



Dosage escalation of intravenous cyclophosphamide in cats with cancer

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ABSTRACT

Cyclophosphamide is an alkylating agent used as chemotherapy for cats with lymphoma, carcinomas and sarcomas. Clinical and pharmacokinetic studies of cyclophosphamide in normal and tumor-bearing cats have shown minimal toxicity and cyclophosphamide at clinically used dosages rarely requires dosage adjustment or treatment delays. Dose intensity appears important for treatment of most cancers; the aim of this study was to perform a modified dose escalation study of cyclophosphamide to establish the maximally tolerated dosage (MTD) for intravenous cyclophosphamide in cats.

The dose limiting toxicity appeared to be neutropenia, and 30% of cats experienced grade 3 or grade 4 neutropenia at a cyclophosphamide dosage of 480 mg/m², which was determined as the MTD. Delayed neutropenia was observed commonly at higher dosages. Thrombocytopenia was less common than neutropenia, and always transient. Gastrointestinal toxicities were uncommon even at MTD. The recommended dosage for single agent cyclophosphamide in cats is 460 mg/m² with a post-treatment interval of three weeks, with hematology performed before any subsequent chemotherapy is administered. This dosage appears safe in combination with prednisolone and L-asparaginase; but has not been evaluated in combination with other chemotherapy agents, or with a post-treatment interval shorter than 3 weeks. Such combinations and shorter intervals are found in some protocols, so this recommended cyclophosphamide dose cannot be considered a direct substitute for cyclophosphamide dosages in existing protocols. There is a suggestion that inadequate renal function may exacerbate the myelosuppression of cyclophosphamide which should be further evaluated.

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Introduction

Cyclophosphamide is a prodrug that is activated by hepatic cytochrome P450-to alkylating metabolites (Stanton and Legendre, 1986). Its main indication in cats is lymphoma treatment, with some activity also reported in carcinomas and sarcomas (Mauldin et al., 1988). Cyclophosphamide's principal toxicity in cats is myelosuppression, affecting neutrophils more than platelets (Okamura et al., 2003). Gastrointestinal toxicities appear rare, except at very high dosages (Fetting et al., 1982), and cyclophosphamide may have antiemetic properties when administered IV to cats at clinically relevant dosages (Bosnjak and Beleslin, 2002). Transient neurotoxicity has been reported within minutes after administration of very high dosages (200–300 mg/kg IV) (Fetting et al., 1982) although ataxia was reported at lower IV dosages (Bosnjak and Beleslin, 2002). Although sterile hemorrhagic cystitis is a common adverse event in dogs and humans, it has not been

convincingly reported in cats (Crow et al., 1977; Henness, 1985). Pharmacokinetic studies of cyclophosphamide in normal cats have shown minimal toxicity following either IV (Stroda et al., 2017) or intraperitoneal (Voorhorst et al., 2014) dosages of 200 mg/m².

Despite wide use over decades, no clear consensus exists regarding the appropriate feline clinical dosage of cyclophosphamide. Single-agent cyclophosphamide was initially reported in cats with lymphoma at an oral dosage of 12.5 mg/kg daily reducing to 6.25 mg/kg every alternate day (Carpenter and Holzworth, 1971), and IV at 10 mg/kg weekly (Ladiges, 1980). In combination protocols, commonly used dosages in the 20th century included 300 mg/m² PO with vincristine and prednisolone in the COP protocol (Cotter, 1983), and 10 mg/kg IV (Jeglum et al., 1987). Some contemporary reports have used 300 mg/m² (Teske et al., 2002), but most use 200–250 mg/m² (Collette et al., 2016). This results in a wide range of recommended doses; for a 5 kg cat, the total dose ranges from 50 mg (10 mg/kg) to 87.6 mg (300 mg/m²). Toxicities are rarely reported at any of these dosages, therefore we designed a modified dosage escalation study to establish the maximally tolerated dosage of cyclophosphamide in tumour-bearing cats.

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Materials and methods

Animals and hematology

Cats with any histologically or cytologically confirmed neoplasia that received IV cyclophosphamide when not neutropenic were evaluable for this study. Hemograms taken before and 7 days after cyclophosphamide were obtained from a specialty veterinary practice (Animal Referral Hospital, Sydney) and case consultations (Veterinary Oncology Consultants). Initially, 20 1-week post-treatment hemograms from 16 cats that received 300 mg/m² cyclophosphamide were retrospectively evaluated as a baseline for dosage escalation.

Hematologic and gastrointestinal toxicities were graded according to VCOG-CTCAE (Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events), (Table 1) (VCOG, 2016). Neutropenia was defined as an absolute neutrophil count < 3.0 × 10³/μL (DeClue and Spann, 2017).

Dosage escalation

From the baseline of 300 mg/m², the cyclophosphamide dosage was escalated by approximately 10% (specifically 330, 360, 400, 440 and 480 mg/m²) in cohorts of at least six cats. If no grade ≥ 3 toxicity was observed, dosage escalation continued. If 33% cats experienced grade ≥ 3 toxicity, dosage escalation would stop, and this dosage was defined as the maximum tolerated dosage (MTD). The next cohort was entered at a dosage halfway between the previous dosage level and the MTD. Because prolonged grade 1 neutropenia was observed in two cats treated at 400 mg/m², the monitoring protocol was subsequently modified to allow for additional hemograms two and three weeks after cyclophosphamide.

Statistical analysis

Student's *t*-test was performed on paired neutrophil counts from individual cats that received cyclophosphamide at the same dosage (440–480 mg/m²) both with and without concurrent L-asparaginase.

Results

Animals and treatments

Ninety-nine cats were evaluable. Ninety-three cats (94%) had lymphoma, five (5%) had mammary carcinoma, and one cat (1%) had sarcoma.

In the initial retrospective analysis of 20 hemograms from 16 cats treated at 300 mg/m² cyclophosphamide IV, three hemograms from three cats showed neutropenia (grade 1 (*n* = 2), grade 4 (*n* = 1)). One cat with neutropenia at week 1 was still neutropenic (grade 1) at week 2.

All cats with lymphoma received cyclophosphamide and prednisolone concurrently; some cats also received SQ L-asparaginase concurrently with the first, but not subsequent, cyclophosphamide doses. At cyclophosphamide dosages of 440–480 mg/m², 38 of the 67 (57%) cyclophosphamide treatments were with L-asparaginase and 29 (43%) with cyclophosphamide alone.

Because cats were sometimes entered before full data were available for previously treated cats, each dosage level included more than the originally planned six cats. In addition, cats that responded to therapy had a second dose of cyclophosphamide, therefore two sets of toxicity data were available for those cats.

Hematologic toxicity

Unless otherwise specified, hemograms were available one week after all treatments.

No cat experienced clinical sepsis/febrile neutropenia or bleeding.

A summary of the neutrophil data for the cats in this study is found in Table 2 and Fig. 1.

Twelve cats received a total of 17 treatments at 330 mg/m². Eleven hemograms showed normal neutrophil counts and six hemograms (from five cats) showed neutropenia (grade 1 (*n* = 5), grade 2 (*n* = 1)).

Nine cats received a total of 11 treatments at a dosage of 360 mg/m². One hemogram showed neutrophilia, four hemograms showed normal neutrophil counts and six hemograms (from five cats) showed neutropenia (grade 1 (*n* = 5), grade 2 (*n* = 1)).

Twelve cats received a total of 19 treatments at a dosage of 400 mg/m². Two hemograms showed neutrophilia, 13 hemograms showed normal neutrophil counts and four hemograms (from two cats) showed neutropenia (grade 1 (*n* = 1), grade 2 (*n* = 3)). Follow-up hemograms were available after three of those four treatments. While the grade 1 neutropenia normalized by week 2, persistent grade 1 neutropenia was seen two and three weeks after treatment in two cats that showed grade 2 neutropenia, although counts normalized by week 4.

Twenty cats received a total of 27 treatments at a dosage of 440 mg/m². Three hemograms showed neutrophilia, 15 hemograms showed normal neutrophil counts and nine hemograms (from nine cats) showed neutropenia (grade 1 (*n* = 5), grade 2 (*n* = 2), grade 3 (*n* = 2)). Two weeks after treatment, 16 hemograms from 14 cats were available (including six from cats neutropenic at week 1). Twelve showed normal neutrophil counts (including three from cats neutropenic at week 1 (grade 1 (*n* = 2), grade 3 (*n* = 1))), four hemograms (from four cats) showed grade 1 neutropenia; of these cats, three had grade 1 neutropenia at week 1, and one was not neutropenic at week 1. Three cats that were neutropenic at weeks 1 and 2, remained neutropenic three weeks after treatment (all grade 1), although counts normalized by week 4.

Ten cats received a total of 15 treatments at a dosage of 480 mg/m². One week after treatment, hemograms were available after 13 treatments (sample not collected one week after second treatment in two cats). One hemogram showed neutrophilia, nine showed normal neutrophil counts and three (from three cats) showed neutropenia (grade 1 (*n* = 1), grade 3 (*n* = 1), grade 4 (*n* = 1)). The cat with grade 4 neutropenia was euthanized due to progressive renal lymphoma causing renal failure. Two weeks after treatment, 12 hemograms were available (including the two surviving cats neutropenic at week 1). Seven hemograms (including the cat with grade 1 neutropenia at week 1) showed normal or high neutrophil counts, and five hemograms (from four cats) showed grade 1 neutropenia (including one cat with grade 3 neutropenia at week 1, and four cats with normal neutrophil counts at week 1). Neutropenia persisted to week 3 in four of the five hemograms (grade 1 (*n* = 3), grade 3 (*n* = 1)). All neutropenic cats had hemograms evaluated at week 4 and all showed normal neutrophil counts.

Table 1

Selected VCOG-CTCAE (Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events) toxicity grading criteria used in this study.

Haematology	Grade 1	Grade 2	Grade 3	Grade 4
Neutrophils (per 10 ³ /μL)	1.5–normal	1.0–1.5	0.5–1.0	<0.5
Platelets (per 10 ³ /μL)	100–normal	50–100	25–50	<25
Anorexia	Coaxing required to maintain appetite	Inappetence <3 days without weight loss	Inappetence >3 days; weight loss >10%; direct intervention required	Life-threatening; total parental nutrition required

Table 2
Neutropenic events and grades following different dosages of cyclophosphamide in tumour-bearing cats.

Dosage mg/m ²	Cats <i>n</i>	Treatments <i>n</i>	Grade of neutropenia (treatments)					Grade 3–4 (treatments) <i>n</i>	Grade 3–4 (cats) <i>n</i>	Cats neutropenic at week 2 <i>n</i>
			0 <i>n</i>	1	2	3	4			
300	16	20	17	2	0	0	1	1 (5%)	1 (5%)	1 (5%)
330	12	17	11	5	1	0	0	–	–	–
360	9	11	5	5	1	0	0	–	–	–
400	12	19	15	1	3	0	0	–	–	2 (17%)
440	20	27	17	6	2	2	0	2 (7%)	2 (10%)	4 (20%)
460	22	25	12	8	1	1	3	4 (16%)	4 (18%)	4 (18%)
480	10	15	8	3	1	2	1	3 (20%)	3 (30%)	4 (40%)

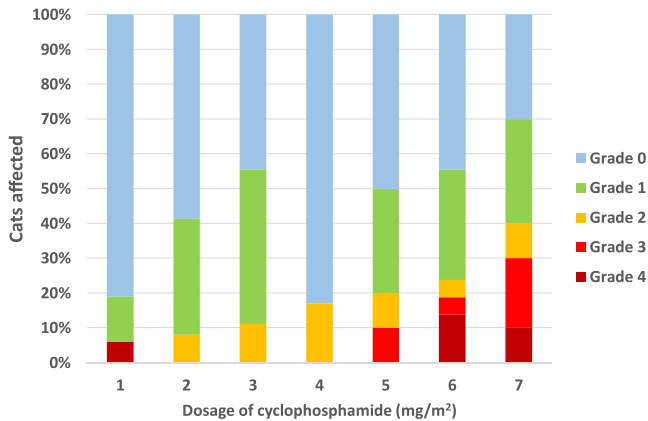


Fig. 1. Grades of neutropenia recorded for cats treated at different cyclophosphamide dosages.

Because three cats (30%) experienced grade 3 or 4 neutropenia after 480 mg/m², an additional cohort was entered at 460 mg/m². Twenty-two cats received a total of 25 treatments at a dosage of 460 mg/m² cyclophosphamide IV. Hemograms were available one week after 23 treatments in 22 cats. One hemogram showed neutrophilia, 12 hemograms (from 11 cats) showed normal neutrophil counts, and 10 hemograms (from 10 cats) showed neutropenia (grade 1 (*n* = 5), grade 2 (*n* = 1), grade 3 (*n* = 1), grade 4 (*n* = 3)). One cat with grade 4 neutropenia was euthanized due to progressive renal lymphoma. Two weeks after treatment, 19 hemograms were available from 18 cats including eight hemograms from cats that were neutropenic at week 1. Neutrophil counts were normal or high in 15, including all cats with grade 1 or 2 neutropenia at week 1, and 4 cats were neutropenic. Two cats that were not neutropenic at week 1 had grade 1 neutropenia at week 2. Additionally, the two surviving cats with grade 4 neutropenia at week 1 had persistent neutropenia. In one cat, neutropenia was grade 1 at week 2 and grade 2 at week 3 before resolving. In the other cat, neutropenia was grade 4 at week 2,

grade 2 at week 3 and grade 1 at week 4 before resolving. Three weeks after treatment, 18 hemograms were available. In addition to the two cats above, three additional cats had grade 1 neutropenia (all three had shown grade 1 neutropenia at week 1, normal counts at week 2 but then showed an apparent “double nadir”). Neutrophil counts were normal at week 4 in three of these four cats, but in the fourth no sample was available.

Among cats that received the same dosage of cyclophosphamide (440–480 mg/m²) more than once, 13 cats received at least one of the treatments with L-asparaginase and at least one without L-asparaginase. The neutrophil counts for these cats were analyzed separately to evaluate whether there was any impact of concurrent L-asparaginase on the neutrophil count. T-test results showed no significant difference with or without L-asparaginase (*P* = 0.899).

Thrombocytopenia was observed but was not dose-limiting. The platelet data is summarized in Table 3.

Other toxicities

Gastrointestinal signs were not reported for any cats treated at 300 mg/m².

At 330 mg/m², one of 12 (8.3%) cats was inappetent (grade 2) after a second dose of cyclophosphamide but was also experiencing progressive lymphoma.

At 360 mg/m², two of nine (22%) cats were inappetent (grade 2); both recovered without intervention.

At 400 mg/m², there were five occurrences of inappetence in three of 12 (25%) cats (grade 2 (*n* = 2), grade 3 (*n* = 3) [mild but lasting 5–7 days]); all recovered without treatment.

At 440 mg/m², five of 18 (28%) cats were inappetent (grade 2 (*n* = 4), grade 3 (*n* = 1) [resulted in owners discontinuing chemotherapy]). Three of the four cats with grade 2 inappetence were given cyclophosphamide again, with mirtazapine (*n* = 1), omeprazole and maropitant (*n* = 1), and maropitant (*n* = 1) and no inappetence was reported.

At 460 mg/m², three of 22 (14%) cats were inappetent (grade 2 (*n* = 2) [improved with mirtazapine or maropitant]; grade 3 (*n* = 1)). In one of the cats with grade 2 inappetence, a second and a third

Table 3
Thrombocytopenic events and grades following different dosages of cyclophosphamide in tumour-bearing cats.

Dosage mg/m ²	Cats <i>n</i>	Treatments <i>n</i>	Grade of TCP at week 1 (treatments)					Grade 3–4 at week 1 (treatments) <i>n</i>	Grade 3–4 at week 1 (cats) <i>n</i>	Cats with TCP at week 2 <i>n</i>
			0 <i>n</i>	1	2	3	4			
300	15	19	13	5	1	0	0	0 (0%)	0 (0%)	ND
330	12	16	11	3	1	1	0	1 (5.3%)	1 (8.3%)	ND
360	9	10	3	4	2	0	1	1 (10.0%)	1 (11.1%)	ND
400	10	13	5	4	3	1	0	1 (7.7%)	1 (10.0%)	ND
440	17	22	12	3	4	0	3	3 (13.6%)	3 (17.6%)	ND
460	17	18	10	4	1	3	0	3 (16.7%)	3 (17.6%)	1
480	10	12	7	3	1	1	0	1 (8.3%)	1 (10.0%)	ND

TCP = thrombocytopenia; ND = not done.

cyclophosphamide dose were given with mirtazapine, and inappetence recurred after the third dose. The cat with grade 3 inappetence had no weight loss but remained inappetent for four weeks; this cat was persistently azotemic before therapy and also had grade 4 neutropenia.

At 480 mg/m², five of 10 (50%) cats were inappetent (grade 2 ($n=4$), grade 3 ($n=1$)). Three of the cats with grade 2 inappetence were given cyclophosphamide a second time with mirtazapine ($n=1$), omeprazole and maropitant ($n=1$) and maropitant ($n=1$) and no inappetence occurred. The fourth cat with grade 2 inappetence was not treated again due to progressive renal lymphoma. The cat with grade 3 inappetence received cyclophosphamide again at 360 mg/m² but weight loss and inappetence continued after the subsequent treatment.

Of all 15 cats with inappetence after any dosage of cyclophosphamide, seven had gastrointestinal lymphoma, three had renal lymphoma, two had hepatic lymphoma, and one each had nodal lymphoma or mammary carcinoma.

Two cats developed azotemia after cyclophosphamide at 440 mg/m²; neither had azotemia within 3 weeks before cyclophosphamide. Azotemia was seen 21 days after the first treatment in one cat, and one week after the fifth dose of cyclophosphamide in a second cat. No clinical evidence for sterile hemorrhagic cystitis (SHC) was observed in this study.

Discussion

In “3+3” Phase 1 trials, the usual acceptable criteria for cessation of dosage escalation is when two of six patients experience dose-limiting toxicity (Le Tourneau et al., 2009). In this study, the toxicity that reached these criteria first was neutropenia. We found that at a cyclophosphamide dosage of 480 mg/m², three of 10 cats (30%) experienced grade 3 or 4 neutropenia, which we felt met the criteria for stopping dosage escalation. At the next dosage level down (460 mg/m²) four of 22 cats (18%) developed grade 3 or 4 neutropenia. In this study, grade 3 and 4 thrombocytopenia occurred, but was less common than neutropenia and was sometimes associated with platelet clumping. Our impression is that at least some of the recorded thrombocytopenia may not reflect true hematologic toxicity, but rather the difficulty of measuring platelet numbers in cats; but in any case, even the recorded thrombocytopenia did not meet criteria for stopping dose escalation. When gastrointestinal toxicity occurred, it was generally mild and responded to anti-nausea medications.

Based on this study, the recommended cyclophosphamide dosage to use in clinical practice when administered as a single agent, would be 460 mg/m²; with a post-treatment interval of three weeks, and hematology collected prior to each subsequent chemotherapy to confirm complete hematologic recovery. Our findings suggest that the MTD for single agent cyclophosphamide in cats is 480 mg/m², with neutropenia the dose limiting toxicity. The majority of neutropenic episodes were recorded one week after cyclophosphamide was administered, but neutropenia was slow to resolve in some cats; and a smaller number of cats developed neutropenia two to three weeks after cyclophosphamide even at lower dosages. This suggests that at the escalated dosage, a post-treatment interval of more than two weeks is needed before a subsequent myelosuppressive treatment. At 480 mg/m², four cats showed an apparent “double-nadir” in neutrophils; the second nadir was grade 1 or 2, but this suggests that, even three weeks after treatment, hematology monitoring is advisable before additional chemotherapy is given.

More than half of the cyclophosphamide treatments in this study were given with concurrent L-asparaginase. To the authors' knowledge no interactions between these two drugs have been

reported in any species. We compared post-treatment neutrophil counts in cats that received the same dose of cyclophosphamide both alone and with L-asparaginase and found no difference. Thus, we do not believe this alters the findings of this study. On the other hand, there are published protocols that combine cyclophosphamide with other chemotherapy agents (such as some COP protocols where vincristine is administered on the same day (Cotter, 1983)). We did not evaluate, and therefore do not recommend, using such combinations with the higher dosage of cyclophosphamide, or with a post-treatment interval shorter than 3 weeks. Cyclophosphamide at a dosage of 460 mg/m² should only be used when administering cyclophosphamide as a single agent, or with L-asparaginase or prednisolone; and subsequent chemotherapy agents should only be administered after 3 weeks and confirmation of normal peripheral neutrophil counts.

Prolonged neutropenia was also reported in Phase 1 evaluation of ifosfamide in cats, (Rassnick et al., 2006); and delayed resolution of neutropenia has also been reported after carboplatin in cats (Kisseberth et al., 2008).

Of the 34 cats treated at 460–480 mg/m², five cats had renal lymphoma, pretreatment azotemia, or both; and three of those five (60%) developed grade 4 neutropenia. In humans, after hepatic metabolism of the parent drug much of the active metabolites and unactivated parent drug are renally cleared (Haubitz et al., 2002); therefore renal insufficiency is sometimes considered a criterion for cyclophosphamide dosage reduction. Although metabolism and elimination of cyclophosphamide in cats is assumed to be similar to that in humans, no detailed pharmacokinetic studies of this drug have been performed in cats to our knowledge. Increased myelosuppression in cats with renal dysfunction, treated with cyclophosphamide, has not been previously reported to our knowledge, but this may be because typically used cyclophosphamide dosages in cats have been well below the MTD. Therefore, it may be prudent to monitor cats with renal insufficiency carefully when giving cyclophosphamide and possibly use a lower dosage; as is recommended for carboplatin therapy (Bailey et al., 2009). Two cats with renal lymphoma treated with cyclophosphamide at 440 mg/m² developed grade 0 and grade 1 neutropenia, and none of the four cats with renal lymphoma treated below that dose became neutropenic.

Based on the non-hematologic toxicities seen, gastrointestinal supportive medication is warranted for mild nausea and/or inappetence that was seen after approximately 25% of treatments at a dosage of 460–480 mg/m². Although responses and treatments varied, improvement in appetite was seen when maropitant or mirtazapine were given. In this study, about half the patients that developed inappetence after cyclophosphamide had gastrointestinal lymphoma (seven of 15; 47%); most cats received the drug early in treatment, while macroscopic lymphoma was present, and it was difficult to separate gastrointestinal signs arising from chemotherapy from those caused by lymphoma. Therefore the prevalence of gastrointestinal toxicities may have been higher than would be observed in clinical practice, in cats without gastrointestinal cancer. No clinical evidence for SHC was observed in this study; this is consistent with the literature where cystitis has only rarely been reported in cats treated with cyclophosphamide, and whether it was SHC in those cases remains unconfirmed. The reason for this is speculative but may relate to different metabolite formation in cats.

Although the individual cyclophosphamide dosage recommended here is markedly higher (300 mg/m² PO versus 460 mg/m² IV), the dose intensity would only be substantially increased if the same post-treatment interval is used, as is the case with most COP protocols that use a 3-week interval (Cotter, 1983). For more dose-dense protocols, such as some protocols where a myelosuppressive agent is given 2 weeks

after cyclophosphamide (Collette et al., 2016), the dose intensity would be similar if giving cyclophosphamide at 300 mg/m² every 2 weeks (150 mg/m²/week) compared to 460 mg/m² every 3 weeks (153 mg/m²/week). However, the similar dose density may be offset by higher peak blood levels of active metabolites achieved with the dosage escalation, which should allow for a higher percentage of tumor cell kill (Blayney et al., 2005). The latter may be clinically important in lymphoma, where cyclophosphamide can be used for induction.

The cyclophosphamide dosage recommended in this study is also approximately twice the recommended canine dosage of 200–250 mg/m². Similarly, the clinically recommended dosage of ifosfamide in cats of 900 mg/m² is almost 2.5 times the clinically recommended dosage for dogs (Rassnick et al., 2006). Ifosfamide metabolism also depends on cytochrome P450 (CYP), particularly CYP3A, for activation, and previous data suggests that feline cytochrome-mediated metabolism differs from that of dogs, horses and humans (Chauret et al., 1997). Recently, novel CYP3A genes were identified in the liver and small intestines of cats (Honda et al., 2011), and may result in the different tolerability of cyclophosphamide in cats.

This study was not designed to assess the clinical efficacy (i.e. tumor response rate) for cats receiving different dosages of cyclophosphamide. A recent report of a combination protocol using a cyclophosphamide dosage of 200–250 mg/m² IV or PO for feline lymphoma reported a 38% complete response rate, and median survival time of 97 days (Collette et al., 2016). Higher response rates have been reported in studies using a cyclophosphamide dosage of 300 mg/m² (Cotter, 1983; Teske et al., 2002); and although speculative, the difference may be partly due to the higher dosage cyclophosphamide. Further studies using this higher cyclophosphamide dosage would yield more information regarding efficacy.

Conclusions

The dose-limiting toxicity of cyclophosphamide in cats is neutropenia, but when administered as a single agent, or in combination with L-asparaginase or prednisolone, the MTD is substantially higher than the dose currently in clinical use. Under these circumstances, cyclophosphamide at 460 mg/m² IV every 3 weeks appears to be an appropriate dosage to use in cats with normal renal function. Further evaluation of tumor response to this higher dosage, and of cyclophosphamide dosing in cats with renal insufficiency may be warranted.

Conflict of interest statement

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