Treatment of Canine Oral Melanoma with Adjuvant Chemotherapy and Immunotherapy

**Gleidice Eunice Lavalle, Carla Emanuela Tertuliano Caires, Stéfane Valgas Teixeira,
Rúbia Monteiro de Castro Cunha & Rubens Antônio Carneiro**

ABSTRACT

***Background*:** Melanoma is the most frequent cancer in the canine oral cavity. It shows an aggressive behavior, characterized by rapid and invasive growth and high metastatic potential. Metastasis is seen in more than 80% of dogs at time of death. Adjuvant therapy should be recommended because of potential recurrence and metastasis. Oral melanoma has a poor prognosis even when adjuvant treatments are used. There are some treatment options, but the high death rate due to the disease is still a challenge. The aim of this study was to assess the overall survival of dogs diagnosed with oral melanoma and treated with adjuvant chemotherapy and immunotherapy.

***Materials, Methods & Results*:** A retrospective analysis was carried out in 20 dogs with oral melanocytic or amelanocytic melanomas. Cases were staged according to a modified World Health Organization clinical staging system for canine oral malignant melanoma. Tumor size (T1: < 2 cm; T2: 2 - 4 cm; T3: > 4 cm), regional metastasis (N0: no metastasis; N1: metastasis) and presence of distant metastasis (M0: no metastasis; M1: metastasis) are evaluated. Then, cases were divided into 4 stages: I (T1 N0 M0), II (T2 N0 M0), III (T3 N0-1 M0, Tx N1 M0) and IV (Tx Nx M1). Diagnoses were confirmed with histopathological exam and immunohistochemistry (IHC) when necessary. In poorly differentiated neoplasms, IHC was performed at the request of the submitting veterinarian using specific markers PNL-2 and Melan-A. Animals were divided into 2 groups: dogs submitted to surgery alone were included in group 1 (G1); dogs submitted to surgery associated with adjuvant chemotherapy with four 21-day cycles of carboplatin (300 mg/m^2) and immunotherapy with six 7-day cycles of interferon-α (3 x 10^6 IU/m^2) were included in group 2 (G2). Twenty dogs diagnosed with oral melanoma were evaluated: 3 were included in G1 and 17 in G2. Considering clinical staging of the dogs: 7 stage II, 12 stage III and only 1 stage IV. There was no stage I patients. In poorly differentiated neoplasias, IHC was performed at the request of the submitting veterinarian using specific markers PNL-2 and Melan-A. Patient follow-up was obtained through the evaluation of patient records and telephone interviews with owners. The overall survival time (OS) was defined by the period (in days) between the date of surgical excision and the death caused by the disease. Median overall survival time was 86 days for animals in G1 and 894 days for animals in G2 ($P = 0.01$).

***Discussion*:** Carboplatin was considered an appropriate cytostatic drug to treat microscopic disease in oral melanoma. INF-α was chosen for immunotherapy in this study because it promotes immune system stimulation associated with an indirect antiproliferative effect on neoplastic cells. The association of INF-α and carboplatin resulted in a significant increase in overall survival, when compared to the literature, suggesting that association of chemotherapy and immunomodulation is an important strategy in the treatment of canine oral melanoma. Controlled prospective randomized trials are necessary to confirm the benefits of chemotherapy and immunotherapy association to treat canine oral melanoma. Adjuvant therapy with chemotherapy and immunotherapy was considered effective to increase overall survival and maintained quality of life of dogs diagnosed with oral melanoma.

Keywords: melanoma, adjuvant treatment, immunotherapy, dogs.

INTRODUCTION

Melanoma is the most frequent cancer in the canine oral cavity. It shows an aggressive behavior, characterized by rapid and invasive growth and high metastatic potential to regional lymph nodes, lungs, and other [13,17]. Adjuvant therapy should be recommended because of potential recurrence and metastasis [18], but the high death rate due to the disease is still a challenge [17]. Oral melanoma has a poor prognosis even when adjuvant treatments are used [17]. Treatment protocols with cytostatic drugs and immunotherapies are being developed for humans and animals to interfere with disease progression [1]. Adjuvant treatment for oral melanoma include chemotherapy, radiotherapy, electrochemotherapy [14,19] and immunotherapy, such as DNA xenogeneic vaccine [21]. The usage of immunomodulators to treat oral melanoma is promising, and dogs are a study model for human treatment [3,14]. Autologous tumor cell vaccines, allogeneic cell vaccines, canine dendritic cell vaccines with melanosomic differentiating antigens and immunostimulatory such as interleukine-2 (IL-2) and tumor necrosis factor alpha (TNF-α) are used as immunotherapy for canine melanomas [14, 23]. Alpha interferon (INF-α) has been used in the treatment of other diseases but has not been reported in the treatment of canine oral melanoma [1].

The aim of this study was to assess the overall survival of dogs diagnosed with oral melanoma and treated with adjuvant chemotherapy and immunotherapy.

MATERIALS AND METHODS

Animals

A retrospective analysis was carried out in 20 dogs with oral melanocytic or amelanocytic melanomas admitted to the Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil. Animals were divided into 2 groups according to 2 different protocols: 3 dogs submitted only to surgical excision of the neoplasm were included in group 1 (G1), and; 17 dogs submitted to surgical excision associated with adjuvant therapy using four 21-day cycles of carboplatin at 300 mg/ m^2 [EV, B - Platin®, injective][1] and six 7-day cycles of INF-α at 3x106 IU/m^2 [IM, Alfainterferona 2a Humano Recombinante, injective][2] were included in group 2 (G2). In case of suspected regional metastasis, cytology and lymph node removal were performed,

and distant metastasis were diagnosed through thoracic radiographs. The animals treated only with surgery, due to the refusal of chemotherapy treatment by the responsible. Cases were staged according to a modified World Health Organization clinical staging system for canine oral malignant melanoma. Tumor size (T1: < 2 cm; T2: 2-4c m; T3: > 4 cm), regional metastasis (N0: no metastasis; N1: metastasis) and presence of distant metastasis (M0: no metastasis; M1: metastasis) are evaluated. Then, cases were divided into 4 stages: I (T1N0M0), II (T2N0M0), III (T3N0-1M0, TxN1M0) and IV (TxNxM1) [3].

Histopathological and Immunohistochemistry evaluation

Diagnoses were confirmed with histopathological exam and IHC when necessary. The tumor samples were collected in a surgical procedure, fixed for 48 h in 10% buffered formaldehyde and wrapped in paraffin. Then, 4 μm histological sections were performed and stained with hematoxylin and eosin[3] (H&E). In poorly differentiated neoplasias, IHC was performed at the request of the submitting veterinarian using specific markers PNL-2[4] and Melan-A[5]. The 4 μm sections were cut from melanoma samples, mounted on gelatin slides, dewaxed, and rehydrated, for immunohistochemical analysis. Staining was obtained according to the method of reaction of the peroxidase with the secondary antibody (Novolink Polymer Detection System)[6]. Antigenic recovery was carried out by incubating, for 20 min, in Citrate pH 6.0 target recovery solution (Dako Cytomation)[5], 2 min in pressurized moist heat, in a Pascal® pressure cooker[7] at 125°C, and cooling to temperature environment. Endogenous peroxidase was blocked by double incubation for 10 min in 3% hydrogen peroxide in methanol solution. Through incubation, for 20 min in the Ready to Use reagent without whey protein block[5], non-specific binding sites of endogenous proteins were blocked. Sections were incubated, for 16 h at room temperature, with primary antibodies with specific dilutions for each, followed by HRP polymer (Novolink polymer detection system)[6] and 1-min incubation with chromogen 3′3-diaminobenzidine (DAB system)[5]. Finally, the slides were contrasted with the Giemsa dye, in the dilution 1: 5, for 30 min and washed with hydrochloric acid, dilution 1: 100, absolute alcohol and isopropyl alcohol, respectively. Then, the melanin pigment becomes greenish, different from the brown hue found in the DAB / primary antibody chromogen reaction.

The negative control was done by incubating the slides with antibody diluent (Antibody Diluent with Bottom Reducing Components)[5]. Positive controls were analyzed in non-neoplastic areas of the samples [22].

Statistical analysis

Patient follow-up was obtained through the evaluation of patient records and telephone interviews with owners. The overall survival time (OS) was defined by the period (in days) between the date of surgical excision and the death caused by the disease. Deaths from unknown causes or causes unrelated to the tumor were censored. For OS evaluation, univariate analysis was used (Kaplan-Meier estimated survival curves). They were considered statistically significant when P-values < 0.05 by the log-rank test (Cox-Mantel). Median survival was determined by the period in which 50% of patients in a group came to death.

RESULTS

Twenty dogs diagnosed with oral melanoma were evaluated: 3 (15%) were included in G1 and 17 (85%) in G2. Considering clinical staging: 7 (35%) stage II, 12 (60%) stage III and only 1 (5%) in stage IV. There was no stage I patients.

According to the histomorphology classification, 4 (20%) of tumors were amelanocytic and 16 (80%) were melanocytic melanomas. A significant increase in overall survival was associated with the proposed adjuvant therapy. Median overall survival time was 86 days for G1 and 894 days for G2 ($P = 0.01$; Figure 1).
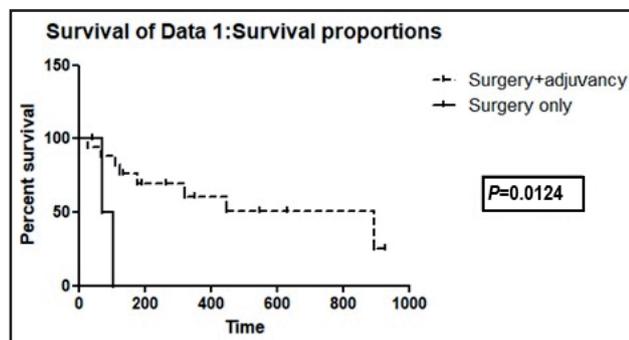
Figura 1. Overall survival for surgery (G1) and surgery associated with adjuvant therapy (G2) dogs.

DISCUSSION

Canine oral melanoma metastasis can be seen in 70% of enlarged lymph nodes and in 40% of clinically normal lymph nodes at diagnosis [19,23]. This shows that most diagnoses are late, as seen in the present study. The low frequency with which tutors check their pet´s mouth and delay in seeking veterinary care could partially justify the frequent late diagnosis. A study was demonstrated an overall survival of 65 days for untreated dogs and 86.5 days for dogstreated with surgery alone, suggesting that surgery alone does not significantly improve survival and that therapeutic complementation may be important in canine oral melanomas [11].

Local and systemic treatments are essential to adequately treat canine oral melanoma [1,7,15,17,20]. One previous study failed to demonstrate improvement in overall survival when adjuvant therapies were associated with surgical excision of the neoplasm [7]. Carboplatin was considered an appropriate cytostatic drug to treat microscopic disease in oral melanoma [10, 17]. A study compared adjuvant radiotherapy and carboplatin, and the drug was considered as the adjuvant treatment of choice by the authors [10]. Only mild to moderate side effects (myelosuppression) were seen throughout the study, and it was considered a safe drug.

Radiotherapy is rarely used in Brazil due to high cost and it is only available in two states, but it can also be considered a treatment option for inoperable tumors, tumors that did not have clean surgical margins and for animals with lymph node metastasis, without distant metastasis [4,9,12]. In 2012, was reported similar overall survival and disease-free interval with usage of adjuvant carboplatin [10]. INF-α was chosen for immunotherapy in this study because it promotes immune system stimulation associated with an indirect antiproliferative effect on neoplastic cells, possibly resulting in an anticancer response [1,6,12]. Median overall survival of dogs in G2 was approximately 2.5 years, far superior to dogs in G1 (86.5 days). An overall survival of 165 days for animals treated with surgery and carboplatin was described, while was described an overall survival of 477 days and 510 days for stage II and III animals treated with Oncept vaccine as adjuvant therapy [8,15,16,21]. Therefore, the association of INF-α and carboplatin resulted in a significant increase in overall survival, when compared to the cited literature, suggesting that association of chemotherapy and immunomodulation is an important strategy in the treatment of canine oral melanoma. It should be noted that Oncept vaccine is not available in Brazil.

Severe side effects were not seen during treatment with carboplatin and INF-α. Mild to moderate myelosuppression was related with usage of carboplatin, which was evaluated with complete blood counts performed at the drug´s nadir. No side effects were attributed to INF-α.

Controlled prospective randomized trials are necessary to confirm the benefits of chemotherapy and immunotherapy association to treat canine oral melanoma.

CONCLUSION

Therapeutic complementation with chemotherapy and immunotherapy was associated with an increase in overall survival, with maintenance of quality of life of dogs diagnosed with oral melanoma. The authors believe that multimodal therapy has a significant beneficial effect in the treatment of canine oral melanoma and its usage should be encouraged.

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