Cancer Chemother Pharmacol (2002) 50: 131–136
DOI 10.1007/s00280-002-0469-8

ORIGINAL ARTICLE

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# STEALTH liposome-encapsulated cisplatin (SPI-77) versus carboplatin as adjuvant therapy for spontaneously arising osteosarcoma (OSA) in the dog: a randomized multicenter clinical trial

Received: 19 November 2001 / Accepted: 3 March 2002 / Published online: 14 June 2002


**Abstract** *Purpose:* This trial was designed to compare the efficacy of adjuvant STEALH liposome-encapsulated cisplatin (SPI-77) to "standard-of-care" carboplatin therapy in dogs with osteosarcoma (OSA) in the context of a randomized study design. *Methods:* The study included 40 pet dogs with spontaneously arising OSA which were randomized to receive SPI-77 (350 mg/m$^2$ i.v. every 3 weeks for four treatments) or carboplatin (300 mg/m$^2$ i.v. every 3 weeks for four treatments) along with amputation of the affected limb. Median disease-free (DFS) and overall survival (OS) were compared using standard life-table analysis. *Results:* The median follow-up was 693 days (range 321–730 days). Of 38 dogs eligible for follow-up, 25 were dead of their disease, 9 were alive and disease-free (8 receiving SPI-77, 1 receiving carboplatin; $P = 0.02$), 2 were free of disease when they were lost to follow-up at 321 and 395 days, and 2 had died of an unrelated disease. The median DFS times for dogs treated with SPI-77 and carboplatin were 156 and 123 days, respectively ($P = 0.19$). The median OS times for dogs treated with SPI-77 and carboplatin were 333 and 207 days, respectively ($P = 0.18$). *Conclusions:* While STEALTH liposome encapsulation of cisplatin allowed the safe administration of five times the maximally tolerated dose of free cisplatin to dogs without concurrent hydration protocols, this did not translate into significantly prolonged DFS or OS. However, a larger proportion of dogs receiving SPI-77 enjoyed long-term DFS when compared with dogs receiving carboplatin.

**Keywords** Stealth liposome · Cisplatin · Carboplatin · Osteosarcoma · Canine

This work was supported in part by a research grant from ALZA Corporation, Mountain View, CA 94043, USA.

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## Introduction

Cisplatin is one of the most widely used chemotherapeutic agents employed today in the clinical practice of human and veterinary oncology. It has a broad spectrum of activity and in particular is the cornerstone of adjuvant systemic therapy for osteosarcoma (OSA) in both humans and pet dogs [5, 12]. In both species, its use is ultimately limited by dose-dependent toxicities, in particular nephrotoxicity [5, 9, 12]. Several strategies have been employed to abrogate or avoid the nephrotoxicity of cisplatin, including the development of less-toxic and/or more-efficacious

platinum analogues, concurrent use of nephroprotectant agents, and the use of time-consuming hydration protocols [3, 9, 13, 18]. Another approach is to encapsulate cisplatin in liposomes in order to beneficially alter the toxicity profile, pharmacokinetics, and biodistribution [20, 22, 25].

SPI-77 is one such liposomal formulation of cisplatin. The long-circulating pegylated liposomes of SPI-77 are sometimes termed STEALTH liposomes because they tend to avoid uptake by the mononuclear phagocytic system [17]. This translates into greatly increased blood circulation times, area under the time-concentration curve (AUC) and delayed plasma clearance [17, 24]. Ultimately, STEALTH liposomes preferentially accumulate in tumor tissues due to increased circulation time, higher vascular permeability relative to nonpegylated liposomes, and the inherently leaky nature of tumor vasculature [6, 26, 27]. Antitumor efficacy has been shown for SPI-77 in two different tumor models, the murine C26 colon carcinoma and the Lewis lung tumor models in mice [14].

The dog has the highest reported incidence of spontaneous OSA of any species, with approximately 12,000 cases developing each year in North America [23]. It afflicts primarily large and giant breed dogs, with distal radius and proximal humeral metaphyseal locations predominating. OSA in dogs has several parallels with its counterpart in humans and has served as a model for novel therapeutics in the past [10, 11]. As in humans, metastatic rates in the dog are high, and primary tumor control by amputation will only cure approximately 10% of cases, with 90% going on to die of metastatic disease to the lungs or other long bones [4, 19]. Similar to the human experience, platinum-based chemotherapeutic agents (cisplatin and carboplatin) are the cornerstone of adjuvant systemic therapy aimed at eradicating micrometastatic disease [3, 5, 21]. Cisplatin therapy following amputation results in survival times of approximately 11 months compared with 3 to 4 months with amputation alone. However, its use in this species is limited, as in humans, primarily by nephrotoxicity. The maximally tolerated dose (MTD) in dogs has been established at 70 mg/m$^2$ and it must be delivered concurrently with a 6-h saline diuresis protocol [15, 21]. In the past decade, carboplatin (MTD 300 mg/m$^2$ in the dog) has supplanted cisplatin as the "standard-of-care" in dogs with OSA because of its ease of delivery, lack of nephrotoxicity, and comparable efficacy [3, 5].

The encapsulation of cisplatin in the SPI-77 formulation results in an ability to safely and repeatedly deliver doses of cisplatin at five times the MTD of free cisplatin, that is 350 mg/m$^2$ intravenously (i.v.) every 3 weeks (unpublished preclinical work in normal dogs). This higher dosing scheme and the established efficacy of free cisplatin in treating spontaneously arising OSA in pet dogs offers a unique model to determine whether SPI-77 would translate into enhanced efficacy over "standard-of-care" therapy. This trial was designed to compare the efficacy of the MTD of adjuvant SPI-77 to the MTD of "standard-of-care" carboplatin therapy in dogs with OSA in the context of a randomized study design.

## Materials and methods

### Subject population

Between December 1998 and August 1999, 40 privately owned pet dogs with previously untreated, histologically confirmed appendicular OSA without evidence of distant metastasis were randomized into this study. Pretreatment evaluation included complete physical examination, complete blood cell count (CBC) with platelet count, biochemistry profile, urinalysis, thoracic and primary tumor radiographs, modified Karnofsky performance score [16], and histopathological assessment of tumor type. Eligible dogs were free of complicating concurrent disease, had adequate hematological and serum biochemical parameters to undergo amputation and chemotherapy, and had a modified Karnofsky score of 2 or lower. Informed consent and agreement to necropsy forms were signed by each dog's owner prior to entry. All experimental procedures and protocols were reviewed and approved by the Institutional Animal Care and Use Committee (University of Wisconsin, School of Veterinary Medicine). Two dogs were ultimately excluded from the study because of an inability to confirm a diagnosis histologically in one case and lack of availability of SPI-77 in the other.

### Formulation of SPI-77

SPI-77 is a STEALTH liposomal formulation of cisplatin, formulated in liposomes that contain a pegylated lipid [*N*-(carbamoyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine sodium salt, MPEG-DSPE], another phospholipid (hydrogenated soy phosphatidylcholine, HSPC), and cholesterol. The total lipid content of the SPI-77 formulation is approximately 71 mg/ml, and the cisplatin concentration is 1 mg/ml. SPI-77 liposomes average 110 nm in diameter.

### Treatment

Dogs were randomized 7 days prior to amputation to receive either SPI-77 or carboplatin adjuvant therapy. Randomization was performed by the coordinating institution (University of Wisconsin). No stratification was used in the randomization; rather each case with histologically confirmed OSA was equally likely to be placed in one of the two treatment groups. All dogs were scheduled to receive four cycles of either SPI-77 (350 mg/m$^2$ i.v.) or carboplatin (300 mg/m$^2$ i.v.) once every 3 weeks, with the first treatment occurring 7 days prior to amputation. The dose of SPI-77 used was based on previous toxicity data in normal dogs and represents five times the established MTD of free cisplatin in the dog [5, 21]. The carboplatin dose used represents the previously established MTD in dogs [3]. Carboplatin (Paraplatin; Bristol-Myers Squibb, Princeton, N.J.) was delivered as a standard 10-min i.v. infusion at a concentration of 10 mg/ml. SPI-77 was diluted from its original concentration of 1 mg/ml cisplatin equivalent to 0.3 mg/ml in normal saline. Administration was initiated at a rate equivalent to 10 mg/m$^2$ per h for the first 15 min of infusion and then the rate was increased to 40 mg/m$^2$ per h.

All dogs underwent amputation (forequarter amputation for lesions of the forelimb; hip disarticulation for lesions of the hind limb) 7 days following the first chemotherapy treatment. The three subsequent chemotherapy treatments were initiated 2 weeks following amputation.

### Subject follow-up

A complete physical examination, CBC, biochemical profile, and urinalysis were performed prior to each chemotherapy treatment. Thoracic radiographs (three projections: right lateral, left lateral and ventrodorsal) were performed at the last chemotherapy treatment and every 2 months following completion of the final treatment. Additional diagnostics and imaging studies (i.e., abdominal ultrasound, computed tomography, bone surveys) were performed when indicated at the discretion of the clinician in charge in order to determine the presence of recurrence or metastasis at extrapleural sites.

### Statistical analysis

Median disease-free (DFS) and overall survival (OS) times were compared between the SPI-77 and carboplatin groups. DFS was defined as the number of days from the time of amputation to the development of detectable metastasis. OS was defined as the number of days from surgery to death or euthanasia due to progressive disease. Because alternative treatments were allowable after gross metastatic disease was documented, DFS was the primary endpoint of the study and OS was a secondary endpoint. Survival curves were generated using the Kaplan-Meier method, which accounted for (i.e. censored) dogs that were alive, lost to follow-up, or had died from unrelated causes at the time of analysis. Treatment groups were compared using the log rank test of significance to determine the significance of differences between survival curves. Assuming a 4-month standard deviation in the DFS, 20 dogs in each group should have been sufficient to measure a 3-month difference in DFS between the treatment groups, with a $P$-value of 0.05 and a power of 0.90.

In addition, the effect of several variables, including age, body weight, tumor location, and serum alkaline phosphatase (AlkP) at diagnosis, were evaluated within treatment groups using the log rank test. If a variable was found to be prognostic for DFS or OS, the distribution of the variable within the two groups was compared using an unpaired $t$-test. The proportion of dogs alive and free of disease at the end of the study (median follow-up time of 693 days) was compared between groups using Fisher's exact test. For all analyses, a $P$-value of less than 0.05 was considered significant.

## Results

### Subject demographics

The subject characteristics by group are listed in Table 1. The 15 breeds represented included 7 Rottweilers, 6 Golden Retrievers, 4 Alaskan Malamutes, 4 Labrador Retrievers, 3 St. Bernards, 3 German Shepherd dogs, 2 Dalmatians, 2 Great Pyrenees, and 1 each of Doberman Pincer, Springer Spaniel, Irish Setter, Great Dane, English Setter, Giant Schnauzer and German Shorthaired Pointer. Serum AlkP values, the only repeatable prognostic factor for dogs with OSA [7, 8], were not significantly different between the groups.

**Table 1.** Subject characteristics by group

| Characteristic | SPI-77 group | Carboplatin group |
|---|---|---|
| Weight (kg) | | |
| Mean | 41.8 | 39.1 |
| Median | 41.6 | 40 |
| Range | 23.5–57.4 | 21.1–52.3 |
| Age (years) | | |
| Mean | 7.4 | 7.6 |
| Median | 7 | 7.5 |
| Range | 2–12 | 2–12.5 |
| Sex | | |
| Male neutered | 9 | 9 |
| Male | 2 | 1 |
| Female spayed | 8 | 8 |
| Female | 1 | 0 |
| Location | | |
| Distal radius | 9 | 7 |
| Proximal humerus | 5 | 5 |
| Distal femur | 3 | 3 |
| Distal tibia | 2 | 1 |
| Proximal tibia | 1 | 1 |
| Distal ulna | 0 | 1 |
| Serum alkaline phosphatase | | |
| Mean | 79 | 219 |
| Median | 134 | 121 |
| Range | 33–546 | 27–937 |
| Cases with levels above normal reference range | 7 | 9 |

### Toxicities

Two dogs developed acute anaphylaxis-like symptoms immediately upon initiation of SPI-77 infusion. In both cases, drug infusion was paused and the dog was given Benadryl (4 mg/kg i.m.) and dexamethasone $NaPO_4$ (2 mg/kg i.v.) and the infusion restarted 30 min later at 5 mg/m$^2$ per h again for 15 to 30 min. At that time the administration rate was increased back to 40 mg/m$^2$ per h. Neither dog experienced further reactions.

No dog in either group required a treatment delay because of significant myelosuppression, diminished performance score, or other toxicity. No clinicopathological evidence of nephrotoxicity or hepatotoxicity was encountered in any dog of either group.

### Outcome

The median follow-up for the dogs in the study was 693 days (range 321–730 days). Of the 38 dogs eligible for follow-up, 25 died of OSA metastatic disease; post mortem materials were available in 19 (75%) and lung metastasis was confirmed radiographically in 5 others. The final case was presumed to have developed vertebral metastasis due to the development of an acute transverse myelopathy, but the clients declined further evaluation in that case. Of the 25 dogs who died of OSA metastasis, 21 had evidence of lung metastasis only, 2 of bone metastasis only, 1 with bone and lung metastasis, and 1 with lung and lymph node metastasis. Nine dogs were alive and disease-free, and of these, eight had received SPI-77 and one had received carboplatin ($P = 0.02$, Fisher's exact test). Two dogs were free of disease when they were lost to follow-up at 321 and 395 days (both received carboplatin), and two dogs died of unrelated disease at 41 days (chemodectoma) and 515 days (fibrosarcoma with pulmonary metastasis). The median

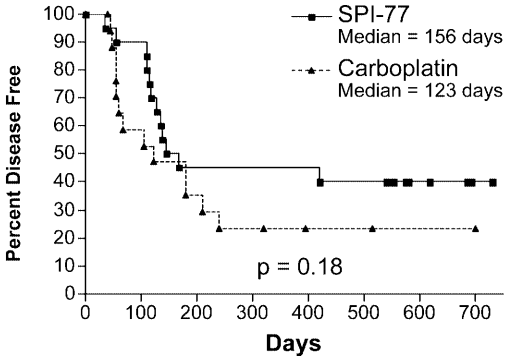


**Fig. 1.** Kaplan-Meier disease free survival duration estimates for dogs with osteosarcoma following treatment with SPI-77 (350 mg/m$^2$ cisplatin equivalent, i.v., every 3 weeks for four treatments) or carboplatin (300 mg/m$^2$, i.v., every 3 weeks for four treatments)

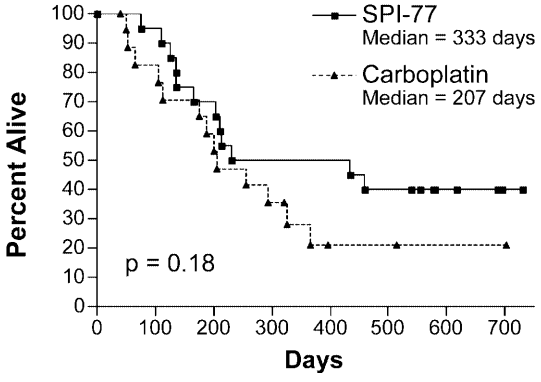


**Fig. 2.** Kaplan-Meier overall survival duration estimates for dogs with osteosarcoma following treatment with SPI-77 (350 mg/m$^2$ cisplatin equivalent, i.v., every 3 weeks for four treatments) or carboplatin (300 mg/m$^2$, i.v., every 3 weeks for four treatments)

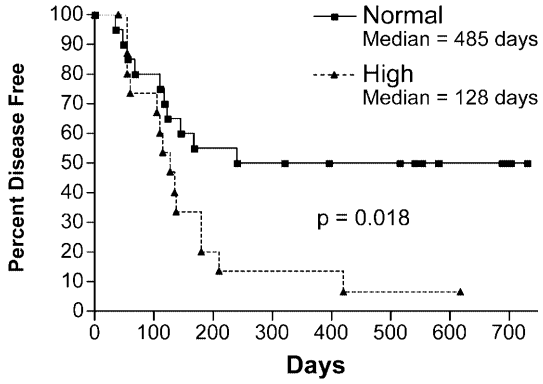


**Fig. 3.** Kaplan-Meier disease-free survival duration estimates for dogs with pretreatment serum alkaline phosphatase levels within the laboratory normal reference range and those above the reference range

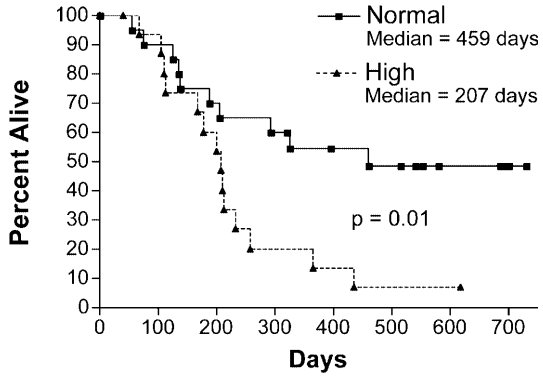


**Fig. 4.** Kaplan-Meier overall survival duration estimates for dogs with pretreatment serum alkaline phosphatase levels within the laboratory normal reference range and those above the reference range

DFS times for dogs treated with SPI-77 and carboplatin were 156 and 123 days, respectively ($P = 0.19$, Fig. 1). The median OS times for dogs treated with SPI-77 and carboplatin were 333 and 207 days, respectively ($P = 0.18$, Fig. 2).

Of the variables analyzed, only serum AlkP levels at the time of diagnosis were predictive of both DFS and OS. Dogs with AlkP levels within the laboratory normal reference range and those with levels above the reference range had DFS times of 485 and 128 days, respectively ($P = 0.018$, Fig. 3). Dogs with AlkP levels within the laboratory normal reference range and those with levels above the reference range had OS times of 459 and 207 days, respectively ($P = 0.010$, Fig. 4).

## Discussion

The two treatment groups compared in this study were typical of historical populations of dogs with OSA with respect to age, sex, body weight, breed, and location of primary tumor [5, 23]. To date, the only repeatable predictor of DFS and OS for dogs with OSA is pretreatment serum AlkP (both total and bone isoenzyme) [7, 8]. The randomization was successful in ensuring similar populations for comparison.

This study has shown that encapsulation of cisplatin in the pegylated STEALTH liposome employed with SPI-77 allows the safe and repeated delivery of doses up to five times the MTD of native cisplatin in tumor-bearing dogs. This dose escalation is possible without the hydration protocols necessary when delivering native cisplatin [15]. However, the systemic delivery of these escalated doses did not translate into enhanced efficacy as measured by DFS or OS: the DFS and OS times in the SPI-77-treated dogs were not significantly different than the times in the carboplatin-treated dogs in this study. Nor did dogs receiving SPI-77 have DFS and OS times longer than those found previously with adjuvant non-liposomal cisplatin in dogs amputated for OSA. The 11-month overall median survival found for SPI-77-treated dogs in this study was nearly identical to that reported in several other studies using native cisplatin at one-fifth the cisplatin-equivalent dose [2, 5, 21]. Of some note, however, is that the tails on the survival curves in dogs receiving SPI-77 were quite long, and indeed there

was a significantly higher proportion of dogs enjoying long-term survival than in the carboplatin group. However, numbers were relatively small in these groups, and the ''gold standard'' of censored life-table survival analysis did not reveal a difference.

The lack of enhanced efficacy when compared to historical cisplatin or prospective carboplatin begs the question ''why is more not better?'' Several possibilities exist. First, the model may not be appropriate for the theoretical advantages of SPI-77. This OSA model is not a gross disease model; rather it is a micrometastatic model. Micrometastatic disease does not have the leaky vasculature that gross macroscopic tumors inherently create, and that result in the preferential accumulation of the long-circulating STEALTH liposomes [6, 26, 27]. Second, while more liposome-encapsulated cisplatin may distribute into tumors and circulate for longer periods in the plasma, is it in a bioavailable form, or released in a form that is not accessible to the tumor cells? There is some preliminary support for the latter theory in a rodent model [1, 28]. In a B16 murine melanoma model, tumor microdialysis has revealed that more total platinum distributes into tumors when SPI-77 is used in comparison to free cisplatin; however, less cisplatin is actually released into the tumor extracellular fluid and fewer platinum-DNA adducts are formed in the tumor tissue [28]. In three other mouse tumor models (M-109 lung carcinoma, J-6456 lymphoma and A-375 melanoma), in vitro release assays have shown a negligible release (below 10%) of platinum from SPI-77 liposomes, and SPI-77 has been shown to be inferior to free cisplatin using in vitro cytotoxicity assays [1]. Based on these studies, it would seem prudent to seek the development of ''leakier'' liposomal formulations. The ideal liposome carrier should become leaky in environments approximating the tumor microenvironment, that is, hypoxic and acidotic tissues.

Finally, this trial also demonstrated the utility of spontaneous tumors in animals as translational models for the evaluation of these and other novel anticancer therapies.

**Acknowledgements** We wish to thank Dr. Valerie Poirier, Linda Beaver, Jean Tillis, and Anne Stewart for their expert technical assistance. Additionally, Drs. Greg MacEwen, Betsy Hershey, Brenda Phillips, Douglas Thamm, Stuart Helfand, Michelle Turik, and Kristine Burgess are acknowledged for case management.

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