A case report: Surgery and Metronomic Chemotherapy with Cyclophosphamide and Piroxicam of High-grade Soft Tissue Sarcoma in A 5-year-old Dog

รายงานสัตว์ป่วย: การรักษาทางศัลยกรรมร่วมกับเมโทรโนมิก คีโมเทอราปี ด้วยไซโคลฟอสฟาไมด์ และไพรอกซิแคมในสุนัข อายุ 5 ปีที่ป่วยด้วยมะเร็งร้ายแรงซอฟท์ทิชชูซาร์โคมา

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ABSTRACT

A 5-year-old, female, neutered mixed breed dog was referred for the treatment of a large high-grade sarcoma mass of the left trunk. This dog with clinical stage $T_4N_0M_0$ (stage IIB) was treated with surgery and metronomic therapy with 2-drug combination consisting of orally administered low-dose cyclophosphamide at 10 mg/m^2 and full-dose piroxicam at 0.3 mg/kg daily coupled with surgery. The dog was re-evaluated at 3-month intervals throughout her treatment period. Thus far, this dog is alive with pleasing on her quality of life. Recurrence or metastases has not been found and disease free interval is more than 24 months. This case report suggested that the 2-drug regimen was very effective in inhibiting the regrowth of tumor in this dog with high grade soft tissue sarcoma.

Key words: sarcoma, dog, surgery, metronomic chemotherapy

บทคัดย่อ

สุนัข เพศเมีย พันธุ์ผสม อายุ 5 ปีป่วยด้วยมะเร็งซอฟท์ทิชชูซาร์โคมาร้ายแรงระดับเกรดสูง มีก้อนมะเร็งขนาดใหญ่ ที่บริเวณข้างลำตัวด้านซ้ายช่วงอก ระยะที่ 2บี เข้ารับการรักษาที่โรงพยาบาลสัตว์ โดยวิธีทางศัลยกรรม ร่วมกับการใช้เมโทรโนมิก คีโมเทอราปี อย่างต่อเนื่องด้วยยาไซโคลฟอสฟาไมด์ ขนาด 10 มิลลิกรัมต่อพื้นที่ผิวตารางมิลลิเมตร และยาไพรอกซิแคม ขนาด 0.3 มิลลิกรัมต่อพื้นที่ผิวตารางมิลลิเมตรทุกวัน สุนัขได้รับการตรวจสุขภาพทุก 3 เดือนตลอดการรักษา ผลที่ได้รับพบว่า สุนัขดูปกติ มีคุณภาพชีวิตที่ดี ทั้งยังไม่

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พบการกลับมาเกิดซ้ำของมะเร็ง หรือการแพร่กระจายไปยังอวัยวะอื่นๆ มานานมากกว่า 24 เดือนของการเฝ้าติดตาม ปัจจุบันสุนัขยังดำรงชีวิตอยู่อย่างปกติ

รายงานสุนัขที่ป่วยด้วยมะเร็งร้ายแรงซอฟท์ทิชชูซาร์โคมานี้ แสดงให้เห็นถึงประสิทธิภาพของการใช้เมโทรโนมิก คีโมเทอราปีของยาทั้งสองตัวร่วมหลังการรักษาทางศัลยกรรม มีผลช่วยยับยั้งการกลับมาเจริญเติบโตและการแพร่กระจายของเซลล์มะเร็งได้อย่างเป็นผลที่น่าพอใจทางคลินิก

คำสำคัญ: ซาร์โคมา สุนัข ศัลยกรรม เมโทรโนมิก คีโมเทอราปี

Introduction

Soft tissue sarcomas (STS) are a heterogenous group of mesenchymal tumors that constitute 14-17 % of all skin and cutaneous tumors in dogs (White, 1986; Liptak and Forrest, 2013). STS are typically characterized as locally invasive into surrounding tissues but generally have a low risk of spreading (Liptak and Forrest, 2013; Chase *et al*., 2009; Williamson and Middleton, 1998). The most common histologic types diagnosed in dogs include fibrosarcoma, schwannoma, neurofibrosarcoma, malignant fibrous histiocytoma and hemangiosarcoma (Mauldin, 1997; Williamson and Middleton, 1998).Other sarcoma including hemangiopericytoma, osteosarcoma, synovial sarcoma, chondrosarcoma and lymphangiosarcoma are usually excluded because of a more aggressive local behavior and a higher metastatic (Kuntz *et al*., 1997; Dernell *et al*., 1998). The risk for distance metastases, most commonly to the lung, range from 11-40 % depending on the histological grade and size of the tumor. STS tend to occur in middle aged to older dogs. There is no breed or sex predilection. The cause of STS is unknown but may be associated with trauma, foreign bodies and parasites.

Surgery is the mainstay of treatment for most sarcomas, possibly combined with radiation. Depending on the site, grading and stage of a sarcoma (Table 1 and 2), the goal of surgery is to remove the entire tumor along with at least 2-3 cm of the normal tissue surrounding the tumor (Liptak and Forrest, 2013). The completeness of this surgical removal is determined microscopically examining all surgical margins. Radiation therapy can be used in the management of residual microscopic local disease after surgery or in a palliative setting for tumors that are not amenable to surgical excision. Chemotherapy is often recommended for high grade sarcomas, given the increased likelihood of metastasis. It may also be considered for patients with incompletely-excised tumors and for whom radiation therapy is not an option or for patients with non-resectable tumors. Chemotherapy for bulky disease has not been shown to be highly effective in STS-cases (Helphan, 1986). It cannot be considered a good option for initial therapy planning. Doxorubicin based treatment protocols are most often used for treating STS but a unique side effect of this drug is its ability to weaken the heart muscle with multiple doses.

In recent years, several studies revealed that metronomic chemotherapy may be an interesting choice of systemic medication. It allows cancer patients to live with the tumor, as long as it is causing few or no problems, rather than suffering from it as it

grows. Use of these medications are also lower the drug doses and lower the risk of adverse effects. Metronomic chemotherapy refers to the chronic administration of low doses of cytotoxic drugs on a continuous or semi-continuous basis without resting pauses, not necessarily reflecting the drug's mechanism of action, but the interval and dose (Loven *et al*., 2013). This therapy has been shown to be an effective means of inhibiting tumor angiogenesis (Gately and Kerbel, 2001; Kerbel and Kamen, 2004; Miller et al.,2001; Shimizu and Oku, 2004). It is inhibition of mobilization of endothelial progenitor cells, which arise from the bone marrow and seed tumor tissues. These bone marrow-derived endothelial cells are a major source of new blood vessels that develop in tumors (Kerbel and Kamen, 2004; Shaked *et al*., 2005; Lam *et al*., 2006; Mancuso *et al.,* 2006). In addition, several researches showed the metronomic chemotherapy stimulated production of thrombospondin-1, a potent endogenous angiogenesis inhibitor (Bocci *et al*., 2003; Folkman, 2004; Kamat *at el*., 2007). Interestingly, studies on continuous low doses of cyclophosphamide have also been appeared to exert substantial positive effects on antitumor immunity (Matar *et al*., 2000; Ghiringhelli *et al*., 2004; Salem *et al*., 2007). So far, several researches indicated this therapeutic concept was to be primarily antiangiogenic, either by direct killing or inhibiting endothelial cells in the tumor vasculature, killing bone marrow derived endothelial progenitor cells and helping the immune system to mount a response against tumor. The alkylating agent cyclophosphamide is one of the most widely cytotoxic drugs used in this way. Besides, there are some drugs such as cyclooxygenase-2 inhibitors (COX-2), metformin, nelfinarvir, nitroxoline, thalidomide and others, which were developed and approved not for anti-cancer treatment but for other indications which later showed anti-angiogenic potency among other effects (Masferrer *et al*., 2000; Gasparini *et al*., 2001; Spieth *et al*., 2003; Gills *et al*., 2007; Gonzalez-Angulo and Meric-Bernstam, 2010; Shim *et al.,* 2010). They also can be given orally daily in a metronomic style. COX-2 inhibitors have been demonstrated to exert an anti-angiogenic effect in cancer cells by blocking COX-2 receptors and stopping the intracellular signaling cascade that lead to production of growth factors such as basic fibroblast growth factor, vascular endothelial growth factor, tumor growth factor β (Gately and Kerbel, 2003). In veterinary oncology, piroxicam, a nonselective COX inhibitor, has been demonstrated to elicit significant antitumor activity in dogs with transitional cell carcinomas of the urinary bladder (Knapp *et al*., 1994). The goal of using both metronomic cyclophosphamide chemotherapy and COX-2 inhibitors is to increase greater anti-angiogenic potential and tumor growth. The objective of this study was to examine the effect of continuous treatment with low-dose cyclophosphamide and full-dose piroxicam effectively delayed tumor recurrence in a dog with incompletely resected high-grade soft tissue sarcoma.

Case presentation

A 5-year-old, female, neutered mixed breed dog was referred for the treatment of a large mass of the left trunk (Figure 1). The owner reported that the mass had been seen for several months. On physical examination the dog was bright, alert and responsive, well

hydrated and in good body condition. On palpation, a firm, no painful and poorly circumscribed mass measuring approximately 20x15x10 cm^3 was present in the left trunk. This mass was located in the soft tissues and seemed to have deep connections with the underlying structures including the ribs. No regional lymphadenopathy was palpable. An incisional biopsy was indentified the mass as a high-grade sarcoma. The dog was underwent a complete blood cell count (CBC), serum biochemical profile, urinalysis, chest radiographs, abdominal ultrasounography and computed tomography scan of the thoracic cavity (Figures 2a-b, Figures 3a-c). All the tests were within normal limits and the thoracic radiography did not reveal any evidence of metastasis. Tumors were staged according to computed tomography (CT) findings using the TNM system for soft tissue sarcoma reported by Owen 1980 (Table 2). Based on the owner's financial and emotional reasons, this dog with clinical stage $T_4N_0M_0$ (stage IIB) was treated with metronomic chemotherapy coupled with surgery. Anesthesia was induced with propofol (4 mg/kg) and maintained with 2% isoflurane and oxygen. A blood sample and urine sample was taken for analysis prior to starting therapy and this was repeated at regular intervals, every 1 to 2 months initially, reducing to every 2 to 3 months with time. A blood sample may also be repeated if the patient is unwell.

Table 1 Soft Tissue Sarcoma grading system (Kuntz *et al*., 1997)

Score	Degree of differentiation	Mitotic index	Necrosis
1	Resemble normal adult mesenchymal tissue	0-9	none
2	Specific histologic type	10-9	<50% necrosis
3	Undifferentiated	>20	>50% necrosis

Grade 1 = cumulative score of $\leq$ 4 for the 3 categories

Grade 2 = cumulative score of 5 or 6

Grade 3 = cumulative score of $\geq$ 7

Table 2 TNM classification for soft tissue sarcoma (Owen, 1980)

T	Primary tumor			
	T1	Tumor < 2 cm maximum diameter		
	T2	Tumor 2-5 cm maximum diameter		
	T3	Tumors > 5 cm maximum diameter		
	T4	Tumor invading other structures such as fasia, muscle, bone or cartilage		
N	Regional lymph nodes (RLN)			
	N0	No histologically verified metastasis		
	N1	histologically verified metastasis		
M	Distant metastases			
	M0	No evidence of distant metastasis		
	M1	Distant metastasis detected (specify sites)		
Stage grouping		T	N	M
I				
IA		T1	N0	M0
IB		T2	N0	M0
II				
IIA		T3	N0	M0
IIB		T4	N0	M0
III				
IIIA		AnyT	N1	M0
IIIB		AnyT	anyN	M1

Surgical technique: Marginal excision involved resection of the overlying attached skin and dissection along the tumor's visible extent (Figure 4a). Dissection demonstrated involvement of the large soft tissue-density mass with calcified at left thoracic wall and left caudal lung lobe consolidation (Figure 4b). Remove the thoracic wall containing the neoplasm and a margin of normal tissue, leaving a rectangular defect. Cut a piece of mesh slightly larger than the defect was put over and sutured to the pleural side of the defect. Fold over the edges of the mesh and suture the double thickness of the mesh to the pleural side of the defect (Figure 4c). Use a simple continuous suture pattern to appose remnants of the muscles. Minimize dead space by apposing the skin and underlying tissues with walking sutures.Place a thoracostomy tube and evacuate air from the thoracic cavity (Figure 4d-e). The patient was monitored closely in the postoperative period for respiratory distress, hemorrhage, hypoventilation, or the development of pneumothorax (Figure 4f). Analgesic therapy is given in this dog. One week later, the dog was scheduled for a follow-up appointment. The dog was decided to treat with a 2-drug combination consisting of PO administered low-dose cyclophosphamide (10 mg/m^2) and full-dose piroxicam (0.3 mg/kg) daily. The dog was re-evaluated at 3-month intervals throughout her treatment period.

The dog recovered uneventfully, and the reconstruction surgery and functional results were good. Radiography of the thorax was performed three times after surgery. There was no evidence of lung metastasis. The owner reported that the dog's quality of life was excellent postoperatively.

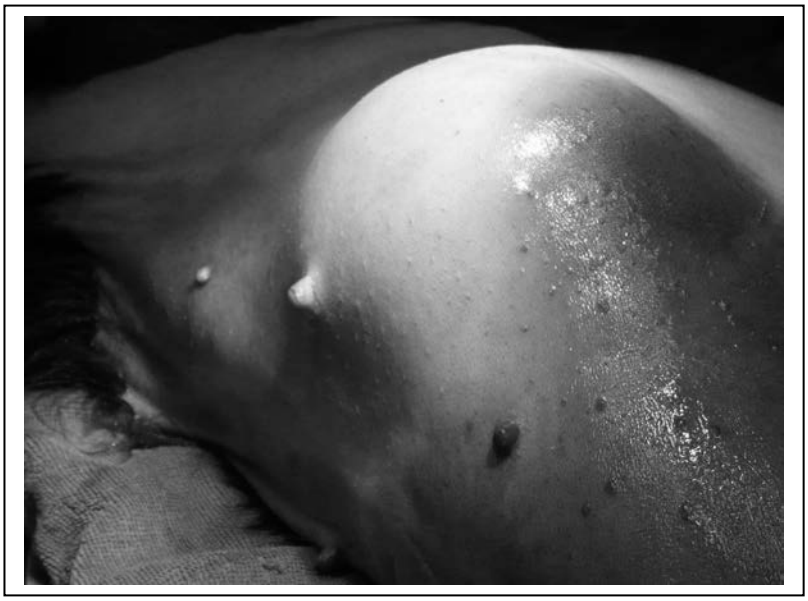

Figure1 A 5-year-old, female, neutered mixed breed dog with a large neoplasm of the left trunk.

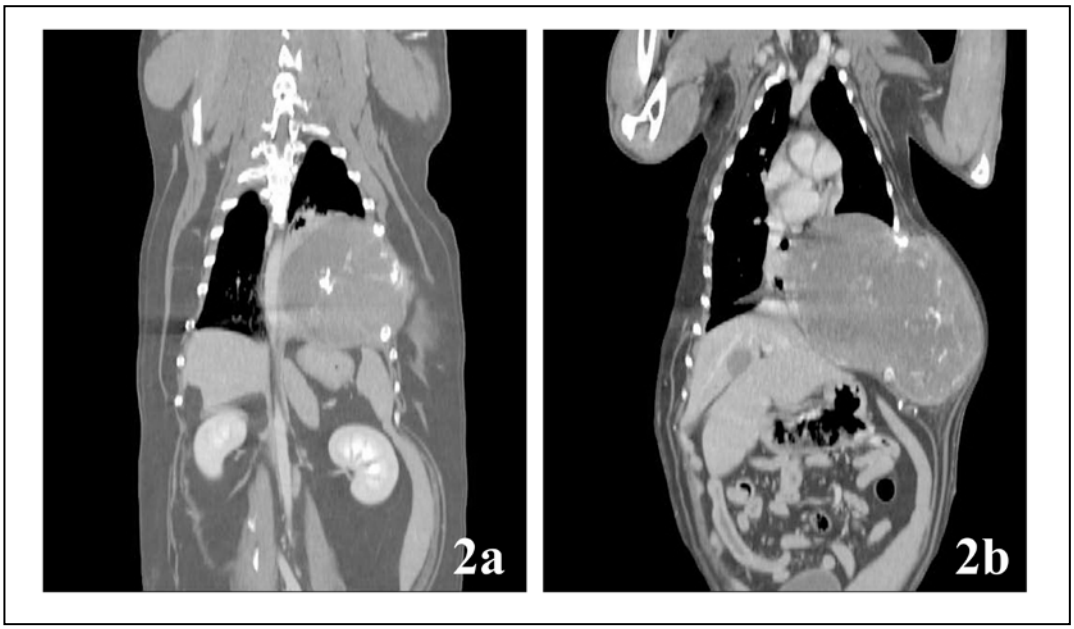

Figure 2 Dorsally reconstructed CT images of this dog showed the calcified mass involving the left thoracic wall musculature and extending into the thorax (a) bone destruction found at ninth rib and the space between the eight and tenth ribs increased (b) the mass came in close contact with the liver and the liver margin still be presented.

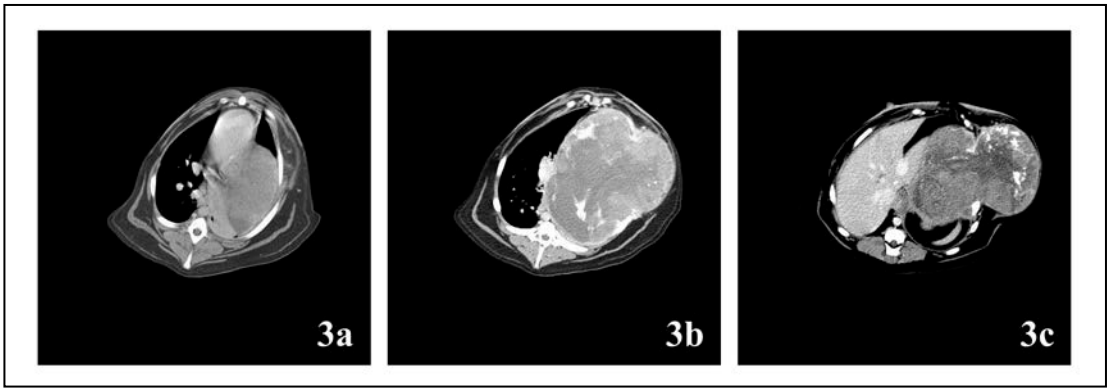

Figure 3 Transverse post-contrast CT images of a dog (a) a large soft tissue-density mass with calcified at left thoracic wall with left caudal lung lobe consolidation (b) the large soft tissue-density mass extends into the thorax (c) the mass extends into the thorax on one side and into the subcutaneous tissues on the other side.

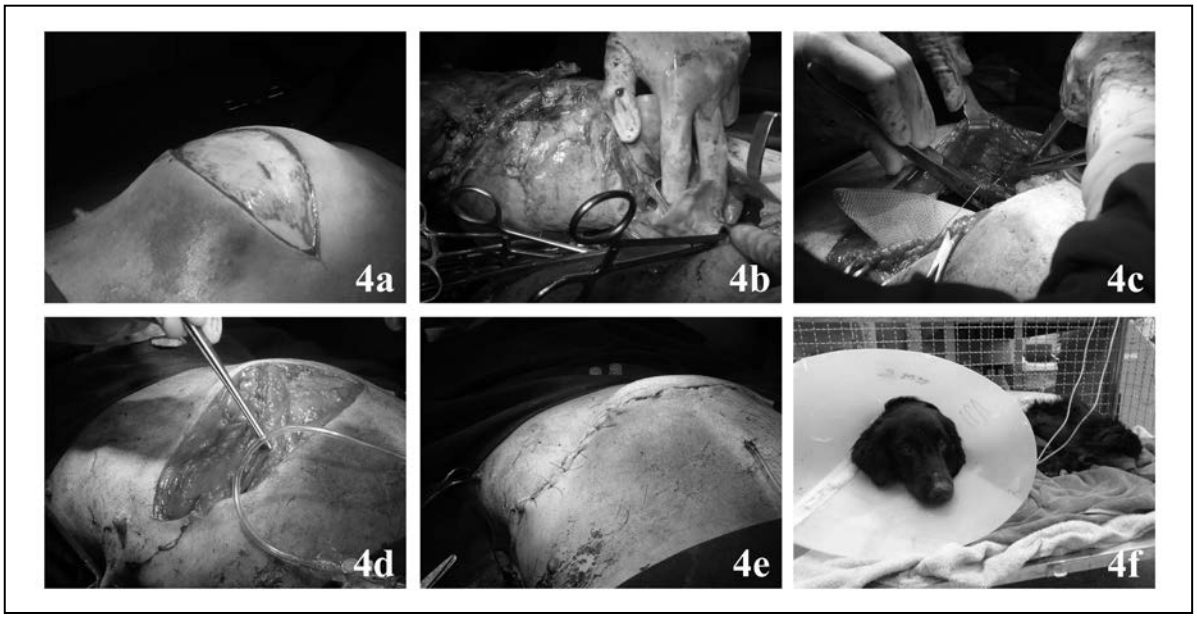

Figure 4 Surgical excision of a large neoplasm of the left trunk in a dog (a) a high grade sarcoma, approximately 20x15x10 cm^3, located on the trunk, incised through the skin overlying the neoplasm (b) the excision involved resection of the overlying attached skin and dissection along all the tumor's visible extent including the large soft tissue-density mass with calcified at left thoracic wall and left caudal lung lobe consolidation (c) a piece of mesh slightly larger than the defect was put over and sutured to the pleural side of the defect, before close the thoracotomy (d) a chest tube was to be placed, before closing the incision (e) Close the incision (f) the dog was monitored closely in the postoperative period for respiratory distress, hemorrhage, hypoventilation, or the development of pneumothorax.

Discussion

As we know, the dogs with high-grade STS are prone to early recurrence in cases of incomplete excision. Therefore, the wide excision; surgical margins of 3 cm lateral to the tumor and one fascial plane deep of STS, is always necessary (Kuntz *et al.,* 1997; Liptak and Forrest, 2013). This dog was not performed the complete marginal excision due to the huge-size of tumor. Other alternative treatments should be considered to use for palliation in macroscopic and may be for limited benefit in eliminating microscopic local or metastatic disease of STS. Therefore, the technique, combined treatments of surgery and metronomic therapy was used in this case in order to control local and systemic disease. This decision making was also based on the refusal of the owner's expense. We found that the striking inhibition of tumor recurrence that was observed in this dog treated with the continuous low-dose cyclophosphamide and full-dose piroxicam treatment regimen. Similarly, this combined treatment has been shown to be effective at treating several scientific data. It has also been shown to be useful for soft tissue sarcomas, transitional cell carcinoma of the urinary bladder and splenic hemangiosarcoma (Knapp *et al*., 1994, Knapp *et a*l., 1995; Greene *et al*., 2007; Lana *et al*., 2007; Elmslie *et al.*, 2008; Schrempp *et al*., 2013).

The treatment in this case was normally well tolerated. Overall, the side effects are usually mild, such as a day or two of vomiting, diarrhea or not wanting to eat, and either stop by themselves or resolve after reducing the dose of the drug. Fortunately, we have not found any side effects during the treated-interval. The routine follow-up at 1, 3, 6, 9, 12, 15, 18… months after surgery and treated with metronomic chemotherapy has been performed. So far, this dog is alive with pleasing on her quality of life. Recurrence or metastases has not been found and disease free interval is more than 24 months. This case report suggested that the 2-drug regimen was very effective in inhibiting the regrowth of microscopic tumor foci in this dog with high grade STS. Nevertheless, more studies using large populations evaluating the effect of combing metronomic chemotherapy with a COX inhibitor chemotherapy are still needed. In fact, the proper treatment planning for STS based on knowledge of typical biologic behavior as well as expected behavior is essential with familiarity with all potential treatment options. Proper postoperative assessment and follow-up is vital for monitoring disease control and prompt treatment of uncontrolled disease.

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