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Adjuvant electrochemotherapy of malignant ocular melanoma in a dog

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Abstract

Melanocytoma and malignant melanoma are the most typical eye tumors in dogs and cats. Due to the presence of melanin, the general appearance is often highly pigmented in a nodular formation with well-defined borders. Electrochemotherapy (ECT) is a therapy combining reversible electroporation and anti-neoplastic drugs to enhance their cytotoxic effects through increasing cellular uptake by the electroporated tumor cells. In this article, the use of adjuvant ECT for the treatment of canine ocular melanoma is reported. The pre-surgical exams (blood count, renal and liver functions, echocardiogram, and electrocardiogram) were within the normal range, as were the chest radiography and abdominal ultrasound without signs of metastases. On the day of the surgery, an excisional biopsy of the tumor was performed without safety margins by keratectomy associated with conjunctivectomy and adjuvant ECT in the surgical site and peri-tumoral region. The animal was followed for approximately 7 months and remained without evidence of tumor recurrence. A complete resolution of corneal opacification was also observed. To our knowledge, this is the first report of the use of ECT in an ocular neoplasm. From a comparative oncology perspective, this report opens the way for future therapeutic approaches in superficial ocular cancers in veterinary and human medicine.

Introduction

Malignant melanoma and melanocytoma are the most common eye tumors in dogs and cats, and their epibulbar and limbal presentations account for 10.00 to 34.00% of all canine melanocytic ocular tumors, respectively.[1,2]

Ocular malignant melanomas originate from melanocytes. The general appearance is typically nodular with well-defined borders and highly pigmented due to the presence of melanin; although, in rare cases it may be non-pigmented (amelanotic).[1,3,4] In dogs, limbal or epibulbar melanomas usually have a less aggressive behavior than their intra-ocular presentation.[5] Gonioscopy and ocular ultrasound help distinguish epibulbar tumors from melanocytic tumors with extra-scleral extensions.[1,2]

Currently, the indicated treatments of ocular cancers are surgery, radiotherapy, cryotherapy, laser therapy, and photodynamic therapy, in human and veterinary medicine,[3,6] resulting in visual disability in some cases, depending on tumor and chosen therapy characteristics.

Electrochemotherapy (ECT) is a technique combining reversible electroporation with anti-neoplastic drugs, enhancing their cytotoxic effects through increasing cellular uptake by the electroporated tumor cells.[7,8] The applied high-intensity electric pulses are delivered to the tumor by electrodes, which may differ in models and intended applications.[7] In some cases, the use of conductive gels is indicated for providing a more homogeneous local electric field distribution between the electrodes and the tumor surface, enhancing the efficiency of ECT.[9,10]

The ECT has been successfully used in the treatment of different tumors such as squamous cell carcinomas, soft tissue sarcomas, and malignant melanoma in human and veterinary medicine as a unique treatment or adjuvant to the surgeries.[7,8] Regarding ocular tumors, only pre-clinical *in vitro* and *in vivo* (chick embryos) uveal melanoma models are described.[11] In peri-ocular regions, the use of ECT has been reported in basal cell carcinomas avoiding the necessity of wide margins and maintaining eyelid function.[12,13]

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In this paper, the use of adjuvant ECT in the treatment of ocular melanoma in a dog is reported. To our knowledge, this is the first report of the use of ECT in an ocular neoplasm until the moment, paving the way for future therapeutic approaches in superficial ocular tumors not only in veterinary medicine but also in human medicine, from a comparative oncology perspective.

Case Description

A 12-year-old male mixed-breed dog with a nodule in the left eye was referred to the veterinary eye care department. During the physical exam, the owners also reported that the animal had mild eye secretion, denying noticing signs of discomfort, itching, pain, visual deficit, or any other behavioral abnormalities.

During the ophthalmic evaluation, both eyes showed normal direct and consensual light reflexes, normal fundoscopy and visual tests, and negative fluorescein assessment, and intra-ocular pressure using a rebound tonometer (Icare, Raleigh, USA) of 19.00 mmHg in both eyes and Schirmer test (DrogaVet, São Paulo, Brazil) of 20.00 mm per min. However, the left eye had mucoid secretion, conjunctival hyperemia, and a pedunculated irregular nodular neoformation with a blackish appearance in the superficial limbal-conjunctival region with corneal involvement measuring approximately 8.00 × 7.00 × 7.00 mm (Fig. 1A), without presenting intra-ocular alterations assessed by pupillary dilation with 10.00 mg mL^{-1} tropicamide (Alcon, Geneva, Switzerland), gonioscopy (Volk, Mentor, USA) and ocular ultrasound (Siemens, Munich, Germany). Lubricating eye drops based on 0.15% sodium hyaluronate (União Química, São Paulo, Brazil) were prescribed.

Cytology was suggestive of a malignant neoplasm of melanocytic origin. Due to the resistance of the tutors to the option of enucleation, surgical excision of the neoformation associated with ECT in the surgical site was indicated.

The pre-surgical exams (blood count, renal and liver functions, echocardiogram and electrocardiogram) were within the normal range, as were the chest radiography and abdominal ultrasound without signs of metastases.

On the day of the surgery, an excisional biopsy of the tumor (10.00 × 8.00 × 9.00 mm) was performed without safety margins by keratectomy associated with conjunctivectomy and adjuvant ECT in the surgical site and peri-tumoral region (Figs. 1B and 1C).

For ECT, intravenous administration of bleomycin (Celon, Hyderabad, India) was performed at a dose of 15,000 IU per m^2. After 8min, an electric field of 1,300 V cm^{-1}, eight electric pulses (100 µSec each), and a frequency of 5.00 kHz (VetCP 125; VetCâncer, São Paulo, Brazil) were applied through a needle electrode, being positioned parallel to the retina to avoid displacement and using of an ultrasound gel (Multigel, São Paulo, Brazil) to allow a homogeneous distribution of the electric field between the ocular surface and the electrode. After ECT, corneal covering was performed with a protective membrane (BioSIS, Philadelphia, USA) and temporary tarsorrhaphy for 15 days. For the post-operative period, oral amoxicillin (Agener União, São Paulo, Brazil), meloxicam (Ourofino, Cravinhos, Brazil) and dypirone (Sanofi, São Paulo, Brazil) were prescribed in addition to topical moxifloxacin (Alcon), tromethamine ketorolac (Genom, São Paulo, Brazil) and sodium hyaluronate.

Later, the histopathological examination using light microscopy confirmed that the lesion was melanoma (Fig. 2). Ten days after the surgical treatment, the patient underwent a new ophthalmic evaluation, where there was a decrease in the presence of mucoid secretion and conjunctival hyperemia and early removal of the temporary tarsorrhaphy by the patient. Despite this intercurrence, there was no need for a new surgical intervention or changes in the therapeutic protocol. The absence of tumor recurrence signs was observed (Fig. 3A). In addition, the presence of a small scar granuloma, neovascularization at the surgical site, and absence of corneal lesions on examination with fluorescein were noted, indicating that the use of electrodes during ECT did not injure the cornea.

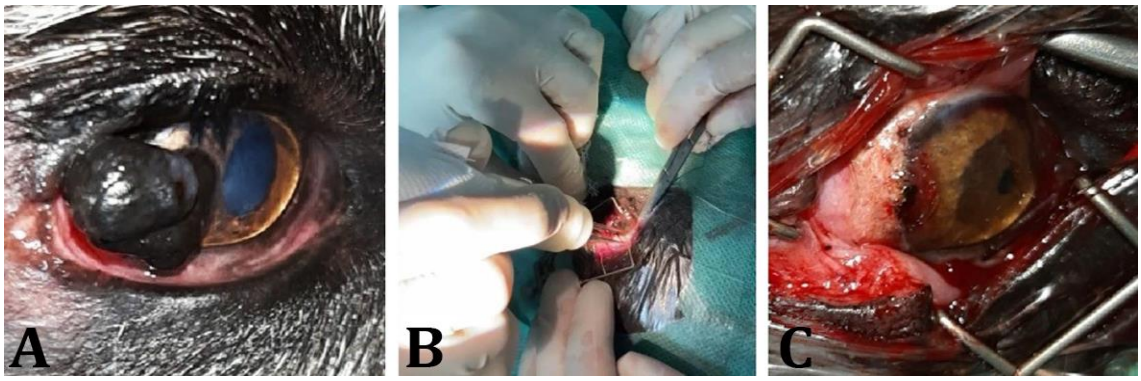

Fig. 1. A) Ocular melanoma before surgical resection associated with electrochemotherapy; **B)** Application of electrochemotherapy in the surgical site; **C)** Immediate post-surgical appearance.

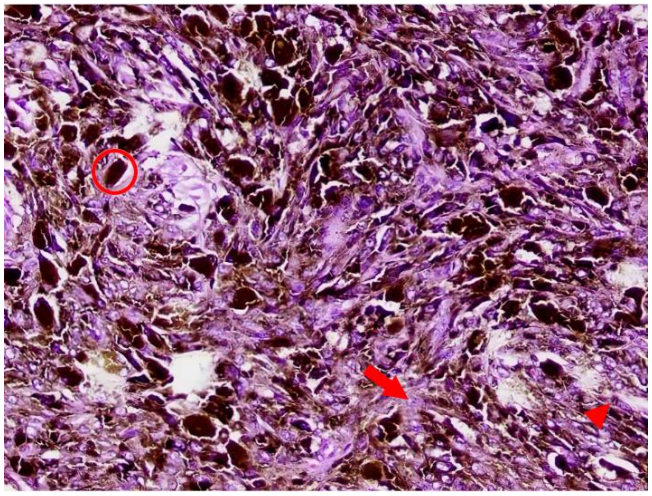

Fig. 2. Photomicrograph of a canine ocular melanoma indicating the presence of fusiform neoplastic cells with oval nuclei and moderate, eosinophilic, and distinct cytoplasm, being variably filled with coarse and black granulation (circle). Nuclei show moderate atypia (arrowhead; elongated nuclei) with chromatin being coarsely aggregated and condensed on the periphery of the nuclear membrane, having evident and central nucleoli (arrow). Binucleation figures are also observed. The presence of intense pigmentation makes it difficult to count mitoses, (Hematoxylin and Eosin staining, 20×).

The use of sodium hyaluronate and moxifloxacin was maintained until further return. Twenty-five days after the initial treatment, the patient presented granuloma formation and inflammatory epithelial hyperplasia at the surgical site (Fig. 3B), absence of corneal lesions on examination with fluorescein and intra-ocular pressure of 19.00 mmHg. Other intra-ocular structures remained within normal aspects. The use of anti-inflammatory eye drops based on fluorometholone acetate (Latinofarma, Cotia, Brazil) was instituted.

The patient underwent a new ophthalmological evaluation 45 days after the initial treatment, which revealed a > 90.00% reduction of the epithelial granuloma and the presence of conjunctivalization close to the treated site (Fig. 3C).

Furthermore, the inflammation was absent due to the use of anti-inflammatory eye drops, with a small opaque area in the cornea, without signs of tumor recurrence. Until further return, sodium hyaluronate-based lubricant was maintained.

Due to the low rate of metastasis of ocular tumors and the normalcy of regional lymph nodes, the use of systemic chemotherapy was not instituted. After 105 days (Fig. 3D), the conjunctivalization proved to be completely healed, and there were no signs of inflammation or tumor recurrence. The slight peripheral corneal opacification was still present, but it did not cause visual axis impairment.

The animal was followed for approximately 7 months and remained without evidence of tumor recurrence. A complete resolution of corneal opacification was also observed (Fig. 3E).

Discussion

The use of adjuvant ECT presented here proved to be effective, keeping the animal free of the disease without the need for ocular enucleation and maintaining the functionality of the affected eye. As this use of adjuvant ECT in the eye was a first approach, further studies are necessary; however, the reported case demonstrates its use as a possible therapeutic option in future cases of eye tumors in humans and animals.

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Conflict of interest

The authors declare no conflict of interest.

Fig. 3. A) Ten days after surgical treatment with adjuvant electrochemotherapy of the canine ocular melanoma. No signs of recurrence and presence of cicatricial granuloma are found; **B)** Presence of conjunctivalization 25 days after treatment. **C)** 45 days after the initial treatment of the canine ocular melanoma, there was a > 95.00% reduction in conjunctivalization and no signs of tumor recurrence were seen; **D)** 105 days after the initial treatment of the canine ocular melanoma, conjunctivalization was completely healed and there were no signs of inflammation or tumor recurrence. A slight peripheral corneal opacification was also observed. **E)** 215 days after the initial treatment of the canine ocular melanoma, no signs of inflammation or tumor recurrence were detected. The total resolution of the corneal opacification was also observed.

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