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Evaluation of Satraplatin in Dogs with Spontaneously Occurring Malignant Tumors

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Background: Satraplatin is the 1st orally bioavailable platinum anticancer drug.

Objective: Our objectives were to evaluate efficacy in vitro against a canine cancer cell line, to determine the maximally tolerated dose (MTD) of satraplatin in tumor-bearing dogs, to identify the dose-limiting and other toxicities in dogs, and to record pharmacokinetics (PK).

Animals: Dogs with macro- or microscopic malignant neoplasia.

Methods: D17 canine osteosarcoma cells first were evaluated in a clonogenic survival assay. Then, dogs with a diagnosis of malignant neoplasia were prospectively entered in standard 3 + 3 cohorts. Additional patients were entered at the MTD to assess efficacy. Total and free platinum (by ultrafiltrate) concentrations were determined with inductively coupled plasma mass spectroscopy.

Results: Satraplatin inhibited clonogenic survival in vitro at clinically relevant and achievable concentrations. Twenty-three dogs were treated, 14 with PK evaluation. The MTD was 35 $mg/m^2/d$ for 5 days, repeated every 3–4 weeks. Bioavailability was 41%. PK variables (mean ± SD) at the MTD included T_{max} 1.8 (± 0.7) hours, C_{max} 72 (± 26) ng/mL, area under concentration $(AUC)_{0-24\ h}$ 316 (± 63) h×ng/mL, and MRT 7 (± 1.3) hours. Higher AUC after the 5th versus the 1st dose suggested drug accumulation. Interestingly, platelets consistently reached nadir sooner than did neutrophils (day 14 versus 19). Myelosuppression was dose-limiting and gastrointestinal toxicity was mild.

Conclusions and Clinical Importance: Satraplatin was well tolerated in tumor-bearing dogs, thus warranting further investigation in a phase II trial.

Key words: Canine; Chemotherapy; Clinical pharmacology; Pharmacokinetics; Pharmacology.

Satraplatin (JM216) is the first PO-delivered platinum chemotherapy drug used in human patients for cancer treatment, developed initially in the mid-1990s.[1] As a platinum (IV) compound, the drug is more lipophilic and stable than other platinum agents, allowing oral delivery and bioavailability. In addition to the oral formulation, satraplatin is unique as compared with other platinum agents in that it causes less neuro- and nephrotoxicity.[2–4] The major metabolite of JM216 is JM118, which itself has significant anticancer activity.[5] The dose-limiting toxicity (DLT) seen in people and rodents to date is myelosuppression, with lowest neutrophil and platelet counts both occurring at 2–4 weeks. Other adverse effects include nausea and diarrhea in approximately one-third of patients.[6] Pharmacokinetics (PKs) and physical properties are known in people.[7–9] As a result of its lipophilic nature and reactivity, satraplatin tends to accumulate in tissues, maintaining detectable concentrations for up to 2 weeks after cessation of therapy.[6] The ideal dosing scheme is once daily for 5 days and repeated every 3–5 weeks.[9] This schedule has been used in people, and a decrease in tumor size has been seen in human patients with ovarian cancer, among others, as well as palliation of tumor-related pain even in the absence of objective tumor response.[10]

Abbreviations:

DLT	dose-limiting toxicity
ICP-MS	inductively coupled plasma mass spectroscopy
MTD	maximally tolerated dosage

Although resistance to other platinum drugs such as cisplatin is a frequent cause of treatment failure, satraplatin creates bulkier adducts in DNA that may be more difficult for cellular machinery to repair.[11] Accordingly, in vitro evidence demonstrates satraplatin efficacy in cisplatin-resistant cell lines.[5,12] Satraplatin enters the cell primarily by passive diffusion, likely because of its enhanced lipophilicity.[13] Satraplatin also has been shown to inhibit cytochrome P450,[14] and resistance to satraplatin may occur by glutathione conjugation.[15]

While in vitro investigations have included both chemosensitive and chemoresistant cell lines of human prostate, colon, breast, leukemia, and ovarian cancer,[5] no published reports exist of in vitro activity of satraplatin or its metabolites against nonhuman cancer. Identification of efficacy in vitro at concentrations that can be achieved in the plasma based on PK evaluation provides preclinical support for the use of a novel chemotherapy agent in vivo.

Satraplatin has not been evaluated in animals with spontaneously occurring neoplasia. Oral chemotherapy agents offer many advantages for treatment of dogs with

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cancer. The purpose of this study was to determine the toxicity and the maximally tolerated dose (MTD) of satraplatin in dogs with cancer. PK data were collected to allow determination of the optimum dose and dosing interval for future clinical studies in order to evaluate efficacy in a prospective manner. Our hypothesis was that nephro- and neurotoxicity would not be dose limiting for satraplatin in dogs and that a dosage resulting in acceptable toxicity would be established. Based on available human data, we hypothesized that gastrointestinal toxicity or myelosuppression would be dose limiting for this drug in dogs.

Materials and Methods

In Vitro Studies

Tumor Cells. The canine osteosarcoma cell line D17 was available at our institution (originally obtained from the American Type Culture Collection, Manassas, VA). D17 cells were originally derived from an osteosarcoma in an 11-year-old Standard Poodle from pulmonary metastatic lesions. Cells were delivered to 12 well plates using RPMI media supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.12 mg/mL gentamicin, and supplemental satraplatin when applicable. Cells were maintained at 5% CO_2 and 37°C. Low plating density was used with 200 cells per well, and cells were allowed to proliferate for 4 days before fixation with formalin and crystal violet staining. Viability was assessed by enumeration of colonies using staining and inverted light microscopy. A colony was considered successful if more than 15 cells were present. After initial plates failed to show inhibition of clonogenic survival except at higher doses, the plates subsequently were covered with aluminum foil for the duration of the experiment to protect the drug from light. Satraplatin concentrations used were as follows: 12.5, 25, 37.5, 50, 75, 100, 150, 200, 300, and 600 nM. These concentrations are equal to 6.3, 12.5, 18.8, 25, 37.5, 50, 75, 100, 150, and 300 ng/mL, respectively. Three experiments were run with overlapping concentrations, some in duplicate and others singly.

In Vivo Studies

Subjects. Dogs with spontaneously occurring neoplasia were recruited from the oncology service at the University of Missouri Veterinary Medical Teaching Hospital. Enrollment criteria included cytologic or histologic diagnosis of cancer, adequate performance status, life expectancy of at least 16 weeks, satraplatin administered as the sole anticancer therapy, adequate renal and bone marrow function as determined by chemistry profile and CBC, and informed owner consent. At the University of Missouri, IACUC approval is not required for clinical trials in animals if owner consent is obtained and the protocol could be performed by general practitioners. Informed owner consent was obtained in all cases.

Pretreatment Evaluation. CBC, chemistry profile, and urinalysis were performed on all dogs as well as 3-view thoracic radiographs and additional staging tests as appropriate for the tumor type such as abdominal ultrasound examination or nuclear scintigraphy. For chemotherapy, normal renal function, a neutrophil count of at least 3,000/μL, and a platelet count of at least 150,000/μL were required within 24 hours of the 1st dose.

Patient Monitoring. The first 3 dogs enrolled had CBCs collected Monday and Thursday of each week for the first 3 weeks to determine neutrophil and platelet nadirs. Such frequent blood sampling is impractical in client-owned animals so we chose not to evaluate the nadir in all subjects. The nadir should be at the same time point regardless of dose, although more profound with higher doses. CBCs were performed at the predicted nadir in subsequent dogs. Biochemical evaluation with urinalysis was performed at baseline and at the time of the third or (most often) the 4th cycle of chemotherapy. Sixteen dogs had at least 1 biochemical profile after receiving satraplatin, 2 died unexpectedly before the 2nd cycle of treatment, 2 dogs exited the study without biochemical evaluation because of disease progression early in the course of treatment, and 3 dogs either were not evaluated because of lack of owner compliance or because the information was not available from other veterinary clinics. Once dogs exited the study, they were not routinely monitored except to recommend thoracic radiographs every 3 months and to request postmortem examination at the time of their death.

Assessment of Response. Although not a primary objective of this study, clinical response was noted for the purpose of justifying a future phase II trial. Complete response (CR) was defined as resolution or disappearance of all clinically detectable disease, partial response (PR) was defined as $>50\%$ reduction in tumor volume (length $\times$ width $\times$ height $\times \pi/6$), stable disease (SD) was defined as $<25\%$ increase and $<50\%$ decrease in tumor volume, and progressive disease (PD) was defined as $>25\%$ increase in tumor volume or the detection of new lesions. Response assessment was made at 3 months after enrollment and objective responses were required to be maintained for at least this period of time. Outcome of patients treated in the adjuvant setting was evaluated with progression-free survival (PFS), defined as the date of enrollment to the date of detectable new lesions. Overall survival (OS) was defined as the date of enrollment to the date of death.

Assessment of Toxicity. Adverse events were graded according to the Common Terminology Criteria for Adverse Events as published by the Veterinary Cooperative Oncology Group (VCOG-CTCAE v.1).[16] For most adverse events, grade 3 or higher toxicity was considered dose-limiting for that individual. However, afebrile, asymptomatic grade 3 neutropenia or thrombocytopenia was not considered dose-limiting, whereas febrile grade 3 neutropenia or grade 4 myelosuppression of any kind constituted a DLT. If a dog experienced DLT, the next lowest dose was used subsequently for that dog so that chronic toxicity still could be evaluated. If a dog experienced only nausea as a DLT, that dog was allowed to receive an antiemetic (mirtazapine) before each treatment for subsequent doses at the same dosage. If grade 3 or higher gastrointestinal toxicity was encountered despite the addition of antinausea medication, the next lowest dose was used for subsequent treatments. Owners were provided with a diary to record any changes in thirst, urination, appetite, stool, or other physical variables at home.

Dosing Scheme. Groups of 3–6 dogs were treated at each dose level until the MTD was reached in a standard 3 + 3 design as detailed below. All dogs meeting the eligibility criteria and receiving at least 1 dose of satraplatin were considered evaluable for toxicity. Dogs were dosed initially at 50 mg/m^2 PO once daily for 5 days. This dosage was selected based upon data from a preliminary study (unpublished data) in which healthy Beagle dogs were treated at various dosages daily for 5 days repeated every 28 days for a total of 7 cycles, and was the dose at which no clinically relevant toxicity was seen. Dosage initially was increased or decreased by 10 mg/m^2/d increments until DLT was encountered, then further titrated at 5 mg/m^2/d increments. Using 10 and 50 mg capsules, dosing was rounded to the nearest 10 mg, without exceeding the calculated dose by more than 10%. The dose was administered to each dog after at least a 5-hour fast, although a small amount of food was allowed if needed to facilitate administration. After dosing, dogs were encouraged to drink, and food was withheld for an additional hour to allow absorption. Because effects of motility-modifying drugs on satraplatin absorption were unknown, metoclopramide was avoided and mirtazapine was used as the initial antiemetic and appetite stimulant, if needed. During the course of the trial, an NK1 receptor antagonist (maropitant) became available and was used as an initial or secondary antiemetic when needed.

Dose Escalation and De-escalation Rules. Enrollment was staggered to ensure safety. Dogs were enrolled in a new cohort concurrently, but at least 3 dogs must have completed the 1st cycle and have been evaluated for toxicity before dose escalation could occur. The standard 3 + 3 design involves dose escalation in the absence of DLT, and cohort expansion by an additional 3 dogs if 1 DLT is seen. If 2 or more DLTs are found at a given dosage, then the MTD has been exceeded. Once the MTD was determined, a minimum of 6 additional dogs were planned to be enrolled at this level to further characterize the safety profile at the MTD dose level. This also would allow preliminary justification for a phase II trial if at least 1 dog showed evidence of efficacy. These dogs did not have PK samples collected. De-escalation was permitted in the event of life-threatening adverse events, even if a cohort was not complete.

PK Evaluation. During the dose escalation phase, all dogs had PK evaluation performed at each dose level with blood drawn to determine concentrations of total and free platinum. Sampling times were as follows: day 1: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 6, 12, and 24 hours after administration; day 5: 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 6, 12, 24, 48, and 72 hours after administration. Prechilled tubes and a precooled centrifuge (4°C) were used to collect EDTA plasma from blood samples at each time point. One milliliter of plasma was transferred into a cryotube and immediately frozen at −80°C. Aliquots of plasma were ultrafiltered with Centrifree 30,000 D ultrafiltration devices[a] in a precooled fixed angle rotor centrifuge (4°C) for 20 minute at 2,000 × *g*. Aliquots (approximately 500 μL total) of the plasma ultrafiltrate (PUF) were immediately frozen at −80°C. The use of these special filters allowed the separation of free platinum because it is considered the biologically relevant portion. However, because the relative value of free and total platinum has been debated, both fractions were evaluated. Accumulation of drug was determined by dividing day 5 PK values by day 1 PK values for maximal plasma concentration (C_{max}) and area under concentration (AUC) time curves.

PK Modeling. The PK parameters of total platinum (Pt_{TOT}) and ultrafiltered platinum (PUF) were calculated by noncompartmental methods and equations by Microsoft Excel software.[17] C_{max} and time to maximal plasma concentration (T_{max}) were determined from the plasma concentration-time profiles. AUC time curves and area under the moment curve (AUMC) were calculated by linear trapezoidal summation. Clearance was calculated as dose/AUC time curves and MRT as AUMC/AUC time curves.

Platinum Determination. Because the anticipated peak serum concentrations of platinum were relatively low (200–400 ng/mL), inductively coupled plasma mass spectrometry (ICP-MS) was used to determine serum platinum concentrations for PK evaluation. ICP-MS is more sensitive than other methods of platinum determination.

Instrument. A plasma-quadrupole inductively coupled plasma mass spectrometer (PQ3 ICP-MS) was used for the measurements. Table 1 shows the instrument settings and operating conditions.

Effect of Sample Handling on Results. Before collection of clinical samples, the effect of various serum conditions was evaluated. Serum from unaffected, healthy animals that was lipemic, hemolyzed, or both, was obtained and spiked with satraplatin. Samples were processed and evaluated by ANOVA for differences among expected results. Additionally, acid balancing was evaluated with 0.9% HCl with 2 different concentrations of HNO_3 (0.7 and 7%).

ICP-MS Platinum Standard Solution. The working standard solutions were prepared every 7 days. These stock standard solutions were prepared by diluting the 1,000 μg/mL platinum standard solution with 0.24 M HCl and stocked at room temperature. The standard solutions had concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20 ng/mL platinum and contained 10 ng/mL iridium as internal standard.

Microwave Digestion. Acid assist closed vessel microwave digestion can be used to denature the plasma protein matrices efficiently to release metals from the matrices. All frozen samples were thawed at room temperature in a hood for 1 or 2 hours and vortexed well. One hundred microliters of aliquots of plasma samples collected at different time intervals were gravimetrically transferred into Teflon insert vials. Afterwards, 1,000 μL of Optima grade HCl (36%) and 100 μL of Optima grade HNO_3 (69%) were added into each sample successively. All samples then were digested by microwave. After digestion and cooling to room temperature, the samples were transferred into 15 mL polypropylene centrifuge tubes and diluted with 18 MΩ ultra pure water to 10 mL. Diluted samples were diluted again 1 + 9 with 0.24 M HCl containing 10 ng/mL iridium. The prepared samples were analyzed by ICP-MS. The parameters of microwave digestion are shown in Table 2.

Heated Sonic Water Bath Digestion. For metal analysis of PUF, a more economical digestion method, heated sonic water bath digestion, was used. The samples were prepared by heating at 60°C with sonication for 30 minutes. One hundred microliters of aliquots of PUF were gravimetrically transferred into 15 mL polypropylene Eppendorf centrifuge tubes. Each sample was mixed with 300 μL of aliquot Optima grade HNO_3 (69%) and 100 μL of aliquot Fluka TraceSelect grade H_2O_2 (30%) and then digested by water bath with sonication. Digested samples then were analyzed by ICP-MS.

Statistical Analysis. A descriptive analysis for breed, sex, age, weight, plasma albumin concentration, target dose, actual dose, dosage, measurable disease, and diagnosis was performed. To evaluate the relationship between PK and hematologic toxicity, regression analysis was conducted between the $t_{1/2}$, T_{max}, C_{max}, and AUC time curves (for Pt_{TOT} and PUF), and the nadir neutrophil and platelet counts. In addition, regression analysis between the actual dose administered and the nadir neutrophil and platelet counts also was performed. Kaplan-Meier analysis was calculated for the PFS and survival time points. A Kaplan-Meier log-rank test was used to calculate the difference in survival between the measurable and nonmeasurable disease osteosarcoma groups. All parameters were considered significant if the *P* value was $<.05$.

Table 1. PQ3 ICP-MS operating conditions and parameters.

Plasma	RF Power	Forward Power (1,300–1,400 W)
		Reflected: <2 W
	Ar gas flow rates	Auxiliary: 1–1.3 L/min
		Nebuliser: 0.9–1.0 L/min
	Voltage	2,400–3,600 V
Vacuum	Analyzer stage	3.0E−6–3.5E−6 mbar
	Expansion stage	0.8–1.0 mbar
Acquisition	Isotopes	^{191}Ir, ^{194}Pt, ^{195}Pt
parameters	Acquisition mode	Peak jump
	Channel per mass	4
	Dwell time	2,000 μs
	Uptake	60 seconds
	Acquisition time	10 seconds
	Rinsing time	60 seconds
	Replicates	10

Table 2. Closed vessel microwave digestion operating parameters.

Sample size	0.1 mL	
Digestion solution	Optima HNO_3	1 mL
	Optima HCl	0.1 mL
Stage	1	2
Power (W)	150	300
Temperature (°C)	140	180
Time (minutes)	10	25

Results

In Vitro Studies

Inhibition of clonogenic survival was observed in D17 cell lines. We concluded that JM216 was light sensitive, because inhibition was markedly more evident when multiwell plates were protected from light with aluminum foil. There was marked inhibition of clonogenic survival at or above concentrations of 37.5 nM (equivalent to 18.8 ng/mL) (Fig 1).

In Vivo Studies

Twenty-three dogs were enrolled, representing 11 breeds as follows: mixed breed (n = 6), Rottweiler (n = 3), 2 each Border Collie, Boxer, Greyhound, Labrador Retriever, and Portuguese Water Dog, and 1 each German Shepherd, Great Pyrenees, Great Dane, and Mastiff. There were 10 castrated males, 12 spayed females, 1 intact male, and no intact females. The median weight was 29 kg (range, 15–65 kg). Tumor types were as follows: osteosarcoma (n = 12, half treated in the adjuvant setting postamputation, and half with measurable disease), squamous cell carcinoma (n = 3, 2 oral, 1 nasal), thyroid carcinoma (n = 2), and 1 each lymphoma, anal sac adenocarcinoma, gastric carcinoma, soft tissue sarcoma, prostatic carcinoma, and nasal hemangiosarcoma. Overall, 14 dogs had measurable and 9 dogs had microscopic disease.

Baseline bloodwork was within normal limits with the exception of a slightly high BUN concentration in 1 dog that was attributed to nonsteroidal anti-inflammatory drug treatment and possible low-grade gastric ulceration, and which resolved after therapy was initiated and concomitant medications discontinued. Because satraplatin, like other platinum compounds, can be protein-bound, we monitored albumin concentration. The median baseline albumin concentration was 3.2 g/dL (range, 2.5–3.6 g/dL), with 17 dogs within normal range, 5 with hypoalbuminemia, and 1 unrecorded. Serum albumin concentration at study enrollment was not associated with toxicity (P = .782). No neuro- or nephrotoxicity was noted.

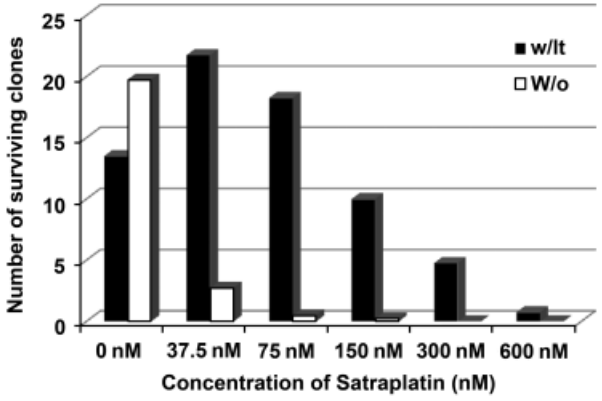

Fig 1. Light exposure decreased efficacy of JM216 (satraplatin pareny drug) in a clonogenic survival assay of canine D17 osteosarcoma cells seeded at 200 cells/well (solid, with light; clear, without light). Thus, this drug should be considered light sensitive. X-axis represents considerations of satraplatin in vitro, which are equivalent to 0, 18.8, 37.5, 75, 150, and 300 ng/mL, respectively.

Because of capsule size, some doses were rounded up if this resulted in less than a 10% increase, or down to the next lowest capsule size, if this was not the case. Overall, including all dose levels, the median target dosage was 35 mg/m^2/d because this was the MTD (range, 30–50 mg/m^2/d), whereas the median actual dosage was 33 mg/m^2/d (range, 24–52 mg/m^2/d). For all 14 patients in the PK evaluation, daily doses were the same each day, although for the remaining 9 patients some modifications were allowed (different doses on different days) to approximate the total dose over 5 days to average as close to 35 mg/m^2/d as possible. Treatment cycles were intended to repeat every 3 weeks, but treatment delays because of myelosuppression resulted in a median treatment interval between 1st and 2nd treatments of 25 days (range, 19–41). The median number of cycles completed was 4 (range, 0.5–4), with 13 dogs completing the intended 4 cycles, and the remaining dogs completing 3 (n = 3), 2 (n = 2), 1.5 (n = 2), 1 (n = 1), and 0.5 (n = 2) cycles.

The 1st dog enrolled experienced grade 4 febrile neutropenia with sepsis, but recovered with appropriate supportive care (IV fluid therapy and broad-spectrum IV antibiotics). Because the adverse event was life-threatening, we did not feel it was ethical to enroll a 2nd dog at this dosage, and felt the MTD had likely been exceeded. We then aimed to treat at a dosage that did not result in a DLT before initiating the 1st full cohort. The dosage thus was de-escalated to 40 mg/m^2/d for 5 days. The 2nd dog enrolled also experienced grade 4 neutropenia without fever and recovered uneventfully with prophylactic antibiotics. Although this dog's DLT was not clearly life-threatening, we felt the most appropriate measure to ensure patient safety was to de-escalate further. Another de-escalation to 30 mg/m^2/d for 5 days was well tolerated with no DLT in the next 4 dogs (1 additional dog was enrolled because 1 dog was suboptimally dosed because of a body weight between 2 doses). The dosage then was escalated to 35 mg/m^2/d for 5 days, and there was a single DLT in the first 3 dogs, leading to enrollment of an additional cohort of 3 dogs in which no DLTs were seen. The dosage was re-escalated to 40 mg/m^2/d for 5 days, and 2 additional DLTs (grade 4 neutropenia) occurred (2 dogs were enrolled concurrently), thus 35 mg/m^2/d for 5 days was selected as the MTD. An additional 9 dogs were enrolled at the MTD to assess preliminary efficacy. In this cohort, DLTs were seen in 3 dogs during the 1st cycle.

The DLT was myelosuppression, typically neutropenia as observed in 14/23 dogs (grade 1 = 5 dogs, grade 2 = 1 dog, grade 3 = 1 dog, grade 4 = 6 dogs). Thrombocytopenia also was seen in 16/23 dogs (grade 1 = 5 dogs, grade 2 = 6 dogs, grade 3 = 3 dogs, grade 4 = 3 dogs). The median neutrophil nadir occurred at day 19 (range, 7–23) and median platelet nadir occurred at day 14 (range, 7–26). Other toxicities were mild. These results are summarized and compared with dose cohort in Table 3.

Table 3. Hematologic adverse events after initial dosing with satraplatin, reported by dosing cohort.

		Severity Grade of Hematologic Toxicity by Patient							
		Neutropenia				Thrombocytopenia			
Dose level (mg/m^2)	No. of Dogs	1	2	3	4	1	2	3	4
30	4						1		
35	15	5	1	1	2	5	5		2
40	3				3			2	1
50	1				1			1	

Gastrointestinal toxicities in 8 dogs included grade 1 in 5 dogs at various dosages, and 1 each of grade 3, 4, and 5. The dog with grade 5 gastrointestinal toxicity had a perforating gastric ulcer after receiving 3 of 5 doses in the 1st cycle of treatment. This dog also had been treated with the nonsteroidal anti-inflammatory drug piroxicam for 3 weeks before enrollment, and this dog also had nasal hemangiosarcoma and was swallowing blood from the nasopharynx, which also could have contributed to ulcer formation. Although satraplatin may have contributed to ulcer formation or progression, a causal association could not be made because these other circumstances could have caused the ulcer. No neurologic or renal toxicity was noted, and 1 dog enrolled with a slightly high BUN concentration maintained this BUN concentration or lower throughout the trial.

Chronic toxicity also was documented, because all dogs were allowed to complete up to 4 cycles total. In subsequent cycles of therapy, only hematologic toxicity was seen with 4 episodes grade 1, 6 episodes grade 2, 4 episodes grade 3, and 3 episodes grade 4 from available data. Owners' assessment of appetite was reported for 928 days of therapy across all dogs, and ranked as excellent for 539 days, good for 211 days, decreased for 104 days, and poor for 57 days.

When dose, neutrophil nadir count, and platelet nadir count were analyzed by linear regression analysis against C_{max}, T_{max}, $t_{1/2}$, and $AUC_{0-24\ h}$ (total platinum and platinum ultrafiltrate days 1 and 5), the only associations that were statistically significant were the neutrophil nadir which correlated inversely with the $AUC_{0-24\ h}$ for PUF on day 1 ($P = .047$, $R^2 = 0.340$, Fig. 2); the dose which correlated directly with C_{max} for total platinum ($P = .028$, $R^2 = 0.341$) and PUF ($P = .029$, $R^2 = 0.34$) on day 1, as well as with the $AUC_{0-24\ h}$ for PUF on day 1 ($P = .024$, $R^2 = 0.358$); and dose which correlated inversely with both neutrophil ($P = .045$, $R^2 = 0.345$) and platelet ($P = .048$, $R^2 = 0.31$) nadir counts. PK variables are presented in Table 4. At the MTD, for PUF, drug accumulation was 1.01 ($\pm$ 0.29) for C_{max} and 1.17 ($\pm$ 0.24) for $AUC_{0-24\ h}$. Total platinum drug accumulation was 1.85 ($\pm$ 0.43) for C_{max} and 2.74 ($\pm$ 0.44) for $AUC_{0-24\ h}$. Compared with PK data generated at 35 mg/m^2/d for 5 days in normal Beagle dogs (data not shown, same sampling times and platinum determination method), our findings indicated a slightly longer T_{max}, slightly lower C_{max}, and almost identical $AUC_{0-24\ h}$ for total platinum and ultrafiltrable platinum, and these findings were consistent across days 1 and 5.

Bioavailability was estimated using the $AUC_{0-24\ h}$ for IV satraplatin in Beagle dogs at a dosage of 5 mg/m^2 which is 859 and 106 h$\times$ng/mL for total platinum and PUF, respectively.[b] The bioavailability of oral satraplatin in this study was estimated at 0.41 and 0.43 for total platinum and PUF, respectively. This value for oral bioavailability is consistent with the data in Beagle dogs which showed oral bioavailability ranging from 0.16 to 0.31 for total platinum and 0.21 to 0.40 for PUF based on the oral formulation.

Objective responses and PFS were evaluated. Response assessments were based on physical examination and, when indicated, imaging evaluation. Among dogs with measureable disease that were treated, there were 0 CR, 1 PR, 5 SD, 6 PD, and 2 were not evaluable for response. Among dogs treated in the adjuvant setting (resolution of all macroscopic disease, leaving only microscopic disease with a high risk of recurrence or metastasis), 1 dog (thyroid carcinoma) died of another cancer at 183 days, and another (oral squamous cell carcinoma) is still alive without evidence of disease at 565 days. The rest were dogs with osteosarcoma and are described below. The median PFS across all dogs was

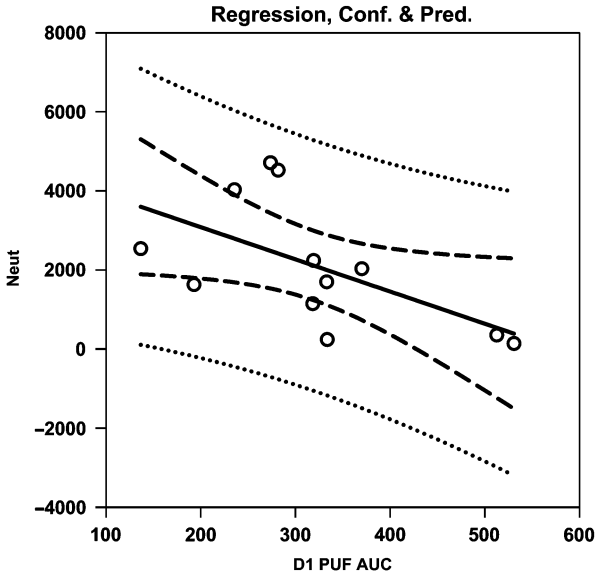

Fig 2. Neutrophil nadir was inversely correlated with the $AUC_{0-24\ h}$ for platinum ultrafiltrate (PUF) on day 1 of treatment ($R_2 = 0.340$, $P = .047$). Neut, neutrophil number per microliter; Conf., confidence intervel (represented by dashed lines); Pred., predicted best fit line (represented by solid line).

Table 4. PUF and Pt in 7 dogs treated at the established MTD (at 35 mg/m^2/d) in tumor-bearing dogs.

PK Parameter	Pt_{TOT} (Day 1)	PUF (Day 1)	Pt_{TOT} (Day 5)	PUF (Day 5)
T_{max} (hours)	2.1 (1.1)	1.8 (0.7)	2.1 (1.2)	1.9 (1.1)
C_{max} (ng/mL)	115 (38)	71.7 (26.1)	206.2 (60)	70.8 (25.1)
$AUC_{0-24\ h}$ (h ng/mL)	1,210 (319)	298 (57)	3,272 (849)	346 (101)
Cl (mL/h/m^2)	15,345 (4,150)	115,092 (26,139)	3,156 (1,221)	91,663 (36,366)
MRT (hours)	36 (14.2)	7 (1.3)	81 (25)	14 (14.3)
$T_{1/2}\ \lambda$	24.7 (11)	5.7 (1.4)	56.2 (17.6)	10.2 (9.7)

PUF, plasma ultrafiltrate (free Pt); Pt_{TOT}, total platinum; T_{max}, time to maximal plasma concentration; C_{max}, maximal plasma concentration; AUC, area under the curve (time–concentration curve); Cl, clearance; MRT, mean residence time; MTD, maximally tolerated dosage.
Values are expressed as mean (standard deviation).

249 days (±60) and median OS was 320 days (±60). Although the variety of tumor types precludes meaningful interpretation for a given disease state, over half of the dogs enrolled were affected by osteosarcoma and this group then was examined separately. Of note, 12 dogs with appendicular osteosarcoma were treated, 6 with measurable disease and 6 in the adjuvant setting. Progression-free and OS times for dogs treated in the adjuvant setting are comparable to other platinum drugs when used in dogs with appendicular osteosarcoma. In the adjuvant setting, 5 dogs developed metastatic disease at 73, 84, 439, 473, and 806 days, surviving 73, 171, 577, 741, and 806 days, respectively, whereas 1 dog was censored as alive and disease-free at 699 days.

Of treated dogs, 20 were dead because of disease, 1 was censored as alive without evidence of disease, and 2 were alive with SD. Of the dogs that died, 7 were evaluated by complete postmortem examination. Histopathology of the kidneys disclosed no abnormalities apart from occasional metastatic neoplasia, renal infarcts in 1 dog, and 1 dog in which a few tubules in the cortex were dilated with lightly eosinophilic material, and in the same dog, a few tubules in the medulla that contained darkly basophilic material. Evaluation of the liver indicated in 1 dog each necrosis, congestion, and hemorrhage (thought in each dog to be related to metastatic neoplasia), and normal histology in 4 dogs. Additional findings were perforating gastric ulcer (clinical aspects described above), metastatic cancer to multiple organs in some dogs, hemangiosarcoma as the cause of death in 1 dog and incidental pulmonary artery chemodectoma in another dog, and myeloid hypoplasia in 1 dog.

Regarding analysis of platinum concentrations, the initial studies were to determine the limits of detection (LOD). Using standard platinum solutions (10 ng/mL iridum as internal standard), concentrations from 0.01 to 20 ppb (ng/mL) in HCl (0.24 M), the calculated LOD of platinum was 0.003 ng/mL in 0.24 M HCl. During processing, samples were acid balanced with HNO_3 and HCl. When 0.7% HNO_3 and 0.9% HCl were used for acid matching, recovery of platinum was 98.3%, but when samples were acid unmatched with 7% HNO_3 and 0.9% HCl, recovery decreased to 90.5%. After ANOVA comparison, there was no difference in platinum recovery from spiked samples in lipemic serum, hemolyzed serum, or both. The analysis of percentage recovery of satraplatin for plasma microwave digestion was 98.4% (95.0% CI, 96.0–100.8%). The analysis of percentage recovery of satraplatin for sonication water bath digestion were 97.4% (95.0% CI, 93.2–101.6%) and 99.4% (95.0% CI, 95.7–103.1%) for PUF and urine, respectively. In normal dogs, 8.7% platinum of administered dose was recovered from the urine over the first 24 hours.

Discussion

We determined the MTD of orally administered satraplatin in tumor-bearing dogs to be 35 mg/m^2/d for 5 days. Because additional DLTs occurred in an extended cohort treated at this dosage, it is reasonable to consider a range of 30–35 mg/m^2/d for 5 days for clinical use. Our in vitro studies showed biologic activity at concentrations that are achievable in vivo as shown by the PK data. Regarding PK, we also demonstrated that nadir neutrophil count was inversely correlated with the $AUC_{0-24\ h}$. Dosage also impacted some PK parameters, as would be expected. During optimization, we were able to show that acid balance is important for accurate determination of platinum concentrations after satraplatin administration.

The platelet nadir occurred before the neutrophil nadir in most cases. This is unusual because neutrophils are shorter lived (6–8 hours) than platelets (6 days) and thus typically are depleted more rapidly. It is unlikely that an early neutrophil nadir was missed because blood counts were assessed twice weekly in the first 3 dogs. Failure to identify the nadir was possible, however, given the wide variability in time to nadir. Possible explanations include selective effects on different stages of precursors for the 2 cell lines, but further studies are required to confirm this hypothesis. Apart from myelotoxicity, the drug was well tolerated with minimal gastrointestinal and no nephro- or neurotoxicity. The 1 dog that developed a perforating gastric ulcer had 2 other risk factors for ulceration, either of which could have caused ulceration alone. Whether satraplatin contributed to the ulcer is unknown but considered unlikely because the dog had only received 3 doses of the first 5-day course. In people, the DLT also is myelosuppression with platelet and neutrophil nadirs at 17 and 21 days, respectively, with recovery by day 28, and a large variability in days to nadir which is very similar to the dogs treated here.[3]

Preliminary efficacy was suggested in this group of dogs. Of note, dogs with appendicular osteosarcoma

treated in the adjuvant setting experienced a median survival time (577 days) comparable or superior to other published outcomes with platinum-based chemotherapy regimens (cisplatin or carboplatin alone or in combination with doxorubicin, median survival 10–14 months).[18–21] Although we studied a small group of dogs, based on previous studies very few dogs with osteosarcoma treated in the adjuvant setting would be expected to live beyond a year if the drug was ineffective, yet 4 of 6 did so in the present study. Orally administered satraplatin may offer an alternative to the costly and time-consuming fluid diuresis required for cisplatin administration in dogs, and to the expense of IV carboplatin.

Satraplatin use in people has produced few objective responses, but has resulted in notable palliation of cancer-related clinical signs in patients with a variety of tumors such as nonsmall cell lung cancer, mesothelioma, and hormone-refractory prostate cancer.[6,10] However, only patients with advanced or refractory disease are evaluated in early phase trials, and satraplatin may be most effective in the minimal disease setting, as is the case for most cytotoxic chemotherapy. Additionally, the palliation of pain and cancer symptoms is valuable even in patients with advanced disease.

In conclusion, orally administered satraplatin was well tolerated in this group of tumor-bearing dogs. Preliminary efficacy in an extended treatment group warrants a larger scale phase II trial.

Footnotes

[a] Centrifree YM-30, Millipore, Billerica, MA
[b] Personal communication, Agennix Inc, Princeton, NJ

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