

APPLICATION OF PLATELET-RICH PLASMA IN NONUNION FEMUR FRACTURE IN A DOG - CASE REPORT

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ABSTRACT

The role of platelet-rich plasma (PRP) as a promoter of bone union remains controversial. The purpose of this study is to investigate the effect of PRP on non-union femur fracture in a dog. This is a case of a 3 year old mongrel dog with femur fracture. Following unsuccessful treatment of the fracture with dynamic compression plate and external bone fixation, PRP was injected through the skin three times every 10 days in the region of the fracture line.

One month after the last injection, there was union between the bone fragments. The administration of PRP in the event of unsuccessful union between bone fragments stimulates bone union.

Key words: dog, bone non-union, platelet-rich plasma

INTRODUCTION

Non-union of long tubular bones is a complication that occurs following traumatic fractures. This complication is more common in the dog than in the cat. The prevalence of femur non-union following trauma in humans is about 2-6% (Tsiridis, E. et al.2007). Prevention of bone non-union is considered the best method of dealing with this problem. This can be achieved by restoring the anatomical integrity of the bone fragments using internal or external fixation (Sanchez, M. et al. 2009). Removing the infected necrotic tissue and restoring bone integrity with intramedullary fixators is one of the methods for dealing with these complications (Megas P.et al., 2009). However, significant part of the complications depend on the clinical condition of the patient. If the defect exceeds the critical size, bone grafting for new bone tissue regeneration is indispensable (Greenbaum M.et al., 1993; Schlegel KA et al 2004).

Autogenous bone grafting is considered the gold standard for bone defect filling, despite significant problems arising from pain at the donor sites and the limited amount of bone tissue obtained from the donor, especially in small dog breeds. The concept of tissue engineering is based on three pillars - scaffold, cells and growth factors. Ceramics is used as a tissue engineering material because it offers three-dimensional support and serves as a scaffold for cell proliferation, cell differentiation and ultimately, for bone recovery. Recently, a new group of resorbable high SSA ceramics is being used that is equally effective in terms of osteogenic differentiation (Kasten P. et al. 2003) and ectopic bone formation (Kasten P. et al. 2004; Kasten P. et al. 2005).

Scaffolds can be combined with mesenchymal stem cells (MSC) and/or growth factors. Growth factors affect chemotaxis, differentiation, proliferation, and synthesis activity of bone cell, thus regulating physiological remodelling and fracture healing. Numerous growth factors, such as bone morphogenetic proteins, platelet derived growth factor, transforming growth factor, and

insulin-like growth factors, have stimulatory effects on bone defect repair (Bostrom MP. et al. 1999). Since PRP can be used alone, it does not pose a risk of transmissible diseases. PRP can easily be obtained on the day of surgery by two centrifugation steps from autogenous whole blood (Tzioupis C. et al. 2007).

Recently there has been discussion on the effect of PRP and there is no consensus on the role of PRP in bone regeneration (Gandhi A. et al. 2006).

MATERIAL AND METHODS

The study was conducted in an 8 year old mongrel dog named Chochka weighing 21 kg. The dog was admitted to the clinic on 05.05.2017 for examination and treatment. Based on radiography, comminuted femoral fracture of the right limb was diagnosed (Figure 1). Anatomical integrity of the bone was restored using internal plate fixation (Figure 2). A few days after surgery, the dog started putting weight on the leg. Twenty days after the surgery, the dog became reluctant to put weight on the leg. Four months after surgery, the plate was removed and bone ends were curetted. Bone fragments were fixed by external fixation, and a Steinman pin was introduced into the medullary canal (Figure 3). Five days after the surgical intervention, 10 pulsed electromagnetic field procedures were carried out. However, no union of bone fragments was detected by radiography, and the dog was still reluctant to put weight on the leg.

In order to stimulate osteogenesis, 5 ml of PRP were injected percutaneously in the fracture line three times every 10 days. Platelet concentration was 1243 ± 145 .



Figure 1



Figure 2



Figure 3



Method of preparation of PRP

Double centrifugation method (Perazzi A.et al., 2013)

The blood was centrifuged for 20 min at 2800 rpm to achieve separation of cell layers. This procedure divides the blood into three basic components: red blood cells, platelet rich plasma (PRP) and platelet poor plasma (PPP). Each vacutainer yielded approximately 4–5 mL of PRP, 80% of it was discarded.

The part containing platelets and mononuclear cells was carefully removed using a spinal needle attached to a syringe and re-suspended in 2 ml of the remaining plasma. The final solution, obtained by mixing the resulting PRP and plasma, was placed in sterile vacutainers and was centrifuged at 1300 rpm for 15 min for better separation of the platelet pellets from the supernatant layer of PPP. The platelet pellets accumulated at the bottom of the container and the PPP on top. The PPP was removed; only the PRP was left in the containers. The platelet pellets were re-suspended within the remaining plasma with a vortex mixer; the final PRP was drawn up with a syringe.

RESULTS

In order to stimulate osteogenesis, 5 ml of PRP were injected percutaneously in the fracture line three times every 10 days. Platelet concentration was 1243 ± 145 .

Healing was monitored by check-up radiographies 10 days after each administration of PRP. Results from check-up radiographies are shown below.



Figure 4. 10 days after the first administration of PRP



Figure 5. 10 days after the second administration of PRP



Figure 6. 10 days after the third administration of PRP

Results show evidence of primary union in the caudal part of the fracture line, ten days after the administration of PRP (Figure 4). Ten days after the second administration of PRP, narrowing of the fracture line and advanced healing were observed (Figure 5). On the tenth day after the third administration, even more advanced healing was observed (Figure 6).

DISCUSSION

Various factors influence fracture healing. These can be grouped as fracture-related factors: open fracture, infection, fracture type; patient-related factors: diabetes mellitus, systemic infection and iatrogenic factors: pre-administration of drugs such as non-steroidal anti-inflammatory drugs and steroids.

Clinical and experimental results in literature on the osteogenic potential of PRP are controversial. A number of authors have reported beneficial effect of PRP on bone regeneration in case reports (Froum SJ et al. 2002; Perazzi A. et al 2013) and experimental studies (Fuerst G. et al. 2004) while others have found no effect of PRP on bone union. Several reasons that could explain the controversial results of clinical and experimental results in PRP administration are given. It is assumed that PRP alone cannot induce bone formation but can support osteogenesis in the presence of precursor cells (Froum SJ et al 2002; Say F. et al 2014).

Cancellous bone grafts taken from the iliac crest have osteogenic, osteoinductive and osteoconductive properties. They are widely used in clinical practice (Elshaha A. et al 2006). As alternative to autologous bone grafting, a number of researchers have reported injecting bone marrow, bone morphogenetic protein, PRP and demineralised matrix (Bielecki, T. et al. 2008; Braly. H. L. et al. 2013; Calori, G. M. et al. 2008; Raghoobar GM et al 2005). PRP was first used in 1987 in cardiac surgery to prevent massive blood transfusion (Ferrari, M. et al. 1987). More than 30 bioactive proteins are present in the platelet alpha granules (Anitua, E. et al. 2004).

Transforming growth factor beta (TGF) and platelet derived growth factor (PDGF) are growth factors that play a major role in fracture healing (Schmitz J. P. et al. 1986). Growth factors activate some of the cells that have a major role in tissue repair, provide soft tissue healing and stimulate bone regeneration (Lucarelli, E. et al. 2003).

Bielecki et al., 2008 in one of their studies, have administered a single dose of PRP in 32 cases of delayed union or non-union fractures. They found union in all cases of delayed union and in 65% of the case of non-union of bone fragments. They recommend the use of PRP within 11 months following surgery.

Unlike them, we administered PRP three times every 10 days. Following administration, the platelets release 70% of the deposited GF within the first 10 minutes and nearly 100% within the first 1 hour. Insignificant amount of GF excretion still continues up to the eighth day, till the platelets die, since under normal conditions lifespan of platelets is 8-10 days.

In another study, Calorie et al. 2008 compared the results from two groups of patients with surgical revision and administration of PRP or BMP (bone morphogenetic protein). In 120 cases of atrophic non-union, complete union was achieved in 86.7% of the patients in the BMP group and 68.3% of the patients in the PRP group.

Griffin et al. 2009 analyse the use of PRP in clinical trials and report that the use of PRP is safe but no clinical evidence has been found on its beneficial effect when used in acute or delayed union fractures. Say F et al., 2014 have administered PRP three times every week in patients with delayed union and non-union and found improvement in 75% of the patients in the delayed union group and no such for the patients in the non-union group.

CONCLUSION

In conclusion, complete bone union was achieved in this study. Although the study was pilot, we can conclude that PRP injections can be administered in patients with non-union fractures.

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