Marginal Resection and Acridine Orange Photodynamic Therapy in a Cat with Recurrent Cutaneous Malignant Melanoma

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ABSTRACT

If a tumor cannot be completely resected, an adjunctive treatment modality is recommended to prevent local recurrence. One treatment option is intraoperative photodynamic therapy (PDT), which uses the reagent Acridine orange (AO). A 9-year-old domestic short-haired castrated male cat previously underwent surgical resection of a malignant melanoma at the left upper eyelid four times at another veterinary hospital. The tumor was resected with a 1 cm margin laterally, but the caudal margin contacted the cranial bone. AO-PDT was performed to control the residual tumor cells. At 9 months postoperatively, local recurrence was detected, and AO-PDT and surgical resection was repeated. At 16 months postoperatively, the cat died, but tumor recurrence was not detected at the original site. There were no adverse effects from the AO-PDT detected at the surgical site at any time during follow-up. This case report suggests that AO-PDT may be useful in combination with marginal resection of malignant tumors in cats, especially when radiotherapy is not performed.

INTRODUCTION

A recurring theme in the surgical management of cancer is that the first surgery has the best chance of achieving a cure.[4] If the tumor cannot be resected completely, then an adjunctive treatment modality is recommended to control the residual tumor cells after surgical resection. Radiation therapy is the treatment of choice for residual solid tumor cells,[3] but only select facilities can administer the treatment. Thus, photodynamic therapy (PDT) is a promising alternative in these cases.

Acridine orange (AO) is a reagent used in photodynamic therapy.[2, 10, 12-13, 23] This treatment has been successfully performed to treat superficial small tumors including mouse epithelial tumors[23] and rat gastric tumors.[22] To treat a large tumor or a tumor located in deep tissue that is unreachable by light, Kusuzaki et al. performed an intralesional or partial marginal tumor excision, followed by the administration of AO solution and xenon light irradiation, a type of photodynamic therapy, to control the residual tumor cells.[11]

In this case report, a cat with a frequently recurring melanoma on the upper eyelid underwent an incomplete tumor resection, which was then followed by intraoperative AO photodynamic therapy (AO-PDT), and achieved a prolonged progression-free interval.

Case

A 9-year-old domestic short-haired castrated male cat had a history of a recurrent mass at the left upper eyelid that was previously resected four times by a veterinarian. The mass was first resected 11 months prior to presentation and recurred three additional times; the cat underwent surgery a total four times, with the most recent surgery performed 1 month previously. The latest recurrence was detected 2 weeks prior to the initial examination. The tumor was diagnosed as a malignant melanoma, and the cat was brought to the Mommy Animal Hospital for evaluation of a recurrent mass at the left upper eyelid.

The mass was located at the left upper eyelid and measured 2.5 × 1.0 cm in diameter (Figure. 1). On the laboratory examination, the complete blood count and blood chemistry were within normal limits, and the FeLV and FIV tests were negative. Cytology of the mass was performed and showed anisokaryosis and nucleolar pleomorphism, which suggested recurrence of the melanoma.

The tumor was resected with a 1 cm margin laterally, and the left eye was removed. The ventral margin of the tumor was adjacent to the cranial bone; therefore, AO-PDT was performed at the tumor bed to control the residual neoplastic cells. AO (Acridine orange hydrochloride solution, 10 mg/mL in H2O; Sigma-Aldrich, St. Louis, MO, USA) was sterilized by microfiltration using a membrane filter (25AS020AS; Advantex MFS, Inc., Dublin, CA, USA). The surgical site was packed with gauze soaked in dilute AO solution (1 μg/ml in saline) for 5 minutes. Thereafter, the site was irradiated with xenon light (Xenon nova 175; Karl Storz Endoscopy Japan K. K., Tokyo, Japan) for 10 min at a 400–700 nm wavelength and a 20.7 (mW/cm2) power density 10 cm from the light source. The power density was measured using a spectroradiometer (USR-45DA-14, Ushio Inc., Tokyo, Japan).

The cat was discharged without any postoperative complications noted. Pathology of the resected mass confirmed a malignant melanoma (Figure. 2). A complete margin was achieved laterally, but the caudal margin was incomplete. Tumor cells

Figure 1: *The original tumor was located along the entire left upper eyelid. The medial half of the eyelid was irregular due to the previous multiple resections.*

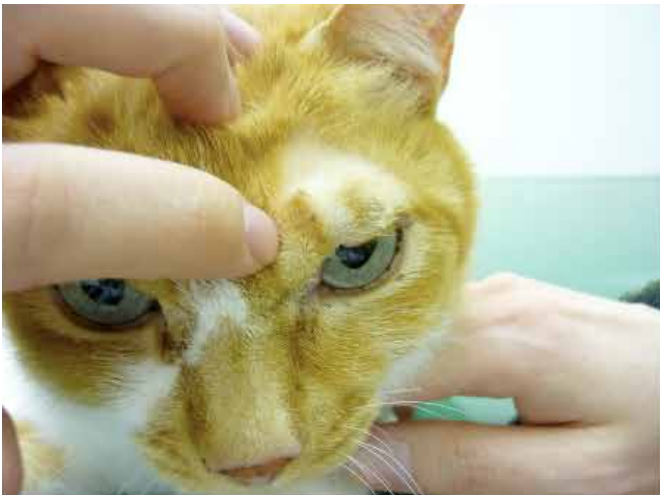
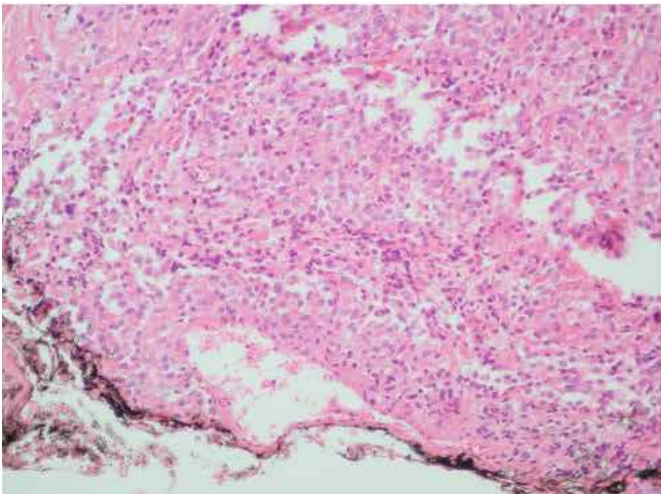


Figure 2: *Histopathology identified the mass as a malignant melanoma. The lateral margin was complete, but the caudal margin was incomplete. Tumor cells were observed invading lymphatic vessels. Mitotic figures were counted at 5–6 per 400× field.*



were observed invading into the lymphatic vessels, and mitotic figures numbered 5–6 per 400× field. Postoperatively, carboplatin chemotherapy was performed at 80–140 mg/m2/3 weeks for 13 months.

At 3 months postoperatively, the left submandibular lymph node was enlarged at 1.2 cm; a fine needle aspirate and cytology revealed melanoma. At 8 months postoperatively, the lymph node increased in size to 4.5 cm, and the cat was referred to the Veterinary Teaching Hospital of Azabu University. The lymph node was excised, AO-PDT was applied to the tumor bed, and the site was irradiated with 12 Gy of a 5 MeV electron beam with a 3 cm cone.

At 9 months postoperatively, tumor recurrence at the upper eyelid was detected. The masses were excised, and AO therapy was performed at the tumor bed. At 12 months postoperatively, multiple 1 cm subcutaneous masses were detected at the left upper lip and the rostral and caudal left ear. The masses were identified as metastases based on cytological evaluation. At 14 months postoperatively, metastases were distributed in the skin throughout the body. At 16 months, the cat died, but on necropsy, recurrence was not detected at the original tumor site. Throughout the entire treatment period, there were no adverse effects associated with the AO-PDT treatments.

DISCUSSION

The present case suggests two important findings. AO-PDT may be effective in reducing residual neoplastic cells following a tumor excision, indicated by the 9- and 7-month periods of tumor control following the incomplete excisions. Moreover, because the therapy does not require any special equipment, AO-PDT is a safe and easy method to perform in clinical veterinary practice.

As the present case demonstrates, AO-PDT may be effective in reducing local tumor recurrence. In general, a wide resection is required to prevent local tumor growth. However, in a study of human sarcoma patients, one group received both marginal surgery and AO-PDT to preserve function in all four limbs,[2, 11 16] while the second group underwent surgical resection with wide margins. The local recurrence rate was similar between both groups at 29% over a 10-year follow-up period.[16] In the current case, the tumor recurred four times and within 2 weeks of the most recent surgery at the initial presentation. Unsurprisingly, the tumor was in contact with the underlying tissue and could not be completely excised. AO-PDT was performed intraoperatively following a marginal resection, and after the AO-PDT, local tumor recurrence was controlled for approximately 9 months. The tumor did recur once more, and the resection and photodynamic therapy was repeated; the tumor was controlled at the site for 7 months until death of the patient. Thus, AO-PDT may be effective in reducing the residual tumor growth in cats as well as in humans.

AO-PDT is also a safe and easy method to perform clinically. The drug is believed to have only mild toxicity. The International Agency for Research on Cancer (IARC) of the World Health Organization considered AO as not classifiable as to its carcinogenicity to humans (class 3).[7] Several studies detail the usage of AO solution at surgical sites in humans[2, 11] and report no toxicity. When administered systemically, AO was safe at an oral dose of 500 mg in humans,[9] at an intravenous (IV) dose of 1 to 10 mg/kg in mice,[5, 15, 19-21] and at an IV dose of 0.1 mg/kg in dogs.[15] Similarly, there were no adverse effects during or after AO-PDT treatment in the present case. Based on the current and previous findings, local administration of a 1 mg/ml AO solution is safe and does not cause any adverse effects in cats.

Cancer cells preferentially utilize glycolytic rather than oxidative pathways to generate energy, producing lactic acid,[18] and as a result, tumor tissues become acidic.[6] AO is able to preferentially accumulate in malignant tumors, and with blue light excitation, it emits brilliant green fluorescence.[1, 8, 12-14, 21] AO accumulation in musculoskeletal tumors depends on the ΔpH between the intracellular and extracellular compartments, or between the intracellular and vacuolar pH.[17]

In the present case, the pH of the tumor tissue was not measured, nor was the emitted fluorescence measured after applying the AO solution onto the tumor tissue. Thus, whether AO accumulated within the residual tumor in this case is unknown. However, the pathology findings showed malignancy of the tissue. Therefore, we suspect that AO accumulated within the malignant tumor, and AO-PDT may in fact be an effective therapy.

In summary, the present case report demonstrates that AO-PDT may be a useful therapy following marginal resection of malignant tumors in cats, especially when radiotherapy is not possible. However, the indications for AO-PDT therapy are not precisely known in animals; therefore, additional case studies investigating this therapy are necessary.

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