Phase I Evaluation of Low-Dose Suramin as Chemosensitizer of Doxorubicin in Dogs with Naturally Occurring Cancers

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Background: Low and nontoxic concentrations (10–50 μM) of suramin, which is a nonspecific inhibitor of multiple growth factors, including fibroblast growth factors, enhances the activities of cytotoxic chemotherapeutic agents, such as doxorubicin and paclitaxel, both in vitro and in vivo. Suramin has not been evaluated as a chemo sensitizing agent in dogs with cancer.

Hypothesis: Nontoxic suramin can be used safely as a chemo sensitizer in dogs.

Animals: Sixteen dogs of various breeds with measurable tumors were treated; 1 dog that had undergone amputation for osteosarcoma received adjuvant therapy.

Methods: The dogs received 53 courses of treatment with suramin in combination with doxorubicin. The suramin dosage was 6.75 mg/kg IV 3 h before standard doxorubicin administration every 2 weeks. The pharmacokinetics and clinical efficacy were determined.

Results: The pharmacokinetics of low-dose suramin followed a 2-compartment model with half-lives of 2 h and 6 days. The dose for each dog was determined from the results of a previous study, which demonstrated that the doxorubicin concentration declined below therapeutic levels by 24 h after the second dose. The dose was increased progressively by 1 mg/kg until the effective concentration of 5 nM was achieved within 24 hours. The latter target concentration was based on the results of a previous study, which demonstrated an increased toxicity. Subsequent phase I/II studies in human patients have indicated no or minimal adverse effects attributable to the low suramin doses, and enhanced drug activity.

Conclusions and Clinical Importance: A fixed, low-dose suramin regimen yields the desired target plasma concentrations in most dogs, and appears to enhance the activity of doxorubicin without enhancing toxicity.

Key words: Clinical trial; Dog disease; Drug resistance; Neoplasms; Osteosarcoma; Prostatic neoplasm.

Suramin, which is a polysulfonated naphtylurea, was developed over 60 years ago for the treatment of parasitic diseases, such as trypanosomiasis and onchocerciasis. Subsequently, suramin was shown to inhibit the reverse transcriptase in RNA tumor viruses. More recently, suramin has been investigated as an antitumor agent. In contrast to the low concentrations required for fibroblast growth factor inhibition, substantially higher concentrations of approximately 200 μM are needed for the antiproliferative activity of suramin. At these high concentrations, suramin has modest to moderate antitumor activity against hormone-refractory prostatic carcinoma, marginal activity against recurrent high-grade gliomas, and no or limited activities against germ cell tumors, breast cancers, and renal carcinomas. At cytotoxic doses, suramin is associated with multiple toxicities, including severe fatigue, neurotoxicity, adrenal insufficiency, anemia, and coagulopathies. These toxicities have led investigators to question the value of this drug as a safe cytotoxic agent, and its commercial development was halted in 1999. Recently, we have shown that acidic and basic fibroblast growth factors (FGFs) confer broad-spectrum chemoresistance upon tumor cells. Furthermore, we have shown that this chemoresistance can be reversed in vitro with either antibodies to aFGF and bFGF or with low nontoxic (10–50 μM) concentrations of suramin, which inhibits numerous growth factors. In subsequent studies, we have found that low doses of suramin enhance the activities of paclitaxel and doxorubicin in mice that bear human prostate, lung, breast, and bladder xenograft tumors, in the absence of increased toxicity. Subsequent phase I/II studies in human patients have indicated no or minimal adverse effects attributable to the low suramin doses, and enhanced drug activity. These encouraging results motivated the present study in dogs.

The pharmacokinetics of suramin, whether in high doses as a therapeutic agent or in low doses as a chemo sensitizer, are well characterized in human patients. However, corresponding data for dogs are not available. The primary objective of the present study was to determine the pharmacokinetics of low-dose suramin in combination with doxorubicin in dogs with naturally occurring cancers. The targeted upper limit of the plasma suramin concentration was 50 μM at the time of the initiation of doxorubicin administration and the lower limit was 10 μM at 24 h after administration of a 2-fold higher dose than that used in the current study.  The secondary objectives were to characterize the toxicities and potential activities of the suramin/doxorubicin combination.
### Materials and Methods

**Inclusion and Exit Criteria**

Dogs that had cytologically or histopathologically confirmed solid tumors or lymphoma for which no effective therapy existed, standard therapy was declined, or with tumors that were resistant to previous chemotherapy treatments, were eligible for this study. Informed owner consent was obtained before the start of therapy. The dogs were expected to have a minimum survival time of 2 weeks. Treatment was continued until disease progression, owner noncompliance, or discontinuation at the owner’s request. The study was approved by the Veterinary Teaching Hospital Board and required signed owner consent.

**Patient Evaluation and Monitoring**

Physical examination and CBC were completed before each treatment. Echocardiograms and other diagnostic tests, which included serum biochemistry profile, thoracic radiographs, and abdominal ultrasound examination, were performed when clinically indicated and at the clinicians discretion. The following criteria were used to determine tumor response: complete response (CR), when all gross evidence of the tumor disappeared; partial response (PR), represented by a >50% but <100% decrease in tumor size; stable disease (SD), when the tumor volume did not decrease by >50% or increased by <25%; and progressive disease (PD), as evidenced by an increase in tumor volume of >25% or the appearance of new neoplastic lesions. Accessible tumors were measured directly using calipers. Measurements were made in the longest dimension and the perpendicular plane. For the remaining tumors, size was determined by measurements from radiographic or ultrasonographic images using calipers. The tumor measurements were compared to those obtained during the previous physical examination. Response duration was calculated from the time CR or PR was achieved until PD developed. Toxicities were graded according to a modification of the National Cancer Institute (NCI) Common Toxicity Criteria (Table 1).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Neutrophils (cells/μL)</th>
<th>Platelets (cells/μL)</th>
<th>Hematological</th>
<th>Gastrointestinal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&gt;1,500</td>
<td>&gt;150,000</td>
<td>No toxicity</td>
<td>No toxicity</td>
</tr>
<tr>
<td>1</td>
<td>1,000–1,500</td>
<td>100,000–150,000</td>
<td>Mild</td>
<td>No toxicity</td>
</tr>
<tr>
<td>2</td>
<td>800–1,000</td>
<td>50,000–100,000</td>
<td>&lt;5% weight loss</td>
<td>1–5/day</td>
</tr>
<tr>
<td>3</td>
<td>500–800</td>
<td>25,000–50,000</td>
<td>5–10% weight loss</td>
<td>6–10/day</td>
</tr>
<tr>
<td>4</td>
<td>&lt;500</td>
<td>&lt;25,000</td>
<td>&gt;10% weight loss</td>
<td>Intractable</td>
</tr>
</tbody>
</table>

Evidence of drug toxicity was monitored by evaluation of the history of the dog obtained from the owners, physical examination, and clinopathological data.

**Drug Administration**

Suramin was provided by NCI (Division of Cancer Treatment and Diagnosis), in sterile, 600-mg, 10-ml vials. Each vial was reconstituted with sterile water to yield a 100 mg/ml solution. The stock solution was further diluted with 0.9% sodium chloride to 21 ml of the desired dosing solution. The drug solution was filtered through a Millex sterilizing filter unit with pore size of 0.22 μm, and was administered to the dog via an indwelling IV catheter at a rate of 1 ml/min over 20 minutes. Doxorubicin hydrochloride was supplied in a 10-ml vial at a concentration of 2 mg/ml; the calculated dosage was diluted in 0.9% sodium chloride to approximately 0.5 mg/ml. Doxorubicin was administered as a 30-minute infusion via the same indwelling IV catheter that was used for suramin administration. Treatment was scheduled at 2-week intervals.

**Dosage**

The suramin dosage was fixed at 6.75 mg/kg throughout the study. The dogs were premedicated with 2 mg/kg diphenhydramine IM 30 minutes before receiving doxorubicin. Doxorubicin at a dosage of 30 mg/m² (or 1 mg/kg if the body weight was <15 kg) was given 3 hours after the end of the suramin infusion. The protocol called for a delay in treatment and a 25% doxorubicin dose reduction if grade 3 or 4 hematological or gastrointestinal toxicity occurred.

**Blood Sampling and Analysis**

Blood samples (3 to 5 ml in volume) were collected from the jugular or cephalic veins into Vacutainer glass tubes. Sampling was carried out before treatment and at 5, 30, 90, 180, and 240 minutes, as well as 1 and 7 days post-infusion. When the day after the infusion fell on a weekend or holiday, samples were drawn on the next workday. The samples were centrifuged at 3000 rpm for 10 minutes, and the plasma was collected and stored in a −70 °C freezer for later analysis. The plasma concentrations of suramin were determined using high performance liquid chromatography, as described previously.

**Pharmacokinetic Analysis**

The plasma concentration-time profiles of suramin were analyzed using the WinNonlin software. Open 2- and 3-compartment linear models with constant infusion input were used, and the concentration data for all treatments from dogs that received >1 treatment were analyzed. The performance of the pharmacokinetic models was compared using the Schwartz and Akaike Information Criteria, as calculated by the WinNonlin software. The pharmacokinetic parameters of doxorubicin were not evaluated in this study.

**Results**

**Patient (Dog) Characteristics**

Seventeen dogs were enrolled in the study between May 2002 and January 2003 (Table 2). Six of the dogs had received no prior therapy. Of the 11 dogs that had prior therapy, 2 were given chemotherapy, 3 underwent surgery, and 6 received a combination of chemotherapy and surgery. Five dogs had received prior treatment with doxorubicin monotherapy or doxorubicin-containing combinations (total of 30 treatments, ranging from 3–12 treatments per dog). Six dogs (35%) had evidence of metastatic disease at the onset of treatment.
Pharmacokinetics of Suramin

Seventeen dogs received 53 courses of the suramin and doxorubicin combination. Four dogs that exhibited worsening clinical signs were withdrawn from the trial within 1 week of the first treatment, at the request of the owner. The remaining 13 dogs received a total of 49 treatment courses (range, 2 to 10 cycles; median, 3 cycles). Figure 1 shows the plasma concentration-time profile of suramin during the first cycle of treatment, and Table 3 summarizes the pharmacokinetic parameters. The Akaike Information Criterion and the Schwartz Criterion showed no significant difference between the 2- and 3-compartment models. These models yielded almost identical total body clearance rates for suramin (±10% difference), which indicates that the 2-compartment open model is adequate in describing the disposition of suramin. The observed major pharmacokinetic half-life of approximately 7 days indicated slow elimination of the drug.

Table 2 shows the plasma concentrations of suramin at the target timepoints. For all 17 dogs, the suramin concentration at 5 minutes post-suramin administration exceeded 50 μM. The suramin concentrations for 38 out of 53 (72%) treatments or 9 out of 17 (53%) dogs fell below the upper target concentration of 50 μM before doxorubicin infusion (ie, 3 hours after the end of suramin infusion), with mean and median concentrations of 44.1 and 44.9 μM, respectively. Two of the 8 remaining dogs had maximal suramin concentrations of <50 μM during the first 4 treatments, with higher concentrations for the later cycles, which was consistent with the accumulation of this slowly eliminated drug after multiple doses. In all the dogs, the maximal suramin concentration was <76 μM, or within 160% of the targeted upper limit of 50 μM. The lower target concentration of 10 μM at 24 hours was achieved in 9 of the 13 dogs for which 24-hour samples were available (Table 2). Two more dogs reached this range in the second and later cycles. For all 13 dogs that received ≥2 treatments, the trough suramin concentrations at 2 weeks after the previous treatments were 0.7–7.3 μM, with an average of 3.0 μM. Overall, the desired plasma concentrations were achieved for most treatments.

Table 2. Patient information.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Response</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>No. of treatments</th>
<th>Suramin (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN = 10</td>
<td>PR = 2</td>
<td>3.75–16</td>
<td>3.8–63</td>
<td>1–10</td>
<td>47.6–223</td>
</tr>
<tr>
<td>FS = 7</td>
<td>SD = 8</td>
<td>3–14</td>
<td>3.5–13</td>
<td>1–10</td>
<td>18.9–75.9</td>
</tr>
<tr>
<td>PD = 2</td>
<td>NA = 5</td>
<td>3–14</td>
<td>3.5–13</td>
<td>1–10</td>
<td>8.6–28.6</td>
</tr>
</tbody>
</table>

Mean | 8.7 | 28.1 | 3.1 | 101 | 45.0 | 14.3 |
Median | 8.8 | 28.5 | 2 | 93.2 | 44.9 | 13.3 |

*Tumor-bearing dogs received 1 to 10 courses of suramin (intravenously over 20 minutes), followed by doxorubicin (intravenously over 30 minutes, 3 hours after completion of suramin administration). The patient and treatment data are summarized. The values shown represent the ranges from multiple cycles.

Abbreviations: FS, female spayed; MN, male neutered; PR, partial response; SD, stable disease; PD, progressed disease; NA, not applicable.

Pharmacokinetics of suramin in dogs

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Range (median)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative AUC, mg·h/ml</td>
<td>2.08–9.16 (3.98)</td>
<td>4.19 ± 1.95</td>
</tr>
<tr>
<td>Alpha half-life, hours</td>
<td>1.57–3.59 (2.10)</td>
<td>2.16 ± 0.54</td>
</tr>
<tr>
<td>Beta half-life, days</td>
<td>3.17–11.5 (5.78)</td>
<td>6.34 ± 2.63</td>
</tr>
<tr>
<td>V1, L/kg</td>
<td>0.0369–0.0939 (0.0537)</td>
<td>0.0563 ± 0.0158</td>
</tr>
<tr>
<td>V2, L/kg</td>
<td>0.121–0.485 (0.255)</td>
<td>0.281 ± 0.112</td>
</tr>
<tr>
<td>Vdss, L/kg</td>
<td>0.174–0.543 (0.321)</td>
<td>0.337 ± 0.119</td>
</tr>
<tr>
<td>Total body clearance, mL/h/kg</td>
<td>0.753–3.25 (1.68)</td>
<td>1.86 ± 0.76</td>
</tr>
</tbody>
</table>

AUC, area under the plasma concentration-time curve; Vdss, volume of distribution at steady state; V1, volume of distribution for the central compartment; V2, volume of distribution for the peripheral compartment.

Suramin at 6.75 mg/kg was infused over 20 minutes, every 2 weeks, for 1 to 10 cycles. Plasma samples for pharmacokinetic analysis were obtained for 53 treatment courses of the 17 dogs. The tabulated data are for 13 dogs (49 cycles); and excluded dogs were treated for only 1 cycle, or <2 terminal half-lives. The pharmacokinetic analysis is based on the data for all the treatments per dog.

Figure 1. Suramin plasma concentration-time profiles. Suramin at a fixed dose of 6.75 mg/kg was infused over 20 min in 17 dogs. Mean plasma concentrations and observed ranges for the first treatments are shown. Data points are connected by straight lines.
The normal values are 3.0–10.4 × 10^9/L or neutrophils and 106–424 × 10^9/L for platelets.

Hematologic and Nonhematologic Toxicities

The toxicities associated with doxorubicin monotherapy include anaphylaxis, myelosuppression, vomiting, hemorrhagic colitis, and dose-dependent cardiomyopathy. We did not observe anaphylaxis or dose-dependent cardiomyopathy in the present study. Other toxicities were within the ranges expected for doxorubicin alone, as reported below.

Table 4 summarizes the hematologic data and lists the numbers of treatments associated with neutropenia and thrombocytopenia 2 weeks after drug treatment. Overall, hematologic toxicity was infrequent, occurring in only 3 out of 37 treatments. One dog had an episode of concurrent grade 1 neutropenia and grade 4 thrombocytopenia at the time of the second treatment. However, these hematologic changes were also present before the first treatment; clinical stage 5 lymphoma (presumably with bone marrow involvement) was suspected as the cause of the hematologic abnormalities. The CBCs for 2 dogs indicated 1 episode each of grade 2 thrombocytopenia. Two dogs required hospitalization because of febrile neutropenic events 7 days after the first treatment. Both dogs recovered uneventfully and had normal neutrophil and platelet counts at the 2-week post-treatment CBC evaluation. The subsequent doses of doxorubicin for these dogs were reduced by 25%. For the 3 dogs that received >4 courses of treatment, no cumulative hematologic toxicities were observed.

The majority of the nonhematologic toxicities involved the gastrointestinal tract, and included mild loss of appetite and self-limiting vomiting. During the course of the 53 treatment courses, 12 of the 17 dogs (71%) experienced a total of 6 episodes of grade 1 anorexia, 2 episodes of grade 3 anorexia, 8 episodes of grade 2 vomiting, and 1 episode of grade 1 diarrhea. Three dogs (18%) were reported by their owners to be lethargic immediately after treatment. Mild alopecia was observed in 2 of the 17 dogs. Cardiotoxic events, which are associated with doxorubicin at cumulative doses >240 mg/m^2, were not observed in the 3 dogs that received cumulative doses of doxorubicin of 240–420 mg/m^2; serial echocardiograms in these dogs indicated normal percentages of fractional shortening with no evidence of cardiomyopathy. Four of the 17 dogs did not experience any nonhematologic adverse effects.

Toxicities associated with high doses of suramin administered as a therapeutic agent, such as peripheral neuropathies, adrenal insufficiency, and coagulopathies, were not observed in any of the dogs.

Response to Therapy

Sixteen dogs were evaluated for their responses to therapy (Table 2). Two dogs (12.5%) had PR, 8 dogs (50%) had SD, and 2 dogs (12.5%) had PD. Four dogs with worsening clinical signs were withdrawn by their owners, and these dogs were not available for follow-up. One dog with PR was a castrated male dog with prostatic adenocarcinoma. This dog received 5 courses of treatment and had a durable remission of 7.5 months. This dog was euthanized 282 days after initial diagnosis due to acute onset neurological clinical signs that appeared to be unrelated to the primary tumor or its treatment. Postmortem examination of the brain indicated no gross lesions and no evidence of ischemia, although arteriosclerosis was observed throughout the body. The examination also showed tumor nodules in the prostate, consistent with the initial diagnosis of prostatic adenocarcinoma, but no evidence of metastasis. The second dog with PR was a castrated male dog that had undergone multiple treatments for SC hemangiosarcoma. This dog, which had previously shown improvement after treatment with doxorubicin and gemcitabine, was placed on the suramin/doxorubicin protocol after attaining SD on the most recent cycle of vincristine, doxorubicin, and cyclophosphamide. The response duration was 2 weeks. Four other dogs progressed on doxorubicin or a doxorubicin-containing therapy before switching to the suramin/doxorubicin combination. All 4 dogs showed SD responses on the suramin protocol.

The majority of the dogs (50%) attained SD. One of these dogs was treated for transitional cell carcinoma of the bladder and had resolution of sublumbar lymphadenopathy. In addition, resolution of the pain associated with secondary hypertrophic osteopathy occurred in a dog with metastatic mammary carcinoma.

Discussion

The primary objective of the present study was to evaluate the pharmacokinetics of low-dose suramin in dogs with spontaneously occurring tumors. The results show that a fixed dosage of 6.75 mg/kg delivered the targeted plasma concentration range of 10–50 μM in approximately 60% of the 53 treatment courses administered to 17 dogs. This targeted plasma concentration
has been associated with chemosensitization and minimal toxicity in previous studies.\textsuperscript{14,18–21} That the fixed dose did not deliver the desired concentrations to all dogs may be attributable to the large (17-fold) variations in body weight and to the slow elimination of suramin (half-life of 6 days). Several approaches may be adopted to improve this therapeutic regimen. First, dose adjustment to compensate for the residual drug concentrations may lower the peak suramin concentration during subsequent treatments. Second, fine-tuning of dose requirements may be possible based on patient parameters that correlate with drug disposition. Using a longer delay between the administration of suramin (given first) and doxorubicin may allow the suramin concentrations to decline to the targeted upper limit of approximately 50 μM. Similar approaches have been used successfully in human patients, in whom the targeted suramin concentrations were achieved for >90% of the treatments.\textsuperscript{22,b} Although the pharmacokinetics of doxorubicin was not analyzed as part of this trial, it is not expected to be altered by suramin because doxorubicin elimination occurs primarily by metabolism\textsuperscript{32} and the metabolism of suramin is minimal. A previous Phase I study in human patients with prostate cancer, combining high-dose suramin treatment with doxorubicin, did not identify any drug-drug interactions by toxicity evaluation.\textsuperscript{13} In a recent evaluation of potential interactions between suramin and paclitaxel (both highly protein-bound drugs), no interactions were observed.\textsuperscript{b}

Our secondary objective was to obtain an initial assessment of drug activity in dogs. The majority of the dogs (11/17 or 65%) had refractory, recurrent, or metastatic disease. One dog with PR was observed among the 3 dogs with prostatic carcinoma (durable response of 7.5 months). Four dogs that had progressed after receiving prior doxorubicin monotherapy or doxorubicin-containing combinations showed SD or an objective response after receiving the suramin/doxorubicin combination. The fifth dog, which had SD after receiving a doxorubicin-containing combination, experienced PR after receiving the suramin/doxorubicin combination. These findings suggest that suramin reverses the clinical resistance to doxorubicin. As CBCs were not obtained routinely at the time of the expected nadir for doxorubicin, the true incidence of hematological toxicity with low-dose suramin in combination with doxorubicin is probably higher than suggested by the day 14 post-treatment CBCs. No toxicity attributable to low-dose suramin was observed, as described previously for human patients.\textsuperscript{22}

In summary, the present study demonstrates that low-dose suramin can be safely co-administered with doxorubicin to dogs with spontaneously occurring tumors. Additional studies to refine the suramin dosing schedules so as to accommodate patients with different body weights are being performed. The preliminary efficacy data for the suramin/doxorubicin combination, together with the apparent lack of suramin-related toxicity, has prompted an ongoing study of this combination in dogs with osteosarcoma or prostatic carcinoma.

\textbf{Footnotes}

\textsuperscript{a}Villalona-Calero MA, et al., Lung Cancer 2003;41 S2:149
\textsuperscript{b}Chen, D. et al., Pharm. Res., 2006, in press
\textsuperscript{c}Millipore Corporation, Bedford, MA
\textsuperscript{d}Benevenie Laboratories, Bedford, OH
\textsuperscript{e}WinNonlin software, version 4.0

\textbf{Acknowledgments}

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\textbf{References}


