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Hepatotoxicity Associated with CCNU (Lomustine) Chemotherapy in Dogs

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One hundred seventy-nine tumor-bearing dogs were treated with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) between 1995 and 2001. CCNU was given as a single dose of 50–110 mg/m^2 body surface area PO. Treatment interval varied, but the minimal interval between CCNU doses was 3 weeks. After treatment, 11 dogs (6.1%) developed hepatic toxicity. The median number of CCNU doses and the median total cumulative CCNU dose were significantly higher in dogs that developed hepatic toxicity (4 doses; 350 mg/m^2) than in dogs without hepatic damage (3 doses; 230 mg/m^2). Median duration to detection of hepatic toxicity from the last dose of CCNU was 11 weeks (range 2–49 weeks). Common biochemical abnormalities were abnormally high serum liver enzyme activities and hypoalbuminemia. Six dogs with CCNU-associated hepatic toxicity had ascites, and 3 dogs had concurrent pleural effusion. Serum concentrations of bile acids were abnormally high in 4 of 5 dogs tested. Percutaneous ultrasound-guided liver biopsies were performed in 10 dogs, and findings were nonspecific and chronic in nature. Seven dogs were euthanized because of progressive liver failure, and their median survival from diagnosis of liver disease was 9 weeks. Three dogs died of other causes and 1 dog of unknown cause. Although clinical signs resolved in 3 dogs, biochemical abnormalities and histopathologic lesions persisted 4 to 38 months from the time of diagnosis of liver disease. Our findings suggest that CCNU can cause delayed, cumulative dose-related, chronic hepatotoxicity that is irreversible and can be fatal.

Key words: Alkylating agent; Cancer; Canine; Liver; Neoplasia; Nitrosourea; Toxicity.

The oral nitrosourea alkylating agent 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), is completely and rapidly absorbed from the gastrointestinal tract. After absorption, CCNU undergoes rapid chemical degradation and enzymatic metabolism by hepatic microsomal enzymes.[1–3] Because it degrades rapidly, antitumor effects probably are produced by one of the metabolites rather than the parent compound.[2,4] Metabolites are excreted primarily by the kidneys, but biliary excretion and enterohepatic circulation also occur. CCNU and its degradation products have low molecular weights and are highly lipid soluble, 2 properties that facilitate their entry into the cerebrospinal fluid.[2]

In humans, CCNU is used mainly to treat primary and metastatic brain tumors and Hodgkin's lymphoma. Activity against non-Hodgkin's lymphoma, melanoma, colonic carcinoma, and small-cell lung cancer also has been reported.[5–9] Clinical reports describing the use of CCNU in dogs are limited. Responses were seen in dogs with brain tumors, mast cell tumors, and lymphoma.[10–12]

The dose-limiting toxicity of CCNU in humans is delayed myelosuppression, with nadirs of both platelets and granulocytes seen 3–5 weeks after administration.[6–8] Acute nausea and vomiting are common but can be diminished by pretreatment with antiemetics.[5–8] Nephrotoxicity can occur when large, cumulative doses of CCNU are given.[1,13] Finally, hepatotoxicity has been described rarely in people treated with CCNU but is not well characterized.[6–8] Another nitrosourea analog, 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU), has caused delayed, dose-related hepatic abnormalities in 26% of human patients.[14]

Preclinical toxicity data in dogs showed that CCNU caused severe bone marrow hypoplasia when used at dosages of 200–600 mg/m^2.[14–16] In tumor-bearing dogs receiving CCNU at 90 mg/m^2 PO, the acute dose-limiting toxicity is neutropenia with a nadir approximately 7 days after treatment. Thrombocytopenia is not observed after a single dose but appears to be a cumulative toxicity in some dogs.[11,12] Other toxicities documented in preclinical trials of CCNU in dogs included gastrointestinal toxicity at high doses, delayed liver damage, and rare renal toxicity.[14,15,17,18] Liver failure was documented in one of the previously reported tumor-bearing dogs treated with 10 doses of CCNU.[11] Since that report, the investigators of the present study have observed hepatotoxicity in additional dogs treated with CCNU.

The aims of this retrospective study were to describe the clinical, biochemical, and histopathologic abnormalities, as well as the outcomes of tumor-bearing dogs with CCNU-induced hepatotoxicity. Another objective was to identify risk factors for development of CCNU-induced hepatotoxicity.

Materials and Methods

Medical records of 303 dogs that were treated with CCNU (Lomustine[a]) at the Harrington Oncology Program between January 1995 and June 2001 were reviewed. These records included dogs from pre-

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vious reports on the use of CCNU.[11,12] CCNU was given as a single dose of 50–110 mg/m^2 body surface area PO. Treatment interval varied depending on the primary disease, response to treatment, and treatment protocol. The minimal interval between CCNU doses was 3 weeks.

Inclusion criteria for this study were: treatment with CCNU, no clinicopathologic signs of hepatic disease before treatment with CCNU, and at least 1 biochemistry profile available after CCNU treatment was initiated. Dogs that met these inclusion criteria were further subdivided into 2 groups. Group 1 was comprised of tumor-bearing dogs that did not develop clinical or biochemical signs of hepatic damage. Group 2 was comprised of dogs that developed hepatic abnormalities after initiation of CCNU treatment, had no evidence of neoplastic disease in the liver, and had hepatic histopathologic abnormalities that were not compatible with changes caused by other concurrent hepatotoxic drugs.

Signalment, primary neoplastic disease, concurrent medications, number of CCNU doses, and cumulative CCNU dose (mg/m^2 body surface) were recorded for all dogs. Dose intensity (mg/m^2/wk) was calculated for dogs with CCNU treatment intervals of ≤3 months. Additional data recorded for dogs with CCNU-induced hepatic toxicity included clinical signs at the time hepatotoxicity was recognized, primary neoplastic disease status at the time of toxicity, clinical pathology results, diagnostic imaging, histopathologic findings from percutaneous liver biopsy and postmortem examination, and outcome. All dogs with CCNU-induced liver toxicity had abdominal ultrasonographic findings at the time of diagnosis of liver disease. One pathologist (JMG) reviewed all liver histopathology slides.

Time to detection of hepatotoxicity was calculated in weeks from the last dose of CCNU to the date of the biochemistry profile documenting hepatic toxicity. Survival was calculated in weeks from the date of the 1st abnormal biochemistry profile that led to diagnosis of hepatotoxicity until death.

Factors examined for their potential influence on risk of developing hepatotoxicity included age, body weight, number of CCNU treatments, cumulative CCNU dose, CCNU dose intensity, breed (mixed breed versus purebred), disease (lymphoma versus mast cell tumor versus other), sex (male versus female), initial CCNU dosage (≥90 mg/m^2 versus <90 mg/m^2), concurrent medications (yes versus no), and prophylactic use of trimethoprim-sulfadiazine (TMS; yes versus no). Comparison of categorical variables between dogs with and without hepatotoxicity were made by the chi-square test of independence or the Fisher's exact test when cell values were <5. Continuous variables were compared by the Wilcoxan rank sum test (for non-Gaussian data) or the Student's *t*-test (for Gaussian data).[19] Statistical significance was set at $P \leq 0.05$.

Results

One hundred seventy-nine dogs treated with CCNU met the inclusion criteria of the study. One hundred sixty-eight dogs were included in group 1. Eleven dogs (6.1%) developed CCNU-induced hepatotoxicity and were included in group 2. The number of CCNU doses given to all dogs was as follows: a single dose (n = 45) and 2 (35), 3 (34), 4 (20), 5 (12), 6 (8), 7 (11), 8 (5), 9 (2), 10 (3), 11 (1), and 12 doses (3). Results of age, weight, primary disease, number of CCNU doses, cumulative CCNU dose, dose intensity, and use of prophylactic TMS for dogs in both groups are given in Table 1. There were 43 mixed breed and 125 purebred dogs in group 1. Group 2 included 1 mixed breed and 10 purebreds. Males were slightly overrepresented in both groups (53% in group 1 and 55% in group 2). A biochemistry profile was done within 1 month of the last CCNU dose in 111 dogs, within 2 months in 54 dogs, 3 months in 23 dogs, 4 months in 16 dogs, 5 months in 12 dogs, 6 months in 6 dogs, 7 months in 4 dogs, 8 months in 7 dogs, 9 months in 4 dogs, 10 months in 2 dogs, 11 months in 1 dog, and 12 months or longer in 4 dogs. Some dogs had more than 1 biochemistry profile after CCNU treatments.

Table 1. Signalment, primary disease, and CCNU treatments for dogs without (group 1) and with (group 2) CCNU-induced hepatotoxicity.

Parameter	Group 1 (n = 168)	Group 2 (n = 11)
Age (years)		
Median	9	7.5
Range	1–15	6–13
Weight (kg)		
Median	33	27
Range	4–69	6–49.5
Primary disease		
Lymphoma	99	4
MCT	50	5
BT	8	1
MH	5	1
Melanoma	2	
Metastatic carcinoma	2	
ALL	2	
No. of CCNU doses		
Median	3	4
Range	1–12	2–10
Cumulative CCNU dose (mg/m^2)		
Median	230	350
Range	70–1,080	180–920
Dose intensity (mg/m^2/wk)[a]		
Median	22	25
Range	7–31 (n = 97)	15–30 (n = 11)
Prophylactic TMS[b]	104 (62%)	9 (82%)

MCT, mast cell tumor; BT, brain tumor; MH, malignant histiocytosis; ALL, acute lymphoblastic leukemia; TMS, trimethoprim-sulfadiazine.

[a] Calculated for dogs with treatment intervals of ≤3 months.

[b] Number of dogs receiving oral prophylactic TMS for 7 days starting 5–7 days after ≥1 dose of CCNU.

There was no statistically significant difference between the 2 groups with respect to age, weight, breed, sex, and primary neoplastic disease. Similarly, there was no significant difference with respect to initial CCNU dosage, CCNU dose intensity, concurrent medications, and use of prophylactic TMS at the time of CCNU treatment. Both number of CCNU doses and cumulative CCNU dose were significantly higher in dogs with CCNU-induced hepatotoxicity (group 2) compared to dogs without hepatotoxicity ($P = 0.044$ and 0.034, respectively; Table 1).

Of the 11 dogs with CCNU-induced hepatotoxicity, 2 were Boxers and 1 each was a Shar-Pei, Bassett Hound, Pointer, Cocker Spaniel, Staffordshire Bull Terrier, Labrador Retriever, Siberian Husky, Miniature Schnauzer, and mixed breed. CCNU was given at a dosage of 90 mg/m^2 every 3–4 weeks in 10 dogs and every 6 weeks in 1 dog.

Two dogs received reduced dosages of 60 and 80 mg/m^2 after the 1st treatment because of severe neutropenia (<500 cells/μL) at the nadir. Concurrent medications were administered to most dogs at the time of CCNU treatments; 9 dogs had received TMS, 6 dogs prednisone, 3 dogs enrofloxacin, 2 dogs cimetidine, 1 dog famotidine, and 1 dog enalapril. Only 5 dogs were receiving other medications at the time of diagnosis of hepatotoxicity; 4 dogs were receiving prednisone, 2 dogs cimetidine, 1 dog ranitidine, 1 dog famotidine, and 1 dog enalapril. Dogs received between 2 and 10 doses of CCNU before detection of hepatotoxicity. The median cumulative CCNU dose was 350 mg/m^2 (range 180–920 mg/m^2), and the median dose intensity was 25 mg/m^2/wk (range 15–30 mg/m^2/wk). Time to detection of hepatotoxicity ranged from 2 to 49 weeks (median 11 weeks). At the time of diagnosis of hepatotoxicity, remission status of the primary neoplastic disease was judged to be complete (100% clinical resolution) in 8 dogs, partial (>50% but <100%) in 2 dogs, and stable in 1 dog.

Before receiving CCNU, all 11 dogs with hepatotoxicity were asymptomatic. Predominant clinical signs of hepatotoxicity were decreased appetite (n = 8), weight loss (5), polyuria/polydipsia (4), occasional vomiting (4), and abdominal distention (3). One dog did not show any clinical signs, but abnormally high serum liver enzyme activities were detected on routine follow-up biochemistry, and serum bile acid concentrations were abnormal. Histopathology led to the diagnosis of CCNU-induced hepatotoxicity, and the drug was discontinued.

Before CCNU treatments, liver and renal clinicopathologic data were judged to be normal in 10 dogs. One dog with lymphoma had renal failure secondary to hypercalcemia. The most common biochemical abnormalities at the time of hepatotoxicity were abnormally high serum activities of alkaline phosphatase (ALP; n = 10, median 1,431 U/L, range 441–10,164 U/L, reference range 20–320 U/L), alanine transaminase (ALT; n = 10, median 1,036 U/L, range 137–10,105 U/L, reference range 10–95 U/L), aspartate transaminase (AST; n = 11, median 131 U/L, range 54–1,333 U/L, reference range 15–52 U/L), γ-glutamyl transferase (GGT; n = 8, median 40 U/L, range 23–416 U/L, reference range 1–10 U/L), and hypoalbuminemia (n = 7, median 2.7 g/dL, range 1.7–2.9 g/dL, reference range 3–4.2 g/dL). Hyperbilirubinemia (n = 4, median 1.3 mg/dL, range 0.6–1.6 mg/dL, reference range 0.1–0.5 mg/dL), hypercholesterolemia (n = 4, median 594 mg/dL, range 367–1,178 mg/dL, reference range 110–314 mg/dL), and decreased blood urea nitrogen (BUN; n = 3, median 6 mg/dL, range 6–7 mg/dL, reference range 8–33 mg/dL) were seen less commonly. Four dogs had mildly increased serum creatinine concentrations (median 2.3 mg/dL, range 2.1–3.4 mg/dL, reference range 0.5–1.5 mg/dL), and 3 of the 4 dogs had concurrently increased BUN (median 61 mg/dL, range 36–62 mg/dL, reference range 8–33 mg/dL). One of the 4 dogs with azotemia had pre-existing renal failure, whereas azotemia was a new finding in the other 3 dogs. Serum globulin concentrations were normal in all 11 dogs.

Abnormalities of CBC at the time of hepatotoxicity included mature neutrophilia in 4 dogs (12.0–15.5 × 10^3 cells/μL, reference range 3.0–11.5 × 10^3 cells/μL), mild anemia in 4 dogs (hematocrit 32–36%, reference range 37–55%), and mild thrombocytopenia in 4 dogs (103–195 × 10^3 cells/μL, reference range 200–500 × 10^3 cells/μL).

Urinalyses were performed on 8 of 11 dogs. Isosthenuria (n = 3) or hyposthenuria (3) were common, but concurrent renal failure (3), furosemide (2), or prednisone (2) therapy made interpretation of the results difficult. Urine protein was negative to trace in all 8 dogs, including the 7 dogs with hypoalbuminemia, and urine protein : creatinine ratio was <1 in 4 dogs in which it was tested. One additional dog developed hypoalbuminemia 8 weeks after diagnosis of liver disease, and urinalysis disclosed a protein concentration of 30 mg/dL with urine specific gravity of 1.043. Four dogs had glucosuria without hyperglycemia. Three had concurrent renal failure, and the 4th developed renal failure 4 months later. One dog with renal failure also had evidence of a urinary tract infection on urine culture, but renal culture was negative.

Five dogs had fasting and 2-hour postprandial serum bile acids (SBA) measured. Both concentrations were abnormally high in 4 dogs (fasting range 19–141 μmol/L, postprandial range 54–166 μmol/L) and were within the normal range in 1 dog (<15 μmol/L). Prothrombin time and activated partial thromboplastin time were within normal reference range in 8 dogs that were tested.

At the time of hepatotoxicity, abdominal ultrasonography was performed in all 11 dogs, and thoracic radiographs were obtained for 6 dogs. The liver appeared enlarged in 1 dog, decreased in size in 3 dogs, and was normal in size in the remaining 7 dogs. Echogenicity of the liver was nonhomogeneous in 4 dogs, diffusely increased in 2 dogs, diffusely decreased in 1 dog, and normal in 4 dogs. Six dogs had evidence of abdominal effusion. Three of the 6 dogs with abdominal effusion had concurrent pleural effusion. Echocardiograms showed normal cardiovascular function in the 3 dogs with pleural effusion and in 1 additional dog with exercise intolerance and abdominal effusion. Fluid analysis was performed on peritoneal (n = 5) and pleural (3) effusions in all 6 dogs, and results were consistent with a pure transudate. All 6 dogs with effusions had hypoalbuminemia, which was only mild in 5 dogs (range 1.7–2.9 g/dL).

Percutaneous ultrasound-guided liver biopsies[b] were obtained in 10 dogs. One dog receiving CCNU to treat a brain tumor (with no concurrent medications) developed hyperbilirubinemia, hypoalbuminemia, ascites, and abnormally high serum bile acid concentrations but did not have a biopsy performed. Histopathologic abnormalities were mostly nonspecific and chronic in nature. The most common findings were multifocal sinusoidal and portal aggregates of hemosiderin-laden Kupffer cells (n = 10), hepatocytes with karyomegaly (8), multifocal mild to moderate cytoplasmic hepatocellular vacuolization (6), mild to moderate neutrophilic and lymphoplasmacytic periportal inflammation (5), bridging fibrosis (3), and mild bile duct hyperplasia (1). There was no evidence of neoplasia in any of the liver biopsy specimens.

Overall, median survival after diagnosis of hepatotoxicity for all 11 dogs was 15 weeks (range 2–167 weeks). Seven dogs were euthanized because clinical signs associated with liver disease progressed 2–42 weeks after diagnostic biochemistry (median 9 weeks). These dogs were the 6 with

effusions and included all 4 dogs with newly diagnosed renal failure and glucosuria. Two dogs died from progressive lymphoma 15 and 23 weeks after diagnosis of hepatotoxicity, and 1 dog died of an unrelated cause after 167 weeks. The remaining dog with a solitary pulmonary histiocytic tumor had partial remission after treatment with CCNU and was asymptomatic, but abnormally high serum liver enzyme activities were documented on a routine biochemistry profile after 2 doses of CCNU. Abnormally high serum bile acid concentrations and lack of ultrasonographic and histopathologic evidence of neoplasia in the liver led to a diagnosis of CCNU-induced hepatotoxicity, and the drug was discontinued. The dog then developed progressive histiocytic disease in the lungs and was euthanized 8 weeks later with evidence of hepatomegaly (with no visible masses) and abdominal effusion. Because a postmortem examination was not performed, it is not known whether the clinical signs before euthanasia were caused by progression of hepatotoxicity induced by CCNU or by progression of the neoplastic disease.

Of the 10 dogs with clinical signs of hepatotoxicity, 7 were euthanized because of liver disease, and signs resolved in the remaining 3 dogs 10–39 days after they developed. Biochemical liver abnormalities, although improved with time, persisted until death in all 3 dogs (15, 23, and 167 weeks from diagnosis). Serum bile acid concentrations remained abnormal in one of these dogs when retested 43 and 95 weeks after diagnosis of hepatotoxicity.

Three dogs had postmortem examinations 3, 23, and 42 weeks after diagnosis of hepatotoxicity, and 1 dog had another liver biopsy 43 weeks after diagnosis. Histopathologic hepatic lesions were persistent and stable in 3 dogs and progressive in 1 dog. The stable changes included increased numbers of sinusoidal and portal aggregates of hemosiderin-laden Kupffer cells (n = 4), hepatocytes with karyomegaly (3), and mild (1) to moderate (1) neutrophilic and lymphoplasmacytic periportal inflammation. The postmortem examination of 1 dog 42 weeks after diagnosis of hepatotoxicity had more severe and extensive portal and bridging fibrosis, as well as areas of acute inflammation, hepatocellular necrosis, and fibrin deposits. The more acute findings were thought to be caused by terminal sepsis or disseminated intravascular coagulopathy.

Discussion

Clinicopathologic evidence of hepatic toxicity was documented in 6.1% (11 of 179) of tumor-bearing dogs treated with varying numbers of CCNU treatments at the hospital from 1995 to 2001. This incidence rate unfortunately is limited by the retrospective nature of the study and could be higher. For example, 124 of 303 dogs treated with CCNU were excluded from analysis because of insufficient follow-up. Also, evaluation of biochemical profiles from dogs treated with CCNU was not standardized. Dogs categorized into group 1 (no hepatotoxicity) might have had only 1 biochemistry profile evaluated 1 month after the last CCNU treatment, and clinical or pathologic changes consistent with hepatotoxicity could have occurred later but were not detected or not reported. Prospective evaluation of dogs treated with CCNU with rigorous monitoring of biochemical profiles and liver function tests is warranted to accurately determine the incidence of hepatic toxicity.

Preclinical studies of CCNU have shown the drug to be a potent hepatotoxin in several species, including rats, dogs, and monkeys.[14,15,17,18,20–24] When rats were given single high doses of CCNU PO, hepatic lesions occurred soon after treatment and consisted primarily of bile duct injury with associated cholestasis. Focal hepatocyte necrosis occurred later and was mainly periportal, suggesting hepatocyte injury was secondary to bile duct injury.[20,21] Ultrastructural changes included alterations of microtubules affecting all hepatocytes and bile canaliculi.[22]

Preclinical studies in dogs receiving CCNU dosages ranging from 40 to 200 mg/m^2 (given all at once or divided over several days) revealed the dose-limiting adverse effect to be hematopoietic toxicity. Dogs treated with lower dosages survived hematopoietic toxicity but exhibited hepatic toxicity, renal toxicity, or both.[14,15] Abnormally high serum ALT activity was seen starting at a CCNU dosage of 40 mg/m^2. The clinically important feature of CCNU-induced hepatotoxicity in dogs was its dose-related delay in onset, because there was an inverse relationship between the size of the dose and the length of time that elapsed before abnormal serum ALT activity was detected. Hepatotoxicity occurred as late as 1 month after CCNU treatment. Liver function abnormalities were reversible at lower dosages, but histopathologic changes were persistent. At higher dosages, biochemical abnormalities also were irreversible.[14,15] Henry and Schein[18] gave normal male Beagles a single 75–85 mg/m^2 dose of CCNU PO and monitored serum biochemistry and serial percutaneous liver biopsies for up to 3 months. None of the dogs showed any clinical signs of hepatotoxicity. Serum ALT activity was increased in all dogs, starting 2 weeks after treatment, and it returned to normal by 2 months. Histopathologic changes first were identified 3–4 weeks after treatment with CCNU and persisted throughout the observation period. Changes were inflammatory in nature and included Kupffer cell hyperplasia, periportal fibrosis, and small foci of individual hepatocyte necrosis with no disruption of the normal lobular architecture of the liver. Liver samples obtained during postmortem examination showed similar changes.[18]

The mechanism of hepatic injury caused by CCNU is not clearly understood, but several mechanisms have been proposed. After absorption PO, CCNU undergoes rapid spontaneous decomposition, as well as microsomal enzymatic metabolism to yield chloroethyl moieties that have alkylating activity and cyclohexyl isocyanates, which can carbamylate amino groups of proteins.[3,25] The role of carbamylation in antitumor activity or normal tissue toxicity is not clear. Metabolites that have long half-lives in plasma and tissues might be responsible for the delayed toxic effects.[14] Alternatively, although the drug and its metabolites are excreted primarily in the urine, biliary excretion and enterohepatic circulation also might occur.[2,14] High concentrations of toxic metabolites in the bile could be responsible for the hepatotoxic effects of CCNU.[2,14] Another possible explanation is that isocyanates carbamylate tubulin in hepatocytes, leading to mitotic spindle inhibition and G2 cell cycle arrest or to disruption of secretory functions such as bile secretion, which in turn results in cholestasis and sec-

ondary hepatocyte injury.[22,24] Finally, alkylating agents cause depletion of glutathione, lipid peroxidation, and cytotoxicity in isolated rat hepatocytes.[26] Use of different thiol compounds shortly after administration of alkylating agents reduced cytotoxicity. Also, use of antioxidants such as butylated hydroxyanisole, α-tocopherol, or the iron chelator desferoxamine prevented lipid peroxidation and delayed hepatocyte cytotoxicity.[26]

In the present study, median time to diagnosis of hepatotoxicity was 11 weeks after the last CCNU dose. When compared to dogs without hepatotoxicity, tumor-bearing dogs with hepatotoxicity received significantly more CCNU doses (4 versus 3) and a significantly higher total cumulative CCNU dose (350 mg/m^2 versus 230 mg/m^2). These findings suggest that CCNU-induced hepatotoxicity is delayed and might be related to cumulative dose. However, because of the small sample size, further studies with additional dogs are needed to substantiate these findings.

Most dogs in group 2 showed clinical signs that prompted clinical evaluation and diagnosis of hepatic toxicity. Biochemical abnormalities reflected chronic hepatotoxicity, with increased serum activities of liver enzymes and hypoalbuminemia being the most common findings. The majority of the dogs had abnormally high serum activities of all 4 measured enzymes, but serum ALT activity was the highest in most dogs, with the median value 11 times the upper limit of the normal reference range. This finding is in agreement with Henry and Schein[18] and suggests primary hepatocellular injury. Four of the 5 dogs tested had abnormal SBA concentrations, with a pattern most consistent with that of intrahepatic cholestasis.[27] Ascites was a common finding in the dogs with hepatotoxicity. Fluid analysis compatible with a pure transudate supports hepatic portal hypertension as opposed to posthepatic portal hypertension, which results more commonly in a modified transudate.[28] Five of the 6 dogs with ascites had only mild hypoalbuminemia (2.4–2.9 g/dL), which could not have been the sole cause of the effusions.[28] Three dogs had concurrent pleural effusion with the same characteristics as the ascitic fluid. All 3 dogs lacked cardiovascular, pulmonary, or thoracic cavity diseases that could explain pleural effusion. Simultaneous pleural and peritoneal effusion is rare in dogs but has been described previously in 2 dogs with liver disease (cirrhosis and chronic active hepatitis).[29] Hepatic hydrothorax occurs in 4–10% of humans with hepatic cirrhosis accompanied by ascites. The most likely mechanism of hepatic hydrothorax is movement of ascitic fluid into the pleural cavity through small defects (usually <1 cm) in the diaphragm. The diaphragmatic defects are formed because of increased intra-abdominal pressure. Fluid moves into the thoracic cavity as a result of an intrathoracic : peritoneal pressure gradient.[30] Although none of the dogs in this study had histologic evidence of cirrhosis, hepatic hydrothorax secondary to increased intra-abdominal pressure is a possible explanation for the presence of a transudative pleural effusion in the 3 dogs.

Histopathologic findings were mild to moderate and supportive of chronic hepatopathy with minimal hepatocellular necrosis, occasional inflammation, and bridging fibrosis. These findings were similar to the histopathologic changes described by Henry and Schein,[18] although periportal fibrosis was less common in these dogs. One possible explanation for this difference is that the needle biopsy liver specimens were too small and did not include enough portal triads, thereby underestimating the severity of the histopathologic lesions or missing unevenly distributed lesions, as suggested in a recent study.[31] The histopathology reported by Henry and Schein,[18] however, also was obtained with percutaneous needle biopsy specimens. Additionally, 3 dogs had larger biopsy specimens obtained at postmortem examination, and the lesions were similar in 2 dogs 3 and 23 weeks after the original biopsy and more severe in 1 dog 42 weeks after the original biopsy. Lack of marked bile duct injury, cholestatic damage, or biliary precirrhotic changes as described in rats might be explained by species differences, dose differences, or possibly, insufficient size of the percutaneous needle biopsy specimens in some of these dogs.[20–24]

Four of the 11 dogs with CCNU-induced hepatotoxicity also developed glucosuria and renal failure. Because of the small number of dogs with renal failure and lack of further clinicopathologic and histologic information, further conclusions could not be made, but such findings could represent CCNU toxicity.

Many dogs in this study were receiving concurrent medications, either at the time of CCNU treatment or at the time hepatotoxicity was diagnosed. Nine of the 11 dogs in group 2 received TMS for 5–7 days after 1 or more CCNU treatments. The percentage of dogs treated with TMS was not statistically different between the 2 groups. TMS has been reported to be hepatotoxic, but it usually causes acute hepatotoxicity, with diffuse and sometimes massive hepatocellular necrosis being a common feature.[32] Most dogs develop toxicity while receiving the antibiotic. The dogs in this study were not receiving TMS for 3 weeks or longer at the time hepatotoxicity was documented, and diffuse necrosis was not seen in any of the liver biopsy specimens. Therefore, TMS is unlikely to have contributed to liver disease in these dogs. Four dogs were receiving prednisone at the time of hepatotoxicity. Clinical, biochemical, and histopathologic abnormalities usually are different with steroid hepatopathy.[33] Cimetidine inhibits microsomal enzymes and impairs metabolism of other drugs.[34] It augments myelosuppression in people treated with BCNU and CCNU, drugs that undergo microsomal detoxification.[35,36] Two dogs in this study (group 2) received cimetidine concurrently with CCNU. Cimetidine might have enhanced CCNU-induced hepatotoxicity in these dogs.

The majority of dogs with CCNU-induced hepatotoxicity died from progressive liver disease. As in the preclinical trials, even in dogs that had resolution of their clinical signs, serum biochemistry abnormalities, SBA abnormalities, and histopathologic lesions persisted for 15–167 weeks after diagnosis of liver disease.[14,17,18] These findings reinforce previous data that CCNU causes irreversible hepatotoxicity that can progress and result in death weeks to months after diagnosis.

Results of this study suggest a delayed, cumulative, dose-related, and irreversible chronic hepatotoxicity associated with administration of CCNU to dogs. Future prospective studies are necessary to determine the true incidence, identify additional risk factors, and evaluate the efficacy of

monitoring regimens and possibly hepatoprotective agents in preventing CCNU-induced hepatotoxicity in tumor-bearing dogs.

Footnotes

[a] CCNU, CeeNU, Bristol-Myers Squibb Co, Princeton, NJ

[b] Bard 18g Biopty-cut biopsy needle, Bard Urological Division, Covington, GA

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