CASE REPORT

Surgical management of an odontogenic tumor in a banded Gila monster (Heloderma suspectum cinctum) with a novel herpesvirus

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An adult, 817 g, male, wild-caught banded Gila monster (Heloderma suspectum cinctum) was evaluated during a quarantine examination. The lizard was obtained from the Arizona Game and Fish Department, after capture as a ‘nuisance’ (Sullivan et al. 2004). Physical examination revealed that the Gila monster was mildly underweight and probably mature based on the presence of bilateral nuclear sclerosis and incipient cataracts. An approximately 4 x 3 mm, broad based, light tan gingival nodule with an irregular surface and unknown depth was identified on the lingual aspect of the cranial right mandible. There was also marked bilateral and symmetric temporal muscle atrophy, of unknown significance. A heparinized whole blood sample was collected, from the ventral coccygeal vein, and submitted for hematologic and biochemical profiles. Blood values were compared to species specific reference ranges from two sources (Cooper-Bailey et al. 2011; International Species Information System 2013). While the majority of values were considered within range per International Species Information System (ISIS), significant hematologic findings according to the study by Cooper-Bailey et al. included a mild leukocytosis (10.0 x 10^9/L) with a mild basophilia (1.6 x 10^9/L) and moderate azurophilia (2.5 x 10^9/L). The biochemical profile was considered unremarkable for the species.

At recheck, four months later, the intraoral mass was larger (8 x 5 mm) (Figure 1(A)). After topical application of 2% lidocaine1 at 1 mg/kg BW, a fine-needle aspirate of the mass was performed using a 22-gauge hypodermic needle and submitted for cytologic examination. The sample was poorly cellular, but revealed mild atypia of squamous epithelial cells, suggestive of either hyperplasia or squamous cell carcinoma. Repeat bloodwork showed an increasing leukocytosis (13.1 x 10^9/L) with a mild basophilia (1.9 x 10^9/L) and mild azurophilia (1.5 x 10^9/L) according to ISIS ranges, but increased in all cell lines according to Cooper-Bailey et al. Values appeared most consistent with an inflammatory leukogram.

Six weeks after initial evaluation, the Gila monster was sedated with midazolam2 at 0.25 mg/kg BW IM and hydromorphone3 at 0.1 mg/kg BW IM to collect an incisional biopsy. Topical 2% lidocaine4 at 2 mg/kg BW was infiltrated around the mass for analgesia. A 5 x 5 x 3 mm biopsy was collected from the surface of the mass using curved iris scissors. A portion of the mass was placed in formalin and submitted for histopathologic evaluation. The remaining section was saved frozen for later molecular analysis, utilizing polymerase chain reaction (PCR). A hemostatic sponge, Gelfoam5,6 and topical epinephrine5 at 0.01 mg/kg BW were applied to the biopsy site to control bleeding. Flumazenil5 at 0.01 mg/kg BW IM was administered to reverse the effects of midazolam2.

Histologic findings included anastomosing epithelial cords extending from the gingiva and were considered most consistent with squamous cell carcinoma, with neoplastic cells extending to the sample margins. Given the progression of the mass and the histologic diagnosis, excision with wide margins was elected.

A pre-operative, whole body, computed tomography (CT) scan was performed under the same sedation protocol as described above (Figure 2). Findings, considered to be incidental, included three round to angular, well-defined mineral attenuating structures at the caudal aspect of the right shoulder, measuring up to 3 mm in length (consistent with scapulohumeral osteochondromas) and a focal interruption of the last right lumbar rib (likely an anatomic variation). A single right mandibular tooth appeared to be laterally displaced; while not identified on CT, this appeared to coincide with the mass. No bony changes were appreciated in the right mandible; however, the images were low resolution due to patient size. Dental radiographs may have offered better detail in this case. Five months...
after original examination, a right segmental mandibulectomy was performed. The Gila monster was sedated with ketamine at 2.4 mg/kg BW IM, midazolam at 0.24 mg/kg BW IM, and hydromorphone at 0.12 mg/kg BW IM. Intubation was performed with a size 2.0, uncuffed, endotracheal tube and anesthesia was maintained on isoflurane in oxygen and nitrous oxide (50%). An elliptical incision was made around the soft tissue mass, including 5 mm of grossly normal tissue. Electrocautery was used for hemostasis and soft tissues were dissected to expose the body of the mandible. Rongeurs were used to resect the mandible (approximately 2 cm) cranial and caudal to the mass and provide gross margins. This was followed by excision of all visible glandular tissue in the area. The wound was lavaged with sterile saline and a small piece of hemostatic sponge (Gelfoam) was placed in the wound prior to closure of the submucosa (3-0 polydioxanone; simple interrupted pattern) and mucosa (3-0 polyglactin 910; cruciate pattern). Flumazenil (0.01 mg/kg BW IM) was given once isoflurane was discontinued.

A portion of the excised tissue, measuring 1.1 × 0.9 × 0.2 cm, was placed in formalin and submitted for histopathologic examination (Figure 3). Histologically, there was an exophytic mass contiguous with the stratum intermedium of an adjacent developing tooth. The mass was composed of anastomosing cords of epithelial cells with palisading, often columnar cells on the periphery and spindloid cells within the center and occasional rests of Malassez or Serres on a loosely fibrillar, collagenous stroma. Frequently, within the mass, there were round structures exhibiting a variably thick ring of homogeneous, intensely eosinophilic and birefringent material, which was occasionally mineralized (predentin and dentin). Within the rings, there was a poorly cellular, loose, and stellate matrix. Rarely, sandwiched between the ring and the stellate matrix was a second row of palisading, columnar cells. The ring centers were also occasionally necrotic. The mitotic index was 5 per ten 400x fields. Minimal inflammation was present. Histologic findings were consistent with a tumor of odontogenic origin.

Post-operative care included subcutaneous lactated Ringer’s solution at 12 ml/kg BW, hydromorphone at 0.1 mg/kg BW IM once daily for three doses, then every other day for two doses, meloxicam at 0.15 mg/kg BW IM every other day for six doses, and ceftazidime at 21 mg/kg BW IM every 72 hours for five doses. The Gila monster was discharged one week following surgery. To reduce the risk of post-operative dehiscence or infection, food was withheld for two weeks. Recheck examination and suture removal were performed three weeks after surgery without complication. At recheck five months later, the Gila monster was acting normal and there was no evidence of tumor recurrence (Figure 1B).

Following surgery, DNA was extracted from the original tissue biopsy using a Maxwell 16 automated...
A nested PCR amplification was performed using a previously described protocol for the DNA-dependent DNA polymerase gene found in herpesviruses, as previously described (VanDevanter et al. 1996). The second round of amplification was modified to obtain longer sequence, using DFA as the forward primer instead of the TGV primer as previously described. The PCR amplicon was resolved in 1% agarose gels, excised and purified using a commercial kit. The PCR amplicon was then sequenced via the Sanger method. Amplicons were sequenced twice in each direction. After editing out primers, a 487 base pair segment of herpesviral DNA-dependent DNA polymerase was amplified. The sequence was submitted to GenBank under accession number KT000388. Using the guidelines set by the International Committee on the Taxonomy of Viruses, this herpesvirus is hereafter referred to as Helodermatid herpesvirus 1 (HeHV1) (Pellett et al. 2011).

Amino acid sequences from the herpesviral DNA-dependent DNA polymerase gene were aligned using MAFFT (Katoh & Toh 2008). Partial homologous amino acid sequences, for which full-length sequences were not available, were included with ambiguities added for unknown amino acids (Yonkers et al. 2015). Bayesian analyses of amino acid alignments were performed using MrBayes 3.1.2 on the CIPRES server, with gamma distributed rate variation and a proportion of invariant sites (Ronquist & Huelsenbeck 2003; Miller et al. 2010). Amino acid substitution models were selected using ProtTest (Abascal et al. 2005), and it was found that the LG model with gamma distribution and a proportion of invariant sites was the best model, followed by the WAG model (Whelan & Goldman 2001). LG is not implemented in Mr Bayes, so the WAG model was used for the Bayesian analysis. The first 25% of 2,000,000 iterations were discarded from the beginning of the chain as a burn-in. Maximum likelihood (ML) bootstrap analyses of each alignment were performed using RAxML on the CIPRES server with gamma distributed rate variation, a proportion of invariant sites, and the LG model (Stamatakis et al. 2008; Miller et al. 2010). Bootstrap analysis was used to test the strength of the tree topology, with 1000 subsets. The Bayesian phylogenetic tree of the herpesviruses is displayed in Figure 4. This virus clusters within the subfamily Alphaherpesvirinae, forming a clade with Iguanid herpesvirus 2 and Gerrhosaurid herpesviruses that is well supported with 98.3% posterior probability and 67.4% ML bootstrap support. Branch lengths in this clade are consistent with a possible novel genus.

Odontogenic tumors are benign, but locally aggressive (Head et al. 2003). Tumors of odontogenic epithelium include ameloblastomas and odontomas,

Figure 3. (A) Proliferative gingival tissue from the mandible. H&E 100× bar = 50 µm. Odontogenic epithelium (OE) arranged in characteristic anastomosing cords invading the gingival epithelium and gingival stroma. (B) Higher magnification of proliferative gingival tissue from the mandible in (A). H&E 200× bar = 30 µm. OE surrounding loosely arranged stellate reticulum and a center of predentin (PD). (C) Proliferative gingival tissue from the mandible. H&E 100× bar = 50 µm. Normal tooth structure in the upper portion of the photomicrograph with a center of stellate reticulum (SR) surrounded a single layer of ameloblasts (A) with the location of the nucleus at the apical pole. A cap of dentin (D) and enamel (E) overlays the ameloblasts. (D) Higher magnification of proliferative gingival tissue from the mandible in (C). H&E 200× bar = 30 µm. OE surrounding loosely arranged SR and a center of PD.
Ameloblastomas are formed strictly from the enamel organ, whereas odontomas produce the full gamut of tooth structures including enamel (from the epithelium) and dentin (from the ectomesenchyme). Ameloblastomas are reported in the veterinary literature, in dogs, cats, horses, cattle, rats, a llama, an alpaca, a black rat snake, and several species of fish (Britt et al. 2005; Grim et al. 2009; Comolli et al. 2015). Given the local damage these tumors can inflict and their propensity to recur if not adequately treated, the current recommendation is surgical resection (Sham et al. 2009). Differentiation of tumor type from surgical biopsies is sometimes difficult; however, fine differentiation is not always necessary for appropriate management. In a case of mandibular ameloblastoma in a mare, initial biopsies were suggestive of a sarcoma and the diagnosis was not made until post mortem examination (Kutzer et al. 2007). Similarly, evaluation of an aspirate and incisional biopsy in the present case was more consistent with squamous cell carcinoma. Incisional biopsies are not always accurate and cases may require examination of multiple sections from different areas within the tumor for diagnosis (Gomes et al. 2010). In the case of odontogenic tumors, however, the progression and management are similar for most variations.

Oral neoplasms described in lizards include squamous cell carcinoma, fibrosarcoma, spindle cell sarcoma, chondroma, and papilloma (Hernandez-Divers & Garner 2003; Garner et al. 2004). The significance of the herpesvirus identified in this case is unknown; however, it should be noted that herpesviruses have been identified in cases of stomatitis and oral squamous cell carcinoma in lizard species (Wellehan et al. 2004, 2005). Herpesvirus-like particles have been found in venom glands of snakes, and the venom glands of...
Gila monsters are located adjacent to the tumor site reported in this case (Simpson et al. 1979). Association with human papillomavirus has been reported in people with ameloblastoma (Sham et al. 2009), and polyomaviruses are used as a model for induction of ameloblastomas in mice (Stanley et al. 1964). An epizootic of ameloblastomas was seen in salmon in which 100 nm particles morphologically consistent with a large icosahedral virus were seen that could not be further characterized (Grim et al. 2009). There is limited evidence that herpesviruses may play a role in ameloblastoma induction; one study found small RNA encoded by Human herpesvirus 4 in 15% of ameloblastomas and none of the controls (Fujita et al. 1997). It is possible that viral infection contributed to disease in this case, but this may also represent herpesviral recrudescence in an animal stressed by tumor formation. Stress is well documented as a cause of herpesvirus reactivation (Stowe et al. 2000; Strachan et al. 2011). Advanced age and immunosuppression related to stress of captivity, or underlying disease, are also possible factors that predisposed this Gila monster to development of a neoplastic process.

This case describes the presentation and treatment of an odontogenic tumor, associated with a novel herpesvirus, in a wild-caught adult Gila monster. Surgical excision with wide margins is the treatment of choice for this neoplasm, and was performed in this case with apparent success.

Notes
1. VetOne®, MWI, Boise, ID 83705, USA.
2. West-Ward, Eatontown, NJ 07724, USA.
3. Hospira, Inc., Lake Forest, IL 60045, USA.
4. Pharmacia and Upjohn Company, Kalamazoo, MI 49001, USA.
5. IMS, Limited, El Monte, CA 91733, USA.
6. Hikma Farmaceutica, Portugal S.A.
7. Zetamine®, VetOne, Boise, ID 83705, USA.
8. Isoflurane® USP, Piramal Healthcare Limited, Andhra Pradesh 502321, India.
9. PDS®, ©Ethicon, LLC, USA.
10. Vicryl®, ©Ethicon, LLC, USA.
11. Baxter Healthcare Corporation, Deerfield, IL 60015, USA.
12. MeloxiMed™, Intas Pharmaceuticals Limited, Ahmedabad 382210, India.
13. Sandoz, Cambé, Brazil 13150-000.
14. Promega, Madison, WI, USA.
15. QIAquick gel extraction kit, Qiagen Inc., Valencia, CA, USA.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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