J Vet Intern Med 2006;20:1389–1397

Response of Canine Cutaneous Epitheliotropic Lymphoma to Lomustine (CCNU): A Retrospective Study of 46 Cases (1999–2004)

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Background: Epitheliotropic lymphoma (ELSA) is an uncommon cutaneous canine malignancy of T lymphocytes. A consensus regarding the therapeutic standard of care is lacking, warranting evaluation of chemotherapeutic agents traditionally employed against canine nodal lymphoma in the treatment of ELSA.

Hypothesis: The purpose of this retrospective, multi-institutional study was to evaluate the efficacy of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in the treatment of ELSA.

Animals: Forty-six dogs with adequate follow-up and treatment response information.

Methods: All cases were diagnosed histopathologically. Immunohistochemisty (CD3, CD79a) was performed on 42/46 samples.

Results: Presenting skin lesions included generalized scales (25/46), plaques or nodules (22/46), mucocutaneous lesions (14/46), and corneal involvement (1/46). Lymph node involvement and Sézary syndrome were documented in 7 and 2 dogs, respectively. The median number of CCNU treatments was 4 (range, 1–11), with a median starting dose of 60 mg/m^2 (range, 30–95). Of the 46 dogs, 15 achieved complete remission, 23 achieved partial remission, 5 had stable disease, and 3 had progressive disease, for an overall response rate of 83%. The median number of treatments to achieve a response was 1 (range, 1–6). The overall median duration of response was 94 days (range, 22–282). Sixteen dose reductions were required because of neutropenia (10/46), thrombocytopenia (1/46), anemia (1/46), increased liver enzyme activity (3/46), or unspecified reasons (1/46).

Conclusions and Clinical Implications: Given the high response rate and well tolerated protocol, prospective studies are warranted to investigate the utility of CCNU alone or in multi-agent protocols for the treatment of ELSA.

Key words: Alkylator; Chemotherapy; Epitheliotropic lymphoma; Mycosis fungoides; Nitrosourea; Skin.

Epitheliotropic lymphoma (ELSA) is an uncommon clinical entity, but it is the most common variant of the cutaneous T-cell lymphomas and generally affects older dogs. No specific etiology or breed predisposition has been reported.[1] Canine cutaneous T-cell lymphoma (CTCL) has been shown to express T-cell receptor (TCR) homology with the human CTCL TCR, suggesting an evolutionarily conserved TCR rearrangement. As a result, ELSA often is referred to interchangeably with the terminology ''mycosis fungoides'' (MF) used in humans.[2] The basis for this misnomer stems from the similarity in lesion distribution, histopathology, and clinical course of both MF and ELSA. However, ELSA more accurately describes a spectrum of cutaneous diseases in dogs including pagetoid reticulosis, mycosis fungoides, and the disseminated T-cell leukemia known as Sézary syndrome.[3] Although canine ELSA appears to be a clinical model for MF, there are 2 striking differences that distinguish ELSA from its human counterpart. First, lymphocyte epitheliotropism is present throughout the spectrum of disease stages encompassed by canine ELSA but is absent in advanced stages of human MF.[2] Furthermore, these 2 diseases are immunophenotypically different. Most canine cutaneous T-cell lymphomas have been shown to consistently express CD3 and CD8 surface markers, contrary to human MF, which typically expresses CD3 and CD4 surface markers.[2] This immunophenotypic difference may simply reflect an operational difference between human and canine skin-associated lymphoid tissue, despite an apparent similarity in the clinical manifestation of these different malignant T-cell populations.[1]

The clinical presentation of canine ELSA is varied and ranges from solitary patches, plaques, or nodules to generalized erythema and scaling with mucocutaneous involvement.[3] At the time of presentation, most animals affected with generalized disease are intensely pruritic. Advanced stage disease with lymphadenopathy or circulating malignant T cells in the peripheral blood (Sézary syndrome) is relatively uncommon.[3] Human MF

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Previously presented in part at the 24th Veterinary Cancer Society Annual Conference in Kansas City, MO, on November 4, 2004.

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Submitted: February 28, 2006; Revised May 1, 2006, May 24, 2006; Accepted June 20, 2006.

0891-6640/06/2006-0016/$3.00/0

patients with >80% of their body surface affected are at increased risk to develop Sézary syndrome, but such an association has not been investigated in dogs.[4] Histopathology is characterized by a neoplastic lymphocyte infiltration exclusively in the epidermis, Pautrier's microabscesses (collections of neoplastic lymphocytes around cutaneous dendritic cells), and a striking tropism for hair follicles and apocrine sweat glands.[3]

Studies of affected humans report a poor prognosis with advanced disease stage, increased tumor burden within a given stage, and the presence of Sézary syndrome.[4] Local cutaneous therapies such as surgical excision, external beam radiotherapy, topical methoxsalen[a] combined with ultraviolet light, nitrogen mustard, and corticosteroids are adequate for lower stage (patch or plaque) or less aggressive disease stages. However, systemic chemotherapy is warranted with advanced stage disease.[4] Several published case reports demonstrate the variability in clinical behavior of ELSA, from an indolent clinical course to a rapidly fatal systemic disease process with reported survival times of 3 to 6 months for dogs with advanced disease.[5–14] Therapeutic approaches have been described in the veterinary literature but a standard of care therapy has not been established for canine ELSA.[5,15–18] Reported response rates to retinoids,[16] cyclosporine,[18] fatty acids,[5] L-asparaginase,[6] dacarbazine,[15] and pegylated doxorubicin[7] generally are <50% and are of short duration. A recent publication evaluating 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in the treatment of ELSA by Williams et al[19] documented an overall response rate of 78%. Consistent with previous reports, the response to CCNU in this study[19] was not durable with a median duration of 106 days. Although limited by its retrospective nature, the Williams et al. study represents the largest report of treatment data for ELSA and suggests that CCNU is a reasonable treatment option for this neoplasm of dogs.

The primary goals of the present study were to characterize a population of dogs with ELSA, to evaluate their response to CCNU, to describe treatment-associated toxicities, and to identify factors predicting the response to treatment or response duration. A secondary aim was to confirm the results of a recent publication[19] in a similar study population.

Materials and Methods

Data Collection

Data sheets were sent to collaborating institutions to collect information regarding signalment, clinical presentation, before and concurrent therapy, response to treatment, duration of response, CCNU (Lomustine[b]) dosage and dose interval, CCNU toxicity, relapse, relapse therapy and response, and cause of death from the medical records or by telephone interviews. Response duration was defined as the time from first administration of CCNU until detection of progressive disease. Hematologic toxicity was graded according to the criteria listed in Table 1.[20] When available, samples from the original tumor were collected for immunohistochemical analysis (immunophenotyping). Criteria for entry in the study included a histologic diagnosis of ELSA, measurable macroscopic disease, adequate follow-up information, and treatment with CCNU.

Table 1. Criteria for grading hematologic toxicities.[20]

Grade	Neutrophil Count (cells/μL)	Platelet Count (cells/μL)
1	1,500–2,449	100,000–LLN[a]
2	1,000–1,499	50,000–99,999
3	500–999	25,000–49,999
4	<500	<25,000

[a] LLN, lower limit of normal.

Statistical Analysis

Continuous variables (age, weight, response duration, drug dosages, duration from diagnosis to CCNU therapy, clinicopathologic findings) were assessed for normality by the Shapiro-Wilks test and visual inspection of the data. Most continuous variables were not normally distributed, and for the sake of consistency median and range were used to describe them. The Wilcoxon rank sum test was used to compare continuous variables among groups. Categorical variables were described using frequencies, proportions, or percentage, and the Fisher's exact test was used to compare these variables among groups. For purposes of comparison, response was assessed in a subjective manner according to the following criteria: complete response (CR) was defined as 100% reduction in measurable tumor burden; partial response (PR) was defined as 50–99% reduction in measurable tumor burden and no new lesion development; stable disease (SD) was defined as <49% reduction or <10% increase in measurable tumor burden and no new lesion development; progressive disease (PD) was defined as >10% increase in measurable tumor burden or detectable new lesions. All statistical analyses were performed using a statistical software package.[c]

Immunohistochemistry

All available tissue samples were reviewed by a single pathologist (JWW). For immunohistochemical analysis, 5-μm paraffin embedded sections were placed on positively-charged slides, air-dried, deparaffinized, and hydrated through a series of alcohols to water. All incubations were performed at room temperature. The slides were covered with a protein blocking reagent[d] for 30 minutes. After a rinse in 0.05 M Tris buffer pH 7.60, the slides were treated with an avidin/biotin blocking solution[e] for 15 minutes. After rinsing with Tris buffer, a second protein blocking step was performed using Super Block[f] for 5 minutes. Excess reagent was blotted off followed by application of the primary antibody. For T and B cell receptors, the primary antibodies recognized CD3[g] (1:1,000, polyclonal) and CD79a[h] (1:500, monoclonal), respectively. Normal canine spleen served as the positive control in the detection of CD3 and CD79a. Negative control slides were incubated with an irrelevant polyclonal antibody.[i] After the 1-hour incubation for CD3 and CD79a, slides were rinsed in Tris buffer and detection was performed with a commercial kit[j] in which a biotinylated anti-rabbit/anti-mouse IgG was applied to each slide for 30 minutes. After a rinse with Tris buffer, each slide was incubated with a streptavidin-alkaline phosphatase for 30 minutes. The final chromagen was developed using a New Fucshin reagent solution according to the package insert,[k] which produced an insoluble red pigment. The slides were washed in water and counterstained with Mayers' hematoxylin, dehydrated in a series of alcohol rinses, and coverslipped before immunohistochemical evaluation.

Results

Signalment

Data were reviewed for 46 dogs with histologically confirmed ELSA diagnosed between May 1999 and

October 2004 at 8 institutions. The study population was composed of mixed breeds (n = 7); Terriers (n = 6); Golden and Labrador Retrievers (n = 4 each); Cocker Spaniels and Shetland Sheepdog (n = 3 each); Bichon Frise, Gordon Setter, Chinese Shar-Pei, Boxer, and Bulldog (n = 2 each); and 1 each of the following breeds: German Shepherd, Siberian Husky, Keeshond, Chihuahua, Pug, Maltese, Alaskan Malamute, Rottweiler, and Pekingese. The male:female ratio was 1:1 with 6 intact and 17 castrated males and 2 intact and 21 spayed females. The median age at diagnosis was 10 years (range, 5.75–19 years) and the median weight was 24.5 kg (range, 3.6–48.8 kg).

Lesion Distribution

The most common lesions reported were generalized scales (n = 25) and generalized plaques or nodules (n = 22). Other clinical presentations included lymphadenopathy (n = 14), mucocutaneous involvement (n = 14), a solitary lesion (n = 8), alopecia (n = 6), and corneal involvement (n = 1). Many dogs were represented in more than 1 lesion category.

Staging

Staging modalities employed varied with respect to the case and clinician and included the following: CBC; serum biochemistry; urinalysis; lymph node aspirate or biopsy and cytology, histopathology, or both; abdominal and thoracic radiography; abdominal ultrasound; and bone marrow aspiration and cytology. All dogs had a histopathologic diagnosis of cutaneous ELSA. Forty-three dogs had a CBC available for analysis before treatment with CCNU. Lymphocytosis and circulating lymphoblasts consistent with Sézary syndrome were noted in 2 dogs after blood smear review by a pathologist. CBC results in the remainder of the study population were within normal limits. Serum biochemistry was performed in 44 dogs and was normal in 31/44. The remaining 13 dogs had liver enzyme activity (alkaline phosphatase [ALP], alanine aminotransferase [ALT], and gamma glutamyltransferase [GGT]) above the laboratory reference ranges, including ALP (n = 7); ALT (n= 2); and ALP, ALT, and GGT (n = 4). In dogs with increased liver enzyme activity, the median values of ALP, ALT, and GGT were 664 U/L (range, 125–3,288 U/L), 105 U/L (range, 88–475 U/L) and 27 U/L (range, 14–61 U/L), respectively. Because of variation in reference ranges among individual laboratories, liver enzyme activity comparison was not performed. Lymphadenopathy was reported in 14 dogs, and further analysis in 7 dogs confirmed a monomorphic population of lymphoblasts, consistent with lymphoma (cytology, n = 5; histopathology, n = 2). Thoracic radiographs were performed in 27/46 dogs. Abnormalities noted included an interstitial or infiltrative pattern (n = 3) and sternal lymphadenopathy (n = 1). The underlying cause of these radiographic findings was not determined in any case. Abdominal radiographs were obtained in 5 dogs. Four of the 5 had normal radiographic findings, and 1/5 had evidence of splenomegaly and loss of detail in the cranial abdomen. No further diagnostics were performed in the latter case. Abdominal ultrasonography (n = 25) identified abnormalities in 15 dogs related to the spleen (n = 3), liver (n = 7), lymph nodes (n = 3), urogenital system (n = 4; renal cyst, prostatic cyst, bladder sediment, and renal infarct), and adrenal glands (n = 1). Some dogs had more than 1 abnormal ultrasonographic finding. The spleen was described as mottled, heterogeneous, or nodular in 3 separate dogs. A splenic aspirate was not performed in any of the aforementioned dogs. Hepatomegaly (n = 2), coarse texture (n = 2), and nodular appearance (n = 3) were described liver abnormalities. Aspirates (n = 2) confirmed vacuolar hepatopathy. Iliac or sublumbar lymphadenopathy was identified in 3 dogs, but was not investigated further. Bone marrow cytology was performed and indicated no abnormality in 7 dogs.

Immunohistochemistry

Thirty-eight archived tissue samples were available during the study period for immunohistochemical analysis. In all samples, the major population of CD3-positive and CD79-negative cells were morphologically identical to the malignant population seen on the hematoxylin and eosin–stained sections. Of the 8 unavailable samples, information regarding immunophenotype was available for 4. All were CD3 positive, and 2/4 were CD79 negative based on immunohistochemistry performed at a separate laboratory.

Previous Therapy

Fourteen dogs received CCNU as the first form of therapy, whereas 32 dogs received some form of therapy before CCNU. Five of 32 dogs received chemotherapy before CCNU consisting of CHOP-based (cyclophosphamide,[l] doxorubicin,[m] vincristine,[n] and prednisone) protocols, MOPP (mechlorethamine,[o] vincristine, procarbazine,[p] and prednisone), or L-asparaginase[q]. One dog received CHOP followed by MOPP, and another dog received CHOP followed by L-asparaginase so that among the 5 dogs, 7 chemotherapy protocols were administered. Responses were noted in 3 dogs receiving CHOP (CR, n = 1; PR, n = 2) and in 2 dogs receiving MOPP (CR, n = 2), whereas no responses were obtained in 2 dogs that received L-asparaginase. The remaining 27/32 received antibiotics (n = 9), prednisone (n = 4), retinoids (n = 2), lyme/sulfur dips (n = 2) and multiagent therapy (n = 10) including prednisone and antibiotics (n = 7/10), prednisone and retinoids (n = 1/10), retinoids, interferon, and safflower oil (n = 2/10). The overall median time from definitive diagnosis until CCNU administration was 14 days (range, 1–346 days).

CCNU Therapy

A total of 212 cycles of CCNU were administered to the study population with a median number of 4 cycles (range, 1–11 cycles). The overall median starting dose of CCNU was 60 mg/m^2 (range, 30–95 mg/m^2) with a median cumulative dose of 239 mg/m^2 (range, 40–784 mg/

Table 2. Information for 46 dogs receiving CCNU therapy.

CCNU	Median	Range
Starting dosage (mg/m^2)	60	30–95
No. of cycles	4	1–11
Cumulative dosage (mg/m^2)	239	40–784

CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

m^2) (Table 2). The typical dosing schedule of CCNU was an oral treatment every 3 weeks in 46 dogs (range, 2–4 weeks) with a few treatment delays or variability because of increased liver enzyme activity (n = 6), neutropenia (n = 4), or thrombocytopenia (n = 1).

Of the 46 dogs receiving CCNU, responses were noted in 38/46 dogs (CR, n = 15 [32.6%]; PR, n = 23 [50%]). Five dogs (11%) had SD, and 3 dogs (7%) experienced PD (Table 3). The overall response rate (CR + PR) was 82.6% (38/46). The overall median number of cycles needed to note a response was 1 cycle (range, 1–6 cycles). The overall median duration of response (CR + PR) was 94 days (range, 22–282). The median duration of response was 132 days (range, 26–258) for the CR dogs, and 94 days (range, 22–282) for the PR dogs. For the SD dogs, the median time to disease progression was 60 days (range, 36–63). The time to disease progression was not statistically significant when comparing the responders (CR + PR) and nonresponders (SD + PD); (P = .07; Table 4). A significant difference was noted in the cumulative dose (P = .002) and number of CCNU cycles administered (P = .002) between responders (300 mg/m^2, 4 cycles) and nonresponders (131 mg/m^2, 2 cycles). A significant difference in the initial CCNU dosage between responders (60 mg/m^2) and nonresponders (56.3 mg/m^2) was not observed (P = .56). There was no significant difference in response (P =.33) or response duration (P = .20) among dogs presented with lymphadenopathy as compared to dogs that did not. The presence of mucocutaneous involvement (P = .65), oral lesions (P = 1.00), or diffusely distributed disease (P = .67) was not predictive of response or response duration.

CCNU Toxicity

Of the 212 cycles of CCNU administered, there were 89 recorded events of increased liver enzyme activity in dogs with previously normal serum biochemistry. Laboratory results were available in the medical record for 84/89 events. Thirty of the 84 events were transient, whereas the remainder (n = 54) persisted on repeated analysis. For all dogs with increased liver enzyme activity, the most common findings were high ALT (median, 204 U/L; range, 74–5360 U/L), AST (median, 150 U/L; range, 82–425 U/L), ALP (median, 567; range, 157–8,804 U/L), and GGT (median, 49; range, 12–200). Increased serum bile acid concentrations were observed in the single dog in which this test was performed. As previously stated, because of the differences in individual laboratory reference ranges, a comparison of increases in liver enzyme activity was not performed. Despite increases in liver enzyme activity and in serum bile acid concentrations in 1 dog, clinical evidence of liver failure (hypoalbuminemia, hypoglycemia, hypocholesterolemia, coagulopathies, hyperbilirubinemia, hyperbilirubinuria) was not identified. There were 44 documented hematologic toxicity events including neutropenia (n = 31) and thrombocytopenia (n = 13). The 31 neutropenic episodes were recorded 1 week after a CCNU dosage of (1) $\geq$70 mg/m^2 and were classified as Grade 1 (n = 4), Grade 2 (n = 1), Grade 3 (n = 1), and Grade 4 (n = 2); (2) 60–69 mg/m^2 and were classified as Grade 1 (n = 2), Grade 2 (n = 1), Grade 3 (n = 4), and Grade 4 (n = 3); (3) 50–59 mg/m^2 and were classified as Grade 1 (n = 2) and Grade 3 (n = 1); and (4) $\leq$50 mg/m^2 and were classified as Grade 1 (n = 3), Grade 2 (n = 5), Grade 3 (n = 1), and Grade 4 (n = 1) with 2/5 Grade 2 neutropenic episodes occurring 1 week after administration of CCNU at a dosage of 20 mg/m^2. Resolution of neutropenia was recorded (n = 10) after discontinuation of therapy or dose reduction at the administration of the next cycle. The 13 thrombocytopenic events were first noted 1 week after CCNU dosages of (1) $\geq$70 mg/m^2 and were classified as Grade 1 (n =1) and Grade 2 (n = 1); (2) 50–69 mg/m^2 and were classified as Grade 1 (n = 2) and Grade 3 (n = 1); and (3) $\leq$50 mg/m^2 and were classified as Grade 1 (n = 1), Grade 2 (n = 4), Grade 3 (n = 2), and Grade 4 (n= 1) with Grade 1 and Grade 3 thrombocytopenia (n = 1

Table 3. Response to CCNU in 46 dogs with ELSA.

		No. Cycles to Obtain Response		Starting Dosage (mg/m^2)		Response Duration (Days)	
Response Group	No. Dogs	Median	Range	Median	Range	Median	Range
Overall (CR + PR)	38	1	1–6	60	40–90	94	22–282
CR	15	1	1–6	60	47–90	132	26–258
PR	23	1	1–2	65	40–80	94	22–282
SD	5	n/a	n/a	51	40–71	60	36–63

CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; ELSA, epitheiotropic lymphoma; CR, complete response; PR, partial response; SD, stable disease; n/a, not applicable.

Table 4. Relationship of examined variables on response duration in 46 dogs following treatment with CCNU.

Variable	No. of Dogs	Median Response Duration	*P* Value
Lymphadenopathy			.20
LSA confirmed	7	75 days	
LSA ruled out	7	105 days	
Not examined	32	90 days	
Mucocutaneous Lesions			.43
Yes	8	75 days	
No	38	60 days	
Oral Cavity Lesions			.42
Yes	6	90 days	
No	40	90 days	
Lesion Distribution			.45
Diffuse	38	90 days	
Localized	8	90 days	
Response			.07
Yes (CR + PR)	38	94 days	
No (SD + PD)	8	46 days	
Prior Therapy			.22
Yes	32	90 days	
No	14	135 days	
Concurrent Prednisone			.15
Yes	27	75 days	
No	19	105 days	

CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; LSA, lymphosarcoma; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

each) recorded 1 week after a CCNU dosage of 20 mg/m^2. All 13 thrombocytopenic events persisted until the time of death.

CCNU Dose Reductions or Discontinuation

Sixteen dose reduction events were recorded for the 212 cycles of CCNU administered. Twelve were attributable to myelosuppression, including neutropenia (10/12), thrombocytopenia (1/12), and anemia (1/12). The neutropenic events were classified as Grade 1 (n = 2), Grade 2 (n = 1), Grade 3 (n = 2), and Grade 4 (n = 5). The thrombocytopenic event was classified as a Grade 1 toxicity (Table 1). The remaining dose reductions were because of increased liver enzyme activity (n = 3) or for unrecorded reasons (n = 1).

CCNU was discontinued in 29/46 dogs because of PD, in 8/46 because of increased liver enzyme activity, and in 4/46 because of thrombocytopenia. In 2 dogs, thrombocytopenia and increased liver enzyme activity or PD were cited as the cause of discontinuation. The median number of cycles resulting in discontinuation of therapy because of thrombocytopenia or increased liver enzyme activity was 8 (range, 5–11) and 3 (range, 1–5) cycles, respectively. Five of 46 dogs were alive at the time of data collection, and 2/5 with SD were receiving CCNU when they were lost to follow-up. Of the 3 dogs not receiving CCNU, 2 were alive with PD and the other had relapsed before data collection.

Concurrent Therapy

Of the 43 dogs in which information regarding concurrent therapy was available, 14 received CCNU monotherapy. More than half of the dogs (27/43) received concurrent corticosteroids. The initial daily prednisone dosage varied (0.9–3 mg/kg), and was tapered and discontinued (n = 6) or maintained (0.4–2 mg/kg q12–24 h) throughout treatment (n = 21). The co-administration of prednisone was not associated with response (P = .54) or response duration (P =.15; Table 4).

L-asparaginase (400 IU/kg) was administered at the initiation of CCNU therapy in 5 dogs, and in 1 dog a total of 4 cycles were administered. Other medications given with CCNU included essential fatty acids (n = 8), nonsteroidal anti-inflammatory agents (n = 3), retinoids (n = 2), and interferon (n = 1).

Rescue Therapy

Twenty-seven dogs with either PD or CCNU discontinuation because of toxicity received rescue therapy. Fourteen dogs received only 1 rescue protocol, and 13 dogs received 2 or more rescue protocols. The most common chemotherapy agents used alone or in combination were prednisone, doxorubicin, vincristine, cyclophosphamide, procarbazine, mechlorethamine, and dacarbazine[r]. Other therapies included mitoxantrone[s] and chlorambucil[t] (n = 1), radiation therapy (n = 3), denileukin ditftitox[u] (n = 1), and temozolamide[v] (n = 2). Responses to rescue therapy varied and were not consistently recorded. However, CR (n = 3) and PR (n = 16) were documented in response to CHOP, MOPP, and L'COP (L-asparaginase, cyclophosphamide, vincristine, and prednisone).

Outcome

Forty-one of 46 dogs were dead or euthanized at the time of writing: 29/41 died or were euthanized because of presumed disease progression, but a postmortem examination was performed in only 3/29 dogs. In 1 dog, lymphoma was documented in peripheral lymph nodes, liver, and skin. In another dog, lymphoma of the skin and multifocal renal tubular necrosis of unknown etiology were noted. The third dog died because of disseminated intravascular coagulopathy and vascular leak syndrome believed to be a consequence to treatment with denileukin diftitox, a suspicion supported by postmortem examination findings. Six deaths occurred during treatment with CCNU (myelosuppression and sepsis, thrombocytopenia, pancytopenia, anorexia, nasal discharge and lethargy, and immune-mediated hemolytic anemia; n = 1 each). An additional dog died of respiratory distress after CCNU was discontinued. Thoracic radiographs obtained 2 days before death disclosed an infiltrative pattern that was presumed to

be pneumonia or PD. Five dogs (including 1 of the dogs with documented progressive skin lesions) were euthanized because of neurologic abnormalities including seizures, ataxia, weakness, and thoracolumbar spinal cord compression. Necropsies were not performed on any of these dogs, and whether they had central nervous system involvement is not known. However, as stated, only 1 dog with seizures also had documented PD. The remaining dog was euthanized because of unknown causes.

Five dogs were alive at the time of study completion. Two of these 5 dogs were lost to follow-up while still being treated with CCNU. One dog had CR with 10 cycles of CCNU. When PD was noted, 2 cycles of doxorubicin followed by 2 additional cycles of CCNU were administered, ultimately yielding SD. The other dog was lost to follow-up after having achieved SD with 4 cycles of CCNU. The response duration for both dogs was 287 and 60 days, respectively. Three dogs were alive with PD but not receiving CCNU at last contact.

Discussion

Canine cutaneous epitheliotropic lymphoma represents a diagnostic and therapeutic challenge to veterinary oncologists. The clinical presentation of ELSA is similar to many autoimmune, allergic, and parasitic skin diseases, which can delay definitive diagnosis and appropriate treatment. The most common clinical presentation noted in this study was diffuse scale or generalized plaques and nodules. Several dogs also presented with lymphadenopathy, mucocutaneous involvement, or T-cell leukemia. Advanced disease stage, increased tumor burden within a given stage, and Sézary syndrome are known poor prognostic factors identified in humans with MF.[4] In contrast, high tumor burden, advanced disease stage, and presence of Sézary syndrome were not identified as poor prognostic indicators in this study of canine ELSA, likely because of the lack of uniform staging performed in this retrospective study and the low number of cases in each prognostic category. For example, as Sézary syndrome is an infrequently reported finding with ELSA,[21] only 2 dogs presented with a lymphocytosis prompting review of blood smears and documentation of circulating malignant T cells. The number of dogs with Sézary syndrome likely was underestimated as a pathologist review of all blood smears was not consistently performed. If a more sensitive tool such as flow cytometry had been used on all blood samples from the study population, the detection rate likely would have improved. For this reason, the authors suggest a minimum staging scheme consisting of a CBC including review of the white blood cell parameters by a pathologist or flow cytometry, serum biochemistry, lymph node aspiration or histopathology, thoracic and abdominal radiography, and abdominal ultrasonography on all dogs newly diagnosed with ELSA until the prognostic importance of disease stage on clinical outcome is identified. Another explanation for the inability to identify the prognostic relevance of disease stage is that description of the affected surface area may have been underestimated in animals with thick haircoats or pretreatment with steroids, resulting in fewer dogs categorized as having widespread disease.

Immunohistochemical analysis performed in this study identified a monomorphic population of round cells in the epidermis that stained positively for a T-cell marker and negatively for a B-cell marker. The inclusion of B- and T-cell immunohistochemical markers as part of a panel for cutaneous round cell tumors may help confirm the diagnosis of ELSA and eliminate treatment delay. The inclusion of additional immunohistochemical markers such as O^6-methylguanine-DNA methyltransferase may be useful to detect drug resistance (inherent to the tumor or induced by earlier chemotherapy) before initiating treatment with CCNU as this is a known mechanism of drug resistance against alkylating agents.[22]

Treatment of ELSA with CCNU was effective in >80% of the study population, resulting in a response rate and duration superior than reported in previous studies on CCNU for the treatment of relapsed multicentric lymphoma[23] and nearly identical to that reported in a recent publication on cutaneous ELSA performed during the same study period.[19] A smaller study[w] on cutaneous ELSA that investigated CCNU at 50 mg/m^2 every 3 weeks found a 100% complete response rate with 2 durable responses of 7 and 15 months. A limited number of cases (n = 7) were studied, and only 5/7 were confirmed to have ELSA, thus comparison with this study is difficult. Documented responses to retinoids, radiation therapy, and high doses of linoleic acid[x] have been noted, but toxicity[16,17] and cost[16] were high. Cyclosporine failed to elicit a response but resulted in marked toxicity in the 3 dogs treated in 1 study.[18] In contrast, CCNU administered at a dosage of 60 mg/m^2 every 3 weeks was well tolerated and resulted in acceptable toxicity in the present study. The most common toxicity noted was myelosuppression, which resolved and did not reoccur upon dose reduction at the next administration in the majority of cases. However, neutropenia (Grade 2; n = 2) and thrombocytopenia (Grade 1, Grade 3; n = 1 each) were documented in a single patient after 2 separate administrations of CCNU at 20 mg/m^2. An initial dosage of 60 mg/m^2 in this patient resulted in Grade 4 neutropenia, and dose reductions (which also resulted in neutropenia varying from Grade 2–4) were made at each subsequent administration until a dosage of 20 mg/m^2 was administered for the last 2 treatments. Myelosuppression is unexpected at this dosage of CCNU and likely reflects either an individual sensitivity to CCNU or cumulative toxicity. Toxicity of this nature is unlikely in the general population of dogs with ELSA. Increased liver enzyme activity and cumulative thrombocytopenia also were noted after a median of 3 and 8 treatments, respectively, in a subpopulation of study dogs (89 and 13 recorded events, respectively of the 212 CCNU administrations). Hepatotoxicity was characterized by increased liver enzyme activity and did not result in documented hepatic failure, although serum bile acid concentrations

were increased in 1 dog in which they were measured. The incidence of CCNU-induced hepatotoxicity in dogs is reported to be 6.1% and may be higher if examined prospectively in study populations undergoing standardized evaluation of serum biochemistry during treatment.[23] The limited results of this study are not intended to support or refute previous data concerning CCNU-related hepatotoxicity and the authors recommend routine monitoring of liver enzymes and hepatic function in any dog receiving CCNU. Because a consensus regarding therapy in the face of increased liver enzyme activity does not exist, it is the obligation of the clinician to determine when CCNU therapy should be discontinued, if at all. However, the toxicity profile of CCNU demonstrated in this study is similar to that reported in a similar study on a similar patient population.[19] Based on these findings, CCNU monotherapy appears to be reasonably safe and economical and should be considered for newly diagnosed cases of canine ELSA.

This study has several limitations inherent to its retrospective nature. Similar to the study by Williams et al,[19] the limited number of dogs precluded the ability to draw meaningful conclusions from the statistical analysis of prognostic factors, and the small sample size may have led to a Type II error (ie, failure to detect significance when it is in fact present). A prospective study including a larger number of dogs with more statistical power may allow the detection of a significant difference in response data for the categorical variables tested (eg, the initial CCNU dosage, stage of disease, response duration in responders versus nonresponders).

Response assessment in the measurable disease setting can be subjective if not based on precise measurements at the time of first examination and upon reevaluation. Because many dogs affected by ELSA present with diffusely distributed lesions, the measurement of initial lesions and their subsequent response to therapy becomes an extremely subjective undertaking unless serial photographs are taken. In cases where accurate measurements were not recorded in the medical record, recall bias may skew result assessment data. Repeat biopsy after treatment is a more objective manner of response assessment in cases of diffusely distributed disease and can confirm histologic remission in true complete responders. In addition, because of the dosing schedule inherent to CCNU, many patients may have been examined only once every 3 weeks. This may have prevented earlier detection of PD, thus falsely increasing remission duration assessment.

The retrospective nature of this study also prevented control over the concurrent use of other medications such as corticosteroids and antibiotics. Although a plethora of T cells is present in the epidermis, their immune function is questionable, predisposing animals to pyoderma and necessitating the use of antibiotic therapy.[4] Prednisone was used in more than half of the dogs and is likely necessary in many patients to alleviate the pruritus often associated with ELSA. The concurrent use of prednisone in this study population provided no detectable improvement in response rate or duration. In fact, dogs concurrently receiving prednisone had a shorter response duration (2.5 months versus 3.5 months) although the difference was not statistically significant. This finding may be a reflection of the small sample size or a result of the tendency to use prednisone in more severely affected animals. Prospective clinical trials investigating the use of CCNU monotherapy to eliminate any noted improvement in clinical signs attributable to resolution of inflammation and infection are warranted. However, this goal may be unrealistic because concurrent medications often are necessary to control these untoward sequelae. The use of L-asparaginase in the treatment of multicentric lymphoma provides no significant improvement in response duration[25,26] or overall survival time but has been shown to shorten the time to obtain a response.[25] L-asparaginase was administered to 5 dogs in the study population and may have improved the response rate, but this was not noted in this study.

A statistical difference was noted when comparing cumulative dose and number of CCNU cycles between responders and nonresponders. This finding is not unexpected and is explained by the fact that dogs that responded to treatment continued to receive additional cycles of CCNU, leading to a higher cumulative dose when compared to the nonresponders in which CCNU was discontinued. However, despite an initial response to CCNU, relapse was noted in a majority of the cases. When attempted, rescue protocols were of variable efficacy, and the number of cases within each rescue protocol category was too small for meaningful conclusions to be drawn. Of the 27 dogs in which rescue therapy was attempted, there were 19 documented responses (CR, n = 3; PR, n = 16) after CHOP, MOPP, and L'COP-based protocols. Although such a high response rate to rescue therapy seems encouraging, these results should be interpreted with caution because the dosages employed, dose intensity, and duration of response after rescue therapy were not consistently reported and could reflect the phenomenon of recall bias. Alternatively, this data may support the consideration of such chemotherapeutic agents in combination with CCNU for the treatment of newly diagnosed or relapsed ELSA.

Survival times were not evaluated in this study because of the aforementioned limitations associated with its retrospective nature including lack of consistent therapeutic modalities before and after treatment with CCNU. However, the cause of death in a majority of ELSA patients presumably was attributable to PD. Postmortem examination was performed in very few dogs. Five of the 46 dogs had evidence of neurologic disease at the time of death. Central nervous system spread of cutaneous lymphoma has been documented,[27] and if postmortem examination had been performed in a majority of the dogs, widespread involvement at the time of death may have been more evident. Survival analysis was beyond the scope of this study and lack of consistent postmortem examination findings is unlikely to have had an impact on the value of information reported here.

In conclusion, CCNU seems to be a safe, well-tolerated chemotherapeutic agent for the treatment of canine ELSA. Although response duration was short, the high response rate observed in this study supports the incorporation of CCNU into protocols to treat ELSA. A randomized prospective analysis is warranted to further investigate the benefit of CCNU in monotherapy or combined with other agents for the treatment of canine ELSA.

Footnotes

[a] Methoxsalen, Oxsoralen Lotion 1%, Valeant Pharmaceuticals, Costa Mesa, CA
[b] CCNU, CeeNU, Bristol-Myers Squibb Co, Princeton, NJ
[c] Stata 8.0 for Windows, Stata Corporation, College Station, TX
[d] Blocking solutions, Invitrogen Corp, South San Francisco, CA
[e] Avidin/biotin blocking kit, Invitrogen Corp, South San Francisco, CA
[f] Super Block, Pierce Chemical Co, Rockford, IL
[g] CD3, Sigma-Aldrich, St. Louis, MO
[h] HM47/A9, Lab Vision, Fremont, CA
[i] *Helicobacter pylori*, Cell Marque Corp, Hot Springs AR
[j] detection kit, Signet Labs Inc, Dedham, MA
[k] New Fucshin reagent solution, Kirkegaard & Perry Labs, Gaithersburg, MD
[l] Cyclophosphamide, Cytoxan, Bristol-Myers Squibb Co, Princeton, NJ
[m] Doxorubicin, Adriamycin, Merck & Co, Inc, Whitehouse Station, NJ
[n] Vincristine, Oncovin, Merck & Co, Inc, Whitehouse Station, NJ
[o] Mechlorethamine, Mustargen, Merck & Co, Inc, Whitehouse Station, NJ
[p] Procarbazine, Matulane, Sigma-Tau Inc, Gaithersburg, MD
[q] L-asparaginase, ELSPAR, Merck & Co, Inc, Whitehouse Station, NJ
[r] Dacarbazine, DTIC-Dome, Bayer Corp, West Haven, CT
[s] Mitoxantrone, Novantrone, Serono Inc, Rockland, MA
[t] Chlorambucil, Leukeran, Merck & Co., Inc, Whitehouse Station, NJ
[u] Denileukin diftitox, Ontak, Seragen Inc, Lexington, MA
[v] Temozolomide, Temodar, Schering-Plough Corp, Kenilworth, NJ
[w] Graham JC, Myers RK. Pilot study on the use of lomustine (CCNU) for the treatment of cutaneous lymphoma in dogs. 17th Annual Internal Medicine Forum, 1999:723 (abstract)
[x] Petersen A, Wood S, Rosser E. The use of safflower oil for the treatment of mycosis fungoides in two dogs. Proceedings of the 15th Annual Meeting of the American Academy of Veterinary Dermatology, 1999:49–50 (abstract)

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