

## Case Report

# Cyclooxygenase Inhibitor Associated with Carboplatin in Treatment of Metastatic Nasal Carcinoma in Dog

**Carlos Eduardo Fonseca-Alves,<sup>1</sup> Aline Gonçalves Corrêa,<sup>1</sup>  
Fabiana Elias,<sup>2</sup> and Sabryna Gouveia Calazans<sup>3</sup>**

<sup>1</sup> *Department of Veterinary Clinic, School of Veterinary Medicine and Animal Science, Universidade Estadual Paulista, Botucatu, SP, Brazil*

<sup>2</sup> *Department of Veterinary Medicine, Federal University of Southern Border, Realeza, PR, Brazil*

<sup>3</sup> *Department of Veterinary Medicine, University of Franca, Franca, SP, Brazil*

Correspondence should be addressed to Carlos Eduardo Fonseca-Alves; [carlooseduardofa@hotmail.com](mailto:carlooseduardofa@hotmail.com)

Received 30 October 2013; Accepted 22 December 2013; Published 12 February 2014

Academic Editors: C. Hyun, C. M. Loiacono, and M. T. Mandara

Copyright © 2014 Carlos Eduardo Fonseca-Alves et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

A 10-year-old, intact male, pinscher was presented with unilateral bloodstained nasal discharge, sneezing, dyspnea, zygomatic arch deformity, submandibular lymph node increase, blindness in right eye, and exophthalmia. After clinical examination, it was found that the animal presented with upper respiratory tract dyspnea origin, possibly caused by an obstructive process. Complete blood count (CBC), ocular ultrasonography, thoracic radiographs, mandibular lymph node, and nasal sinus fine needle aspiration were performed. The right mandibular lymph node excisional biopsy was conducted and a tumor sample was obtained through the nasal fistula at hard palate. The material was processed, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Immunohistochemical staining for cytokeratin (AE1/AE3), vimentin, and COX-2 was performed. After histopathological evaluation nasal carcinoma diagnosis was obtained. Chemotherapy was established with carboplatin 300 mg/m<sup>2</sup> intravenously—four cycles with intervals of 21 days—and firocoxib 5 mg/kg orally every 24 hours for 7 months. After 7 months the treatment started, the animal presented with ataxia, vocalization, hyperesthesia, and anorexia. Due the clinical condition presented, the animal owner opted for performing euthanasia. The chemotherapy protocol was effective causing the disease stagnation, minimizing the clinical signs, and extending patient survival and quality of life.

## 1. Introduction

Nasal tumors are uncommon and represent approximately 2% of all tumors in dogs [1]. Distant metastasis are rarely observed and when occur, prognosis is poor [2]. Older dogs have increased risk for nasal tumors, but it has been observed in dogs younger than six months to greater than 16 years old [3]. Nasal tumors are usually malignant and local invasion is common, with secondary extension to paranasal sinuses [4]. Clinical signs include unilateral or bilateral bloodstained discharge, dyspnea, facial deformity, and exophthalmos. In cases of cranial involvement, tumor can also cause neurological signs, especially seizures, but it only occurs in 20% of cases [5]. In these cases, the animal may present with pain, head pressing against the obstacles, and behavioral changes such as aggressiveness [6, 7]. Nasal tumor histopathological

evaluation is essential to establish the definitive diagnosis and computerized tomography is important for tumors extension evaluation in the nasal cavity [6]. The clinical stage is based on the tumor's size (T), in the World Health Organization (WHO) and TNM (tumor, node, and metastasis) classification system. Tumor staging can be based on skull radiographs or computerized tomography, thoracic radiographs, fine-needle aspiration, and cytological examination of enlarged palpably lymph nodes. The *in vitro* studies have demonstrated the possible role of cyclooxygenase (COX) inhibitor as a single agent to prevent the occurrence of tumors. Recent studies in spontaneous canine tumors and experimentally induced mouse tumors have shown that COX-2 inhibitors have anti-tumor and chemopreventive effects in several different types of cancer. However, no prospective studies evaluating the efficacy of COX-2 inhibitors drugs in cases of nasal carcinomas

were conducted. The mechanisms by which COX inhibitors exert their antitumor effects are not completely understood, but studies have shown that COX-2-derived prostaglandins contribute to tumor cell resistance to apoptosis, new blood vessel formation, and tumor cell proliferation [8]. This paper reports clinical approach and describes a chemotherapy protocol in a dog with a primary nasal carcinoma presenting pulmonary and regional lymph node metastasis.

## 2. Case Report

A 10-year-old, intact male dog, pinscher was presented with unilateral nasal bloodstained discharge, sneezing, dyspnea, zygomatic arch deformity, submandibular lymph node increased, loss of vision in right eye, and exophthalmos. Physical examination revealed hard palate and oronasal fistula commitment. The animal has been presenting these clinical signs for three months and it was previously treated with amoxicillin 20 mg/kg, PO, BID, and prednisone 1 mg/kg, PO, BID. After clinical examination it was found that dyspnea origin was from the upper respiratory tract, possibly caused by an obstructive process. Submandibular lymph node cytological evaluation showed polyhedral shaped cells with oval to elongated nucleus with evident nucleoli; the cytoplasm was basophilic and well defined. There were intense anisocytosis, anisokaryosis, multinucleated giant cells, macrophages, and few lymphocytes, suggesting an epithelial tumor metastasis. Ocular ultrasound revealed a hypochoic structure with uniform texture and irregular contours in retrobulbar space. The chest thoracic radiographs showed nodular interstitial pattern, suggestive of pulmonary metastasis. The skull radiographic evaluation revealed widespread loss of the shells details and small radiolucent areas on increased radiopacity areas. Excisional biopsy was performed on the right mandibular lymph node and the tumor sample was obtained through the nasal fistula at hard palate. Due to the limitation to surgically access of the chest, lung nodules biopsies were not performed. Material was processed, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Histological evaluation of the nasal tumor revealed proliferation of round cells with oval and round nucleus, dispersed chromatin, prominent nucleoli, and bounded eosinophilic cytoplasm. These cells were arranged in palisades forming islands or robes. The tumor histopathology was compatible with the basal cell carcinoma (Figure 1), and metastasis with the same histological pattern of the primary tumor was detected in the submandibular lymph node. Immunohistochemical staining for cytokeratin antibody to confirm the diagnosis of carcinoma and COX-2 antibody to assess the expression of the primary tumor and lymph node metastasis was performed. Immunohistochemical staining was performed using peroxidase method and DAB. Slides were dewaxed in xylol and rehydrated in graded ethanol. For antigen retrieval, the slides were incubated in citrate buffer (pH 6.0) for 30 s in a pressure cooker (Pascal; Dako, Carpinteria, CA, USA). The sections were treated with freshly prepared 3% hydrogen peroxide in absolute methanol for 20 min to inhibit endogenous peroxidase activity and washed in Tris-buffer saline. Three primary antibodies were used: AE1/AE3 (monoclonal mouse,

Neomarkers, Fremont, CA, USA), vimentin (polyclonal mouse, Abcam, Cambridge, UK), and COX-2 (monoclonal mouse, Dako, Carpinteria, CA, USA), applied at dilutions of 1:50, 1:50, and 1:50, respectively, at 4°C temperature overnight. A signal amplification system with enzyme labeled polymer conjugated to a dual secondary antibody (Dako Envision System, Dako, Carpinteria, CA, USA) was used for AE1/AE3 and vimentin antibodies. For COX-2 antibody a similar signal amplification system (Advance, Dako, Carpinteria, CA, USA) was used. After each step in the process the slides were rinsed with Tris-buffered saline. The slides were developed with 3'-diaminobenzidine tetrahydrochloride (DAB, Dako, Carpinteria, CA, USA) for 5 min and counterstained with Harris haematoxylin. Positive and negative controls were performed for all antibodies by omitting the primary antibody and substituting with Tris-buffered saline. Positive stain for cytokeratin antibody in primary nasal tumor (Figure 2) and lymph node metastasis (Figure 3) and negative stain for vimentin antibody and strong expression of COX-2 protein in primary nasal tumor (Figure 4) and lymph node metastasis were found. Due to high expression of COX-2 in the primary tumor, it was decided to introduce the COX-2 inhibitors in the treatment protocol. Clinical staging of patient was conducted before start the chemotherapy protocol. The patient presented clinical stage T3N1M1 in accordance with WHO and stage T3 in accordance with Owen [7]. Chemotherapy with carboplatin 300 mg/m<sup>2</sup> intravenously—four cycles with intervals of 21 days—and firocoxib 5 mg/kg orally every 24 hours for 7 consecutive months were established. To evaluate the chemotherapy and COX-2 inhibitor treatment side effects, clinical and laboratory examinations (serum biochemistry and CBC) were carried out every 21 days. The patient presented no adverse effects. After the second chemotherapy cycle, the patient presented no more dyspnea, however, still presented unilateral bloody nasal discharge. After 7 months, the animal started with ataxia, vocalization, hyperesthesia, and anorexia. Due to the clinical condition presented, the animal owner requested euthanasia. Necropsy was performed and presence of a tumor in the nasal cavity was found, affecting palate, paranasal sinus, and retrobulbar space with optic nerve involvement, causing compression of the central nervous system. The lung parenchyma presented some nodules suggestive of metastasis. Fragments of lung nodules, sinus, and retrobulbar space were collected for histopathology. The histopathological analysis of specimens collected at necropsy confirmed the diagnosis obtained with biopsies performed previously and confirmed the presence of pulmonary metastases of nasal carcinoma. We performed immunohistochemical staining for COX-2 antibody of primary nasal tumor after treatment with carboplatin and COX-2 inhibitor. Immunohistochemistry showed low expression for the protein COX-2 (Figure 5).

## 3. Discussion

Some papers in the literature evaluated COX-2 expression in canine tumors, but few papers evaluated the expression of this specific protein associated with treatment using

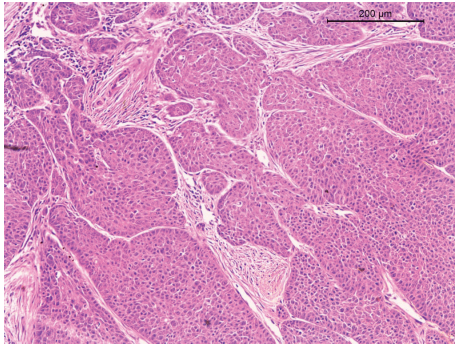


FIGURE 1: Histopathological appearance of the canine nasal carcinoma. Note the proliferation of epithelial cells. Note moderate cellular pleomorphism. Hematoxylin and eosin (HE). Bar = 200  $\mu\text{m}$ .

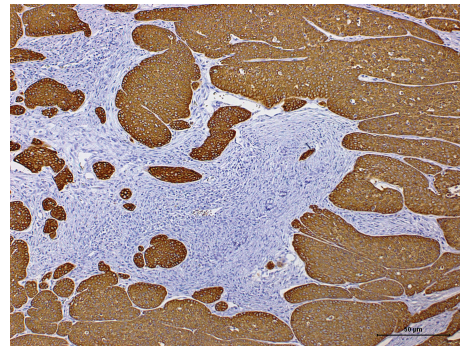


FIGURE 3: Immunolocalization of AE1/AE3 in lymph node metastasis of nasal carcinoma. Positive signal can be observed as the brown color in the cytoplasm of the cells or in the cytoplasm with hematoxylin counterstain. Bar = 50  $\mu\text{m}$ .

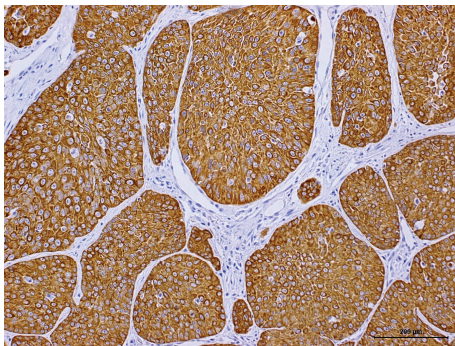


FIGURE 2: Immunolocalization of AE1/AE3 in nasal carcinoma. Positive signal can be observed as the brown color in the cytoplasm of the cells or in the cytoplasm with hematoxylin counterstain. Bar = 200  $\mu\text{m}$ .

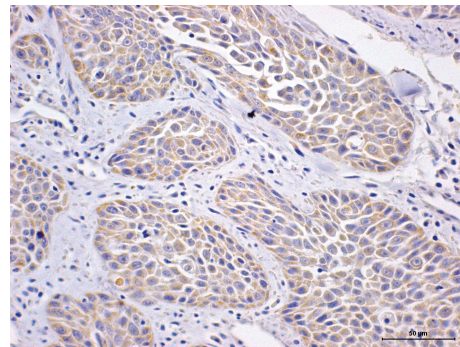


FIGURE 4: Immunolocalization of COX-2 in nasal carcinoma before treatment. Positive signal can be observed as the brown color in the cytoplasm of the cells or in the cytoplasm with hematoxylin counterstain. Bar = 50  $\mu\text{m}$ .

COX-2 inhibitor. The immunohistochemistry found that the primary tumor and lymph node metastasis showed high expression of COX-2. Overexpression of COX-2 has been demonstrated in various canine neoplasms. Rassnick et al. [9] and Borzacchiello et al. [10] provided evidence of similar overexpression in canine nasal carcinomas. Recent studies in rodents, dogs, and humans indicated that COX-2 inhibitors may have chemopreventive and antitumor activity [10]. There is also evidence that COX-2 may increase tumor invasiveness and metastasis and this is important in angiogenic factors production [4].

The prognosis for dogs with nasal carcinomas that did not receive treatment other than palliative medications is poor [2]. Nasal carcinoma is uncommon in dogs and treatment is both difficult and controversial. The median survival time is 3.1 months, and the probability of surviving up to 1 and 2 years after diagnosis was only 12% and 2%, respectively [9]. Vanherberghen et al. [2] considered epistaxis as a poor prognosis; once the dogs had epistaxis at the time of diagnosis, the survival time was 88 days. Radiotherapy is the treatment that provides longer survival for these patients. Median survival times for dogs treated with radiation therapy range from 7.4 to 47.7 months. In this case the association between firocoxib

and carboplatin presented a survival rate closer to that one found in dogs treated with radiotherapy [11].

Based on studies that many different carcinomas express COX-2 and present increased PGE2 levels, COX-2 blockade has become an important component in antineoplastic therapy [12]. It is known that COX-2 may contribute to tumor aggressiveness by suppressing apoptosis, promoting angiogenesis and tumor invasion, and by tumor cell proliferation stimulation. Based on progression of epithelial tumor in humans, COX-2 showed a potentially powerful impact factor; therefore, COX-2 inhibition in canine tumors can be considered a strategy to improve outcome disease. The use of selective COX-2 inhibitors, could improve a variety of canine tumors response. However, tumors primarily treated with radiation therapy, as selective COX-2 inhibitors have been shown to improve the irradiation effect [13].

Despite the negative prognostic factors, after chemotherapy antineoplastic associated with nonsteroidal anti-inflammatory therapy the animal presented remission of clinical signs. In another study cisplatin combined with COX-2 inhibitors has been examined in many tumors. In transitional cell carcinoma, this combination increased the apoptotic index and demonstrated a positive effect on survival [14].

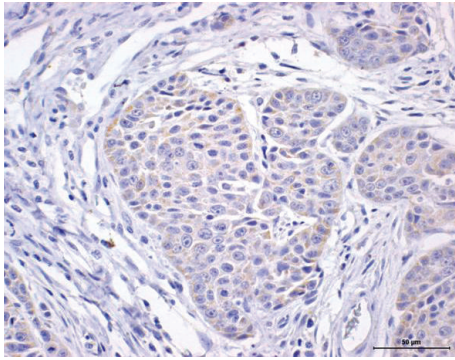


FIGURE 5: Immunolocalization of COX-2 in nasal carcinoma after treatment. Positive signal can be observed as the brown color in the cytoplasm of the cells or in the cytoplasm with hematoxylin counterstain. Bar = 50  $\mu$ m.

Sorenmo et al. [12] evaluated the effect of carprofen or piroxicam administration in dogs with prostatic carcinoma. The authors found that COX-2 inhibitors associated with carboplatin use can offer positive effects on median survival times. Untreated dogs had a median survival time of 0.7 months with poor prognosis and treated dogs had a median survival time of 6.9 months, demonstrating the efficacy of COX-2 inhibitors.

#### 4. Conclusion

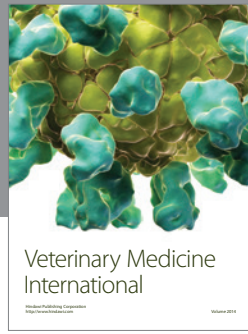
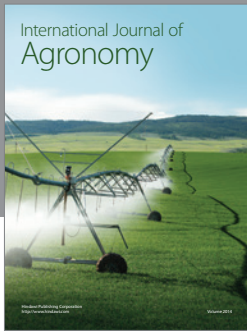
The chemotherapy protocol used was effective providing the disease stagnation, minimizing the clinical signs, and increasing patient survival and quality of life.

#### Conflict of Interests

The authors report no conflict of interests. The authors alone are responsible for the content and writing of the paper.

#### References

- [1] F. Millanta, S. Citi, D. Della Santa, M. Porciani, and A. Poli, "COX-2 expression in canine and feline invasive mammary carcinomas: correlation with clinicopathological features and prognostic fmolecular markers," *Breast Cancer Research and Treatment*, vol. 98, no. 1, pp. 115–120, 2006.
- [2] M. Vanherberghen, M. J. Day, F. Delvaux, A. Gabriel, C. Clercx, and D. Peeters, "An immunohistochemical study of the inflammatory infiltrate associated with nasal carcinoma in dogs and cats," *Journal of Comparative Pathology*, vol. 141, no. 1, pp. 17–26, 2009.
- [3] D. W. Wilson and D. L. Dungworth, "Tumors of the respiratory tract," in *Tumors of Domestic Animals*, D. J. Meuten, Ed., pp. 365–377, Iowa State Press, Ames, Iowa, USA, 2002.
- [4] J. C. Woodard, "The respiratory system," in *Veterinary Pathology*, T. C. Jones, R. D. Hunt, and N. W. King, Eds., pp. 947–973, Williams & Wilkins, Baltimore, Md, USA, 6th edition, 1996.
- [5] L. W. C. Chow, W. T. Y. Loo, and M. Toi, "Current directions for COX-2 inhibition in breast cancer," *Biomedicine & Pharmacotherapy*, vol. 59, supplement 2, pp. S281–S284, 2005.
- [6] F. Ninomiya, S. Suzuki, H. Tanaka, S. Hayashi, K. Ozaki, and I. Narama, "Nasal and paranasal adenocarcinomas with neuroendocrine differentiation in dogs," *Veterinary Pathology*, vol. 45, no. 2, pp. 181–187, 2008.
- [7] L. N. Owen, *TNM Classification of Tumors in Domestic Animals*, vol. 149, World Health Organization, Geneva, Switzerland edition, 1980.
- [8] S. I. Mohammed, P. F. Bennett, B. A. Craig et al., "Effects of the cyclooxygenase inhibitor, piroxicam, on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer," *Cancer Research*, vol. 62, no. 2, pp. 356–358, 2002.
- [9] K. M. Rassnick, C. E. Goldkamp, H. N. Erb et al., "Evaluation of factors associated with survival in dogs with untreated nasal carcinomas: 139 cases (1993–2003)," *Journal of the American Veterinary Medical Association*, vol. 229, no. 3, pp. 401–406, 2006.
- [10] G. Borzacchiello, O. Paciello, and S. Papparella, "Expression of cyclooxygenase-1 and -2 in canine nasal carcinomas," *Journal of Comparative Pathology*, vol. 131, no. 1, pp. 70–76, 2004.
- [11] C. J. Henry, W. G. Brewer Jr., J. W. Tyler et al., "Survival in dogs with nasal adenocarcinoma: 64 cases (1981–1995)," *Journal of Veterinary Internal Medicine*, vol. 12, no. 6, pp. 436–439, 1998.
- [12] K. U. Sorenmo, M. H. Goldschmidt, F. S. Shofer, C. Goldcamp, and J. Ferracone, "Evaluation of cyclooxygenase-1 and cyclooxygenase-2 expression and the effect of cyclooxygenase inhibitors in canine prostatic carcinoma," *Veterinary and Comparative Oncology*, vol. 2, no. 1, pp. 13–23, 2004.
- [13] C. Petersen, S. Petersen, L. Milas, F. F. Lang, and P. J. Tofilon, "Enhancement of intrinsic tumor cell radiosensitivity induced by a selective cyclooxygenase-2 inhibitor," *Clinical Cancer Research*, vol. 6, no. 6, pp. 2513–2520, 2000.
- [14] S. I. Mohammed, B. A. Craig, A. J. Mutsaers et al., "Effects of the cyclooxygenase inhibitor, piroxicam, in combination with chemotherapy on tumor response, apoptosis, and angiogenesis in a canine model of human invasive urinary bladder cancer," *Molecular Cancer Therapeutics*, vol. 2, no. 2, pp. 183–188, 2003.



# Hindawi

Submit your manuscripts at  
<http://www.hindawi.com>

