TREATMENT OF A SQUAMOUS CELL CARCINOMA IN A SULPHUR CRESTED COCKATOO (*CACATUA GALERITA*) WITH THE EXPERIMENTAL DRUG EBC-46

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INTRODUCTION

Squamous cell carcinomas (SCC) are uncommon in psittacine birds (Leach, 1992; Pye et al., 1999). Carboplatin therapy has been the drug of choice for therapy (Filippich et al., 2005) and is very effective when injected intralesionally, often resulting in complete resolution of the neoplasm (Filippich, pers comm). Therapy is often used to reduce the size of a lesion prior to surgical excision. In this case, surgical excision of the lesion was not possible due to its location and size (on the dorsum of the pygostyle). Another factor limiting surgical excision of the pygostyle was that the patient had very little use of the legs due to previous healed fractures and was reliant on its tail for balance and perambulation. Thus carboplatin was injected intralesionally, mixed 50/50 with sesame oil, at three weekly intervals for six cycles. Further treatment options were pursued after recurrence of the neoplasm,m and it was elected to trial therapy with an experimental anti-cancer agent EBC-46. This drug shows great promise for the local treatment of solid tumours in companion animals.

CASE REPORT

A 45 year old female sulphur crested cockatoo was found as a juvenile with a fractured left leg and unable to fly (Fig. 1). The bird has had a dysfunctional left leg since that time. In 2004, the right leg fractured at the distal tibiotarsus and healed with the distal fragment and tarsus laterally deviated, thus preventing perching. Radiographs at this time showed that the bird had numerous shotgun pellets lodged within the body, one being responsible for the permanently damaged left leg. A wedge osteotomy and type II fixature failed to restore full function to the right leg. This has resulted in the bird being unable to perch, relying greatly on the tail for balance. The bird has been on daily Metacam (Boehringer Ingelheim - 0.2 mg/kg q24h) since 2006.

The bird presented on the 21st August 2009 with a region of crusted excoriation 25 x 10 mm over the dorsal pygostyle, incorporating the central rectrices (Fig. 2). The owner had recently observed blood around the tail and on the beak but was unsure how long the lesion had been present. The feathers surrounding the lesion were blood stained and ungroomed. Initially the lesion was thought to be due to self mutilation, due to the fact that the bird was not mobile and now sat on a platform that the owners had constructed, and from which it could not leave. Enrichment was instituted with a foraging tray for pellets, another for a few

sunflower seeds daily and a baffle cage for fruit and vegetables. A revisit was scheduled for 4 weeks to assess any improvement. On 25th August the clients reported that the bird had accepted the baffle cage and the food-based enrichment. A revisit on the 28th August showed no change in the lesion (Fig. 3) and two punch biopsies were performed under masked induction with isoflurane anaesthesia.

Histology*: There was thickening and dysplasia of the epithelium with occasional infiltrating cords of neoplastic squamous epithelium. Areas showed mixed mononuclear cell inflammation and small numbers of eosinophils. Mitotic figures were not seen. Multiple fragments of pale tissue consisting of large amounts of irregularly arranged keratin contain aggregates of bacteria and small numbers of degenerated inflammatory cells. Squamous cell carcinomas of the skin are locally aggressive and frequently reoccur following inadequate excision. They tend to grow slowly and may become large if not treated. They can arise anywhere in the skin.*

Figure 1. The bird on its platform

It was elected to treat the bird with Carboplatin intralesionally at 6.5 mg (10 mg/kg) mixed 50/50 with sesame oil for 6 cycles at three weekly intervals. It is thought that by incorporating the drug in an oily suspension it remains at the site of injection for longer before entering the systemic circulation and so the local concentration persists longer. Treatment was commenced on the 7TH October and completed on the 21st January 2010. A complete blood count (CBC) was performed before each treatment to assess myelosuppression. The CBC parameters remained with normal limits during treatment. The lesion had reduced in size and was crusted but the bird still appeared irritated by the lesion and periodically cleaned/irritated the area with its beak (Fig. 4). Two biopsies were taken and forwarded for histopathology to assess the treatment efficacy.

Report: *Beneath the hyperplastic epidermis, the dermis is infiltrated by mild fibrovascular hyperplasia with an accompanying mild infiltrate of mainly heterophils and lymphocytes.*
This is a fibrosing dermatitis. There is no evidence of squamous cell carcinoma in these sections. The fibrosing lesion may be secondary to the previous squamous cell carcinoma or to the effects of chemotherapy.

A revisit was scheduled for six months to reassess the lesion. When the bird presented on the 4th April 2010 the lesion was crusted and excoriated but larger (25 x 20 mm), with a hyperkeratotic nodule between the rectrices (~ 6 x 8 mm) (Fig. 5). General anaesthesia was performed with masked induction with isoflurane. Two punch biopsies were obtained and there were forwarded for histopathology.

Report: *Beneath the crusted and hyperplastic epithelium, the dermis is infiltrated by variably sized*

trabeculae and islands of pleomorphic squamous epithelial cells with prominent intercellular bridges and individual cell keratinisation. Additional changes include multifocal fibroplasia and secondary inflammation. This is a cutaneous squamous cell carcinoma. This indicates recurrence of the previously diagnosed squamous cell carcinoma. Squamous cell carcinomas are locally infiltrative, may recur and are relatively slow to metastasise.

Surgery (pygostyle amputation) was not considered as a treatment option due to the bird's functional disability and its reliance on the tail for balance and support. A second series of intralesional carboplatin therapy was considered, perhaps in a surfactant base to facilitate a longer presence in the local tissue. Another option was to trial EBC-46. This is an experimental anti cancer drug produced from a North Queensland rainforest tree. EBC-46 has been used by injectable and topical application. The owners opted for a treatment trial with EBC-46 and this was commenced by injectable therapy on the 29th June, again under isoflurane anaesthesia. A conservative dose rate was calculated allometrically from known effective doses in mammals (0.41mg/kg) (Reddell, pers comm.) as this was the first trial in an avian case. The drug was injected into the lesion and its periphery. The drug was released whilst slowly withdrawing the 25g needle. Within three hours the lesion and the surrounding tissue appeared darkened and poorly perfused. Within 24 hours the lesion had reduced in size and was dry and crusted (Fig. 6). These observations are consistent with initial responses to EBC-46 that have been observed in mammals. This may have been due to the therapy or an absence of self mutilation whilst in the hospital. The periphery of the lesion appeared firm and there was minor oedema of the pygostyle. The patient remained clinically normal after this therapy.

One week later, the lesion had reduced in size to 25 x 15 mm and the surrounding border appeared less raised and reactive. The lesion was essentially dry. The response to therapy was not as great as that seen in mammalian cases (Reddell, pers comm.) A second application of EBC-46 was applied topically at this time.

At a revisit one week later the owners advised that the bird was vocalising more during the day and appeared more content than for some time, but was still self mutilating the wound. The lesion appeared unchanged in size and was centrally excoriated.

Two weeks later, haemorrhage was noticed by the owners and repeat biopsies performed two days later (29th July 2007). Histology showed that there had been no response to the EBC-46 therapy (Fig. 7).

Report: *Two sections of skin/stratified squamous mucosa were examined. Each revealed extensively ulcerated and reveal numerous, anastomosing aggregates of moderately pleomorphic ovoid to polyhedral cells which demonstrated individual cell keratinisation as well as occasional keratin pearl formation. The cells have moderate amounts of amphophilic cytoplasm and moderately sized, round to ovoid nuclei with vesicular chromatin and typically single prominent nucleoli. Occasional mitoses are apparent within the cell population. Intervening between the cellular aggregates are accumulations of maturing fibrovascular tissue. Also evident are extensive aggregates of assorted inflammatory cells. The sections reveal a squamous cell carcinoma in keeping with the preceding biopsy.*

DISCUSSION

Carboplatin therapy has been used traditionally for the treatment of squamous cell carcinoma in birds (Pye et al., 1999; Filippich et al., 2005). It was selected to treat this patient with intralesional

carboplatin in sesame oil to reduce the systemic effects of chemotherapy and to achieve a persistent local drug concentration (Filippich per comm., 2009). The failure of the tumour to respond to carboplatin and the absence of a surgical option for excising the tumour led to a trial therapy with the experimental anticancer drug EBC-46.

EBC-46 is a small molecule derived from a North Queensland rainforest tree. It is a new class of drug which destroys tumours by locally activating the innate immune system via protein kinase C (PKC). Because of this mode of action, EBC-46 potentially has broad efficacy against a wide range of tumour types. Research to date at the Queensland Institute of Medical Research (Parsons, pers comm.) indicates that EBC-46 works in mammals by activation of three PKC-dependent signal pathways which act to:

1. cause rapid degranulation of tumour-associated neutrophils, resulting in localised release of reactive oxygen and proteases, which disrupt and destroy tumour cells in the immediate vicinity (Houghton 2010);

2. initiate, in the lesion, a secondary signalling cascade (involving cytokines and chemokines) that activates adhesion receptors (especially intracellular adhesion molecules) on the walls of the tumour vasculature near to the sites of injection. These adhesion receptors attract the rolling neutrophils in the bloodstream which then extravasate across the vasculature wall and degranulate. This degranulation, plus damage to the vascular walls associated with the intensity of extravasation, result in highly localised hypoxia and haemorrhagic necrosis of the lesion, usually evident within 24 hours of treatment. In essence, the drug causes a localised neutrophil/heterophil 'storm' in the lesion; and

3. cause, in and surrounding the tumour, a localised inflammatory response which results in further leucocyte infiltration and activation of neutrophils present in that infiltrate.

Depending on the nature of individual tumours (e.g. whether it has a significant pre-treatment leucocyte infiltrate), one or more of the above mechanisms are involved in tumour destruction. There is also evidence of a highly localized primary necrosis (non PKC-dependent) in tumour cells caused by EBC-46. This occurs only in the immediate vicinity (± 0.2cm) of the site(s) of injection and is due to disruption of tumour cell plasma membranes, a rapid loss of mitochondrial membrane potential and mitochondrial swelling.

Chicken heterophils have been found to respond to the same PKC-dependent chemical signals as neutrophils (Farnall et al., 2003) so it is highly likely that EBC-46 would have the same effect on heterophils as neutrophils.

Administration of EBC-46 is by a single dose given intralesionally or by topical application of a gel for thin ulcerative lesions (Reddell, per comm. 2010). The drug acts very rapidly, with the outcomes of the treatment in tumour ablation evident within five to 15 days. There have not been any significant side effects in any patients.

Both formulations of EBC-46 have been used on this patient. The injectable was formulated in 30% propylene glycol in saline as the excipient, while the topical gel was in isopropanol and Carbomer 940 (for use on thin ulcerative squamous cell carcinomas as tissue permeation is limited to ~ 5mm).

CONCLUSION

Carboplatin has been the most appropriate therapy for squamous cell carcinomas in psittacine birds and should result in a significant reduction in tumour size or complete resolution of the neoplasm. A factor that may have contributed to the poor response in this case was the relatively large size of the lesion and failure to effectively infiltrate the entire lesion with the chemotherapeutic agent. Additionally, the difficulty in mixing the small volume of carboplatin with the sesame oil to create the suspension may have reduced its efficacy. This would have resulted in a reduced concentration of carboplatin at the injection site.

There appeared to be some initial response to the EBC-46 therapy consistent with its known mode of action and effects in mammals but it is also possible that because the bird was hospitalised after treatment, this influenced its behaviour and reduced self mutilation. This case is ongoing and it is planned to administer EBC-46 at a higher dose rate to that previously used.

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Figure 2: The lesion at first presentation

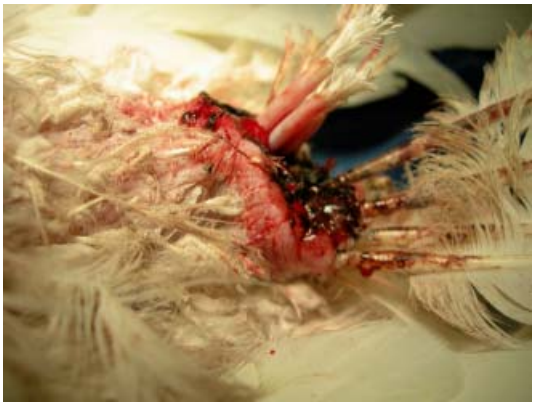

Figure 3: Untreated lesion at first biopsy prior to treatment

Figure 4: Tumour after six cycles of carboplatin

Figure 5: Tumour before EBC-46 therapy

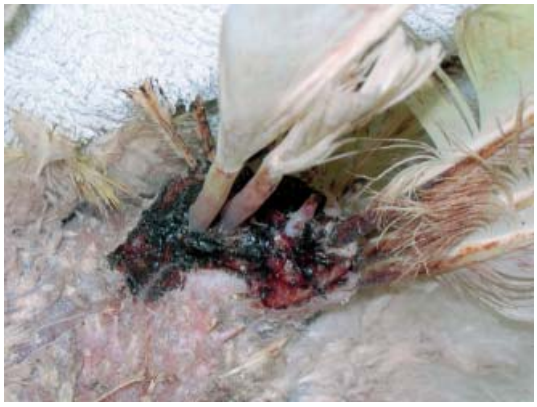

Figure 6: Lesion 24h after EBC-46 therapy

Figure 7: Biopsy on 29th July 2010