J Vet Intern Med 2006;20:1384–1388

Single Agent Gemcitabine Chemotherapy in Dogs with Spontaneously Occurring Lymphoma

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Background: Gemcitabine has been shown to be effective as a single agent in a variety of tumors including nonHodgkin's lymphoma. Its use in veterinary medicine has been limited and to date this drug has not been used as a first-line therapy in dogs with lymphoma.

Hypothesis: Gemcitabine as a single agent may be efficacious in dogs presented for the first time with lymphoma.

Animals: Twenty-four dogs with spontaneously occurring lymphoma.

Methods: All dogs were clinically staged and given gemcitabine at 400 mg/m^2 over a 30-minute intravenous infusion weekly for 3 weeks and then given 1 week off treatment before starting a second cycle.

Results: A single dose of gemcitabine lowered both neutrophil count (decrease in mean neutrophil count from 10,640 cells/μL to 3,140 cells/μL) and platelet count (decrease in mean platelet count from 201,290 cells/μL to 139,190 cells/μL) 7 days after administration. Consequently gemcitabine dosage was reduced at the second treatment in 8 of 21 dogs or a dose delay of 1–7 days and a reduction of dosage was used in 7 of 21 dogs. Seven dogs completed the assigned 4-week cycle. Two of these dogs had progressive disease and 5 had stable disease. No objective responses were seen in dogs treated with a second cycle of gemcitabine.

Conclusions and Clinical Importance: Gemcitabine administration as a single agent resulted in hematologic toxicity and did not reduce lymphoma burden. If gemcitabine is to be used in veterinary medicine, additional prospective pharmacologic studies should be done to determine the appropriate dosage, regimen, and schedule of use before it can be recommended for use in the treatment of dogs with lymphoma as a single agent.

Key words: Anticancer agents; Chemotherapy; Gemzar; Lymphosarcoma; Nucleoside analog.

Gemcitabine HCl[a] is a cell cycle phase specific nucleoside analog that mimics the pyrimidine base cytosine and is classified as an antimetabolite.[1] It primarily kills cells undergoing DNA synthesis and also blocks the progression of cells through the G_1-S phase checkpoint. It is metabolized intracellularly to the monophosphate form 2′,2′-difluorodeoxycytidine monophosphate (dFdCMP) by deoxycytidine kinase. Nucleoside kinases convert dFdCMP to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides.[1,2] The cytotoxic effects of gemcitabine are attributed to a combination of the two actions of the diphosphate and the triphosphate nucleosides. These include inhibition of DNA synthesis and masked-chain DNA termination. Cellular increases in the diphosphate and triphosphate nucleosides also contribute to cell death.

Cozzi et al[3] investigated intravesical gemcitabine to establish the toxicology and pharmacokinetics necessary for clinical trials. At all intravesical doses, significant systemic absorption was seen. The plasma half life of gemcitabine in the dog is short (1.4 hours) and the area under the curve (AUC) is one-half that observed with an IV infusion.[4] Plasma protein binding is negligible and the major route of elimination is via the urine in 24 hours.

Gemcitabine is approved for use in human beings for inoperable, locally advanced, or metastatic non-small-cell lung cancer[5–7] and locally advanced or metastatic adenocarcinoma of the pancreas.[8–10] Other reports indicate it may have value for relapsed B cell chronic lymphocytic leukemia,[11–13] nonHodgkin's lymphoma,[14–16] advanced hepatocellular carcinoma,[17] bladder cancer,[18] advanced stage ovarian cancer,[19] breast cancer,[20–22] and as a radiosensitizer.[23]

The value of gemcitabine in veterinary oncology has yet to be determined. There have been limited studies evaluating the maximally tolerated dose in tumor-bearing dogs. Cozzi et al[3], demonstrated that animals receiving 1 g of intravesical gemcitabine had severe systemic toxicity. Postmortem examination in these animals disclosed multiorgan toxicity including severe bone marrow hypoplasia, transmural hemorrhage, and necrosis in the small intestine and severe ulcerative hemorrhagic cystitis. Recently, its use as a single agent in spontaneous canine malignancies[24] or as a radiosensitizer in head and neck tumors[25] has been reported. The objective of this study was to evaluate the safety and efficacy of gemcitabine as a single agent in dogs with spontaneously occurring lymphoma.

Material and Methods

Dogs presented with histologically confirmed lymphoma were entered into a prospective, open-label, multi-institutional study to evaluate the safety and efficacy of gemcitabine as a single chemotherapeutic agent in the treatment of lymphoma. Inclusion

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Submitted October 16, 2005; Revised March 24, 2006; Accepted May 16, 2006.

0891-6640/06/2006-0015/$3.00/0

criteria for this study included informed owner consent, a recent diagnosis of lymphoma of any stage or grade, clinically measurable disease, and adequate organ function including bone marrow reserve (absolute neutrophil count of >3,000 cells/μL; platelet count >100,000/μL), hepatic transaminase activities (aspartate aminotransferase [AST] and alanine transferase [ALT] not to exceed three times the upper limit of normal), and adequate renal function (serum creatinine concentration <3 mg/dL). Urine specific gravity was not considered in evaluation of renal function because prerenal azotemia would exclude a patient from this study. Exclusion criteria included any concurrent disease state that would require additional therapy and potentially result in life expectancy of <6 months, active systemic infection, pregnancy, previous treatment with any chemotherapeutic agent for lymphoma, systemic treatment with glucocorticoids during the 2 months before entry in the study, previous radiation therapy to target lesions or any previous hormonal, immunologic, or biologic therapy directed at the lesions.

All dogs were staged according to World Health Organization guidelines[26] with a CBC, serum biochemistry, abdominal ultrasonography, thoracic radiography (3 views), abdominal radiography (2 views), bone marrow aspirate and cytology, bone marrow core biopsy, and lymph node core biopsy.

To quantify total lymphoma burden for each patient, measurement of all lesions were taken and scored. This measurement then was recorded as a comprehensive lesion measurement (CLM). Measurements were done by the most appropriate method: palpation, radiography, ultrasonography, or computed tomography as determined by the clinician. Calipers or a metric ruler were used to determine the dimensions of all identified lesions. All abnormal findings (enlarged lymph nodes, organomegaly, cutaneous lesions) were documented and measured in 2 dimensions. This measurement then was expressed as a product. All measurements then were summated to give the CLM for that patient. This value was used to determine the total disease burden of each patient and to determine response to treatment. Tumor response was calculated by dividing the CLM of each individual patient after treatment by the CLM taken before the first treatment with gemcitabine. This value was expressed as a percentage of tumor response. As disease responds to therapy, this percentage response approaches zero (no clinical evidence of disease). In this study, complete response (CR) was defined as a tumor response equal to 0%. Partial response (PR) was defined as tumor response ≤50% with no increase in size of any previously documented lesion or any new lesion development. Stable disease (SD) was defined as tumor response between 51 and 124% with no increase in size of any previously documented lesion or any new lesion development. Progressive disease (PD) was defined as tumor response >125%. Response to treatment or lack thereof was assessed only after the full cycle (3 doses of gemcitabine) was administered.

After clinical staging, all dogs were treated with gemcitabine at an initial dosage of 400 mg/m^2 delivered over a 30-min IV infusion. 200-mg vials were reconstituted with 5 mL of 0.9% NaCl. This dilution yielded a gemcitabine concentration of 38 mg/mL. A treatment cycle consisted of a single gemcitabine dose repeated weekly for 3 weeks followed by 1 week of rest. A total of 4 cycles were planned for each patient. Physical examinations, CBC, and serum biochemistry were performed weekly during the study period.

Toxicity and clinical response to treatment was scored on a modified version of the Eastern Cooperative Oncology Group (ECOG)[27] performance grading scheme (Table 1). According to Food and Drug Administration (FDA) study guidelines, performance grades ≥2 resulted in a gemcitabine dosage reduction to 300 mg/m^2 to maintain weekly administration schedule of the drug. When determined by the clinician, a treatment delay of <7 days also could be instituted if the investigator deemed it to be in the patient's best interest. If treatment was delayed, a CBC was repeated within 1–6 days as determined by the clinician and a reduced dosage of 300 mg/m^2 was administered if an acceptable performance score was obtained at the next follow-up examination. If a treatment was delayed for >7 days, the scheduled dose of gemcitabine was omitted for that week.

Statistical Methods

CBC results were analyzed among various sample collections periods (eg, before each gemcitabine administration) using a paired test and ANOVA.

Results

Twenty-four dogs were considered eligible for treatment in this study. Twenty-one of the 24 dogs presented for treatment had complete hematologic data available for review on day 7. Of the 3 dogs excluded from evaluation, 1 dog never returned for follow up, 1 dog returned at 11 days because of poor owner compliance

Table 1. Modified Version of the ECOG[a] Performance Grading Criteria.

	0	1	2	3	4
Hematologic Toxicity Score					
Neutrophils cells/μl	≥ 3000	1,500–2,999	1,000–1,499	500–999	≤ 499
Platelets/μl	≥ 200,000	100,000–199,000	50,000–99,999	15,000–49,999	≤ 14,000
Creatinine mg/dl	≤ 1.5	1.6–3.0	3.1–4.0	4.1–5.0	≥ 5.1
Non–Hematologic Toxicity Score					
Hemorrhage	None	Mild	Moderate	Debilitating	Life Threatening
Infection	None	Mild/No reaction	Moderate/Required Rx	Debilitating	Life Threatening
Vomiting	None	Sporadic/Self limiting	Frequent	Intractable Continuous	—
Diarrhea	None	Mild	Moderate	Severe	—
Body Temp (°F)	< 102	102–102.9	103–103.9	104–104.9	> 104.9
Body Temp (°C)	< 38.9	38.9–39.4	39.5–39.9	40.0–40.5	> 40.5
Injection site reaction	None	Pain	Phlebitis	Ulceration	Slough
Alopecia	None	Mild	Moderate	Severe	—
Performance Status					
Grade	0	1	2	3	4
Criteria	Fully active	Restricted	Severely compromised	Completely disabled	Dead

[a]ECOG, Eastern Cooperative Oncology Group.

Table 2. Distribution of neutropenic toxicity in 21 dogs 7 days following a single dose of gemcitabine.

Toxicity score	0	1	2	3	4
Neutrophils/μL	≥3000	1,500–2,999	1,000–1,499	500–999	≤499
No. of dogs	11	5	2	2	1
Percentage of dogs	52%	24%	9%	9%	5%

and was excluded from evaluation in this data set, and 1 dog was removed 5 days after receiving the initial dose of gemcitabine because of complications related to disease progression. Of the 21 dogs evaluated, there were 8 neutered males, 6 neutered females, 6 sexually intact males, and 1 sexually intact female. The most common breeds were mixed breed dogs (3), Golden Retrievers (2), Labrador Retrievers (2) Rottweillers (2) and Shetland Sheepdogs (2).

Of the 21 dogs included in this study, 7 of 21 had stage III lymphoma, 10 of 21 had stage IV lymphoma, and 4 of 21 had stage V lymphoma. All dogs with stage V lymphoma had bone marrow involvement. Eighteen dogs were classified as substage a and 3 dogs were classified as substage b at their initial presentation.

At day 7, gemcitabine administration resulted in a decrease of the mean neutrophil count of 10,640–3,140 cells/μL ($P < .01$). Ten of the 21 dogs (48%) demonstrated ≥ grade 1 neutropenia (ie, an absolute neutrophil count <3,000 cells/μL; Table 2). Gemcitabine resulted in a decrease of the mean platelet count from 201,290 cells/μL to 139,190 cells/μL ($P < .01$) 7 days after the initial treatment in the first cycle. Fifteen of 21 (71%) dogs demonstrated > grade 1 thrombocytopenia. (ie, a platelet count <200,000 cells/μL; Table 3). Using the modified ECOG Toxicity Scoring System, 48% (10/21) of dogs had neutropenia with a toxicity score of ≥1, 71% (15/21) of the dogs had thrombocytopenia with a toxicity score of ≥1, and 38% (8/21) of dogs had both neutropenia and thrombocytopenia with a toxicity score of ≥1 after initial treatment.

Five of the 21 dogs were given the planned second weekly gemcitabine dosage of 400 mg/m^2 according to original study guidelines. Eight of the 21 dogs were given gemcitabine at a reduced dosage of 300 mg/m^2. Seven of the 21 dogs were given their second dosage at a later time at a reduced dosage of 300 mg/m^2 and 1 of the 21 dogs was removed from the study and not given its second dose because of the owner's wishes.

Seven dogs completed the initial 4-week treatment cycle. These dogs had their current CLM value compared to the initial CLM. Five of the 7 dogs had SD and 2 dogs had PD. No dogs had CR or PR. No objective responses were seen in dogs that received a subsequent cycle of gemcitabine. Three dogs died acutely after receiving doses of gemcitabine. One dog received 3 doses of gemcitabine but continued to have PD and was removed from the trial according to the owner's request and was given asparaginase at 10,000 IU/m^2 IM. This dog died on the day it received asparaginase. The second dog presented after a third dose of gemcitabine with PD and signs of disseminated intravascular coagulation and sepsis. It died shortly after being presented to the hospital. The third dog was removed from the trial after receiving 3 doses of gemcitabine despite PD. This dog was given doxorubicin chemotherapy at 30 mg/m^2 and then presented 3 days later in septic shock and died shortly thereafter. The study was concluded at 7 weeks because of the poor response to treatment and the acute death of 3 dogs during the study period.

Discussion

In this study, gemcitabine given to dogs with spontaneously occurring lymphoma at a dosage of 400 mg/m^2 by a 30-minute IV infusion decreased blood neutrophil and platelet counts and resulted in no clinical remissions. The intent of the study was to enroll a minimum of 50 dogs per study site. Twenty-four dogs were available for evaluation because of early termination of the study. The acute death of 3 dogs prompted the investigators to reconsider the original study design and investigate possible causes for the unexpected deaths. An exhaustive search of the medical literature did not identify a direct cause and effect relationship between gemcitabine and asparaginase or anthracyclines associated with death. Adverse reactions have been seen with gemcitabine in combination with high-dose cisplatin[c] causing severe neutropenia and renal failure resulting in death. Species differences of undetermined nature may be the cause of the acute toxicity seen with gemcitabine followed by asparaginase or doxorubicin.

The starting gemcitabine dosage of 400 mg/m^2 was chosen with the intent to increase dosage at the beginning of the second 4-week cycle if there was no clinically relevant toxicity or PD. This dosage represented 30% of the 1200 mg/m^2 dose given to normal Beagle dogs[b]. Although decreases in neutrophil and platelet counts are anticipated with most chemotherapeutic agents, the toxicity associated with gemcitabine

Table 3. Distribution of thrombocytopenic toxicity 7 days following a single dose of gemcitabine.

Toxicity score	0	1	2	3	4
Platelets/μL	≥200,000	100,000–199,999	50,000–99,999	15,000–49,999	≤14,999
No. of dogs	6	5	7	3	0
Percentage of dogs	29%	23%	33%	14%	0%

administration to these dogs with lymphoma was not anticipated and was more severe than was seen in preliminary studies performed in normal Beagle dogs.[b] A single administered dosage of gemcitabine and additional administered dosages did not reduce disease burden in this study.

For the purposes of this study, we used an additive formula to provide an objective, numerical method to categorize and describe the overall response to chemotherapy in each dog. Traditionally, CR or remission is defined as return to normal lymph node size, PR is defined as reduction of lymph node size by $\geq$50% and NR is defined as $<$50% reduction in lymph node size. For the purposes of this study, a more specific, quantitative measurement was needed to fully evaluate the therapeutic response to gemcitabine. Consequently, the CLM was developed in an attempt to better categorize response.

A gemcitabine administration schedule similar to that described in this study has been evaluated in humans for single agent treatment of nonHodgkin's lymphoma.[12–14] One study investigated gemcitabine as a single agent for treatment of refractory or low grade nonHodgkin's lymphoma in 36 patients.[14] Gemcitabine was administered on days 1, 8, and 15 at a dosage of 1,000 mg/m^2 IV over 45 min on a 28-day schedule. This protocol resulted in severe hematologic toxicity and grade 3 of 4 (National Cancer Institute Common Toxicity Criteria) leukopoenia in 33% of patients and grade 3 of 4 thrombocytopenia in 50% of patients. CR was observed in 2 patients and PR was observed in 7 patients. Although the toxicoses seen in this study were manageable and the drug proved to be efficacious, this study concluded that a delivered dosage of 1,000 mg/m^2 resulted in frequent hematologic toxicity.

We postulate that the poor response observed in our study may be due, in part, to the frequent necessity of dose reduction or delay as a result of the hematologic toxicosis after the first administered dose of gemcitabine. We also postulate that the overall treatment time and number of doses may have an effect on response. In our study, no dog received a full second cycle of chemotherapy. In humans with nonHodgkin's lymphoma, the interval to best response ranged from 15 to 90 days with an average of 45 days to best response.[13] Our study did not allow sufficient time to continue therapy in the subset of animals that met criteria for continuing gemcitabine. Additional doses of gemcitabine may have resulted in better therapeutic response if these animals had continued treatment.

Historically, gemcitabine has been used to treat solid and metastatic tumors.[10,28] However, the treatment regimen for gemcitabine used in this study was based, in part, on previously reported treatment protocols in human patients with lymphoma.[12–14] Plasma concentrations of gemcitabine reach plateau values 15 minutes after infusion in human beings with nonhematologic malignancies.[29] The AUC is proportional to the dose whereas clearance of the parent drug is not dose dependent. In normal Beagle dogs, the pharmacokinetics of gemcitabine were consistent with those reported previously in humans and no additional toxicosis was observed in these dogs[b]. It also has been shown that intravesical administration of gemcitabine at dosages up to 1,000 mg/m^2 is well tolerated with minimal systemic toxcicity.[3] The pharmacokinetics of gemcitabine may have been influenced by the hematologic and systemic nature of lymphoma in our dogs. Disease, specific alterations in pharmacokinetics have been described for dogs and may increase the AUC for gemcitabine.[30,31] Such disease-specific alterations may have augmented the myelosuppressive effects of gemcitabine in these patients. Interestingly, reports describe the use of gemcitabine in dogs not afflicted with lymphoma at higher dosages without similar toxicosis.[24] The neutropenia and thrombocytopenia observed in this study may be related to this postulated interaction between drug and disease condition and may have led to the unexpected toxicity observed in our study. This toxicity is seen immediately because of the rapid turnover of these cells lines and their sensitivity to chemotherapeutic agents.[32]

Studies of the efficacy and toxicity of gemcitabine in the medical literature are conflicting.[11–14] Its use in veterinary oncology still is new and its place has yet to be determined. Additional studies need to be conducted to evaluate the safety, efficacy, and pharmacokinetics of gemcitabine in dogs with lymphoma. Alternative treatment regimens should be explored such as longer or shorter infusion protocols, or alternative administration schedules. The unique properties of this drug and its synergism with other drugs such as the platinum compounds,[7] topoisomerase I inhibitors,[11,21] vinca alkaloids,[20,22] the taxanes,[8] and anthracyclines[33] suggests that its use in combination protocols may be of some benefit in lymphoma and other tumor types in humans. This combination approach should be explored in veterinary patients because it has been documented in studies of human patients that the combination of different drug classes or the combination of cell-cycle specific drugs and cell-cycle nonspecific drugs is superior to single agent therapy.[34] As additional information about gemcitabine is obtained in our veterinary patients, efforts can be made to develop a safer single- or multi-agent protocol with reduced hematologic toxicity and improved efficacy.

Footnotes

[a] Gemcitabine HCl, Eli Lilly and Co, Greenfield IN

[b] Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN

[c] Gemcitabine HCl product insert and post marketing experiences, Eli Lilly and Co, Greenfield IN

Acknowledgments

The authors wish to thank the following people for their support and assistance: G. King, A. Porter, A.

Jurgens (Gulf Coast Veterinary Oncology); M. Carothers (Akron Veterinary Internal Medicine/Oncology); J. Turner, L. Fulton, S. Sheaffor, C. Wood, J. Peterson, K. Arrington, L. Bravo (Animal Cancer Institute, LLC Columbia, MD,.); Jean A. Wright, T. Campi, M. Langley, A. Hodge, M. Jackson, (Elanco Animal Health); H. McAllister (Research Consultant).

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