

Incidence of sterile hemorrhagic cystitis in tumor-bearing dogs concurrently treated with oral metronomic cyclophosphamide chemotherapy and furosemide: 55 cases (2009–2015)

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OBJECTIVE

To determine the incidence of sterile hemorrhagic cystitis (SHC) in tumor-bearing dogs concurrently treated with oral metronomic cyclophosphamide chemotherapy and furosemide.

DESIGN

Retrospective case series.

ANIMALS

55 dogs.

PROCEDURES

Record databases of 2 specialty practices were searched to identify dogs treated with oral metronomic cyclophosphamide chemotherapy in conjunction with furosemide for a minimum of 28 days between January 2009 and December 2015. Information extracted from the records included signalment, tumor diagnosis, cyclophosphamide and furosemide dosages, and concurrent medications. Confirmed SHC was defined as the presence of gross or microscopic hematuria and clinical signs associated with lower urinary tract disease in the absence of infection or neoplasia of the urinary tract; the definition for suspected SHC was the same, except the absence of infection or neoplasia of the urinary tract was not confirmed.

RESULTS

Cyclophosphamide dosage varied from 6.5 to 18.6 mg/m² once daily to 6.3 to 49.2 mg/m² every other day. Median duration of cyclophosphamide administration was 272 days (range, 28 to 1,393 days). Median cumulative dose of cyclophosphamide administered was 2,898 mg/m² (range, 224 to 14,725 mg/m²). Median furosemide dose was 1.4 mg/kg (0.64 mg/lb). Confirmed or suspected SHC was identified in 2 of 55 (3.6%) dogs. Cyclophosphamide administration was discontinued for the dog with confirmed SHC but not the dog with suspected SHC.

CONCLUSIONS AND CLINICAL RELEVANCE

Results indicated that oral administration of furosemide in conjunction with oral metronomic cyclophosphamide chemotherapy was associated with a low incidence of SHC, which suggested that furosemide may protect against cyclophosphamide-induced SHC. (*J Am Vet Med Assoc* 2016;249:1408–1414)

Cyclophosphamide is a widely used alkylating agent for the treatment of various cancers in both people and dogs. The indications for its use in dogs include lymphoma,^{1–3} sarcomas,^{4,5} and carcinomas.⁶ Sterile hemorrhagic cystitis is a well-documented adverse effect associated with cyclophosphamide administration in both humans⁷ and dogs.^{1–6,8–13,a–c} Sterile hemorrhagic cystitis is a painful condition char-

acterized clinically by signs of lower urinary tract disease such as hematuria, pollakiuria, and stranguria without evidence of a urinary tract infection.^{6,7,9} It can be a debilitating and sometimes irreversible and fatal consequence of cyclophosphamide toxicity that necessitates discontinuing administration of the drug.^{4,7,8,c} The pathogenesis of SHC involves the formation and accumulation of acrolein and 4-hydroxymetabolites (by-products of cyclophosphamide metabolism)¹⁴ in the urine, which cause submucosal edema, hemorrhage, necrosis, and fibrosis of the urinary bladder mucosal epithelium.^{7,8,12,15}

In both human patients and dogs, the incidence of SHC is associated with the cyclophosphamide dose. Sterile hemorrhagic cystitis has been reported

ABBREVIATIONS

MTD	Maximum tolerated dose
SHC	Sterile hemorrhagic cystitis
VCOG-CTCAE	Veterinary Cooperative Oncology Group–Common Terminology Criteria for Adverse Events

following oral or IV administration of conventional MTDs of the drug.^{6,7,11-13} It has also been reported in patients receiving chronic (metronomic) oral administration of cyclophosphamide,^{4,5,7,8,16,a-c} and the risk of a patient developing SHC increases as the cumulative dose of cyclophosphamide administered increases.^{7,9,16-18,b} In dogs, the reported incidence of SHC ranges from 3.8% to 12.1% following administration of the MTD of cyclophosphamide (200 to 300 mg/m²) and decreases to 1.2%² and 1.8%¹¹ when cyclophosphamide is administered concurrently with IV or oral administration of furosemide, respectively.

Metronomic cyclophosphamide chemotherapy is defined as long-term administration of cyclophosphamide, generally at doses lower than the MTD, at equally spaced intervals without extended rest periods (ie, periods when the drug is not administered).¹⁹ In dogs, metronomic cyclophosphamide chemotherapy is commonly used to treat soft tissue sarcoma⁴ and splenic hemangiosarcoma.⁵ In contrast to administration of the MTD of cyclophosphamide, which frequently results in direct cytotoxicosis, metronomic dosing of cyclophosphamide is believed to stimulate the immune response by suppressing regulatory T cells,^{20,21} increasing interferon γ secretion,²⁰ and suppressing angiogenesis through modulation of the secretion of vascular endothelial growth factor and thrombospondin-1.²² Metronomic cyclophosphamide chemotherapy is an attractive treatment option for many owners because they can administer the drug orally to their dogs at home, the risk of gastrointestinal and hematologic toxicoses is low, and the cost is relatively low, compared with other treatment options. The incidence of SHC in dogs treated with metronomic cyclophosphamide chemotherapy without concurrent administration of furosemide ranges from 6.9% to 32% (**Online Supplement S1**, available at avmajournals.avma.org/doi/suppl/10.2460/javma.249.12.1408).^{4,5,8,a-c}

Several approaches have been suggested to decrease the incidence and severity of SHC in dogs receiving cyclophosphamide such as administering the drug in the morning and allowing the dog free access to water and frequent opportunities to urinate,^{6,10} in addition to concurrent administration of furosemide^{2,10,11} or glucocorticoids^{2,6,8} to promote diuresis and minimize the duration of contact between acrolein metabolites and the bladder epithelium. In human medicine, mesna (2-mercaptoethane sulfonate sodium), a sulfhydryl compound, is routinely used to prevent SHC in patients receiving high-dose cyclophosphamide chemotherapy.^{16,23-25} Mesna binds to and detoxifies urotoxic metabolites such as acrolein in the kidneys and urinary tract.^{14,25} Although there is evidence that administration of mesna¹¹ or furosemide^{2,11} decreases the incidence of SHC in dogs receiving the IV or oral MTD of cyclophosphamide, concurrent administration of furosemide to dogs receiving oral metronomic cyclophosphamide chemotherapy has yet to become standard practice.^{4,5,8,a-c}

The purpose of the study reported here was to determine the incidence of SHC in tumor-bearing dogs concurrently treated with oral metronomic cyclophosphamide chemotherapy and furosemide. We hypothesized that the incidence of SHC in dogs receiving oral metronomic cyclophosphamide chemotherapy in conjunction with furosemide would be lower than that reported in other studies^{4,5,8,a-c} for dogs receiving oral metronomic cyclophosphamide chemotherapy without furosemide.

Materials and Methods

Case selection

The medical record databases for the Animal Referral Hospital in Sydney, NSW, Australia, and Veterinary Oncology Consultants in Wauchope, NSW, Australia, were searched to identify records for tumor-bearing dogs that received oral metronomic cyclophosphamide chemotherapy in conjunction with furosemide between January 2009 and December 2015. Dogs were included in the study if they were orally administered cyclophosphamide and furosemide once daily or every other day for a minimum of 28 days. Dogs enrolled in the study also had to have urinalysis results that were within reference limits prior to initiation of cyclophosphamide and furosemide treatment (baseline) and have a urinalysis performed 4 to 6 weeks after treatment initiation and then every 6 to 12 weeks thereafter while receiving cyclophosphamide.

Medical records review

For each dog enrolled in the study, information extracted from the medical record included signalment (age, sex, and breed), body weight, tumor diagnosis, cyclophosphamide dosage (dose, frequency, and duration), furosemide dosage, and dosage of any other medications (eg, corticosteroids, NSAIDs, toceranib, doxycycline, tamoxifen, and any other chemotherapeutic agents) concurrently administered. The total number of doses and cumulative dose of cyclophosphamide administered were calculated.

Confirmed SHC was defined as the presence of gross or microscopic hematuria (as determined by urinalysis) and clinical signs associated with lower urinary tract disease (eg, hematuria, pollakiuria, and stranguria) without evidence of infection (as determined by negative results for bacteriologic culture of a urine sample at baseline) or neoplasia of the urinary tract (as determined by an abdominal ultrasonographic examination). Suspected SHC was defined as the presence of gross or microscopic hematuria (as determined by urinalysis) and clinical signs associated with lower urinary tract disease without confirmation of the absence of infection or neoplasia of the urinary tract (ie, bacteriologic culture of a urine sample or abdominal ultrasonographic examination did not need to be performed at baseline). For each dog with confirmed or suspected SHC, the severity of

the condition was retrospectively graded on the basis of VCOG-CTCAE version 1.1 criteria,²⁶ and the time to the development of confirmed or suspected SHC (number of days from initiation of cyclophosphamide administration to diagnosis of confirmed or suspected SHC; duration to SHC), additional laboratory findings, and follow-up information were recorded.

Cyclophosphamide and furosemide preparations

Cyclophosphamide powder was compounded into capsules of 3 different strengths (5, 10, and 20 mg) by an Australian compounding pharmacy^d in accordance with the Australian Code of Good Manufacturing Practice. For each batch of raw cyclophosphamide powder, analyses for potency, sterility, and active ingredients were performed with high-performance liquid chromatography at an external laboratory. Additionally, each batch of powder came with a certificate of analysis to verify that the active ingredient was of appropriate identity and purity. Potency was considered acceptable if it ranged between 97.0% and 103%. At the compounding pharmacy, cyclophosphamide powder was digitally weighed and placed into capsules; the capsules were confirmed to contain the appropriate amount of powder by 2 pharmacists who used software on 2 separate occasions. The expiration date for each batch of capsules was set 180 days after the date of compounding. The stability of the compounded cyclophosphamide capsules was not routinely tested. Furosemide was administered orally as commercially available 20- or 40-mg scored tablets.^{e-h}

Results

Study population

The initial record search identified 80 dogs that were treated with metronomic cyclophosphamide chemotherapy during the 7-year observation period. Twenty-five dogs were subsequently excluded from the study because of insufficient information ($n = 7$), they were not administered furosemide in conjunction with the cyclophosphamide (8), or they received cyclophosphamide and furosemide for < 28 days (10). Thus, the study population consisted of 55 dogs. The median duration of follow-up was 272 days (range, 28 to 1,393 days) after initiation of cyclophosphamide and furosemide administration.

The study population consisted of 21 castrated males, 7 sexually intact males, 25 spayed females, and 2 sexually intact females with a median age of 10 years (range, 4 to 17 years) and body weight of 23.7 kg (52.1 lb; range, 5.7 to 53.0 kg [12.5 to 116.6 lb]). Twenty breeds were represented, with the most common being mixed ($n = 15$), Labrador Retriever (9), Golden Retriever (4), and Rottweiler (4); all other breeds were represented by ≤ 4 dogs. Diagnoses included soft tissue sarcoma ($n = 26$), hemangiosarcoma (13), lymphoma (4), osteosarcoma (3), squa-

mous cell carcinoma (2), and apocrine gland anal sac adenocarcinoma, histiocytic sarcoma, infiltrative lipoma, melanoma, multiple myeloma, small intestinal adenocarcinoma and extraskelatal osteosarcoma, and thyroid carcinoma (1 each).

Cyclophosphamide and furosemide treatment protocols

Cyclophosphamide was administered orally once daily to 43 dogs and every other day to 7 dogs. Four dogs received cyclophosphamide once daily initially and then were switched to every-other-day administration. One dog received cyclophosphamide every other day initially and then was switched to once-daily administration. The median dose of cyclophosphamide was 12.7 mg/m² (range, 6.5 to 18.6 mg/m²) for dogs receiving the drug once daily and 14.2 mg/m² (range, 6.3 to 49.2 mg/m²) for dogs receiving the drug every other day. For the overall population, the median cyclophosphamide dose was 12.8 mg/m² and the median cumulative dose of cyclophosphamide administered was 2,898 mg/m² (range, 224 to 14,725 mg/m²). The median dose of furosemide orally administered with each dose of cyclophosphamide was 1.4 mg/kg (0.64 mg/lb; range, 0.80 to 2.4 mg/kg [0.36 to 1.09 mg/lb]). The median treatment duration was 272 days (range, 28 to 1,393 days), and the median number of doses of cyclophosphamide administered was 233 doses (range, 16 to 1,393 doses).

Other medications administered concurrently

While receiving cyclophosphamide and furosemide, 2 dogs also received prednisolone (0.7 and 1.4 mg/kg [0.32 and 0.64 mg/lb], PO, every other day, respectively). Nonsteroidal antiinflammatory drugs were administered to 47 of the 55 (85.5%) dogs, of which 44 received piroxicam (0.25 to 0.31 mg/kg [0.11 to 0.14 mg/lb], PO, once daily or every other day), 2 received firocoxib (4.0 and 5.2 mg/kg [1.82 and 2.36 mg/lb], PO, once daily, respectively), and 1 received meloxicam (0.12 mg/kg [0.05 mg/lb], PO, once daily). Four dogs received toceranib (median dose, 2.6 mg/kg [1.18 mg/lb; range, 2.3 to 3.0 mg/kg {1.05 to 1.36 mg/lb}], PO with food, every Monday, Wednesday, and Friday). One dog received chlorambucil at a dose of 6.6 mg/m², PO, every other day, and 1 dog received chlorambucil at alternating doses of 5 and 2.5 mg/m², PO, every other day. One dog received L-asparaginase (10,000 U/m², SC, twice 4 weeks apart). Twenty-eight dogs received doxycycline (median dose, 5.1 mg/kg [2.32 mg/lb; range, 4.4 to 6.6 mg/kg {2.0 to 3.0 mg/lb}], PO, twice daily with food) and tamoxifen (median dose, 1.1 mg/kg [0.5 mg/lb; range, 0.84 to 1.3 mg/kg {0.38 to 0.59 mg/lb}], PO, once daily).

SHC

Sterile hemorrhagic cystitis was confirmed in 1 dog (VCOG-CTCAE grade 4) and suspected in 1 dog (VCOG-CTCAE grade 2). Thus, the incidence rate of

SHC within the study population was 3.6% (2/55). Three other dogs had microscopic hematuria as determined by a dipstick ($n = 1$) or urinalysis performed by a reference laboratory (2), but did not have clinical signs of lower urinary tract disease and therefore did not meet the definition of SHC. Those 3 dogs continued to receive cyclophosphamide without developing clinical signs of SHC, and results of all subsequent urinalyses performed for those dogs were within reference limits.

The dog with confirmed SHC was a 9-year-old 40-kg (88-lb) spayed female Labrador Retriever with an incompletely resected grade I soft tissue sarcoma that was administered cyclophosphamide (8.5 mg/m², PO, once daily) and furosemide (1.0 mg/kg [0.45 mg/lb], PO, once daily) in addition to piroxicam (0.25 mg/kg, PO, once daily with food). The dog was examined 511 days after initiation of cyclophosphamide administration (cumulative dose of cyclophosphamide administered, 4,344 mg/m²) because of a 3-week history of pollakiuria, stranguria, and dark yellow urine. A free-catch urine sample was submitted to a reference laboratory for urinalysis, which revealed $> 100 \times 10^6$ RBCs/L but no bacteria. Bacteriologic culture of a urine sample obtained by cystocentesis yielded negative results. An abdominal ultrasonographic examination revealed a focal area (diameter, 0.6 cm) of thickened wall at the right dorsal or apical region of the bladder. Cyclophosphamide administration was discontinued. Three-view thoracic radiographs were obtained, and no remarkable abnormalities were detected. A laparotomy was performed. Grossly, the thickened area in the bladder wall was ulcerated, and white material covered the mucosal surface. The abnormal portion of the bladder wall was resected and submitted for histologic evaluation. Results revealed submucosal edema, hemorrhage, necrosis, fibrosis, and ulcerative neutrophilic cystitis, all of which were consistent with SHC. The hematuria and other clinical signs of lower urinary tract disease resolved (ie, urinalysis results were within reference limits, and bacteriologic culture of a urine sample yielded negative results) during the 8 weeks after surgery. Cyclophosphamide administration was not resumed, and the dog did not develop any clinical signs associated with lower urinary tract disease during the 673 days after surgery during which it was monitored.

The dog with suspected SHC was an 8.5-year-old 37-kg (81.4-lb) spayed female Rottweiler with an incompletely resected grade I soft tissue sarcoma that was administered cyclophosphamide (10.2 mg/m², PO, once daily) and furosemide (1.1 mg/kg, PO, once daily) in addition to piroxicam (0.27 mg/kg [0.12 mg/lb], PO, once daily with food), doxycycline (6.1 mg/kg [2.77 mg/lb], PO, twice daily with food), and tamoxifen (1.1 mg/kg, PO, once daily). The dog had acute onset of pollakiuria 106 days after initiation of cyclophosphamide administration (cumulative dose of cyclophosphamide administered, 2,264 mg/m²). Results of a dipstick analysis of a free-catch

urine sample revealed $> 250 \times 10^6$ RBCs/L; microscopic evaluation and bacteriologic culture of a urine sample were not performed. The cyclophosphamide dosage was changed to 10.2 mg/m², PO, every other day. The clinical signs resolved spontaneously within 1 week after the dosage change. The dog remained free of clinical signs of lower urinary tract disease for 232 days after the dosage change, and results of all subsequent urinalyses were within reference limits.

Discussion

In the present study, the overall incidence rate of SHC was 3.6% (2/55) for dogs that were orally administered metronomic cyclophosphamide chemotherapy in conjunction with furosemide, which was lower than that (6.9% to 32%) reported in other studies^{4,5,8,a-c} for dogs that were orally administered metronomic cyclophosphamide chemotherapy without furosemide. However, it should be noted that the criteria used to diagnose SHC in the present study were stricter than the criteria used to diagnose SHC in those other studies.^{4,5,8,a-c} The incidence rate of SHC ranges from 1.2% to 1.8% in dogs treated with the MTD of cyclophosphamide (PO or IV) in combination with furosemide.^{2,10,11} If the criteria used to diagnose SHC in those studies^{2,10,11} had been used in the present study, the dog with suspected SHC would not have been identified as having SHC, and the SHC incidence rate would have been 1.8% (1/55), which is consistent with that reported in those studies.^{2,10,11}

None of the 8 dogs that were excluded from the present study because they received metronomic cyclophosphamide and furosemide for < 28 days developed SHC. However, 2 of the 10 dogs that were excluded because they received metronomic cyclophosphamide without furosemide developed confirmed (VCOG-CTCAE grade 4) or suspected (VCOG-CTCAE grade 2) SHC at 557 and 120 days, respectively, after initiation of cyclophosphamide administration. Although conclusions cannot be drawn regarding the risk of SHC in the small number of dogs that were excluded from the study, we found it interesting that the incidence rate of confirmed or suspected SHC in dogs that did not receive furosemide in conjunction with metronomic cyclophosphamide chemotherapy (3/10 [30%]) was substantially greater than that (2/55 [3.6%]) for dogs that did receive furosemide in conjunction with metronomic cyclophosphamide chemotherapy.

The risk of SHC is positively associated with the cumulative dose of cyclophosphamide administered in both human patients and dogs.^{7,9,16-18,b} In a data-driven review¹⁶ of 11 studies of human patients with confirmed SHC, the mean cumulative dose of cyclophosphamide administered was $> 100,000$ mg (range, 2,000 to 531,000 mg) over a mean of > 2.5 years (range, 1 month to 12 years). In many studies^{1,2,4,5,11,a,c} involving dogs, it is difficult to determine the nature of the relationship between the cumulative dose of cyclophosphamide and risk of SHC because the cumulative

dose of cyclophosphamide administered was either not calculated or analyzed as a risk factor for SHC. However, the cumulative dose of cyclophosphamide administered to dogs with confirmed SHC has been reported by investigators of other studies.^{2,8,9,11,27,b} In a study⁸ of 14 dogs that developed SHC after receiving metronomic cyclophosphamide chemotherapy, the median cumulative dose of cyclophosphamide administered was 3,600 mg/m² (range, 1,600 to 10,400 mg/m²), whereas in a study⁹ of 22 dogs that developed SHC after receiving the oral MTD of cyclophosphamide, the median cumulative dose of cyclophosphamide administered was 1,570 mg/m² (range, 494 to 6,545 mg/m²). In another study,¹¹ the median cumulative dose of cyclophosphamide administered was 200 mg/m² (range, 175 and 225 mg/m²) for 2 dogs that developed SHC after receiving the oral MTD of cyclophosphamide (200 or 250 mg/m²) and 700 mg/m² (range, 200 to 800 mg/m²) for 4 dogs that developed SHC after receiving the IV MTD of cyclophosphamide (200 mg/m²). For dogs receiving the IV MTD of cyclophosphamide, SHC developed after administration of a median of 2 doses (range, 1 to 6 doses; n = 22 dogs) in 1 study² and after a median of 2.5 doses (range, 1 to 3 doses; 6 dogs) in another study.²⁷ There was a significant positive association between the cumulative oral dose of cyclophosphamide administered and the risk of SHC for dogs of 2 studies.^{9,b} In the present study, the median cumulative dose of cyclophosphamide administered was 2,898 mg/m² (range, 224 to 14,725 mg/m²) for all study dogs; the cumulative dose of cyclophosphamide administered was 4,453 mg/m² for the dog with confirmed SHC and 2,264 mg/m² for the dog with suspected SHC. Because only 2 of the 55 dogs of the present study developed confirmed or suspected SHC, we did not perform any statistical analyses to evaluate the association between specific risk factors such as the cumulative cyclophosphamide dose administered and the development of SHC.

In the present study, the duration from initiation of cyclophosphamide administration to diagnosis of SHC (duration to SHC) was 511 days for the dog with confirmed SHC and 106 days for the dog with suspected SHC, which were similar to the median duration to SHC (122 to 216 days) reported by investigators of other studies^{4,5,8,a-c} involving dogs that received oral metronomic cyclophosphamide chemotherapy without furosemide. It is possible that the risk of SHC increases as the duration of oral administration of cyclophosphamide increases. Unfortunately, the association between the duration of cyclophosphamide administration and the risk of SHC could not be assessed for the dogs of the present study, and the nature of such a relationship remains speculative.

Oral metronomic cyclophosphamide chemotherapy dosages reported in the veterinary literature^{4,5,8,a-c} range from 10 to 25 mg/m² once daily to 10 to 50 mg/m² every other day. Although the cyclophosphamide dosage (50 mg/m², PO, every other day) administered to the dogs in the 1977 Crow et al⁸ study was

not described as metronomic (the term had not been coined yet), we used it for comparison with the present study because the dosage was in accordance with the definition of metronomic chemotherapy that was subsequently developed and accepted by the medical community.¹⁹

In the present study, the dose of cyclophosphamide ranged from 6.5 to 18.6 mg/m² (median, 12.7 mg/m²) for the once-daily protocols and from 6.3 to 49.2 mg/m² (median, 14.2 mg/m²) for the every-other-day protocols. Unfortunately, most of the scientific literature of SHC in dogs treated with cyclophosphamide is retrospective in nature, which means that the cyclophosphamide dosages are not standardized and the cumulative dose of cyclophosphamide administered and duration of treatment and follow-up for affected dogs are frequently not reported. Additionally, SHC is not clearly defined in multiple studies,^{1,4,5,a-c} which makes comparisons among studies difficult.

Furosemide tablets for oral administration are readily available and fairly inexpensive. The mechanism by which furosemide prevents SHC has not been elucidated but is believed to be associated with its diuretic activity, which decreases the concentrations of acrolein and 4-hydroxymetabolites of cyclophosphamide in the urine and the duration of contact that those metabolites have with the bladder mucosa, thereby decreasing their toxic effects on the mucosa.

Most of the dogs of the present study received medications in addition to cyclophosphamide and furosemide, which prevented us from assessing adverse effects aside from SHC. On the basis of the data obtained from the records of the dogs of the present study, it appeared that oral administration of cyclophosphamide and furosemide was well tolerated. The most frequently reported adverse effects associated with furosemide administration include dehydration; an increase in the excretion of sodium, potassium, calcium, and magnesium; and an increase in BUN and plasma creatinine concentrations,²⁸ and those changes were not noted in any of the dogs of the present study. One dog developed adverse gastrointestinal effects (VCOG-CTCAE grade 2), and its owner discontinued cyclophosphamide and furosemide administration; however, it was also receiving piroxicam, which was a more likely cause of the adverse gastrointestinal effects than either cyclophosphamide or furosemide.

The majority (47/55 [85.5%]) of dogs in the present study received NSAIDs in addition to cyclophosphamide and furosemide. Long-term administration of furosemide may exacerbate the nephrotoxic potential of concurrently administered NSAIDs because furosemide is a diuretic, which may induce dehydration. Although none of the dogs of the present study developed clinically apparent renal dysfunction, renal function should be closely monitored in patients concurrently treated with NSAIDs and furosemide.

The dog with suspected SHC in the present study received tamoxifen in addition to cyclophosphamide and furosemide. Tamoxifen is a synthetic antiestro-

genic compound that has antiangiogenic and both proestrogenic and antiestrogenic effects in dogs.²⁹⁻³¹ It is possible that the hematuria detected in that dog was a result of tamoxifen-induced vaginitis and was not associated with cyclophosphamide toxicosis.

It has been suggested that glucocorticoids may provide protection against SHC because they promote water intake and thus diuresis.^{2,8} In a study⁸ of 203 dogs and 32 cats treated with cyclophosphamide, the overall incidence rate of SHC was 4.8% (8/168) for patients that received prednisone and 10.4% (7/67) for patients that did not receive prednisone. In the present study, only 2 dogs received prednisolone (0.7 and 1.4 mg/kg, PO, every other day) concurrently with cyclophosphamide and furosemide. Neither of those dogs developed SHC. Thus, the effect of glucocorticoid administration on the development of SHC could not be evaluated in the present study, and the role of glucocorticoids in the prevention of SHC remains undefined. Further prospective studies are necessary to compare the incidence of SHC between dogs concurrently administered cyclophosphamide and furosemide and dogs concurrently administered cyclophosphamide and a glucocorticoid.

The limitations of the present study were those typical for retrospective studies and included a small number of dogs with confirmed or suspected SHC (which prevented statistical assessment of risk factors associated with the development of SHC), the lack of a standardized treatment protocol, and the lack of a control group. Additionally, data collection may have been hindered by incomplete medical records and recall bias by referring veterinarians. Another potential limitation of this study was the administration of compounded cyclophosphamide capsules. Although all capsules were manufactured in accordance with the Australian Code of Good Manufacturing Practice by a nationally recognized compounding pharmacy, which used internal quality controls for the cyclophosphamide potency of the capsules, no stability validation studies were performed and no independent quality controls were used to assure the cyclophosphamide potency of the capsules, which could raise concerns about dosage variability. Compounded capsules are commonly used in clinical practice, despite increasing concerns regarding the potency and stability of compounded chemotherapeutics.³² Future studies involving the use of compounded cyclophosphamide preparations should include quality controls, and investigators should report the stability and potency data obtained from the compounding pharmacy.

A strength of the present study was that serial urine samples were obtained for standardized urinalysis from all dogs throughout the duration of cyclophosphamide treatment, which likely increased the sensitivity for detection of SHC. Serial urinalyses were not routinely performed in other studies,^{2,3,11,a-c} and patients frequently had clinical signs of lower urinary tract disease before further evaluation for SHC was performed. It is likely that patients with mild

clinical signs of lower urinary tract disease were not identified in those studies,^{2,3,11,a-c} which could have resulted in underestimation of the incidence of SHC.

A review¹⁶ of 14 studies involving human patients who developed bladder cancer after receiving cyclophosphamide orally on a daily basis revealed that the cumulative dose of cyclophosphamide administered was > 100,000 mg for most of those patients. The incidence of cyclophosphamide-induced SHC in human patients ranges from 0.7% to 7.5% (median, 2.5%).^{7,16,25} For white persons in the United States, the estimated annual incidence rate of bladder cancer is 40 cases/100,000 men and 10 cases/100,000 women¹⁶; thus, it appears that oral administration of cyclophosphamide on a daily basis to human patients confers a substantial, independent, and probably dose-related increase in the risk for bladder cancer that may persist for many years after administration of the drug is discontinued.¹⁶ Additionally, results of most studies^{7,16,33,34} that evaluated the risk of bladder cancer in human patients following daily oral administration of cyclophosphamide indicate that the risk of bladder cancer was positively associated with the presence of hematuria and cystitis during cyclophosphamide administration. Occasionally, dogs that develop SHC subsequently develop transitional cell carcinomas³⁵⁻³⁷ or urinary tract infections.^{2,8} Unfortunately, because only 2 dogs developed confirmed or suspected SHC in the present study, we could not accurately evaluate the incidence of transitional cell carcinoma and urinary tract infection subsequent to SHC. Long-term follow-up of a large number of dogs is necessary to determine whether, similar to human patients, those that develop SHC are at an increased risk for transitional cell carcinoma, compared with those that do not develop SHC.

Sterile hemorrhagic cystitis is a potentially debilitating complication that can limit the quality of life for dogs treated with oral metronomic cyclophosphamide chemotherapy. Results of the present study indicated that oral administration of furosemide in conjunction with oral metronomic cyclophosphamide chemotherapy was associated with a low incidence of SHC, which suggested that furosemide may protect against cyclophosphamide-induced SHC. A prospective clinical trial with a larger study population than that of the present study is necessary to definitively determine whether the incidence of SHC for dogs concurrently treated with metronomic cyclophosphamide chemotherapy and furosemide is lower than that for dogs treated with metronomic cyclophosphamide chemotherapy without furosemide.

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Footnotes

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