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Original Contribution

EFFICACY AND TOXICITY OF DOXORUBICIN AND CYCLOPHOSPHAMIDE CHEMOTHERAPY IN DOGS WITH SPONTANEOUS MAMMARY TUMOURS

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ABSTRACT

The aim of the present study was to follow up the effect and toxicity of doxorubicin and cyclophosphamide chemotherapy combined with operative treatment of malignant mammary tumours in dogs. The study was performed on 6 bitches, aged 9-14 years, from various breeds. These dogs presented mammary adenocarcinoma confirmed by histopathological investigation. The animals underwent partial mammectomy. Ten days after the intervention, chemotherapy with doxorubicin and cyclophosphamide was scheduled.

Blood samples were obtained prior to and following the operation, and after each course of chemotherapy to monitor the changes in some principal haematological and biochemical parameters. The efficacy of the treatment was evaluated by thoracic radiography for the absence of metastases or the regression of already existing ones.

The studies showed that the operative intervention combined with sequential regimen of doxorubicin and cyclophosphamide chemotherapy suppressed effectively the development of new neoplasm and metastases, but was accompanied by general adverse reactions as lethargy, anorexia, vomiting, hair loss, fever, hypochromic anaemia and strong immunosuppresion.

Key Words: dog, adenocarcinoma, mammary gland, doxorubicin, cyclophosphamide

INTRODUCTION

Mammary gland tumours are among the commonest neoplasm in bitches [1] – about 52% of all tumours can be found among them [2] It must be stated also that 41% to 53% of mammary neoplasm are malignant [2, 3]. They occur also in male dogs, but the prevalence is only 1% [3]. The average age of onset is 10–11 years, but cases in dogs under 4 years of age are also reported [3].

Breeds as Poodle, English Spaniel, English Setter and Terriers are at higher risk for mammary tumour development whereas the incidence in other breeds (Boxer, Chi-hua-hua) is minimal [4, 5].

About 65 % of mammary gland tumours affect the caudal mammary complexes [4].

The retrospective study on tumour aetiology among pets showed that mammary neoplasm prevailed in dogs, with highest incidence occurring among Bologneses over 8 years of age, and with 60% malignancy [6]. Canine mammary tumours are hormonally dependent. The additional studies about the hormonal aetiology of tumorigenesis show that 50% – 60% of all malignant mammary neoplasm contained receptors for oestrogen (ERS), progesterone (PRS) – in more than 30 per cent; receptors for androgen – in more than 20% of cases and that these receptors were present in about 70% of benign mammary tumours [7, 8, 9]. The treatment of dogs with hormonal preparations, such as oestrogens and progesterone, increases the risk of mammary tumour development because these hormones have a strong stimulating effect on canine mammary neoplasm [10]. That is why castration is a means of preventing tumour growth. The risk

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of mammary tumour development in dogs is 0, 05% if they are castrated prior to the first oestrus (about the age of 6 months), 8 % - after the first oestrus and 26 % after the second one [11]. Intact females develop mammary neoplasm 7 times more often than dogs castrated at the age of 2 years or earlier [12, 13].

From all three types of treatment – surgical, radiotherapy and chemotherapy, only the last could manage a systemic or unknown metastatic disease.

During the last years, the studies about the effect of preparations and combinations used for chemotherapy, including anticancer antibiotics, alkylating agents, vinca alkaloids, glucocorticoids, antioxidants etc., are increasing. The chemotherapy is, however, accompanied by various side effects such as vomiting, diarrhoea, anorexia, lethargy, fever, alopecia, anaemia, neutropenia etc. [14, 15]. The comparative studies with different combinations on the basis of doxorubicin showed that some of them differed insignificantly with regard to their haematological toxicity while in others, it was clearly manifested [16]. Various investigators evidenced the efficacy of different combinations of chemotherapeutics through the obvious regression of tumour growths, delayed appearance of metastases and prolonged duration of patient's life [14, 17, 18, 19].

The aim of the present investigation was to monitor the effect and the toxicity of a chemotherapy protocol with doxorubicin and cyclophosphamide combined with operative treatment of malignant mammary tumours in dogs.

MATERIAL AND METHODS

The present study was performed on 6 bitches with mammary neoplasm. They were aged 9-14 years and weighed 4–15 kg. The breed distribution of these dogs was as follows: 2 Bologneses, 2 Cocker Spaniels, 1 Miniature Pintcher and 1 mixed-breed dog.

Initially, the animals were subjected to partial mammectomy under halothane general anaesthesia. After the removal of the tumour mass, histopathology was done on it.

Ten days after the operation, sequential chemotherapy of doxorubicin (Adriblastina®, 10 mg, Pharmacia&Upjohn, Milan, Italy) and cyclophosphamide (Endoxan®, 200 mg, Asta Medica, Frankfurt) was administered using the following schedule:

- Doxorubicin, intravenously at 20-30 mg/m^2 once weekly, for 3 consecutive weeks.
- Cyclophosphamide, intravenously at 100 mg/m^2 3 days after the doxorubicin administration, for 3 consecutive weeks.

Blood was sampled from the cephalic antebrachial vein for analysis of the following haematological and biochemical parameters:

- Haematological parameters: red blood cell counts (RBC, T/l) – in a Bürker's chamber, haemoglobin (Hb, g/l) using colorimetry; haematocrit (Hc, l/l) – via microcentifugation; total white blood cell counts (WBC, G/l) – in a Bürker's chamber; differential WBC counts (%) – on stained blood smears.
- Biochemical parameters: total protein (g/l), aspartate aminotransferase (ASAT, U/l), alanine aminotransferase (ALAT, U/l), urea (mmol/l), creatinine (μmol/l) –colorimetrically with kits of Human Diagnostica, Germany.

Other investigations done in order to determined the efficacy and toxicity of chemotherapy were:

- Clinical: body temperature (BTT, °C), heart rate (min $^{-1}$), respiratory rate (min $^{-1}$) - using routine techniques.
- Electrocardiography – with a microprocessor single-channel electrocardiograph MAIMEX-ECG 1222 ASB (Bulgaria). ECGs were obtained prior to and after the end of chemotherapy. Six leads were registered: 3 standard and 3 augmented in right lateral recumbency using a thermal ECG paper at a rate of 50 mm/s.
- Radiography – with a stationary unit TUR 800 (Germany). Radiographic surveys (lateral views; 55-60 kV, 10-16 mAs and 100 cm film-focus distance) were done immediately prior to surgery and after the end of chemotherapy.

The data were statistically processed by the Student's t-test using a software(Statistica, version 6.0, StatSoft Inc., 2001).

RESULTS

Clinical state of patients prior to, during and after the end of the treatment

The body temperature in 5 dogs was within the normal range (37,5-39,3) and in one – subnormal (36,3 °C). In 2 dogs the heart rate was higher than normal (120 min $^{-1}$). The

respiratory rate in all patients was higher than the reference values (30 min $^{-1}$) (**Table 1**).

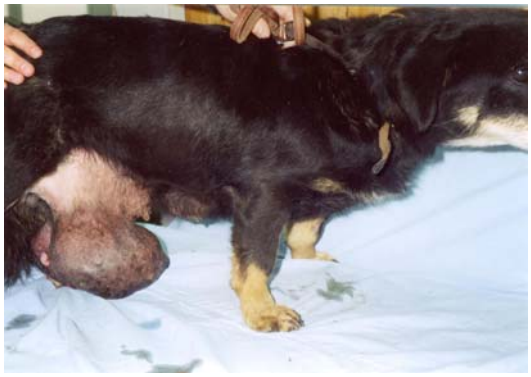

Figure 1. *Mammary tumours involving IV and V mammary complexes*

The general condition of 5 dogs was good, the appetite was preserved, the urination and defecation – normal. One bitch was lethargic, with uncoordinated movements, difficult respiration, refusal of water and food, incontinence.

The inspection of the mammary gland revealed formations with size of 2–20 cm, located primarily in IV and V mammary complexes (**Figure 1**). In one patient the growth was ulcerated. The palpation showed a hard and elastic consistency, non-temperate, non-painful formations that were adhered to the abdominal and thoracic wall in two dogs. In one dog, the abdominal wall was highly painful. In another patient, 2nd degree of lameness was present in the left forelimb because of the specific growth of the neoplastic mass. The regional lymph nodes in these cases were enlarged, non-painful and non-temperate with hard elastic consistency.

The analysis of data about the clinical signs during the different periods of the complex therapy – body temperature, heart and respiratory rates, did not show considerable changes (**Table 1**).

Table 1. *Changes in some clinical parameters in dogs with malignant mammary neoplasm prior to the operation (time period 0), after the operation (time period 1), after the 1st, 2nd and 3rd courses of chemotherapy (time periods 2, 3 and 4, respectively). Data are presented as mean ± SEM.*

Parameter	***Time period***				
	0	**1**	**2**	**3**	**4**
Body temperature, °C	38,00±1,096	38,72±0,54	38,67±0,36	38,67±0,20	38,44±0,24
Heart rate, min $^{-1}$	118,00±12,96	122,33±12,68	121,00±10,49	122,00±14,42	121,20±13,54
Respiratory rate, min $^{-1}$	49,17±10,59	46,00±11,45	44,00±12,33	50,33±9,24	17,60±7,54

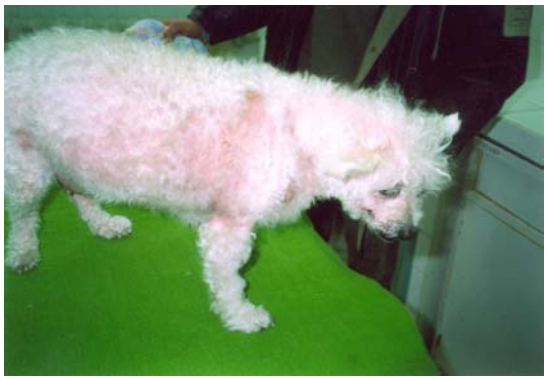

Figure 2. *Alopecia occurring 20 days after the initiation of chemotherapy.*

During the chemotherapy, there were gastrointestinal troubles in 3 patients – anorexia (for 3-4 days after the injection of the doxorubicin), vomiting (for 3 days following the administration of the doxorubicin onward), and diarrhoea (10 days after the beginning of the chemotherapy, continuing on the average 3 days with dark red colour and mucus). Alopecia appeared in a patient 20 days after the beginning of the chemotherapy (**Figure 2**) and faded away 1 month after the end of the treatment.

Blood laboratory investigations

The results from the investigations of some haematological and blood biochemical parameters prior to the combined therapy did not show any deviations from the reference values (**Table 2**).

The blood analyses showed significant decrease in RBC counts during the 2nd time period (4.87±0.79; $p<0.05$ vs baseline of 6,55±1,52) and WBC counts by time period 2 (8,57±4,76; $p<0.05$), time period 3 (7,24±4,42; $p<0.01$) and 4 (4,63±1,39; $p<0.01$) compared to baseline (18,03±8,28). The changes in the serum biochemical liver and kidney profiles (urea, creatinine, ASAT, ALAT and total protein) were not significant.

- Histopathological investigations;
- The histopathological diagnosis in all patients was mammary adenocarcinoma;
- Functional studies.

Table 2. *Changes in some haematological and blood biochemical parameters in dogs with malignant mammary neoplasm prior to the operation (time period 0), after the operation (time period 1), after the 1st, 2nd and 3rd courses of chemotherapy (time periods 2, 3 and 4, respectively). Data are presented as mean ± SEM.*

Parameter	***Time period***				
	0	**1**	**2**	**3**	**4**
Hb, g/l	141,67 ±28,16	124,17± 28,10	122,33±27,65	124,17±39,21	126,40±45,00
Hcr, l/l	0,42±0,09	0,36±0,08	0,35±0,08	0,36±0,11	0,35±0,12
Er, T/l	6,55±1,52	5,19±1,09	4,87±0,79*	4,96±1,52	5,14±1,49
Lc, G/l	18,03±8,28	11,87±6,42	8,57±4,76*	7,24±4,42**	4,63±1,39**
St, %	6,17±5,08	6,67±3,08	12,00±4,90	12,50±5,58	7,50±3,42
Sg, %	56,67±5,35	51,17±12,40	40,00±24,72	52,50±11,72	51,50±8,35
Ly, %	35,33±7,87	35,83±8,11	28,33±18,65	32,17±14,81	37,50±6,81
Mo, %	1,50±1,22	2,66±3,01	1,66±2,33	1,50±1,52	3,20±2,16
Eo, %	0,50±0,84	3,67±4,59	5,20±5,40	1,17±1,33	2,00±1,83
Total protein, g/l	50,5±6,44	49,50±3,33	52,17±12,29	49,33±12,63	50,00±12,39
Urea, mmol/l	5,03±1,88	6,64±3,87	4,64±1,68	6,53±5,39	3,88±1,09
Creatinine, µmol/l	87,33±39,15	72,83±13,76	72,33±9,91	76,33±15,81	73,20±6,83
ASAT, U/l	22,33±17,92	31,50±53,90	7,17±8,30	14,83±8,84	11,00±7,52
ALAT, U/l	24,17±26,53	37,83±54,05	30,50±35,74	30,63±31,44	17,76±19,65

** p<0.05; **p<0.01- significant vs baseline values (period 0).*

Radiography

The radiography of lungs showed identical changes in the radiological findings in three dogs (**Figure 3a**). They consisted of multiple sharply outlined nodular densities with various size and intensity.

In the other 3 dogs, radiology did not reveal any visible metastases (in lesions < 5 mm, a negative finding is possible).

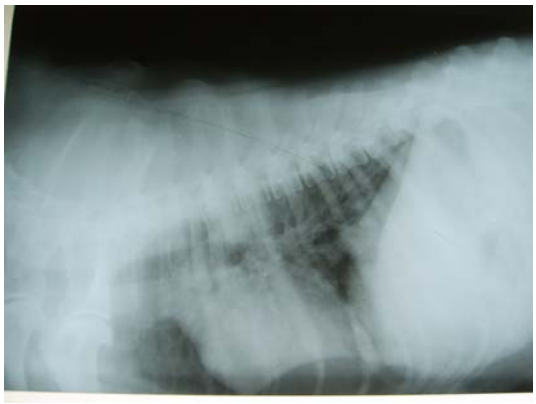

Figure 3a. *Lateral pulmonary radiograph prior to the chemotherapy. The arrows show multiple sharply delineated nodular densities with various size and intensity.*

The comparison of radiographs of dogs with lung metastases prior to and after the therapy (**Figure 3a** and **3b** respectively), a clear reduction in lung field density was observed as well as decreased size and intensity of densities. In one dog this change occurred as early as the second week after the beginning of the chemotherapy.

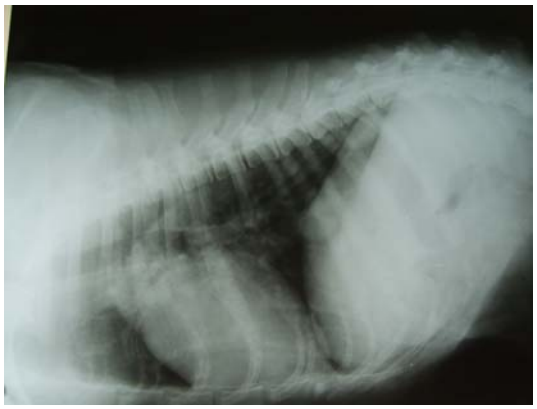

Figure 3b. *Lateral pulmonary radiograph after all courses of the chemotherapy. The arrows show more transparent lung fields, reduced number, size and intensity of densities.*

ECG study

In all studied animals, a sinus rhythm was registered both prior to and following the chemotherapy. Some of the dogs showed a physiological respiratory arrhythmia. The cardiac contraction rate varied considerably from one individual to another, but was within the normal range in both studied periods. The deviations in studied ECG parameters after the chemotherapy were insignificant vs the baseline values. No rhythm, conductivity or repolarisation changes were present.

Recurrence and time of survival

In two dogs, the neoplastic growth recurred at the site of the primary tumour about 1.5 month after the chemotherapy.

The survival time varied greatly – from 2 months to 1,5 years after the treatment. In the bitch with the lowest survival time, the death occurred in the course of a second operative intervention performed on the occasion of another disease. In one dog, the death occurred as a result of rapidly developing sepsis.

One year after the performed treatment, one of the patients is in a good physical condition.

DISCUSSION

Chemotherapy is a principal method of treatment in human and, recently, in veterinary oncology. It prevents the tumour dissemination by controlling the early metastases that tend to proliferate quickly and is hardly likely to possess resistant cells. The metastasis is the main cause of death in cancer patients regardless of the treatment option. Malignant mammary tumours in the dog metastatise in regional lymph nodes and lungs, just like in men [20]. Metastases are observed in 77% of dogs with mammary carcinomas [21]. The radiographic survey in our study revealed lung metastases in 3 of our patients and we followed up their regression prior to (**Figure 3a**) and after the end of the chemotherapy (**Figure 3b**). Regression of lung metastases is observed not earlier than 2 weeks after the beginning of chemotherapy [22].

The early detection of neoplasm in dogs and cats and the quickly initiated therapy generally prevent the local and distant metastases [23]. It is clear that the better efficacy of chemotherapy is in the control of the dissemination of the disease, the longer survival and better life quality of patients. However, it is accompanied by a number of side effects that stop both owners and veterinarians to use it in cancer therapy.

The drugs that are effective in the treatment of human breast cancer, doxorubicin and cyclophsophamide, could also be used in the therapy of canine mammary neoplasm [13]. That explains why we used them in this study.

Cyclophosphamide is the most commonly used alkylating agent in veterinary practice. One to two weeks after its application, leukopenia is observed. Although more rarely, its continuous use could result in anaemia and thrombocytopenia [24, 25]. Most anticancer drugs are excreted through the kidneys and the liver. If these organs are not functioning properly, their rapid accumulation could result in a severe, non-controllable intoxication.

Doxorubicin belongs to the group of anticancer antibiotics. This cytostatic has a marked haematological and gastrointestinal toxicity and cardiotoxicity, and in cats – nephrotoxocity as well [26, 27]. Because doxorubicin is metabolised by the liver, the doses should be reduced when a concomitant liver damage is present. During the infusions, the detection of anxiety, face oedema, trembling of the head could be a sign for a too rapid infusion or occurring anaphylaxis.

The boundary between efficacy and toxicity in chemotherapy is very narrow. The toxicity of the therapy with doxorubicin and cyclophosphamide is accompanied by moderate neutropenia and clinical signs as lethargy, anorexia, vomiting, diarrhoea, fever [15, 19, 28], that were observed in our study as well. The commonest problems are the gastrointestinal signs, bone marrow suppression and immunosuppression [16, 25, 29]. Vomiting and anorexia could be due to damage of the gastrointestinal epithelium or CNS effects. These problems are not generally life-threatening and the use of antiemetic drugs has a good effect on them. Other, more rarely encountered problems are: diarrhoea, stomatitis, oesophagitis, and gastroduodenal ulcers [30].

Although in our study, only the changes in total RBC and WBC counts were significant, there was a clear tendency towards reduction of all blood cells from the myeloid stem lineage except for segmented neutrophils and eosinophils. This leukopenia with a right shift showed that there was a total suppression of the bone marrow activity. The eosinophilia could be either due to accidental infection with parasites, or allergic systemic reaction to cytostatics without clinical manifestation.

The myeloid toxicity leads to leukemia and immunosuppression affecting both humoral and cell-mediated immunity that is a very serious disadvantage of chemotherapy. The myeloid toxicity could affect all blood cell components. The anaemia and thrombocytopenia could become life-threatening. Because of the shorter half-life and the lower WBC stores, the leukopenia and the related risk of infections are the first and the commonest problems. Chemotherapy should be postponed until the minimum WBC counts are restored.

More rarely, a toxicity involving other systems could be observed. Cyclophosphamide, used for breast cancer treatment, could result in haemorrhagic cystitis, so the prolonged use of this drug is restricted [31]. Also, cyclophosphamide has been related to urinary bladder carcinoma in dogs [32]. Doxorubicin has a potential nephrotoxiciy in cats [26].

Cardiotoxicity could be observed during the application of doxorubicin and it is a very characteristic feature of this cytostatic [15, 33]. The cardiotoxocity of doxorubicin consists in progressive myocardial degeneration, myocytolysis, vacuolisation and fibrosis [33]. The probability of cardiac failure increases with accumulation of doses, although the beginning of this failure could occur several weeks after the last dose. The arrhythmias and conductivity troubles happen quickly but are not necessarily connected with the occurring congestive heart failure [33]. In numerous studies, cardiac troubles were observed in 18% of dogs and congestive heart failure – in 4% [33].

In a study of Hammer et al [15] 2 out of 5 dogs treated with doxorubicin, vincristine and cyclophosphamide, developed sepsis that appeared also in one in our patients with a lethal issue.

Skin reactions and alopecia accompanying the chemotherapy are less common in veterinary practice than in human oncology [34]. A higher chance of developing alopecia is observed in shorthair and curly-haired breeds (Shepherd dogs, Poodles, Afghan Hounds and some Terriers) [34]. Alopecia was present in one Bolognese from our patients.

The lungs, liver, heart and the central nervous system could also be injured, but the clinical manifestations were considerably more rarely observed than the other complications [35].

The survival time and the efficacy of performed chemotherapy depend on various factors like: the stage of the disease, the histological type and the degree of tumour differentiation, the presence of relapses and metastases, the tumour size, the early diagnostics and the rapidly initiated therapy [13, 23, 36].

The clear regression of tumours, the delayed metastases, the increased life duration and improved life quality of patients are a proof for the efficacy of the chemotherapy [17, 18, 19].

In conclusion it could be suggested that the combination of a surgical intervention with a chemotherapy protocol is effective for treatment of dogs with malignant mammary gland neoplasm. It could also be considered that the tested combination of cyclophosphamide and doxorubicin suppressed effectively the development of new tumour growths and metastases but was accompanied by general adverse reactions such as lethargy, anorexia, hair loss, fever, hypochromic anaemia and marked immunosuppression.

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