Treatment of a degloving lesion in a green bush rat snake *(Gonyosoma prasinum)* with surgical approach and single application of Thrombocyte-Leukocyte-Rich Plasma (TLRP)

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A one-year-old green bush rat snake *(Gonyosoma prasinum)* was presented for a severe degloving lesion in the cervical region, exposing the underlying subcutaneous and muscular tissue, along with the oesophagus and right jugular vein. After patient stabilization, surgical debridement was performed. Before closure, the wound was irrigated with heterologous Thrombocyte-Leukocyte-Rich Plasma (TLRP) in order to adjuvate wound healing. Thirty days after treatment the patient shed its skin, revealing complete re-epithelialization underneath. No significant reduction in healing time was noted but TLRP may have contributed to avoid common complications such as suture dehiscence and wound site infection. Platelet-rich products are widely used to promote wound healing in human and veterinary medicine; literature is still lacking, but such products are revealing themselves as safe and promising tools in exotic animal regenerative medicine as well, and their application in reptile traumatology should be further investigated.

INTRODUCTION

Traumatic injuries are perhaps the most common cause of degloving lesions in captive snakes.[1] Trauma can result from incorrect physical containment, from bites or scratches from live preys or from the subject rubbing against sharp objects or furniture in the terrarium when shedding its skin.[2] In snakes, and specifically in Colubridae – which are frequently held in captivity -, the difficulty in applying an effective durable bandaging, the low tolerance to handling and the reduced volume/surface ratio (resulting in an increased risk of dehydration) are factors that often make the surgical approach preferable to conservative treatment with healing by second intention.[16] Within the context of a multimodal approach to snake trauma management the growing interest towards modern regenerative medicine techniques can therefore be of interest: in recent years, preparations based on

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platelet concentrates such as Platelet-rich plasma (PRP) and Thrombocyte-Leukocyte-rich plasma (TLRP) have become increasingly popular in pet and unconventional animal medicine and their use appears to be particularly useful in accelerating and adjuvating the wound healing process in such species.[3-5]

CLINICAL CASE

A young, one-year-old male green bush rat snake (*Gonyosoma prasinum*), weighing 55 g, was presented for a severe degloving skin lesion in the cervical region, located about 2 cm from the cephalic extremity, caudally. The lesion involved the skin and subcutaneous tissues; it revolved by more than 180° around the longitudinal axis of the animal, exposing part of the trunk muscles, the right jugular vein and the oesophagus, the integrity of which could not be correctly assessed by manual containment alone.[a] The degloving lesion was of recent onset, as the owner reported that it was not present 5 hours earlier. It was assumed that the lesion was caused by rubbing of the skin against the terrarium furniture during ecdysis, as the animal was about to shed its skin. At inspection, the lesion was dehydrated and necrotic skin flaps were present, with no exudate or contamination by foreign material. At physical examination, the subject was alert, active and in a good state of nutrition and hydration. The last meal, consisting of a baby mouse about 5 days old, had been given a week earlier and was eaten without any hesitation. The owner reported that the subject was housed in a 1.5 m long, 1 m high and 80 cm deep glass terrarium. The furniture consisted of pieces of wood and branches, which allowed the serpent to climb; the substrate was 4 cm deep and consisted of acidic sphagnum moss; a drinking water dish was present and the water was replaced every 3 days. No UVB lamps were present in the tank. A 70 W heating spotlight outside of the terrarium was used to maintain a daily constant temperature of 27 °C. The day/night temperature excursion was of 3 °C, guaranteeing a constant 24 °C during the night. The patient was immediately placed in a box in a warm chamber heated to 28 °C and with 80% humidity. The bottom of the hot chamber consisted of a padded drape of absorbent material moistened with lukewarm saline, so as to keep the subject and the wound hydrated. Given the small size of the animal and in order to avoid any additional stress, no samples were taken for haematological or biochemical tests. The initial rehydration therapy consisted of 0.4 ml of Ringer lactate[6,M] administered subcutaneously (SC) in the body's lateral fold, between the ventral and costal scales. In order to perform a complete debridement of the lesion the animal was premedicated with butorphanol[A] 0.5 mg/kg intramuscularly (IM), followed after 30 minutes by the IM administration of ketamine[B] 10 mg/kg and medetomidine[C] 50 µg/kg.[7,8] Due to the small size of the subject and the lack of adequate instrumentation the anaesthesia was monitored only clinically, with the evaluation of reflexes, respiration and cardiac impulse.[7] The maintenance of spontaneous ventilation throughout the entire procedure (average respiratory rate of 15 breaths per minute) and the need to work around the circumference of the cervical area led to the decision of recurring to endotracheal intubation only in case of need. Once an adequate level of sedation was reached (decreased muscle tone and absence of pain response), a 6 Fr PVC tube was inserted into the oesophagus, in order to assess its integrity and to isolate it from the surgical suture.[a] The patient was placed on a padded drape over a heating pad, the lesion was cleaned with sterile saline and iodopovidone solution[I] and a gentle couretage of the necrotic skin flaps was then performed. Before closure, the degloving lesion was irrigated with 0.2 ml of heterologous Thrombocyte-Leucocyte-Rich Plasma (TLRP) obtained from a non-conspecific donor snake (*Python regius*), in accordance with the protocol described by Di Ianni *et al.*[3,L] The donor was selected based on the size, negativity for the most frequent infectious diseases of the species and a good health status at physical examination; 0.9 ml of blood was drawn via cardiocentesis and was immediately collected in a tube with sodium citrate. A series of centrifugations were performed to separate the platelet and leukocyte fraction from the remaining corpusculate fraction, using Histopaque® 1077.[L] The skin flaps were then approxi-

In reptiles, particularly in Squamata, skin incision margins tend to invert.[10,11] For this reason, extroflecting stitches[10,11] are to be preferred (i.e. horizontal "U" suture) and absorbable sutures recommended (polyglecaprone,[25] polyglyconate[11]).

Figure 1 - Patient under sedation. A 6 Fr PVC tube (asterisk) was inserted in order to distend the oesophagus (black arrow) and assess its integrity. The right jugular vein (red arrow) can be seen under the lacerated skin.

The reptile patient consumed its meal with no difficulty and without apparent pain or discomfort as the prey passed by the surgical suture.

mated and sutured with an extroflecting vertical U-shaped mattress suture,[9-11] using resorbable Monocryl®[D] 5/0 monofilament suture.[b] Prior to antagonizing the effect of medetomidine[C] with atipamezole[E] 0.25 mg/kg IM, and reawakening the subject, a layer of collagenase- and chloramphenicol-based ointment[F] and a thin layer of oxytetracycline spray[c,G] was applied on the suture in order to prevent post-surgical infections with environmental

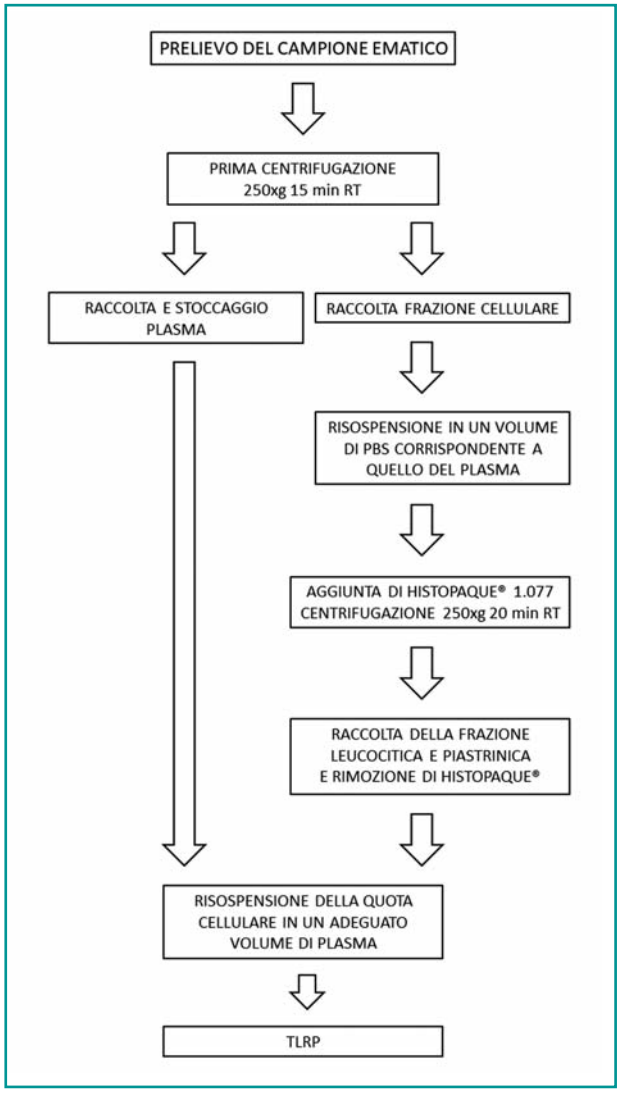

Diagramma di flusso che illustra la preparazione del TLRP come descritto da Di Ianni *et al*. Il sangue è stato ottenuto tramite cardiocentesi e trasferito in una provetta con sodio citrato. Dopo la prima centrifugazione, le frazioni cellulari sono state risospese in Histopaque®[L] di densità 1.077 e ricentrifugate per ottenere il TLRP come prodotto finale.

Figure 2 - Patient under sedation. The skin flaps were sutured with interrupted stitches using non-absorbable Monocryl® 5/0 monofilament.

Figure 3 - Patient under sedation. After suturing of the lesion, a collagenase and chloramphenicol ointment and a thin layer of oxytetracycline spray were applied on the suture.

bacteria[12] that could result from the rubbing of the lesion on the bottom of the tank. The owner was recommended to house the subject in isolation, at temperatures between 25-27 °C and relative humidity between 70-80%, in an unfurnished tank and using newspaper as substrate in order to avoid wound soiling or dehiscence. Antibiotic therapy with enrofloxacin[H] 5 mg/kg SID[13] SC was established for 10 days. Follow-up visits were scheduled at 15, 20 and 30 days after surgery.

FOLLOW-UP

At day 5 after surgery the subject was alert and active and there was no evidence of wound dehiscence. A gentle courettage was performed with the animal under sedation with butorphanol[A] 0.5 mg/kg IM in order to remove the small amount of eschar formed and to revive the tissue, followed by cleaning and disinfection of the wound using water for injectable preparations and iodopovidone[I] in a 3:1 ratio. The cleaning procedure was repeated at day 15 and no necrosis was detected. At day 20 the animal was offered a small meal consisting of a newborn mouse of a diameter slightly larger than that

In snakes, the use of TLRP as an adjuvant therapy for tissue regeneration appears to be very promising even after a single application on the lesion;[5,6] however, further studies on the use of a standardized protocol in reptile patients are warranted.[5,6]

of the patient's cervical segment; the meal was eaten with

Figure 4 - Assessment of the lesion at day 15 after surgery, before disinfection. The tissue repair process was evaluated positively and sepsis was excluded.

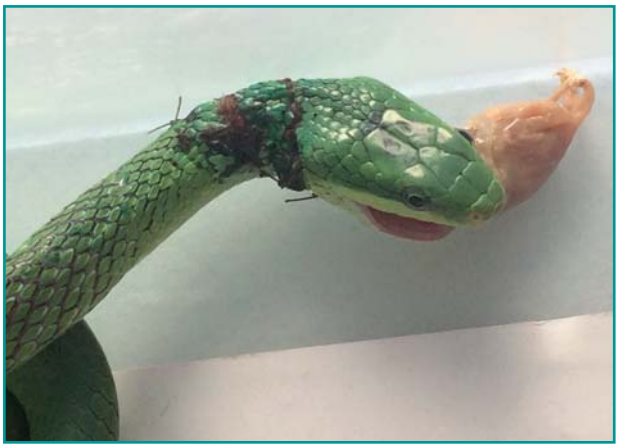

Figure 5 - First meal at day 20 after surgery.

no difficulty or discomfort when the prey passed by the surgical suture. At day 30 the subject underwent its first ecdysis.[f] The owner reported to have helped the subject by mechanically and gently removing the residual molting residues next to the lesion. Twenty-four hours after the shedding of the skin, the deeper part of the lesion was apparently largely made up of fibrous connective tissue.[g] On the same date a second meal was offered, again eaten with no difficulty.[g]

DISCUSSION

In view of the high incidence of traumatic lesions in snakes, in recent years reptile veterinarians have shown an increasing interest and focus on regenerative medicine.[3-5] In these animals the tissue repair process is similar to that in mammals,[14] however the completion of the process usually takes longer, requiring 4 to 6 weeks;[10,14] a key role in the speed of the repair process is also played by temperature, with the patient requiring to be kept within the correct preferred optimum temperature zone (POTZ).[11,14] The prolonged healing time makes reptiles particularly prone to complications such as wound infections or surgical suture dehiscence; in this regard, TLRP may be considered a promising resource to accelerate and adjuvate reptile repair processes. TLRP is easily obtainable in a clinical setting,

Figure 6 - First skin shedding at day 30 after surgery.

Figure 7 - Second meal at day 30 after surgery. With the healing complete, it is now possible to observe that the bottom part of the lesion is largely made up of fibrous connective tissue.

production costs are low, the product can be stored for a long time by freezing and it is available for heterologous use.[4] The main obstacle against an informed and effective use of platelet concentrates lies in the limited number of reptile cases available to clinicians as well as in the lack of a validated classification system in the literature that takes into account the numerous protocols available for the preparation of such products.[5,15] Therefore, aim of this work was to increase the case record available to clinicians and specifically for a type of snake (colubrids) in which this approach has not yet been described; an already validated protocol[3] was used in order to allow harmonization of the data obtained with what already present in the literature.

CONCLUSION

In the case examined, a correct surgical approach and management in combination with a single TLRP application allowed the complete healing of the affected skin lesion. Although the healing time was not significantly accelerated compared to what reported in the literature,[10,14] the excellent post-operative course and the absence of complications (such as suture dehiscence and wound infection) in an area subject to severe stress, in a particularly neurotic and active species, suggest that TLRP may be a promising adjuvant in the treatment of traumatic lesions in these animals. In any case, further studies on the standardization of the treatment protocol in different reptile species are warranted.

KEY POINTS

- In captive snakes, trauma is perhaps the most common cause of degloving wounds.[1]
- In reptiles, the medical and surgical approach to degloving wounds involves the use of premedication and analgesia.[9,10]
- In reptiles, particularly in Squamata, skin incision margins tend to invert.[10,11]
- Temperature also seems to play an important role in the healing process.[3,4].

Trattamento chirurgico di una lacerazione cutanea in un esemplare di *Gonyosoma prasinum* (colubro verde della boscaglia) con ausilio di TLRP (Trombocyte-Leucocyte Rich Plasma)

Summary

Un giovane esemplare maschio di Gonyosoma prasinum *di un anno (green bush rat snake) è stato portato in visita a causa di una grave lesione cutanea ("degloving" o sguantamento). Tale lesione interessava cute e sottocute, esponendo parte dei muscoli del tronco, la vena giugulare di destra e l'esofago. Dopo aver stabilizzato il paziente, è stato effettuato un delicato curettage della ferita. Prima di suturare la lesione, la ferita è stata irrigata con Thrombocyte-Leukocyte Rich Plasma (TLRP) eterologo, per favorirne la guarigione. Trenta giorni dopo il trattamento con TLRP il paziente ha effettuato l'ecdisi, rivelando una completa riparazione tissutale. Non è stato rilevato una significativa riduzione dei tempi di cicatrizzazione rispetto a quanto riportato in letteratura, ma il trattamento con TLRP può aver contribuito ad evitare complicazioni comuni come infezioni o la deiscenza della sutura chirurgica. I derivati piastrinici come il TLRP sono ampiamente utilizzati in medicina veterinaria per promuovere la rigenerazione tissutale. Nella medicina degli animali esotici la letteratura è ancora carente, ma si stanno rivelando strumenti sicuri e promettenti. La loro applicazione nella traumatologia dei rettili merita quindi di essere approfondita.*

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[A] Dolorex® Inj. vial 10 ml, Msd Animal Health Srl, Milan (MI), Italy
[B] Ketavet® 100 mg/ml, Intervet production Srl, Aprilia (LT), Italy
[C] Domitor® IM IV SC, 1 vial 10 ml, FI-02200 Espoo, Finland
[D] Monocryl®, Ethicon, Johnson & Johnson S.p.A, Pomezia (RM), Italy
[E] Atidorm® 5 mg/ml, FATRO S.p.A, Ozzano Emilia (BO) Italy
[F] Iruxol® Collagenase+Chloramphenicol, Smith & Nephew Srl, Agrate Brianza (MB), Italy
[G] Spray Caf®, MSD Animal Health S.r.l, Segrate (MI), Italy
[H] Baytril® 25 mg/ml, Bayer S.p.A, Milan (MI), Italy
[I] Betadine® 10% Cutaneous Solution, Meda Pharma S.p.A, Milan (MI), Italy
[L] Flow chart illustrating TLRP preparation as described by Di Ianni *et al.* The blood was obtained by cardiocentesis and transferred into a tube with sodium citrate. After the first centrifugation, cell fractions were resuspended in Histopaque®[N] adjusted to a density of 1.077 g/ml and then recentrifuged to obtain the TLRP final product.
[M] Ringer's Lactate, SALF S.p.A, Via Guglielmo Marconi, 2, 24069 Cenate Sotto (BG), Italy
[N] Histopaque® 1077, Sigma Aldrich S.p.A Via Gallarate, 154, 20151 Milan (MI), Italy.