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ORIGINAL ARTICLE

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Preclinical study of dolastatin-10 in dogs with spontaneous neoplasia

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Abstract *Purpose*: To evaluate the short-term adverse effects of administration of dolastatin-10 (Dol-10) to dogs with spontaneously occurring malignant tumors. *Methods*: A total of 34 tumor-bearing dogs were given Dol-10 as a rapid intravenous bolus every 14 days at starting dosages ranging from 200 to 350 μg/m^2. Acute and short-term adverse effects, antitumor response, and duration of response were characterized. *Results*: The maximum tolerated dose varied greatly from patient to patient, but a reasonable starting dose for further studies was established at 300 μg/m^2. The median number of treatments per dog was 2 (range 1 to 17). Granulocytopenia was the dose-limiting toxicity. The overall response rate was 3%, consisting of a complete and durable (30 months) response in a dog with high-grade malignant lymphoma that was refractory to standard therapy. Two minor or transient responses were observed, and two dogs experienced disease stabilization for 8 and 16 weeks. *Conclusions*: Dol-10 appears to be well tolerated in tumor-bearing dogs at doses approaching those tolerated by humans. The clinical activity observed in dogs with non-Hodgkin's lymphoma warrants further investigation.

Keywords Dolastatin-10 · Dogs · Spontaneous neoplasia

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Introduction

Dolastatin-10 (Dol-10) is a highly lipophilic, linear pentapeptide isolated from the marine shell-less mollusk *Dolabella auricularia*, with a primary structure consisting of four novel amino acids [1]. Dolastatin-10 has antiproliferative activity against a variety of tumor cell types in the subnanomolar range in vitro, and has potent activity against various human tumor xenografts [1, 2, 3]. Dolastatin-10 appears to exert its antitumor effect via inhibition of tubulin polymerization, leading to metaphase arrest and, at higher concentrations, the complete disappearance of intracellular tubulin [1].

Dol-10 binds to the tubulin molecule, competitively inhibiting the binding of the vinca alkaloids but not colchicine [4]. It may induce cytostasis or apoptosis, depending on the cell line studied and experimental conditions [2, 5, 6, 7]. As is the case with many antitumor natural products, Dol-10 resistance can be conferred by P-glycoprotein expression [8]. The results of preclinical pharmacologic studies suggest extensive hepatic metabolism of the parent compound, and extensive protein binding in human, dog and mouse plasma [9]. Additional murine data suggest that Dol-10 may exert in vivo antitumor effects in part by inducing an acute reduction in tumor blood flow [10].

The results of two phase I trials of Dol-10 in humans with advanced solid tumors have been recently reported. In these studies, the maximum tolerated dose was 400 μg/m^2 in minimally pretreated patients and 300 μg/m^2 in more heavily pretreated patients, with granulocytopenia being the dose-limiting toxicity. Rapid distribution and an elimination half-life of 320 min have been identified in pharmacokinetic

studies. The pharmacokinetics appear to vary considerably within and between patients. No antitumor responses have been noted [11, 12]. A recent phase II study of Dol-10 in human prostate cancer demonstrated disease stabilization in several patients [13].

The dog is an excellent translational model for the investigation of novel antineoplastic therapies. Unlike murine models, the canine model utilizes relatively outbred, immunocompetent animals with spontaneously occurring tumors, with a spectrum of tumor histotypes and biologic behavior similar to those found in humans. The relatively large size of canine tumors when compared with murine tumors may more closely approximate human solid tumors with respect to important biologic factors such as hypoxia and clonal variation, and allows serial sampling of tumor tissue over time. Finally, the relatively rapid time course of disease progression, when compared with human cancer, allows for more rapid assessment of therapeutic endpoints than is possible in many human clinical trials [14].

The purpose of this study was to prospectively evaluate the acute and short-term toxicities associated with Dol-10 administration in a population of client-owned (pet) dogs with spontaneously occurring neoplasia of various histologic types and sites in the context of a dose-escalation trial. Preliminary data regarding antitumor efficacy were also obtained.

Materials and methods

Patient population

Client-owned dogs from the patient population presenting to the Veterinary Medical Teaching Hospital at the University of Wisconsin-Madison, from June 1997 to February 2000, were studied. Clients were offered Dol-10 therapy for their companion animals in cases of advanced disease where no meaningful standard of care therapy existed, or where standard therapy had failed or been declined. Histologic confirmation of diagnosis (or cytologic diagnosis in the case of some patients with lymphoma) was obtained in all patients. Staging methods employed varied depending on the histologic type and anatomic site of the tumor, and the clinical status of the dog. These included, but were not limited to, physical examination, complete blood count, serum biochemistry profile, urinalysis, and thoracic radiographs. Dogs were eligible for the study provided they had adequate performance status and hematologic and serum biochemical parameters to undergo chemotherapy, and were free of complicating concurrent disease. Dogs in the study had not received chemotherapy within 2 weeks prior to Dol-10 administration, nor received any concurrent antineoplastic therapy. All of the dogs had measurable disease at the time of entry. Tumors were measured either by physical assessment (i.e. caliper measurements) or by serial examination of radiographs.

Drug administration

Dol-10 (NSC 376128) was synthesized at the Arizona State University Cancer Research Institute, reconstituted to a concentration of 200 μg/ml in 0.1 *M* sodium phosphate buffer, pH 7.1, and stored at room temperature. Written approval for the clinical protocol was obtained from the University of Wisconsin Institutional Animal Care and Use Committee, and signed informed consent and consent to necropsy were obtained from owners prior to study entry. The Dol-10 was administered as a rapid intravenous bolus every 14 days. The starting dosage and dosing interval were chosen based on data generated in normal dog toxicity studies [15]. The objective was to escalate the dosage in 50 $\mu g/m^2$ increments to the point where fewer than one-third of the dogs experienced clinically significant toxicosis necessitating dosage adjustment or treatment discontinuation. Treatment was continued until the pa#132;tient developed progressive disease, developed significant toxicity, or until quality of life was diminished to a point deemed unacceptable by the owner and attending oncologist.

Toxicity assessment

Complete blood counts were obtained prior to each treatment, and 7 days following Dol-10 administration. A minimum neutrophil count of 2500/μl and platelet count of 75,000/μl were required for administration of Dol-10. At each 14-day re-evaluation visit, a thorough history and physical examination were performed to determine any clinically adverse effects of the drug treatment, and a complete blood count was obtained. Hematologic toxicity was assigned a severity grade according to a modification of the NCI Common Toxicity Criteria [16] (Table 1). Dogs receiving four or more doses of Dol-10 were assessed for cumulative trends in hematocrit and platelet count.

Parameters for evaluation of therapeutic response

Tumor measurements were recorded for each dog immediately before, and then again 14 days following each Dol-10 treatment. Three-dimensional caliper measurements were obtained in accessible tumors, or in the case of radiographs or ultrasound, the longest diameter and a perpendicular diameter at the widest portion of each tumor were measured. A complete response (CR) was defined as complete regression of all measurable tumors for at least 6 weeks. A partial response (PR) was defined as at least a 50% decrease in the measurable tumor volume, with no evidence of new tumors for at least 6 weeks. Stable disease (SD) was defined as less than 50% decrease, or less than 25% increase in the measurable tumor volume, without the development of new lesions for at least 6 weeks. Progressive disease (PD) was defined as greater than 25% increase in the measurable tumor volume, or the appearance of new tumors. The duration of response or disease stabilization was defined as the time from achievement of response until subsequent progression of disease, loss to follow-up, or death. Whenever possible, complete post-mortem examinations were performed at the time of death or euthanasia.

Results

Patient characteristics

A total of 34 dogs were enrolled in the study. The patient characteristics are presented in Table 2. Tumor types included malignant lymphoma (similar to human high-grade non-Hodgkin's lymphoma, 15 dogs), malignant mast cell tumor (four), malignant melanoma

Table 1 Modified NCI Common Toxicity Criteria for canine neutropenia

Grade 0	Neutrophils $>1500/\mu l$
Grade 1	Neutrophils 1000–1500/μl
Grade 2	Neutrophils 800–1000/μl
Grade 3	Neutrophils 500–800/μl
Grade 4	Neutrophils $<500/\mu l$

Table 2 Patient characteristics

Age (years)	
Median	8
Range	3–13
Weight (kg)	
Median	26
Range	2.6–50
Sex	
Female	20
Male	14
Breed	
Golden retriever	8
German shepherd	6
Labrador retriever	2
German short-haired Pointer	2
Mixed breed	2
Other (one each)	14
Tumor type	
Lymphoma	15
Mast cell tumor	4
Apocrine gland carcinoma	3
Melanoma	2
Squamous cell carcinoma	2
Bronchogenic carcinoma	2
Hemangiosarcoma	2
Other (one each)	4
Prior therapy	
Chemotherapy	33
Immunotherapy	4
Radiotherapy	2

(two), oral squamous cell carcinoma (two), bronchogenic carcinoma (two), hemangiosarcoma (two), apocrine gland carcinoma (three), and one each of osteosarcoma, mammary gland carcinoma, fibrosarcoma, and multilobular osteochondrosarcoma. No patient had disease confined to the primary site, eight had disease confined to the regional lymph nodes, and 26 had distant metastasis (or systemic disease in the case of lymphoma) at the time of entry. Of the 34 patients, 33 had prior chemotherapy, 2 had prior radiotherapy, and 4 had prior immunotherapy. Drugs previously received included doxorubicin (22 dogs), cyclophosphamide (20), asparaginase (14), vincristine (13), CCNU (7), mitoxantrone (4), vinblastine (4), paclitaxel (4), carboplatin (3), melphalan (2), cisplatin (1), Doxil (1), and actinomycin D (1). The median number of prior chemotherapy protocols was one, with a range of zero to four.

Treatments

A total of 82 treatments were given at 14-day intervals, at starting dosages of 200 $\mu g/m^2$ (19), 250 $\mu g/m^2$ (7), 300 $\mu g/m^2$ (7), and 350 $\mu g/m^2$ (1). Dogs received from 1 to 17 Dol-10 treatments, with a median of two treatments per dog. Intrapatient dose escalation occurred in 12 dogs, and dose reduction was necessary in three as a result of hematologic toxicity.

Table 3 Neutropenia following dolastatin-10 administration

Dose ($\mu g/m^2$)	No. of patients (first dose)	Total no. of doses	Modified NCI toxicity grade			
			1	2	3	4
160	–	3	–	–	–	–
180	–	1	–	–	–	–
200	19	31	1	2	–	1
250	7	15	1	–	–	2
300	7	27	2	1	–	–
350	1	2	–	–	–	–
400	–	1	–	–	–	–

Hematologic toxicity

Ten dogs experienced neutropenia 7 days following Dol-10 treatment (Table 3), and all dogs had neutrophil counts in the normal range 14 days following Dol-10. One dog experienced grade 1 thrombocytopenia (platelet count 100,000–150,000/μl) following one treatment at 250 $\mu g/m^2$, and following one of seven treatments at 200 $\mu g/m^2$. Grade 2–4 neutropenia (neutrophil counts less than 1000/μl) occurred in five dogs. This occurred at a dose of 200 $\mu g/m^2$ in two dogs, 250 $\mu g/m^2$ in two, and 300 $\mu g/m^2$ in one. Patient weight did not correlate with likelihood or severity of neutropenia. None of the dogs with neutropenia developed fever or other signs consistent with infection or sepsis. None of the four dogs receiving four or more Dol-10 doses demonstrated any significant decreases in hematocrit or platelet count over time.

Gastrointestinal toxicity

Four dogs experienced gastrointestinal toxicity. Two owners reported mild inappetance persisting for 3–7 days following treatment. Two owners reported mild self-limiting diarrhea, and one dog experienced mild vomiting for 2 days immediately following treatment. Gastrointestinal side effects occurred in three dogs at a dose of 200 $\mu g/m^2$, and in one dog at a dose of 300 $\mu g/m^2$. No gastrointestinal effects were dose-limiting, or required treatment delays or specific therapy.

Therapeutic response

Of the 34 patients, 33 were evaluable for response. One patient with malignant lymphoma experienced a durable CR, for an overall response rate of 3%. Two patients experienced minor or transient responses, and two patients experienced SD. The patient experiencing a CR had failed previous multiagent chemotherapy with asparaginase, doxorubicin, cyclophosphamide, prednisone and vincristine (CHOP). CR was achieved after 2 Dol-10 treatments at 200 and 250 $\mu g/m^2$, and the dog went on to receive an additional 15 doses of Dol-10 at 300 $\mu g/m^2$ every other week. Treatment was discontinued

at that time while the dog was still experiencing CR. The dog developed a biopsy-confirmed hepatocellular carcinoma 14 months after Dol-10 initiation, and further treatment was declined. The dog was euthanized, still in clinical CR from lymphoma, 30 months after initiation of Dol-10 therapy. Complete post-mortem examination revealed no gross or microscopic evidence of lymphoma.

One dog with lymphoma that had failed four previous chemotherapy drugs/protocols (CHOP, CCNU, actinomycin D, and mitoxantrone) experienced a 48.5% reduction in lymph node volume after a single injection of Dol-10 at 200 $\mu g/m^2$. A second injection of 250 $\mu g/m^2$ was given, and the dog was euthanized by the referring veterinarian for respiratory distress of undetermined origin 7 days later. Post-mortem examination was not performed, and no further clinical information was provided. Another dog with WHO stage III malignant mast cell tumor that had been previously treated with vinblastine and CCNU experienced a greater than 50% reduction in the volumes of the primary mass and regional lymph node metastasis, but this response persisted for only 4 weeks and thus could not, by definition, be considered a PR. The two dogs with apocrine gland carcinoma of the anal sac experienced disease stabilization for 8 and 16 weeks. Both had previously failed chemotherapy with melphalan and an anthracycline-based protocol. Ultrasound examination of the abdomen in one of these dogs suggested marked cavitation of an enlarged median sacral lymph node after two treatments that was not present prior to Dol-10 administration.

Post-mortem findings

Post-mortem examination was carried out on 14 dogs. No changes attributable to Dol-10 administration were identified in any dog. Four dogs had evidence of mild to moderate hepatic vacuolar degeneration, but three of these had been treated with corticosteroids, which could have been responsible for the changes observed. Two dogs had evidence of renal interstitial fibrosis, varying from mild to severe.

Discussion

The acute and short-term toxicities associated with Dol-10 administration to dogs with a wide variety of tumors were evaluated in a dose-escalating fashion. No signs of acute reaction were noted in any patient during or immediately following drug delivery.

When delivered as a rapid intravenous bolus every 14 days, the maximally tolerated dose of Dol-10 appeared quite variable in the canine tumor-bearing patients treated in this study. Although pharmacokinetic studies were not carried out as part of this trial, data from human subjects suggest that the plasma clearance of Dol-10 can vary considerably within and between patients [11]. It is possible that the range of tolerable Dol-10 doses observed in this study was a result of a similar phenomenon. Based on the variability observed in this group of patients, a starting dose of 300 $\mu g/m^2$ appears advisable, with dose escalation or reduction applied as necessary based on individual toxicity.

Myelosuppression was the most frequent toxicity encountered in canine patients treated with Dol-10. It was also the dose-limiting toxicity in two phase I trials of Dol-10 in humans [11, 12]. Gastrointestinal signs were infrequent, mild and self-limiting. Although neurologic complications and injection site irritation have been also reported in humans, no clinical evidence of these was observed in dogs receiving Dol-10. No association could be made between the amount or type of prior chemotherapy and the development of any toxicity.

A wide variety of tumor histotypes were treated in this study. These included carcinomas, sarcomas and round-cell tumors, and represent a more diverse gamut of tumor types than is often encountered in many human phase I clinical trials. Although the primary focus of this study was to characterize the short-term toxicoses associated with Dol-10 administration, preliminary information regarding antitumor activity was also generated. The overall response rate of 3% is low, but the one responding patient with multicentric lymphoma experienced a CR that persisted for 30 months, until the time of death from an unrelated disease. This is exceptional in that the rate of "cure" in canine lymphoma is less than 5% with standard CHOP-based chemotherapy, and that failure to experience durable CR to first-line therapy is an extremely poor prognostic factor for long-term disease-free survival [17].

Transient or minor responses were also seen in two patients with lymphoma and malignant mast cell tumor. Interestingly, all the patients in which clinical activity was observed had failed prior treatment with vinca alkaloids. Given that Dol-10 is capable of competitively inhibiting vinca alkaloid binding to the tubulin molecule [4] and can be handled via P-glycoprotein [8], it was surprising that clinical activity was observed in these patients, and perhaps lends support to the notion that Dol-10 may exert some of its in vivo antitumor effect via targeting of the vascular compartment.

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References

1. Bai R, Roach MC, Jayaram SK, Barkoczy J, Pettit GR, Ludena RF, Hamel E (1993) Differential effects of active isomers, segments, and analogs of dolastatin 10 on ligand interactions

with tubulin: correlation with cytotoxicity. Biochem Pharmacol 45:1503–1515
2. Turner T, Jackson WH, Pettit GR, Wells A, Kraft AS (1998) Treatment of human prostate cancer cells with dolastatin 10, a peptide isolated from a marine shell-less mollusc. Prostate 34:175–181
3. Pettit GR, Kamano Y, Herald CL, Fujii Y, Kizu H, Boyd MR, Boettner FE, Doubek DL, Schmidt JM, Chapuis J-C, Michel C (1993) Isolation of dolastatins 10–15 from the marine mollusc *Dolabella auricularia*. Tetrahedron 49:9151–9170
4. Bai R, Pettit GR, Hamel E (1990) Dolastatin 10, a powerful cytostatic peptide derived from a marine animal: inhibition of tubulin polymerization mediated through the vinca alkaloid binding domain. Biochem Pharmacol 39:1941–1949
5. Beckwith M, Urba WJ, Longo DL (1993) Growth inhibition of human lymphoma cell lines by the marine products, dolastatins 10 and 15. J Natl Cancer Inst 85:483–488
6. Maki A, Diwakaran H, Redman B, Al-Asfar S, Pettit GR, Mohammad R, Al-Katib A (1995) The *bcl-2* and p53 oncoproteins can be modulated by bryostatin 1 and the dolastatins in human diffuse large cell lymphoma. Anticancer Drugs 6:392–397
7. Hu Z-B, Gignac SM, Quentmeier H, Pettit GR, Drexler HG (1993) Effects of dolastatins on human B-lymphocytic leukemia cell lines. Leuk Res 17:333–339
8. Toppmeyer DL, Slapak CA, Croop J, Kufe DW (1994) Role of P-glycoprotein in dolastatin 10 resistance. Biochem Pharmacol 48:609–612
9. Newman RA, Fuentes A, Covey JM, Benvenuto JA (1994) Preclinical pharmacology of the natural marine product dolastatin 10 (NSC 376128). Drug Metab Dispos 22:428–432
10. Chaplin DJ, Pettit GR, Parkins CS, Hill SA (1996) Antivascular approaches to solid tumour therapy: evaluation of tubulin binding agents. Br J Cancer 27 [Suppl]:S86–S88
11. Madden T, Tran HT, Beck D, Huie R, Newman RA, Pusztai L, Wright JJ, Abbruzzese JL (2000) Novel marine-derived anticancer agents: a phase I clinical, pharmacologic, and pharmacodynamic study of dolastatin 10 (NSC 376128) in patients with advanced solid tumors. Clin Cancer Res 6:1293–1301
12. Pitot HC, McElroy EA, Reid JM, Windebank AJ, Sloan JA, Erlichman C, Bagniewski PG, Walker DL, Rubin J, Goldberg RM, Adjei AA, Ames MM (1999) Phase I trial of dolastatin-10 (NSC 376128) in patients with advanced solid tumors. Clin Cancer Res 5:525–531
13. Vaishampayan U, Glode M, Du W, Kraft A, Hudes G, Wright J, Hussain M (2000) Phase II study of dolastatin-10 in patients with hormone-refractory metastatic prostate adenocarcinoma. Clin Cancer Res 6:4205–4208
14. Vail DM, MacEwen EG (2000) Spontaneously occurring tumors of companion animals as models for human cancer. Cancer Invest 18:781–792
15. Mirsalis JC, Schindler-Horvat J, Hill JR, Tomaszewski JE, Donohue SJ, Tyson CA (1999) Toxicity of dolastatin 10 in mice, rats and dogs and its clinical relevance. Cancer Chemother Pharmacol 44:395–402
16. Boyce KL, Kitchell BE (2000) Treatment of canine lymphoma with COPLA/LVP. J Am Anim Hosp Assoc 36:395–403
17. MacEwen EG, Young KM (1996) Canine lymphoma and lymphoid leukemias. In: Withrow SJ, MacEwen EG (eds) Small animal clinical oncology, 2nd edn. Saunders, Philadelphia, pp 451–479