Combined Gemcitabine and Carboplatin Therapy for Carcinomas in Dogs

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Background: Response and adverse reactions to combined gemcitabine (GEM) and carboplatin (CARBO) therapy in dogs with carcinomas are not documented.

Hypothesis: GEM and CARBO are safe for the treatment of dogs with carcinomas.

Animals: Thirty-seven dogs with histologically or cytologically confirmed carcinomas.

Methods: Prospective clinical trial. Dogs were treated with GEM (2 mg/kg, 20–30-minute infusion IV) on Days 1 and 8 and 4 hours later, CARBO (10 mg/kg IV) on Day 1. The cycle was repeated on Day 22.

Results: Thirty-seven dogs (29 with measurable tumor) received a median of 2 cycles (range 0.5–6) for a total of 101 cycles administered. Twelve dogs (32%) developed neutropenia (3 Grade 3, and 5 Grade 4) and 9 (24%) thrombocytopenia (2 Grade 3, and 1 Grade 4). Dogs >20 kg were twice as likely to develop thrombocytopenia ($P = .023$). Twenty-seven dogs (73%) had evidence of gastrointestinal (GI) toxicosis, but most signs were of mild to moderate severity and self-limiting. One dog died of treatment-related complications. Overall tumor response rate was 13%. One dog with metastatic prostatic carcinoma achieved a complete remission and 1 dog with intestinal adenocarcinoma and 1 with tonsillar squamous cell carcinoma achieved partial remission. Twelve dogs achieved stable disease for a median of 72 days.

Conclusion and Clinical Importance: GEM and CARBO combination causes mild to moderate hematologic and GI toxicosis in dogs with carcinoma. Response rate in this study was modest, and optimization of dosing of this combination is required.

Key words: Antimetabolite; Canine; Chemotherapy; Platinum agent; Synergism.

Gemcitabine (GEM, 2′,2′-difluorodeoxycytidine) is a deoxycytidine analog with structural similarities to the pyrimidine antimetabolite cytosine arabinoside. GEM has complex pharmacokinetic and pharmacodynamic properties related to dose and duration of infusion.[1–4] GEM exhibits cell cycle phase specificity, exerting its cytotoxic effect in S phase, and causes G1/S arrest.[5,6] GEM is licensed for the treatment of human pancreatic and lung cancer, improves patient performance status and quality of life even in advanced stages of disease. However, GEM is most often used in combination chemotherapy protocols where it has apparent synergism with platinum agents, anthracyclines, alkylators, and taxanes.[7] GEM also has potent radiosensitizing properties.[5,6,8,9]

GEM has been infrequently used in animals, and the optimal dosing regimen has not been established. However, from recent reports describing the use of GEM in dogs with cancer, it appears that toxicity profiles parallel those observed in humans, but single agent efficacy has been disappointing. GEM is not toxic when administered at doses ranging from 300 to 675 mg/m^2 every 2 weeks. A dog with oral malignant melanoma and another with perianal squamous cell carcinoma (SCC) had beneficial response to GEM administered for more than 8 weeks.[10] Clinically important hematologic toxicosis, but no reduction in tumor burden, occurred with GEM administered (400 mg/m^2 weekly) to dogs with lymphoma.[11] The use of GEM combination chemotherapy protocols in dogs has not been reported.

Carboplatin (CARBO, *cis*-diammine-1,1-cyclobutane decarboxylate platinum [II]) is an analog of cisplatin with similar biologic effects and antitumor activity.[12,13] CARBO is less nephrotoxic and emetogenic than cisplatin, but its myelosuppressive effects are greater.[14] Myelosuppression is characterized by neutropenia and thrombocytopenia with nadirs in the range of 10–21 days.[15] In people, CARBO has efficacy against a variety of tumors. CARBO is used extensively in multidrug chemotherapy regimens for the treatment of osteosarcoma, melanoma, rhabdomyosarcoma, brain tumors, and non-Hodgkin's lymphoma in people. Pharmacokinetically guided dosing schemes are most often used for CARBO, where the total dose administered is calculated based on the patient's renal function assessed by glomerular filtration rate and desired target AUC of drug to be achieved. Therefore, consistent patient drug concentration exposures are maintained, resulting in better efficacy and less toxicosis.[16,17] CARBO is customarily administered to dogs every 3 weeks at dosages between 250 and 300 mg/m^2 for the treatment of osteosarcoma,[18] malignant melanoma,[19] transitional cell carcinoma (TCC),[20] and SCC.[21]

Synergism between GEM and platinum agents has been well documented. Synergy is believed to occur because of the increased propensity of platinum-DNA adduct formation after GEM triphosphate incorporation into the growing DNA strand.[22,23] GEM can also inhibit repair of platinum adducts and crosslinks.[24] In vitro studies have demonstrated that the added benefit is time dependent where sequential drug exposure, GEM

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first and platinum agent second, exerts the greatest cytotoxic effect.[22,25] In fact, other experiments have shown that simultaneous drug exposure could result in an antagonistic effect.[22,26,27] While the ideal time between GEM administration and platinum drug exposure ("wait period") can vary among cell types, a minimum of 4 hours is recommended between administration of these drugs for superior cytotoxic effects to occur.[22,26,27] In people, phase II/III trials of GEM-platinum combinations have yielded response rates in the range of 30–75% for various tumor types including carcinoma of the cervix, breast, nasopharyngeal region, and bladder.[28–33] While GEM-platinum combinations appear to be well tolerated, hematologic, renal, and gastrointestinal (GI) toxicoses are commonly reported. Hematologic toxicoses, manifested as neutropenia and thrombocytopenia, are dose limiting. A small percentage of human patients have cumulative hematologic toxicoses, in the form of anemia and persistent neutropenia, with increased number of cycles completed. Renal toxicosis is usually manifested as mild to moderate increases in serum creatinine concentration and GI toxicosis is usually manifested in the form of diarrhea and increases in serum activity of hepatic enzymes.[7,28–33]

To the authors' knowledge, the use of GEM and CARBO combination chemotherapy for the treatment of dogs with cancer has not been reported. Based on the previous information and favorable response rates attained in human trials, the goal of this prospective study was to evaluate the toxicity and efficacy of GEM/CARBO doublet therapy for the treatment of dogs with tumors of epithelial origin. There are several issues concerning the design of a protocol using these drugs, in particular dose, sequence, and interval between drugs. Given the number of variables we designed a protocol with a constant sequence and interval and intended to manipulate only drug dose. There is ambiguity in the data concerning optimum sequence and interval of GEM-platinum combinations. As a starting point we decided to evaluate GEM administered 4 hours before CARBO.

Materials and Methods

Dogs

Client-owned dogs with histologically or cytologically confirmed carcinoma that were presented to the Veterinary Teaching Hospital at Michigan State University are included in this prospective study. Dogs previously treated with a platinum agent or GEM were not excluded. Pretreatment evaluation consisted of CBC, serum biochemistry panel, urinalysis, tumor measurements, and regional lymph node evaluation via fine needle aspirate and cytology or biopsy and histopathology. All dogs had 3-view thoracic radiographs and abdominal ultrasound performed as part of their staging evaluation process. Dog age, breed, sex, weight, tumor type, disease status, presence or absence of metastatic disease, concurrent use of a nonsteroidal anti-inflammatory drug (NSAID) or antiemetic medication, and neutrophil and platelet count at the start of chemotherapy were all recorded. Prior treatment with chemotherapy and total number of GEM/CARBO cycles administered were also recorded.

Treatment Protocol

Dogs were treated with GEM/CARBO combination chemotherapy as follows. On Day 1, GEM[a] was administered at 2 mg/kg (diluted in 100 mL of 0.9% sodium chloride) by slow IV infusion over a 20–30-minute period, followed by CARBO[b] at 10 mg/kg (maximum of 300 mg/m^2) as an IV bolus 4 hours after GEM administration. On Day 8, dogs were administered GEM at 2 mg/kg as a slow IV infusion over a 20–30-minute period. No chemotherapy was administered on Day 15, and the cycle was repeated at Day 22 until tumor progression or a total of 6 cycles were administered. The GEM dose at 2 mg/kg was based on the "no toxic effect" dose in preclinical studies performed at Eli Lilly, which would have represented a range of 40–80 mg/m^2 for most of the expected oncology patient population at our institution.[34] GEM dose escalation between groups of 3 animals after a modified Fibonacci scheme $(2, 3, 5, \ldots)$[35] was planned. Use of an NSAID (piroxicam,[c] 0.3 mg/kg PO q24h; deracoxib,[d] 1 mg/kg PO q24h; or meloxicam,[e] 0.5 mg/kg PO q24h) during the study was at the discretion of the attending clinician. Dose reductions were allowed at the discretion of the attending clinician in cases with unacceptable (Grade 3 or 4) toxicoses, although dose reduction of only 1 drug at a time was encouraged. When indicated by signs of intoxication, a 25% reduction of the GEM dose was attempted 1st. If the dog continued to have evidence of a toxic effect after 25% GEM dose reduction, then the dose of CARBO was similarly reduced.

Toxicoses

Hematologic intoxication resulting from GEM/CARBO was monitored by weekly CBC evaluation during treatment, renal intoxication was monitored by serum biochemistry panel every 3 weeks and GI toxicosis was monitored through a client questionnaire at each visit. GI toxicosis, neutrophil, and platelet counts were graded using a modified National Cancer Institute Common Toxicity Criteria presented in Table 1.[36] Depending on the severity of clinical signs, symptomatic therapy was initiated, including metoclopramide,[f] 0.4–0.5 mg/kg PO q6–8h famotidine,[g] 0.5 mg/kg PO q12–24h or both for vomiting. Hospitalization with IV fluid support, antibiotics, and symptomatic treatment was initiated at the discretion of the attending clinician.

Follow-Up

All dogs with measurable disease were evaluated every 7–14 days. Physical examination with caliper measurements was used to evaluate palpable tumors. Three view thoracic radiographs were used to evaluate intrathoracic disease and ultrasonography was used to evaluate intra-abdominal disease. Dogs with microscopic disease were evaluated every 6–8 weeks. Additional information was gathered through review of patient records and telephone conversations with the referring veterinarian.

Response

Response was assessed by 3-dimensional measurements for palpable tumors and by 2-dimensional measurements from thoracic radiographs or abdominal ultrasound for internal lesions. Response to therapy was categorized as complete response (CR; 100% resolution of all measurable disease for a period ≥21 days), partial response (PR; ≥50% but ≤100% resolution of measurable tumor for a period ≥21 days), stable disease (SD; ≤50% resolution in measurable tumor and ≤25% increase in measurable tumor with no new lesion formation for a period ≥21 days), and progressive disease (PD; >25% increase in measurable tumor or development of new lesions). Time to progression (TTP) was defined as the time from achievement of maximal tumor response until development of

Table 1. Scoring system for signs of toxicoses applied to dogs with carcinomas treated with gemcitabine and carboplatin.[a]

Grade	Clinical Sign
	Neutropenia
0	>2,000/μL
1	1,500–2,000/μL
2	1,000–1,500/μL
3	500–1,000/μL
4	<500/μL
	Thrombocytopenia
0	> 155,000/μL
1	75,000–155,000/μL
2	50,000–75,000/μL
3	25,000–50,000/μL
4	< 25,000/μL

Gastrointestinal signs

	Vomiting	Diarrhea	Anorexia
0	None	None	None
1	1/day	1–5/day	≤ 24 h
2	2–5/day	5–7/day	24–48 h
3	6–10/day	> 7/day	> 48 h/d
4	> 10/day, hosp	> 7/day, hosp	> 48 h, hosp

[a]Modified National Cancer Institute Common Toxicity Criteria.

PD. Dogs lost to follow-up or dead while in remission were censored for TTP.

Statistical Analysis

The effects of age, weight, sex, disease status (gross versus microscopic), presence or absence of metastatic disease, neutrophil and platelet count, use of supportive medications (NSAIDs, metoclopramide and/or famotidine), prior chemotherapy, and total number of cycles administered were evaluated as categorical variables for association with and the development of toxicity by the Fisher's exact test. Results were considered significant at $P < .05$. TTP was calculated using the Kaplan-Meier method. Statistical analysis was performed by a commercially available computer software package.[h]

Results

Thirty-seven dogs were treated with GEM/CARBO (Table 2).

Tumor Types and Characteristics

Eighteen tumor types were treated (Table 3). At the outset, 29 of the 37 dogs (78%) treated with GEM/CARBO had macroscopic disease at the primary site. Seven dogs had macroscopic intrapulmonary metastatic disease; 1 dog with bronchoalveolar carcinoma had macroscopic disease but no gross metastasis; and 1 dog with bronchoalveolar carcinoma had the primary tumor and affected tracheobronchial lymph nodes surgically resected before the start of chemotherapy. Of the 9 dogs with tumors of the genitourinary system, 8 had macro-

Table 2. Dog characteristics and other therapies.

Age (years)	
Median	11.2
Range	6.6–17.3
Weight (kg)	
Median	23.5
Range	3.2–60
Sex	
Male neutered	22
Male intact	2
Female spayed	12
Female intact	1
Breed	
Mixed breed	13
Cocker Spaniel	2
German Shepherd	4
Doberman Pinscher, Pekingese, Bichon Frise, West Highland White, Terrier, Long Haired Daschund, Keeshond, Sheltie, Chihuahua, Golden Retriever, Afghan Hound, Shar Pei, Portuguese Water Dog, Toy, Poodle, Beagle, Affenpinsher, Jack Russell Terrier, Labrador Retriever, Bouvier des Flanders	1
Previous therapy	
Surgery	18
None	15
Chemotherapy	8
Radiation therapy	2
Concurrent therapy	
NSAID	30
Metoclopramide	27
Pamidronate	2
Radiation therapy	1

scopic disease at the primary site, 1 had renal carcinoma with microscopic disease after surgical resection of the primary tumor, and 4 had measurable visceral organ metastatic disease (3 prostatic carcinoma and 1 renal carcinoma). Five of the 8 dogs with tumors of the GI system had macroscopic disease and metastatic disease (2 hepatocelluar carcinoma, 1 rectal carcinoma, 1 intestinal carcinoma). Three of these 5 dogs with GI tumors had regional lymph node metastasis, 1 had regional lymph node and lung metastasis, and 1 had visceral organ as well as lung metastasis. Two dogs with mammary tumors were treated (1 solid adenocarcinoma, 1 inflammatory carcinoma), and both had macroscopic disease and measurable metastasis. The dog with solid adenocarcinoma had regional lymph node metastasis and the dog with inflammatory carcinoma had regional lymph node and lung metastasis. Eight of 9 dogs with miscellaneous tumors (Table 3) were treated while having macroscopic primary disease and all had measurable metastatic disease. One dog with an anal sac apocrine gland adenocarcinoma and sublumbar lymph node metastasis started chemotherapy while affected by presumed microscopic residual disease after surgery and radiation therapy.

Eight of the 37 dogs (22%) treated with GEM/CARBO were treated based on negative prognostic factors after surgical resection of their primary tumor. These negative prognostic factors included large primary tumor size (n = 3), high tumor grade (n = 1), incomplete resection (n = 1), confirmed lymph node metastasis (n =

Table 3. Tumor location, type, and stage.

Anatomic Location	n	Tumor Type	Disease Status	Metastasis
Respiratory	9	5 bronchoalveolar CA	2 macro, 3 micro	1 RLN, 1 lungs
		2 pulmonary CA	2 macro	2 lungs
		1 adenosquamous CA	1 macro	1 pleura
		1 nasal ACA	1 macro	1 RLN
Genitourinary	9	5 prostatic CA	5 macro	1 RLN, 1 RLN and lungs, 1 lungs and pleura
		2 TCC	2 macro	None
		2 renal CA	1 macro, 1 micro	1 lungs
Gastrointestinal	8	4 hepatocellular CA	2 macro, 2 micro	1 RLN and lungs, 1 lungs and visceral organs
		2 rectal CA	1 macro, 1 micro	1 RLN
		1 gastric CA	1 macro	1 RLN
		1 intestinal CA	1 macro	1 RLN
Mammary	2	1 solid ACA	1 macro	1 RLN
		1 inflammatory CA	1 macro	1 RLN and lungs
Miscellaneous	9	3 SCC	3 macro	1 RLN, 1 bone
		3 CUPS	3 macro	2 RLN, 1 RLN and visceral organs
		1 adrenocortical CA	1 macro	1 liver
		1 apocrine gland ACA of skin	1 macro	1 RLN
		1 apocrine gland ACA of anal sac	1 micro	1 RLN

CA, carcinoma; ACA, adenocarcinoma; TCC, transitional cell carcinoma; SCC, squamous cell carcinoma; CUPS, carcinoma of unknown primary site; macro, macroscopic disease; micro, microscopic disease; RLN, regional lymph nodes.

2), vascular or lymphatic invasion on histopathology (n = 1). Toxicoses and TTP only were assessed in these dogs.

Previous Therapies

Of the 37 dogs, 18 had surgery before starting GEM/CARBO, 2 had radiation therapy (1 palliative, carcinoma of unknown primary site; 1 curative-intent, apocrine gland adenocarcinoma of the anal sac gland) and 8 had PD after prior chemotherapy. The median time from previous therapy to initiating GEM/CARBO was 14 days (range 1–90 days). Four of the 8 dogs who received prior chemotherapy had been given only single agent therapy. Drugs previously used included doxorubicin (n = 3), cyclophosphamide (n = 3), mitoxantrone (n = 2), cisplatin (n = 1), vincristine (n = 1), GEM (n = 1), and mitotane (n = 1).

Other Therapies

Curative-intent radiation therapy was delivered between the 2nd and 3rd GEM/CARBO cycle for 1 dog with tonsillar SCC metastatic to the retropharyngeal lymph nodes.

Treatments

A total of 101 cycles of GEM/CARBO were administered to 37 dogs. The median number of cycles administered was 2 (range, 0.5–6). Two dogs completed 6 cycles, 5 dogs completed 5 cycles, 6 dogs completed 4 cycles, 5 dogs completed 3 cycles, 8 dogs completed 2 cycles, and 5 dogs completed 1 cycle. One dog with a metastatic hepatocellular carcinoma completed 1 cycle and received the 1st dose of the 2nd cycle, but was euthanized 4 days after hospitalization for vomiting. Postmortem evaluation was not performed in this dog. It is unclear whether vomiting in this dog was the result of chemotherapy toxicity or tumor progression. Five

dogs did not complete a full 21-day cycle of GEM/CARBO. Two dogs had CUPS (macroscopic disease and metastasis), 1 dog had TCC (macroscopic disease and no metastasis), 1 dog had renal CA (macroscopic disease and metastasis), and 1 dog had hepatic CA (macroscopic disease and metastasis). Of these, 2 were euthanized because of extensive disease at the outset with evidence of progression on therapy (1 renal CA and 1 hepatic CA). Another dog developed complications of PD soon after the 1st day of treatment and was euthanized because of urinary obstruction from tumor (CUPS). One dog died at home with no necropsy (CUPS), and 1 dog had treatment discontinued because of owner financial concerns (TCC).

Hematologic Toxicoses

Twelve dogs (32%) experienced neutropenia for a total of 17 episodes (17% of cycles). Grade 3 neutropenia occurred in 3 dogs (8%) and Grade 4 neutropenia (neutrophil count < 500/μL) occurred in 5 dogs (14%). Neutropenia was evident at Day 15 of the 21-day cycle in 9 of 12 dogs. The median neutrophil count at the start of chemotherapy was $9.2 \times 10^3/\mu L$ (range; $4.5–27.9 \times 10^3/\mu L$). The median neutrophil count at Day 15 was $5.46 \times 10^3/\mu L$ (range; $0.04–23.63 \times 10^3/\mu L$). All dogs that developed neutropenia, except the one who died of neutropenic sepsis, recovered within 7 days, and had neutrophil counts within the reference range by Day 21. Of the 4 other dogs that developed neutropenia, 2 had Grade 1 neutropenia and 2 had Grade 2 neutropenia. No significant correlation was found between dog variables evaluated and development of neutropenia.

One dog with tonsillar SCC was hospitalized for neutropenic sepsis and thrombocytopenia after completion of the 3rd cycle of chemotherapy. This dog had also completed full course radiation therapy 3 weeks before the start of a 3rd cycle of GEM/CARBO. Radiation

therapy was administered between the 2nd and 3rd cycle for this dog. Despite normalization of neutrophil count and resolution of fever the dog was euthanized based on continued worsening of clinical condition and coagulopathy consistent with disseminated intravascular coagulopathy (DIC; thrombocytopenia, increased prothrombin and activated partial thromboplastin times, high fibrinogen and positive D-dimers). DIC and presence of metastatic SCC to the retropharyngeal lymph node was confirmed on postmortem evaluation. Extramedullary hematopoiesis and severe pancytopenia of the bone marrow were also noted. This dog was the only treatment-associated death in this study group.

Nine dogs (24%) experienced thrombocytopenia for a total of 19 episodes (19% of cycles). Thrombocytopenia was evident at Days 7 and 15 of the protocol in 5 of 9 dogs. Grade 3 thrombocytopenia was seen in 2 dogs while 1 dog had Grade 4. The rest had Grade 1 or 2 thrombocytopenia. Dogs $>20\,kg$ were twice as likely to develop thrombocytopenia ($P = .023$; 95% CI 1.28–3.37). The median platelet count at the start of chemotherapy was $435 \times 10^3/\mu L$ (range; $81–1,212 \times 10^3/\mu L$). The median platelet count at Days 7 and 15 was $364 \times 10^3/\mu L$ (range; $26–1,112 \times 10^3/\mu L$). All dogs that experienced thrombocytopenia recovered within 7 days.

No cumulative neutropenia or thrombocytopenia was recorded in dogs receiving >2 cycles of GEM/CARBO. The number of cycles administered (≤ 2 versus >2 cycles) was not significantly correlated with development of neutropenia ($P = .17$), thrombocytopenia ($P = .71$), or GI toxicity (0.27). Clinically relevant anemia was not observed in any dog.

GI and Other Toxicoses

Twenty-seven dogs (73%) had signs of GI toxicosis for a total of 51 episodes (51% of cycles). The most commonly recorded sign was mild to moderate vomiting of 1–2 days duration (Grade 1 or 2). Diarrhea was recorded in 2 dogs and neither required medical intervention. Five dogs had Grade 3 or 4 toxicosis, but 4 did not require hospitalization. Of the 5 dogs that had Grade 3 or 4 toxicosis, 3 were receiving metoclopramide and a NSAID, 1 received only metoclopramide, and 1 received only a NSAID. One dog with a metastatic bronchoalveolar carcinoma experienced 2 episodes of Grade 4 GI toxicosis requiring hospitalization and IV fluid therapy. Both episodes occurred after GEM and CARBO were administered the same day (Day 1) of the 2nd and 3rd cycles resulting in a 7-day treatment delay for both episodes. The dog was administered famotidine PO and no further toxicosis was observed during subsequent cycles. Twenty-two of 37 dogs (59%) were given metoclopramide PO prophylactically during chemotherapy. Of the 22 dogs that received metoclopramide, 18 (82%) experienced GI toxicosis despite prophylactic antiemetics. Nine of 15 dogs (60%) that did not receive prophylactic metoclopramide experienced GI toxicosis. No correlation was found between the prophylactic use of metoclopramide and the occurrence of GI toxicosis ($P = .26$). Dogs > 11 years of age were twice as likely to experience GI toxico-

sis than those <11 years, but this was not statistically significant ($P = .07$). No renal or hepatic toxicosis was observed.

Dose Reductions and Treatment Delays

Three dogs had drug dose reduction and 8 dogs had treatment delay. Two dogs (67%) had a reduction in their GEM dose, 1 as a result of Grade 3 neutropenia and 1 as a result of concurrent Grade 4 neutropenia and Grade 4 thrombocytopenia. One dog (33%) had a 25% dose reduction of both CARBO and GEM because of concurrent Grade 3 neutropenia and Grade 2 GI toxicosis. Seven dogs (88%) had a 7-day delay in their treatment as a result of toxicosis: 1 (13%) as a result of Grade 4 neutropenia with concurrent Grade 4 GI toxicosis; 1 (13%) as a result of Grade 3 neutropenia with concurrent Grade 2 GI toxicosis; 1 (13%) as a result of concurrent Grade 3 neutropenia, Grade 3 thrombocytopenia and Grade 2 GI toxicosis; 1 (13%) as a result of concurrent Grade 4 neutropenia, Grade 3 thrombocytopenia, and Grade 3 GI toxicosis; 1 (13%) as a result of Grade 3 neutropenia; 1 (13%) as a result of Grade 3 thrombocytopenia; and 1 (13%) as a result of Grade 3 GI toxicosis. One dog had a 7-day treatment delay because of noncompliant owners. No GEM dose escalations were performed because 2 of the 1st 3 dogs treated, had Grade 4 toxicosis. Four large breed dogs received a reduced dose of CARBO, based on an intent-to-treat rule of CARBO, not to exceed $300\,mg/m^2$.

Concurrent Use of NSAIDs and Other Drugs

Thirty of 37 dogs (81%) were administered a NSAID PO concurrent with GEM/CARBO. Fifteen dogs started NSAID therapy before Day 1 of treatment, 12 dogs started NSAID therapy on Day 1 (same day) of treatment, and 3 dogs started NSAID after initiating chemotherapy. Twenty-seven dogs received piroxicam, 2 deracoxib, and 1 meloxicam. No statistical significance was found between the use of a NSAID and the likelihood of neutropenia ($P = .9$), thrombocytopenia ($P = .9$), or GI toxicity ($P = .9$). Two dogs (1 with metastatic SCC and 1 with CUPS) received 1 dose of pamidronate[i] for bone metastasis concurrently with GEM/CARBO on Day 1 of the 1st cycle. Prophylactic use of metoclopramide was reported in "GI and other toxicoses."

Treatment Response

Twenty-nine of 37 dogs had measurable tumor and 23 were evaluated for response. Of those not evaluated for response, 5 with measurable tumor were euthanized before tumor reevaluation, 1 discontinued therapy because of owner financial concerns and 8 had microscopic disease. One dog with metastatic prostatic carcinoma achieved a CR for 104 days and 2 dogs (1 with metastatic SCC and 1 with intestinal carcinoma) achieved a PR for 65 and 107 days, respectively. Therefore, the overall response rate was 13%. In addition, 12 dogs with macroscopic disease (2 prostatic carcinoma, 2 bronchoalveolar carcinoma, and 1 each nasal adenocarcinoma,

apocrine gland adenocarcinoma, TCC, gastric carcinoma, CUPS, mammary carcinoma, SCC, adrenocortical carcinoma) achieved SD for a median TTP of 72 days (range; 35–162 days). Nine of these 12 dogs also had metastatic disease. Eight (67%) owners of these 12 dogs reported that clinical signs attributed to the tumor were no worse than before instituting GEM/CARBO and 4 (33%) owners reported clinical signs had improved when compared with those before starting GEM/CARBO. All 8 dogs with microscopic disease are alive at the time of this writing: 3 with bronchoalveolar carcinoma at 136, 644, and 756 days; 2 dogs with hepatocellular carcinoma at 168 and 570 days; and 1 of each with anal sac apocrine gland adenocarcinoma, renal carcinoma, and rectal carcinoma at 547, 644, and 743 days, respectively.

Discussion

Thirty-seven dogs with various types of carcinoma were treated with GEM/CARBO combination chemotherapy on a 21-day cycle. It appears that hematologic toxicity in the form of neutropenia and thrombocytopenia is dose limiting when using this combination in dogs. GI toxicity, while frequent, is usually mild and self-limiting. Eighteen different tumor types were treated, and the majority of dogs had macroscopic and metastatic disease at the time of treatment. We conclude that, when used in combination on a 21-day schedule as described in this study, the dosages of GEM and CARBO should not exceed 2 and 10 mg/kg, respectively. Incorporation of other drugs that exhibit synergism with GEM and/or CARBO, without overlapping toxicity, and with different mechanisms of action and drug resistance might be attempted for improved efficacy against epithelial tumors in dogs. It is clear that further in vitro and in vivo investigations of this combination are necessary to determine the optimum dosing scheme and schedule in dogs, which is beyond the scope of this pilot study. Because of the responses seen in prostatic carcinoma, SCC and intestinal carcinoma, a prospective, phase II study of GEM/CARBO warrants further investigation in this subset of patients.

The primary goal of this study was to evaluate the toxicity and response rate to GEM/CARBO doublet therapy on a 21-day administration cycle in dogs with carcinomas. We have demonstrated that, at low dosages, GEM can be safely combined with other chemotherapeutic drugs for the treatment of cancer in dogs. It is possible that altering aspects of combinations, rather than dose escalation of GEM, may yield superior results to single agent therapy, especially because the optimal dosing regimen of GEM in dogs has yet to be established. A major limitation of our study is the low number of dogs treated (n = 37) with GEM/CARBO, which may have underscored the degree and incidence of toxicity. Response to treatment could have also been negatively affected by the number and percentage of dogs with advanced stage of disease, which resulted in short follow-ups and in some cases abbreviation of the 21-day chemotherapy cycle because of progression in other cases.

The 21-day protocol and schedule used in this study was extrapolated from human protocols used to treat various types of epithelial tumors, coupled with evidence that 28-day regimens resulted in significant and sometimes excessive levels of hematologic toxicity.[29,37–39]

Previously we treated 7 dogs with a different GEM/CARBO combination, which appeared to be too toxic (unpublished data). In designing the protocol used here, the starting dosages of 2 mg/kg of GEM and 10 mg/kg of CARBO were selected based on several considerations. Our rationale for choosing a starting dosage of 10 mg/kg of CARBO was based on published dosages in the range of 200–300 mg/m² for the treatment of various canine malignancies.[18–20] We reasoned that dosing our patients on the basis of body weight rather than body surface area would allow for a more physiologically appropriate range of the CARBO dose, regardless of patient weight or condition status. However, for those patients >25 kg, dosing at 10 mg/kg would have exceeded the standard dose of 300 mg/m² used in previous studies.[13–15,18–21] In contrast, we chose to start the GEM dose at 2 mg/kg was based on the "no toxic effect" dose in preclinical studies performed at Eli Lilly, which would have represented a range of 40–80 mg/m² for most of the expected oncology patient population at our institution.[34] However, it is important to note that the optimal dose regimen and schedule for GEM administration in tumor-bearing dogs has yet to be established.

We initially planned to perform GEM dose escalations in cohorts of 3 dogs in a standard trialing design, but found that important toxicoses was observed in the 1st 2 dogs treated (1 Grade 4 GI toxicosis and 1 Grade 4 neutropenia). We therefore concluded that the initial chosen dose was at or near to the maximum tolerated dose of GEM when administered in combination with CARBO on a 21-day schedule. In addition, 10 dogs required either a chemotherapy dose reduction or treatment delay because of the development of toxicoses, further supporting that higher drug doses would not be tolerated on a 21-day protocol of this combination. Dog accrual was thus continued at dosages of 2 and 10 mg/kg (not to exceed 300 mg/m²) for GEM and CARBO, respectively, and further dose escalation was not attempted.

Overall, GEM/CARBO resulted in 36% hematologic and 51% of dogs having sings of GI toxicosis, respectively. Most frequently, hematologic toxicosis was observed on Days 7 and 15 of the 21-day cycle of chemotherapy described in this study. This is consistent with nadirs reported for GEM single agent (7–10 days) and CARBO single agent (10–21 days). Despite the fact that about 32% of the dogs in this study developed neutropenia and about 24% had thrombocytopenia, when the number of cycles administered is considered, only 17% of the cycles administered resulted in Grade 3 or 4 toxicosis and 19% of the cycles resulted in Grade 1 or 2 hematologic toxicity. This demonstrates that the protocol was fairly well tolerated by the dogs in this study.

GI toxicosis was important in this study, where 51 total episodes (51%) of GI toxicosis were observed in 27 dogs. While most episodes (45%) were mild to moderate

and did not require medical intervention, 5 dogs (5%) experienced Grade 3 or 4 toxicosis, and 2 of these required hospitalization for IV fluid therapy and supportive care. The prophylactic use of metoclopramide in our study was also very common. More than half of the dogs (22 of 37) were given metoclopramide PO while on chemotherapy as a means to prevent or minimize nausea and vomiting. Therefore the incidence and degree of GI toxicosis may be underestimated. However, when comparing dogs that received metoclopramide with those that did not, no statistical difference was observed in the incidence of GI toxicosis. The prophylactic use of other antiemetic medications, or use of a combination of antiemetic drugs might be indicated to prevent nausea and vomiting that might be induced by GEM/CARBO. It is of interest to note that 30 dogs (81%) in this study were administered an NSAID PO along with GEM/CARBO for the treatment of their carcinoma, or for pain control. Piroxicam was the NSAID most often used in our study. Piroxicam is commonly used in the treatment of veterinary carcinomas because of its reported inhibitory effects on the COX-2 enzyme, which can mediate multiple biologic processes including apoptosis, angiogenesis, and chemotherapy resistance.[40] However, concurrent inhibition of COX-1 activity also occurs, so possible adverse effects of piroxicam use include GI irritation and renal toxicity.[41] While no dog experienced renal toxicosis, it is possible that piroxicam or NSAID use may have at least contributed to the GI signs, including vomiting and inappetence, observed in our study group. Comparisons among groups are difficult, because piroxicam was administered to 27 of the 30 dogs who received an NSAID concurrently with GEM/CARBO, while 2 others received deracoxib and 1 meloxicam. Seven of 37 dogs in the study did not receive an NSAID. Needless to say, the incidence of GI toxicosis may have been falsely increased by the use of NSAIDs.

The overall response rate in our study was 13%, with 18 different histologic types of carcinoma treated. Because of the small number of dogs with each tumor type, we could not determine if any difference in response or likelihood of toxicity was associated with different tumor types. Response was observed for 3 tumor types, a prostatic carcinoma, an intestinal carcinoma, and a SCC. All 3 dogs had macroscopic and metastatic disease. Another 12 dogs with macroscopic tumor burden achieved SD for a median of 72 days. In the majority of these cases, dogs had inadequate response to established protocols for their disease and the achievement of SD with GEM/CARBO was subjectively important in that it appeared to change the rapid progression of the relapse. It is also worth mentioning, however, that 81% of the dogs in this study were also receiving an NSAID (ie., piroxicam), thus we cannot rule out the possible contribution of these drugs to the overall response rate or improvement in patient quality of life. It is also impossible to draw conclusions on the potential benefit that GEM/CARBO might have provided to those patients treated in the adjuvant setting, because it is conceivable that some of these dogs might have been cured of their disease by surgery.

When evaluating our study design, we recognize that a major limitation is the fact that an optimal dosing regimen of GEM in dogs has not been established and that activity against canine malignancies has been modest. Traditionally, drugs have not been tested in combination unless single agent efficacy has been demonstrated, but some contemporary trials and drug combinations have been designed in an attempt to exploit synergistic or additive interactions.[7,25] Other guidelines have also been established for the development of drug combinations including the use of drugs with different mechanisms of action, the use of drugs that do not show cross-resistance, and the use of drugs with no overlapping toxicities. While the GEM/CARBO regimen used in this study does not meet all of these criteria, we still attempted the use of this combination in dogs based on compelling data on the use of GEM/CARBO for the treatment of epithelial malignancies in humans and the fact that the synergy of the combination has been shown to overcome resistance to the individual components. We believe that the true utility of GEM in this setting is likely to be as a potentiator of CARBO cytotoxicity.

Footnotes

Acknowledgment

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