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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Mechanical properties of tibial bone surgery treated with autogenous platelet-rich plasma (PRP) in rabbit

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Abstract

Platelet-rich plasma (PRP) is a volume of plasma fraction of autologous blood having platelet concentrations above baseline. The aim of this study was to determine the effect of the platelet-rich plasma (PRP) on mechanical property in the defect of tibial bone in rabbit. In this study, 24 female adult New Zealand rabbits were divided into two groups (I and II) of 12 rabbits each. After general anesthesia, a hole (1×4 mm) in diameter and depth was created in the middle of the right tibia shaft with low speed dental bit. In group I defect was left empty and the rabbits in this group received no PRP. In group II defect was filled with autogenous PRP gel. After 50 days of surgery, the bone defect was evaluated by means of mechanical factor. There was a significant increase in compression properties of tibial bone in group II as compared to the other group (I). The results of this study showed that the PRP can increase the mechanical property of tibial defect in rabbit.

Keywords: Tibial bone; platelet-rich plasma; mechanical properties; rabbit

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Introduction

Although mechanical stabilisation has been a hallmark of orthopaedic surgical management, orthobiologics are now playing an increasing role. Platelet-rich plasma (PRP) is a volume of plasma fraction of autologous blood having platelet concentrations above baseline. The use of platelet-rich plasma (PRP) in tissue regeneration is a developing area for clinicians and researchers and has been employed in various fields of surgery. Although the growth factors and mechanisms involved are still poorly understood, the easy application of PRP in clinical practice and its possible beneficial outcome, including bone regeneration, reduction of bleeding and rapid tissue healing, hold promise (Man et al., 2001). PRP is defined as an "autologous concentration of platelets in a small volume of plasma" and is considered to be a rich source of autologous growth factors (Marx, 2004). The contribution of PRP to the bone healing process is thought to be based on the growth factors (GFs) stored in it. The growth factors reported to be present in PRP are platelet derived growth factor (PDGF), transforming growth factors-β (TGF-β), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), insulin growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), as well as three blood proteins known to act as cell adhesion molecules for osteoconduction (Ganio et al., 1993; Einhorn, 1998; Sanchez et al., 2003; Tozum and Demiralp, 2003). PRP is a new approach in tissue regeneration for clinicians and researchers. It is used in various surgical fields, including head and neck surgery, orthopedic surgery, otolaryngology, cardiovascular surgery, as well as oral and maxillofacial surgery (Man et al., 2001). The filling of osseous defects after trauma, infection, non-union or tumour resection still represents a major problem in orthopedic

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surgery. Segmental bone loss or non-union results in the vast demand for new bone to replace and restore the function of the lost bone (Bauer and Smith, 2002). The objective of this investigation was to evaluate the effect of PRP on bone healing and its mechanical property.

Materials and Methods

Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the United States guidelines (United States National Institutes for Health Publication no. 85-23, revised in 1985) and our ethical committee on animal care approved the protocol. In this study, 24 New Zealand, 26-28 week old and weighing 2.5-3 kg female rabbits were divided into two groups (I and II) of 12 rabbits each. All rabbits were kept in individual cage during the whole experimental period, under strict hygienic conditions and fed with standard ration for rabbits and water ad labium.

Autogenous PRP Preparation

With the animal under anesthesia, 4.5 ml blood sample was collected by means of cardiac puncture, which was placed in an appropriate assay tube and centrifuged during 15 minutes at 1800 rpm. Heavier red blood cells were collected deep in the tube, while plasma remained on top. Plasma was including four layers: first layer above red blood cells was the platelet-very rich plasma (PVRP); the second layer was the platelet-rich plasma (PRP), the third layer was the plasma with moderate platelet content (PMP), the fourth layer was constituted of poor platelet content (PPP). PVRP and PRP layers were aspirated and added by 10 µl of 10% calcium chloride solution for inducing coagulation. PRP gel was ready to fill bone defect in experiment group. The whole PRP preparation procedure was performed under strictly sterile conditions (Kobaiashi Wilson et al., 2006).

Surgical Procedure

Under intramuscular Diazepam (1mg/kg) premedication and intravenous Ketamine hydrochloride (35mg/kg) and Xylazine (5mg/kg) general anesthesia, right tibia was routinely prepared for surgery. A 3cm longitudinal skin incision was made. The space between extensor and flexor muscles groups was dissected, providing a wide view of tibial bone and a hole of 1×4 mm in diameter and depth was created in the middle of the right tibia shaft with low speed dental bit. The cavity then was washed carefully with a physiological saline solution. In group I defect was left empty and the rabbits in this group received no PRP. In group II defect was filled with autogenous PRP gel. Muscle, fascia and skin were closed by routine suturing.

Post Operative Care

Antibiotics (penicillin G procaine 40000 IU/kg IM, bid), dexamethasone (0.6 mg/kg, IM) and analgesic such as tramadol hydrochloride (5 mg/kg, IM, bid) were administered as post-operative care for three days. No operative or postoperative complications were encountered. All of the rabbits tolerated surgery well and survived until the final experimental time. No wound opening or infections were observed.

Compressive Pressure Analysis

All rabbits were euthanized with an intravenous injection of an over dosage of thiopental sodium, causing a quick and painless death, at 50th day of operation. The tibia bones were taken out from each animal and then were sent to the laboratory to examine Mechanical Properties. All mechanical testing were performed using a Zwick/Roell 2005 (Fig. 1) with a crosshead speed of 0.01 mm/s. A load-distance curve was recorded to obtain the mechanical properties. Load bearing was obtained with maximum load recorded of the linear portion of the load-distance curve. All specimens were tested for each group, and data were represented as mean±standard deviations (SD).

Fig. 1: Mechanical machine (Zwick/Roell)

Table 1: Load bearing of groups I and II (Mean ± SD)

Group	Load (N)
I	86.35±3.05^{b}
II	122.8±2.15^{a}

Statistical analysis

Statistical analysis was carried out on the load bearing data using one way analysis of variance with the software program SPSS for Windows, version 16. $P<0.05$ was considered to be statistically significant.

Results

There was a significant increase in compression properties of tibial bone in group II in 50 days after surgery as compared to other group. The biomechanical testing showed 86.35±3.05N in group I and 122.8±2.15N in group II recorded at 50th day after surgery.

Discussion

Most have been related to the use of PRP in oral and cranial surgery. Currently, it is common to combine the platelet-rich material with autograft, allograft, demineralised bone matrix or other graft material to fill bony defects in the mandible or cranium (Marx et al., 1998). There are few clinical studies examining the role of PRP in bone healing after orthopaedic trauma (Fennis et al., 2004; Eppley et al., 2006). Although platelet concentrates have been used to promote bone healing, the underlying cellular-level mechanisms remain poorly understood. The effect of PRP on bone cells may not be due to the action of a single growth factor but instead, to the synergistic effects of the many growth factors derived from platelets. It has been shown that addition of either PDGF or TGF in cell cultures did not induce an enhanced cell proliferation similar to that observed in the presence of a platelet concentrate (Castelnovo et al., 2000). Also, applying antibodies against PDGF suppressed only partially human bone cell proliferation induced by platelet-derived growth factors (Gruber et al., 2002). Tayapongsak et al. (1994) showed a 50% decrease in time for remodeling and graft incorporation with the addition of autologous fibrin adhesive to particulate cancellous bone and marrow grafts in major mandibular reconstruction surgery (Tayapongsak et al., 1994). PRP stimulates bone formation through its osteoinductive effects with its rich source of growth factors. Gandhi et al. (2006) evaluated the effects of local PRP at mid-diaphyseal femur fracture on a diabetic rat model. Gandhi et al. (2006) found a significant decrease in growth factors at the fracture site of these diabetic rats when compared with healthy and nondiabetic rats. Furthermore, PRP delivery at the fracture site normalized the early parameters of cellular proliferation and chondrogensis while improving the late parameters of mechanical strength of patient with diabetes (Gandhi et al., 2006). The histomorphometric evaluation showed a tendency for slightly more bone when PRP was combined with autogenous bone as compared to autogenous bone alone, but this difference was not significant.

Plachokova et al. (2007) showed that PRP had no effect on early bone healing in addition to an osteoconductive material (dense HA/β-TCP particles) in a rat model. Lynch et al. (1991) reported on the effects of combined recombinant PDGF-IGF on bone formation around roughened titanium implants in the dog model. Direct application to the implants stimulated the regeneration of bone in periimplant sites in the early phase of healing. Both the implant-bone contact and fill of the periimplant spaces were improved. An immediate extraction- implant placement dog study observed a twofold increase in implant-bone contact and in areas of bone adjacent to the implant surface when using a recombinant PDGF and IGF-I gel beneath an e-PTFE membrane (Lynch et al., 1991). Because PRP is prepared from autologous blood it is inherently safe, and any concerns regarding transmission of diseases such as HIV, hepatitis, or Creutzfeld-Jakob disease, or of immunogenic reactions that exist with preparations of allograft or xenograft, are eliminated (Man et al., 2001). Although *in vitro* studies have shown a significant relationship between the application of PRP and the proliferation of adult mesenchymal stem cells, the proliferation of fibroblasts and the production of extracellular matrix (Slater et al., 1995; Liu et al., 2002; Schliephake, 2002; Lucarelli et al., 2005), its use in trauma and orthopaedic procedures still lacks the support of randomized controlled trials. Further research in basic science and clinical use is needed to define the treatable musculoskeletal conditions, methods of administration and the ideal patient population.

Conclusion

The results of this study show that the platelet rich plasma can increase the mechanical property in tibial defect in rabbit.

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