Recent advances on canine mammary cancer chemotherapy: A review of studies from 2000 to date

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SUMMARY

Research performed in the field of mammary cancer chemotherapy significantly improved survival rates in women. Chemotherapy is, therefore, part of breast cancer management along with surgery in humans. In small animals, however, mammary cancer chemotherapy is not routinely performed, except for some cases of distant metastases or tumours not amenable to surgery. Clinical research on adjuvant chemotherapy provided only a few promising results on dogs. On the other hand, many novel agents have been evaluated in canine mammary cancer cell lines, as dog is considered a good model for human breast cancer research. This study reviews recent (the last 15 years) clinical and experimental research on canine mammary cancer chemotherapy (systemic therapy), providing interpretation of the results in correlation to humans along with a brief description of the anticancer agents used.

Keywords: Anticancer agents, Chemotherapy, Dog, Mammary cancer, Systemic therapy

RESUME

Progrès récents chimiothérapie des tumeurs mammaires canines: examen des études de 2000 à ce jour

Les recherches effectuées dans le domaine de la chimiothérapie du cancer du sein ont considérablement amélioré le taux de survie chez les femmes. La chimiothérapie est, par conséquent, une partie de traitement du cancer du sein avec une intervention chirurgicale chez l'homme. Chez les petits animaux, cependant, la chimiothérapie des tumeurs mammaires n'est pas systématiquement effectuée, sauf dans certains cas de métastases ou de tumeurs qui ne se prêtent pas à une intervention chirurgicale. La recherche clinique sur la chimiothérapie fourni quelques résultats prometteurs chez le chiens. D'autre part, beaucoup d'agents anticancéreux ont été évalués dans des lignées de cellules de cancer mammaires chez le chien, car le chien est considéré comme un bon modèle pour la recherche sur le cancer du sein humain. Cette étude analyse les articles récents de recherche clinique et expérimentale sur la chimiothérapie des tumeurs mammaires canines (thérapie systémique), en fournissant une interprétation des résultats en corrélation avec les résultats chez l'homme ainsi qu'une brève description des agents anti-cancéreux utilisés.

Mots-clés: agents anticancéreux, chimiothérapie, chien, tumeur mammaire

Introduction

Tumours of the mammary gland are the most common tumours in intact female dogs. Approximately 35% to 50% of these tumours are malignant having the tendency to metastasize mainly via lymphatics to regional lymph nodes and/or distant sites (mainly the lungs) [26, 43]. The biologic behaviour of canine mammary tumours resembles that in humans and dog is regarded as a useful model for human breast cancer research [29, 32, 43].

Surgical treatment remains the treatment of choice, except for inflammatory carcinoma or presence of distant metastases. Recent years, more conservative mastectomy techniques are recommended in both humans [44] and canines, as survival in dogs is not influenced by the extent of mastectomy [15, 43]. The protective role of early (before the 1st or even 2nd oestrus) ovariohysterectomy (OHE) on the development of mammary tumours is unequivocal [36]. On the other hand, the results of studies evaluating the effect of concurrent or post mastectomy OHE on survival are controversial; the fact is that OHE could eliminate the harmful influence of female sex hormones on the already existing mammary gland tumours (in case they express oestrogen receptors) or could prevent pyometra [26, 30].

In women with breast cancer, adjuvant postoperative chemotherapy is usually performed to increase survival rates, and there have been many advances in that field [15]. In dogs, however, postoperative chemotherapy is not routinely used. In animal patients with mammary cancer, chemotherapy has been usually advocated in cases of advanced disease (distant metastases or inoperable tumours) that may otherwise lead to death or euthanasia shortly. Only few studies on the efficacy of adjuvant postoperative chemotherapy on reducing recurrence and prolonging survival have been performed in dogs with mammary cancer [41].

The aim of the study reported here was to review the clinical, as well as the experimental, data appeared from 2000 to date on canine mammary cancer chemotherapy (systemic therapy in general), to describe the drugs used, to interpret the results in correlation to humans and to address the most promising agents in canine mammary cancer treatment.

The search for studies included in this review was performed in PubMed and Web of Science using the terms "canine mammary cancer" and "chemotherapy" or "adjuvant chemotherapy" or "systemic therapy". Furthermore, relevant references found in these studies or in recent publications of books that include chapters on mammary tumours in small animals [15, 43] were searched. We included clinical and experimental studies on canine mammary cancer chemotherapy, as well as the most significant studies on systemic therapy with non-chemotherapeutic drugs including cyclooxygenase (COX) inhibitors, desmopressin, anti-hormonal agents and monoclonal antibodies, published the last 15 years. The discussed data of each study were extracted from its summary enriched with some necessary clarifications from the main document.

Studies

The anticancer agents used in the studies included in this review are presented in Table I.

The clinical studies are reported first, followed by the experimental *in vitro* and *in vivo* studies. The presentation of clinical studies begins with the first article published after 2000, continues with studies according to chemotherapeutic drug category, and finishes with studies evaluating the effect of non-chemotherapeutic agents on canine mammary cancer and on inflammatory carcinoma particularly. The experimental studies are presented according to the category of drugs evaluated for their anticancer effect; chemotherapeutic agents firstly and thereafter various non-chemotherapeutic agents and particularly COX inhibitors and tyrosine kinase inhibitors (monoclonal antibodies).

CLINICAL STUDIES

In 2001 Karayannopoulou and colleagues [18] documented for the first time in the literature the effect of adjuvant postoperative chemotherapy on the survival of bitches with clinical stage III mammary cancer. The chemotherapeutic protocol was based on a 5-fluorouracil (5-FU) and cyclophosphamide combination (150 mg/m^2 and 100 mg/m^2 of body surface area, respectively, given intravenously once weekly for four consecutive weeks). The drugs 5-FU and cyclophosphamide are usually part of first-line protocols used for early breast cancer treatment in women [11]. The agent 5-FU belongs to antimetabolites, structural analogues of normal cellular molecules, which by interacting with enzymes inhibit their activity. 5-FU is a pyrimidine analogue, which interferes with DNA and RNA synthesis; thus by inhibiting cell growth and division leads to cell death (S-phase-specific drug). Cyclophosphamide is the most frequently used alkylating agent in veterinary medicine. Alkylating agents induce their cytotoxic effect by

Anticancer agent	Drug category
5-Fluorouracil	Antimetabolite
Cyclophosphamide	Alkylating agent
Gemcitabine	Antimetabolite
Doxorubicin	Antitumor antibiotic
Paclitaxel, Docetaxel	Taxanes
Mitoxantrone	Antitumor antibiotic
Tamoxifen	Estrogen antagonist
Carboplatin, Cisplatin	Platinum agents
Benzene-polycarboxylic acids complex with *cis*-diammineplatinum (II) dichloride (BP-C1)	Platinum
Desmopressin (DDAVP)[a]	Synthetic derivate of vasopressin
Aglepristone	Progesterone antagonist
Tarantula cubensis extract (TCE)	Homeopathic medication
Meloxicam, Piroxicam, Deracoxib	NSAIDs[b] (COX[c] inhibitors)
Vincristine, Vinblastine	Vinca alkaloids
Hexadecylphosphocholine (Miltefosine)	Phospholipid
4-methylumbelliferone (4-MU)	Hyaluronan synthesis inhibitor
Migrastatin	Natural product of microbial origin
Melatonin	Hormone
Curcumin	Plant derived-bioactive substance
Tolfenamic acid	NSAID (COX inhibitor)
Masitinib	Tyrosine kinase inhibitor
Cetuximab, Trastuzumab	Monoclonal antibodies

[a]DDAVP, 1-deamino-8-D-arginine vasopressin - [b]NSAIDs, non-steroidal anti-inflammatory drugs - [c]COX, cyclooxygenase

Table I: Anticancer agents used in clinical, as well as experimental *in vitro* and *in vivo* studies, on mammary cancer systemic therapy in dogs during 2000-2015.

replacing a hydrogen atom on a biologically active molecule with an alkyl radical (R-CH_2-CH_3+) interfering with DNA replication and RNA transcription. Alkylating agents are not cell cycle phase specific drugs [33]. In this prospective nonrandomized study, a significant improvement in disease-free interval and survival time ($P < 0.05$) was demonstrated in eight dogs treated with adjuvant chemotherapy compared to eight dogs treated with surgery alone.

Another antimetabolite used for treating women with advanced breast cancer is gemcitabine [6]; it is a pyrimidine analogue and its cytotoxic effect is induced through inhibition of DNA synthesis [14]. In dogs, the anticancer effect of gemcitabine has been evaluated in a prospective clinical study [28]. Ten dogs with aggressive mammary carcinomas (clinical stages IV and V) were treated with surgery and adjuvant weekly gemcitabine (800 mg/m^2 intravenously) chemotherapy for at least four cycles, whereas nine dogs had surgery alone. No significant differences were found in time to local recurrence, time to distant metastases and overall survival between the two groups. Adjuvant gemcitabine chemotherapy in dogs with aggressive mammary carcinomas did not establish benefit.

Although antitumor antibiotics (doxorubicin) or taxanes (paclitaxel and docetaxel) are considered as powerful drugs in the treatment of early or advanced human breast cancer [6, 52], their use in dogs did not establish benefit. Antitumor antibiotics are natural products derived from fermentation of soil fungi. These cytotoxic drugs bind to nucleic acids by intercalating between DNA strands and thereby inhibit DNA or RNA synthesis. They are cell cycle phase nonspecific drugs. The anthracycline doxorubicin, although cardiotoxic, has been the most frequently used antitumor antibiotic in veterinary medicine [14, 33]. Taxanes belong to antimicrotubule agents and act by inhibiting microtubules disassembly leading to mitotic arrest. Hypersensitivity reactions are among toxicities associated with taxanes complicating their use [14]. In dogs, in a prospective clinical study [38], the efficacy of adjuvant postoperative chemotherapy with doxorubicin or docetaxel was evaluated in 31 dogs with malignant mammary tumours of histological stages II and III (vascular or lymphatic invasion, regional lymph node metastasis or distant metastasis). Nineteen of these dogs were treated with surgery alone and the remaining 12 dogs were randomly subdivided into two groups receiving five doses of either doxorubicin or docetaxel (30 mg/m^2 as intravenous infusions, three weeks apart). No significant differences in the recurrence-free interval, time to metastasis and overall survival were found between the surgery group and the adjuvant chemotherapy groups, although dogs treated with chemotherapy had a tendency towards higher long-term local control (no tumour presence in another mammary gland) and survival rates. In another study [31], the efficacy and toxicity of paclitaxel (Taxol) was evaluated in 25 dogs with various malignant tumours including those of mammary gland (three). In this retrospective study, 64% of the dogs, although pre-medicated with corticosteroids, exhibited allergic reactions. Partial response was observed in 20% of the animals for a median duration of 53 days. Toxicity was unacceptable at a dose of 165 mg/m^2. Further investigation on paclitaxel efficacy and toxicity in dogs is needed. Recently, a novel nanoparticle formulation of paclitaxel that limits hypersensitivity reactions has been investigated for use in dogs with mammary carcinoma [19].

Another antitumor antibiotic, mitoxantrone, a synthetic analogue of doxorubicin with similar cytotoxic activity [14], is among drugs that are considered useful in treating women with advanced breast cancer [6]. In dogs, its anticancer effect has been evaluated in two studies. In one of them [3], the effect of mitoxantrone (Oncotron) plus tamoxifen (Mammofen) on mammary tumour regression was examined and compared to mitoxantrone chemotherapy alone. Neoadjuvant (before surgery) chemotherapy is often used to shrink tumours that are locally advanced, so that they can be later excised by less extensive surgery [43]. Endocrine therapy with tamoxifen in a neoadjuvant or adjuvant setting is usually performed in women with hormone-dependent breast tumours [11, 25]. Tamoxifen is an anti-oestrogenic drug (an oestrogen receptor antagonist), which by binding to oestrogen receptors prevents oestrogen stimulation of existing cancer cells. It has shown, however, both anti-oestrogenic and oestrogenic effects causing pyometra in dogs [15, 41, 43, 45]. In the aforementioned prospective randomized study [3], 12 bitches with mammary tumours (maximum diameter less than 3 cm) were divided into group I treated with mitoxantrone alone (5 mg/m^2 intravenously on days 0, 21 and 42) and group II treated with mitoxantrone plus tamoxifen (tablets 10 mg, twice daily for three months). The regression percentage was 66.20% in group I and 69.56% in group II. Concluding, tamoxifen supplementation showed no significant advantage over mitoxantrone chemotherapy alone in achieving mammary tumour regression. The second study evaluating the use of mitoxantrone in dogs [47] is a retrospective study in 94 dogs with mammary carcinomas; survival data of 36 dogs treated with surgery plus adjunct chemotherapy and of 58 dogs treated with surgery alone were evaluated and compared. The regimens used included doxorubicin or mitoxantrone plus a platinum agent (carboplatin), with or without piroxicam. Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) that can block the activity of COX-1 and COX-2 (nonspecific COX inhibitor), which are important enzymes involved in tumour development and progression in both humans and dogs [32, 41]. None of the regimens used had a positive effect on survival. However, in a subgroup of five out of 26 dogs with advanced disease (clinical stage IV or lymphatic invasion) and complete surgical margins, the use of mitoxantrone and carboplatin showed encouraging results (mean survival time 1139 days compared to 347 days of seven dogs that received doxorubicin and/or carboplatin, and to 343 days of 14 dogs that received no chemotherapy at all). Therefore, as suggested, adjuvant treatment with mitoxantrone alone

or in combination with carboplatin may benefit dogs with mammary cancer.

Platinum agents, such as cisplatin and the aforementioned carboplatin, exhibit their cytotoxicity by blocking the replication and transcription of DNA [14]. In women, platinum agents and especially carboplatin are drugs used in treatment protocols for early, but most often for advanced, breast cancer [6]. Due to severe dose-dependent side effects, however, their use in veterinary medicine is limited [14, 21]. In a prospective study in bitches bearing mammary tumours of advanced clinical stage, adjuvant carboplatin chemotherapy had a positive effect on survival [23]. Specifically, in 29 dogs treated with different therapy protocols including surgery, chemotherapy (carboplatin) and COX inhibitors, the overall survival periods were evaluated and compared. The use of COX inhibitors in cancer treatment could be beneficial, taking into consideration that higher amounts of the enzyme COX-2 have been found in several tumours including those of the mammary gland and have been associated with increased cell proliferation and differentiation, inhibition of cell apoptosis, increased angiogenesis and metastasis [22, 32, 41]. Indeed, in the aforementioned study, dogs with low COX-2 scores survived longer than dogs with high COX-2 scores. In addition, therapy protocols with carboplatin or COX inhibitors as adjuvant to surgery led to a significant longer overall survival when compared to surgical treatment alone.

The pharmacokinetic profile of a novel platinum agent, Benzene-polycarboxylic acids complex with *cis*-diammineplatinum (II) dichloride (BP-C1), was studied in seven dogs with metastatic or inoperable mammary tumours, in order to evaluate any antitumour effects and to determine optimal doses and possible side effects [21]. BP-C1 is composed of a polymer complex of carbonic and oxicarbonic acids rich in carboxyl-groups that replace the chloride ions. These carboxyl-groups bind platinum more tightly than the chloride ions and thus toxicity may be reduced compared to the most common platinum drugs, carboplatine and cisplatin. BP-C1 perhaps acts in a different way than cisplatin inducing apoptosis. In the aforementioned study, BP-C1 was injected subcutaneously in each dog daily for seven days, in three design levels. No overall significant antitumour efficacy of BP-C1 was found, but a significant reduction in toxicity was observed. The maximum tolerated dose was above 0.46 mg/ kg.

A non-chemotherapeutic agent, desmopressin, induced significant improvement in survival of bitches with advanced mammary carcinomas (clinical stage III and IV) [17]. Desmopressin (1-deamino-8-D-arginine vasopressin; DDAVP) is a synthetic derivate of the antidiuretic hormone, with haemostatic and anti-metastatic properties [41]. The perioperative use of DDAVP could improve survival by inhibiting tumour cell dissemination during mastectomy. In the aforementioned prospective randomized study, 18 bitches received perioperative desmopressin treatment (1 μg/kg intravenously) and 10 bitches received saline solution (controls). En bloc mastectomy was performed in all animals. Particularly in dogs with histological grade II or III carcinomas, the use of DDAVP induced a significant increase in disease-free survival ($P < 0.001$) and overall survival ($P < 0.01$) times compared to animals treated with normal saline. These results were in agreement to those found previously by the same authors in 21 bitches with malignant mammary tumours [16].

A study on neoadjuvant endocrine therapy was performed in dogs with mammary carcinomas for evaluating the antiproliferative and apoptotic effect of aglepristone [12]. Aglepristone is a progesterone receptor antagonist, which has shown antitumour activity in humans with breast cancer. In dogs, it is currently used for pregnancy termination and pyometra treatment [41]. In the described study, 22 out of 27 intact bitches with mammary carcinomas were treated with two doses (on 1st visit and eight days later) of aglepristone (20 mg/kg), whereas five bitches with oil placebo (control). Tumour samples were received before and 15 days after treatment and examined by immunohistochemistry for progesterone receptor (PR) expression, proliferation index (PI) and apoptotic index (AI), using antibodies against PR, and Ki67 or cleaved lamin A antigens for PI or AI respectively. The percentage of PR-positive tumours decreased after aglepristone treatment. In addition, the 61.5% of PR-positive tumours had a reduction in PI ≥20%, but no change in mean AI was observed. Aglepristone induced a PR expression-related inhibiting effect on proliferation of canine mammary carcinoma cells and could become a useful agent for neoadjuvant treatment of PR-positive mammary tumours.

In a neoadjuvant setting, the antiproliferative and apoptotic effect of *Tarantula cubensis* extract (TCE), which is a homeopathic medication, in 13 dogs with mammary adenocarcinoma of clinical stage II was evaluated [13]. TCE was administered subcutaneously between the shoulder blades at weekly intervals for three times (3ml in each dog). Approximately 10 days after the third injection, complete unilateral mastectomy was performed. Punch biopsies of tumour tissues were taken pre- and post-treatment with TCE. The apoptotic index B-cell lymphoma 2 (Bcl-2) and the cell proliferation index Ki-67 were assessed by immunohistochemistry. A significant decrease in the expression of Bcl-2 and Ki-67 was found after treatment with TCE indicating that this agent may help in controlling tumour growth in canine mammary adenocarcinoma cases.

In dogs with inflammatory mammary carcinoma, systemic therapy with COX inhibitors, plus chemotherapy or not, was found to induce stability of clinical condition leading in longer survival times. Specifically, in one study [8], the survival outcome of seven dogs treated with the NSAID piroxicam was compared with that of three dogs treated with traditional chemotherapy (doxorubicin and cyclophosphamide with or without 5-FU) that caused severe toxic effects. The result was that piroxicam palliative treatment

significantly increased survival time ($P < 0.01$). In another study in dogs with inflammatory mammary carcinoma [7], the use of chemotherapy plus palliative therapy in seven dogs gave better results than palliative treatment alone used in 23 dogs. Taking into consideration that COX-2 expression has not been investigated in inflammatory breast cancer in women [32] and that the use of COX inhibitors as palliative treatment in dogs with inflammatory mammary carcinomas gave encouraging results, further research on this prognostic marker in humans with inflammatory breast cancer may become a useful tool in its management.

Summarizing, the results of the aforementioned studies regarding the effect of the evaluated anticancer agents on survival of dogs with mammary cancer are presented in Table II. However, since these results have derived from studies that had different study designs, inclusion criteria and efficacy assessment, comparisons between different therapeutic strategies should be considered with reserve. Generally, studies on canine mammary cancer patients should be based on standardized procedures as proposed by Matos and colleagues [29], in order to facilitate reproducibility and assessment of the results. In most of the clinical studies included in the present review, the cases were selected based mainly on TNM clinical staging and sometimes on tumour histological grading or lymphatic invasion. In human breast cancer patients, however, some prognostic markers [oestrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor 2 (HER2/new) expressions] are routinely used for classification of cancer and selection of the most appropriate therapeutic approach for each patient [1, 4]. In canines, on the other hand, although the expression of these markers shows similarities to humans [32], their evaluation before treatment is not performed routinely but only occasionally for research purposes.

EXPERIMENTAL IN VITRO AND IN VIVO STUDIES

In an experimental *in vivo* study, canine mammary gland tumour (solid carcinoma) was subcutaneously transplanted in severe combined immunodeficiency mice. Subsequently, the chemosensitivity of six chemotherapeutic agents (5-FU, cyclophosphamide, doxorubicin, cisplatin, vincristine and vinblastine) given intravenously as a single injection was evaluated and their clinical effectiveness was predicted [51]. Vincristine and vinblastine, two chemotherapeutic drugs that we have not described yet, are plant alkaloids extracted from *Vinca rosea*. They belong to antimicrotubule agents, which cause mitotic arrest by interfering with the polymerization or depolymerization of microtubules that play a critical role in cell function and division [14, 33]. In this experimental study, it was found that only cyclophosphamide, 5-FU and cisplatin could be effective in treating the original patient (dog) with mammary cancer, as it was also proved in clinical studies [18]. In an *in vitro* study in cell cultures of 30 excised canine mammary gland tumours, the 50% inhibitory concentration of cisplatin, carboplatin or doxorubicin was investigated [37]. Growth inhibition of cultures, which was assessed via DNA measurements 24, 48 and 72 h after treatment, was higher in platinum agents than doxorubicin. The advantage of platinum drugs (specifically carboplatin) over doxorubicin was also proved in clinical studies described previously [23, 47].

Various non-chemotherapeutic drugs were also examined *in vitro* for evaluating their anticancer activity. Hexadecylphosphocholine (HePC, Miltefosine) is a phospholipid used against leishmaniasis in both humans and dogs [9]. It has also shown antitumor activity inducing apoptosis in human breast cancer cells, although the mechanism of action is not clear. Upon addition of HePC in a canine mammary tumour cell line originated from a spontaneous primary canine mammary osteosarcoma, the tumour cells rapidly exhibited features of a type of cell death that resembles apoptosis, though the process was more rapid than in other tumour cell lines [10]. Another drug evaluated *in vitro* is 4-methylumbelliferone (4-MU), which is a hyaluronan (HA) synthesis inhibitor. HA is a basic constituent of extracellular matrix, whereas the HA synthases (HAS)1-3 are involved in its synthesis. HA mediates the growth and metastasis of tumour cells, especially of mesenchymal-like cells. The antitumour effect of 4-MU has been shown against various malignant tumours. In canine mammary tumour cells, 4-MU inhibited HA synthesis via

Anticancer agents inducing:		
significant survival improvement	weak survival improvement	no survival improvement
5-Fluorouracil	Doxorubicin	Gemcitabine
Cyclophosphamide	Docetaxel	
Carboplatine	Mitoxantron	
Desmopressin (DDAVP)[a]		
COX[b] inhibitors (NSAIDs)[c]		

[a]DDAVP, 1-deamino-8-D-arginine vasopressin - [b]COX, cyclooxygenase - [c]NSAIDs, non-steroidal anti-inflammatory drugs

Table II: The effect of anticancer agents on survival evaluated in clinical studies performed on canine mammary cancer systemic therapy during 2000-2015.

downregulation of HAS2 mRNA levels. In addition, 4-MU inhibited cell proliferation and induced apoptosis of the examined cells. These cytotoxic findings suggest that 4-MU may serve as a therapeutic agent against mammary cancer in dogs [34, 35]. The natural product migrastatin is a new metastasis inhibitor of microbial origin, firstly isolated from *Streptomyces* sp. Some more effective analogues of migrastatin have been synthesized and evaluated in mouse models and *in vitro* as potential anti-metastatic drugs. In cell lines isolated from canine mammary adenocarcinomas and their lung metastases, the effect of six migrastatin analogues (MGSTA-1 to 6) on tumour cell migration and invasion was investigated. Two of these analogues, MGSTA-5 and MGSTA-6, caused potent inhibition of cancer cell migration and invasion and were considered as promising anti-metastatic compounds for both dogs and humans [27]. The anticancer value of the hormone melatonin was examined in cell cultures of canine mammary tumours [24]. Melatonin showed an oncostatic effect by decreasing cell proliferation and inducing apoptosis, mainly in ER-positive tumours, and thus it could be a potential therapeutic agent for these tumours. A plant derived-bioactive substance, curcumin, was investigated in canine mammary cancer cell lines, in combination with cyclophosphamide, and a synergistic growth inhibitory activity via induction of apoptosis and cell cycle arrest was revealed [2]. This result provides evidence that the development of natural compounds as novel anticancer agents, as well as their use in combination with cytotoxic agents for lowering their dose, may lead in more specific and less toxic therapeutic approaches for treating dogs with mammary cancer.

The anticancer effects of various NSAIDs on canine mammary carcinoma cells were also investigated *in vitro*. The nonspecific COX inhibitor piroxicam was found to reduce the growth of canine mammary carcinoma cells, but at doses that would induce severe toxicity *in vivo* [20]. Deracoxib is a selective COX-2 inhibitor used in dogs for management of pain and inflammation in cases of osteoarthritis and orthopaedic surgery. Deracoxib also exhibited cytotoxic properties *in vitro* and reduced the growth of canine mammary cancer xenografts in mice [42]. In an *in vitro* study [48], piroxicam and deracoxib were used at various concentrations, as single or combined treatment, on a canine mammary carcinoma cell line. High drug concentrations significantly decreased viable cells and increased apoptotic cells, after 72 h incubation. Combined treatment gave better results. It was thus suggested that combined use of these two NSAIDs may contribute in the treatment of canine mammary carcinomas. The anticancer effect of tolfenamic acid (TA) was investigated in six canine tumour cell lines including 2 osteosarcomas, 2 mammary carcinomas and 2 melanomas [50]. TA is a NSAID (COX-2 inhibitor), which has a different mechanism of action than traditional anticancer NSAIDs; it inhibits specificity proteins (Sps) that are important oncogenic factors. Sps were highly expressed in all six cell lines, whereas TA decreased their expression. In addition, after exposure to TA, the number of tumour cells undergoing apoptosis was significantly increased ($P < 0.05$) in a dose-dependent manner. Concluding, TA is a promising anticancer NSAID, but further, clinical investigation is needed. Finally, the effect of deracoxib and doxorubicin, used as single agents or in combination, was evaluated on a canine mammary carcinoma cell line [49]. The viability of cells, as well as oxidative and antioxidant parameters [Malondialdehyde (MDA), nitric oxide (NO), and activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and total glutathione (GSH)], were determined. Doxorubicin-induced oxidative damage to carcinoma cells was increased compared to control cells. On the contrary, deracoxib induced no significant changes. In combination treatment, however, deracoxib attenuated the doxorubicin-induced effect. Therefore, the use of a COX inhibitor in conjunction with a chemotherapeutic agent may benefit the mammary cancer patient.

Tyrosine kinase inhibitors, as masitinib, target tyrosine kinase receptors, such as the epidermal growth factor receptor (EGFR, HER-1 or ErbB-1) and the HER2/new (ErbB-2), which are associated with increased angiogenesis, tumour growth and metastasis [4, 5]. Apart from its tyrosine kinase inhibition activity, masitinib, in an *in vitro* study, exerted its potential for chemosensitization; it strongly sensitized canine mammary carcinoma cells to the chemotherapeutic agent gemcitabine, enhancing the antiproliferative activity of the drug [46]. The monoclonal antibodies cetuximab and trastuzumab target the ErbB-1 and ErbB-2 tumour-associated antigens and thus they are used for passive immunotherapy in human cancer patients (trastuzumab is used in the treatment of HER2/new positive breast tumours) [4, 39]. In an *in vitro* study, ErbB-1 and ErbB-2 homologues were identified in canine mammary carcinoma cell lines and were recognized by cetuximab and trastuzumab antibodies leading in growth inhibition of neoplastic cells [39]. Furthermore, a canine anti-EGFR (ErbB-1) antibody was generated by Singer and colleagues (2014) and induced significant tumour cell growth inhibition in canine mammary carcinoma cell lines [40].

Summarizing, the evaluation of anticancer agents such as miltefocine, 4-MU, migrastatin analogues and tolfenamic acid suggested that their use may be beneficial in the treatment of mammary cancer in dogs and perhaps also in humans, since in both species mammary cancer demonstrates many similarities in risk factors, carcinogenetic pathways, clinical course, histological characteristics and prognostic factors [29, 32, 43]. Regarding targeted therapy and especially immunotherapy, its use in veterinary medicine needs to be established further.

Conclusions

In veterinary medicine, more efforts are made for preventing rather than treating canine mammary tumours, by performing ovariohysterectomy early in the animal's life. Research on mammary cancer chemotherapy in dogs has not been as extensive as in humans and usually follows it.

To our opinion, some possible reasons for that include: 1) the unwillingness of many pet owners to continue or even to proceed in chemotherapy, especially in non-developing countries, 2) more effective but increased dosages of some drugs, such as doxorubicin, may cause unacceptable toxicities (cardiotoxicity), 3) toxicities induced by some drugs (paclitaxel) eliminate their use and 4) difficulties in drug administration (paclitaxel). Nevertheless, research on canine mammary cancer sometimes precedes that in humans and may contribute in human breast cancer research and treatment (for example the evaluation of anticancer activity of novel agents such as migrastatin analogues or the investigation of COX-2 expression in inflammatory breast carcinoma and the use of COX inhibitors for its management).

Regarding the design of studies and the criteria used, more standardized procedures are needed in order to facilitate comparisons between studies. Furthermore, in canine mammary cancer research and routine management, we suggest that veterinarians should invest in predictive prognostic markers, such as ERs or the enzyme COX-2, which could contribute to better treatment strategies and increased survival rates.

To our opinion, minimally invasive systemic therapy performed as adjuvant to surgery or as palliative, by using promising chemotherapeutic drugs or other agents such as desmopressin or COX inhibitors could benefit dogs with early or advanced mammary cancer and should be advocated routinely. We also suggest further investigation on canine mammary cancer systemic therapy, in order to evaluate or develop novel cancer therapeutics, and to find out appropriate drug dosages or possible toxic effects, focusing on increasing survival rates in both animals and humans, as dog is considered a good model for human breast cancer research.

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