NOTE *Internal Medicine*

Phase I Dose-Escalation Study of Nimustine in Tumor-Bearing Dogs

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ABSTRACT. Nimustine (ACNU) is an alkylating agent of the nitrosourea and can be an antineoplastic agent in dogs. But, there has been no report on its dose-limiting toxicity (DLT) in dogs. This study was a phase I dose-escalation clinical trial to determine the maximum tolerated dose (MTD) and DLT of ACNU in tumor-bearing dogs. The starting dosage was 25 mg/m^2, and subsequent dosages were administered in increments of 5 mg/m^2 in cohort of 3 dogs. Eight dogs were included, the MTD was determined to be 25 mg/m^2, DLT was neutropenia, and the optimal interval was considered to be 21 days. The data herein provide a basis for the subsequent phase II trial of ACNU in dogs.
KEY WORDS: ACNU cancer, canine, nitrosourea, phase I.

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Nitrosourea (3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride, ACNU) compounds, such as lomustine (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, CCNU) and carmustine (1,3-bis (2-chloroethyl)-1-nitrosourea, BCNU), have been reported to possess a high antitumor activity in dogs with a variety of tumors [4, 6, 7, 9, 10]. ACNU was developed as a nitrosourea-derived anticancer agent for humans in Japan and has been reported to have an equivalent or higher cytotoxic activity than CCNU against the murine lymphoid leukemia cell line L-1210 [5]. A phase II study in humans revealed that ACNU was effective against lung cancer, brain tumors, alimentary tract cancer and tumors of hematopoietic organs [8]. A pharmacokinetic study showed that, as a result of its hydrophilic nature, up to 30% of ACNU passes through the blood–brain barrier, which is a unique advantage of ACNU [13]. In humans, major adverse events associated with ACNU are neutropenia and thrombocytopenia with no apparent sign of hemorrhage. Subjectively, the symptoms were mild and were primarily nausea, vomiting and diarrhea. No adverse events were observed in the central nervous, circulatory and respiratory systems or in the liver or kidney [1].

In veterinary oncology, CCNU and BCNU are commonly used to treat dogs with relapsed lymphomas, mast cell tumors and histiocytic sarcomas. There are several reports in the literature on the optimal dose regimen and possible side effects of these drugs [4, 6, 7, 9, 10]. In tumor-bearing dogs orally treated with CCNU at 90 mg/m^2, neutropenia was the acute dose-limiting toxicity (DLT) observed at approximately 7 days after treatment. Thrombocytopenia was not observed after a single dose, but there appeared to be cumulative toxicity following multiple administrations in some dogs. At a higher dose or after multiple administrations of the drug, gastrointestinal toxicity, cumulative and irreversible chronic hepatotoxicity, and rare renal toxicity were also documented in preclinical trials of CCNU [2–4, 6]. Bone marrow toxicity, such as neutropenia and thrombocytopenia, and vascular pain during BCNU treatment were reported in lymphoma-bearing dogs receiving BCNU treatment [7]. Although the nitrosourea drugs, CCNU and BCNU, are not currently commercially available in Japan, ACNU and MCNU (ranimustine) are available for use, and several institutions, including our group, have used ACNU for the treatment of malignancies in dogs. However, to date, there are no published reports on the DLT of ACNU in dogs. The purpose of this prospective study was to determine the maximum tolerated dose (MTD) and DLT for a single administration of ACNU in tumor-bearing dogs.

Client-owned dogs bearing histologically or cytologically confirmed tumors that had been referred to the Veterinary Medical Center of the University of Tokyo were included in this study. Dogs were eligible to participate, if they had failed to respond adequately to conventional antineoplastic treatments or if treatment had been discontinued due to economic reasons of the clients. Dogs that received myelosuppressive chemotherapy, surgery or radiation therapy within 2 weeks of referral were excluded from this study. In addition, dogs included in this study were expected to survive for at least 4 weeks without treatment, showing no hematological or serum biochemical abnormalities and weighing over 5.0 kg. Dogs with concurrent diseases were permitted at the discretion of the attending clinician as long as the unrelated illness was deemed not to increase the likelihood or severity

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of ACNU-associated toxicity. Client consent was obtained before enrollment into this phase I trial. The Institutional Animal Care and Use Committee at the Veterinary Medical Center of Tokyo University approved the study protocol.

Before administration of ACNU or on the day of treatment, all dogs underwent baseline physical examination, complete blood count (CBC), serum biochemical examination and urinalysis. Each dog was administered a dose of ACNU as an intravenous (IV) bolus injection. Thereafter, the dogs were re-admitted to our hospital in order to undergo regular physical examination and CBC every week for at least 3 weeks. Examination was continued, if any abnormality was observed until it returned to normal level. At each visit, owners were asked to provide information on any gastrointestinal or other adverse events observed at home. Toxicities were graded according to the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events v1.0 (Appendix Table 1A) [12]. DLT was defined as any grade 4 hematological toxicity or as a grade 3 or 4 adverse event that developed after a single dose of ACNU.

This clinical trial was designed based on the standard method of dose escalation scheme that was reported Vail *et al.* [11]. The initial ACNU dose was set at 25 mg/m^2 based on the preliminary results of our clinical experience. Doses were serially increased in a cohort of 3 dogs. If none of 3 dogs in a given cohort experienced severe toxicity, the dosage for the next cohort was escalated by 5 mg/m^2. If 1 of the 3 dogs experienced severe toxicity, additional 3 dogs were treated at the same dosage. If none of the additional dogs experienced severe toxicity, the escalation was resumed. If 2 dogs in a cohort experienced severe toxicity, additional enrollment in that cohort was discontinued. MTD was defined as the highest dose level in which no more than 1 of 6 dogs developed DLT [11].

Although evaluation of antitumor activity of ACNU was not the primary endpoint of this study, dogs with measurable disease were evaluated for response using Response Evaluation Criteria in Solid Tumors. Response to antitumor activity was categorized as follows: complete response, complete disappearance of all measurable disease; partial response, >30% but <100% reduction in measurable disease; stable disease, <50% reduction or no change in measurable disease without the appearance of new neoplastic lesions; and progressive disease, an increase of >20% in the sum of the longest diameters of measurable tumors or appearance of new neoplastic lesions [11]. All assessments were performed over 21 days or more. A decrease in tumor size for a short duration was reported as stable disease.

Eight dogs enrolled in this study were referred to our department with various tumors between March 2009 and April 2010. The following breeds were represented: Welsh Corgi Pembroke (n=4), Golden Retriever (n=1), Miniature Dachshund (n=1), Shih Tzu (n=1) and mixed breed (n=1). There were 5 male and 3 female dogs. Dogs ranged in age from 5 to 14 years (mean, 9 years; median, 9 years) and weighed between 6.4 and 30.4 kg (mean, 13.7 kg; median, 12.7 kg). The tumors were lymphoma (n=4), histiocytic sarcoma (n=1), nasal adenocarcinoma (n=1), oral melanoma (n=1) and tonsillar squamous cell carcinoma (n=1). Two dogs had not been treated previously (25%), 1 had undergone surgery (12.5%), and 4 had been treated with the following chemotherapeutic agents (62.5%): vincristine (n=4), cyclophosphamide (n=4), doxorubicin (n=4), CCNU (n=1), mitoxantrone (n=1) or dacarbazine (n=1). All 4 dogs with lymphoma enrolled in this study were found to be resistant to the aforementioned chemotherapeutic agents. Seven dogs, not including 1 dog with oral melanoma, had macroscopic tumors at the time of ACNU treatment.

As summarized in Table 1, a total of 6 dogs were treated with ACNU at a dosage of 25 mg/m^2. Because one of 3 dogs in the first cohort experienced severe toxicity characterized by grade 4 febrile neutropenia, additional 3 dogs were treated with the same dose of ACNU. Grade 4 neutropenia resolved after supportive therapy with antibiotic administration and fluid therapy. After receiving 25 mg/m^2 of ACNU, mild neutropenia was observed in 1 dog (grade 2), and mild thrombocytopenia was observed in 2 dogs (grade 1, n=1; grade 2, n=1). The ACNU dose was increased to 30 mg/m^2 in remaining 2 dogs, both of which experienced severe toxicity characterized by asymptomatic grade 4 neutropenia that resolved following hospitalization and supportive care. Accordingly, the MTD of ACNU was determined to be 25 mg/m^2, and the DLT of ACNU was considered to be neutropenia. A decrease in neutrophil counts to varying degrees was observed in all 8 dogs that received ACNU with nadir occurring 7 days after administration. The median and mean neutrophil counts at the nadir for all dogs were 4,000 cells/μl and 5,500 cells/μl, respectively (range, 0–18,700 cells/μl). Neutrophil counts recovered to within the normal reference range in all 4 dogs with neutropenia within 7 days after the nadir. Decreased thrombocyte counts were also noted in all 8 dogs. The platelet count nadir occurred between days 7 and 21 after treatment (day 7, n=2; day 14, n=3; and day 21, n=3). In the 8 dogs that received ACNU treatment, the median and mean platelet count nadirs were 284,000 cells/μl and 229,000 cells/μl, respectively (range, 51,000–586,000 cells/μl). Thrombocyte counts recovered to normal levels within 7 days after the nadir in 3 of 4 dogs with thrombocytopenia. One dog that developed profound thrombocytopenia died due to tumor progression without recovery of the thrombocyte count. On the basis of the results of the thrombocyte count nadir, the recommended dosing interval for single-agent ACNU was determined to be 21 days. Hematological abnormalities other than neutropenia and thrombocytopenia were not observed in this study.

Gastrointestinal toxicity of any kind was identified in 4 of the 8 dogs (50%) as summarized in Table 2. No dog experienced severe gastrointestinal toxicity, and all clinical signs resolved following supportive care, except in 1 dog with anorexia due to tumor invasion. Vomiting (grade 1, n=2; grade 2, n=1) and anorexia (grade 1, n=3) were the most common toxic events, observed in 3 of the 8 dogs. Mild diarrhea was observed in 1 of the 8 dogs (grade 1, n=1). These gastrointestinal toxicities occurred within 7 days after ACNU treatment. Medications, such as H2 blocker (5 of 8 dogs), prednisolone (4 of 8 dogs), antibiotics (4 of 8 dogs), angiotensin-converting enzyme inhibitors (1 of 8 dogs) and

Table 1. Adverse hematologic events following administration of a single dose of ACNU

ACNU Dose (mg/m^2)	# of dogs	Grade of neutropenia*					Grade of thrombocytopenia*				
		0	1	2	3	4	0	1	2	3	4
25 (1st cohort)	3	2	0	0	0	1	1	1	1	0	0
25 (2nd cohort)	3	2	0	1	0	0	3	0	0	0	0
30	2	0	0	0	0	2	0	1	1	0	0

*See Appendix Table A1 for grading criteria.

Table 2. Adverse gastrointestinal events following administration of a single dose of ACNU

ACNU Dose (mg/m^2)	# of dogs	Grade of anorexia*					Grade of vomiting*					Grade of diarrhea*				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
25 (1st cohort)	3	1	2	0	0	0	1	2	0	0	0	2	1	0	0	0
25 (2nd cohort)	3	2	1	0	0	0	2	0	1	0	0	3	0	0	0	0
30	2	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0

*See Apendix Table A1 for grading criteria.

metoclopramide (1 of 8 dogs), were prescribed for dogs in this study. No dog received any prophylactic antiemetic agent.

Serum biochemical measurements were repeated every 7 days after ACNU treatment in all 8 dogs until 21 days post-treatment. No hepatic or renal adverse events were observed, except in 1 dog that developed azotemia, which was likely due to tumor progression. An increased creatinine level from 0.9 to 2.7 mg/d*l* and a decreased urine-specific gravity from 1.055 to 1.020 were observed in this dog. No dog experienced vascular pain during ACNU infusion.

Seven of the 8 dogs had a measurable tumor mass before the administration of ACNU. The antitumor efficacy of single-dose ACNU was evaluated in 7 of the 8 dogs, 21 days after the start of treatment. Response to ACNU treatment was identified as partial response in 1 dog (lymphoma), stable disease in 5 dogs (lymphoma, n=3; histiocytic sarcoma, n=1; nasal adenocarcinoma, n=1) and progressive disease in 1 dog (lymphoma).

ACNU is a nitrosourea compound commercially available in Japan. Therefore, evaluating its optimal dosage in dogs would be beneficial. According to the results of our study, the MTD of ACNU was determined to be 25 mg/m^2, and neutropenia was the DLT. Because gastrointestinal toxicity was mild in dogs after administration of ACNU in this study, it may not be necessary to prescribe any prophylactic antiemetic or antidiarrheal agent in dogs that undergo ACNU treatment. All dogs underwent repeated serum biochemistry profile examination until 21 days after the administration of ACNU in order to evaluate potential organ toxicity. No severe abnormalities were noted, except in 1 dog with tumor progression. Liver damage has been reported to be cumulative and may occur up to 1 month after CCNU treatment in dogs [6]. Additional studies with longer follow-up times and bone marrow and liver function monitoring are warranted to evaluate multiple ACNU courses. Vascular pain during BCNU infusion was reported to resolve by increasing the infusion time to approximately 60 min [7]. Of note, there were no signs to suggest vascular pain in the 8 dogs that received intravenous bolus ACNU injections. This may be due to the amphiphilic nature of ACNU, which does not require dilution in alcohol, in contrast to BCNU which need to be diluted in alcohol.

As with CCNU, the neutrophil count nadir was observed 7 days after ACNU administration. The neutrophil count recovered to within the reference range by day 14 in all dogs [4]. The platelet count nadir was identified between days 7 and 21 after ACNU administration. In 3 of the 4 dogs with thrombocytopenia, platelet counts recovered to normal levels from nadir within 7 days. In 1 dog, thrombocytopenia (51,000 cells/μl) was not resolved due to an increased platelet consumption following tumor progression and disseminated intravascular coagulation, as determined by the prolonged prothrombin and activated partial thromboplastin times and an increased production of fibrin/fibrinogen degradation products. On the basis of these results, in particular the platelet nadir, the recommended dosing interval for single-agent ACNU at 25 mg/m^2 was indicated to be 21 days with an acceptable level of toxicity observed at MTD according to the standards of clinical veterinary oncology. In humans, ACNU induces delayed and cumulative bone marrow toxicity consistent with observations reported after CCNU treatment. This represents a principle limitation for the clinical application of the nitrosourea-based antitumor agents [5]. In our study, we were unable to determine whether cumulative myelosuppression would also occur in dogs after multiple ACNU administrations because adverse events following only a single-dose treatment were evaluated in this study. Cumulative toxicity associated with ACNU administration, especially bone marrow toxicity, should be investigated in the future.

Since this report is a phase I trial, anti-tumor response was not evaluated as a primary endpoint. However, it is of note that 1 of 4 dogs with lymphoma that acquired resistance to a CHOP (cyclophosphamide/doxorubicin/vincristine/prednisolone)-based protocol achieved partial remission after ACNU treatment. Furthermore, 1 dog with lymphoma demonstrated (in less than 21 days) a decrease in tumor size

with ACNU treatment (25 mg/m^2), an animal that had previously shown progressive disease after previous CCNU treatments (64.5 mg/m^2). This disparity may be a result of the difference in antitumor activity between ACNU and CCNU or a consequence of variance in bioavailability between orally administrated CCNU and intravenously administered ACNU.

CCNU products are only available for oral administration in capsules of 10 mg, 40 mg or 100 mg. Therefore, it would be difficult to adjust the dose for each case. It would also be difficult to administer the drug in dogs that experience vomiting, because gastrointestinal lesions may decrease bioavailability. In contrast, since ACNU is a water-soluble drug suitable for IV injection, the abovementioned challenge in CCNU administration is not an issue.

In summary, ACNU appears to be safe and well tolerated when administered via IV injection as a single dose of 25 mg/m^2 to tumor-bearing dogs. The DLT of ACNU treatment was neutropenia. Since the neutrophil and platelet nadirs typically occurred during the first 2 weeks after drug administration, the treatment interval was recommended to be 21 days. To determine the therapeutic efficacy of ACNU in tumor-bearing dogs, a dose of 25 mg/m^2 administered as an IV injection every 3 weeks should be considered for subsequent phase II studies with the aim of treating diseases, such as lymphoma, mast cell tumors and histiocytic sarcomas.

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Appendix Table A1. Grading criteria used to assess adverse hematologic and gastrointestinal events following administration of a single dose of ACNU to dogs (Veterinary co-operative oncology group-common terminology criteria for adverse events v 1.0 [VCOG-CTCAE] [12]

Adverse Event Grade		Criteria
Neutropenia		
	0	≥3,000 neutrophils/*μl*
	1	1,500–2,999 neutrophils/*μl*
	2	1,000–1,499 neutrophils/*μl*
	3	500–999 neutrophils/*μl*
	4	<500 neutrophils/*μl*
Thrombocytopenia		
	0	≥200,000 platelets/*μl*
	1	100,000–199,999 platelets/*μl*
	2	50,000–99,999 platelets/*μl*
	3	15,000–49,999 platelets/*μl*
	4	<15,000 platelets/*μl*
Anorexia		
	0	None
	1	Coaxing or dietary change required to maintain appetite
	2	<3 days duration, no significant weight loss
	3	3–5 days, weight loss, nutritional supplementation needed
	4	>5 days, life-threatening consequences
	5	Death
Vomitting		
	0	None
	1	<3 episodes in 24 hr
	2	3–5 episodes in 24 hr, <3 episodes/day for 2–5 days, SC/IV fluids for <1 day
	3	>5 episodes in 24 hr, vomiting >4 days, IV fluids for >24 hr
	4	Life threatening (eg, hemodynamic collapse)
	5	Death
Diarrhea		
	0	None
	1	Increase of <2 stools/day over baseline
	2	2–6 stools/day over baseline, SC/IV fluids <24 hr
	3	>6 stools/day over baseline, incontinence, IV fluids >24 hr
	4	Life threatening (eg hemodynamic collapse)
	5	Death