

Successful Rescue of an Apocrine Gland Carcinoma Metastatic to the Cervical Lymph Nodes by Mitoxantrone Coupled with Trains of Permeabilizing Electrical Pulses (Electrochemotherapy)

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Abstract. *Canine apocrine gland carcinoma is a locally aggressive neoplasm that can occasionally lead to metastatic spread, thus mimicking the behavior of their human counterpart. In this paper we describe the successful treatment of a cervical metastatic spread of this neoplasia by using mitoxantrone selectively driven within the tumor cells by trains of biphasic pulses. The dog experienced tumor reduction from the first cycle of electrochemotherapy (ECT) and complete remission by the time of its fourth session. Neither systemic or local toxicities were detected during the whole course of therapy. The dog is in complete remission after six months from his last treatment. Electrochemotherapy is a safe and efficacious therapy for metastatic carcinoma and warrants further investigation.*

Electrochemotherapy (ECT) is an anticancer treatment that involves the association of chemotherapy drugs with the delivery of permeabilizing electrical pulses, leading to improved local control with lack of systemic toxicities (1). In the past, our group has treated several cohorts of companion animals with spontaneous neoplasms by selectively driving anticancer agents by means of trains of biphasic pulses (2-13). To date, the use of ECT has been limited to the treatment of locoregional disease; however, there have been certain responses resulting in long-term

survival, observed in dogs with melanomas and cats with soft tissue sarcomas, which indicate that ECT could also play a role in the delay and even the prevention of distant metastases (4, 5).

Case Report

A-ten-year-old male intact husky dog was referred for two masses located in the right submandibular site and right prescapular area. The owner was unable to provide a time frame for the two growths. At presentation, the dog was bright, alert and responsive; a 5x4x2 cm mass was easily palpable on the right side of the mandible, while a 15x6x5 cm mass was located near the right shoulder, roughly in the area of the right prescapular lymph node. A fine-needle aspiration biopsy was performed and was suggestive of carcinoma, presumably of adnexial origin (data not shown).

At this time, several options were offered to the owner: surgery, surgery followed by systemic chemotherapy, surgery coupled with adjuvant electrochemotherapy (4-14). The owner elected the dog to be treated with the combination of surgery with ECT. The tumor was staged with a complete blood cell count (CBC), serum biochemical profile, urinalysis, chest radiographs (three projections) and abdominal ultrasonography. All the tests were within reference limits and the imaging studies showed no evidence of metastatic spread (data not shown).

Anesthesia was induced with a combination of medetomidine (DOMITOR; Pfizer-Italia, Pomezia Italy) and propofol (DIPRIVAN; Astra Zeneca, Milan, Italy) as per manufacturers' instructions followed by maintenance with isoflurane and the tumors were surgically marginally excised. At this time, the tumor bed was pretreated with a combination of hyaluronidase and lidocaine (LIDO-HYAL

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Figure 1. A) Microscopic appearance of the primary lesion at the time of surgical excision (haematoxylin/eosin; original magnification x40). B) M-mode ultrasonographic imaging of the cervical district of the dog showing lymph node enlargement following metastatic spread. C) Histopathological appearance of one cervical metastasis after three cycles of mitoxantrone-based ECT (haematoxylin/eosin; original magnification x40).

B; Laboratori Farmaceutici Giovanni Ogna & Figli S.p.A., Milan, Italy), to dissolve the ground substance and to increase local analgesia (5-10). Five minutes after the injection of hyaluronidase, the tumor and 1 cm of normally appearing margins were infiltrated with cisplatin (Platinex vial 50 mg/100 ml; Bristol-Myers Squibb, Sermoneta (LT), Italy) at a concentration of 0.5 mg/ml (total dose 15 mg). Five more minutes after the infiltration of the antineoplastic agent, trains of 8 biphasic electric pulses (EP) lasting 50 + 50 µs each, with 1 ms interpulse intervals, were delivered by means of needle electrodes (3). The dog recovered from the treatment and received a second session one week later, using caliper electrodes with the adherence of the electrodes being increased by using an electroconductive gel, as extensively described in the past (2-14).

The excised biopsy tumour specimens were fixed in 10% buffered-formalin and paraffin embedded. Sections of 5 µm were stained with haematoxylin-eosin, haematoxylin-van Gieson, and PAS-haematoxylin. The histopathology examination showed that the neoplasm was subdivided into lobules by fibrous trabeculae; the neoplastic cells had an eosinophilic cytoplasm and nuclei hyperchromatic with prominent nucleoli (Figure 1A). The histological characteristics of these tumors were consistent with carcinomas originating from the cutaneous apocrine glands.

The dog was scheduled for bi-monthly follow-up examinations and discharged. The dog remained in complete remission for one year. At that time, the dog returned following the discovery of cervical masses by the owner. Physical examination showed that there were several bilateral cervical nodules as well as a 3x2 cm submandibular mass. An ultrasonographic examination showed several cervical masses of approximately 3x3 cm in size (Figure 1B). Fine-needle aspiration was suggestive of metastatic spread (not shown).

Treatment options were mainly limited to systemic chemotherapy with anthracyclines or platinum compounds; however, with the aim of boosting drug efficacy it was

proposed to combine chemotherapy with electroporation (15). The dog was scheduled to receive, under sedation with medetomidine and propofol, systemic mitoxantrone (Novantrone 10 ml vial (2 mg/ml); Wyeth Lederle, Aprilia, Italy) at a dose of 6 mg/m² every three weeks, followed by the application of permeabilizing pulses. The dog had a CBC examination one week after each therapy. The dog tolerated the therapy without significant side-effects with the exception of one episode of neutropenia (700 neutrophils/µl) that required antibiotic therapy. The masses showed a remarkable reduction since the first therapy and by the end of the third cycle, only one cervical mass (2x2 cm) was still appreciable. The dog was scheduled to have the nodule excised together with its fourth dose of mitoxantrone. The node was excised and processed for histopathology. Compared with the first carcinoma, the tumor treated with the novel protocol showed a less aggressive phenotype with proliferation of multiple layers of epithelial cells lining a lumen; the neoplastic cells had hyperchromatic nuclei with little pleomorphism or mitotic activity. (Figure 1C). The recovery was uneventful and the dog was scheduled for bi-monthly follow-up consisting of a physical exam and thoracic radiographs. The patient has been in complete remission after six months from the completion of the rescue protocol and is followed-up on a three monthly basis.

Discussion

Apocrine gland carcinoma has been frequently described in veterinary medicine (14) and its treatment is based on surgical excision, eventually followed by adjuvant radiation therapy, especially for apocrine gland carcinomas of the anal sac. Chemotherapy has been confined to the treatment of aggressive apocrine gland carcinomas of the anal sac in dogs (15, 16) and its role can currently be considered as palliative (15). At present, the standard approach for metastatic carcinomas of apocrine origin is surgery (17-19),

however, the data are partially biased by the fact that, again, most of the patients had metastases originating from the anal sac gland (18).

To the best of our knowledge, this is the first report of successful eradication of metastatic spread by employing systemic chemotherapy plus electroporation; so far ECT has been confined to the palliation of metastatic spread of apocrine carcinoma of the anal sac in a cat that experienced a partial remission (2). The choice of mitoxantrone was made considering the lower toxicity of this agent compared to other anticancer agents, and on the basis of a previous successful report in a small cohort of human patients with breast carcinoma (15). The tolerability of this novel ECT protocol and the successful response observed in our patient with relapsing and advanced disease (T2, N1, M0) suggest a potential role of this therapy for the treatment of locoregional metastatic diffusion. Further studies are needed to assess the potential ECT control of metastatic cancer by further investigating this protocol.

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