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A Phase II Clinical Trial of Vinorelbine in Dogs with Cutaneous Mast Cell Tumors

I.A. Grant, C.O. Rodriguez, M.S. Kent, G. Sfilgoi, I. Gordon, G. Davis, L. Lord, and C.A. London

Background: Few effective drugs are available to treat dogs with locally aggressive or metastatic mast cell disease.

Hypothesis: Vinorelbine, a semisynthetic derivative of vinblastine, is an effective drug for the treatment of canine mast cell tumors (MCT).

Animals: Twenty-four dogs with cutaneous MCT.

Methods: Dogs with at least 1 measurable, cytologically confirmed, and previously untreated cutaneous MCT received a single treatment with vinorelbine at the previously established dosage of 15 mg/m^2 IV. Tumor measurements and CBC were evaluated before and 7 days after treatment. Adverse events were graded according to Veterinary Cooperative Oncology Group (VCOG) guidelines.

Statistics: Data were accrued in accordance with a Simon's 2-stage design with a noninteresting response rate of .05, a target response of .25, and α and β values of .10.

Results: Three of 24 dogs (13%) had a response to treatment, including 1 measurable complete response and 1 measurable partial response. The 3rd dog had microscopic complete response to treatment with stable measurable disease. Twenty other dogs (83%) had stable disease and 1 dog (4%) had progressive disease. Neutropenia occurred in 13 dogs (54%) (grade 1, n = 4; grade 3, n = 6; grade 4, n = 3). Gastrointestinal toxicity occurred in 11 dogs (46%) (anorexia: grade 1, n = 3; grade 2, n = 1; grade 3, n = 1; diarrhea: grade 1, n = 2; grade 3, n = 1; vomiting: grade 1, n = 5; grade 3, n = 1).

Conclusions and Clinical Importance: Vinorelbine was associated with an overall response rate of 13% and a high prevalence of neutropenia. Additional studies are indicated to determine if repeated dosing of vinorelbine or combination of vinorelbine with other drugs increases the observed biologic activity against canine MCT.

Key words: Chemotherapy; Navelbine; Vinca alkaloid.

Unlike other domestic species, dogs commonly develop cutaneous mast cell tumors (MCT).[1] Prognosis and treatment for MCT primarily are based on results of tumor staging and histologic assessment of tumor grade.[2–6] Additional factors often considered when developing a therapeutic plan include the breed of the patient,[2] growth rate,[2] anatomic location of the tumor,[6–8] and proliferation indices (mitotic index,[9,10] AgNOR,[11–13] PCNA,[14,15] and Ki67[13,14,16]) after histopathologic evaluation of a tumor biopsy specimen. Chemotherapy typically is used in the management of high-grade tumors, nonresectable measurable disease, advanced clinical stage disease, and, anecdotally, tumors that demonstrate a natural history suggestive of an aggressive phenotype.[17]

Only a limited number of drugs have been shown to exhibit biologic activity in dogs with MCT. Prednisone alone has been associated with a response rate of 20%[18] and CCNU (lomustine) alone has been associated with a response rate of 42%[19] in dogs with measurable disease. Based on these responses to single agent therapy, combination protocols also have been evaluated. Prednisone in combination with vinblastine produced an overall response rate of 47%[5] and in 1 report combining prednisone, cyclophosphamide, and vinblastine, a 64% overall response rate was observed.[20] Interestingly, vincristine, a compound that is closely related to vinblastine in terms of its chemical structure, appeared to be minimally effective in the management of this disease (response rate of 7%).[21] However, in combination with cyclophosphamide, hydroxyurea, and prednisolone, a measurable response was recorded in 60% of the dogs treated.[22] There are currently no reports in the veterinary literature that describe the response of canine MCT to treatment with vinblastine alone. Based on this small number of studies, the response rate and median response duration reported with lomustine, vinblastine, and prednisone have favored their use as the standard of care for the treatment of canine MCT. Combination therapy using all 3 drugs has been reported to result in an overall response rate of 83% in affected dogs for a median response duration of 10 months (Bennet et al, personal communication). Given the limited number of drugs available to treat canine MCT, new drugs that could be used with or in place of existing drugs are greatly needed.

Vinorelbine (Navelbine) is a semisynthetic, 2nd generation vinca alkaloid derived from vinblastine.[23] In preliminary clinical studies in human medicine,

From the Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California-Davis, Davis, CA (Grant, Rodriguez, Kent, Sfilgoi, Gordon, Davis, London); and the Department of Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH (Lord). Dr Grant and Dr London are currently affiliated with the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH. *This study was performed at the Veterinary Medical Teaching Hospital at the University of California, Davis and The College of Veterinary Medicine, The Ohio State University, Columbus, OH. Presented at the 26th Annual Conference of the Veterinary Cancer Society, Callaway Gardens Resort, Pine Mountain, GA, October 19–22, 2006.*

Corresponding author: Dr Cheryl A. London, DVM, PhD, Associate Professor, Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 454 VMAB, 1925 Coffey Road, Columbus, OH 43210; e-mail: london.20@osu.edu.

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vinorelbine had broader antitumor activity than vinblastine, possibly as a result of greater intracellular drug accumulation and peak intracellular drug concentrations.[24] Intracellular drug reservoirs are proposed to exist for vinorelbine, enabling gradual drug release and prolonged antitumor effects with low toxicity.[24] Recently, a Phase I study of vinorelbine was conducted in dogs with a variety of tumors.[25] Unfortunately, a single dog treated for multiple grade 3 MCT with lymph node metastases was lost to follow-up. A dosage of 15 mg/m^2 administered IV was recommended for phase II trials, and neutropenia was the dose-limiting toxicity.

Given the proposed activity of vinblastine in the treatment of canine MCT and the potential for enhanced antitumor activity of vinorelbine over vinblastine in studies in human medicine, the aim of the present study was to evaluate the observed response rate and toxicity associated with the use of vinorelbine alone for the treatment of dogs with measurable MCT.

Materials and Methods

Patient Selection

The study was a prospective Phase II clinical trial carried out at the Veterinary Medical Teaching Hospital (VMTH) of the University of California-Davis and at the College of Veterinary Medicine of The Ohio State University (OSU). Client-owned dogs were eligible for inclusion in the study if they had $\geq$ 1 measurable cutaneous MCT, confirmed by cytologic examination of a fine needle aspirate. A tumor was defined as being measurable if its longest diameter could be determined with calipers. Dogs of any age, breed, sex, weight, or neuter status were included. All dogs initially were referred for evaluation and staging to determine their eligibility for surgical excision of their MCT. For patients in which surgery was recommended, informed consent was sought from the owner to delay treatment for 1 week to allow a single administration of vinorelbine followed by reevaluation of tumor size and drug-associated toxicity 7 days later. If owners declined surgery, based on cost or tumor-related prognosis, consent still was sought for patient inclusion in the study. Tumor grade was available only for patients that underwent surgery. When additional chemotherapy was recommended for any reason, dogs received the standard of care protocol used at our institution. Dogs were excluded from the study if they currently were receiving treatment with glucocorticoids or had received chemotherapy with a vinca alkaloid at any time in their previous history.

After obtaining a medical history, each dog underwent complete physical examination. A CBC was evaluated before treatment with vinorelbine at a dosage of 15 mg/m^2 IV. Criteria for treatment included a baseline neutrophil count of >3,000 cells/μL and, on this basis, all dogs were eligible for treatment. An IV catheter was placed and connected to a free-flowing infusion of 150 mL 0.9% NaCl. The prescribed vinorelbine dose was diluted to 1 mg/mL in 0.9% NaCl and administered over 6–10 minutes through the side port of the fluid administration set in accordance with instructions for administration in the package insert.[a] After the occurrence of grade 4 febrile neutropenia (neutrophils <500 cells/μL) 6 days after treatment of the 3rd dog entered into the study, owners subsequently were discharged with amoxicillin-clavulanate (13.75 mg/kg PO q12h) to administer at home for 5 days beginning on the 3rd day after treatment.

Assessment of Tumor Response

For the purposes of this study, tumor measurements were made and response to treatment was evaluated based on response evaluation criteria in solid tumors (RECIST) guidelines. Before treatment, 2 attending veterinarians independently measured tumors with calipers. Each recorded the longest diameter of the lesion, and the measurement used was the average of the 2 readings. In the case of multiple tumors, measurements were made of up to 3 lesions and added to generate the sum of the longest diameters. All pretreatment measurements were performed before fine needle aspiration to eliminate the effect of local histamine release and peritumoral edema on tumor size. Dogs were reexamined 7 days after treatment and measurements were repeated to determine tumor response. Whenever possible, the same 2 veterinarians carried out both sets of measurements. Response to treatment was calculated by the formula: tumor response = [(posttreatment measurement − pretreatment measurement)/pretreatment measurement] × 100%. Responses were categorized as a complete response, CR (no measurable disease); partial response, PR (at least a 30% decrease in tumor size); progressive disease, PD (at least a 20% increase in tumor size); and stable disease, SD (neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD).

Assessment of Drug-Induced Toxicity

All owners completed a pretreatment questionnaire documenting any episodes of lethargy, anorexia, diarrhea, or vomiting in the 3 days before presentation. The time frame for this pretreatment evaluation was arbitrarily assigned but was considered long enough that if patients demonstrated any of these clinical signs after treatment, it was most likely a direct adverse effect of the treatment rather than continuing clinical signs associated with underlying disease at the time of treatment. A CBC was performed in all dogs before treatment and 7 days after treatment to evaluate hematologic toxicity. Other toxicities were evaluated based on a physical examination and a posttreatment questionnaire that owners completed, documenting the occurrence and severity of lethargy, decreased appetite, and episodes of vomiting or diarrhea in the week after treatment. All toxicities were graded in accordance with guidelines produced by VCOG for chemotherapy induced toxicity in dogs.[26]

Statistical Methods

Overall response rate was defined as the number of dogs achieving CR or PR divided by the number of dogs treated. A 2-stage design was used to calculate a sample size of 24 dogs based on our goal of detecting a lower overall response rate of .05 and a target overall response rate of .25 by use of an $\alpha = .10$ and $\beta = .10$.[27] For stage 1, 1 dog in the 1st 9 treated needed a confirmed response (either PR or CR) for the study to proceed to the 2nd stage, and a minimum of 3 dogs with a confirmed response from the total of 24 dogs treated was needed to support future testing of this drug. Summary statistics were used to characterize information about the patients and hematologic toxicity by a standard software package.[b] Comparisons were made between responders and nonresponders for categorical data by a χ^2 test. A Fisher's exact test was used for categorical data when the expected value of a given cell in the comparison was <5. Comparisons were made between responders and nonresponders for continuous variables by the Wilcoxon rank sum test. Standard statistical software was used.[c] Statistical significance was established as $P < .05$.

Results

Patient Characteristics

Twenty-four dogs were treated. Median age was 7.5 years (reference range, 3.3–16.6 years). Fifteen dogs were female (14 spayed) and 9 were male (4 neutered). There were 7 mixed breed and 17 purebred dogs. Seven of the

17 purebred dogs were Labrador Retrievers, 2 were Rottweilers, and the remainder was made up of 1 of each of the following breeds: Jack Russell Terrier, Bernese Mountain Dog, Boxer, German Short Haired Pointer, Golden Retriever, American Foxhound, Pug, and Rhodesian Ridgeback. The median weight of the dogs was 33.3 kg (reference range, 5.4–51.5 kg). Six dogs (25%) had more than 1 tumor and 4 dogs (17%) had both local disease and regional lymph node metastasis based on evaluation of aspiration cytology results of an enlarged regional lymph node. Three of the 4 dogs with lymph node metastasis had tumors on extremities, and the remaining dog had a tumor on its lip that had metastasized to the mandibular lymph node. Tumor locations were extremities (50%, 12 of 24), trunk (21%, 5 of 24), head and neck (17%, 4 of 24, including 1 tumor on the lip), inguinal region (4%, 1 of 24), axilla (4%, 1 of 24), and peri-anal region (4%, 1 of 24). The median pretreatment (or summated pretreatment) dimension was 3.9 cm (reference range, 0.65–26.0 cm). Grading was available in 54% of cases (13 of 24). Of these, 11 were grade 2 tumors and 2 were grade 3 tumors.

Response to Treatment and Toxicoses

Response to treatment was evaluated in all 24 dogs. Two of 24 dogs had a measurable response to treatment that could be classified as CR in the 2nd dog treated and PR in the 11th dog treated. In addition, 1 dog (the 24th dog treated) experienced histopathologic resolution of neoplastic disease despite tumor measurements indicating SD. The dog with the PR was a 7.5-year-old, castrated male Labrador Retriever with a SC grade 2 MCT measuring 4.6 cm at its longest diameter, located over the sternum. The owner of this dog elected surgery 21 days after recheck examination (28 days after vinorelbine treatment) during which time there was no further change in the diameter of the tumor. The dog with a CR was a 12.5-year-old, spayed female Pit Bull Terrier cross with a SC grade 2 MCT measuring 5.0 cm at its longest diameter, located over the lateral aspect of the left thigh. After dramatic response to treatment, chemotherapy was continued starting 1 week later (2 weeks after vinorelbine treatment) by a multidrug protocol adapted from one commonly used in our hospital for MCT patients. Vinorelbine was administered in place of vinblastine on an alternating schedule every 2 weeks with lomustine administered PO. Prednisone was given according to protocol guidelines. A CR was maintained throughout the protocol (210 days), but the dog was euthanized 117 days after completing chemotherapy because of urinary tract obstruction secondary to a transitional cell carcinoma.

The 24th dog treated was a 6-year-old, spayed female Labrador Retriever with 2 tumors, one located over the proximal right antebrachium measuring 1.2 cm at its longest diameter and a 2nd tumor located over the right body wall measuring 1.5 cm at its longest diameter. A 24% reduction in the sum of the longest diameter of the tumors was recorded at the time the dog was reassessed for treatment response indicating the presence of SD. Surgery was performed 12 days after treatment and no histopathologic evidence of neoplastic mast cells was found at either tumor site, and the presence of stromal and inflammatory cells was indicative of tumor regression. The dog had been presented to OSU with a cytology report from an external laboratory service confirming the presence of its MCT, and the diagnosis was verified by evaluation of fine needle aspiration cytology at the time of pretreatment assessment. Therefore, despite measurements indicating SD, total disappearance of all neoplastic mast cells indicated CR. Surgery was not performed in all dogs and therefore tumor grade could not be assessed for all patients and was not included in the statistical analysis. Twenty other dogs (83%) had SD and 1 dog (4%) had PD. There was no substantial difference between responders and nonresponders with respect to age, sex, pretreatment tumor size, and severity of hematologic toxicity.

The grade of treatment-associated toxicities was evaluated in all 24 dogs using the criteria in Table 1. The median neutrophil count 7 days after treatment was 1,481 cells/μL (reference range, 170–7,534 cells/μL). Neutropenia occurred in 13 dogs (54%) (grade 1, n = 4; grade 3, n = 6; grade 4, n = 3). The 3 dogs with grade 4 neutropenia weighed 30.1, 42.2, and 51.4 kg, respectively, and with the exception of a Pug and Jack Russell Terrier, the patients with grade 3 neutropenia weighed ≥29 kg. No dogs developed thrombocytopenia. One dog became febrile and developed grade 3 anorexia, vomiting, and diarrhea 6 days after treatment, necessitating admission

Table 1. Treatment associated toxicities adapted from VCOG-CTCAE guidelines.

Toxicity	Definition
Neutropenia	
Grade 1	1,500–3,000 cells/μL
Grade 2	1,000–1,499 cells/μL
Grade 3	500–999 cells/μL
Grade 4	<500 cells/μL
Anorexia	
Grade 1	Coaxing/dietary change needed
Grade 2	Oral intake altered <3 days
Grade 3	Oral intake altered 3–5 day duration
Grade 4	Life threatening
Vomiting	
Grade 1	<3 episodes in 24 hours
Grade 2	3–5 episodes in 24 hours
Grade 3	>5 episodes in 24 hours
Grade 4	Life threatening
Diarrhea	
Grade 1	>2 feces in 24 hours over baseline
Grade 2	2–6 episodes in 24 hours
Grade 3	>6 episodes in 24 hours
Grade 4	Life threatening
Lethargy	
Grade 1	Mild lethargy over baseline
Grade 2	Moderate lethargy over baseline
Grade 3	Severely restricted in daily activities
Grade 4	Disabled

VCOG, Veterinary Cooperative Oncology Group; CTCAE, Common Terminology Criteria for Adverse Events.[26]

to an emergency hospital. A CBC indicated grade 4 neutropenia (neutrophils, 170 cells/μL). The dog was treated for sepsis and recovered with supportive care. No other dogs developed signs consistent with sepsis. No statistically significant association was identified between body weight and occurrence of neutropenia. Lethargy (grade 2) occurred in 5 dogs, mild anorexia (grade 1) in 3 dogs and moderate anorexia in 1 dog (grade 2), mild diarrhea (grade 1) in 2 dogs, and 5 dogs vomited once after treatment (grade 1 toxicity).

Discussion

In this Phase II study evaluating the efficacy of vinorelbine as a novel chemotherapeutic agent for the treatment of 24 dogs with measurable MCT, an overall response rate of 13% and a high prevalence of drug-induced neutropenia were observed. Based on Simon's 2-stage design, neither the null hypothesis nor the alternative hypothesis was true, and further investigation of vinorelbine thus is warranted.

Only 3 peer-reviewed studies in the veterinary literature examine the efficacy of single agent chemotherapy for the treatment of canine MCT in the setting of measurable disease. A number of other studies report on combination protocols, and the 47% overall response rate reported for combined therapy with vinblastine and prednisone has favored its selection as a standard of care for the management of this disease. In this phase II study, vinorelbine, a compound structurally related to vinblastine and with potentially broader antitumor activity, was associated with an overall response rate of only 13%. This approximates the 7% overall response rate reported for vincristine, and raises the question as to the efficacy of the vinca alkaloids in the absence of corticosteroids for the management of canine MCT.

This prospective study allowed the tumor response and treatment-associated toxicities to be evaluated in an identical manner for every patient. However, there are inherent limitations in our study design. First, dogs were treated only once, and the response to treatment was evaluated only at 1 time point, 7 days later. The decision to treat each patient once was based on the high cost of the drug and the limited budget available to perform the study. Multiple treatments given to individual dogs would have substantially decreased the number of dogs treated overall and resulted in insufficient statistical power. We also assumed that a response to therapy would be observed within 7 days. Our treatment group was principally made up of dogs scheduled to undergo surgical resection of their tumors, and we sought consent to delay treatment for a period of time that was acceptable to owners and clinicians and that we considered sufficiently long to demonstrate a response to therapy. In previous studies, authors have reported on the response of MCT to chemotherapy after 28 days of treatment[18,21] or 7 and 21 days after each cycle of treatment[19] although none of these authors commented directly on the time to treatment response. If more than 1 treatment was required to yield the best tumor response and optimal time to response was >7 days, we may have underestimated the efficacy of the drug. To evaluate treatment response, the percentage change in the longest or summated longest tumor diameter was measured, according to RECIST unidimensional measurement guidelines.[28–30] In the dog that experienced PR, tumor response was sustained for 21 days. The dog experiencing CR received additional chemotherapy and the tumor response was sustained for 7 days before receiving the 1st follow-up dose. Owing to the duration of these responses, we believe that they were true response to the treatment and not merely random fluctuations in tumor size, typical of the biologic behavior of MCT.

A 2nd limitation of the study was that all dogs did not undergo surgical removal of their tumors. Consequently, the tumor grade could not be assessed in each patient, and therefore the impact of tumor grade on response to therapy could not be determined.

An unexpected finding of this study was that in at least 1 case, there was no evidence of neoplastic mast cells after surgical removal of the tumors despite insufficient change in tumor diameter to classify the dog as a responder based on our defined response criteria. Mast cell granules contain cytokines, including basic fibroblast growth factor. An exuberant proliferation of fibroblasts at the site of this dog's tumors may account for the presence of measurable disease at the time of surgery. This finding suggests that response criteria based solely on change in tumor diameter may be of limited use for determining response to treatment in phase II studies of novel chemotherapies for MCT. Consequently, we felt it legitimate to classify this outcome as a positive response to treatment.

No significant differences were found between responders and nonresponders in the categories analyzed. This finding was not surprising based on the low number of responses recorded. Interestingly, the dogs with PR and CR had no hematologic toxicity. As previously noted, however, there was no statistically significant association between response to treatment and severity of hematologic toxicity.

With the exception of the dog that developed febrile neutropenia and sepsis, treatment with vinorelbine generally was well tolerated and antibiotics were used to decrease the risk of additional occurrences of sepsis. The use of antibiotics allowed us to safely treat all dogs at the prescribed target dosage rather than introduce inconsistency by dose reduction. To the best of our knowledge, antibiotics are not reported to have antitumor effects in canine MCT although this possibility cannot be ruled out. The lack of response in the dogs receiving vinorelbine together with antibiotics suggests that this effect is unlikely.

In a previous phase I study, 32% of dogs developed neutropenia, with a nadir at 7 days, typically after their 1st treatment with vinorelbine.[25] Grade 2–4 neutropenia was observed in only 21% of those patients, including 2 dogs that had received vinorelbine at an escalated dosage of 20 mg/m^2. The prevalence and severity of toxicity were higher in our study. Neutropenia occurred in 13 dogs (54%) (grade 1, n = 4; grade 3, n = 6; grade 4, n = 3) and was moderate to severe in 37% of dogs, including 6 of 24 dogs (25%) with grade 3 neutropenia and 3 of 24 dogs (12%) with grade 4 neutropenia. The median weight

of dogs in our study population was higher than in the previous study and neutropenia was most severe in larger dogs. However, no statistically significant association between body weight and severity of hematologic toxicity was identified. One difference between the 2 studies was the time over which the drug was administered. We chose a 6–10-minute infusion in accordance with protocols used in humans compared with the 5-minute infusion used in the previous study, and this protocol may account for the increased incidence and severity of neutropenia. In summary, this study demonstrated neutropenia as a dose-limiting toxicity associated with the use of vinorelbine and confirmed the maximum tolerated dosage for dogs to be 15 mg/m^2 in accordance with previous recommendations.[25]

Within the limitations of the study design, the observed overall response rate to vinorelbine is sufficient to justify additional testing of the drug in the management of canine MCT. Future studies should include serial treatments with vinorelbine, treatment response assessment over longer periods of time, and cytologic or biopsy-based assessment of residual disease in measurable lesions to fully evaluate the efficacy of vinorelbine as a cytotoxic agent in canine MCT. In vitro cytotoxicity assays with vincristine, vinblastine, and vinorelbine against malignant canine mast cells may be helpful to further investigate their antitumor activity.

Considering the 47% response rate reported for vinblastine combined with prednisone, it is not possible to determine the relative contribution of each drug to treatment response. Recently, the in vivo activity of single agent vinblastine was evaluated for the 1st time. In a multiinstitutional study, Henry et al reported a relatively low response rate (defined as >50% reduction in tumor size) of 17.6% in 49 dogs with measurable MCT after repeated dosing,[31] a finding that approximates to the response rate reported in our study. The antitumor activity of vinorelbine may be superior to that of its parent compound when used in combination therapies that include prednisone, lomustine, or both for this tumor type.

Footnotes

[a] Bedford Laboratories, Bedford, OH
[b] Microsoft Office Excel 2003, Microsoft Corporation, Redmond, WA
[c] Stata Version 9.1, StataCorp, College Station, TX

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