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# Review Article
# Metronomic Chemotherapy in Small Animal Practice: An Update

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## Abstract

Metronomic chemotherapy can be considered a new chemotherapy modality involving continuous administration of low doses of cytostatic agents which targets endothelial cells. Endothelial cells are vital for the development of new blood vessels and are essential for tumour growth. Furthermore, these cells are genetically stable, divide at a high rate and do not acquire resistance to cytotoxic drugs as tumour cells. Therefore, they are sensitive to low doses of cytostatics. Because of the importance of metronomic chemotherapy in veterinary medicine, this review provides an update of the status of metronomic chemotherapy in small animal practice. In dogs with cancer, metronomic chemotherapy can be considered a good treatment option and a first-line therapy. Further studies evaluating the levels of regulatory T cells Tregs and tumour microvessel density are needed to better understand the role of this therapeutic modality in different canine tumours.

**Key words:** Regulatory T cells, angiogenesis, tumour microenvironment, cyclophosphamide, COX-2

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## INTRODUCTION

The epidemiological factors, such as environmental influences and somatic DNA mutations which are associated with development of cancer, a very common disease in humans and animals are shared across species (Fonseca-Alves *et al*., 2013). The tumour microenvironment is crucial to cancer progression and is affected by different growth factors (Liu *et al*., 2011), such as Platelet Derived Growth Factor (PDGF), Fibroblast Growth Factor (FGF) and Vascular Endothelial Growth Factor (VEGF) (Byrne *et al*., 2015).

The blood vessels associated with tumours are an attractive target for anticancer therapy, as endothelial cells are less susceptible to cancer chemotherapy resistance and are more genetically stable than tumour cells (Liu *et al*., 2011). Metronomic chemotherapy, proposed in 2000 is a relatively new chemotherapy modality in amurine model (Hanahan *et al*., 2000). Metronomic chemotherapy is based on the use of low doses of cytotoxic chemotherapy with the aim of modulating the tumour micro environment and stimulating the immune system by inhibition of angiogenesis and regulatory T cells (Hanahan *et al*., 2000). In contrast, traditional chemotherapy is based on the use of cytotoxic drugs that inhibit or kill rapidly dividing cells. These drugs are mostly administered only once or as part of therapy cycles at the highest dose that does not result in fatal toxicity ("Maximum Tolerated Dose [MTD]) (Kerbel and Kamen, 2004).

Neoplastic cells divide faster than normal cells and are targeted by MTD chemotherapy; however, this therapy causes some unintended effects in normal cells. For tissue recovery, a specific interval is needed between doses to allow the recovery of vulnerable normal tissues, such as the intestinal epithelial and bone marrow precursor cells. During this period, it is important to identify adverse effects and institute supportive therapy if required (Mutsaers, 2009). The definition of metronomic chemotherapy can vary but typically involves a continuous oral administration of cytotoxic chemotherapy drugs which target endothelial cells of vessels supplying the neoplastic cells (Kerbel and Kamen, 2004). In this context, low-dose chemotherapy may be considered an example of anti-angiogenic therapy. This form of therapy has become popular in small-animal oncology (Mutsaers, 2009). This review provides an update on the status of metronomic chemotherapy in small-animal practice.

## TUMOUR ANGIOGENESIS

Angiogenesis is defined as the formation of new blood vessels from pre-existing vasculature (Fantasia, 2015), a process that is essential for normal development and tissue homeostasis. Under physiological conditions, angiogenesis is active in embryogenesis, tissue development, ovulation, corpus luteum formation and the healing process (Folkman, 2007). In contrast, pathological angiogenesis occurs in a variety of disorders and is characterized by neovascularization as seen in proliferative retinopathy, age-related macular degeneration, rheumatoid arthritis, psoriasis, diabetes, tumour growth and metastatic spread (Fantasia, 2015).

Angiogenesis is an important mechanism in tumour development and is responsible for nutritional support that facilitates proliferation of cancer cells and in establishing favourable conditions for metastatic spread (Tian *et al*., 2015). It is a complex, multistage process that involves remodelling of the extracellular matrix, endothelial cell migration, proliferation, differentiation and capillary anastomosis (Fantasia, 2015). The anti-angiogenic effect of metronomic chemotherapy is associated with rapidly dividing tumour-associated endothelium (Mutsaers, 2009). In conventional chemotherapy, the long intervals between treatment cycles promote the survival and regeneration of endothelial cells, allowing tumour angiogenesis to continue (Hanahan *et al*., 2000). In metronomic chemotherapy, however, the use of daily doses does not permit the replication of these endothelial cells (Mutsaers, 2009).

Several angiogenic stimulatory molecules have been characterized, including basic Fibroblast Growth Factor (bFGF), growth factors bound to heparin, growth factor derived from platelets (PDGF), Tumour Necrosis Factors (TNF and TNFβ), Transforming Growth Factor (TGFα) and Vascular Endothelial Growth Factor (VEGF) (Pasquier *et al*., 2010). The VEGF is a particularly potent mitogen in angiogenesis and its function seems to be related to the endogenous regulation of this process (Kerbel and Kamen, 2004).

The main target of metronomic chemotherapy is the endothelial cells of tumour blood vessels. Metronomic chemotherapy results in inhibition of blood vessel growth by selective inhibition of proliferation and/or induction of apoptosis of activated endothelial cells, selective inhibition of endothelial cell migration and a steady decrease in the levels and viability of circulating endothelial progenitor cells (Pasquier *et al*., 2010) leading to inhibition of tumour growth. In comparison, classic chemotherapy allows the mobilization of endothelial progenitor cells and the formation of resistance to chemotherapy (Liu *et al*., 2011).

## IMMUNE SYSTEM

Besides the inhibition of angiogenesis, another mechanism responsible for the anti-tumour effect of metronomic chemotherapy involves the stimulation of the immune response by regulation of circulating levels of regulatory T cells (Tregs) (Mutsaers, 2009). Tumour cells may be destroyed by lymphocytes, particularly cytotoxic T lymphocytes, T helper cells and/or Natural Killer (NK) cells. However, this immune response can be down regulated by Treg cells. Treg cells normally are involved in the prevention of autoimmune diseases and the inhibition of anti-tumour immune responses but in the tumour microenvironment, these cells negatively modulate the immune system (Fig. 1) (Mutsaers, 2009). Regulatory T cells (Treg cells) in the tumour microenvironment cause suppression of the immune response by lymphocytes and Natural Killer (NK) cells (Fig. 1).

Several studies have shown high levels of Treg cells in different tumour types which are associated with tumour development and lack of treatment response (Pasquier *et al*., 2010). The immunostimulatory effect of metronomic chemotherapy involves suppression of circulating Treg cell levels, leading to proliferation of peripheral T cells in the tumour microenvironment and destruction of the tumour cells (Burton *et al*., 2011).

Low doses of cyclophosphamide used as metronomic chemotherapy were able to reduce CD4+/CD25+ clusters of differentiated T cells (Treg cells) in human patients with cancer (Pasquier *et al*., 2010). A study of immune responses during chemotherapy treatment in dogs with lymphoma and osteosarcoma also showed a decrease in the number of T cells and antibody responses, supporting the concept that this form of chemotherapy induces immunosuppression (Lana and Dobson, 2011).

Recent studies suggested that circulating Treg cells are increased in dogs with various cancers, including melanoma, lymphoma and osteosarcoma (Biller *et al*., 2007). According to Burton *et al*. (2011), dogs with soft tissue sarcoma showed higher levels of Treg cells than healthy dogs. When dogs with soft tissue sarcomas received cyclophosphamide orally at a dose of 15 mg $m^{-2}$ daily, the number of Treg cells decreased over a period of 28 days (Burton *et al*., 2011). This treatment was also associated with a significant decrease in blood vessel density within tumour tissues.

## DRUGS USED IN METRONOMIC CHEMOTHERAPY

Although often considered experimental, metronomic treatment protocols use common chemotherapeutic drugs, such as capecitabine, cyclophosphamide, lomustine, methotrexate, thalidomide, paclitaxel and prednisone among others and have produced encouraging results (Mutsaers, 2009). These drugs are classified as cytostatic agents, cytotoxic drugs and anti-angiogenic drugs. Among the cytotoxic agents, cyclophosphamide is the most commonly used in metronomic therapy (Fonseca-Alves *et al*., 2015); however, several studies have evaluated other neoplastic drugs, such as chlorambucil, lomustine and methotrexate (Tripp *et al*., 2011).

In addition to the agents commonly used in metronomic chemotherapy protocols, other drugs are used because of their anti-angiogenic properties. The main representatives

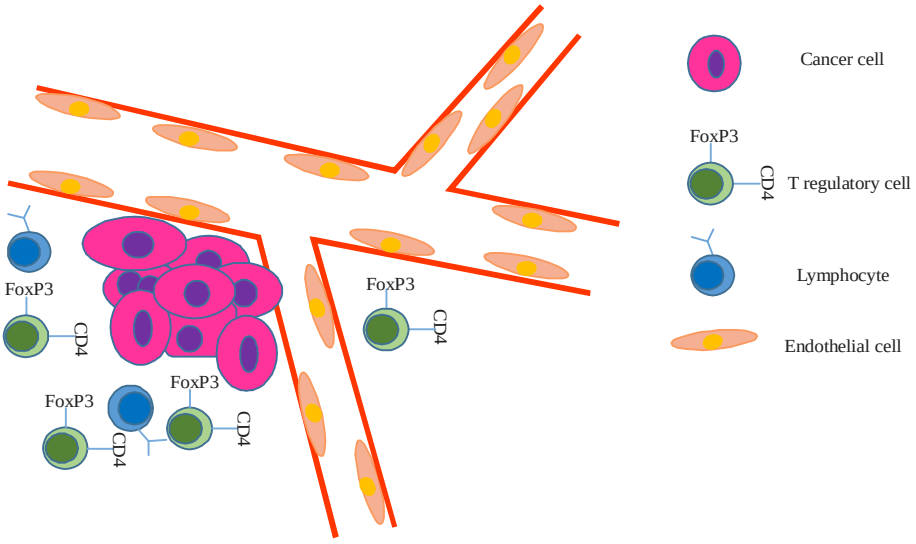


Fig. 1: Graphic representation of the tumour microenvironment

of this group are the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), although anti-vascular agents, such as monoclonal antibodies and inhibitors of receptor tyrosine kinases are also used (Masferrer *et al*., 2000).

## NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

The NSAIDs inhibit cyclooxygenase enzymes 1 and 2 (COX-1 and COX-2), thus impeding the eicosanoid synthesis pathway of the arachidonic acid cascade. They vary in their specificity, thereby reducing the synthesis of prostaglandins which play an important role in inflammation. The NSAIDs are widely used as adjuvants for controlling pain in cancer patients (Lorimier and Fan, 2010).

In addition to the analgesic effects of NSAIDS, their anti-neoplastic potential has been investigated more recently (Fonseca-Alves *et al*., 2014). Several reports on the effect of COX-2 and/or PGE2 on various types of epithelial and non-epithelial tumours, such as colorectal cancer, carcinoma, melanoma and oral osteosarcoma have been published in recent years (Lana and Dobson, 2011). According to Elmslie *et al*. (2008), COX-2 is expressed in tumour cells or during tumour stromal development and stimulates angiogenesis through the increased production of VEGF and bFGFs.

Early studies using COX-2 inhibitors demonstrated the anti-angiogenic effects of these drugs (Mutsaers, 2009). Some authors concluded that COX-2 may play a key role not only in the production of Treg cells but also in their activities (Elmslie *et al*., 2008). Among the NSAIDs, piroxicam has been most widely studied but meloxicam and carprofen are also used. When piroxicam was administered to dogs with transitional cell carcinoma of the bladder, the tumours in 12 of 18 treated animals regressed (Mohammed *et al*., 2002).

The most frequent adverse effects of NSAIDs are gastrointestinal disorders and kidney or liver toxicity and these should be monitored via laboratory tests during treatment (Masferrer *et al*., 2000). To evaluate the influence of COX-2 on tumour angiogenesis and tumour growth, Masferrer *et al*. (2000) noted that the inhibitory effect of COX-2 inhibitors was dose-dependent with better results observed with higher doses. The anti-angiogenic activity was evaluated using inhibitors of isoforms of COX-2, COX-1 as well as non-selective drugs that act on both COX-1 and COX-2 (Mohammed *et al*., 2002).

## ALKYLATING ANTINEOPLASTIC AGENTS

Alkylating agents contain reactive alkyl groups; these agents react with purine bases and pyrimidine in DNA causing crosslinks that prevent the normal function of the nucleic acid. These drugs are not specific to the cell cycle and are lethal to latent and dividing cells, although dividing cells are more sensitive to the drugs. This allows them to be effective against both slow and rapid growing tumours. This class of drugs includes the nitrogen mustards (cyclophosphamide and chlorambucil), nitrosourea (lomustine and carmustine) and others (Sasaki and Shimoda, 2015).

**Cyclophosphamide:** Cyclophosphamide is a nitrogen-alkylating agent from the group of oxazophorines that alter the structure of DNA. This agent is widely administered via oral or intravenous routes for treating carcinomas, sarcomas and lymphomas (Webb *et al*., 2015). Burton *et al*. (2011) treated dogs with soft tissue sarcoma using cyclophosphamide at a dose of 15 mg $m^{-2}$ and found a decreased density of blood vessels in the tumour microenvironment. Cyclophosphamide promotes anti-angiogenic and immunomodulatory effects when used alone or when combined with other substances (Dobson, 2014). However, long-term administration causes a potential adverse effect of sterile hemorrhagic cystitis as observed in dogs treated with metronomic chemotherapy (Leach *et al*., 2012). This disorder can result from a bladder irritation caused by acrolein which is produced during cyclophosphamide metabolism by cytochrome P450 in the liver. Additionally, cyclophosphamide is associated with other changes, such as bone marrow suppression and gastrointestinal effects (Collette *et al*., 2015).

**Chlorambucil:** Chlorambucil is an alkylating agent that can be used as an alternative treatment as it does not cause sterile hemorrhagic cystitis. The absence of adverse effects during long-term administration and the possibility of administration via the oral route make this drug an attractive option for use in metronomic chemotherapy (Leach *et al*., 2012). Schmidt *et al*. (2009) observed a negative effect of chlorambucil on vascular endothelial precursors. Moreover, there was a decrease in the number and viability of progenitor endothelial cells. Chlorambucil is indicated for the treatment of chronic lymphocytic leukemia, lymphoma and myeloma, exhibits minimal toxicity to bone marrow cells and gastrointestinal tract and is used in metronomic chemotherapy protocols (Tian *et al*., 2015).

**Lomustine:** Lomustine is a highly lipophilic drug that is easily distributed throughout the central nervous system. It is metabolized in the liver and its active and inactive metabolites

are excreted by the kidneys (Heading *et al*., 2011). The adverse effects reported with the use of lomustine in dogs are liver toxicity and severe myelosuppression. Some animals may develop acute hepatitis after 5-7 months of administration of lomustine (personal observation). Tripp *et al*. (2011) evaluated the effects of lomustine in dogs treated with metronomic chemotherapy for resectable tumours that are refractory to conventional chemotherapy. Some dogs were reported to suffer gastrointestinal effects, myelosuppression, hepatotoxicity and nephrotoxicity upon administration of metronomic doses of lomustine.

**Tyrosine-kinase inhibitors:** Tyrosine-kinases are essential for the regulation of cellular processes; they phosphorylate certain proteins and modulate enzymatic activity. They are located on the cell surface and once activated by a mutation, tyrosine kinases may promote gene amplification and over expression of these receptors causing cell growth. Tyrosine-kinase inhibitors prevent phosphorylation of the substrate and resulting in inhibition of downstream signal transduction. Examples of tyrosine-kinase receptors are the free cytokine receptors KIT, VEGFR2 and VEGFR which are altered in various types of neoplasms (London, 2009). Tyrosine-kinase inhibitors compete with ATP for binding to the catalytic site of several oncogenic tyrosine kinases. These agents have a safe therapeutic profile and can be associated to other forms of chemotherapy or radiation.

## METRONOMIC CHEMOTHERAPY IN DOGS

Metronomic chemotherapy is widely used in veterinary medicine; however, most published reports only evaluated clinical responses to therapy without investigating the anti-tumour mechanisms of this therapy. Additionally, most studies did not use metronomic chemotherapy as first treatment option, thus making it difficult to assess the response of tumours to metronomic chemotherapy. Most metronomic chemotherapy protocols use COX-2 inhibitors in conjunction with low doses of a cytotoxic chemotherapeutic agent, a regimen that provides anti-angiogenic therapy and modulates circulating Treg cells. Previously, COX-2 inhibitors proved effective for the treatment of nasal carcinoma (Fonseca-Alves *et al*., 2014) and prostate cancer (Fonseca-Alves *et al*., 2015) in dogs.

We have found that as a first therapeutic option for metastatic prostate cancer with bone metastasis in dogs, metronomic chemotherapy yields a good initial response with decreased pain and an overall survival advantage compared to dogs treated with conventional therapies (unpublished data). This clinical trial is in its final phase and we expect that our findings may help to clarify the appropriate role for metronomic chemotherapy in cancer treatment in animals. We expect that metronomic chemotherapy is associated with high expression levels of COX-2 in these tumours a characteristic that is associated with intense vascularization of metastatic tumours.

Burton *et al*. (2011) evaluated the response of two different doses of cyclophosphamide in metronomic therapy regimens in dogs with soft tissue sarcomas; the researchers evaluated the levels of circulating Treg cells and the tumour microvessel density. They concluded that a dose of 12.5 mg $m^{-2}$ $day^{-1}$ did not reduce Treg cell levels or tumour microvessel density. However, at a dose of 15 mg $m^{-2}$ $day^{-1}$, decreases in Treg levels and tumour microvessel density was seen over a period of 28 days (Burton *et al*., 2011). Marchetti *et al*. (2012) used first-line metronomic chemotherapy in canine metastatic spontaneous tumours and found that 40% of animals (6/15) showed either a complete response or stabilization of disease and dogs with a good response to therapy showed low levels of basal plasma VEGF, compared with dogs with no response to therapy which had high plasma VEGF levels.

Low-dose cyclophosphamide administered in conjunction with a full dose of piroxicam was shown to be effective for the control of local recurrence of incompletely resected soft tissue sarcomas (Elmslie *et al*., 2008). These results indicated that first-line metronomic chemotherapy could be an important treatment option for other canine tumours, such as haemangiosarcomas and melanomas.

## METRONOMIC CHEMOTHERAPY IN CATS

In veterinary medicine, little is known about the efficacy of metronomic chemotherapy in cats as only one study has previously evaluated metronomic therapy in cats. Therapeutic protocols used in that study are described in Table 1.

Owners and veterinarians may experience difficulty in administering metronomic therapy in cats, a challenge that may possibly affect the in frequent use of metronomic chemotherapy as a treatment option for these patients. Most drugs used in metronomic chemotherapy in cats and dogs are administered orally and many cats are not tolerant to the administration of oral drugs (Fig. 2). Thus, careful and skillful collaborations between owners and veterinarians may be essential for therapy administration if metronomic therapy is to be used in cats.

According to Leo *et al*. (2014) the main adverse effects of metronomic chemotherapy were reported at 4 weeks after therapy initiation. Commonly reported adverse effects are gastrointestinal changes and adverse hematological effects.

Fig. 2: Cat showing intense salivation after oral administration of lomustine

Table 1: Metronomic chemotherapy protocols previously reported

| Protocol* | Dose |
|---|---|
| Cyclophosphamide | 10-15 mg $m^{-2}$ every 24 or 48 h |
| Cyclophosphamide+piroxicam | Cyclophosphamide: 8.5 mg $m^{-2}$+piroxicam 0.3 mg $kg^{-1}$ every 24 or 48 h |
| Cyclophosphamide+firocoxib | Cyclophosphamide: 10 mg $m^{-2}$ every 24 or 48 h+firocoxib 1 mg $kg^{-1}$ every 48 h |
| Cyclophosphamide+meloxicam | Cyclophosphamide 10-17 mg $m^{-2}$+meloxicam 0.05 mg $kg^{-1}$ every 24 or 48 h |
| Cyclophosphamide+toceranib+meloxicam | Cyclophosphamide from 15-17 mg $m^{-2}$+meloxicam 0.05 mg $kg^{-1}$ every 24 or 48 h toceranib+2.5 mg $kg^{-1}$ three times a week |
| Cyclophosphamide+thalidomide+piroxicam | Cyclophosphamide: 6-10 mg $m^{-2}$+piroxicam 0.3 mg $kg^{-1}$ every 24 or 48 h+thalidomide 5 mg every 24 h |

*Protocols based on Leo *et al.* (2014) publication

The most important adverse effect after chronic therapy is renal failure; Leo *et al.* (2014) reported that 20% of cats (3/15) developed severe renal failure. Thus, metronomic chemotherapy can be an important therapeutic modality for the treatment of cats with various malignancies; however, further studies are necessary to establish therapeutic doses as well as the possible adverse effects in cats.

## CONCLUSION

Metronomic chemotherapy can be considered a good first-line treatment option for dogs with cancer. New studies evaluating the levels of Treg cells and tumour microvessel density are required for better understanding of this therapeutic modality in different canine tumours. Metronomic chemotherapy appears to be an attractive treatment option for cats; however, further research studies with larger numbers of animals are needed to evaluate therapeutic responses and toxicity in cats.

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