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APPLICATION OF PLATELET RICH PLASMA (PRP) IN TREATMENT OF A CONTUSED LACERATED WOUND IN A DOG: A CLINICAL CASE

Konstantin Aminkov

University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
E-mail: kaminkov@ltu.bg

ABSTRACT

Second intention wound healing may be impaired by wound and host factors and thus more advanced therapies are required for a fast and satisfactory outcome. Platelet-rich plasma (PRP), rich in growth factors and cytokines essential for tissue repair, could improve wound healing.

The use of regenerative therapies is becoming increasingly popular due to the low-invasive procedures needed to apply them. Platelet-rich plasma (PRP) is gaining interest due to its potential to stimulate and accelerate the wound healing process. The cytokines and growth factors forming PRP play a crucial role in the healing process.

The purpose of this article is to evaluate the effect of locally injected autologous PRP on second intention healing of contused lacerated wound in dog.

Platelet Rich Plasma (PRP) therapy was used in treating of contused lacerated wound in an 5 years old dog. The wound edges were infiltrated with 5 ml of platelet rich plasma three times over 10 days. The wound healed completely 25 days after treatment. This clinical case indicates that autologous Platelet Rich Plasma (PRP) can be successfully used in treating of contused lacerated wound in dog.

Key words: Platelet rich plasma (PRP), contused lacerated wound, platelet, plasma, dog.

Introduction

Cutaneous wound healing is a complex process, which commences immediately after injury and aims at a structurally and functionally desirable wound repair within a reasonable period of time. Second intention wound healing, which occurs when wound edges cannot be approximated, may be impaired by wound and host factors including poor blood supply, defect size, local infection, underlying systemic disease (diabetes), administration of drugs (corticosteroids, chemotherapeutics) and malnutrition, resulting in delayed (impaired) healing (Fossum 2013; Beldon, 2010).

In full-thickness skin defects, the loss of subdermal plexus blood supply due to subcutaneous tissue removal results in a reduced healing outcome (Bohling et al., 2006).

In all the above cases, achieving a fast and satisfactory healing outcome may require employing more advanced treatment modalities (Schreml et al., 2010; Fossum 2013; Li J et al., 2007).

Platelet-rich plasma (PRP) is a volume of plasma of autologous blood having platelet levels above peripheral blood concentration (Panjeshahin et al., 2009). The concentration of platelets may provide a higher amount of several bioactive growth factors reported to promote healing, but the methods used vary considerably, affecting the overall quality of the platelets, outcome, and costs (Lee et al., 2011; Staudenmaier et al., 2009).

Activated platelets, upon contact with exposed endothelium within wounds or damaged tissues, are known to release key wound healing factors including platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), and epidermal growth factor (EGF) (Everts et al., 2006; Scott et al., 2000).

All of these factors set the stage for tissue healing which involves intricate overlapping processes that are often categorized into haemostasis, inflammation, proliferation, and remodelling.

Once tissue injury occurs, a haematoma forms at the site of tissue damage, platelets adhere to exposed collagen creating a clot, and the inflammatory phase begins with activation of platelets resulting in release of growth, bioactive, and haemostatic factors (Scott et al., 2000).

Each factor plays a unique but important integrating role in the early stages of the intrinsic and extrinsic pathways of the clotting cascade. Access to the wound site by neutrophils and macrophages occurs within hours of injury thereby initiating the phagocytosis of tissue debris. Within a few days of injury, the proliferative phase begins and this is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. Finally, the remodelling phase involves collagen maturation and apoptosis of excess cells which can take from several weeks to months after an injury depending of the degree of damage. Based on the above described model, acceleration of wound healing by addition of PRP is based on various platelet growth factors (PGFs) that stimulate different stages of the wound cascade. Compared to application of single, recombinant growth factors, which are applied in supraphysiological concentrations, PRP has the advantage of offering multiple, synergistically working growth factors at the wound site and in concentrations that are biologically and physiologically more pertinent (Akhundov et al., 2012). Traditionally platelets have been used therapeutically to treat the thrombocytopenia and platelet dysfunction (Sensebe et al., 2005).

Wounds in animals usually do not heal or heal slowly due to poor blood flow, decreased oxygen supply, insufficient inflammatory response to trauma and others (Lin et al., 1997; Mazzuco et al., 2004).

The purpose of this case report is to describe our experience using PRP in treating complicated and difficult to heal wounds in dog.

Clinical Case

The patient – a 5 years old female mix breed dog named Hera – The patient was admitted to the clinic after being hit by a car. After clinical examination and X-ray examination, a fracture of the left femur and a wound on the dorsal surface of the proximal part of the metatarsus were found.

The wound was without exudates with uneven wound edges and bottom and without tendency to heal. We applied conservative treatment for 10 days, but without positive result. The wound was atrophic with a dry bottom and thickened wound edges and covered with necrotic tissue. The tissue defect was 2 cm by 6 cm in size (Fig. 1).

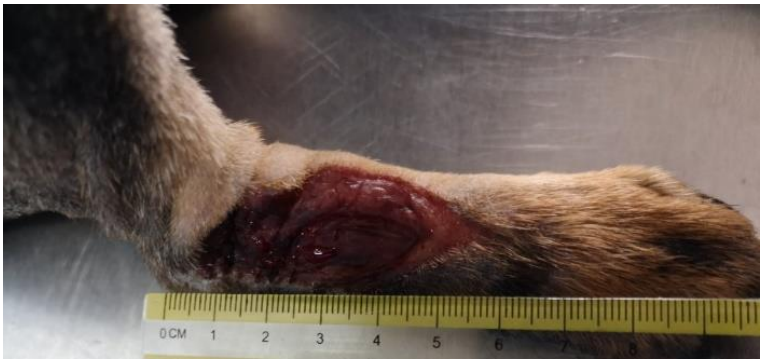

Figure 1: Lacerated wound

Materials and methods

PRP is derived from blood samples obtained from venous blood. 40 ml of venous blood produces 3–5 ml of PRP. The amount depends on the baseline platelet count and the method used for PRP production.

The obtained blood was centrifuged for 7 min at 2700 rpm to achieve cell separation into cell layers. This procedure divides the blood into three main components: red blood cells, platelet rich plasma (PRP) and platelet poor plasma (PPP).

From each vacuum container are received about 10 ml of PRP, 75% of which was discarded. The portion containing platelets and mononuclear cells was carefully removed with a spinal needle attached with a syringe and resuspended with 5 ml of the remaining plasma.

The final solution obtained from the mixing of PRP and plasma was placed in sterile vacuum containers and centrifuged at 3600 rpm for 15 min to better separate the platelets from the supernatant layer of PPP.

The platelets were accumulated at the bottom of the container and PPP at the top. The PPP was removed so that only the PRP remained. The PRP was resuspended with the remaining plasma with a Vortex mixer. The final PRP was drawn up with a syringe.

Each step of the method was carried out using sterile disposables (Perazzi et al., 2013). No topical or systemic drugs, antibiotics or anti-inflammatory drugs were administered to dogs during the PRP treatment.

Results

First application

In the first application, we infiltrated the wound edges and the bottom with 5 ml autologous PRP (Fig. 2).

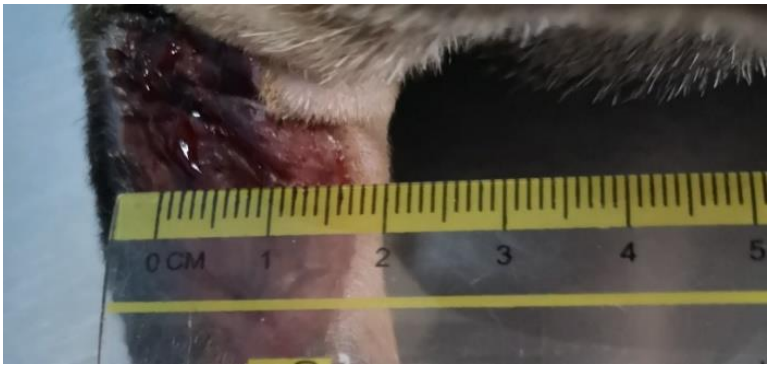

Figure 2: Application I

5 days after the first treatment, it was found that the wound edges were covered with pink, coarse-grained granulation tissue and necrotic tissue was found in separate areas.

The wound was 1.8 / 5.3 cm in size. We found a gradual decrease in the size of the wound and the amount of necrotic tissue (Fig. 3, 4).

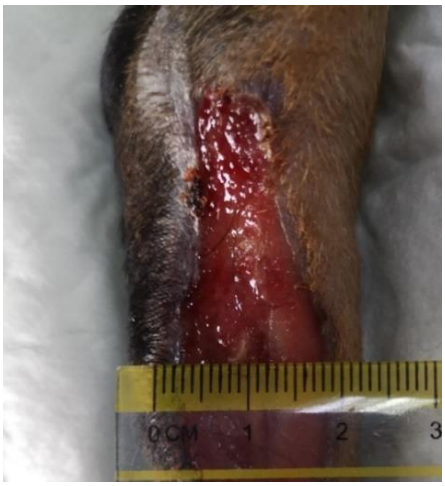

Figure 3: 5 days after the Application I

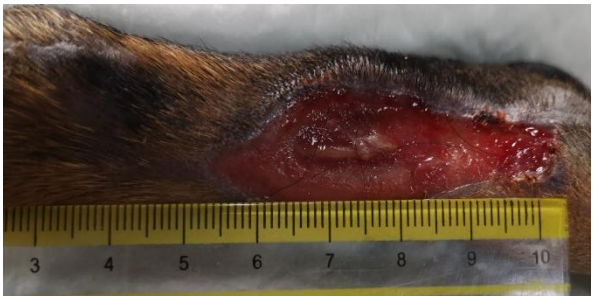

Figure 4: 5 days after the Application I

Second application

In the second PRP application, the wound was found to be filled with granulation tissue and the wound edges were grayish-colored, indicating active epithelization and reduction of wound size - 1.2/3 cm (Fig. 5, 6).

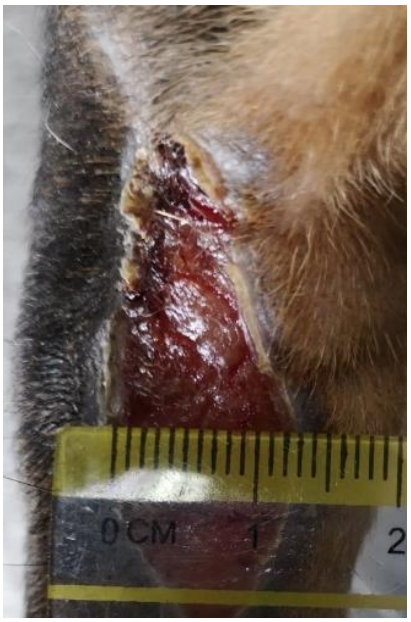

Figure 5: 10 days after the Application I

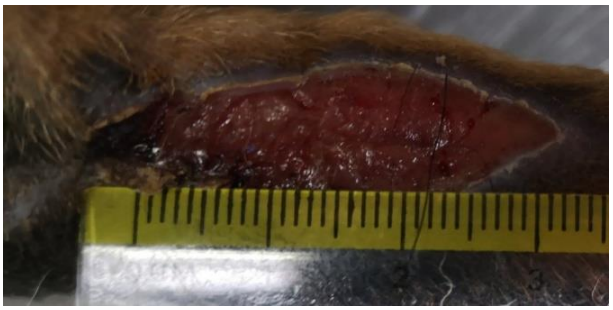

Figure 6; 10 days after the Application I

5 days after the 2nd application, the size of the wound was 1/2.5 cm (Fig. 7, 8).

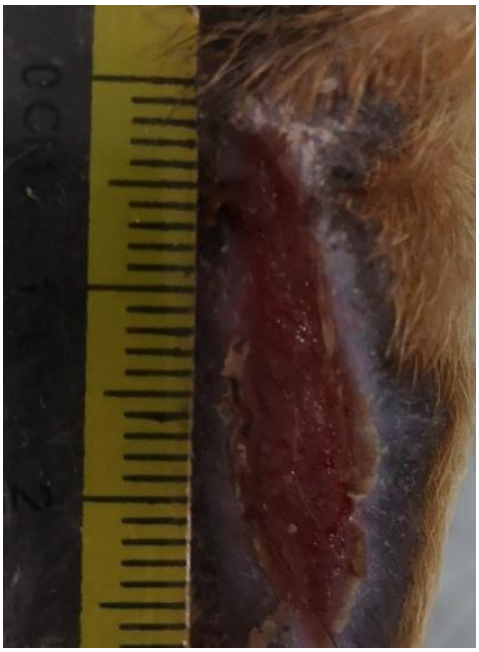

Figure 7: 5 days after the Application II

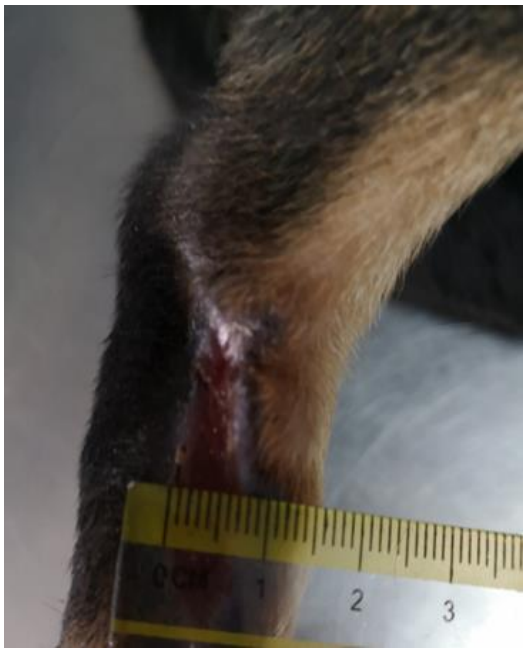

Figure 8: 5 days after the Application II

Third application

In the third PRP application, the epithelialization was found to be highly pronounced and the wound defect was barely noticeable (Fig. 9, 10).

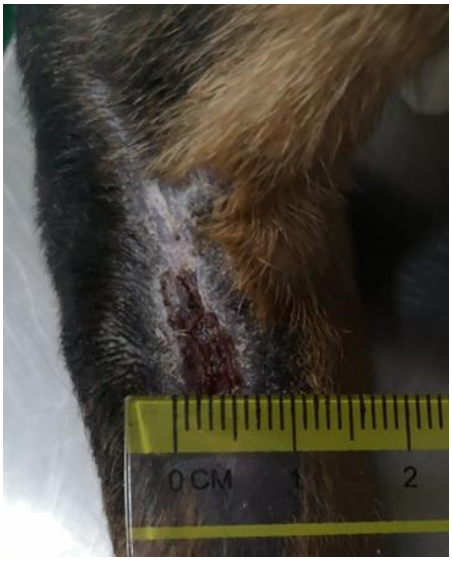

Figure 9: 10 days after the Application II

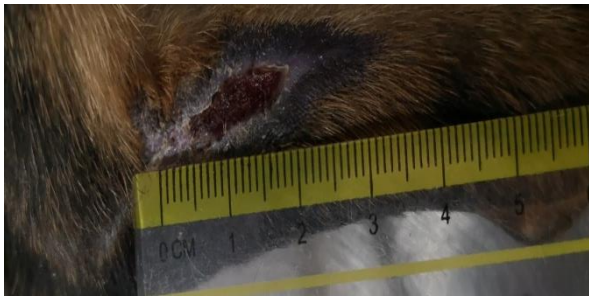

Figure 10: 10 days after the Application II

25 days after the last application of PRP, complete wound healing was detected (Fig. 11).

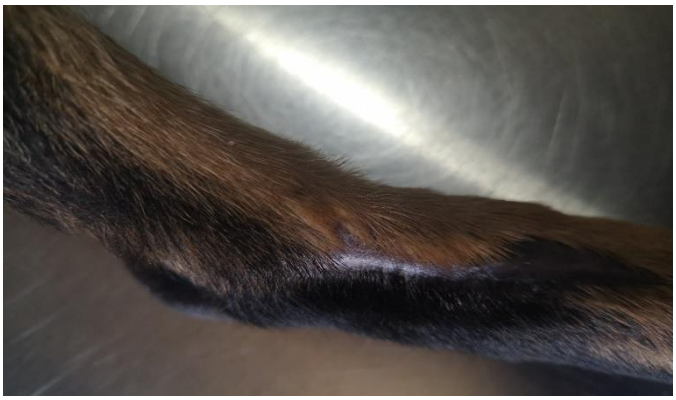

Figure 11: 25 days after the Application III

Discussion

In recent years, PRP and platelet-derived products have gained a strong attention in veterinary medicine since they are supposed to enhance tissue regeneration and wound healing (Harman, 2013).

A number of clinical studies in animals and humans have shown the important role of platelets in the process of wound healing when applied topically. PRP accelerates healing due to release of growth factors (GF) contained in platelets (Mazzuco et al., 2004).

Platelet rich plasma is a well-known biological product typically used in the autologous application settings for therapeutic purposes (Anitua et al., 2004; Man et al., 2001).

The medical use of platelet GF has been described for eye drops (Yoon, 2014), platelet gel (Parazzi et al., 2010) and supplements of culture media (Romanov et al., 2017) and for advanced therapy medicinal products (ATMPs), amongst others. In this sense, there are several recently described clinical applications of autologous PRP, including chronic wound healing (Fréchette et al., 2005; Driver et al., 2006), skin and soft tissue repair (Everts et al., 2006) and treatment of inflammatory pathologies.

Large cutaneous lesions, where poor blood supply, tissue necrosis, excessive scarring, inflammation, and bacterial contamination are possible complications, would take significant advantages from the application of regenerative therapies (Murphy et al., 2012).

Platelet-rich plasma should contain a platelet concentration at least 4-5 times over blood values in order to have a therapeutic effect (Marx, 2004). In our clinical case, platelet numbers in PRPs were many-fold higher (5 to 8-fold), as in other similar studies (Eppley et al., 2004; Dionyssiou et al., 2013). Concentrations higher than 5-fold, however, may not necessarily enhance the wound healing effect (Lacci et al., 2010), since PRP platelet counts are not predictive for growth factors levels released from PRP (Weibrich et al., 2002). The release of bioactive proteins (growth factors and cytokines) from platelets and consequently the effectiveness of PRP depends on platelets activation (Eppley et al., 2004; Weibrich et al., 2002). In most studies evaluating the effect of PRP on second intention wound healing (Hom et al., 2007; Lee et al., 2008; Sardari et al., 2011; Tambella et al., 2014), PRP was just applied on the wound surface; in those cases, activation of platelets was achieved by the addition of thrombin or calcium chloride before application. In the present case, we used the intralesional technique for PRP application. This technique offers the advantage of preventing PRP loss. Furthermore, the endogenous activation of PRP via contact with elements of

injured endothelium, such as collagen and endothelial cells, results in a slower release of bioactive proteins that better benefits wound healing (Mehta et al., 2008; Beldon 2010).

In this article I report the case of a contused lacerated wound in a dog hit by a car. The area involved in the lesion was located in a place where the skin has little elasticity to allow successful reconstructive surgery. The wound was highly contaminated and the area of necrosis extended. Consequently, we opted for a regenerative therapy based on PRP application. Since the main lesion was particularly extended to reach a homogeneous distribution of the therapeutics, we injected the whole injured area with PRP. The application of PRP probably contributed to the positive outcome, inducing a prompt formation of granulation tissue, a strong adhesion of the wound edges to the underlying tissue and a rapid tissue growth, starting from wound margin. Furthermore, no sign of excessive scarring, tissue contraction, and fragile re-epithelialization, sometimes associated to second intention healing, was observed.

In experimental study in dogs Karayannopoulou et al., 2015 applied the intralesional technique for PRP treatment of experimentally created skin defects. They monitored the rate of tissue defect healing on days 0, 4, 10, 15, and 20. They found that on day 4 of the aplication of PRP that the treated wounds increased in size by about 5%, on day 10 they found a reduction in the size of the tissue defect by 60%, on day 15 90% and on day 20 completely healing of treated skin wounds. In my clinical case, the healing rate was reported on days 0, 5, 10, 15, 20 and 25. On day 5 of the first PRP application, I found that the wound size decreased by 10%, on day 10 by 50% , Day 15 by 60%, day 20 by 85%, and on day 25 of the first PRP injection the wound was completely healed.

In experimental studies in horses (Carter et al., 2003; Carter et al., 2011) on PRP gel treatment of cutaneous wounds of the extremities, which are resistant to healing resembling chronic ulcers in humans, Carter et al., (2003) found that PRP improved the quality of healing by producing organized collagen bundles and by accelerating epithelial differentiation (Carter et al., 2003), whereas Monteiro et al., (2009) reported that PRP slowed wound healing significantly (less exuberant granulation tissue) but no significant differences in histological variables were revealed between PRP-treated and untreated wounds (Monteiro et al., 2009).

However, some controversies exist about the efficacy of PRP application (Fréchette et al., 2005). While some authors reported the effectiveness of PRP gel in the treatment of nonhealing chronic wounds, others did not report any improvement (Monteiro et al., 2009; Kazakos et al., 2009, Ganio et al., 1993; Josifova et al., 2001). This might be due to differences in experiment (animal, human), wound defect model, differences in PRP biology among species, differences in PRP preparation techniques, differences in PRP activity and differences in investigated time points (Plachokova et al., 2009).

In dogs treated with dexamethasone (impaired healing), in which the efficacy of PRP on the healing of 2x2 cm full-thickness skin wounds was examined (Sardari et al., 2011), no significant differences were found between treated wounds and untreated controls in the healing rate.

These results suggest that PRP is indeed an effective therapeutic option to manage soft tissue wounds where a large amount of tissue is destroyed, especially when surgery alone cannot guarantee satisfactory results (Zubin et al., 2015).

Conclusion

The results of this clinical case suggest that,the PRP method is an effective therapeutic method in treatment of a contused lacerated wound in a dog. Regenerative therapy can be applied in order to improve the quality of tissue regeneration and the rate of wound healing in dogs.

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