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Tolerability of Gemcitabine and Carboplatin Doublet Therapy in Cats with Carcinomas

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Background: This study was performed to determine the toxicity of gemcitabine-carboplatin doublet therapy in cats with carcinomas.

Hypothesis: Gemcitabine and carboplatin are safe in tumor-bearing cats.

Animals: Twenty cats with spontaneously occurring carcinomas.

Methods: A cohort of 6 cats received gemcitabine (2 mg/kg IV) on days 1, 8, and 15 and carboplatin (10 mg/kg IV) immediately after gemcitabine on day 1 of a 21-day cycle. A 2nd cohort of 14 cats received carboplatin 4 hours after gemcitabine on day 1 and gemcitabine on day 8 but not day 15. The cycles were repeated every 21 days.

Results: Cats in the 1st cohort received a median of 3.75 cycles per animal (range, 1–6). Two cats (33.3%) developed grade 3 or 4 neutropenia, 1 (16.7%) grade 4 thrombocytopenia, and 1 (16.7%) grade 3 gastrointestinal toxicity. Gemcitabine dose reductions and treatment delays occurred in 1 and 4 cats, respectively. Cats in the 2nd cohort received a median of 2 cycles per animal (range, 0.5–10). Two cats (14.3%) had grade 3 or 4 neutropenia and 1 (7.1%) had grade 3 and 4 gastrointestinal toxicity. One cat required gemcitabine dose reduction and 6 had treatment delays. In the 2nd cohort, of 11 cats with measurable tumors, there was 1 complete response (pancreatic carcinoma) and 1 partial response (squamous cell carcinoma, receiving concurrent nonsteroidal anti-inflammatory drugs).

Conclusions and Clinical Importance: Gemcitabine-carboplatin combination appears moderately well tolerated in tumor-bearing cats. Minimal patient benefit suggests that alternative schedules or combinations of gemcitabine with other agents should be explored.

Key words: Chemotherapeutics; Chemotherapy; Drug interactions; Oncology treatment.

Gemcitabine (2′,2′-difluorodeoxycytidine) is a pyrimidine nucleoside analogue that resembles deoxycytidine[1] and has substantial single-agent clinical activity against solid tumors and hematologic neoplasms in humans.[2–5] The mechanism of action involves incorporation into strands of replicating DNA, in competition with deoxycytidine triphosphate, leading to "masked-chain termination" and inhibition of DNA replication and repair.[1,6] This process is cell cycle phase-specific and blocks G_1-S progression. Additionally, gemcitabine impairs DNA synthesis by inhibition of ribonucleotide reductase and depletion of deoxynucleotide triphosphate pools,[1,6,7] an effect that is also believed to mediate its strong radiosensitizing properties.[8] Clinical studies of gemcitabine in veterinary medicine are limited[9–12] and results are not readily comparable because of diverse dosing schemes and durations of infusion that may influence pharmacokinetic parameters.[13,14] Myelosuppression is the dose-limiting toxicity in dogs, with neutrophil and platelet nadirs at 7–10 days.[9–11] A study of twice weekly gemcitabine used as a radiosensitizer in cats indicated a neutrophil nadir at 8–15 days,[11] but no studies have been conducted using different schedules or combination chemotherapy protocols. Reported antitumor activity of single-agent gemcitabine in dogs also is limited[9,10] and may be explained in part by suboptimal dosing and treatment schedules.

Carboplatin (*cis*-diammine-1,1-cyclobutanedicarboxylato platinum [II]) is an analogue of cisplatin, with activity against several solid tumors in humans.[15] It is cell cycle phase nonspecific and binds irreversibly to the N7 position of guanine (or occasionally adenine), leading to the formation of intra- and interstrand DNA crosslinks, triggering cell death by apoptosis.[16] Carboplatin is widely used in veterinary medicine because of its favorable toxicity profile compared with that of cisplatin and has demonstrated activity and acceptable toxicity as a single agent in tumor-bearing cats.[17–21,a] Myelosuppression also is dose limiting for carboplatin and the drug particularly affects neutrophils and platelets, with nadirs in cats of 14–21 days.[17,18]

Gemcitabine potentially is synergistic with drugs that damage DNA. Combination with platinum agents has demonstrated synergism in rodent and in vitro models.[22–25] In humans, clinical efficacy has been documented against carcinomas of the ovary, pancreas, lung, and thyroid gland.[26–31] Gemcitabine facilitates formation of platinum-DNA adducts[22] and may inhibit repair of adducts and crosslinks.[25] Beneficial effects of the combination appear to depend on schedule and time of drug administration, but controversy remains regarding an optimal treatment

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schedule. Whereas sequential gemcitabine followed by platinum administration produced synergism in early studies,[25] additional research has yielded conflicting results.[22,23]

To our knowledge, evaluation of gemcitabine as a single agent or in combination chemotherapy protocols has not been reported in cats, other than in radiosensitization studies.[11,12] Although phase I studies of single-agent gemcitabine in cats are lacking, the limited expected single-agent activity of this drug,[9,10] along with its unique mechanisms that are well suited for combination therapy,[22–25] make it a good candidate for evaluation in a combination protocol. Based on neutrophil nadirs, administration of gemcitabine with carboplatin may prevent temporal overlap of hematologic toxicity. Previous evaluation of this combination in tumor-bearing dogs resulted in mild to moderate toxicity and modest antitumor activity.[b] The purpose of this study was to determine the tolerability of 2 distinct gemcitabine-carboplatin doublet treatment protocols in cats with spontaneously occurring carcinomas by presenting toxicity data. Observed tumor responses were also recorded.

Materials and Methods

Patient Selection

Cats presented to the Veterinary Teaching Hospital of Michigan State University from 2003 to 2006 with a histologic or cytologic diagnosis of carcinoma were eligible for inclusion in this study. Additionally, 1 or more of the following criteria were required: unresectable tumor, incompletely excised tumor with microscopic residual disease, histologically high-grade tumor, or presence of gross metastasis. Because the endpoint of the study was evaluation of toxicity, cats receiving nonsteroidal anti-inflammatory drugs (NSAIDs) at the time of initiation of the study were also included and prescription of NSAIDs was allowed after study inclusion. Patients with life-limiting intercurrent disease and those not expected to survive for 6 weeks with their malignancy were excluded from participation. Cats were staged with a CBC, serum biochemistry profile, urinalysis, 3-view thoracic radiographs, and, in most cases, abdominal ultrasound examination. Information recorded included age, breed, sex, body weight, tumor type, presence of macroscopic versus microscopic disease, presence or absence of metastasis, prior treatment, and concurrent use of NSAIDs.

Treatment Protocols

Cats were treated with a combination of gemcitabine[c] and carboplatin[d] in 2 distinct fixed-dose doublet protocols. One cohort of cats was treated with the original protocol design and received gemcitabine (2 mg/kg) as a 20-minute IV infusion every 7 days and carboplatin (10 mg/kg IV bolus) immediately after gemcitabine administration on day 1 of a 21-day cycle. A modified protocol was used in the 2nd cohort of cats. Modifications consisted of a delay in carboplatin administration (10 mg/kg IV bolus) to 4 hours after gemcitabine infusion on day 1 of the cycle and administration of gemcitabine (2 mg/kg) as a 20-minute IV infusion on days 1 and 8 only, with a chemotherapy break on day 15. The cycles were repeated every 21 days until tumor progression or for an intended 6 cycles. Additional cycles were permitted if incomplete tumor responses were observed. Dose reductions and treatment delays were allowed based on observed toxicity at the discretion of the attending clinician. When dose modifications were deemed necessary, the gemcitabine dose was reduced by 25%.

Table 1. Modified National Cancer Institute common toxicity criteria grading scheme.

	Hematologic toxicity		
Grade	WBC (μL)	Neutrophils (μL)	Platelets (μL)
0	>3,000	>2,000	>100,000
1	2,000–3,000	1,500–2,000	75,000–100,000
2	1,500–2,000	1,000–1,500	50,000–75,000
3	1,000–1,500	500–1,000	25,000–50,000
4	<1,000	<500	<25,000

	Gastrointestinal toxicity		
	Anorexia	Vomiting	Diarrhea
0	None	None	None
1	Partial inappetence	1/day	1–4/day
2	Significantly decreased	2–5/day	5–7/day
3	Requires fluid therapy	6–10/day	>7/day
4	Requires feeding tube	>10/day, hospitalization	>7/day, hospitalization

WBC, white blood cells.

Toxicity

Cats were evaluated for toxicity by physical examination and owner assessment by use of a questionnaire at each visit and a CBC every 7 days while receiving chemotherapy. Serum biochemistry profiles were repeated in cats receiving more than 1 cycle of chemotherapy. Hematologic and gastrointestinal toxicities were graded according to a modified National Cancer Institute Common Toxicity Criteria scheme (Table 1).[32,e]

Response

Although the objective of this study was evaluation of toxicity, measurement of macroscopic tumors was performed on initiation of the study and the observed responses were documented. External tumors were measured with calipers at each visit and internal tumors were evaluated with 3-view thoracic radiographs or abdominal ultrasound examination every 6–8 weeks. Only cats with measurable tumors were included in response calculations. Response to treatment was categorized as follows: complete response (CR), 100% disappearance of all gross measurable disease; partial response (PR), ≥50% but <100% reduction in the volume of measurable disease plus no development of new lesions; stable disease (SD), <50% reduction in the volume of measurable disease or <25% increase in the volume of measurable disease plus no development of new lesions; and, progressive disease (PD), ≥25% increase in the volume of measurable disease or appearance of new lesions. Responses were required to persist a minimum of 21 days to be counted. Time to progression (TTP) was defined as the time from maximal tumor response or from beginning of chemotherapy for microscopic tumors and those with SD until tumor progression.

Results

First Patient Cohort

Patient and Tumor Features. Six cats comprised the 1st cohort. The median age for these cats was 13 years. Both sexes were represented equally. Additional patient features are listed in Table 2. Tumor types included 1 each of mammary adenocarcinoma, hepatic carcinoma, biliary adenocarcinoma, intestinal adenocarcinoma,

Table 2. Patient characteristics.

	1st Cohort	2nd Cohort
Age (years)		
Median	13	13
Range	10–20	7–23
Sex		
Male neutered	3	7
Female spayed	3	7
Weight (kg)		
Median	4.1	4.0
Range	3.6–5.8	2.9–6.6
Breed		
Domestic short hair	4	9
Domestic long hair	1	1
Siamese	1	1
Persian	0	1
Devon Rex	0	1
Bengalese	0	1
Tumor type		
Mammary adenocarcinoma	1	2
Hepatic carcinoma	1	0
Biliary adenocarcinoma	1	0
Intestinal adenocarcinoma	1	0
Nasal carcinoma	1	0
Apocrine gland adenocarcinoma	1	0
Squamous cell carcinoma	0	5
Pancreatic carcinoma	0	3
Renal carcinoma	0	1
Adrenocortical carcinoma	0	1
Ceruminous gland adenocarcinoma	0	1
Carcinoma of unknown primary site	0	1
Tumor features		
Gross disease	5 (83.3%)	11 (78.6%)
Metastasis	5 (83.3%)	6 (42.8%)
Previous therapy		
Surgery	4	7
Chemotherapy	1	2
NSAID initiated before study	0	3
NSAID initiated at the time of study	0	4
Intended dose intensity (mg/kg/wk)		
Carboplatin	3.333	3.333
Gemcitabine	2	1.333
Received dose intensity (mg/kg/wk)		
Carboplatin	2.865 (85.9%)	3.189 (95.7%)
Gemcitabine	1.603 (80.1%)	1.254 (94.1%)

NSAID, nonsteroidal anti-inflammatory drug.

nasal carcinoma, and apocrine gland adenocarcinoma. Four cats had cytoreductive surgery before inclusion in the study. Five (83.3%) cats had measurable tumors, whereas 1 cat with biliary adenocarcinoma and regional lymph node metastasis had microscopic disease at the initiation of gemcitabine-carboplatin chemotherapy. Five (83.3%) cats had metastasis to lymph nodes (n = 2) or distant sites (n = 3). One cat with metastatic mammary adenocarcinoma had received single-agent mitoxantrone and carboplatin before inclusion in the study. One cat with intestinal adenocarcinoma had a history of chronic renal failure.

Treatment. Twenty-one cycles of the original protocol were administered to 6 cats. Individual cats received a median of 3.75 cycles, with a range of 1–6 cycles per animal. No cats received prior or concurrent NSAIDs.

Hematologic Toxicity. Neutropenia developed in 3 cats (50%) and thrombocytopenia in 2 (33.3%) (Table 3). Eleven neutropenic episodes were recorded and consisted of 2 grade 1 (n = 1), 3 grade 2 (n = 2), 1 grade 3 (n = 1), and 5 grade 4 (n = 2) events. Grade 3 or 4 neutropenia affected 2 (33.3%) cats and all episodes occurred on day 21. In 2 of 3 cats, neutropenia resolved within 7 days of detection. One cat with grade 4 neutropenia required 3 weeks to recover a normal neutrophil count and experienced grade 4 neutropenia in the next cycle despite a 25% gemcitabine dose reduction. At that time, the cat presented with clinical signs suggestive of infection or sepsis (eg lethargy, fever) and was hospitalized for supportive care, but confirmatory diagnostic tests were not performed. The dose of gemcitabine was further reduced subsequently and the cat experienced an additional 4 episodes of grade 1 or 2 neutropenia. This cat had received previous chemotherapy for recurrent mammary adenocarcinoma, including mitoxantrone 3 years earlier, and single-agent carboplatin 3 weeks before the start of gemcitabine-carboplatin chemotherapy. None of the other cats with neutropenia demonstrated clinical signs suggestive of infection or sepsis. Five episodes of thrombocytopenia developed in 2 (33.3%) cats and consisted of 3 grade 1 (n = 2) and 2 grade 4 (n = 1) events. Both cats had concurrent neutropenia. Platelet counts returned to normal within 1 week of detection of thrombocytopenia. Five cats had serum biochemical profiles monitored during treatment and there was no evidence of renal toxicity. One cat with previously diagnosed chronic renal failure had stable blood urea nitrogen and creatinine concentrations after 5 cycles of gemcitabine-carboplatin therapy.

Gastrointestinal Toxicity. All 6 (100%) cats from the 1st cohort developed gastrointestinal toxicity (Table 3). There were 12 grade 1 episodes (n = 6), 5 grade 2 episodes (n = 3), and 2 grade 3 episodes (n = 1). Toxicity consisted of 11 occurrences of vomiting, 10 of anorexia, and 6 of diarrhea. Grade 3 or 4 toxicity affected 1 (16.7%) cat, which had 2 grade 3 episodes. This cat experienced an episode of grade 3 anorexia concurrent with grade 4 neutropenia and possible sepsis, starting 4 days after gemcitabine administration on day 15 of cycle 2. A 2nd episode of grade 3 anorexia developed in this cat after administration of gemcitabine and carboplatin on day 1 of cycle 4.

Treatment Delays and Dose Reductions. One (16.7%) cat required 2 consecutive 25% gemcitabine dose reductions because of grade 4 neutropenia and thrombocytopenia. Thirteen treatment delays were recorded in 4 (66.7%) cats. The intended dose intensity for carboplatin and gemcitabine as well as the average dose intensity received by cats in this cohort are listed in Table 2. There were no treatment-related deaths.

Response. Five cats had measurable tumors and were evaluable for response. No objective responses were

Table 3. Toxicity and response of 1st cat cohort.

		Toxicity Grade			
	Overall	1	2	3	4
Hematologic					
Neutropenia	3 (50%)	1 (16.7%)	2 (33.3%)	1 (16.7%)	2 (33.3%)
Thrombocytopenia	2 (33.3%)	2 (33.3%)	0 (0%)	0 (0%)	1 (16.7%)
Gastrointestinal	6 (100%)	6 (100%)	3 (50%)	1 (16.7%)	0 (0%)
Cats with NSAIDs	0 (0%)	0	0	0	0

	Response			
	CR	PR	SD	PD
All cats	0 (0%)	0 (0%)	4 (80%)	1 (20%)
Cats with NSAIDs	0	0	0	0

NSAIDs, nonsteroidal anti-inflammatory drugs; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

observed (Table 3). Four of the 5 cats achieved SD for a mean and median TTP of 88 and 98 days, respectively (range, 36–119 days). Tumor types in patients that achieved SD included mammary adenocarcinoma (n = 1), intestinal adenocarcinoma (n = 1), nasal carcinoma (n = 1), and apocrine gland adenocarcinoma (n = 1) and all had measurable metastatic disease. One cat with hepatic carcinoma had PD despite treatment. One cat with metastatic biliary adenocarcinoma reduced surgically to microscopic disease received 6 cycles of chemotherapy and had a TTP of 260 days.

Second Patient Cohort

Patient and Tumor Features. Fourteen cats were included in the 2nd cohort. The median age was 13 years and both sexes were represented equally. Additional patient information is listed in Table 2. Types of tumors included oral or tonsilar squamous cell carcinoma (n = 5), pancreatic carcinoma (n = 3), mammary adenocarcinoma (n = 2), and 1 each of renal carcinoma, adrenocortical carcinoma, ceruminous gland adenocarcinoma, and carcinoma of unknown primary site. Seven cats had cytoreductive surgery before inclusion in the study. Eleven (78.6%) cats had measurable tumors and 3 (21.4%) had microscopic disease, including 1 ceruminous gland adenocarcinoma with histopathologic evidence of vascular invasion, and 1 each of adrenocortical carcinoma and renal carcinoma with neoplastic cells breaching the capsule. Six (42.8%) cats had metastasis to lymph nodes (n = 4), distant sites (n = 4), or both (n = 2). One cat had carcinoma in the mesentery and multiple SC tissues with no obvious primary tumor. Two cats with mammary adenocarcinoma and metastasis had previously received chemotherapy for their tumors, consisting of a combination of doxorubicin and cyclophosphamide.

Treatment. Fourteen cats were treated with the modified gemcitabine-carboplatin protocol. Cats received a median of 2 cycles per animal (range, 0.5–10) for 38 cycles administered. Twelve cats received between 1 and 6 cycles. One cat with pancreatic carcinoma did not complete the 1st cycle of gemcitabine-carboplatin because of PD evident on day 13. One cat with pancreatic carcinoma received 10 cycles because of slow but continuous response to treatment. Seven (50%) cats received NSAIDs concurrent with gemcitabine-carboplatin and included cats with oral or tonsilar squamous cell carcinoma (n = 5), mammary adenocarcinoma (n = 1), and pancreatic carcinoma (n = 1). Three of these cats had been receiving an NSAID before inclusion in the study, whereas the remaining 4 cats had the NSAID initiated at the time of the 1st gemcitabine-carboplatin administration.

Hematologic Toxicity. Neutropenia developed in 4 (28.6%) cats and was characterized by 2 grade 1 (n = 2), 1 grade 2 (n = 1), 1 grade 3 (n = 1), and 1 grade 4 (n = 1) episodes (Table 4). Grade 3 or 4 neutropenic events affected 2 (14.3%) cats and occurred on days 8 and 21. Two cats that had received previous chemotherapy had 1 grade 1 neutropenic event each. All episodes of neutropenia resolved within 7 days. No cats developed thrombocytopenia. Serum biochemical profiles were repeated during treatment in 9 cats and none of the cats developed azotemia.

Gastrointestinal Toxicity. Seven (50%) cats experienced gastrointestinal toxicity, which included 16 grade 1 (n = 7), 1 grade 3 (n = 1), and 1 grade 4 (n = 1) episodes (Table 4). Grade 3 or 4 toxicity developed in 1 (7.1%) cat, which had 1 episode of each. This cat had pancreatic carcinoma and experienced lethargy and anorexia 2 weeks after receiving gemcitabine on day 8 of cycle 4. A 2nd episode of anorexia, vomiting, and diarrhea occurred in this cat 2 days after receiving gemcitabine on day 8 of cycle 9. Three of 7 cats receiving NSAIDs had 4 episodes of grade 1 gastrointestinal toxicity. One of these cats had been receiving an NSAID before inclusion in the study whereas the other 2 had the NSAID initiated at the initiation of gemcitabine-carboplatin therapy.

Treatment Delays and Dose Reductions. One (7.1%) cat required a 25% gemcitabine dose reduction because of neutropenia and 6 (42.8%) cats experienced 7 treatment delays. The intended dose intensity for carboplatin and gemcitabine in this cohort and the average dose intensity actually received by cats are listed in Table 2. There were no treatment-related deaths.

Table 4. Toxicity and response of 2nd cat cohort.

		Toxicity Grade			
	Overall	1	2	3	4
Hematologic					
Neutropenia	4 (28.6%)	2 (14.3%)	1 (7.1%)	1 (7.1%)	1 (7.1%)
Thrombocytopenia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Gastrointestinal	7 (50%)	7 (50%)	0 (0%)	1 (7.1%)	1 (7.1%)
Cats with NSAIDs	3 (21.4%)	3 (21.4%)	0 (0%)	0 (0%)	0 (0%)
Before study		1	0	0	0
At the time of study		2	0	0	0

	Response			
	CR	PR	SD	PD
All cats	1 (9.1%)	1 (9.1%)	3 (27.3%)	6 (54.5%)
Cats with NSAIDs	0	1	2	4
Before study	0	0	1	2
At the time of study	0	1	1	2

NSAIDs, nonsteroidal anti-inflammatory drugs; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Response. Eleven cats had measurable tumors and were evaluated for response. One cat with biopsy-confirmed pancreatic carcinoma and tumor infiltration of peripancreatic fat achieved a CR (Table 4). This cat received 10 cycles of chemotherapy because of slow but continual tumor regression during treatment, and was not treated with NSAIDs. There was no evidence of disease on last evaluation 532 days after starting chemotherapy. One cat with oral squamous cell carcinoma had a PR for 27 days. This cat received an NSAID, initiated concurrently with gemcitabine-carboplatin. Three cats experienced SD for a mean and median TTP of 73 and 71 days, respectively (range, 30–118 days). Tumor types in cats that achieved SD included squamous cell carcinoma (n = 2) and mammary adenocarcinoma (n = 1). Five cats had PD during treatment, including 2 with mammary adenocarcinoma, 2 with pancreatic carcinoma, and 1 with squamous cell carcinoma. One cat with tonsilar squamous cell carcinoma did not return for additional chemotherapy after the 1st cycle and died at day 50, before tumor reevaluation. This cat was considered a nonresponder.

Tumor responses in 7 cats receiving NSAIDs included 1 PR (1 squamous cell carcinoma), 2 SD (2 squamous cell carcinoma), and 3 PD (1 squamous cell carcinoma, 1 mammary adenocarcinoma, 1 pancreatic carcinoma). One cat with squamous cell carcinoma died before tumor reevaluation (Table 4).

Three cats with microscopic disease were excluded from response evaluation. Two cats had TTP of 671 days (ceruminous gland adenocarcinoma) and 430 days (adrenocortical carcinoma), whereas 1 cat with a surgically resected renal carcinoma had no evidence of tumor recurrence at 194 days and was lost to follow-up. None of these cats had received NSAIDs concurrently.

Discussion

Carcinomas are common tumors in cats and therapeutic standards have not been well defined. Although surgical resection or radiation are indicated for local tumor control, the role of chemotherapy is unclear and requires additional study.[17,33,34] Gemcitabine has demonstrated synergistic in vitro and clinical antitumor activity in carcinomas of humans, particularly when combined with platinum agents,[25–31] even in tumors resistant to single-agent protocols of either drug.[26,27] Synergism is due in part to gemcitabine triphosphate incorporation into DNA, which induces structural changes in the DNA helix that favor platinum binding, resulting in increased adduct formation.[22] In addition, gemcitabine may inhibit repair of platinum-DNA adducts and crosslinks.[25] Other than radiosensitization studies,[11,12] to our knowledge, there are no reports evaluating gemcitabine in cats, either as a single agent or in combination protocols. For this reason, we initiated this study to determine the toxicity of gemcitabine-carboplatin combination in tumor-bearing cats.

The treatment designs used in this study were based on in vitro data and current practice in human medicine[25–28,30] and modeled after a treatment scheme previously reported for dogs.[b] Given the lack of preclinical gemcitabine data in cats, the weekly dose was extrapolated from radiosensitization studies[11,12] and decreased further to avoid additive myelosuppression with the use of multiple drugs in combination. The carboplatin dose was empirically selected to be less than the maximally tolerated dose reported in cats (240 mg/m^2).[17] Doses of both drugs were calculated based on body weight to minimize variation among patients. Our rationale for separating gemcitabine and carboplatin administration on day 1 of the cycle in the 2nd cohort of cats originated from research suggesting that synergism is dependent on specific time and schedule of drug administration. The antitumor synergy associated with both gemcitabine and platinum exposure is supported by many lines of evidence, but the optimal dose and scheduling for these agents is poorly understood.[22,23,25–30]

Toxicity was high in the 1st cohort of 6 cats treated with the original protocol, in which 50% developed hematologic toxicity and 100% developed gastrointestinal toxicity. Grade 3 or 4 hematologic toxicity affected 33.3% of the cats. Of the cats that experienced higher grade toxicity, 1 with mammary adenocarcinoma that experienced 2 separate episodes of grade 4 neutropenia and prolonged recovery times had received chemotherapy previously. In this case, cumulative myelotoxicity may have contributed to the higher grade toxicity and delayed marrow recovery. All episodes of severe hematologic toxicity occurred on day 21 in this cohort. We hypothesize that weekly administration of gemcitabine with no breaks may result in temporal overlap with myelosuppression induced by carboplatin and that this may have been a cause of the high frequency of neutropenia observed in these cats. In addition, all cats in this cohort experienced gastrointestinal toxicity. However, gastrointestinal toxicity did not appear to be severe and most often was manifested as self-limiting grade 1 or 2 events. Only 1 cat experienced repeated episodes of grade 3 toxicity that required hospitalization for fluid therapy and supportive care. Based on the observed degree of hematologic and gastrointestinal toxicity, 13 treatment delays were required in 66.7% of the cats and 2 gemcitabine dose reductions in 16.7% of the cats in this cohort. Although there were no treatment-related deaths, we considered this outcome to be unacceptable toxicity. In consequence, based on toxicity and lack of observed tumor responses, we concluded that a protocol modification was needed for continued evaluation of gemcitabine-carboplatin in tumor-bearing cats.

Protocol modifications intended to reduce toxicity consisted of discontinuing gemcitabine on day 15. This would allow patients to have additional time for bone marrow recovery and possibly less myelosuppression at the start of subsequent gemcitabine-carboplatin cycles. Additional modification consisted of delaying carboplatin administration on day 1 of each cycle until 4 hours after gemcitabine infusion. This 2nd modification was introduced based on data suggesting that synergism between gemcitabine and carboplatin depends on time and schedule of drug administration.[25–29] Supporting this hypothesis, in vitro research has demonstrated synergism with pretreatment of tumor cells with gemcitabine,[22,25] which may allow sufficient time for gemcitabine triphosphate accumulation and incorporation into DNA by the time of platinum administration. Moreover, gemcitabine uptake and activation may be inhibited by pre-exposure to platinum drugs.[25] No changes were introduced on day 8. With these modifications, we expected the new protocol to be better tolerated than the original protocol, because of changes in dose intensity, and hypothesized that the drug combination would be more synergistic and lead to objective responses in the form of CR or PR. Dose intensity, or the amount of drug given per unit of time, is an important concept in cancer chemotherapy and is correlated with response rates in several solid tumors and hematologic malignancies in humans.[35] The received dose intensity is more clinically relevant than is the intended dose intensity because it reflects the impact of dose reductions and treatment delays in clinical practice. This concept, usually expressed as mg/m^2/wk, also allows differences among chemotherapy protocols to be identified. The high frequency of toxicity in cats treated with the original protocol resulted in a substantial difference between the intended and received dose intensities for carboplatin and gemcitabine in this cohort, as can be seen in Table 2 (expressed as mg/kg/wk).

Toxicity was moderate in the 2nd cohort of 14 cats, treated with the modified protocol, where 28.6% developed hematologic and 50% developed gastrointestinal toxicity. Most episodes were lower grade and the frequency of grade 3 or 4 hematologic and gastrointestinal toxicity was considered acceptable, affecting 14.3 and 7.1% cats, respectively. Although gastrointestinal toxicity was typically mild, 1 cat with pancreatic carcinoma experienced 1 grade 3 and 1 grade 4 episode. One of these episodes was characterized by lethargy and anorexia 2 weeks after receiving gemcitabine. It is unknown whether this episode was a result of chemotherapy, an effect of the tumor, or a consequence of some other unrelated cause. Adverse effects in the 2nd cohort triggered 7 treatment delays in 42.8% of the cats and 1 gemcitabine dose reduction in 7.1% of the cats, which resulted in a mild decrease in the received dose intensity for carboplatin and gemcitabine compared with the original intent (Table 2). Although the lower cumulative dose of gemcitabine and temporal separation of gemcitabine and carboplatin in the modified protocol may have played a role in the observed differences in toxicity, the frequency and severity of adverse effects after protocol modification suggest that we may be at or near the maximally tolerated dose of each drug using this particular combination and schedule. Further optimization of this doublet protocol with a dual dose escalation and schedule attenuation phase I study is required to determine a doublet maximally tolerated dose for each drug that could be used in future studies.

This study was not designed to determine efficacy. The modified protocol however demonstrated minimal but objective antitumor activity inducing a CR in a cat with gross pancreatic carcinoma, which was continuous at 532 days. A 2nd cat with squamous cell carcinoma experienced PR, but this cat received a concurrent NSAID, which introduces confusion regarding the true origin of the tumor response because of the potential for an antitumor effect of the NSAID. The cat with CR of a pancreatic carcinoma developed diabetes mellitus a short time before diagnosis, requiring insulin treatment. Along with tumor regression, exogenous insulin requirements decreased until complete discontinuation and resolution of diabetes. Interestingly, pancreatic carcinoma is a tumor type for which gemcitabine-platinum combinations appear to demonstrate the most synergism in humans.[28,31,36] Although our observation of CR is anecdotal, the absence of an effective chemotherapy protocol against this tumor in cats, along with results of studies in humans, may make this disease an attractive setting for future evaluation of gemcitabine-carboplatin combinations in cats.

Three cats from the 2nd cohort had microscopic disease and were treated in an adjuvant setting. Hence,

it is not possible to adequately assess the impact of chemotherapy on tumor control. These cats had TTP of 671, 430, and at least 194 days and did not receive concurrent NSAIDs. Although these tumors may have had inherently slow growth rates, the duration of time before progression suggests a positive effect of the combination protocol in delaying tumor recurrence.

Seven cats in the 2nd cohort received an NSAID during chemotherapy. This type of medication may increase the likelihood of gastrointestinal toxicity from chemotherapy, but such an effect is not supported by our findings of only 3 of 7 cats on NSAIDs developing gastrointestinal toxicity and all episodes being mild (grade 1). Another source of confusion related to NSAIDs in this study is the potential antitumor activity of these drugs, and response cannot be objectively evaluated in patients receiving NSAIDs. Cats on NSAIDs were allowed in the study because the goal was to compile toxicity data. The PR observed in a cat with squamous cell carcinoma may have been influenced by concurrent NSAID administration, but the CR of a pancreatic carcinoma was not affected by other potentially active drugs and is attributable to the chemotherapy protocol.

There were several limitations in this study. First, gemcitabine pharmacokinetic or pharmacodynamic studies have not been reported in cats. Thus, it is impossible to determine optimal dose and schedule, particularly in combination with other drugs. We extrapolated our dose of gemcitabine from limited reports in cats and based on mild to moderate toxicity observed in dogs that received a similar protocol. Phase I studies of single agent gemcitabine must be conducted in cats to determine the maximally tolerated dose and optimize the dosing scheme, but our results suggest that the doses and schedule used in the original protocol are associated with unacceptable toxicity. Second, we treated a small number of cats, many of which had advanced-stage cancer. Such patient selection may have negatively influenced the duration of time that cats received chemotherapy, which may underestimate both the potential cumulative toxicity of the protocol as well as the potential for improved response rate if cats had received additional cycles of chemotherapy. The small sample size in both cohorts may also decrease the relevance of our findings. Third, there are no reports demonstrating incorporation of gemcitabine in feline (or canine) DNA and thus we cannot conclude that observed responses were because of the effect of drug combination versus carboplatin alone. In vitro studies with feline tumor cell lines would be required to evaluate drug efficacy. Lastly, we treated a heterogeneous population of cats with carcinomas of different types and clinical stage, including many with metastasis and several that received previous treatment and concurrent NSAIDs. For these reasons, this study is not suitable for an accurate evaluation of response and questions regarding the potential efficacy of this drug combination (or lack of efficacy) cannot be answered from our data. Additional evaluation of more homogeneous groups of cats and tumors would be required for this purpose.

In conclusion, results of our pilot study suggest that a gemcitabine-carboplatin combination, as outlined in the modified protocol, can be administered to cats with naturally occurring carcinomas, which induces moderate but acceptable toxicity. Minimal patient benefit suggests that optimization of this combination, with alternative schedules or combinations of gemcitabine with other drugs, should be explored to establish adequate dosing regimens and to accurately evaluate the potential for efficacy.

Footnotes

[a] Wood CA, Moore AS, Frimberger AE, et al. Phase I evaluation of carboplatin in tumor-bearing cats. Proceedings of the Veterinary Cancer Society 16th Annual Conference 1996;39–40 (abstract)

[b] Dominguez PA, Dervisis NG, Cadile CD, et al. Open phase pilot study: Gemcitabine and carboplatin doublet therapy in canine patients with carcinomas. Proceedings of the Veterinary Cancer Society 25th Annual Conference 2005;2 (abstract)

[c] Gemcitabine hydrochloride, Gemzar, Eli Lilly Co, Indianapolis, IN

[d] Carboplatin, Paraplatin, Bristol-Myers Squibb Co, Princeton, NJ

[e] Cancer Therapy Evaluation Program, Common Toxicity Criteria, Version 2.0, DCTD, NCI, NIH, DHHS, March 1998

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