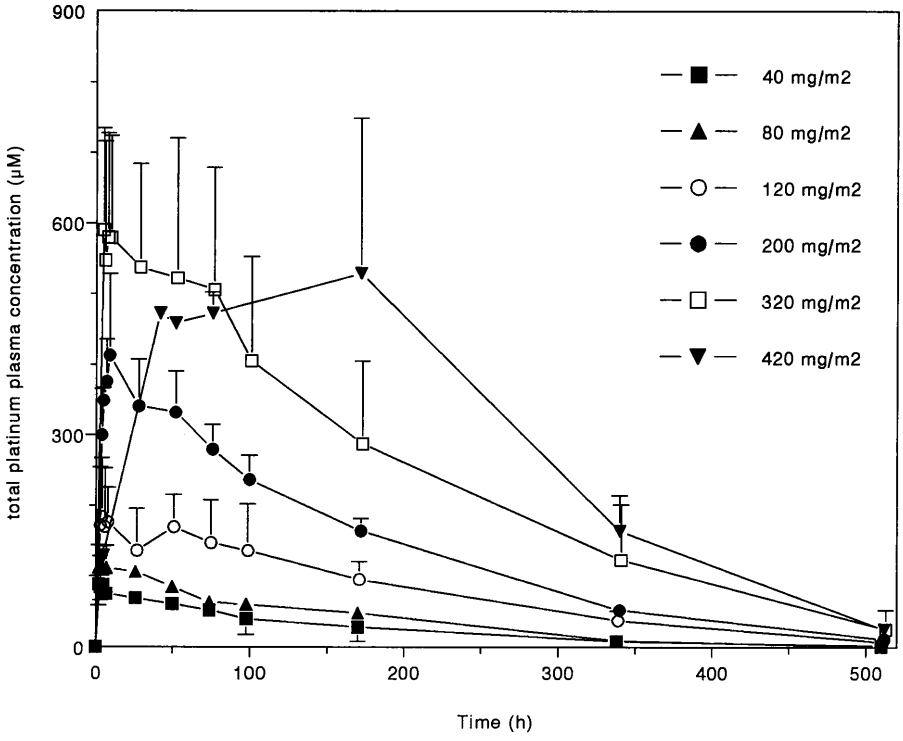


**Fig. 1** Plasma concentration-time curves for total platinum. Values are means ± SD. Each curve was derived from the data from at least three patients. SPI-77 was administered over 2–5 h

**Table 3** Pharmacokinetic parameters. Values are means ± SD. For abbreviations see Methods section

| Dose (mg/m$^2$) | No. of patients | $AUC_{0-\infty}$ (h·m*M*) | $C_{max}$ (μ*M*) | Cl (ml/h) | $t_{1/2}$ (h) | $V_{ss}$ (l) |
|---|---|---|---|---|---|---|
| 40 | 6 | 13.6 ± 1.5 | 100.8 ± 22.7 | 19.8 ± 2.5 | 95 ± 15 | 2.68 ± 0.37 |
| 80 | 2 | 18.2 ± 0.2 | 117.2 ± 24.8 | 30.0 ± 2.8 | 79 ± 4 | 3.45 ± 0.17 |
| 120 | 3 | 44.5 ± 6.5 | 193.5 ± 68.9 | 17.0 ± 2.7 | 132 ± 35 | 3.19 ± 0.41 |
| 200 | 3 | 71.1 ± 2.5 | 423.1 ± 97.7 | 17.7 ± 1.0 | 103 ± 3 | 2.63 ± 0.10 |
| 320 | 9 | 145.4 ± 73.8 | 642.8 ± 141.7 | 18.1 ± 10.8 | 116 ± 55 | 2.86 ± 2.03 |
| 420 | 2 | 163.3 ± 6.7 | 589.1 ± 135.1 | 14.0 ± 0.1 | 145 ± 107 | 2.98 ± 2.24 |

Fig. 2. Pharmacokinetics of total platinum were linear up to a dose of 420 mg/m$^2$ (Fig. 2). Low interpatient variability was observed at all dose levels, except for the dose of 320 mg/m$^2$. The calculated AUC values of total platinum after administration of SPI-77 in our study were approximately 100-fold higher than reported AUC values for total platinum after administration of cisplatin at the comparable dose of 100 mg/m$^2$ [7].

The volume of distribution of total platinum after SPI-77 administration ranged from 2.6 to 3.5 l. Mean elimination half-lives after 2- to 5-h infusions of SPI-77 ranged from 80 to 145 h, and were dose-independent. Total body clearances of SPI-77 were 14–30 ml/h, which is extremely low compared to the reported clearance values for cisplatin, which average 34 l/h [1, 17].

Free (ultrafilterable) platinum levels were below the lower limit of quantification in patients receiving SPI-77 doses of 40 and 80 mg/m$^2$. At the higher dose levels, free platinum levels were measurable, but highly variable. Maximum measured concentrations ranged from 0.4 to 140 μ*M* and were independent of dose and time.

In addition, several ascites samples were collected from one patient and analysed for platinum content. Total platinum levels in the ascites ranged from 8 to 74 μ*M* and free platinum levels ranged from 0.6 to 1.8 μ*M*.

## Lipid profile analysis

The large amount of lipids present in the membrane bilayer of the liposomes may influence the lipid profile of patients receiving SPI-77, especially at high doses. Therefore, serum total cholesterol and triglyceride levels were monitored in all patients. Serum cholesterol levels in patients were highly variable, but increased with increasing doses of SPI-77. In addition, particularly at the higher doses of SPI-77, a fluctuating pattern became apparent. Cholesterol levels tended to increase shortly after administration of SPI-77 and gradually decreased over the 4 weeks following the administration. Maximum cholesterol levels ranged from 5.0 to 20.9 m*M*, which represented increases from baseline values of 23 to

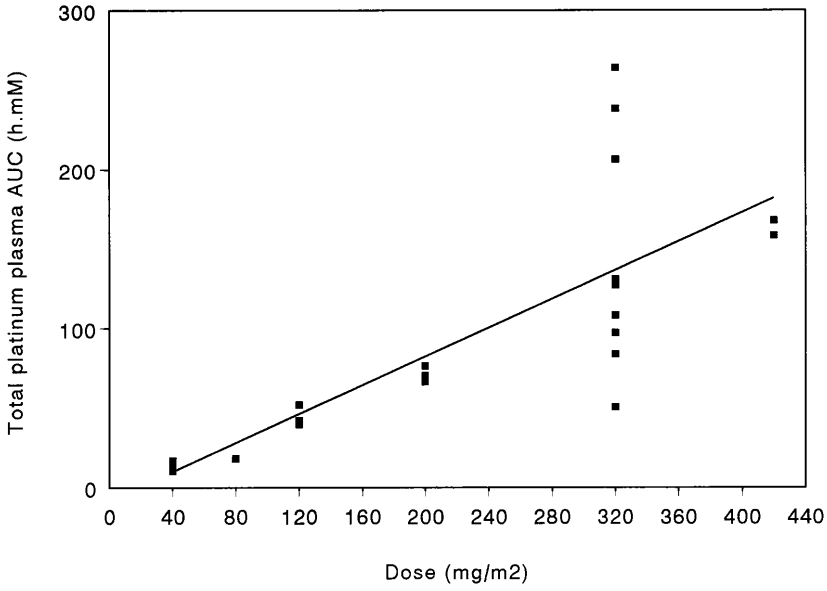


**Fig. 2** AUC values of total platinum (derived from the curves in Fig. 1) plotted against the dose of SPI-77. The solid line is the regression line ($R = 0.81$)

214%, the median increase being 64%. Fluctuations in cholesterol levels are shown in Fig. 3. Maximum increases in serum triglyceride levels as a percentage of the baseline values ranged from 38 to 350%, the median increase being 75%. Maximum triglyceride levels varied widely from 1.4 to 12.3 m*M*.

### Pt-DNA adduct formation

Pt-DNA adduct formation was quantified in 19 patients. Pt-GG and Pt-AG adducts, the two major Pt-DNA adducts, were measured. Adducts were determined in three patients receiving SPI-77 at a dose of 40 mg/m$^2$, three a dose of 80 mg/m$^2$, three a dose of 120 mg/m$^2$, three a dose of 200 mg/m$^2$, five a dose of 320 mg/m$^2$ and two a dose of 420 mg/m$^2$ during the first course. Pt-GG adduct levels ranged from 0.02 to 4.13 fmol/μg DNA and Pt-AG adduct levels ranged from 0.02 to 1.27 fmol/μg DNA. After administration of SPI-77 at a dose of 80 mg/m$^2$, maximum Pt-GG levels ranged from 0.4 to 0.9 fmol/μg DNA and maximum Pt-AG levels ranged from 0.1 to 0.3 fmol/μg DNA. Levels of DNA adducts (Pt-AG + PT-GG) at a dose of 420 mg/m$^2$ ranged from 2.1 to 4.9 fmol/μg DNA. The mean concentration-time curves for all doses are shown in Figs. 4 and 5.

AUC values are presented in Table 4. In Fig. 6, plots of the Pt-DNA adduct AUC versus the administered dose of SPI-77 are shown. A correlation was observed between the AUC values of total plasma platinum and GG-bound platinum ($R = 0.52$, $P = 0.025$), but not between the AUC values of total plasma platinum and AG-bound platinum. No correlation was found between the $C_{max}$ of free platinum and the AUC of Pt-DNA adducts. However, a plot of the AUC of Pt-DNA adducts versus the dose of SPI-77, either in milligrams or in milligrams per square millimetre, revealed a correlation between the Pt-GG adduct AUC and the administered dose of SPI-77 (Fig. 6; $R = 0.74$, $P = 0.0004$; as total dose in milligrams: $R = 0.68$, $P = 0.002$). Total Pt-DNA adduct AUC levels were also significantly correlated with the administered dose of SPI-77 (as milligrams per square millimetre: $R = 0.74$, $P = 0.0004$; as milligrams: $R = 0.67$, $P = 0.002$). No significant correlation was found between the very low Pt-AG adduct AUC and the administered dose of SPI-77.

In addition, it was possible to determine Pt-DNA adduct levels in tumour cells of two patients, one with ovarian carcinoma and one with melanoma. An insufficient amount of DNA was obtained from the third patient (with cervix carcinoma) to allow the measurement of DNA adducts. The Pt-DNA adduct levels (Pt-GG + Pt-AG) in the tumour cells from ascites (in the patient with ovarian carcinoma) ranged from 2.1 to 5.2 fmol/μg DNA, and were approximately tenfold higher than the adduct levels in the WBC from this patient at corresponding time-points (end of infusion, 96 h infusion, and 1, 2, 3, 4, and 5 weeks after infusion) ranging from 0.1 to 0.6 fmol/μg DNA. In the tumour cells from the melanoma biopsy, the Pt-DNA adduct level (Pt-GG + Pt-AG) was 4.4 fmol/μg DNA, compared to 2.9 fmol/μg DNA in the WBC.

### Urinary excretion

The cumulative excretion of cisplatin in urine up to 96 h after administration of SPI-77 was measured in 12 patients and ranged from 2.0 to 6.1% of the total dose administered, with a median of 3.3%, which was dose-independent.

## Discussion

The liposomal formulation of cisplatin, SPI-77, was developed in order to target cisplatin delivery to tumour

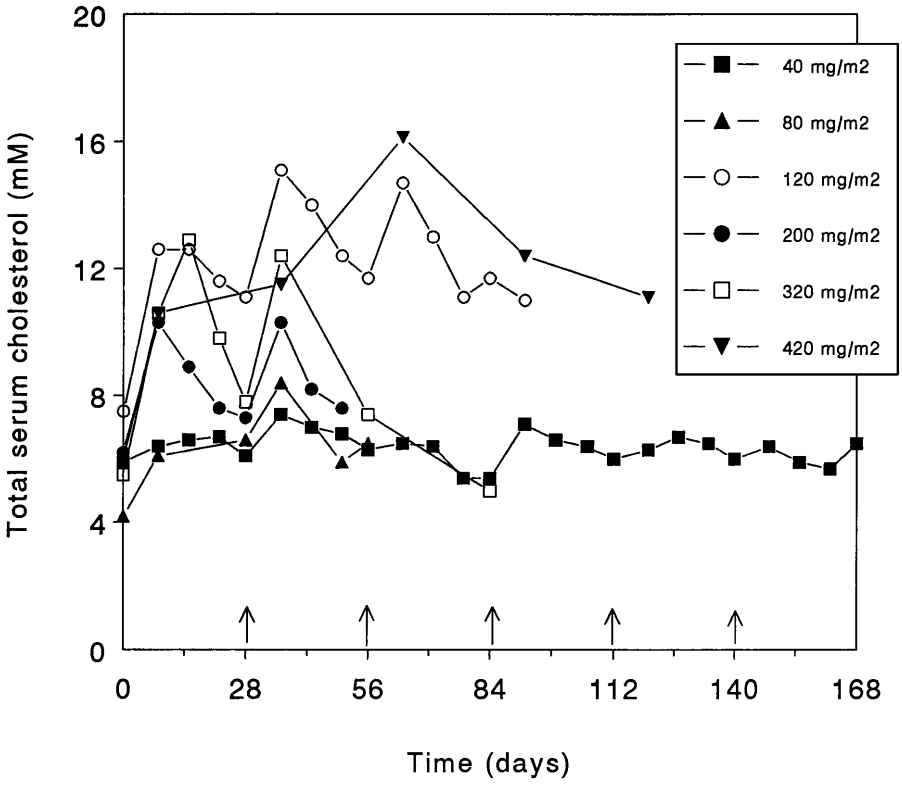


**Fig. 3** Mean serum concentration-time curves for total cholesterol. SPI-77 was administered at t = 0, and at 28, 56 and 84 days. The *arrows* indicate the times of administration of SPI-77

cells while reducing the systemic toxicity associated with free cisplatin. The favourable pharmacokinetic properties of Stealth liposomes should facilitate selective release of cisplatin at the tumour site. Encouraged by the preclinical results in various animal species, we performed a phase I study in cancer patients not amenable to other treatment to investigate the pharmacokinetics and toxicity of SPI-77. Tumour biopsies are preferred to investigate whether platinum accumulates in tumours after administration of liposomal cisplatin. However, a validated method for this does not exist currently, and due to ethical and practical considerations tumour biopsies are not routinely obtained from patients.

In blood, three different forms of cisplatin are present following drug administration: free (i.e. active), protein-bound and erythrocyte-bound [10, 14, 18]. After administration of SPI-77 at doses of 40–80 mg/m$^2$, free, ultrafilterable platinum levels were below the lowest limit of quantification. Administration of SPI-77 at higher doses resulted in free platinum levels varying widely from 0.4 to 140 $\mu M$. Apparently, release of cisplatin from the liposomes occurs at a slow rate, resulting in low systemic exposure to free cisplatin. Our analytical method did not distinguish between liposomal and protein-bound platinum. However, as the plasma protein binding is irreversible, the free fraction is mainly the result of liposomal release. Erythrocyte-bound platinum was not measured because in vitro blood partitioning studies have indicated that SPI-77 resides in the plasma and that affinity for erythrocytes is negligible [13]. The volume of distribution of SPI-77-derived platinum was significantly lower than the volume of distribution of total platinum after cisplatin administration (11–24 l) [2, 7], and approximately equal to the plasma volume.

Elimination of cisplatin is usually described by two-compartment models. After administration of cisplatin, elimination half-lives of 42 h to 7.3 days have been reported for total platinum [2, 7, 10, 14, 22, 23, 24]. Plasma concentration versus time data of total platinum after SPI-77 administration were better characterized by a one-compartment model than by a two-compartment model, which is consistent with the assumption that the plasma pharmacokinetics are largely determined by the liposomes, not by the encapsulated material. However, since we observed considerable variation in the shape of the concentration-time curves, non-compartmental methods were applied to calculate pharmacokinetic parameters.

Slow release of cisplatin from the liposomes, rendering the drug unavailable for renal clearance mechanisms, may be responsible for the low total body clearance found in our study. Clearance of standard cisplatin is largely the result of renal processes such as glomerular filtration and tubular secretion. Ultimately, nearly 90% of cisplatin is excreted into the urine following intravenous administration of cisplatin, with most of the drug excreted within the first few days [10]. The nephrotoxicity associated with cisplatin administration may be due to tubular damage caused by high local pharmacologically active platinum concentrations [8]. In our study, the median urinary excretion of cisplatin was comparable to the results from other investigators, and represents a significant reduction in comparison to standard cisplatin administration [1].

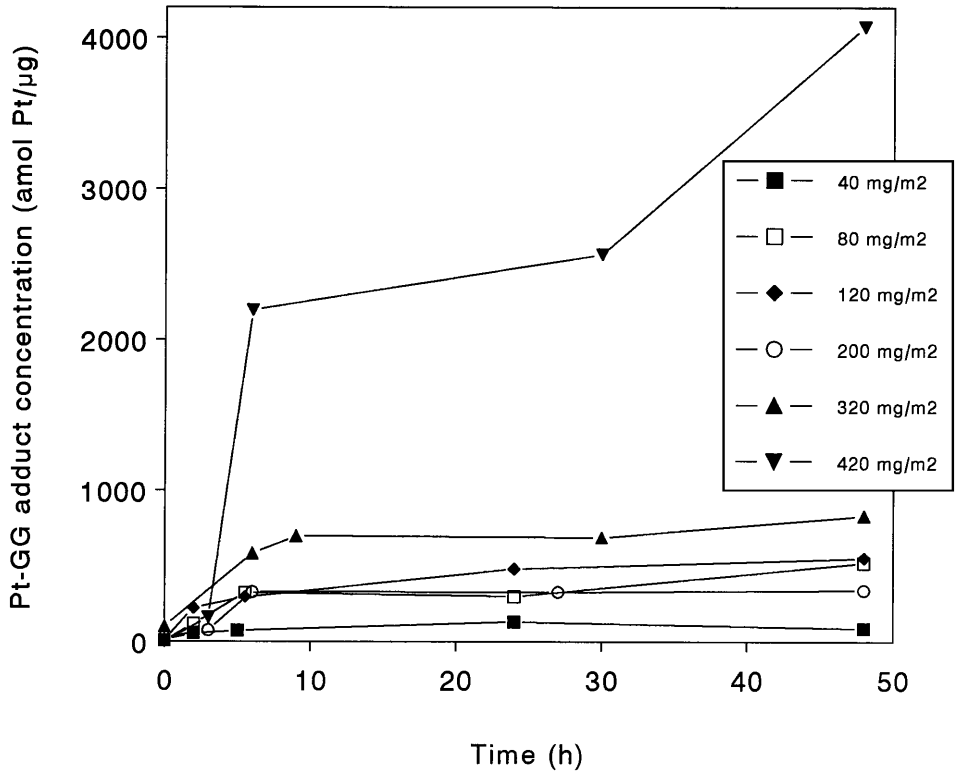


**Fig. 4** Mean concentration-time curves for Pt-GG adducts in WBC. Each curve was derived from the data from three patients, exceptforthe420 mg/m$^2$ dose which was derived from two patients

The two major Pt-DNA adducts, i.e. Pt-GG and Pt-AG, were found in WBC DNA even at the lowest dose levels, indicating that pharmacologically active cisplatin was released from the liposomes in the blood compartment, at least to some extent. As anticipated, Pt-GG adduct levels were higher than Pt-AG levels, but no correlation was found between the AUC of GG- and AG-bound platinum. It has previously been found that cisplatin doses of 70–80 mg/m$^2$ result in maximum Pt-DNA adduct levels of $6.6 \pm 2.05$ fmol/µg DNA [11, 19]. After administration of SPI-77 at a dose of 80 mg/m$^2$, we found that maximum levels of Pt-DNA adducts were on average tenfold lower than after an equivalent dose of standard cisplatin. It is noteworthy that levels of DNA adducts (Pt-AG + PT-GG) at a dose of 420 mg/m$^2$ were high compared to the levels at lower dose levels. A correlation was observed between the AUC values of total plasma platinum and GG-bound platinum and between the Pt-GG adduct AUC and the administered dose of SPI-77. No significant correlation was found between the very low Pt-AG adduct AUC and the administered dose of SPI-77.

Pt-DNA adduct levels in tumour cells were determined in two patients. In one patient tumour cells were derived from ascites and in this patient the Pt-DNA adduct levels in the tumour cells were approximately tenfold higher than the Pt-DNA adduct levels in WBC. In the second patient the Pt-DNA adduct levels, determined in tumour cells from a melanoma biopsy, were in the same range of the Pt-DNA adduct levels in the WBC of this patient.

The increase in triglyceride and cholesterol levels after administration of SPI-77 and the subsequent decrease during the following 4 weeks was likely to have been a result of the administration of the liposomes, which contain high levels of cholesterol. The maximum levels of cholesterol detected of 5.0 to 20.9 m*M* represented considerable increases from baseline values. These levels returned to baseline values after discontinuation of the study.

In contrast to cisplatin administration, gastrointestinal toxicity after administration of SPI-77 was limited, eliminating the need for prophylactic antiemetic therapy. No renal toxicity was observed and pre- and/or post-hydration was unnecessary. Infusion-related reactions were observed in four patients, but it was possible to control these by lowering the infusion rate. Two patients experienced severe muscle weakness, which in one patient was possibly related to the administration of SPI-77, although no objective abnormalities were observed on the electromyelogram and the symptoms were reversible. Extensive neurological measurements were performed in all subsequent patients, but no other neurological side effects were observed. Toxicities were not related to the administered dose of SPI-77.

The study was discontinued after the dose of 420 mg/m$^2$ because platinum accumulation in tumour cells (measured as DNA-bound platinum) and in peripheral tissues was lower than expected, as demonstrated by the low levels of free platinum in ascites, the Pt-DNA adduct levels in tumour cells derived from ascites, and the Pt-DNA adduct levels in tumour cells obtained from tumour biopsies. Although platinum-DNA adduct levels

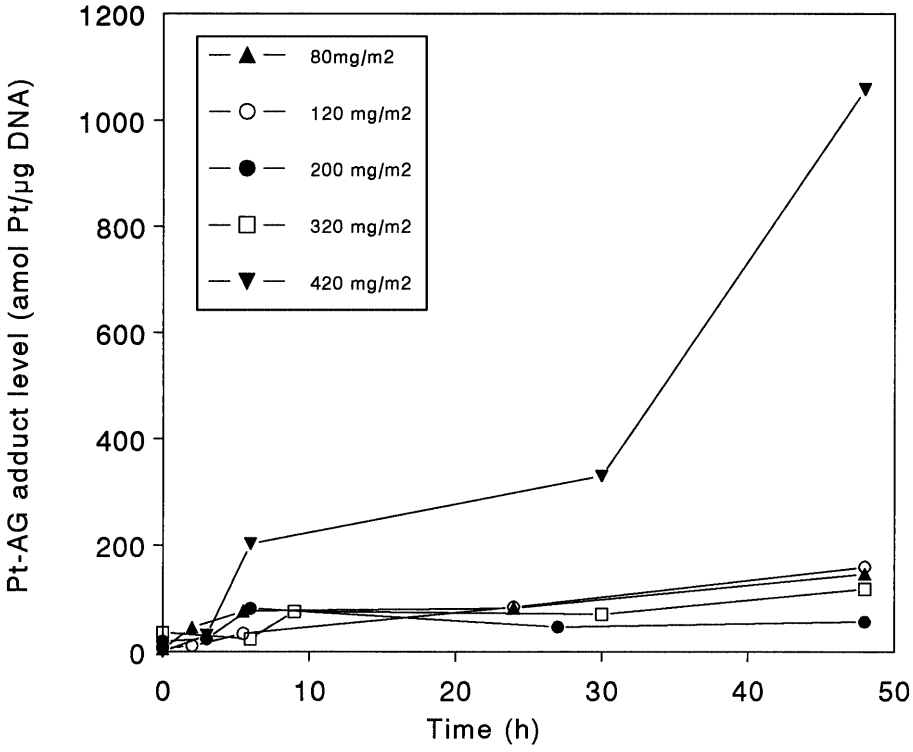


**Fig. 5** Mean concentration-time curves for Pt-AG adducts in WBC. Each curve was derived from the data from three patients, except for the 420 mg/m$^2$ dose which was derived from two patients

**Table 4** AUC values of Pt-GG and Pt-AG adducts

| Dose (mg/m$^2$) | No. of patients | AUC (fmol·h/µg) | |
|---|---|---|---|
| | | Pt-GG | Pt-AG |
| 40 | 3 | 4.3 ± 0.39 | –[a] |
| 80 | 3 | 16.3 ± 4.81 | 4.5 ± 1.00 |
| 120 | 3 | 20.6 ± 11.51 | 4.3 ± 4.03 |
| 200 | 3 | 14.6 ± 6.19 | 4.0[b] |
| 320 | 5 | 30.7 ± 10.04 | 4.6 ± 1.70 |
| 420 | 2 | 120.9 ± 7.81 | 19.5 ± 5.60 |

[a]Actual level too low for calculation
[b]$n = 2$

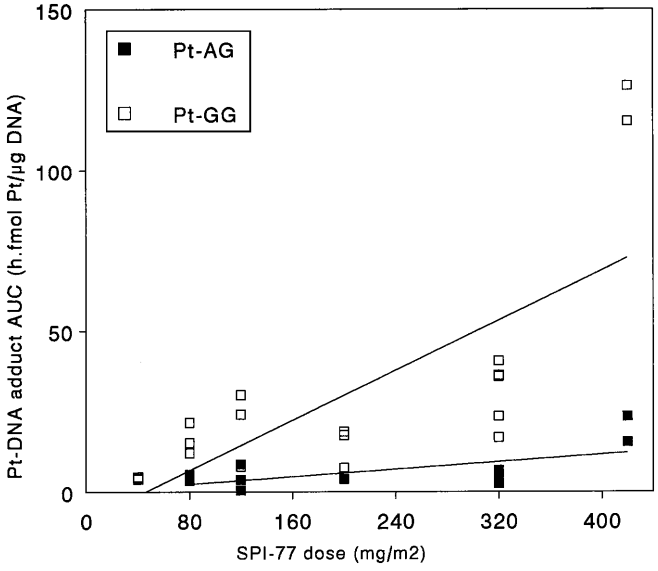


**Fig. 6** AUC values (derived from Figs. 3 and 4) of Pt-GG and Pt-AG adducts in WBC plotted against the dose of SPI-77. The lines represent the correlation between the AUC values with $R = 0.75$ ($P = 0.0004$) for the Pt-GG AUC values and $R = 0.61$ (not significant) for the Pt-AG AUC values

in ascites were relatively high compared to adduct levels in WBC of the same patient, Pt-DNA adduct levels were still low compared to Pt-DNA adduct levels in WBC after administration of standard cisplatin [11, 19]. Recent in vivo studies have shown a positive correlations between Pt-adduct levels in tumour tissue and response to cisplatin treatment [26, 27]. Thus, combining these results, we concluded that there was insufficient release of cisplatin from the liposomes due to their prolonged circulation time, resulting in low concentrations of free platinum in plasma and low levels of Pt-DNA adducts in WBC. The second reason for discontinuation of the study was the need for disproportionately high doses of SPI-77 to achieve satisfactory platinum concentrations, resulting in unpredictable large increases in plasma cholesterol values.

In conclusion, we report the results of a phase I study with liposomal encapsulated cisplatin. SPI-77-derived platinum displayed a markedly different pharmacokinetic behaviour, mainly dominated by the liposomes, as compared to cisplatin. Consequently, its toxicity profile differs largely from that of free cisplatin. However, despite the favourable pharmacokinetic properties, enhanced accumulation of platinum in tumour cells following administration of SPI-77 could not be demonstrated. Increased tumour selectivity may be achieved, for example, by using the altered physiological conditions

at the tumour site, such as lower pH, in combination with an acid-sensitive linker at the liposome. Future studies will be performed with Stealth liposomes which have a more favourable release pattern of cisplatin, and with other drugs encapsulated in the liposomes.

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