**Introduction**

Metronomic chemotherapy (MC) describes the administration of chronic, uninterrupted doses of chemotherapy agents much lower than the maximum tolerated dose (MTD)([Kerbel and Kamen 2004](#_ENREF_16)). MC has several purported mechanisms of action including anti-angiogenesis, promoting host anti-tumour immunity and induction of tumour dormancy ([Biller 2014](#_ENREF_3)). Inhibition of angiogenesis occurs through the inhibition of rapidly dividing endothelial cells within the tumour micro-environment together with inhibition of endothelial cell migration and the up-regulation of the anti-angiogenic factor thrombospodin-1 ([Bocci and others 2003](#_ENREF_4), [Damber and others 2006](#_ENREF_9), [Kerbel and Kamen 2004](#_ENREF_16), [Pasquier and others 2013](#_ENREF_22)). Host anti-tumour immunity has been shown to be suppressed in a variety of neoplasia through the induction of regulatory T lymphocytes (TREG) and myeloid derived suppressor cells (MDSCs) ([Toh and others 2012](#_ENREF_25), [Umansky and Sevko 2013](#_ENREF_26)). These two factors suppress both innate and adaptive immunity in the host, and high TREG counts have been correlated with poor prognosis in a variety of tumours ([Kono and others 2006](#_ENREF_18)). MC has been shown to decrease TREG in certain tumours and to increase lymphocyte proliferation, thus stimulating anti-tumour immunity ([Banissi and others 2009](#_ENREF_1), [Burton and others 2011](#_ENREF_6), [Ghiringhelli and others 2007](#_ENREF_14)).

MC has become more widespread in human oncology over the past ten years and it has been used to treat a variety of advanced neoplasia including breast, ovarian and prostate cancers together with multiple myeloma and melanoma ([Barroso-Sousa and others 2015](#_ENREF_2), [Borne and others 2010](#_ENREF_5), [El-Husseiny and others 2016](#_ENREF_11), [Papanikolaou and others 2013](#_ENREF_21), [Perroud and others 2016](#_ENREF_23)). MC may be ideally suited to veterinary oncology given the ease of administration, supposed minimal toxicity and low cost. However the evidence supporting its efficacy in the canine literature is currently limited, with the majority of studies focusing on its use in soft tissue sarcomas ([Burton and others 2011](#_ENREF_6), [Cancedda and others 2015](#_ENREF_7), [Elmslie and others 2008](#_ENREF_12)). Furthermore, there is very little data regarding toxicity in canine patients – the majority of studies so far report low toxicity: however drugs, dosing regimens, monitoring and reporting are not standardised.

The main toxicity of concern for dogs treated with metronomic doses of cyclophosphamide is sterile haemorrhagic cystitis (SHC), which is caused by the interaction of acrolein (a breakdown product of cyclophosphamide) and the bladder endothelium. The frequency of SHC in canine patients treated with metronomic cyclophosphamide is not well understood – it was reported in as few as 7% of dogs in one study ([Elmslie and others 2008](#_ENREF_12)) compared with 22% in another ([Lana and others 2007](#_ENREF_19)). In the latter study the dose of cyclophosphamide was not standard and the study size was very small (9 dogs in total). Moreover, monitoring and reporting of SHC is also not standardised. Sterile haematuria without clinical signs of cystitis may represent a precursor step to clinical SHC but the incidence of haematuria in dogs treated with metronomic cyclophosphamide is not known. In man haemorrhagic cystitis is defined as a spectrum from microscopic haematuria to fulminating haemorrhagic cystitis, which can be life threatening ([Hassan and others 2007](#_ENREF_15)) and the Veterinary Comparative Oncology Group similarly grades cystitis from microscopic haematuria to catastrophic bleeding ([VCOG 2011](#_ENREF_27)). The incidence of SHC in human patients treated with metronomic cyclophosphamide is also not well reported. It has previously been shown that the concurrent administration of furosemide to dogs receiving MTD cyclophosphamide reduces the risk of developing SHC but again the use of furosemide in a chronic metronomic setting has not been reported ([Charney and others 2003](#_ENREF_8)).

The aim of this study was to retrospectively analyse a cohort of dogs treated with metronomic cyclophosphamide to assess toxicity. Our suspicion was that dogs developed SHC (defined as microscopic or macroscopic haematuria in the absence of infection) at a much greater incidence than has previously reported. A second aim was to assess whether cessation of cyclophosphamide therapy on detection of haematuria would prevent development of clinical signs of cystitis in most cases

**Materials and Methods**

The study design was approved by the University Research Ethics Committee. The clinical records of the hospital were reviewed using the keywords ‘dog’ and ‘cyclophosphamide’. Dogs were eligible for inclusion if they received metronomic doses of cyclophosphamide (defined as between 5 and 15 mg/m2/day or every other day) for treatment of neoplasia. Dogs were excluded if they were concurrently receiving MTD chemotherapy. The following patient data was collected; age, sex, breed, previous therapy, dose of cyclophosphamide, duration of treatment and concurrent drugs received. The dogs must have had a cytological or histological diagnosis of neoplasia and baseline blood tests and urinalysis within seven days prior to treatment to exclude any significant pre-existing abnormalities. Urinalysis was either complete laboratory analysis or dipstick analysis to exclude pre-existing haematuria. Monitoring was performed once every four weeks at either the hospital or the referring veterinary practice and included haematology (to assess for myelotoxicity) and dipstick urinalysis to assess for haematuria. In the majority of cases (59/65) owners were also provided with urine dipsticks and instructed on how to monitor for haematuria at home: owners performed dipstick tests weekly or biweekly. The decision to dispense urine dipsticks was made by the individual clinician at the time. Three different brands of urine dipstick were dispensed to the owners over the time period of the study.

The diagnosis of SHC was based on detection of haematuria in the absence of a urinary tract infection: on detection of haematuria (with or without clinical signs of cystitis) urine culture was performed on a sample obtained by cystocentesis. Should the urine culture be sterile then a diagnosis of SHC was made and cyclophosphamide was discontinued. The presence of urinary tract signs at diagnosis, or in a four week follow up period, were recorded where possible. Other toxicities were recorded in the clinical notes and, where appropriate, were retrospectively assigned a VCOG-CTE score ([VCOG 2011](#_ENREF_27)). Dogs without at least one set of follow-up data were excluded.

**Results**

The database search returned 500 dogs that had received cyclophosphamide between 2007 and 2015. Of these, 95 dogs had received metronomic doses of the drug. From these 95 dogs, 30 had insufficient data to be included, leaving 65 dogs that met the inclusion criteria. The median age was 10 years (range 2 - 14.3) and the median weight was 25.4kg (range 5-59). The most commonly treated breeds were cross breeds (n= 17), Labradors (n=12), border collies (n=5) and boxers (n=4). The majority of dogs (52/65) had received some form of previous treatment, including surgery (32/65), MTD chemotherapy (including carboplatin, epirubicin, mitoxantrone and 5-FU; 7/65 dogs) and radiotherapy (13/65). Five of 65 dogs received surgery and radiotherapy prior to metronomic chemotherapy. The most common tumour types were soft tissue sarcomas (n=18), haemangiosarcoma (n=14, comprising 6 splenic, 4 subcutaneous and 2 cardiac) and osteosarcoma (n=4). Other tumour types consisted of 7 oral tumours (3 fibrosarcomas, 2 sarcomas, 1 carcinoma and 1 squamous cell carcinoma), 6 nasal tumours (4 carcinoma, 2 sarcoma) and 4 mesotheliomas (figure 1).

The median dose of cyclophosphamide administered was 10.37mg/m2 per treatment (range 4.54-16.34). Twenty-two of the dogs received every other day dosing; the remaining 43 were treated daily. The majority (51/65) of dogs received meloxicam as the concurrent non-steroidal anti-inflammatory (NSAID); the remainder received piroxicam (11/65), carprofen (1 dog), firocoxib (1 dog) and 1 dog did not receive NSAID therapy due to pre-existing gastrointestinal disease. The median duration of treatment was 70 days (range 7-686). Seven dogs received treatment for over twelve months.

Toxicity occurred in 32 out of 65 dogs (figure 2). The most common toxicity was SHC which occurred in 16 dogs, based on documentation of haematuria and a negative urine culture. Six of the dogs developed clinical signs of cystitis including dysuria, pollakiuria and nocturia. In these six dogs the clinical signs persisted for a median of three months (range 1-8). In two dogs, the clinical signs developed after microscopic haematuria was detected and cyclophosphamide therapy was withdrawn. Four of the six dogs received therapy for SHC including N-acetyl D-glucosamine (Cystaid®, VetPlus) in 3 dogs and intravesicular dimethyl sulfoxide (DMSO) in one dog. In all dogs cyclophosphamide was discontinued on the discovery of haematuria, and for the dogs with no clinical signs of cystitis no treatment was given for the cystitis. The median time to development of SHC was 110 days (range 7-686).

Twelve out of 65 dogs had some form of gastro-intestinal toxicity which in all cases was attributed to the NSAID rather than cyclophosphamide treatment, as the signs resolved on cessation of the NSAID while cyclophosphamide treatment continued. Four dogs had VCOG grade I-II small intestinal diarrhoea while six dogs developed VCOG grade I-II vomiting. One dog became inappetent. None of the dogs required hospitalisation and in all cases the signs resolved with symptomatic therapy. However, in two cases of vomiting the owners elected to discontinue therapy. One dog developed a suspected gastric ulcer (based on clinical signs and ultrasonography) which resolved with symptomatic therapy. Concerning other toxicity, four dogs developed suspected bacterial infections during treatment (one case each of lymphadenitis, cutaneous lesions, abscesses and septic cholecystitis). One dog also developed severe (VCOG grade 3) proteinuria which improved on cessation of therapy. Myelotoxicity was not detected in any of the dogs.

**Discussion**

The results of the study have reported a relatively high incidence of toxicity (49%) for dogs treated with metronomic doses of cyclophosphamide, though these toxicities were generally low grade and often had minimal expected impact on quality of life. Almost 25% of dogs developed sterile haemorrhagic cystitis, which is a higher incidence than has previously been reported in similar sized cohorts.. Only one study has reported a similar incidence although it involved only nine dogs and non-standard doses of metronomic cyclophosphamide. Routine urine dipstick was also not performed in this study ([Lana and others 2007](#_ENREF_19)). In human medicine, length of treatment with low-dose cyclophosphamide has been associated with the development of SHC ([Yilmaz and others 2015](#_ENREF_29)). This has also been shown in dogs receiving MTD cyclophosphamide for lymphoma ([Gaeta and others 2014](#_ENREF_13)). It is unclear why the incidence of SHC was higher in this study compared with those previously reported although it may be due to the more vigilant monitoring for haematuria by owners and clinicians than in other studies. Previous studies have reported SHC only when the dog has shown clinical signs and indeed in this study the majority of dogs (10/16) that developed SHC did not show clinical signs of stranguria or pollakiuria. These cases were diagnosed with SHC based on the presence of microscopic or macroscopic haematuria in the absence of bacterial infection. Urinary bladder ultrasound was not performed in every case and we cannot exclude other potential causes of haematuria e.g. urolithiasis, though most cases had long follow up and co-morbidities would likely have become apparent.

In man SHC is known to be a spectrum of disease and the presence of haematuria is significant due to the deleterious effects of acrolein on the bladder mucosa. Given the potentially deleterious effects of clinical SHC on canine quality of life we would recommend close monitoring for haematuria in dogs treated with metronomic cyclophosphamide. Withdrawal of cyclophosphamide appeared to prevent the development of clinical signs in most cases, but the development of clinical signs in two dogs following the withdrawal of cyclophosphamide is of concern and warrants prompt action on the discovery of microscopic haematuria.

Gastro-intestinal side effects were also encountered in this study, and were attributed to the non-steroidal anti-inflammatory medication. Both meloxicam and piroxicam are reported to cause vomiting and diarrhoea – the incidence of gastrointestinal side effects in dogs chronically receiving meloxicam is reported to be between 7.6 and 10.%% ([Doig and others 2000](#_ENREF_10), [Wernham and others 2011](#_ENREF_28)). The incidence of gastrointestinal side effects in this study (16.9%) would therefore be expected and the addition of cyclophosphamide does not seem to significantly increase the risk of developing gastrointestinal toxicity. We cannot however completely exclude cyclophosphamide as a cause of vomiting and diarrhoea in our cohort due to the retrospective nature of the study and the concomitant administration of both drugs. In the majority of dogs that developed gastrointestinal toxicity, the signs resolved with a break from the NSAID medication (whilst continuing cyclophosphamide) and the treatment could subsequently be restarted. However in two dogs the owners elected to discontinue metronomic chemotherapy due the incidence of gastro-intestinal side effects and owners should be properly counselled prior to the start of treatment regarding the possible risk of this toxicity.

 Four dogs developed non-urinary tract bacterial infections while receiving treatment which is a side effect not previously reported. In man there are reports of patients developing infections which are presumed to be secondary to immunosuppression although these are infrequent considering the numbers of patients treated with metronomic chemotherapy ([Kong and others 2010](#_ENREF_17), [Reardon and others 2009](#_ENREF_24)) and often involve patients receiving more myelosuppressive drugs. Two studies regarding metronomic cyclophosphamide in geriatric human patients did not report infection as a potential side effect ([Borne and others 2010](#_ENREF_5), [Mir and others 2011](#_ENREF_20)). None of the dogs in the study developed neutropenia and the infections in these dogs may have been coincidental. Nevertheless, further investigation is needed to identify whether this is a genuine problem in veterinary patients treated with metronomic chemotherapy.

**Conclusions**

The incidence of toxicity in this group of dogs treated with metronomic chemotherapy was higher than previously reported. Gastro-intestinal effects were mild and consistent with those seen in dogs treated for an extended period with a non-steroidal anti-inflammatory drug. The incidence of sterile haemorrhagic cystitis was higher than previously reported which may be due to increased vigilance. Further studies are needed to assess the possible connection between metronomic chemotherapy and opportunistic infection.

**Conflict of interest statement**

No conflicts of interest have been declared

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