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USE OF THROMBOCYTE-LEUKOCYTE RICH PLASMA TO TREAT CHRONIC ORAL CAVITY DISORDERS IN REPTILES: TWO CASE REPORTS

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Disorders of the oral cavity represent common conditions in pet reptiles.1 Multiple predisposing factors including immunosuppression, chronic stressful conditions
inappropriate husbandry, disruption of normal oral tissue, or systemic disease may lead
ultimately to infectious stomatitis. Treatment usually involves correction of
environmental parameters, appropriate systemic and topical antimicrobial therapy, and surgical debridement or resection of affected areas.1,2,5 Periodontal disease is another common oral 6
condition, especially in acrodont lizards such as agamids and chameleons.

The major cause of periodontal disease are considered inappropriate diets characterized by soft fruit or wax worms excess that facilitate bacterial colonization, plaque development, and gingivitis. Treatment consists in calculus removal along with topical irrigation with antibacterial solutions.1,6
Both conditions may progress to osteomyelitis with pathologic fractures and/or systemic infections,1,2,6,7 leading to spontaneous decease2,7 or to euthanasia.3,4,8
The progressive reduction of antibiotic efficacy and the search for novel therapeutical approaches have raised interest in regenerative medicine compounds such as platelet-rich blood derivates. Platelet-rich plasma (PRP) is a biologically active product composed of platelets obtained by centrifugation of patient's whole blood and re-suspended in plasma characterized by elevated concentrations of growth factors and chemotactic molecules.9-15 PRP has been mostly applied to orthopedical, dermatological, and ophthalmological conditions in horses and dogs and,16-19 have recently gained interest in exotic animal practice.20-22 Our aim was to evaluate the efficacy of thrombocyte-leukocyte rich plasma (TLRP), a PRP compound, in chronic oral cavity disorders in reptiles. We illustrate 2 cases of reptiles treated with a single injection of heterologous TLRP, specifically 1 ball python (Python regius) with chronic stomatitis, and 1 veiled chameleon (Chamaeleo calyptratus) with severe maxillary osteolysis secondary to chronic periodontal disease.

Case 1

A 4-year-old male ball python presented with facial swelling of 1-year duration but still regularly feeding on adult mice. The snake was housed at 30° C (86°F) in a rack system with another adult conspecific. Clinical examination revealed severe soft swelling of the right maxillary and periorbital region encompassing the right eye. Oral cavity examination revealed hyperemia and swelling of
the right maxillary region with loss of the teeth of the secondary arcade, and moderate presence of mucus. An excisional biopsy was submitted for histopathology, with a diagnosis of moderate chronic multifocal granulomatous stomatitis with fibrosis (Fig. 1). Klebsiella spp. and Pseudomonas aeruginosa sensitive to the most common
antibiotics were isolated from superficial and deep sterile swabs obtained during surgical biopsy procedure. A blood sample taken by cardiocentesis was characterized by 9% of immature erythrocytes, while other hematological and biochemical values were unremarkable. A head computed tomography (CT) scan revealed marked heterogenous swelling of the soft tissues of the
right portion of the skull with minimal osteolysis of the postfrontal right orbital region (Fig. 2). Therapy consisted of surgical debridement and TLRP irrigation since, the granulomatous, and fibrosing lesions were unlikely to

respond to local or systemic antibiotics. Heterologous TLRP was obtained from a healthy conspecific according with a previously described protocol (Fig. 3).20

Debridement was accomplished under general anesthesia by an intraoral approach. Granulomas had substituted periocular tissues and engulfed the eye thus, globe enucleation was also performed. A longitudinal incision was made between the primary
and secondary maxillary arcades, tissues were bluntly dissected, and granulomas were removed with a mixed technique of sharp and blunt dissection (Fig. 4). Following debridement, the wound was irrigated with 0.5 mL of TLRP. The oral side was closed with appositional single 3.0 polydioxanone sutures,a while the cutaneous side was closed with an everting single 3.0 coated polyglycolic sutures.b Meloxicamc 0.5 mg/kg SC was administered after surgery while, due to the antimicrobial properties of TLRP, antibiotic therapy was posponed. The snake was hospitalized for 70 days and maintained between 27°C to 30°C (80.6°F to86°F)23,24 with no additional therapies. On the tenth day, a small mouse was accepted, and dehiscence of the cutaneous and oral sutures
occurred during swallowing, revealing normal granulation tissue in the wound bed (Fig. 5a).
Margins of the cutaneous wound side were about 10 mm apart, while margins of the
mucosal wound were approximately 15 mm distant. The following day the oral mucosa had
spontaneously closed by secondary intention with complete re-epithelization (Fig. 5b). A
thick scab covered the cutaneous wound side. CT scan was repeated after 30 days showing no significant bone changes. Forty-eight days after surgery, the cutaneous scab detached during
ecdysis. Almost complete re-epithelization was
documented (Fig. 5c). At day 66, cutaneous wound margins were 6 mm apart, while the epithelization was complete without scale
formation (Fig. 5d).

Case 2
A 2.5-year-old male veiled chameleon was presented for anorexia and adipsia. The reptile was
housed in a glass terrarium with poor environmental enrichment, with a correct UVB light spot, but without any hot spot or monitoring of temperature and humidity. The patient was fed mainly wax worms and crickets with no mineral or vitamin supplementation. Forced feeding was initiated 4 days before presentation. At clinical examination a weight of 152 g, a body condition score between 2 and 3 out of 5,25 minimal dehydration (<2,5%) and right enophthalmos
were observed. Severe right maxillary periodontitis, plaque accumulation, gingival swelling, and necrotic debris characterized the oral cavity. Necrosis extended to vomer and palatine bones with oral asymmetry (Fig. 6). A mucosal swab was taken for microbiological examination including sensitivity tests, with isolation of Proteus spp. and Aeromonas spp. Therapy consisted of metronidazoled 20 mg/kg per os q48h,26 marbofloxacine (later confirmed by sensitivity test)
10 mg/kg SC q48h,27 local detersion with diluted chlorhexidinef q12h2 and oral rehydration. After
ten days, there was no clinical improvement. A complete blood work and a CT scan were performed. Marked leukocytosis with heterophilia and lymphocytosis was observed, while most of the
other values were within normal ranges (Table 1). Since reference intervals reported in literature regarding uricemia and phosphatemia are discordant,28-30 it was resorted to integrate therapy with allopurinolg 20 mg/kg per os q24h31 and aluminium hydroxideh 100 mg/kg per os q24h.32
CT scan showed a oro-orbital fistula with massive osteolysis of the right portion of the rostral skull, involving the rostro-ventral and rostro-dorsal portion of the frontal bone, the caudal portion of the jugal bone, and ventral portion of the maxillary bone (Fig. 7). After 1-month, no improvement was noted, and antimicrobial therapy was interrupted. To preserve anatomical integrity and chewing physiology surgery was not feasible. Heterologous TLRP (0.2 mL) was obtained from a healthy conspecific as previously described and injected with a 26 G syringe directly into the necrotic bone to promote bone healing. The patient was dismissed with indication of daily oral cavity detersion and allopurinol therapy. Seven days after injection, the owner reported clinical improvement, weight gain (171 g) and reprise of spontaneous drinking. At clinical examination reduction of soft tissue swelling, of necrotic discharge, and improved mobility of the right eye were documented (Fig. 8a). At day 26, there was a marked improvement of clinical conditions, with considerable reduction of the oral mucosal swelling and necrosis, resolution of right vomer, and palatine bone asymmetry (Fig. 8b). Enophtalmos was still present but the

eye was open and mobile. The same day a control CT scan showed no bone lysis progression. Complete return to spontaneous feeding was achieved
27 days after TLRP injection. Uric acid and phosphate returned to acceptable levels after 60 days of allopurinol that was suspended. Seventy-seven days after treatment, a third CT scan was performed with the same results of the previous exam.

Discussion

PRP and other platelet-concentrates have been extensively applied in human ophthalmology, orthopedics, dermatology, and dentistry regenerative medicine. Despite the extensive scientific literature, their efficacy is still under debate. Controversies lie on the large numbers of

protocols available not always comparable, the lack of a validated classification system and the lack of standardized products with great variability between single preparations in platelet, leukocyte and growth factor contents.[13,33-35] The aim of this work was to assess the potential role of platelet-concentrates, on the basis of their regenerative and antimicrobial properties, in treating reptilian oral disorders, and halting chronic bone lysis. TLRP was obtained following a previous protocol[20] with thrombocyte-leukocyte counts paralleling those previously documented.[20] TLRP was preferred to simple PRP since, platelets and leukocytes release growth factors and chemotactic molecules including interleukin-6 and -4 (IL-6, IL-4), tumor necrosis factor-a (TNF-a), transforming growth factor-b (TGF-b), insulin-like growth factor 1 (IGF-1), basic-fibroblast growth factor (b-FGF), vascular endothelial growth factor (VEGF), or platelet-derived growth factor (PDGF)[9,12,36] able to stimulate proliferation of mesenchymal cells including fibroblasts, osteoblasts, and endothelial cells. Although scientific literature refers mostly to mammal species, recent publications confirm that these molecules play similar roles in reptile wound healing. Evidence derives from studies regarding growth factors under physiological conditions such as IGF-1 being widely expressed in reptile blood and tissues,[37-40] FGF playing a major role in crocodile teeth replacement,[41] crocodile oil containing TGF-b at concentrations proven to enhance wound healing and reduce scar formation.[42] Under pathological conditions, tissue IL-6 increases during inflammation in turtles,[43] PDGF is highly expressed in lizards spinal cord after tail amputation,[44] and VGEF contributes to gecko's wound healing and tail regrowth.[45,46] Since activated platelets release biological factors that have been proven to promote bone regeneration by osteo-competent cells,[47] our main aim was to stop bone lysis and reabsorption and induce healing and new bone formation in both patients. Noteworthy, platelet concentrates have been observed to stimulate hard tissue healing and regeneration both in vitro[14] and in vivo, for example after oro-maxillary surgery[13,48] or orthopedic procedures in human patients.[18,19,49,50]

Furthermore, platelet concentrates have been reported to have antimicrobial properties both in vitro and in vivo[49-51] even if, to date, their mechanism of action remains poorly understood. Theories include antimicrobial properties attributed to platelet a-granules components such as complement and complement-binding proteins.[51] Likely, a major role is also played by prestored antimicrobial peptides and enzymes contained in leukocyte granules.[36]

In the ball python, wound healing was not significantly accelerated by TLRP administration[23] however, TLRP antimicrobial properties were thought to prevent secondary infections during the clinical monitoring, making it possible to avoid postsurgical antibiotic administration.

In the chameleon, 1-month of antibiotic therapy was ineffective for oral cavity infection clearing and, surgery was considered too invasive and demolitive. Strikingly, following a single TLRP injection, osteolysis stopped with a gradual reduction of oral inflammation. More so, restitutio ad functionem was achieved in approximately 1-month following TLRP injection with no additional therapies. In both cases, control CT scans showed no progression of bone or soft tissue lesions pointing to the likely efficacy of TLRP. TLRP should be considered a safe and possibly reliable tool to stop osteomyelitis progression even without surgical removal of dead bone. Unfortunately, new-bone formation was not observed in either of these cases. Probably, one administration of TLRP may not enable new bone deposition in the observed period of time. Additional injections were not administered due to the lack of sceintific data regarding multiple applications of

TLRP in reptiles and especially due to the fact that both patients were reasonably improving withouit further therapies. This apporach was valuable to demonstrate the possible efficacy of the treatment without a secondary role of tissue injury (trauma) associated to multiple injections providing clues for a direct correlation of TLRP in tissue repair.

Conclusion

This work should be considered a preliminary report assessing the possible role of a single TLRP administration in bone and wound healing in
2 reptiles. Platelet concentrates could represent an engaging frontier in reptile regenerative medicine since, they are relatively economic, easy to obtain with essential equipment that is easily available in most facilities and can be frozen and stored for heterologous use. In our experience, clinical outcome suggested that TLRP therapy is safe and may contribute to tissue healing by means of its antimicrobial and regenerative properties, even in nonresponding chronic lesions previously approached with traditional therapies, as in the second case described herewith. Further studies will be necessary to assess TLRP mechanism of action in reptiles and to determine more efficient protocols, for example by combination with other
regenerative techniques or by multiple administrations, as reported in human and mammal medicine.[19,52]

Manufacturers

a. PDS II, Ethicon, Johnson & Johnson SpA, Pome- zia, Italy
b. Assufil, Assut Europe SpA, Magliano dei Marsi, Italy
c. Metacam 5 mg/mL, Boehringer Ingelheim, Milano, Italy
d. Flagyl 250 mg, Zambon Pharma, Bresso, Italy
e. Marbocyl FD 10 mg/mL, Vetoquinol, Bertinoro, Italy
f. Stomodine gel, ICF srl, Palazzo Pignano, Italy g. Allopurinolo, Teva Italia srl, Milano, Italy
h. Maalox, Sanofi spa, Milano, Italy

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