Treatment of Eyelid and Corneoconjunctival Squamous Cell Carcinoma using Photodynamic Therapy

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Abstract

Squamous cell carcinoma is the most common tumor affecting the eye (cornea, conjunctiva, and nictitating membrane) and eyelids in the horse. Squamous cell carcinoma is a locally invasive neoplasia that is generally slow to metastasize. Breed predilections have been identified in Haflinger and Draft breeds, but any breed can be affected. Horses are more likely to be affected unilaterally, but bilateral involvement is not uncommon. There are a wide variety of treatment options available to treat squamous cell carcinoma. They include topical (5-fluoruracil, mitomycin C) and intralesional chemotherapy (cisplatin, carboplatin), immunotherapy (i.e., mycobacterium cell-wall extracts, whole cell bacilli Calmette-Guérin (BCG), and propionibacterial cell wall extracts), surgical debulking or excision followed by cryotherapy, hyperthermia, CO2 laser ablation, photodynamic therapy, radiotherapy, or brachytherapy. Treatment success and non-recurrence of the tumor are dependent upon the time of intervention, the treatment protocol selected, post-operative monitoring and immediate intervention if recurrence is suspected.

Surgical excision of the squamous cell carcinoma followed by infracyanine green (InfraCG, EmunDo®) or indocyanine green (ICG) has the advantages of being a one-time treatment and does not require the use of harmful chemotherapeutic agents.

Key words: equine, squamous cell carcinoma, photodynamic therapy, indocyanine/infracyanine green, eyelids, ocular

INTRODUCTION

Squamous cell carcinoma (SCC) is the most common tumor affecting the eye (cornea, conjunctiva, and nictitating membrane) and eyelids in the horse.[1,2] Squamous cell carcinoma is a locally invasive neoplasia that is generally slow to metastasize. Breed predilections have been identified in Haflinger, Appaloosa and Draft breeds, but any breed can be affected. Horses may be affected unilaterally or bilaterally, with the latter being less common. Several environmental factors are associated with an increased prevalence of SCC, and include increased longitude, decreased latitude, increased altitude, and increased mean annual solar radiation exposure.[3] Additionally, increased age, breed predilection, and horses with decreased skin pigmentation or light hair color are at higher risk of developing SCC.[4] The prognosis for eyelid SCC is worse than for corneal or conjunctival SCC. [4] Many treatment options are available to treat the various types of ocular and periocular (i.e., eyelid) SCC in the horse. Variable treatment responses are common, as is recurrence. Recurrent SCC is inherently more difficult to control, or eradicate. Thus, a combination of generous tissue resection of the mass in combination with aggressive adjunctive therapy is more likely to control the disease and prevent recurrence. Photodynamic therapy is an ideal form of adjunctive therapy, as the photosensitive agent can be injected or applied locally to the surgical excision site and eliminates the need for additional chemotherapeutic agents. Additionally, photodynamic therapy is generally effective following a single treatment.

DIFFERENTIAL DIAGNOSIS AND CONFIRMATION OF SQUAMOUS CELL CARCINOMA

Because SCC may present in a variety of clinical manifestations, it is should be suspected with any erosive, erythematous or raised ocular or adnexal lesion.[5] Differential diagnosis for SCC may include other neoplasia (papilloma, melanoma, mastocytoma, basal cell carcinoma, schwannoma, ademona and adenocarcinoma, hemangioma and hemangiosarcoma, lymphoma and lymphosarcoma), inflammatory lesions of the conjunctiva (lymphoid hyperplasia, follicular conjunctivitis) or eyelids (abscesses, granulation tissue, foreign body reaction, solar-induced lesions), and parasites (*Habronema*, *Onchocerca*, *Thelazia*).[6]

Squamous cell carcinoma is subdivided into four different categories based on its histologic appearance. **Plaque**, or carcinoma *in situ*, primarily involves proliferation within the stratum spinosum, but all layers of the epithelium may be proliferative. When underlying connective tissue invades hyperkeratotic epithelium, the SCC is referred to as **papillomatous**. Malignant transformation of the basilar layer of the epithelium and development of hyperchromatic nuclei, increased mitotic figures, pleomorphism and loss of polarity are present in **noninvasive SCC**. When neoplastic tissue with variable degrees of differntiation extends beyond the basal epithelium into the subepithelial tissue it is referred to as an **invasive SCC**. Keratin pearls (i.e., eosinophilic areas of keratinized foci), the formation of whorls, and intercellular bridges are used to characterize well-differentiated neoplasms. In contrast, neoplastic cells of poorly differentiated SCC form cords or nests, but have minima cellular keratinization.[6]

TREATMENT OF SQUAMOUS CELL CARCINOMA

There are a wide variety of treatment options available to treat SCC. Treatment option selection is based on factors such as size and location of the tumor, initial or recurrent presentation, and cost. Treatment options include topical application (5-fluoruoracil, piroxicam) or intralesional injection (cisplatin, carboplatin) of a chemotherapeutic agent for plaque or papillomatous and non-invasive eyelid SCC, respectively. Unless the tumors are relatively small or they are undergoing treatment for the first time, surgical excision paired with an adjunctive treatment option to target residual tumor cells, is recommended. The type of adjunctive therapy is generally based on tumor location, rates of non-recurrence, surgeon preference, and cost. Adjunctive treatment options that are frequently combined with surgical excision include cryotherapy, hyperthermia, CO^2-laser ablation, strontium (^{90}Sr) beta irradiation, and photodynamic therapy.[4,6]
Brachytherapy is a treatment option that relies on the placement of radioactive sources within and around the neoplasm. These sources are left in place for short periods of time while the horse is isolated in specially designed lead-lined stalls. Brachytherapy has a very high non-recurrence rate, however, the high costs, licensure requirements, limited availability, and risks of radiation exposure to humans has severely limited its use.[6]

PRINCIPLES OF PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) relies on specific photochemical reactions resulting from the interactions of light, photosensitive agents and oxygen. [7] There is an increased uptake and retention of the photosensitive agent within the target (i.e., neoplastic) and

microvascular endothelial. Selective irradiation of this area using a specific wavelength of light (coinciding with the specific photosensitive agent) leads to the initiation of necrosis and apoptosis of the target cells, vascular shutdown, and inflammation via the formation of toxic singlet oxygen and free radicals.[3,7] The selective treatment effect associated with PDT occurs due to the selective retention of the photosensitive agent by neoplastic cells and the targeted delivery of light to a highly specific area.[3,7] Photosensitive agents are typically administered via intravenous (IV) injection in humans. However, the IV route of administration is not currently seen as feasible in the horse. Periocular neoplasia have been successfully treated via local PDT.[8-10]

PROGNOSIS

Recurrence rates for surgical excision alone have been reported to range between 50% - 66.7% within a year of treatment, and were between 25% - 67% with ancillary irradiation or cryotherapy.[11,12] The recurrence rate following surgical excision of ocular SCC followed by radiofrequency hyperthermia has been reported to be 42.4%. [13] Dugan, et al. (1991) reported overall recurrence rates of 30.4% for surgical excision alone, or followed by ^{90}Sr beta irradiation, cryotherapy, radiofrequency, ^{137}cesium interstitial radiofrequency, and or immunotherapy.[4] In the same study, the prognosis was found to be poorer for SCC of the eyelids compared to SCC of the third eyelid, nasal canthus, or limbus.[4] Additional factors negatively influencing the prognosis and resulting in lower survival times were larger sized tumors, orbital extension, and recurrent SCC.[4] Metastasis can be most commonly detected within the submandibular lymph nodes, salivary glands, and thorax, and as an expansion of the primary tumor into the orbit,

sinus, or calvarium. Local tumor invasion results in significant ocular discomfort accompanied by ulcerative necrosis and inflammation.[6] Long-term survival, ocular health, and visual outcomes may be affected by either the SCC itself, or complications associated with the specific mode of treatment.[6]

INFRACYANINE GREEN (EMUNDO®) AND INDOCYANINE GREEN BASED PHOTODYNAMIC THERAPY

Infracyanine green (EmunDo®, A.R.C. Laser, GmbH, Nürnberg, Germany) or indocyanine green are photosensitive agents with an absorptive spectrum of between 800-830 nm**.[14] To** achieve activation of the photosensitive agent, diffuse infrared laser energy is applied via a diode laser (Fox, A.R.C. Laser) using a light-diffusing handpiece attached to a 300 micrometer diameter laser fiber. The laser energy protocols for corneoconjunctival and eyelid SCC differ significantly.

SEDATION AND LOCAL ANALGESIA AND AKINESIA

Surgical excision of corneoconjunctival or eyelid SCC in horses at Auburn University were performed under standing sedation and local anesthesia (eyelid and retrobulbar blocks). Sedation was achieved using an intravenous injection of detomidine hydrochloride (0.01-0.02 mg/kg/IV; Dormosedan®, Zoetis US, Parsippany, NJ) and butorphanol tartrate (0.01-0.02 mg/kg/IV; Torbugesic®, Zoetis US, Parsippany, NJ) in combination with an intramuscular depot injection of butorphanol tartrate (0.02-0.04 mg/kg/IM) to minimize involuntary head movements. Frontal and auriculopalpebral eyelid blocks were achieved via 1 ml and 1.5 ml, respectively, subcutaneous (SC)

injections of 2% mepivacaine (Carbocaine®, Zoetis US, Parsippany, NJ) using a 3 ml syringe and a 26 gauge needle. Perilesional line blocks were performed as needed using a variable amount of 2% mepivacaine SC.[15]

Retrobulbar nerve blocks were performed, following clipping and aseptical preparation of the skin in the orbital fossa, as previously described, in any horse undergoing surgical excision of a corneoconjunctival SCC and in individual cases of horses with eyelid SCC.[15] A total volume of 10 ml of 2% mepivacaine was injected into the retrobulbar cone through a 22-gauge, 2.5 inch spinal catheter needle (BD, Franklin Lakes, NJ) through the skin in the orbital fossa.[15]

CORNEOCONJUNCTIVAL OR LIMBAL SQUAMOUS CELL CARCINOMA

Following complete surgical excision of the corneoconjunctival SCC vis superficial lamellar keratectomy and conjunctivectomy, 1% EmunDo®, reconstituted using 1 ml of demineralized water injected into the vial containing the lyophilized infracyanine green vial, is applied to the surface of the exposed stroma and sclera via a 30 gauge needle/1 ml syringe combination with the needle broken off. A digital infrared image of the surgical site is taken using a Nikon Z6 full frame mirrorless camera converted for full-spectrum photography to ensure confluency of the dye.[16] Due to the absorption of infrared light, the dye appears black in the images. Following application of the dye, diffuse infrared light is applied to the surface of the cornea via the diode laser and light diffusing handpiece. The light is applied in 30 second cycles using a meandering pattern of application until between 75J (one quadrant) and 300J (entire cornea, four quadrants) of energy are delivered to the dye applied to the surgical site.

PERIOCULAR (EYELID) SQUAMOUS CELL CARCINOMA

Complete excision with clear (i.e., lack of identifiable neoplastic cells) surgical tissue margins is key to achieving non-recurrence in horses with SCC. The lack of excess periorbital skin and the necessity to maintain a functional postoperative eyelid margin decreases the likelihood of preserving functional vision, or even the globe, in cases of advanced SCC progression.[17]

Surgical excision of the SCC followed by the application of topical EmunDo® or 2% compounded indocyanine green (ICG) and subsequent 2-phase treatment results in a tissue scaffold that allows for accelerated healing of the surgical wounds while minimizing the formation of scar tissue postoperatively. Additionally, even when the eyelid margin must be removed due to invasion of neoplastic tissue, this postoperative scaffold coupled with fusion of the palpebral conjunctiva to the leading edge of the surgical skin excision, results in a functional postoperative eyelid margin. This minimizes secondary corneal complications and maintains a good level of postoperative comfort which allows the surgical site to heal in an uncomplicated manner.

To achieve the above result, eyelid SCC are treated in two phases. First, the neoplastic lesion is excised with clear visible margins (2-5 mm) followed by the topical application of the infracyanine- or indocyanine green dye to the surgical wound bed. Additionally, the dye is injected into the cut edges of the skin incision to allow for treatment of the tissue immediately surrounding the excision. Following application of the dye, diffuse light energy (500 mW) is applied as described above, via a diode laser (810 nm) with a light-diffusing handpiece attached to a 300 micrometer diameter laser fiber. The energy is

applied in 30s intervals at a rate of 75J-90J/cm^2. Second, dye is reapplied topically and the laser energy level is increased to 2.5W-3W. Activation of the dye using this level of energy leads to coagulation and vaporization of deeper tissue and carbonization (visible charring) of the surface. The tissue is treated to effect until visible tissue charring has occurred, with applied energy levels reaching a two- to three-fold increase, from the initial application based on the size and depth of the lesion (150J-300J/cm^2).

Post-operative treatment for both corneoconjunctival and eyelid/periocular SCC is limited to the topical application of a broad-spectrum antibiotic ophthalmic ointment and systemic application of flunixin-meglumine (1.1 mg/kg per os q12h)(Banamine®, Merck Animal Health, Madison, NJ) at a tapering dose over the course of 7-21 days, for eyelid or corneoconjunctival SCC, respectively.

SUMMARY

Photodynamic therapy using infra- or indocyanine green photodynamic therapy for the treatment of corneoconjunctival or eyelid SCC is may allow us to achieve non-recurrence using single treatment applications without the need for chemotherapeutic drugs or the application of radiation, thus minimizing the risks for both the horses, and the humans handling and treating them.

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