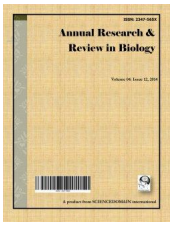

Annual Research & Review in Biology

26(1): 1-13, 2018; Article no.ARRB.40829
ISSN: 2347-565X, NLM ID: 101632869

Non-Steroidal Anti-Inflammatory Drugs as Chemopreventive Agents: Evidence from Cancer Treatment in Domestic Animals

Bianca F. Bishop[1] and Suong N. T. Ngo[1*]

[1]School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, SA 5371, Australia.

Authors' contributions

This work was carried out in collaboration between both authors. Author BFB performed the collection and analysis of the data. Author SNTN designed the study, managed the analyses and interpretation of the data and prepared the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/40829
Editor(s):
(1) David E. Martin, Martin Pharma Consulting, LLC, Shawnee, OK, USA.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Fulya Ustun Alkan, Istanbul University, Turkey.
(2) Thompson Akinbolaji, USA.
(3) Ramesh Gurunathan, Sunway Medical Center, Malaysia.
(4) Mohamed Ahmed Mohamed Nagy Mohamed, El Minia Hospital, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/24385

Review Article

Received 10th February 2018
Accepted 21st April 2018
Published 30th April 2018

ABSTRACT

Aims: This study aims to systematically review currently available data on the use of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of cancer in domestic animals to evaluate the efficacy of different treatment protocols and to suggest further recommendations for future study.
Methodology: Literature data on the use of NSAIDs in domestic animals as chemo-preventive agents in the last decade were collected and critically reviewed. Some older sources from the primary literature search have also been included to determine the background information leading to current rationale behind NSAID use in oncology.
Results: *In vitro* inhibitions of tumour cell proliferation by both piroxicam and meloxicam have been demonstrated only at higher concentrations than those achievable *in vivo*. However, remission rates ranging from 7% to 71% have been observed when piroxicam is administered orally, either alone or in conjunction with other anticancer agents for treatment of transitional cell carcinoma of the urinary

**Corresponding author: E-mail: suong.ngo@adelaide.edu.au;*

bladder of dogs. Piroxicam has also had positive results for multicentric lymphoma and nasal tumour, with remission rates of 79% and 75% respectively. In many cases, NSAID treatment showed increased median survival times and an improved quality of life of treated animals.
Conclusion: NSAIDs have shown potential as an adjunctive therapy for the treatment of some cancers in domestic animals. This review highlights the major limitation of current studies on the role of NSAIDs in cancer treatment, including limited sample size in most cases and mainly by retrospective studies. A recommendation for future study is the investigation of multi-institutional animal trials to increase case numbers and allow for better statistical analysis with adequate control groups.

Keywords: NSAIDs; chemoprevention; carcinomas; cancer; domestic animals; dogs.

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used therapeutic agents for the treatment of animals' pain and inflammation often associated with post-surgical procedures and osteoarthritis [1]. More recently, research has lead to the study of NSAIDs as chemo-preventive agents in animal oncology [2-4]. NSAIDs work by inhibition of cyclooxygenases (COXs), the enzymes responsible for the conversion of arachidonic acid to the eicosanoids: prostaglandins, prostacyclins and thromboxanes [5]. Two COX isoforms have been identified, including the constitutively expressed COX-1, expressed in most tissues and responsible for a number of homeostatic and physiological functions, and the inducible COX-2, induced by stimuli such as serum growth factors and cytokines [3] and associated with pathological presentations such as pain and inflammation [1].

It has been hypothesised that COX-2 is linked to tumour production and propagation via the associated increase in prostaglandins produced by COX-2 producing cells [2-4]. Prostaglandins are necessary to tumour biology in that they mediate processes essential to tumour pathology, such as increasing cell proliferation and angiogenesis [2,6,7]. Along with promoting tumour growth it is suspected that COX-2 also inhibits tumour destruction by promoting the expression of Bcl, an anti-apoptotic proto-oncogene [4,8]. COX-2 has been associated with the inhibition of apoptosis, thereby allowing ageing cells to proliferate past their biological end date and acquire the genetic mutations that lead to carcinoma [9]. A degree of immunosuppression is also involved, as it has been found that prostaglandin E2 will inhibit the activity of immunoreactive T cells, B cells and Natural Killer cells, cumulating in the blockade of tumour necrosis factor and interleukin-10, both essential to the body's defence against tumour development [4]. NSAIDs have been studied extensively in human clinical trials, most notably as chemopreventive therapies in colorectal cancer [4,10]. Colonic adenomas in humans show elevated COX-2 expression in 40% of cases [10] and in carcinogen-induced colonic tumours of rats COX-2 was also increased, while it remains undetectable in normal colorectal mucosa [11]. COX-2 expression has been demonstrated in a number of animal carcinomas, including but not limited to malignant canine melanocytic tumours [12,13] canine mammary tumours [14-16] feline oral squamous cell carcinoma [17,18] canine ovarian carcinoma [19] canine prostatic carcinoma [20] and transitional cell carcinoma [21].

Studies on immuno-histochemical expression of canine mammary tumour showed increased COX-2 expression with tumours of malignancy, associating this over-expression with the increased aggression and angiogenesis of these tumours [14,15]. The work by Lavalle *et al* (2009) has reported that canine mammary carcinoma patients with increased COX-2 expression had shorter survival time [6]. Furthermore, canine osteosarcoma has been shown to express COX-2, and it has been shown more aggressive tumours with a poor prognosis show an elevated level compared to less aggressive tumours [22]. It is the discovery of the expression of COX-2 by tumours that has highlighted the potential for NSAIDs to form part of a multi-drug chemotherapy [3,4]. Experimentally, early evidence has suggested NSAIDs could offer a protective mechanism against the development of tumours in the gastrointestinal tract, as shown in studies on rodents [23]. When sulindac, a non-steroidal anti-inflammatory drug, was added to the feed of males rats with azoxymethane-induced colonic carcinogenesis, the total volume of colon tumours was reduced by greater than 52-62%, and reduced levels of prostaglandin E2 in the colonic mucosa [24]. Other studies have

evaluated the use of various NSAIDs against neoplastic cell lines, and the translation to clinical studies *in vivo*. Reports on the use of meloxicam to treat osteosarcoma *in vitro* have yielded insignificant results from a clinical perspective [25] although piroxicam, as the first of the oxicam NSAIDs available clinically [10] had shown positive results for treatment of multicentric lymphoma [26] and nasal tumor [27] with remission rates of 79% and 75% respectively. Other positive effects of NSAID therapy reported include improved quality of life and increased median survival time post-diagnosis. Although there has been some focus on the use of NSAIDs as single-agents, their efficacy is often assessed as an adjuvant therapy to anticancer drugs, with a predominant focus on transitional cell carcinoma of the urinary bladder as a human model [28-30].

Despite varied studies to determine efficacy, the mechanism by which NSAIDs induce remission and/or increase median survival time are yet to be fully understood. *In vitro* studies on cancerous cell lines display an apoptotic effect of both piroxicam and meloxicam, yet only at supra-pharmacological concentrations [31]. Other proposed mechanisms of efficacy include a direct or indirect consequence to immune effector cells [31] or the dampening of tumour-mediated immunosuppression to prevent the pro-inflammatory state induced by tumours [9]. While several studies have been conducted, a systematic review to analyse currently reported data is lacking. The aim of this study is to critically review and evaluate current scientific evidence on the use of NSAIDs in the treatment of cancer in domestic animals to establish guidelines for their use and to provide recommendations for future study.

2. METHODS

2.1 Study Design

Literature data regarding the use of NSAIDs in management of carcinomas in domestic animals in the last decade were systematically collected and reviewed. The primary search terms including *NSAIDs, oncology, carcinoma, tumour, domestic animals* were used to initially source all peer-reviewed articles published any year, with results being filtered to obtain relevant articles published in English over last decade. Some older sources have been utilised to determine the background information leading to current rationale behind NSAID use in oncology.

2.2 Data Source

Sources were found using several search engines (PubMed, CAB Abstracts, Web of Knowledge, Google Scholar). All sources were searched appropriately to ensure that they were of the standard of evident-based medicine, including predominantly primary research papers and also relevant secondary sources on the topic.

3. RESULTS

A total of 22 studies were identified, evaluated, and discussed in this review. Of which, 14 studies examined the treatments of both COX-2 selective and non-selective NSAIDs, including piroxicam, firocoxib, deracoxib, and meloxicam in dogs with cancers, as adjunctive or mono therapy, and 5 studies investigated the effect of NSAIDs in dog carcinoma cell lines. Transitional cell carcinoma (TCC) of the urinary bladder was noted to be most extensively investigated carcinoma in dogs. The main findings of these studies are summarised in Table 1. The chemo-protective roles of NSAIDs in other selected tumours, such as oral squamous cell carcinoma, mammary carcinoma, mammary gland adenocarcinoma, and oral malignant melanoma have also been summarised in Table 2.

Of the 8 studies investigated the effect of NSAIDs in various carcinoma cell lines, 3 studies investigated the effect of meloxicam on D-17 canine osteosarcoma cells [25,31,43] either as monotherapy [31,43] or adjunct therapy with doxorubicin [25] one of these studies also examined the effect of piroxicam [31]. Two studies investigated NSAIDs as monotherapy, including piroxicam, deracoxib, and meloxicam, on canine mammary carcinoma cells CMT-7 [31], or CMT-U27 [44]. Another study examined the effect of piroxicam and deracoxib on different canine osteosarcoma cell lines HMPOS, POS and COS31 [45]. Overall, the *in vitro* inhibition of tumour cell proliferation by both piroxicam and meloxicam was observed only at higher concentrations, compared to those achievable *in vivo*. Findings of studies investigated NSAIDs in dog carcinoma cell lines are further highlighted in Table 3.

4. DISCUSSION

Much of the rationale for NSAID treatment of carcinoma has its basis from studies of *in vitro* cell lines. Often, NSAIDs are prescribed to cancer patients for their analgesic properties, as

is the case with appendicular osteosarcoma, and so any additional benefits of this medication could be considered advantageous. The pathway of NSAID inhibition of cyclooxygenase, and the subsequent anti-inflammatory and antipyretic effects is well documented, although to date there are numerous theories on the mechanism by which tumour growth is inhibited by COX inhibition. The demonstration of cyclooxygenase expression in tumour cell lines has identified the enzyme as a target for NSAID therapy.

When piroxicam and meloxicam were each tested on canine cell lines, it was found that lymphoma, osteosarcoma, and mammary carcinoma lines were affected by both NSAIDs in a dose-dependent manner, but all at concentrations greater than the maximum that can be achieved *in vivo* [31]. This has been previously determined as 1.3 μM for meloxicam in dogs when administered orally [49]. The response varied dependent on the cell type, which led the authors to hypothesise that there may be effects on immune effector cells along with anti-apoptotic mechanisms that have effects on tumour angiogenesis [31]. Study by Wolfesberger and colleagues also found meloxicam to inhibit osteosarcoma cell proliferation, but only at suprapharmacological concentrations of 50 μM, 100 μM and 200μM. Unexpectedly, at lower concentrations of meloxicam (1 μM, 2 μM, 4 μM and 10μM) a significant increase in the viable cell number was observed [25]. Doxorubicin was assessed in conjunction with meloxicam, and a narrow window for synergistic effects was observed, at 240 nM doxorubicin with 4 μM to 50 μM of meloxicam, leading the authors to conclude that based on this study alone, NSAIDs do not exert a great enough effect on cell proliferation to be used effectively in the treatment of osteosarcoma [25]. Likewise, it was found that deracoxib, a COX-2 selective NSAID, would inhibit osteosarcoma cell growth at intermediate and high concentrations, and had the promising effect of sparing fibroblasts, although the concentrations necessary for cytotoxicity were higher than plasma concentrations achievable, at ≥50 μM [45].

Thus the common conclusion is that current studies on cell lines are limited by the achievable concentration *in vivo*, which is considerably less than the concentration, which achieves apoptosis and inhibition of proliferation *in vitro*. This contributes to the confusion over the exact anti-tumour mechanisms, as a direct cytotoxic or apoptotic effect appears to be unattainable *in vivo*, leading to the speculation that there may be a direct effect on immune effector cells [31]. Transitional cell carcinoma (TCC) is the most common neoplasm of the urinary bladder in dogs and cats [21] with surgery not viable due to the high incidence of metastasis at the time of diagnosis (20%). It has been extensively studied as a model of human invasive bladder cancer. A standard treatment in domestic animals is chemotherapy and NSAID treatment, commonly in combination [37]. Treatment with surgery alone produces median survival times in dogs of 86 days to 106 days [21]. Study by Greene *et al* trialled a combination of cisplatin and piroxicam in canine patients, reporting a median survival time post-diagnosis of 307 days, a minimum improvement of 127 days compared to the 130-180 day range recorded for single therapy with chemopreventive drugs [33]. However, the use of combined cisplatin and piroxicam in dogs was found to show a high incidence of renal toxicity [33], and was not considered efficacious. The observed remission rate was 7%, which is highly contradictive of the remission rate of 71% obtained in an earlier 2000 study of piroxicam and cisplatin in combination. This study recorded a median survival time of only 146 days in comparison to the 307 days, with the same incidence of renal toxicity [29].

In a 2013 clinical trial, which compared the efficacy of cisplatin versus firocoxib versus a combination of the two, positive antitumour effects associated with firocoxib were reported. In this study, 57% of dogs receiving a combination of the two medications showed remission of the cancer based on a common standard, and, although subjective, owners reported 67% of dogs receiving firocoxib alone, and 91% of those received combination therapy, showed an improved quality of life [37]. It is suggested that the decreased toxicity is due to the COX-2 selectivity of firocoxib compared to non-selective piroxicam, as the kidney has a high expression of COX-1 [37]. Piroxicam as part of a multi-drug therapy for TCC has shown remission rates of 35% and 40% when used adjunctively with mitoxantrone and carboplatin respectively [36]. When a carboplatin-piroxicam combination was given, 74% of dogs experienced gastrointestinal toxicity, and 35% showed neutropaenia and/or thrombocytopaenia [36]. While the authors concluded that the remission rate observed was greater than that observed with carboplatin alone (<10%), the toxicity was considered high and survival rate was closely

associated with TNM (tumour, node, metastasis) stage and any prostatic involvement recorded at the beginning of the study. Piroxicam in combination with mitoxantrone, a doxorubicin derivative, showed measurable responses of complete and partial remission in 35% of dogs in the study [28] compared with only a 9% partial response to treatment seen in dogs given a piroxicam-doxorubicin combination [35] although the latter study had only half of the subjects. Both of these studies show favourable results for a piroxicam adjuvant therapy, as it was previously shown that piroxicam alone induced remission in only 17% of TCC cases [32].

Marconato *et al* have proposed the use of a gemcitabine-piroxicam combination for the treatment of transitional cell carcinoma of the urinary bladder [34]. Their result showed no adverse renal or gastrointestinal affects, but a clinical improvement of presenting signs of stranguria, haematuria and pollakiuria. Transitional cell carcinoma in cats has also been shown to some extent to respond to treatment with cyclooxygenase inhibitors. The median survival time for cats in a 2011 survey of eleven TCC cases was 311 days, which is comparable to that noted in dogs treated with piroxicam [32] and deracoxib, although the authors questioned the strength of expression of COX-2 by the carcinoma in cats and hence the efficacy of meloxicam in comparison to other NSAIDs, which was not concluded due to the small sample size [21].

Another important model in the study of NSAIDs in small animal oncology is mammary carcinoma. Inflammatory mammary carcinoma has an estimated prevalence of 7.6% of all mammary tumours in dogs, and is attributed to the poorest survival rates of mammary tumours, with a previous study showing a mean survival post-diagnosis of 25 days [39]. In this retrospective study, both the extent of COX-2 expression in inflammatory mammary carcinoma, along with the response to treatment with piroxicam were examined. Histology slides were also prepared and assessed with antibodies against COX-2, and then the expression was assigned a percentage. All specimens showed strong staining, which correlated with the positive response to piroxicam, increasing the mean survival time to 174 days [39]. Mammary tumours account for 17% of neoplasia in the female cat. These tumours show high growth and metastatic potential in close to 90% of cases, making the suggested efficacy of meloxicam treatment especially important. However, the use of a retrospective study in a hospital of Spain showed that meloxicam given to cats in conjunction with surgical mastectomy and chemotherapeutic drugs had similar survival times to studies without the use of NSAIDs, and hence found them ineffective, with emphasis on their small sample size [40]. From these studies, it is evident that NSAID therapy may have a place in the treatment of mammary carcinoma, especially in dogs, although conclusive evidence is limited by small sample size.

Piroxicam, the most common NSAID model, has shown high remission rates in both multicentric lymphoma and nasal tumour, with 79% and 75% respectively [26,27]. In each case it has been an adjuvant therapy to doxorubicin. Despite the highest remission rates observed with NSAID chemotherapy, the clinical evidence of these results is limited in both cases. In the study on multicentric lymphoma, the work by Mustaers and co-workers has found that treatment with doxorubicin alone showed a remission rate of 74%, which was not statistically significantly different to the result achieved when piroxicam was added to the treatment regime [26]. The study on nasal tumour was limited by the small sample group, along with a deficit in comparative data on other treatments for nasal tumour, as no previous studies established remission rates for this carcinoma treated with piroxicam or doxorubicin alone [27]. However, these results may be statistically significant if more studies are conducted in the future, as COX-2 expression has been seen in 81% of biopsied nasal carcinomas, which raises the question of whether increased expression can be used prognostically to determine the efficacy of NSAID chemotherapy [50].

Canine prostatic carcinoma was also tested simultaneously for COX expression and NSAID efficacy by a study of Sorenmo *et al*, which reported 94.1% of tumour cells expressed COX-1, and 88.1% expressed COX-2 [20]. In this study, dogs receiving piroxicam or carprofen treatment showed a median survival time of 6.9 months, which was approximately 207 days longer than the 0.7 month median survival time recorded for dogs in a control group [20]. When piroxicam was tested as a single agent for treatment of oral squamous cell carcinoma, the remission rate seen was 18% [41]. This is much less than that observed when it was used in conjunction with other anticancer agents, such as cyclophosphamide and cisplatin (55.6%) [42,51].

Table 1. NSAID therapy for transitional cell carcinoma (TCC) of the urinary bladder

Author	NSAID	Adjunctive therapy	No	Dosage	Remission (%)	Survival	Notes
Knapp et al. [32]	Piroxicam	None	34 Dog	Piroxicam 0.3 PO q24hrs	17	181d	Inhibition of tumour growth occurs only at concentrations ≥400 µmol/L)
Greene et al. [33]	Piroxicam	Cisplatin	14 Dog	Cisplatin 50 mg/m^2 IV, q 3 wks Dosage decreased for 9 dogs receiving 40 mg/m^2 Piroxicam 0.3 PO q 24 hrs	7	307d	Renal toxicity: 12/14 dogs *No significant difference in ADRs between different doses
Knapp et al. [29]	Piroxicam	Cisplatin	14 Dog	Cisplatin 60 mg/m^2 IV q 21 days Piroxicam 0.3 PO q 24 hrs	71	146d	Dose-limiting renal toxicity observed in 12/14 dogs
Marconato et al. [34]	Piroxicam	Gemcitabine	38 Dog	Gemcitabine 800 mg/m^2 IV q7d Piroxicam 0.3 mg/kg PO q 24 hr	27	230d	
Robat et al. [35]	Piroxicam	Doxorubicin	34 Dog	Doxorubicin 30 mg/m^2 IV q 21d (25 mg/m^2 dogs < 15 kg) Piroxicam 0.3 mg/kg PO q24 hrs	8.7	168d	Response data available in 23 dogs
Boria et al. [36]	Piroxicam	Carboplatin	31 Dog	Carboplatin 300 mg/kg IV q3wks Piroxicam 0.3 mg/kg PO q24 hr	40	196d	
Henry et al. [28]	Piroxicam	Mitoxantrone	55 Dog	Mitoxantrone 5 mg/m^2 IV q21d Piroxicam 0.3 PO q 24 hrs	Measurable response in 35.4%	291d	GI side effects of diarrhoea and/or haematochezia in 18%
Knapp et al. [37]	Firocoxib	Cisplatin	44 Dog	Dogs received either firocoxib alone (5 mg/kg PO q24 hr) or a combination of firocoxib and cisplatin (cisplatin at 60 mg/m^2 IV q21d)	57% remission in dogs received combined Thx; 20% with firocoxib alone	179d	One third of subjects received cisplatin alone, with a median survival time post-diagnosis of 338d
McMillan et al. [38]	Deracoxib	None	26 Dog	Deracoxib 3 PO q 24 hrs	17% showed partial remission	323d	GI signs observed in 5 dogs
Bommer et al. [21]	Meloxicam	None	11 Cat	Meloxicam 0.09 mg/kg q24 hr for 3-5 days as induction dose, with maintenance of 0.04 mg/kg q24 hr thereafter	Not measured as an endpoint	311d	COX expression occurred in only 37% of feline TCC, less potential for NSAID efficacy

Table 2. NSAID therapy for selected tumours

Author/ Cancer type	NSAID	Adjunctive therapy	No	Dosage	Remission (%)	Survival	Note
Souza et al. [39] Inflammatory mammary gland carcinoma	Piroxicam	None	7/12 Dog	Piroxicam 0.3 PO q 24hr	Not measured as an endpoint	185d	A strong varied expression of COX-2 in all 12 dogs (65.72% positive cells). All responded well to piroxicam, with increased survival rates, quality of life
Borrego et al. [40] Mammary gland adenocarcinoma	Meloxicam	Surgery & concurrent doxorubicin treatment	23 Dog	Doxorubicin (1 mg/kg IV), Vincristine (0.7 mg/m^2 IV) or Cyclophosphamide (250 mg/m^2I V) Meloxicam: 0.2 mg/kg 1d, 0.1mg/kg 5d, 0.025 mg/kg remaining Tx	Not measured as an endpoint	460d	
Schmidt et al. [41] Oral squamous cell carcinoma	Piroxicam	None	17 Dog	0.3 Piroxicam PO q 24 hrs	18	Measured as time to failure (i.e. time from start of treatment to death). Median time was 180d for dogs with remission, 102d for dogs with stable disease	Time to failure was found to be positively associated with tumour response and negatively associated with tumour size
Boria et al. [42] Oral malignant melanoma	Piroxicam	Cisplatin	11 Dog	Piroxicam 0.3 mg/kg PO q24 hrs Cisplatin 50 mg/m^2 IV q21 d	18	119d	This study aimed to determine the maximum tolerated dose (MTD) of cisplatin when administered with piroxicam, before adverse renal toxicity occurred. The base dose was found to be the MTD
  Oral squamous cell carcinoma	Piroxicam	Cisplatin	9 Dog	Piroxicam 0.3 mg/kg PO q24 hrs Cisplatin 50 mg/m^2 IV q21 d	55.6	237d	

Table 3. *In vitro* effect of NSAID therapy on cell lines

Author/ Cell type	NSAID/ Conc.	Effect on cell proliferation	Effect on apoptosis	Time	Note
Alkan et al. [44] Canine mammary carcinoma CMT-U27	Piroxicam Deracoxib Both tested at 50, 100, 250, 500 and 1000 µM	Deracoxib: Reduced cell viability at 250, 500 and 1000µM: 16.49%, 16.64%, 40.69% vs control level (100%) Piroxicam: Reduced cell viability at 1000µM	Deracoxib: Apoptotic cells increased at ≥250 µM Piroxicam: Apoptosis cells increased at 1000 µM	72 hrs Incub-ation	Concluded that combining two NSAIDs increased the inhibitory response above that observed with single agents Proliferation suppressed in a dose-dependent manner
Wolfesberger et al. [25] D-17 canine osteosarcoma	Meloxicam +/- Doxorubicin Meloxicam: 1, 2, 4, 10, 50, 100 and 200 µM Doxorubicin: 60, 120, 240, 480, 960 and 1920 nM	Meloxicam: A significant anti-proliferative effect observed at ≥100 µM Doxorubicin: All concentrations of inhibited cell proliferation. Synergistic effects observed with 240 nM doxorubicin in combination with 4-50 µM meloxicam	Not directly evaluated	72 hrs	*An unexpected, significant increase in viability of osteosarcoma cells observed at meloxicam concentrations of 1, 2, 4 and 10 µM
Knottenbelt et al. [31] D-17 canine osteosarcoma CMT-7 canine mammary carcinoma Canine 3132 B cell lymphoma derived	Piroxicam Meloxicam (Assessed individually) Meloxicam: 0.25-160 µg/ml Piroxicam: 1-320 µg/ml	Piroxicam: Showed significant inhibition at 10µg/ml Meloxicam: Similar results Meloxicam: Showed dose-dependent inhibition of proliferation at >10µg/ml, with maximum effect at 160µg/ml Piroxicam: Inhibited cell growth in a dose-dependent manner at >1µg/ml Meloxicam: Showed dose-dependent inhibition of proliferation at >10µg/ml, with maximum effect at 160µg/ml Piroxicam: Inhibited cell growth in a dose-dependent manner at >1µg/ml	Meloxicam + Piroxicam: Apoptotic cells increased, reached statistical significance at 10 µg/ml	8d	A concentration-dependent inhibition of proliferation was observed in all cell lines Canine lymphoma and mammary carcinoma cell lines appeared to be more sensitive to both drugs
Royals et al. [45] Canine osteosarcoma cell lines HMPOS, POS and COS31	Deracoxib + Piroxicam Deracoxib: 500, 250, 100, 25, 5, 1 µM Piroxicam: 1000, 500, 250, 50, 10, 2.5 µM	Deracoxib: Reduced viability of HMPOS cells at ≥50 µM, POS and COS31 cells at ≥100 µM Piroxicam: Reduced viability of HMPOS and COS31 cells at ≥500 µM, POS cells at ≥250 µM	Not measured as an endpoint	72 hrs	Intermediate and high concentrations of deracoxib were reported to inhibit cell growth, but the intermediate range had minimal effect on non-neoplastic fibroblasts that were also tested as a control

Author/ Cell type	NSAID/ Conc.	Effect on cell proliferation	Effect on apoptosis	Time	Note
Wolfesberger et al. [43] Canine D17 osteosarcoma cell line	Meloxicam: 10, 50, 100, 200, 400, 600 µM	µM seen with 200, 400 and 600 µM after 48 and 72 hr incubation	Apoptosis observed at 400 and 600 µM after 48 hr incubation		
Yoshitake et al. [46] 26 different canine cancer cell lines Canine melanoma cell line	Robenacoxib Carprofen Piroxicam (Assessed individually)	Inhibited cell growth only at concentrations much higher than the concentrations required for inhibition of COX function (Main aims were molecular mechanisms of tested NSAIDs action for anti-carcinogenesis: correlation between COX expression and NSAID sensitivity, not anti-proliferative efficacy as an endpoint measurement)	Not measured as an endpoint	24hrs	*Up-regulation of COX-independent pathway genes *SLC16A6, PER2, SLC9A8, HTR2B, and BRAF* observed in NSAIDs treated canine melanoma cells
Pang et al. [47] Canine osteosarcoma cell lines KTOSA5, CSKOS, and J3T (glioma)	Mavacoxib: 0 µM-1 mM Carprofen: 50, 100 µM	Mavacoxib: Significant inhibition of cell invasion at both 50 µM and 100 µM (P < .02). Inhibition of KTOSA5 stem cell colonies at both 50 µM (P < .001). Cell proliferation inhibition IC50 = ~100 µM Carprofen: Dose-dependent inhibition of cell invasion only at 100 µM (P = .04) At 100 µM: No KTOSA5 stem cell colonies formed. At 50 µM: 20% inhibition of KTOSA5 stem cell colonies Cell proliferation inhibition IC50 = ~170 µM	Mavacoxib: Apoptosis (~40%) observed in CSKOS at both 50µM and 100 µM (P < .001) Carprofen: Apoptosis observed in CSKOS only at 100 µM (P < .001)	48 hrs	*Mavacoxib found to be more effective compared to carprofen
Tamura et al. [48] AZACB canine mammary tumour cells	Celecoxib: 10, 25, 45, 50, 75, 100 µM Meloxicam: 10, 25, 50, 100 µM Etodolac: 10, 25, 50, 100 µM	Celecoxib: Significant inhibition of AZACB cell proliferation observed at both 75 and 100 µM (P < .05) Meloxicam: No significant inhibition of AZACB cell proliferation at 100 µM Etodolac: No significant inhibition of AZACB cell proliferation at 100 µM	Apoptosis observed in celecoxib-treated AZACB cells at 100µM No changes in apoptosis observed in meloxicam-treated or etodolac-treated AZACB cells at 100 µM	24hrs	*Celecoxib inhibited cell proliferation mainly via COX-2-independent mechanisms

From these results it appears that piroxicam is a successful adjuvant therapy in the treatment of oral squamous cell carcinoma, but the difference in sample size must be taken into consideration, as piroxicam was tested as a single agent with 17 cases, compared to only 9 cases when combined with cisplatin.

Moreover, in a recent study by Yoshitake and colleagues (2017) [46] 26 different canine cancer cell lines were tested for COX expression and NSAID sensitivity, no significant correlation was observed between COX expression and sensitivity to treatment. Same results were obtained for expression of COX-pathway related molecules, including prostaglandins (PGs), PGD2, and PGE2 [46]. Piroxicam, carprofen, and robenacoxib were also found to inhibit cancer cells growth only at *in vitro* concentrations much higher than the concentrations required for inhibition of COX function as consistently reported in the literature. The authors therefore concluded that the molecular mechanisms of NSAID action in carcinogenesis might be independent of COX/PG pathways. In this study, up-regulation of other genes, including *SLC16A6, PER2, SLC9A8, HTR2B, and BRAF* were also observed in melanoma cancer cell line treated with the tested NSAIDs, suggesting their potential role in the COX/PG-independent mechanisms of NSAID action in carcinogenesis. Similarly, study by Tamura and co-workers (2015) reported that COX-2 selective NSAID celecoxib inhibited AZACB canine mammary tumour cell proliferation mainly also via COX-2-independent mechanisms [48] further suggesting the importance of COX- independent pathway(s) in NSDAID mechanisms of anti-carcinogenesis.

Adverse effects are an important clinical consideration when prescribing drugs with overlapping toxicity profiles. Gastrointestinal toxicity has been recorded in a number of the studies assessed, with the predominant clinical signs being vomiting and diarrhoea. NSAIDs that are not selective for COX-2 are well known to cause renal toxicity, as they inhibit the constitutively expressed COX-1 enzyme responsible for the production of prostaglandins, which allow for vasodilation in the face of increased blood pressure. When prostaglandin synthesis is inhibited, the kidneys suffer haemodynamic injury. This is a particular problem when combined with cisplatin, also an inhibitor of renal perfusion. A study to determine the maximum tolerated dose (MTD) of cisplatin when combined with piroxicam yielded poor results, with the MTD equal to the base dose [42]. Identical doses of both drugs were used in a study of transitional cell carcinoma of the bladder, with renal toxicoses found in 85.7% of dogs included in the study [33]. This figure was identical to the percentage of renal toxicity observed when cisplatin was used at 60mg/m2 to treat TCC in combination with piroxicam [29]. Knapp (*et al*) discovered that the use of firocoxib in combination with cisplatin to treat urinary TCC is also limited by renal toxicoses, although it was noted that the NSAID/cisplatin combination has greater antitumour activity but no more renal toxicoses than cisplatin used as a sole treatment [37] possibly due to the COX-2 selectivity of firocoxib.

In addition, certain chronic inflammatory conditions have been well reported to predispose susceptible cells to neoplastic conditions in both humans and animals. Most of the resulting tumours are thought arisen from epithelial cell origin (i.e. carcinomas). The most well reported associations include colon cancer associated with inflammatory bowel disease or chronic ulcerative colitis and Crohn's disease, esophageal adenocarcinoma associated with reflux esophagitis or Barrett's oesophagus, hepatitis predisposing to liver cancer, schistosomiasis resulting in an increased risk of bladder and colon cancers, and chronic Helicobacter infection leading to stomach cancer. Some increase in the incidence of lymphoma has also been found, in particular mucosa-associated lymphoid tissue (MALT) lymphoma [52]. Thus, early detection and treatment of these chronic conditions would therefore play an important part in cancer treatment and prevention.

Considering that oncology often involves a palliative approach, an improvement of quality of life is extremely important for establishing a basis for further clinical trials. A limitation of previously reported studies that hinders further comparison is the low number of clinical cases to prove efficacy. Many studies are also retrospective, combining the diagnostic work of various veterinarians and pathologists, much of which is subjective and therefore becomes inconsistent. There have been many varied studies of various drug combinations against various forms of neoplasia, thus resulting in limited additive information. The presence of COX expression by tumour cells has been established, and there are many strong links between intensity of COX-2

expression and tumour aggressiveness, although no conclusive evidence to suggest a particular treatment protocol is most efficacious. Dogs are also over-represented in studies of NSAID use in oncology, although this may be due to the higher incidence of adverse affects and predisposition to toxicity seen in cats, or a decreased number of feline subjects.

5. CONCLUSION

In conclusion, the use of NSAIDs in the treatment of cancers in domestic animals, mainly dogs and cats, has been shown to have some therapeutic value when used as part of a multi-drug protocol. While some specific combinations, such as cisplatin-piroxicam for TCC of the urinary bladder, can be ruled out on the basis of combined toxicity, no drug combinations have been trialled with a considerable number of treated animals that results establish definite guidelines for their use. Important considerations are the adverse reactions seen, when considering the combined effects of anticancer agents with the commonly known complications of long-term inhibition of cyclooxygenases, even when selective COX-2 inhibitors are trialled. Thus multi-institutional studies are highly encouraged in order to achieve adequate sample size.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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