Studies on the epidemiology and treatment in canine neoplastic diseases

(犬の腫瘍性疾患における疫学と治療に関する研究)

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Contents

General Introduction

Great efforts has been paid to conquer the neoplastic diseases in veterinary medicine, especially in small animal practice, and a number of therapeutic options, including chemotherapy, radiation and surgical techniques, became available against many types of tumors [13]. However, we have not completely overcome neoplastic diseases in treatment strategy and it is still to be a major problem in the field of small animal medicine. Previous studies reported that neoplastic disease is one of the most common causes of death in dogs [2, 21].

Many types of tumors are observed in dogs. Biological behaviors, in terms benign and malignant behaviors, are related to the type of tumor in some degree and different in each tumor [8]. In addition, different clinical features and prognosis may be shown even though a same pathological diagnosis was given [8]. This might be explained by clinical staging or pathological grading in a tumor. Therefore, it is essential to understand the clinical characteristics of tumors for the planning of therapeutic strategy and estimating a prognosis.

The concept of canine histiocytic disorders was first described in the late 1970s and consist of several types of histiocytic diseases such as reactive histiocytosis, systemic

histiocytosis, histiocytoma, histiocytic sarcoma (HS) originating from intestinal dendritic cells or macrophages [16]. Canine reactive histiocytosis, systemic histiocytosis (SH) and cutaneous histiocytosis, are complex inflammatory diseases derived from underlying immune dysregulation. Canine reactive histiocytosis is found as a local skin lesion (cutaneous histiocytosis) or a multiple organ involvement (systemic histiocytosis). Canine cutaneous histiocytoma is a benign epitheliotropic neoplasm composed of intraepidermal dendritic antigen-presenting cells (APC) also called as Langerhans cells. Most of canine cutaneous histiocytomas show spontaneous disappearance. HS is a highly aggressive neoplasm and the prognosis of HS-bearing dogs is shown to be poorer than those in other canine histiocytic disorders [3, 16]. HS is found as a local lesion at first, and then finally forms systemic lesions including livers, spleen, lungs and bone marrow [3]. The HS is also known as a highly breed-specific disorder. Bernese Mountain Dog, Rottweilers and Flat Coated Retrievers in Europe and the United States are reported to show a predilection for the development of HS [1, 3]. However, epidemiological information concerning HS in Japanese dogs is not currently available. The population of dog breeds would be quite different between western countries and

Japan. Therefore, it would beneficial for Japanese dogs to understand incidence and breed predilection of HS by comparing with those in dogs in Europe and the United States.

Treatment of the HS is aimed at both local and systemic control. Surgical resection of the lesions should be considered in order to obtain local control of the tumor. However, it has been reported that dogs treated with surgery alone succumbed to disease within 6 months from diagnosis [23]. Although information about clinical characteristics of HS and clinical efficacy of systemic treatment against HS in dogs is limited, the chemotherapy with lomustine (CCNU), one of alkylating compounds of nitrosourea, has been used to treat HS, and CCNU is the only drug effective against HS has been scientifically confirmed [23]. However, CCNU is not commercially available in Japan. A phase II study in humans revealed that nimustine (ACNU), a similar alkylating compound which was developed in Japan, was effective against lung cancer, brain tumors, alimentary tract cancer and tumors of hematopoietic organs. In addition, a usefulness of ACNU against brain tumors is shown because this drug passes through the blood brain barrier to exert its effect [23, 33]. Furthermore, ACNU is also used to

melanoma as an alternative drug of CCNU or carmustine (BCNU) in combination chemotherapeutic protocols [29]. However, to date, there are no published reports on the dose limiting toxicity (DLT) and maximum tolerated dose (MTD) of ACNU in dogs.

In a series of this study, I focused on HS in dogs, and carried out investigations to provide novel insights for understanding clinical characteristics of HS and creating a new therapeutic strategy for its control. The present study is comprised of two chapters. In Chapter I, I investigated clinical epidemiology of HS in Japanese dogs especially focusing on the clinical feature, outcome and prognostic factors. In Chapter II, I determined the DLT and MTD for a single administration of ACNU in tumor-bearing dogs through dose-escalation study in order to establish a novel therapeutic protocol using ACNU against HS bearing dogs.

Chapter I

Clinical Characteristics and Prognostic Factors in Dogs with Histiocytic Sarcoma in Japan

Abstract

Canine histiocytic sarcoma (HS) is a rare neoplasm that originates from dendritic cells or macrophages, and there have been a number of cases experienced in Japan. To identify the characteristics and prognostic variables that determine outcome in dogs with HS in Japan, medical records of 73 dogs with HS were retrospectively analyzed. Signalment, clinical signs, complete blood count (CBC), blood chemistry profiles, treatment, response to treatment and overall survival (OS) were analyzed. Diagnosis of HS was determined histologically in 44 cases and cytologically in 29 cases. The most frequently diagnosed breeds were Flat-Coated Retrievers (*n* = 16, odds ratio [OR] 62.0), Pembroke Welsh corgis (*n* = 15, OR 9.7) and Bernese Mountain dogs (*n* = 14, OR 45.0). Median survival time for all dogs in this study was 43 days. In the dogs that received no treatment or only symptomatic treatment, the median OS was 12 days (range 2–254 days) compared with that of dogs that received surgical treatment and/or chemotherapy (85 days, range 4–360 days). Univariate analysis identified anemia, thrombocytopenia, hypoalbuminemia, hypoproteinemia and not receiving antitumor treatment (chemotherapy and/or surgery) as factors significantly associated with shorter OS.

Multivariate analysis confirmed that platelet counts, localized/disseminated lesional pattern and whether the dog received antitumor treatment were significantly predictive of survival.

Introduction

Reactive and neoplastic histiocytic disorders have been described in dogs. These include reactive histiocytosis, systemic histiocytosis, histiocytoma, localized histiocytic sarcoma (HS), and disseminated HS. Canine HS is a rare round cell neoplasm originating from dendritic cells or macrophages [3, 15] and localized HS and disseminated HS are malignant histiocytic tumors. In addition, hemophagocytic HS was recently described as a different subtype of HS that arises from macrophages and has an aggressive clinical course [15].

Localized HS is reported to occur most commonly in the bone, joints, skin, and subcutaneous tissues [27]. The majority of dogs with localized HS eventually develop distant metastases to the spleen, liver, lymph nodes, bone marrow and lung, even if the primary tumor is treated with localized therapies (surgery and/or radiation) [23, 28]. Therefore, chemotherapy is usually administered for both localized and disseminated HS. However, studies evaluating responses to chemotherapy in canine HS are limited. Although there have been several reports on responses to chemotherapy using

doxorubicin [30], liposomal doxorubicin [30], paclitaxel [20] and CCNU [23, 27, 28], survival time of dogs with HS has been short so far despite the use of aggressive treatments. In addition, no study has compared the prognosis in canine HS between dogs that received antitumor treatments with surgery and/or chemotherapy and those that did not receive such antitumor treatment. Knowledge of the prognostic factors in canine HS also remains limited to date, although one study has reported that anemia, thrombocytopenia, hypoalbuminemia and splenic involvement were associated with a worse prognosis in dogs with HS [27].

The Bernese Mountain dog, Rottweiler, Dobermann, Golden Retriever, Labrador Retriever and Flat-Coated Retriever exhibit a higher prevalence of HS than other breeds [3, 4, 26]. In addition, it has been shown that systemic histiocytosis and HS are inherited in the Bernese Mountain dog with a hereditary coefficient of 0.298 [19]. One study of subdural HS in Japan reported that Pembroke Welsh corgi was the most frequently diagnosed breed [11]. However, epidemiological studies on the incidence of canine HS in Japan are limited. The objective of the present study was to examine the clinical characteristics, outcome and prognostic factors of canine HS in Japan.

Materials and methods

Animals: Medical records of dogs that were referred to the Veterinary Medical Center at the University of Tokyo (UT-VMC) from April 2007 to July 2012 and diagnosed with HS by cytology or histopathology were reviewed. All histopathological examinations were performed by one pathologist (K. UCHIDA). Cases were diagnosed with HS based on the morphological features described in a report by Affolter and Moore [3]. Immunohistochemical staining was performed when possible for confirmation of the diagnosis. Antibodies against human leukocyte antigen (HLA)-DR alpha-chain were used in 5 dogs (mouse anti-human monoclonal antibody; Dako Japan, Tokyo, Japan), ionized calcium-binding adaptor molecule 1 (Iba1) in 3 dogs (rabbit anti-human polyclonal; Wako., Osaka, Japan), CD3 in 2 dogs (rabbit anti-human polyclonal., Dako Japan), CD204 in 1 dog (mouse anti-human monoclonal antibody; TransgGenic. Inc., Kumamoto, Japan) and CD20 in 1 dog (rabbit anti-human polyclonal., NeoMarkers, Fremont, CA, U.S.A.). Cytochemical staining for alpha-naphthyl butyrate esterase (α-NBE) and inhibition of the enzyme by sodium fluoride were also performed when

possible (in 7 dogs) as a marker of monocyte/macrophage lineage.

Clinical information: Information extracted from medical records included signalment, clinical signs, complete blood cell counts (CBCs), blood chemistry profile, diagnostic methods, treatments, response rates and survival times. Adverse events during chemotherapy were evaluated using the Veterinary Cooperative Oncology Group - Common Terminology Criteria for Adverse Events v1.0 (VCOG-CTCAE) [32]. Dogs with measurable lesions were evaluated for response using Response Evaluation Criteria in Solid Tumors [18]. Responses to chemotherapy were categorized as follows: complete response (CR); complete disappearance of all measurable disease, partial response (PR); >30% but <100% reduction in the sum of the longest diameters of measurable tumors, stable disease (SD); <30% reduction or <20% increase in the sum of the longest diameters of measurable tumors without the appearance of new neoplastic lesions, and progressive disease (PD); an increase of >20% in the sum of the longest diameters of measurable tumors or the appearance of new neoplastic lesions [18]. Overall survival (OS) was defined as the duration from the date of diagnosis to the date of death from any cause and response duration was defined as the duration from the

documentation of response (CR or PR) to the date of relapse or progression. OS and response duration for dogs lost to follow-up were censored at the date they were last known to be alive.

Statistical analysis: Survival probabilities were estimated using the Kaplan–Meier product limit method. In the examination of prognostic factors, log-rank tests were used to determine whether each factor as assessed at diagnosis influenced survival. In addition, a forced entry Cox proportional hazards model was developed to assess the independent contributions of various prognostic factors. Several prognostic factors yielding a p value of less than 0.1 were included in the hazards model. A value of $p < 0.05$ was considered to be significant in all statistical tests. Data were analyzed using commercially available statistics software (JMP, version 4, The Statistical Discovery Software, SAS Campus Drive, Cary, NC, U.S.A.).

Results

Animals: Medical records of 73 dogs with HS were reviewed in this study. The mean age was 9.6 years (range, 1.9–15.2 years), and the mean body weight was 23.5 kg (range 2.6–55 kg). Twenty-two dogs were intact males, 14 were castrated males, 13 were intact females, and 24 were spayed females. There were 16 Flat-Coated Retriever, 15 Pembroke Welsh corgis, 14 Bernese Mountain dogs, 8 Golden Retrievers, 6 Labrador Retrievers, 3 Shih Tzu, 2 Shetland sheepdogs and 1 each of Beagle, Pointer, French Bulldog, Rottweiler, Maltese, Yorkshire Terrier, Miniature Dachshund, Toy Poodle and mixed breed dogs. The odds ratios (ORs) of those dogs against all dogs admitted to UT-VMC for the same period were 62.0 (95% confidence limits [CL], 37.6–102.3) for Flat-Coated Retrievers, 9.7 (95% CL 5.6–17.0) for Pembroke Welsh corgis, 45.0 (95% CL 26.3–77.2) for Bernese Mountain dogs, 5.0 (95% CL 2.4–10.3) for Golden Retrievers and 3.0 (95% CL 1.3–7.0) for Labrador Retrievers (Table1-1).

Diagnoses were obtained histologically in 44 cases and cytologically in 29 cases. In cytological examinations, the majority of cells had abundant and lightly basophilic

cytoplasm, and some had multiple small cytoplasmic vacuoles. These cells had pleomorphic nuclei with vesicular chromatin and multiple nucleoli. Multinucleated giant cells, atypical mitotic figures and phagocytosis were commonly observed, although the frequencies of those observations varied among the cases. Histopathologically, the features of HS were also characterized by the irregular proliferation of pleomorphic histiocytic cells and multinucleated giant cells combined with various inflammatory reactions. In 7 dogs where cytochemical analysis was performed, the tumor cells were α-NBE-positive, and the positive staining was inhibited by the addition of sodium fluoride, indicating the tumor cells originated from the monocyte/macrophage lineage. In the immunochistochemical staining performed in 6 dogs, the tumor cells were positive for HLA-DR, Iba-1 and/or CD204. Forty-one dogs were diagnosed with localized HS, and 32 were diagnosed with disseminated HS.

Clinical characteristics: Frequent clinical signs at presentation were anorexia (34%, *n* = 25), lameness (29%, *n* = 21), lethargy (23%, *n* = 17), cough (15%, *n* = 11), presence of one or more palpable masses (11%, *n* = 8), diarrhea (8%, *n* = 6), dyspnea (5%, *n* = 4), and vomiting (5%, *n* = 4). CBC and blood biochemistry profile were examined in 73

dogs. Clinicopathologic abnormalities found at the first presentation included anemia (HCT < 30%, 18/73), thrombocytopenia (PLT < 100,000/μ*l,* 18/73), hypoalbuminemia (Alb < 2.6 g/d*l*, 18/49), azotemia (BUN > 29.2 mg/d*l*, 18/72), hypercreatininemia (CRE > 1.4 mg/d*l*, 5/72), hyperbilirubinemia (T-Bil > 0.5 mg/d*l*, 18/19), increased liver enzymes (ALT > 78 U/*l,* 30/72), increased C-reactive protein (CRP > 1.0 mg/d*l,* 54/67) and increased fibrin/fibrinogen degradation products (FDP) (FDP > 5.0 μg/m*l*, 7/33). Radiographic and ultrasonographic examinations were performed in 68 and 44 dogs, respectively, before treatment. In addition, computed-tomography (CT) and magnetic resonance imaging (MRI) were performed in 26 and 5 dogs, respectively.

The distribution of the tumor lesions examined in this study is summarized in Table 1-2. Nineteen of the 73 (26%) dogs had lesions in the spleen, 16 (22%) in the lung, 14 (19%) in lymph nodes, 14 (19%) in bones and/or joints, 13 (18%) in skin/soft tissue, 5 (7%) in the liver, 4 (5%) in the central nervous system, 4 (5%) in the kidney, 3 (4%) in the mediastinum, 3 (4%) in the gastrointestinal tract and 1 (1%) in the oral cavity. In addition, frequently affected organs were examined in each of the breeds including the Flat-Coated Retriever, Pembroke Welsh corgi and Bernese Mountain dog. Of 16

Flat-Coated Retriever, 9 (56%) had localized HS and 7 (44%) had disseminated HS, and the frequently affected organs in this breed were the skin/soft tissue (7 dogs, 44%), lymph nodes (6 dogs, 38%) and lung (5 dogs, 31%). Of 15 Pembroke Welsh corgis, 13 (86%) had localized HS and 2 (14%) had disseminated HS, and the frequently affected organs in this breed were the lung (8 dogs, 53%) and spleen (3 dogs, 20%). Of 14 Bernese Mountain dogs, 9 (64%) had localized HS and 5 (36%) had disseminated HS, and the frequently affected organs in this breed were the spleen (6 dogs, 43%) and bone/joint (4 dogs, 29%). Of the 73 dogs in this study, 11 were considered to have a hemophagocytic subtype based on hematologic and clinicopathologic abnormalities. However, it was difficult to diagnose definitively, because immunohistochemical staining (MHC class II and the leuko-integrin CD11d/CD18, etc.) using fresh or frozen samples would have been required for a confirmed diagnosis of this subtype.

Treatments: Treatment information was available for 69 of the 73 dogs in the present study. Twenty-two (30%) received no treatment or only symptomatic treatment after diagnosis, and 1 dog was euthanized before treatment. Surgical treatment was performed in 16 dogs (21%), of which 10 received adjuvant chemotherapy. Thirty

(41%) received only chemotherapy. Chemotherapy protocols using CCNU (lomustine) were performed as a single agent (23 dogs), or in combination with ACNU (nimustine; 2 dogs) or doxorubicin (1 dog). ACNU was used as a single agent (12 dogs) or in combination with L-asparaginase (2 dogs). The mean dosage of CCNU was 63.7 mg/m^2 (range, 23.2–93.0 mg/m^2), and the median number of administrations was 2.5 (range, 1–10). Among the 26 dogs that received CCNU, 9 experienced neutropenia (grade 1, 2 dogs; grade 2, 1 dog; grade 3, 2 dogs; grade 4, 4 dogs), 2 experienced thrombocytopenia (grade 1, 1 dog; grade 4, 1 dog), 2 experienced vomiting (grade 1, 2 dogs), 8 showed elevation of liver enzyme activity (alanine aminotransferase, ALT) (grade 2, 1 dog; grade 3, 5 dogs; grade 4, 2 dogs), and one exhibited grade 1 diarrhea, as adverse events after CCNU treatment. The mean dosage of ACNU was 30 mg/m^2 (range 25–40 mg/m^2), and the median number of administrations was 1.5 (range, 1–8). Of the 16 dogs that received ACNU, 3 experienced neutropenia (grade 1, 1 dog; grade 4, 2 dogs), one exhibited grade 2 vomiting, and 2 showed elevation of ALT activity (grade 1, 1 dog; grade 3, 1 dog), as adverse events after ACNU administration. There was no significant relationship between the kind of chemotherapeutic agent administered and the

occurrence of adverse events.

Among the dogs that received treatment with chemotherapy alone, response to treatment could be objectively measured in 17 dogs. In the 11 dogs that received CCNU as a single agent, the response rate was 55% (6/11) (CR, 1; PR, 5), and the median response duration was 111 days (range, 35–291 days). In the 6 dogs that received ACNU as a single agent, the response rate was 50% (3/6) (CR, 1; PR, 2), and the median response duration was 48 days (range, 29–99 days). There was no response recorded after treatment with L-asparaginase or doxorubicin.

Outcomes: Of the 73 dogs analyzed in the present study, 12 were censored, because of loss to follow up. The median OS for all dogs in the study was 43 days (range, 2–468 days). In the dogs that had no treatment or only symptomatic treatment, the median OS was 12 days (range, 2–254 days) compared with that of dogs who received surgical treatment and/or chemotherapy (85 days, range, 4–360 days) (Fig. 1-1). For the dogs that underwent surgery only (n = 6), chemotherapy only (n = 30), and both surgery and chemotherapy (n = 10), median OS was 91, 76 and 62.5 days, respectively. Univariate analysis identified anemia, thrombocytopenia, hypoalbuminemia, hypoproteinemia and

not receiving antitumor treatment (chemotherapy and/or surgery) as factors significantly associated with shorter survival times (Table 1-3). Median OS of anemic dogs (PCV < 30%) was 24 days compared with 61 days in dogs without anemia (p = 0.0097), and median OS of dogs with thrombocytopenia (platelets < 100,000 /μ*l*) was 10 days, compared with 66 days without thrombocytopenia (p = 0.0005). Median OS of dogs with hypoalbuminemia (Alb < 2.6 g/d*l*) was 18 days compared with 64 days in dogs without hypoalbuminemia (p = 0.0302), and median OS of dogs with hypoproteinemia (TP < 5.0 g/d*l*) was 18 days compared with 64 days in dogs without hypoproteinemia (p = 0.0007). The significant prognostic factors were then determined from combinations of the five factors; thrombocytopenia, disseminated distribution of lesion, no antitumor treatment, hypoalbuminemia and anemia, using Cox's proportional hazards modeling. The results in this study suggest that a combination of thrombocytopenia (OR, 5.7), no antitumor treatment (OR, 3.5) and existence of disseminated lesion (OR, 2.0) may be the most appropriate for prognostication (Table 1-4). There were 11 dogs that were conceivably affected with hemophagocytic HS (OS 18 days, range 1–64 days), and their prognoses tended to be poorer than those of the other dogs, even though 6 of the 11

received chemotherapy or surgery.

Discussion

In the present study, clinical characteristics of dogs with HS in Japan were investigated. The signalments of the dogs included were generally similar to those reported in previous studies of canine HS [23, 27, 28]. However, the present study revealed that the Pembroke Welsh corgi was a breed at comparatively high risk of HS in Japan. A previous study including 15 cases of subdural HS in Japan [11] reported that the breed most frequently diagnosed with subdural HS was the Pembroke Welsh corgi (7 of the 15 dogs). It may be necessary to recognize the Pembroke Welsh corgi as a breed at comparatively high risk of developing HS, not only subdural but also other types of HS, in Japan. Although the mode of inheritance of canine HS is not well understood and the genes likely to be involved are unknown, it has been proposed that systemic histiocytosis and disseminated HS (previously called malignant histiocytosis) have familial aspects, and it has been suggested that these diseases may have a genetic basis in the Bernese Mountain dog [4, 19]. Genetic predisposition may also be involved in Pembroke Welsh corgi in Japan.

The distribution of HS in the present study most commonly included the spleen, lung, lymph node, bone/joint skin and/or soft tissues. These results are similar to those reported by Skorupuski *et al.* [27]. Some studies have reported that distribution of the lesions tended to be different between the breed and generally localized in Flat-Coated Retrievers and Golden Retrievers, which was in contrast to Bernese Mountain dogs and Rottweilers, in which HS was invariably disseminated [3, 7, 9]. In the present study, Pembroke Welsh corgis were generally affected with localized HS, while Bernese Mountain dogs and Flat-Coated Retriever did not exhibit a clear tendency with regard to the distribution of lesions.

CCNU was reported to be effective as a chemotherapeutic agent for dogs with HS, and it is the only drug with proven efficacy against HS [27, 28]. One retrospective study reported that 46% of dogs with HS that were treated with CCNU responded to the agent, and the median remission duration was 85 days. In the present study, a similar response rate (55%) was obtained in dogs that received CCNU as a single agent. In addition, clinical responses were obtained in 50% of dogs treated with ACNU as a single agent in the present study. There is currently no literature on the clinical use of ACNU and

associated adverse events in veterinary medicine. In the present study, among severe adverse events, grade 4 hematological toxicity was confirmed in only 2 of the dogs (13%) that received ACNU administration, compared to 5 of the dogs (19%) that received CCNU. Hepatotoxicity shown as elevation of ALT was observed after administration of ACNU and CCNU; however, its frequency tended to be lower after ACNU administration. In contrast to CCNU, which is currently available as capsules for oral administration, ACNU can be intravenously injected. Thus, ACNU is easy to administer in cases with vomiting or with gastrointestinal lesions, and doses can be reduced for dogs that experience adverse events. Because of the retrospective nature of the present study, doses and intervals between treatments with ACNU were variable, and adverse events were not fully evaluated; however, it may be worthwhile evaluating dose limiting toxicity, maximum tolerated dose and the efficacy of ACNU administration in the treatment of canine HS.

Of the prognostic factors, hypoalbuminemia, anemia, thrombocytopenia and not receiving surgery and/or chemotherapy were significantly associated with a poor prognosis. These results are similar to those of a previous report on canine HS that

investigated treatment with CCNU [27], with the exception of not receiving surgery and/or chemotherapy. Hypoalbuminemia, anemia and thrombocytopenia as negative prognostic factors might be associated with hemophagocytic subtype. In a previous study, hemophagocytic HS arising from macrophages was reportedly marked by an aggressive clinical course dominated by splenomegaly, regenerative anemia, thrombocytopenia, hypoalbuminemia and hypocholesterolemia [15]. It was reported that hypoalbuminemia was significantly more severe in the dogs with hemophagocytic HS than in those with nonhemophagocytic HS [15]. Another possibility reported was that hypoalbuminemia might indicate severe inflammation due to tumor invasion. Thrombocytopenia may also result from reduced production due to tumor invasion into bone marrow, increased platelet consumption due to hypercoagulability and immunologic platelet destruction. However, the association of these abnormalities with thrombocytopenia could not be examined in the present study, because the number of dogs where bone marrow aspiration and blood coagulation tests (prothrombin time, activated partial thromboplastin time and FDP) were performed at first presentation was small. Only 4 of 33 dogs that had coagulation tests were diagnosed as suspected-DIC

(Disseminated Intravascular Coagulation) based on diagnostic criteria described by Carr AP *et al.* [5] and bone marrow aspiration was performed in 1 dog. Multivariate analysis confirmed that platelet counts, localized/disseminated lesional pattern, and receiving antitumor treatment were independent prognostic factors. The results obtained in the present study suggested that complete staging is needed before treatment, to predict prognosis. In addition, it was also indicated that prolonged survival could be expected in dogs with HS that were treated with surgery and/or chemotherapy as compared with only symptomatic treatment or no treatment. However, receiving antitumor treatment was a prognostic factor that was independent of other prognostic factors, and there may be an inherent bias in the selection of treatment based on the degree of clinical symptoms. Short survival time (11 days) in the dogs with no treatment or only symptomatic treatment in this study, as reported in a previous study on Flat-Coated Retrievers [9], confirmed that canine HS is rapidly progressive with a grave prognosis. However, the small numbers of subjects enrolled in each treatment (chemotherapy alone, surgery alone, chemotherapy and surgery) made it impossible to confirm which was the best treatment for dogs with HS.

One limitation of the present study was the lack of immunohistochemical staining using anti-CD18 and CD11d antibodies for confirmation of the diagnosis of HS. However, in cases where it was difficult to diagnose HS cytologically or histopathologically, cytochemical analysis for alpha-naphthyl butyrate esterase staining and inhibition of this enzyme by sodium fluoride or immunohistochemistry were assessed to acquire support for a diagnosis of HS, and all were deemed consistent with HS.

In conclusion, HS is an aggressive disease and the survival times of dogs with HS might be very short if only symptomatic treatment or no treatment is performed. In Japan, it is necessary to recognize that the Pembroke Welsh corgi is a breed at comparatively high risk of HS. Anemia, hypoalbuminemia and thrombocytopenia are negative prognostic indicators as has been previously reported [27]. In addition, the present study revealed that receiving treatment including surgery and/or chemotherapy (CCUN or ACNU) improved the prognosis of dogs with HS as compared with dogs that received only symptomatic treatment or no treatment.

Chapter II

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

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, and 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 provides a basis for the subsequent phase II trial of ACNU in dogs.

Introduction

Nitrosourea 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 [14, 22, 24, 27, 28]. Nimustine ((3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl) 1-nitrosourea hydrochloride, 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 [17]. A phase II study in humans revealed that ACNU was effective against lung cancer, brain tumors, alimentary tract cancer and tumors of hematopoietic organs [25]. 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 [33]. 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 [6].

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 [14, 22, 24, 27, 28]. 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 [10, 12, 14, 22]. Bone marrow toxicity, such as neutropenia and thrombocytopenia, and vascular pain during BCNU treatment were reported in lymphoma-bearing dogs receiving BCNU treatment [24]. 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 my 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 MTD and DLT for a single administration of ACNU in tumor-bearing dogs.

Materials and methods

Client-owned dogs bearing histologically or cytologically confirmed tumors that had been referred to the Veterinary Medical Center of the University of Tokyo (UT-VNC) 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 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 my 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 (Table 2-1) [32]. 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.* [31]. The initial ACNU dose was set at 25 mg/m^2 based on the preliminary results of my 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 [31].

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 [31]. All assessments were performed over 21 days or more. A decrease in tumor size for a short duration was reported as stable disease.

Results

Eight dogs enrolled in this study were referred to UT-VMC 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 2-2, 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/μ*l* and 5,500 cells/μ*l*, respectively (range, 0–18,700 cells/μ*l*). 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/μ*l* and 229,000 cells/μ*l*, respectively (range, 51,000–586,000 cells/μ*l*). 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 2-3. 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 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).

Discussion

ACNU is a nitrosourea compound commercially available in Japan. Therefore, evaluating its optimal dosage in dogs would be beneficial. According to the results of this 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 [22]. 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 [24]. 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 [14]. 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/μ*l*) 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 [17]. In this study, I was 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.

Conclusion

Malignant tumor is important disease in the field of clinical veterinary medicine [2, 21]. Some of them significantly shorten the survival time of affected dogs because of the lack of effective therapeutic strategy [31]. HS in dogs is one of the highly aggressive neoplasms and its biological behavior is quite malignant [16]. HS-bearing dogs show poor prognosis and this is also due to the lack of specific remedy [16]. In this series of study, I focused on canine HS and investigated its biological characteristics. In addition, I tried to establish a novel therapeutic strategy against HS.

In Chapter I, the clinical characteristics, outcome and prognostic factors of canine HS in Japan were evaluated. This study revealed that the Pembroke Welsh corgi should be recognized as a breed at comparatively high risk of HS in Japan. This finding is very important for small animal clinicians to confront the disease. Although this breed has not been recognized as a breed with predilection, a molecular analysis might uncover genetic backgrounds for the development of HS and breed predisposition like Bernese Mountain Dog [4, 19]. Anemia, hypoalbuminemia and thrombocytopenia are negative prognostic factors as previously reported [27]. In addition, the present study revealed that the treatment with surgery and/or chemotherapy (CCNU or ACNU) improved the

prognosis of dogs with HS as compared with dogs that received only symptomatic treatment or no treatment. Because CCNU is not available in Japan, it may be worthwhile to evaluate efficacy of ACNU for the treatment of canine HS through prospective study. However, there are no reports defining the appropriate dosage of ACNU in dogs.

Based on the findings of Chapter II, I evaluated and determined the MTD and DLT for a single dose of ACNU in tumor-bearing dogs through dose escalation clinical trial. This study clarified that ACNU can be administered safely to tumor-bearing dogs at a dose of 25 mg/m^2 intravenously every 3-week. Neutropenia was the DLT and gastrointestinal toxicity was mild. Subsequent phase II studies would be necessary to evaluate antitumor activity against HS and cumulative toxicity of ACNU in dogs.

I believe that findings obtained in this series of study contribute to veterinary oncology and provide useful information in the treatment for canine neoplastic diseases, including HS.

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Tables

Table 1-1. The dog breeds most commonly diagnosed with HS in this study and the associated Odds ratio values.

Breed	Number of dogs	Odds ratio	95% CL
Flat-Coated Retriever	16	62.0	37.6–102.3
Pembroke Welsh corgi	15	9.7	5.6–17.0
Bernese Mountain dog	14	45.0	26.3–77.2
Golden Retriever	8	5.0	2.4–10.3
Labrador Retriever	6	3.0	1.3–7.0
Others	14		

CL: Confidence Limits

Table 1-2. Distribution of documented tumor lesions (localized HS and disseminated HS)

Organ affected	Number of dogs	
	Localized HS	Disseminated HS
Spleen	8	11
Lung	6	10
Lymph node（as primary site）	5 (1)	9 (0)
Bone/joint	11	3
Skin and soft tissues	9	4
Liver	1	4
Central nervous system	2	2
Kidney	0	4
Others	3	4

Table 1-3. Association of variables with survival after a logrank test (significance, $p < 0.05$)

Variable	Risk factor (upside)	Number of dogs	Median survival days	p value
Age	> 8 years old	20	56	0.5783
(n = 73)	≤ 8 years old	53	47	
Sex	Male	36	56	0.7307
(n = 73)	Female	37	47	
Hematocrit	HCT < 30%	17	24	0.0097
(n = 73)	HCT ≥ 30 %	56	61	
WBC	WBC > 17,000 /μl	32	18	0.0576
(n = 73)	WBC ≤ 17,000 /μl	41	70	
PLT	PLT < 100,000 /μl	18	10	0.0005
(n = 72)	PLT ≥ 100,000 /μl	54	66	
TP	TP > 5.0 g/dl	10	18	0.0007
(n = 72)	TP ≤ 5.0 g/dl	62	64	
Alb	Alb < 2.6 g/dl	18	18	0.0302
(n = 49)	Alb ≥ 2.6 g/dl	31	64	
BUN	BUN > 29.2 mg/dl	18	16	0.5744
(n = 72)	BUN ≤ 29.2 mg/dl	54	61	
CRE	CRE > 1.4 mg/dl	5	24	0.5773
(n = 72)	CRE ≤ 1.4 mg/dl	67	56	
ALT	ALT > 78 mg/dl	29	46	0.7284
(n = 72)	ALT ≤ 78 mg/dl	43	56	
CRP	CRP > 1.0 mg/dl	53	33	0.2164
(n = 66)	CRP ≤ 1.0 mg/dl	13	61	
FDP	FDP > 5 mg/ml	7	43	0.4917
(n = 33)	FDP ≤ 5 mg/ml	26	33	
Distribution	Localized HS	41	56	0.0692
(n = 73)	Disseminated HS	32	43	
Treatment	No treatment or symptomatic treatment	23	11	< 0.0001
(n = 69)	Chemotherapy and/or surgery	46	85	

WBC, White Blood Cell; PLT, platelet count; TP, total plasma protein; Alb, albumin; BUN, blood urea nitrogen:CRE, Creatinine; ALT, Alanine aminotransferase ; CRP, C-reactive proteinm; FDP, fibrin/fibrinogen degradation products;

Table 1-4.Variables included in the model of survival produced by multivariate Cox proportional hazard analysis.

	HR[a)]	95% CL[b)]	*p* value
Thrombocytopenia	5.7	2.3–14.8	0.0002
Disseminated HS	2.0	1.0–4.0	0.0456
Hypoalbuminemia	1.4	0.7–2.9	0.3151
Anemia	0.8	0.4–1.9	0.6729
No antitumor treatment	3.5	1.6–7.4	0.0015

a) HR; Hazzard Ratio

b) CL; Confidence limits

Table 2-1. 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]

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 hours
	2	3-5 episodes in 24 hours, <3 episodes/day for 2-5 days, SC/IV fluids for <1 day
	3	>5 episodes in 24 hours, vomiting >4 days, IV fluids for >24 hours
	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 hours
	3	>6 stools/day over baseline, incontinence, IV fluids >24 hours
	4	Life threatening (eg hemodynamic collapse)
	5	Death

Table 2-2. 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 Table 2-1 for grading criteria

Table 2-3. 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 Table 2-1 for grading criteria

Legend for Figure

Fig. 1-1. Kaplan–Meier curve showing the difference in survival between the dogs that received antitumor treatments (chemotherapy and/or surgery) and the dogs that received only symptomatic treatment or no treatment.

Figure

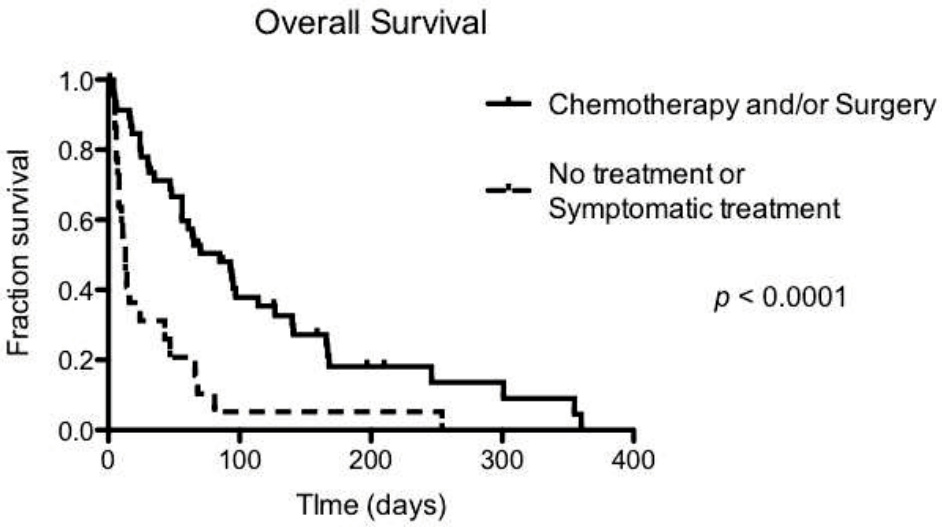
Overall Survival
Fraction survival
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
TIme (days)
Chemotherapy and/or Surgery
No treatment or
Symptomatic treatment
p < 0.0001

Fig. 1-1