Case Report

Application of Platelet-Rich Gel to Enhance Wound Healing in the Horse: A Case Report

Ilaria Iacopetti DVM, PhD, Anna Perazzi DVM, PhD, Vanni Ferrari DVM, PhD, Roberto Busetto DVM

Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, University of Padua, Padua, Italy

A B S T R A C T

A large torn wound of the dorsal elbow region was observed in a 17-year-old Arabian mare. Surgical reconstruction was performed with the horse in standing sedation, but suture dehiscence occurred 2 days later. Autologous platelet-rich gel (PRG) was then applied to the wound every 3 weeks for a total of 3 administrations to accelerate good-quality healing. The wound had healed rapidly and completely within 5 months of the first PRG treatment, without chronic effects or formation of exuberant tissue granulation and with minimum scarring. This case report suggests that topical treatment with autologous PRG, as additional therapy, might be considered beneficial in the management of large-wound healing in horses, and it can be regarded as safe and inexpensive treatment that can be used in field.

1. Introduction

In both animal and human patients, proper wound healing depends on several variables, including blood supply, size of wound, tension and mobility of wound margins, susceptibility to infection, and type and condition of underlying tissue [1]. Advanced age, malnutrition, and disease (such as diabetes) can negatively influence the healing process [3]. This process is regulated by a sequence of events, including coagulation, inflammation, formation of granulation tissue, epithelialization, and tissue remodeling [4,5], mediated by interacting molecular signals, primarily on the part of cytokines and growth factors (GFs), which stimulate and modulate the main cellular activities underlying healing [6-8].

Platelet-rich plasma (PRP) is a concentrate of cytokines and GFs released from platelet alpha-granules at the site of tissue injury. GFs released after platelet degranulation [4] include transforming growth factor-β, platelet-derived growth factor, and vascular endothelial growth factor. Transforming growth factor-β induces chemotaxis of neutrophils and monocytes in the wound site; platelet-derived growth factor leads to fibroblast recruitment and proliferation, and matrix remodeling; vascular endothelial growth factor is a vascular permeability factor that influences extravasation of plasma proteins, creating a support for epithelial and endothelial cells [6].

The effects of PRP have been demonstrated in several studies, in both nonhealing and healing wounds [3,9] in humans [10,11] and animals [7]. The fact that platelets secrete GFs and active metabolites means that their applied use can have a positive influence in clinical situations requiring rapid healing and tissue regeneration [10]. Platelets enhance hemostasis, wound healing and reepithelialization, angiogenesis, growth, and vascular fibroblast proliferation and increase extracellular collagen matrix synthesis and deposition [3,4,8,9,12,13]. To achieve these effects, platelet concentration must be more than four to five times the baseline intravascular platelet count [11,14-17], which is considered the minimal concentration for accelerated epithelialization and granulation [18]. The
methods for PRP sampling and preparation may be manual or automatic [17], but both achieve the required concentration. In horses, skin lesions are relatively frequent, but healing often requires long recovery times and may lead to chronic wounds or exuberant tissue granulation [3,18]. These consequences are more frequent in large wounds on limbs and in every other anatomical region subject to movement, as wounds of the trunk or head usually heal uneventfully [18].

Several mechanisms are responsible for delayed repair in equine’s limbs, such as poor blood supply, reduced oxygen supply, inefficient inflammatory response to trauma, the slightly lower temperature of limbs, GF imbalance, collagen synthesis prevailing over lysis, and deficient apoptosis of the cell components of granulation tissue [3,18].

For all these reasons, current therapy such as plastic surgery or skin transposition in large lesions on equines’ limbs does not always give satisfactory results and may require general anesthesia.

The aim of this clinical case is to describe an alternative or an addition to traditional treatment that could be considered in similar clinical cases to accelerate a physiological mechanism of healing. Platelet-derivatives represent a promising therapeutic modality, offering opportunities for treatment of wounds and soft tissue injuries [8]. Platelet-rich gel (PRG) stimulates the normal healing process, may reduce recovery time for the animal, is safe, and, because the manual method requires minimal technical skill, can be used in the field, with low-cost treatment for owners [19,20].

2. Case Report

A 17-year-old Arabian mare weighing 370 kg was presented at the Department of Veterinary Clinical Science, University of Padua, for evaluation of a large torn wound on the left forelimb, which had occurred a few hours before, while the horse was in a paddock.

2.1. Clinical Findings

Clinical signs included grade 3 of 5 lameness of the left limb and a very large recent injury, torn and bleeding, in the region of the elbow and left forelimb. External examination revealed the absence of foreign bodies or bone fragments inside the wound. Distal to the lesion were edema and swelling, but the foot and metacarpal region were warm on palpation. No other abnormalities were found on general physical examination. The horse was in good health: body temperature, mucous membranes, and capillary refill time were all within normal limits. A complete blood count and serum biochemistry profile showed no abnormalities except a modest blood loss.

2.2. Diagnosis

The wound, still bleeding, extended from approximately 10 cm proximal to the left axillary region to the middle third of the left forelimb, covering >50% of the circumference of the elbow (Fig. 1). Medially, the brachiocephalic and descending pectoral muscles were exposed. Cranially, the extensor carpi radialis was exposed but intact. The biceps brachii and brachial muscles appeared to have been involved in the injury. The elbow joint was not affected. Cranially, in view of the considerable depth of the wound, the median artery was exposed on its junction point with the transverse cubital artery (radial proximal artery) [2]. The skin was torn into a large flap, everted in distal direction: no necrotic areas were detected, and the surface appeared to be still vital, even in its most marginal portions.

2.3. Treatment

A surgical approach was decided based on the characteristics of the wound, the bleeding, the absence of necrosis, the large flap present, and the time of onset. Tetanus antitoxin (5,000 iu; Gellini International, Milan, Italy) and benzylpenicillin (9,000 i.u./kg body weight [bwt])-dihydrostreptomycin (11.25 mg/kg bwt; Pfizer, Latina, Italy) were administered intramuscular (i.m.) preoperatively. Saline solution was infused through a 14-gauge jugular catheter at a rate of 10 ml/kg bwt/h. The horse was given butorphanol (0.02 mg/kg bwt i.v.; Ati, Bologna, Italy) and detomidine (20 µg/kg bwt intravenous [i.v.]; Fatro, Bologna, Italy), so that the wound could be sutured in standing sedation. Lidocaine 2% (total 20 mL; Fort Dodge Animal Health, Aprilia, Italy) was given as subcutaneous local anesthetic. Before suturing, all debris was removed from the wound, which was cleaned under pressure with saline solution. The muscle ends were debrided and stitched with Vicryl EP 0 (Ethicon, Johnson & Johnson, Roma, Italy), with simple interrupted sutures. The edges and bed of the injury were debrided with a large Volkmann’s spoon. Finally, the skin incision was closed with staples. Two passive drainage tubes were added proximodistally to facilitate the escape of serum (Fig. 2). Phenylbutazone (3 mg/kg bwt i.v.; Acme, ReggioEmilia, Italy) was administered after surgery and continued for 3 days. Benzylpenicillin-dihydrostreptomycin, at the aforementioned doses, was administered i.m. for 7 days. No bandages were used to cover the wound after treatment. However, on day 2 after surgery, the sutures were dehiscent: the distal cutaneous strip was completely everted, exposing the muscle layer (Fig. 3).

To reduce the amount of tissue surface exposed, after gentle curettage, the skin flap was stabilized with several
sutures to facilitate adhesion of the flap to the underlying tissue. On day 4, the subcutaneous tissue and muscle were still viable. Although present in the granulation tissue of more remote portions of the wound, focal areas of fibrin and necrotic tissue were also observed. On day 5, more necrotic areas appeared, with a loss of continuity between the subcutaneous tissue and underlying muscles at some points. Systemic antibiotic therapy and daily dressings had been carried out since day 1, and continued during the following week.

Although the horse was in good condition and showed no signs of infection for 2 weeks, the wound showed slow tendency to heal (Figs. 4, 5). Therapy was therefore discontinued and replaced with applications of autologous PRG, obtained from PRP activated with calcium gluconate, every 3 weeks for a total of 3 administrations.

The PRG was prepared by the tube method in commercially designed platelet sequestration tubes with sodium citrate (BD Vacutainer CPT). Whole blood was collected atraumatically through a single jugular venipuncture with an 18-gauge needle in six 8-mL tubes. The blood was centrifuged (Labofuge 400, Heraeus Holding, Hanau, Germany) for 20 minutes at 2,800 rpm to achieve separation of cell layers: red blood cells were isolated from the overlyinguffy coat and plasma by the gel-like plug within the tubes. Thus, 8 mL of blood from each tube yielded approximately 4-5-mL of platelet-poor plasma, of which 80% was discarded. The buffy coat of each tube, containing mononuclear cells and platelets, were then carefully removed with a pipette and resuspended in 0.75-1.0 mL of the remaining plasma. The final solution, obtained by mixing different buffy coats in a sterile 15-mL Falcon tube,
was centrifuged twice at 1,300 rpm for 15 minutes for good separation of platelet pellets in the supernatant layer. The final solution (2 mL of PRP) contained a mean concentration of $9.67 \times 10^7$ platelets L$^{-1}$. Immediately before being applied to the wound surface, the PRP was gelled according to the following procedure: 1 mL of calcium gluconate 10% (Monico Spa, Venezia, Italy) was added to the PRP, and the resulting mixture was gently blended in a sterile Petri dish until it reached a gelatinous consistency. The PRG was then applied to the wound to be treated.

2.4. Outcome

Three milliliter of PRG was gently applied manually to the surface of the lesion with a sterile glove on day 0, and a large covering bandage was then applied to reduce the risk of wound contamination. The bandage was changed every 3 days: the wound became slightly exudative. Saline was used to clean it of serous secretions, but no other topical medication was applied. No antibiotics or anti-inflammatory drugs were given after PRG applications.

No signs of adverse reactions were detected; body temperature, mucous membranes, and capillary refill time were all within normal limits. The horse never presented hyperthermia. Only slight edema of the limb, distal to the wound, could be observed in the first few days after treatment. The wound showed immediate and significant clinical improvement; granulation tissue was formed, with decreased production of fibrin and serous secretions.

On day 14, the wound appeared as shown in Figure 6. In view of the significant size of the wound, a second dose of PRG was then applied on day 21 (3 weeks after the first treatment), with the same good results. At this point, saline cleansing was reduced to once a week. On day 36, swelling was markedly reduced, and no crusts or erosions were noted. The newly formed tissue adhered well to the subcutaneous tissue, even at the edges of the wound (Fig. 7). A third dose of PRG was then applied on day 42 (3 weeks after the second treatment), and the wound again showed marked clinical improvement (Fig. 8).

Clinical examinations were performed 4 months (Fig. 9) and 8 months after the first treatment. Wound healing was complete 5 months after the first treatment, with slight scar formation and quite good cosmetic effect. Twelve months after treatment, hair growth was complete, with good cosmetic effect, and the owner reported that the horse was well and showed no lameness (Fig. 10).

3. Discussion and Conclusion

Several clinical studies, in both human and veterinary medicine, on the restoration of tissue integrity have shown the positive role of platelets in natural wound healing [5,9,18]. When locally applied, platelets accelerate healing of normal tissue and promote healing of impaired wounds [1,3] because of GF liberation, as already widely demonstrated [3,13].

GF can also modulate the inflammation and proliferation phases of the wound-healing process; thus, reducing the risk of keloid and hypertrophic scar formation [21].
characteristic is very important in horses, in which chronic nonhealing wounds or exuberant granulation tissue often develop, especially when the wounds are located on the distal limb [18]. These conditions occur more frequently in old horses in poor health or if the wound is located at a point subject to movement, and may limit an equine's athletic career or delay recovery time [18].

Wounded skin in horses has also been characterized as displaying a weak but protracted inflammatory response. Attempts to ameliorate wound repair in horses have been disappointing. Indeed, costly treatments often fail to prevent or resolve chronic wounds or the development of exuberant granulation tissue [18].

In this case study, PRG was used to treat a large subacute wound in the elbow region, which, after dehiscence of sutures, showed poor prospects of healing, likely because of the size of the wound and at a point subject to continual movement.

PRG, activated with calcium gluconate, was directly applied to the surface of the wound improving adhesion to vertical wound surfaces, such as those of the limbs: the consistency of the gel was good and allowed proper adhesion, although the wound surface was vertical and now oriented on the ventral aspect of the pectorals.

There are many methods for sampling and preparing PRG as follows: test tubes, quadruple blood bags, apheresis, manual preparation in open or closed systems, and automatic preparation devices [6]. Most of these methods, also used in human clinical applications, are automated or semi-automated, and require expensive and sophisticated technical equipment. Manual preparation, together with a double centrifugation tube method, as used in the present case, has the advantage that it does not require expensive equipment, is simple and fast, and can also satisfactorily concentrate equine platelets.

In our case, the number of platelets in the final solution was on average 5.2 times the baseline intravascular platelet count. That is, it was four to five times higher than the baseline count, which is considered the minimal concentration required to accelerate epithelialization and granulation, as frequently reported [14-16,18]. No systemic or local drugs were used after PRG treatments. Nonsteroidal anti-inflammatory drugs (NSAIDS) in particular were avoided, as they would have inhibited the effect of the treatment. Body temperature was monitored frequently, and no alterations due to infection were recorded.

Bandages were used after each treatment only to keep the wound clean and to avoid overdrying of newly granulated tissue. No clinical complications were observed after PRG treatment or during the healing process. Only a slight swelling of the distal limb was observed on day 1 after treatment, without pain or lameness. This swelling was no longer observable on days 3-4 after treatment. Gradual improvement of the wound was noted already on day 1 after autologous topical PRG application, and the effect of treatment lasted 3 weeks. This may be explained by the fact that, although release of secretory proteins by platelets begins within 10 minutes of clotting, with >95% of pre-synthesized GF secreted within 1 hour, platelets continue to synthesize and secrete additional proteins for the rest of their lifetime (5-10 days) [18].

Before each treatment, the wound was cleaned to remove any necrotic tissue or debris. In particular, before the first treatment, care was taken to clean the wound in depth because tissue healing depends to a great extent on early debridement, particularly when necrosis is present. Complete healing of the wound was observed about 5 months after the first PRG application. This case report suggests that topical treatment with autologous PRG as additional therapy can enhance tissue healing considering
the extent of the initial wound and the anatomical position. PRG does not expose the patient to iatrogenic infections or immune reactions. It is safe, fast, cheap, and simple to produce, and can be used to treat acute and subacute wounds in areas subject to movement of equine’s limbs, as well as in cases of delayed wound healing and long-term nonhealing wounds [3]. It can be used in the field and is far less expensive than other treatments [22].

Further investigation of PRG efficacy in large clinical trials is recommended, to confirm the good results seen in this case report.

References