J Vet Intern Med 2011;25:838–845

Prospective Randomized Clinical Trial Assessing the Efficacy of Denamarin for Prevention of CCNU-Induced Hepatopathy in Tumor-Bearing Dogs

K.A. Skorupski, G.M. Hammond, A.M. Irish, M.S. Kent, T.A. Guerrero, C.O. Rodriguez, and D.W. Griffin

Background: Increases in liver enzymes occur in up to 86% of dogs receiving CCNU and can result in treatment delay or early discontinuation of treatment. Denamarin contains S-adenosylmethionine and silybin, both of which have been investigated as treatments for various liver diseases.

Hypothesis: Dogs on CCNU receiving Denamarin have lower alanine aminotransferase (ALT) activity than dogs not receiving Denamarin. Dogs on Denamarin are less likely to require treatment delay because of hepatopathy and are more likely to complete their prescribed course of CCNU.

Animals: Dogs with lymphoma, mast cell tumor, or histiocytic sarcoma that were prescribed CCNU with or without corticosteroids and with normal ALT activity were eligible for enrollment.

Methods: Dogs were prospectively randomized to receive either concurrent Denamarin during CCNU chemotherapy or to receive CCNU alone. Liver-specific laboratory tests were run before each dose of CCNU.

Results: Increased liver enzyme activity occurred in 84% of dogs receiving CCNU alone and in 68% of dogs on concurrent Denamarin. Dogs receiving CCNU alone had significantly greater increases in ALT, aspartate aminotransferase, alkaline phosphatase, and bilirubin and a significantly greater decrease in serum cholesterol concentrations than dogs receiving concurrent Denamarin. Dogs receiving CCNU alone were significantly more likely to have treatment delayed or discontinued because of increased ALT activity.

Conclusions: Increased liver enzyme activity occurs commonly in dogs receiving CCNU chemotherapy. These results support the use of concurrent Denamarin to minimize increased liver enzyme activity in dogs receiving CCNU chemotherapy. Denamarin treatment also increases the likelihood of dogs completing a prescribed CCNU course.

Key words: Lomustine; S-adenosylmethionine; Silybin.

CCNU (lomustine) is a nitrosourea alkylating agent used in veterinary medicine to treat a variety of canine cancers including lymphoma (LSA), mast cell tumor (MCT), and histiocytic sarcoma (HS).[1–5] CCNU has been shown to be hepatotoxic both in preclinical models as well as in clinical patients.[6–11] The exact mechanism of toxicity is not fully understood, but glutathione depletion and lipid peroxidation have been implicated.[7,8,12] Rodents treated with very high doses of CCNU develop acute biliary stasis and hepatocyte necrosis leading to death.[13] The toxicity of CCNU was further investigated in healthy beagle dogs given a single dose of 4 mg/kg.[9] Histopathologic evaluation of liver biopsy samples showed Kupffer cell hyperplasia, cloudy swelling of hepatocytes, and periportal fibrosis in all treated dogs. Serum biochemistry results in these dogs showed significant increases in the activity of liver enzymes, with alanine aminotransferase (ALT) activity increasing significantly in 4 of 5 dogs. The increases in liver enzyme activity were delayed at least 2 weeks after treatment in this study, and some dogs reached peak ALT activity after 4 weeks.[9]

In a clinical setting, increases in liver enzyme activity occur in up to 86% of cancer-bearing dogs receiving therapeutic doses of CCNU.[2,3,10,11] Serious liver injury causing clinical signs, however, occurs in only 3–6% of dogs. Liver biopsy samples taken from dogs with CCNU-induced hepatopathy showed changes including multifocal aggregates of hemosiderin-laden Kupffer cells, hepatocyte karyomegaly, hepatocellular vacuolization, periportal inflammation, bridging fibrosis, and bile duct hyperplasia.[11] Previously published retrospective studies describing the use and toxicity of CCNU in dogs reported inconsistent measurement of liver enzyme activity during therapy, making the true incidence and

Abbreviations:

ALKP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	blood urea nitrogen
GGT	gamma-glutamyl transferase
HS	histiocytic sarcoma
LSA	lymphoma
MCT	mast cell tumor
SAMe	S-adenosylmethionine

From the Department of Surgical and Radiological Sciences (Skorupski, Kent, Guerrero) and the Veterinary Medical Teaching Hospital, University of California, Davis, Davis, CA (Hammond, Irish, Rodriguez); and Nutramax Laboratories Inc, Edgewood, MD (Griffin). Dr Hammond is presently affiliated with VCA Sacramento Veterinary Referral Center, Sacramento, CA. Dr Irish is presently affiliated with VCA Veterinary Referral Associates, Gaithersburg, MD. *This research was presented, in part, at the 2009 ACVIM Forum & Canadian VMA Convention, and the Veterinary Cancer Society meeting in 2010.*

Corresponding author: K. A. Skorupski, DVM DACVIM (Oncology), Department of Surgical and Radiological Sciences, University of California, Davis, Room 2112 Tupper Hall, 1 Shields Avenue, Davis, CA 95616; e-mail: kskorups@ucdavis.edu.

Submitted December 8, 2010; Revised March 29, 2011; Accepted May 3, 2011.

10.1111/j.1939-1676.2011.0743.x

severity unclear.[2,3,10,11] Additionally, some dogs in these previous studies had increased liver enzyme activity before starting CCNU therapy, further confounding results. Nonetheless, increased liver enzyme activity remains a clinically relevant problem for veterinarians who prescribe CCNU. Although most dogs will not develop hepatopathy that requires medical intervention, treatment with CCNU may be delayed or discontinued because of concerns of impending liver failure.

S-adenosylmethionine (SAMe) is a compound that functions widely in the body as a group donor or enzymatic inducer during transmethylation, transsulfuration, and aminopropylation.[14,15] The liver synthesizes the majority of SAMe in the body, and patients with liver disease have low glutathione concentration and decreased ability to scavenge free radicals.[16] SAMe can be supplemented PO, is used commonly to treat liver disease in both humans and dogs, and appears to be most effective in the treatment and prevention of toxin-induced hepatopathy.[17,18] Specifically, administration of SAMe PO is known to minimize alcohol-induced hepathopathy in humans.[17]

Silymarin, the standardized extract of the fruit of the milk thistle plant, *Silybum marianum*, contains several flavonoids, including silybin, silydianin, and silychristin. Silybin is the most potent of these flavonoids, but is poorly absorbed unless complexed with phosphatidylcholine.[19–23] Silybin is thought to be hepatoprotective by several mechanisms, including activity against lipid peroxidation, cytochrome P450 inhibition, and the inhibition of transformation of stellate hepatocytes into myofibroblasts that leads to cirrhosis.[19–23] Like SAMe, silybin is commonly used to treat patients with liver disease and is especially effective at preventing toxic hepatopathies. Silybin has been shown to prevent hepatic damage caused by Amanita mushroom intoxication in dogs when given IV immediately after intoxication.[24] Additionally, silymarin was associated with increased survival in a placebo-controlled clinical trial in alcoholic liver disease in humans.[25] Denamarin is a commercially available veterinary product containing a stable salt of SAMe and silybin in a phosphatidylcholine complex, both with established bioavailability in dogs.[a,26,27]

There is limited evidence to support the use of SAMe, silybin, or both in cancer patients with or at risk for hepatotoxicity from chemotherapy. In a small cohort of human cancer patients that developed hepatoxicity from chemotherapy, treatment with SAMe was shown to significantly decrease ALT and aspartate aminotransferase (AST) activity and permitted patients to receive chemotherapy on schedule.[28] In another study, silybin was studied in children with acute lymphoblastic leukemia with or at risk for increased liver enzyme activity.[29] Silybin was effective at significantly decreasing AST but not ALT activity during chemotherapy, and increased adherence to the prescribed chemotherapy protocol. A possible role for SAMe, milk thistle extract, or both in the prevention of chemotherapy hepatotoxicity in dogs has not been explored.

Therefore, the purpose of this study was to determine whether concurrent Denamarin administration decreases the risk of clinicopathologic and clinical hepatopathy in dogs receiving CCNU chemotherapy. We hypothesized that dogs receiving Denamarin would have lower ALT activity than dogs not receiving Denamarin and that dogs on Denamarin would be more likely to complete their prescribed CCNU course.

Methods

Dogs with a histologic or cytologic diagnosis of LSA, HS, or MCT that were prescribed CCNU chemotherapy at the Veterinary Medical Teaching Hospital at the University of California, Davis were eligible for enrollment in the trial, which was approved by the institution's Animal Care and Use Committee. CCNU could be prescribed alone or in a combination protocol with vinblastine, but had to be prescribed at a treatment interval of no more than 4 weeks between CCNU doses. Dogs were excluded if they had an increased ALT activity at the time of enrollment or a previous history of hepatopathy. Dogs with known or suspected hepatic involvement of their cancer were allowed, and a recent hepatic fine needle aspirate was not required for enrollment. Corticosteroid use was allowed at the discretion of the veterinarian, and dogs with increased alkaline phosphatase (ALKP) activity at enrollment were not excluded. Vitamin and antioxidant supplements were not allowed during the study period, but no other specific drugs were prohibited.

Informed owner consent was obtained and dogs were randomly assigned to 1 of 2 treatment groups of equal size in a sequential manner by a computer generated randomization list. Group A dogs were dispensed Denamarin[b] at the time of the first dose of CCNU. Their owners were instructed to administer Denamarin once daily on an empty stomach at the dosage recommended on the package insert throughout chemotherapy. Group B dogs were treated with CCNU without Denamarin. Dogs that developed a grade 4 increase in ALT activity ($\geq$10$\times$ the upper limit of normal; $\geq$670 IU/L) had CCNU treatment temporarily or permanently discontinued at the discretion of the treating clinician. Dogs in group B that developed a grade 4 increase in ALT activity were started on Denamarin, and dogs in group A with a grade 4 increase in ALT activity continued Denamarin therapy.

Liver function tests were performed with the Hitachi 917 chemistry analyzer.[c] Liver function tests were performed on each dog before starting CCNU and before each additional dose of CCNU. Liver function tests consisted of ALT, AST, ALKP, gamma-glutamyl transferase (GGT), total bilirubin, albumin, cholesterol, and blood urea nitrogen (BUN). Serum glucose concentration was measured inconsistently because of changes in laboratory policy over time.

Dogs were considered off-study when they completed the prescribed CCNU course if their ALT activity was within the reference range. If the ALT activity was not within the reference range at completion of CCNU therapy, liver function tests were performed regularly until normalization of ALT activity occurred for up to a maximum of 6 months after CCNU therapy completion. Dogs taking Denamarin continued on Denamarin until they were off-study.

Power analysis was performed before enrollment to determine sample size using an α of 0.05 and a power of 0.9. Assuming a difference in mean ALT activity of 135 IU/L between groups and a standard deviation of 100 IU/L within groups, a total of 38 dogs would be required to detect a statistically significant difference between groups. To account for the possibility of a wider standard deviation and dogs lost to follow-up, a total enrollment of 50 dogs was planned. Toxicities were graded using the Veterinary Cooperative Oncology Group Common Toxicity Criteria for Adverse Events.[30] Tumor responses were documented using response evaluation criteria in solid tumors.

To look for differences between groups, continuous data were compared by a 2-sided *t*-test whereas categorical data were compared by Fisher's exact test or χ^2-test depending on the number of cases in each group. Factors evaluated for an overall difference between groups included age, sex, breed, weight, tumor type, treatment setting, dose, number of doses, dose adjustments, corticosteroid use and dose, vinblastine in the protocol, response to CCNU, neutropenia, and reason for CCNU discontinuation. For the purposes of statistical analysis, dose adjustments were defined as increases or decreases in CCNU dose for reasons other than increased liver enzyme activity. Therefore, dogs discontinuing CCNU because of grade 4 increases in ALT activity were not considered to have had a dose adjustment. Dogs that received only 1 dose of CCNU were excluded from analysis of dose adjustments. Only dogs with measurable disease were included in analysis of response to CCNU. A 2-sided *t*-test was used to look for differences in the pretreatment results of ALT, AST, ALKP, GGT, bilirubin, albumin, cholesterol, blood glucose, and BUN. Differences in changes in biochemical variables were tested for normality by graphing data on a histogram and assessing the quantile-quantile plot. For normal data, a 2-sided *t*-test was performed and, for nonnormally distributed data, nonparametric analysis by a Mann-Whitney test was done. Changes in ALT, AST, ALKP, GGT, bilirubin, albumin, cholesterol, blood glucose, and BUN were assessed. All statistics were performed by a commercially available software program.[d]

Results

Fifty dogs were enrolled in the clinical trial between April 2007 and October 2009. Twenty-five dogs were assigned to group A and 25 dogs to group B. Age, sex, breed distribution, and weight were not significantly different between groups (Table 1). When tumor types were compared between groups, they also were not significantly different (Table 2). Overall, there were 20 dogs with MCT, 18 with LSA, and 12 with HS. One dog's tumor was reclassified from HS to metastatic histiocytoma after immunohistochemical staining. This dog was included in the HS group during statistical analysis.

Of the 20 dogs with MCT, 9 had grade 2 tumors, 6 had grade 3 tumors, and 4 were ungraded. Three of the 4 ungraded tumors were mucosal in origin. Of the 18 dogs with LSA, 14 were multicentric, 3 were cutaneous epitheliotrophic, and 1 was splenic. Nine were B-cell, 8 were T-cell, and 1 was not phenotyped. Of the 12 dogs with HS, 6 had localized HS and 6 had disseminated HS. Nineteen of the 50 dogs were treated with CCNU in the adjuvant setting and the remaining 31 dogs were treated for gross disease. There was no difference in treatment setting between groups (Table 2).

The mean starting CCNU dose was 70.6 mg/m^2 (median, 71.4 mg/m^2; range, 54.9–81.3 mg/m^2) and the median number of CCNU doses administered was 3 (range, 1–16). Seven (28%) dogs in group A and 9 (36%) dogs in group B received CCNU every 28 days alternating with vinblastine. The remaining dogs received CCNU every 21 days. There were no differences between groups in prescribed CCNU dose, number of doses administered, or inclusion of vinblastine in the protocol (Table 2). Eleven dogs (7 in group A, 4 in group B) were excluded from analysis of dose reductions because they received only 1 dose of CCNU. Reason for discontinuing treatment after 1 dose was PD in 10 dogs and sepsis and owner preference in the remaining dog. Six (15%) dogs required dose reductions and 6 (15%) dogs had dose escalations during their treatment period. The reason for

Table 1. Comparison of patient characteristics between groups.

	Group A	Group B	*P*-Value
Mean age (years)	8.9	8.3	.42
Sex			
F	0	1	.06
FS	5	11	
M	6	1	
MC	14	12	
Breed			
Mixed breed	4	6	.07
Labrador Retriever	1	6	
Golden Retriever	5	1	
Rottweiler	1	3	
Bernese Mountain Dog	1	2	
Other purebred	13	7	
Mean weight (kg)	28.4	29.1	.85

Table 2. Comparison of treatment characteristics between groups.

	Group A	Group B	*P*-Value
Tumor type			
LSA	10	8	.76
HS	5	7	
MCT	10	10	
Treatment setting			
Adjuvant	7	12	.15
Gross	18	13	
Mean CCNU			
Dose (mg/m^2)	70.0	71.0	.56
Mean number of CCNU doses	3.5	3.9	.55
Dose adjustment*			
No	14	13	.56
Reduction	2	4	
Escalation	2	4	
Steroid use			
Yes	17	17	1.00
No	8	8	
Vinblastine			
Yes	7	9	.54
No	18	16	
Response to CCNU			
CR	1	1	.81
PR	6	6	
SD	4	3	
PD	7	3	
Neutropenia			
None	7	9	.28
Grade 1	2	3	
Grade 2	7	2	
Grade 3	1	4	
Grade 4	6	5	

HS, histiocytic sarcoma; LSA, lymphoma; MCT, mast cell tumor.
*In dogs receiving more than one CCNU dose.

Table 3. Highest ALT distribution by group using VCOG criteria[30] and alternative criteria proposed by Hosoya et al.[10]

	VCOG-CTCAE ALT Toxicity Grade[30]					Alternative ALT Toxicity Grading Scheme[10]			
ALT (IU/L)	0 ≤67	1 68–100	2 101–134	3 135–669	4 >670	0 ≤134	Mild 135–335	Moderate 336–670	Severe >670
Group A dogs	8 (32%)	6 (24%)	3 (12%)	7 (28%)	1 (4%)	17 (68%)	4 (16%)	3 (12%)	1 (4%)
Group B dogs	4 (16%)	2 (8%)	4 (16%)	8 (32%)	7 (28%)	10 (40%)	4 (16%)	4 (16%)	7 (28%)

ALT, alanine aminotransferase; VCOG-CTCAE, Veterinary Cooperative Oncology Group Common Toxicity Criteria for Adverse Events.

dose reduction was grade 4 neutropenia with or without sepsis in 5 dogs and bloody diarrhea in 1 dog.

Thirty-four (68%) dogs received prednisone during the study period at a median highest dose of 0.79 mg/kg/d (range, 0.3–1.3 mg/kg/d). The number of dogs treated with prednisone in both treatment groups was equal, and doses were not significantly different between groups. Twenty-three of these dogs started corticosteroids before the study period, 9 started at the same time as CCNU, and 2 started during treatment. The median duration of prednisone administration in the 23 dogs that started prednisone before the study period was 36 days (range, 8–185 days). There were no significant differences in tumor response to CCNU or neutropenia between groups (Table 2).

There were no significant differences between starting ALT, AST, ALKP, GGT, total bilirubin, albumin, cholesterol, BUN, or glucose between groups. Seventeen dogs (68%) in group A and 21 dogs (84%) in group B experienced an increase in ALT activity above the reference range (Table 3). One dog in group A developed a grade 4 increase in ALT activity compared with 7 dogs in group B. Of the dogs with a grade 4 increase, 6 of these increases were found acutely after several doses with normal or near normal ALT activity noted on the most recent previous liver function testing. A median of 3 (range, 0–6) CCNU doses were administered to dogs before the highest ALT activity was documented. The mean highest ALT activity was 173 IU/L (range, 24–1,018) in group A dogs and 692 IU/L (range, 43–5,552) in group B dogs. Dogs in group B had significantly greater posttreatment increases in ALT ($P = .003$), AST ($P = .01$), ALKP ($P = .009$), and bilirubin ($P = .02$) (Fig 1). Dogs in group B had significantly greater decreases in posttreatment cholesterol concentration ($P = .02$). Significant differences between posttreatment GGT, albumin, BUN, and glucose were not found, although GGT had a *P*-value of .054.

The majority of dogs with increases in liver enzyme activity did not undergo diagnostic testing to further define the extent or cause of liver disease, but 6 of 8 dogs with grade 4 increases had some additional testing. Only 1 dog (group B) had bile acid concentrations measured and results were markedly increased at 143.3 μmol/L fasting and 318 μmol/L postprandial. Six dogs (1 in group A, 5 in group B), all with grade 4 ALT increases, had abdominal ultrasound examinations at the time increased liver enzyme activities were noted. Two dogs had ultrasonographically normal livers and 4 dogs had mild hepatomegaly without specific changes suggesting a cause of the increased liver enzyme activities. Two dogs in group B had fine needle aspirates of their livers performed. One had hepatocytes with voluminous cytoplasm and irregular shapes, cytoplasm with abundant lipofuschin, and hepatocyte karyomegaly. These cellular changes were considered consistent with what is seen histologically in dogs with CCNU-induced hepatopathy. The second dog undergoing liver aspiration had results suggestive of moderate vacuolar hepatopathy. This dog underwent liver biopsy collection 6 months after discontinuing CCNU because of recurrent increases in liver enzyme activity, and results showed mild neutrophilic cholangitis and hepatitis, mild lobular collapse with nodular hyperplasia, and vacuolar degeneration.

Seven dogs (28%) in group B required temporary or permanent discontinuation of CCNU treatment because of a grade 4 increases in ALT activity whereas only 1 dog (4%) in group A had treatment discontinued for this reason. This difference was statistically significant ($P = .02$). Six of these dogs, including the group A dog and 5 group B dogs, were receiving corticosteroids. By study design, the 7 dogs in group B that developed a grade 4 increase in ALT activity were started on Denamarin. ALT activity decreased to <300 IU/L within 3 weeks of stopping CCNU and starting Denamarin in 4 of these dogs and within 12 weeks in 2 additional dogs. One dog in group B that developed grade 4 hepatopathy died with features consistent with liver failure during the study period. A necropsy was not performed. This dog was being treated in the adjuvant setting for a grade 3 MCT. Three of the dogs in group B that developed grade 4 increases in liver enzyme activity continued to have chronic hepatopathy as determined by persistent increases in liver enzyme activity requiring medical management. One of these dogs died suddenly 9 months after completing chemotherapy of causes unrelated to cancer or hepatopathy. At necropsy, multiple extrahepatic shunts were noted as well as hepatocellular vacuolation, mild chronic lymphoplasmacytic hepatitis, hemosiderin-laden Kupffer cells, and mild portal fibrosis. These findings were considered supportive of a chronic CCNU hepatotoxicity. Two dogs in group B that developed grade 4 hepatopathies were restarted on CCNU chemotherapy while taking Denamarin after their ALT activity decreased to <300 IU/L. One of these dogs did not develop further increases in liver enzyme activity, but the other had recurrence of a grade 4 increase in ALT activity and had to permanently discontinue CCNU chemotherapy.

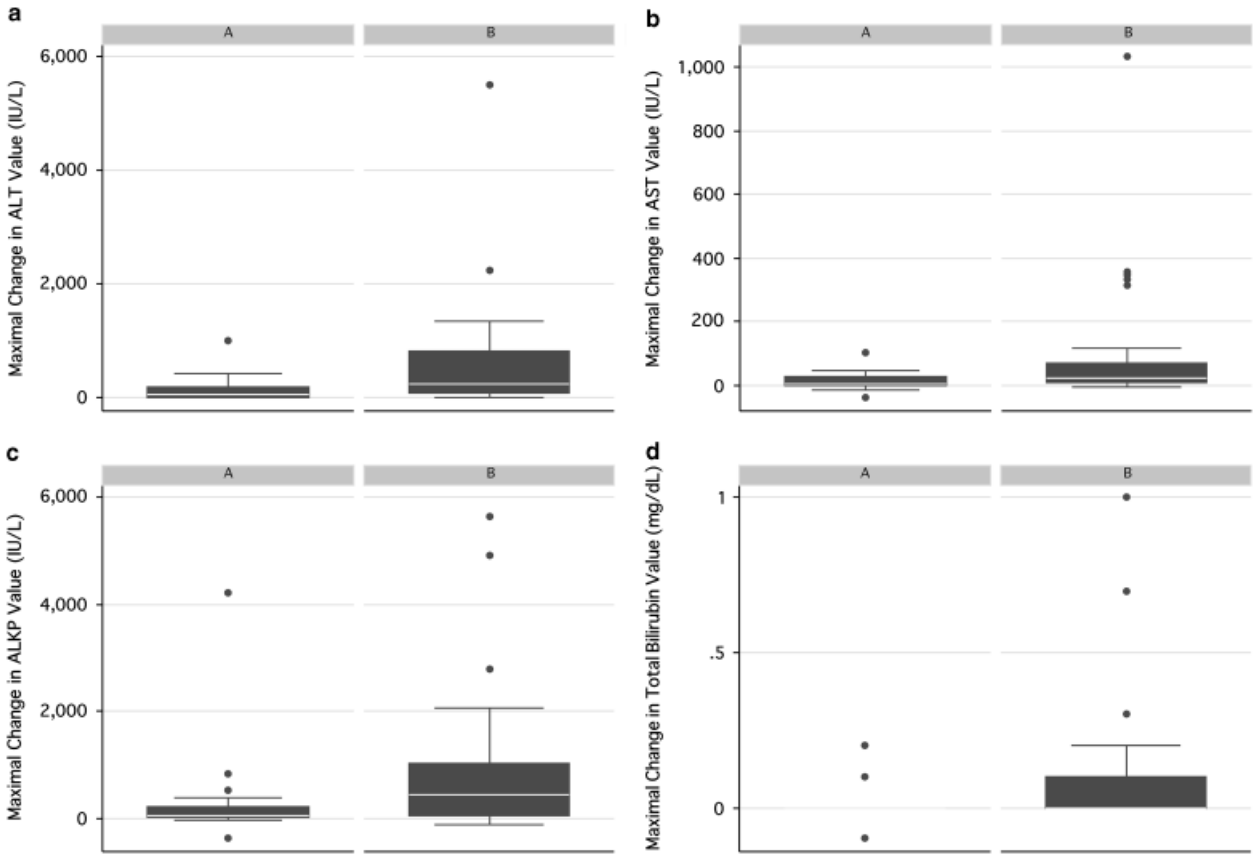

Fig 1. Box and whisker plots of serum chemistry values for dogs receiving CCNU treated with Denamarin (group A) or not receiving Denamarin (group B) with regard to maximum change in alanine aminotransferase (ALT). (**a**), aspartate aminotransferase (AST) (**b**), alkaline phosphatase (ALKP) (**c**), and bilirubin (**d**). Central lines represents the median, boxes represent 25th and 75th percentiles, and whiskers represent 5th and 95th percentiles. Statistically significant differences were found for all 4 of these parameters.

Discussion

The results of this study support those of previous studies suggesting that clinicopathologic evidence of hepatotoxicity is a common finding in cancer-bearing dogs receiving CCNU chemotherapy. Twenty-one of the 25 (84%) control group dogs developed some increase in ALT activity during the course of therapy. This finding is similar to findings of 2 previous retrospective studies. Hosoya et al[10] reported on 109 dogs treated with CCNU for a variety of cancers and found that 83% developed some increase in ALT activity over their starting result. Williams et al[2] reported on 36 dogs with cutaneous LSA treated with CCNU and found that 60% of dogs tested after treatment had increases in ALT activity, and 86% of dogs that had more than 1 dose of CCNU had increases in ALT activity. Given the high incidence of increases in liver enzyme activity in dogs receiving CCNU and the acute onset of severe increases noted in some patients, frequent liver-specific laboratory testing should be considered during CCNU-based therapy in these dogs.

The results of this clinical trial also suggest that Denamarin administration may lessen the severity of CCNU-induced liver enzyme increases in tumor-bearing dogs. Although a high number of dogs receiving the nutraceutical and chemotherapy concurrently (68%) still developed increases in ALT above the reference range, these increases were significantly less severe than in dogs that did not initially receive Denamarin (group B). Likewise, dogs in group B had significantly greater increases in AST, ALKP, and bilirubin, suggesting that Denamarin can minimize the effects of CCNU on both hepatocellular damage and biliary dysfunction.

A major limitation of this study is the low number of cases that underwent additional liver testing with bile acids, abdominal ultrasound examination, and liver biopsies. Although liver fine needle aspirates or biopsies were recommended by treating clinicians in all cases when grade 4 increases were documented, most dog owners opted to take a conservative approach given that the overall prognosis for their dog was guarded and considering the risk associated with liver biopsy. It is impossible, therefore, to eliminate the possibility that some dogs in this study experienced changes in liver function tests because of progression of cancer into the liver or because of other liver diseases.

The incidence of severe liver disease was low in this study. One dog died of liver failure apparently secondary to CCNU administration. An additional 3 dogs that

developed grade 4 liver enzyme increases continued to have increased liver enzyme activities and were placed on long-term treatment with hepatoprotectants. None of these 3 dogs, however, developed clinical signs consistent with liver disease. In this study, dogs experiencing grade 4 liver enzyme increases were allowed to cross over into the Denamarin treatment group. All but one of the dogs that crossed over experienced substantial reductions in liver enzymes within 2–12 weeks of discontinuing CCNU and starting Denamarin. These interventions may have decreased the overall incidence of severe clinical liver disease secondary to CCNU in this study. This low incidence also may have affected results comparing changes between groups in BUN, albumin, cholesterol, and glucose, which are measures of liver function that change only when the organ is severely affected. It is also unclear whether discontinuing CCNU or starting Denamarin may have contributed differentially to reduction in liver enzyme increases in dogs that crossed over. This study was designed to evaluate Denamarin's ability to prevent or lessen, not reverse CCNU-induced hepatopathy and additional study into the role of Denamarin in dogs with pre-existing CCNU hepatopathy is necessary.

The results of this clinical trial suggest that dogs receiving concurrent Denamarin and CCNU are less likely to require treatment discontinuation or delay because of ALT increases and are more likely to complete their prescribed chemotherapy course. Only 1 dog in group A had treatment discontinued because of increased liver enzymes compared with 7 dogs in group B. For the purposes of this study, dogs that developed a grade 4 or higher increase in ALT activity (670 IU/L) were required to have a treatment delay or discontinuation. There is considerable variability among oncologists as to what extent of increase in liver enzyme activity will trigger treatment alteration, and this threshold was chosen to maximize the chances of detecting a difference between treatment groups while still maintaining a level of caution. Treatment delay and discontinuation may lead to tumor relapse and, possibly, decreased survival in patients. Therefore, effective hepatoprotection may result in improved outcome in a subset of dogs with various cancers treated with CCNU.

Dogs in this study were randomly assigned to 1 of 2 treatment groups. The treatment groups were similar with regard to patient characteristics, treatment characteristics, and pretreatment laboratory test results. Additional limitations to this study are the lack of placebo in the control group and lack of blinding of clinician as to which group each dog was assigned. The major endpoints of the study were laboratory test results, however, and the laboratory staff that performed the liver function tests were blinded to each dog's treatment group. Therefore, the results were subject to minimal bias.

The entry criteria for this clinical trial required that dogs had normal ALT activity to minimize the effects of pre-existing liver disease on study results. Dogs were not required to have completely normal liver function tests because this would have resulted in exclusion of a high number of dogs. ALT is the most specific liver enzyme that is routinely measured in the clinic and AST and ALKP may be affected by factors such as bone disease, muscular disease, endocrinopathies, and corticosteroid use. Additionally, past research suggests that increased ALT activity is the most important change in both preclinical models of CCNU toxicity and clinical patients receiving the drug.[9,10] Nonetheless, it is possible that some dogs with occult pre-existing liver disease were enrolled into the study, which may have affected results.

Corticosteroid use was allowed at the discretion of the treating veterinarian in this study. Because LSA and MCT are both routinely treated with corticosteroids, patient accrual would have been substantially affected if dogs on these drugs had been disallowed. The use of corticosteroids likely contributed to increased liver enzyme activity in many patients in this study, but both groups contained an equal number of dogs treated with similar doses of corticosteroids. ALKP was probably most affected because of the steroid isoenzyme with the other measured liver test results affected to a lesser degree. Center et al[26] previously conducted a study evaluating the effect of SAMe on the hepatic effects of prednisolone in dogs. SAMe was not found to influence serum biochemical results that typically increase because of prednisolone administration. The effect of silybin on steroid hepatopathy has not been investigated in the dog, however, and it remains possible that the less severe increases in liver enzyme activity in group A dogs occurred because of Denamarin protection against both CCNU and steroid hepathopathies.

Because SAMe and silybin both have antioxidant properties, some have expressed concern that these nutraceuticals could reverse the antitumor effects of chemotherapy. SAMe and silybin have not been specifically implicated as inhibitors of chemotherapy efficacy, but there is preclinical data to suggest that *N*-acetylcysteine can protect against carmustine-induced myelosuppression.[31] Additionally, a clinical case report exists suggesting that *N*-acetylcysteine combined with vitamin E may reverse the antimelanoma effects of fotemustine.[32] Although tumor response was not considered a primary endpoint of the study presented here, there was no statistical difference in tumor response between groups when looking at dogs treated for gross disease. Similarly, there was no difference in myelosuppression found between groups. Additional study is necessary to determine more definitively the effect of SAMe and silybin on CCNU's antitumor and myelosuppressive properties.

In this study, we chose to use a veterinary product that combines SAMe and silybin with known bioavailability in the dog. Because the combination was studied instead of individual products, it is not possible to determine which nutraceutical, if any, may be a more effective hepatoprotectant. Additional study into the individual effects of SAMe and silybin in this setting is warranted. Also, because nutraceutical content and bioavailability can vary substantially among products, the findings presented here cannot necessarily be applied to other formulations of SAMe and milk thistle extract.

In conclusion, the results of this study suggest that increases in liver enzyme activity are common in dogs

receiving CCNU and that clinicians should consider frequent liver-specific laboratory tests during therapy. The results also suggest that Denamarin coadministration should be considered in all dogs with cancer that are prescribed CCNU chemotherapy with or without corticosteroids. Treatment is likely to limit increases in liver enzyme activity in this population and increase the likelihood of a dog completing the prescribed course of therapy, which can lead to improved survival. Additional study into the role of the individual nutraceuticals contained in Denamarin in protection against CCNU hepatotoxicity is necessary.

Footnotes

[a] Griffin DW, Whalen MO, Filburn CR. Bioavailability of a novel formulation of s-adenosylmethionine in Beagle dogs. ACVIM Forum 2009 (abstract)
[b] Nutramax Laboratories Inc, Edgewood, MD
[c] Roche, Indianapolis, IL
[d] Stata 10.0, Stata Corporation, College Station, TX

Acknowledgments

Funding for this study was provided by Nutramax Laboratories Inc and by the Toni Wiebe Memorial Fund.

Conflicts of interest: This study was funded, in part, by Nutramax Laboratories Inc, which produces Denamarin. Dr Griffin is employed by Nutramax Laboratories Inc. Drs Skorupski and Hammond have accepted honoraria, reimbursement for travel expenses, or both for presenting the results of this research in abstract form or providing continuing education to veterinarians regarding the use of nutraceuticals, including Denamarin, in veterinary cancer patients.

References

1. Moore AS, London CA, Wood CA, et al. Lomustine (CCNU) for the treatment of resistant lymphoma in dogs. J Vet Intern Med 1999;13:395–398.

2. Williams LE, Rassnick KM, Power HT, et al. CCNU in the treatment of canine epitheliotropic lymphoma. J Vet Intern Med 2006;20:136–143.

3. Risbon RE, de Lorimier LP, Skorupski K, et al. Response of canine cutaneous epitheliotropic lymphoma to lomustine (CCNU): A retrospective study of 46 cases (1999–2004). J Vet Intern Med 2006;20:1389–1397.

4. Rassnick KM, Moore AS, Williams LE, et al. Treatment of canine mast cell tumors with CCNU (lomustine). J Vet Intern Med 1999;13:6010–6015.

5. Skorupski KA, Clifford CA, Paoloni MC, et al. CCNU for the treatment of dogs with histiocytic sarcoma. J Vet Intern Med 2007;21:121–126.

6. Viotte G, Lahouel M, Ducastelle T, et al. Hepatotoxicity of (chloro-2-ethyl)-1-cyclohexyl-3-nitroso-1-urea (CCNU) in the rat. Pathol Biol (Paris) 1986;34:40–47.

7. Ahmed AE, Grissom M, el-Azhary R, et al. Studies on the mechanism of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)-induced hepatotoxicity II. Biochemical and morphological characterization of the injury and its prevention by Phenobarbital. Cancer Chemother Pharmacol 1987;19:103–108.

8. Kretschmer NW, Boor PJ, el-Azhary RA, et al. Studies on the mechanism of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU)-induced hepatotoxicity III. Ultrastructural characterization of bile duct injury. Cancer Chemother Pharmacol 1987;19:109–117.

9. Henry MC, Davis RD, Schein PS. Hepatotoxicity of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in dogs: The use of serial percutaneous liver biopsies. Toxicol Appl Pharmacol 1973;25:410–417.

10. Hosoya K, Lord LK, Lara-Garcia A, et al. Prevalence of elevated alanine transaminase activity in dogs treated with CCNU (lomustine). Vet Comp Oncol 2009;7:244–255.

11. Kristal O, Rassnick KM, Gliatto JM, et al. Hepatotoxicity associated with CCNU (lomustine) chemotherapy in dogs. J Vet Intern Med 2004;18:75–80.

12. Khan S, Ramwani JJ, O'Brien PJ. Hepatocyte toxicity of mechlorethamine and other alkylating anticancer drugs. Role of lipid peroxidation. Biochem Pharmacol 1992;43:1963–1967.

13. Ducastelle T, Raguenez-Viotte G, Fouin-Fortunet H, et al. The hepatotoxicity of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in rats. Ultrastructural evidence of a delayed microtubular toxicity. Cancer Chemother Pharmacol 1988;22:153–162.

14. Friedel HA, Goa KL, Benfield P. S-adenosyl-L methionine: A review of its pharmacological properties and therapeutic potential in liver dysfunction and affective disorders in relation to its physiological role in cell metabolism. Drugs 1989;38:389–416.

15. Stramentinoli G. Pharmacologic aspects of S-adenosylmethionine. Pharmacokinetics and pharmacodynamics. Am J Med 1987;83:35–42.

16. Center SA, Warner KL, Erb HN. Liver glutathione concentrations in dogs and cats with naturally occurring liver disease. Am J Vet Res 2002;63:1187–1197.

17. Cederbaum AI. Hepatoprotective effects of S-adenosyl-L-methionine against alcohol- and cytochrome P450 2E1-induced liver injury. World J Gastroenterol 2010;16:1366–1376.

18. Webster CR, Cooper J. Therapeutic use of cytoprotective agents in canine and feline hepatobiliary disease. Vet Clin North Am Small Anim Pract 2009;39:631–652.

19. Wellington K, Jarvis B. Silymarin: A review of its clinical properties in the management of hepatic disorders. BioDrugs 2001;15:465–489.

20. Fraschini F, Demartini G, Esposti D. Pharmacology of silymarin. Clin Drug Investig 2002;22:51–65.

21. Salmi HA, Sarna S. Effect of silymarin on chemical, functional, and morphological alterations of the liver. Scand J Gastroenterol 1981;17:517–521.

22. Kidd P, Head K. A review of the bioavailability and clinical efficacy of milk thistle phytosome: A silybin-phosphatidylcholine complex (Siliphos®). Altern Med Rev 2005;10:193–203.

23. Comelli MC, Mengs U, Schneider C, Prosdocimi M. Toward the definition of the mechanism of action of silymarin: Activities related to cellular protection from toxic damage induced by chemotherapy. Integr Cancer Ther 2007;6:120–129.

24. Vogel G, Tuchweber B, Trost W, Mengs U. Protection by silibinin against *Amanita phalloides* intoxication in Beagles. Toxicol Appl Pharmacol 1984;73:355–362.

25. Ferenci P, Dragosics B, Dittrich H, et al. Randomized controlled trial of silymarin treatment in patients with cirrhosis of the liver. J Hepatol 1989;9:105–113.

26. Center SA, Warner KL, McCabe J, et al. Evaluation of the influence of S-adenosylmethionine on systemic and hepatic effects of prednisolone in dogs. Am J Vet Res 2005;66:330–341.

27. Filburn CR, Kettenacker R, Griffin DW. Bioavailability of a silybin-phosphatidylcholine complex in dogs. J Vet Pharmacol Therap 2007;30:132–138.

28. Santini D, Vincenzi B, Massacesi C, et al. S-adenosylmethionine (AdoMet) supplementation for treatment of chemotherapy-induced liver injury. Anticancer Res 2003;23:5173–5179.

29. Ladas EJ, Kroll DJ, Oberlies NH, et al. A randomized, controlled, double-blind, pilot study of milk thistle for the treatment of hepatotoxicity in childhood acute lymphoblastic leukemia (ALL). Cancer 2010;116:506–513.

30. Vail DM. Veterinary Co-operative Oncology Group-Common toxicity criteria for adverse events (VCOG-CTCAE) following chemotherapy or biologic antineoplastic therapy in dogs and cats v1.0. Vet Comp Oncol 2004;2:194–213.

31. El-Sayed el-SM, Abdel-Aziz AA, Helal GK, et al. Protective effect of *N*-acetylcysteine against carmustine-induced myelotoxicity in rats. Food Chem Toxicol 2010;48:1576–1580.

32. Webster GJ, Kurtovic J, Singh-Grewal I, et al. Letter to the editor: Prevention of hepatotoxicity but loss of antimelanoma effect with combined fotemustine and anti-oxidant treatment. Int Med J 2004;34:298–299.