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Original article

EFFICACY OF PLATELET-RICH PLASMA IN EXPERIMENTAL INSTRUMENTED INTERBODY SPINAL FUSION

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Summary

Introduction. This study aimed to analyze the influence of Platelet-Rich Plasma (PRP) on bone growth in experimental instrumented interbody spinal fusion (IISF).

Methods. 16 adult sheep underwent IISF at L3–L4 level using a cylindrical threaded expanding titanium cage with morselized iliac crest cancellous autograft. In 8 animals (Group I) this was augmented with PRP, in the remaining 8 (Group II), it was not. Radiographs of the spine were taken preoperatively and at 1, 3, and 6 months, moreover autoptic vertebral samples were obtained and evaluated histologically and by CT scan at 8 months.

Results. Histological analysis revealed more evident new bone formation with bony bridge into the cages in Group I than Group II. There were relevant differences between the groups with regard to interbody fusion calculated using trabecular bone score ($p<0.05$).

Introduction

The most commonly studied platelet-released growth factors in spinal fusion are platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-b), along with their isomers [1,2]. Furthermore, epidermal growth factor, basic fibroblast growth factor, fibronectin, insulin-like growth factor-1, PDGF, serotonin, TGF-b1, and thrombospondin-1 may play a role in enhancing osteoblast proliferation [3]. Platelet-Rich Plasma (PRP) is currently used in different surgical procedures with the assumption that platelet activation may release several growth factors that are involved in the bone-healing process. In recent years, several investigators have shown beneficial effects of PRP in bone and soft-tissue healing processes in oral, maxillofacial, and orthopedic surgery [4,5,6,7]. The results in spinal fusion applications are limited and controversial. Weiner and Walker reported a detrimental effect of platelet-rich plasma

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on autograft in patients who had posterolateral spine fusion [8]. In a recent review article, platelet gel was given a grade 2B recommendation (weak recommendation; alternative approaches likely to be better) as an enhancer of the effect of the autograft for both posterior lumbar fusion and anterior lumbar interbody fusion [9]. However, the rate of fusion seems to depend, to a large extent, on the investigator's interpretation [10,11]. Given that there is no single definition of what constitutes fusion, it is difficult, if not impossible, to compare the results of different studies. Moreover, it is difficult to determine radiographically whether fusion has occurred. Therefore, in this study, the most stringent criteria for fusion have been used, along with a classification that allows for the distinction of minor changes on CT-scan. The purpose of the current study is to explore the potential of PRP to increase the rate of bone formation in a graft and enhance the density of the bone formed at 8 months in lumbar instrumented interbody fusion.

Material and Methods

Animal and Experimental Design

This study was approved by the Institutional Animal Care and Use Committee of the University of Sassari. Eight skeletally mature sheep, weighing between 37 and 46 Kg were used. One cylindrical threaded expanding titanium cage (Proconcept-SA, Orange, France) was inserted into each spine and packed with morselized iliac crest cancellous autograft (Figure 1). The graft material included autologous spongy bone harvested from iliac crest in 8 animals augmented with PRP (Group I) and 8 animals with the same autologous bone graft without PRP (Group II). Radiographs of the lumbar spine (anterior and lateral views) were taken preoperatively and postoperatively at 1, 3, and 6 months, respectively (Figure 2). All sheep were humanely euthanized at 8 months postoperatively. The lumbar spines were harvested *en bloc* and evaluated by, helical CT scanning (Toshiba Aquilon Multislice, four slice per rotation), and histologically. The CT scanning parameters were as follows: slice thickness, 0.5 mm; voltage, 135 kV; amperage, 250 mA; and rotation time, 1.5 sec. The image data were reformatted on a SUN computer workstation using G.E. Advantage Window 3.1 software. CT -scans of the fused segment were taken in both the coronal and the sagittal planes and in 3-D view. We developed a classification to optimally determine the presence of bridging trabecular bone between the vertebral bodies. The status of the interbody fusion was quantified using

Figure 1: One cylindrical threaded expanding titanium cage (Proconcept-SA, Orange, France) was inserted into each spine and packed with morselized iliac crest cancellous autograft.

the 'bridging trabecular bone scale': visual rating from CT-reformatted images using a percentage based on the total length of the device/graft-vertebra interface superiorly and inferiorly. Rating was determined by combining both the superior and inferior edges of the device/graft-vertebra interface to yield an overall percentage of bridging bone (Table 1). Fusion was regarded as complete if there was no evidence of nonunion on plain radiograph and if the bridging trabecular bone score was 3, 4 or 5.

PRP Preparation

Immediately prior to surgery, 4 mL of venous blood were collected from the sheep in the study group and placed under vacuum for 30 minutes to obtain autologous procoagulant (thrombin). A second blood sample of 54 mL was taken and mixed with 6 mL of anticoagulant citrate dextrose-A. It was then submitted to a centrifugation process for 14 minutes (2500 and 2300 rpm for 3 and 9 min, respectively, with 2 min of interval between the 2 phases) to generate about 10 mL of PRP. During surgery, PRP was mixed with 0.7 mL of autologous thrombin and 0.3 mL of 10% calcium chloride and loaded onto autologous iliac crest spongy bone. Samples of PRP and venous blood were submitted for machine platelet counts and a smear with Giemsa staining for a manual count. Two additional PRP smears were stained with monoclonal antibody stains (Santa Cruz Biotechnology, Santa Cruz, Calif.). One

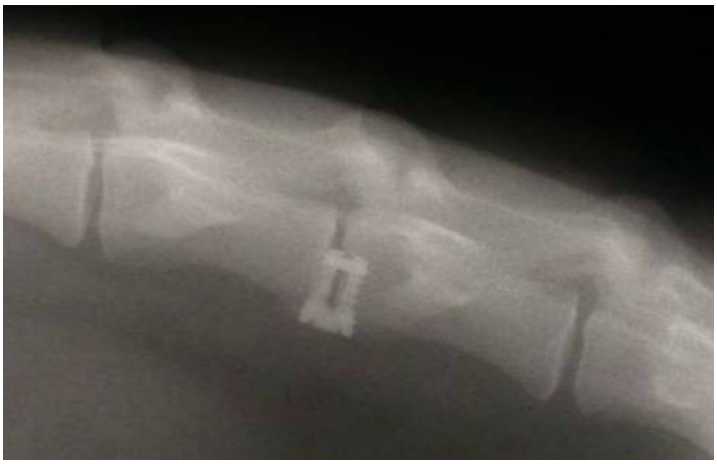

Figure 2: Radiographs of the lumbar spine (anterior and lateral views) were taken preoperatively and postoperatively at 1, 3, and 6 months, respectively.

5	100%(complete bridging)
4	76–99%
3	51–75%
2	26–50%
1	1–25%
0	0% (no bridging)

Table 1: Bridging trabecular bone scale. Visual rating from CT-reformatted images using a percentage based on the total length of the device/graft-vertebra interface superiorly and inferiorly. Rating was determined by combining both the superior and inferior edges of the device/graft-vertebra interface to yield an overall percentage of bridging bone

was stained for PDGF and the other for TGF-b. A sample of the autogenous graft material was placed in formalin, processed with a slow formic acid decalcification and stained with monoclonal antibodies to identify PDGF receptors (PDGFr) and TGF-b receptors (TGF-br)

Statistical Analysis

The number of sheep with union were presented with 95% confidence intervals (95% CI), and the differences between the efficiencies of union in the normal arthrodesis group and the PRP group at 1, 3 and 6 months after surgery were analyzed using the Mann-Whitney U test. All the other data were expressed as means and standard deviations. The correlation of bone volume and the state of bone union was described by the correlation ratio. The differences of bringing trabecular bone score were analyzed using unpaired t tests. The criterion for significance was $p < 0.05$.

Results

There were neither intraoperative nor postoperative complications related to the surgical procedures. There was no difference between groups in interbody healing on plain radiographs achieved at 1, 3, and 6 months ($p = 0.741$, $p = 0.663$, $p = 0.951$), respectively. At 8 months, CT imaging of specimens from Group I showed that disc space had begun to consolidate more completely with the filling in of trabecular bone around the implant (Figure 3); specimens harvested from this group showed a mean of 4.0 on bringing trabecular bone score. Microscopic examination revealed complete fusion with continuous bony bridge from anterior and posterior vertebral endplates through the fenestration inside the implant (Figure 4). CT imaging of specimens from Group II sheep taken at the same time, revealed moderate bridging callus within and outside the cage; specimens harvested from this group showed a mean of 3.62 on bringing trabecular bone score. Microscopic evaluations showed that lamellar bone increased within the cage; immature bone starting from vertebral endplates penetrating through the fenestration of the cage was noted, but a continuous bony bridge had not formed (Figure 5). There were relevant differences between

Figure 3: On CT imaging at 8 months specimens from Group I sheep showed that the disc space had begun to consolidate more completely with the filling in of trabecular bone around the implant

the groups with regard to interbody fusion, calculated using trabecular bone score ($p<0.05$). Processed core bone specimens of each group at 6 months demonstrated a continued production of TGF-b. Monoclonal antibodies identified TGF-b but not PDGF by marrow stem cells and endosteal osteoblasts. The TGF-b positive cells were seen to be concentrated on the trabecular bone endosteal surface, on the periosteal surface, and within active marrow stem cells. The results of this study suggested that PRP addition accelerated the rate of bone formation and the degree of bone formation in a bone graft for at least the first 8 months. This study also showed that cancellous marrow grafts contain cells bearing PDGF and TGF-b receptors as the probable targets of PRP, which are intimately involved in the bone regeneration process. The endosteal location probably represents osteoblasts or preosteoblasts, which are known to be activated by PDGF and TGF-b.

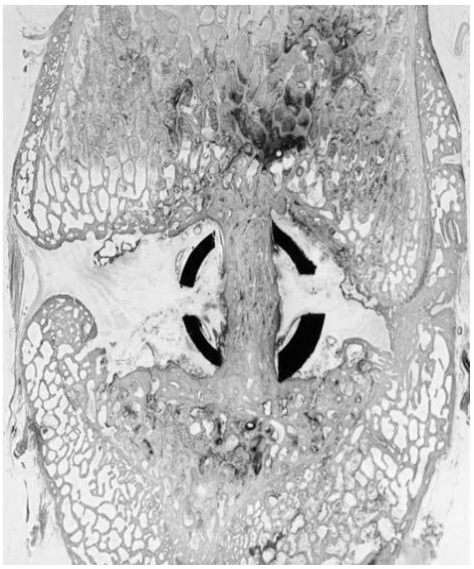

Figure 4: Microscopic examination revealed complete fusion with continuous bony bridge from anterior and posterior vertebral endplates through the fenestration inside the implant.

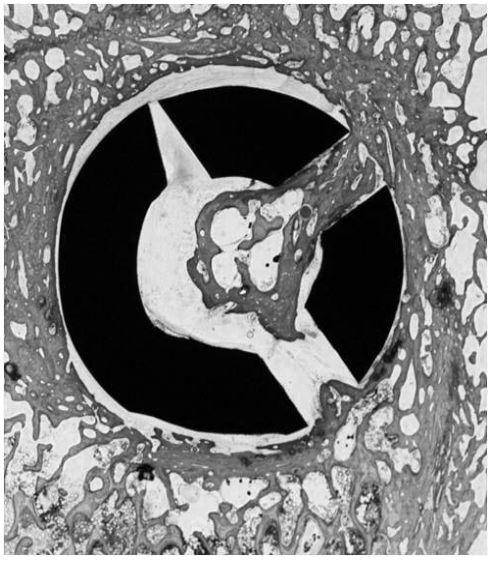

Figure 5: Immature bone starting from vertebral endplates penetrating through the fenestration of the cage was noted, but a continuous bony bridge had not formed.

Discussion

The enhancement of healing by the placement of a concentration of autologous platelets at the site of surgery is supported by basic science studies [12,13,14,15]. Research has revealed that the role of platelets is much more complex than simply 'plug' formation; they are responsible for actively extruding growth factors, which initiate bone formation. PDGF is a glycoprotein who was first described in the alpha granules of platelets; it seems to be the first growth factor present in a wound, and it initiates connective tissue healing, including bone regeneration and repair. The most important specific activities of PDGF include mitogenesis, angiogenesis and macrophage activation. The term transforming growth factor beta (TGF-b) is applied to the superfamily of growth and differentiating factors of which the bone morphogenetic protein (BMP) family, containing at least 13 described BMPs, is a member [16]. Like PDGF the TGF-bs are synthesized and found in platelets and macrophages; when released by platelet degranulation or actively secreted by macrophages, they act as paracrine growth factors, affecting mainly fibroblasts, marrow stem cells and the preosteoblasts. However, each of these target cells has the ability to synthesize and secrete its own TGF-b proteins to act on adjacent cells in a paracrine fashion or act on itself as an autocrine growth factor [17]. TGF-bs therefore represent a mechanism for sustaining a long-term healing and bone regeneration module and even evolve into a bone remodeling factor over time. The most important functions of TGF-bs seem to be the chemotaxis and mitogenesis of osteoblast precursors, and they also have the ability to stimulate osteoblast deposition of the collagen matrix of wound healing and of bone [18]. In addition, TGF-bs inhibit osteoclast formation and bone resorption, thus favoring bone formation over resorption by two different mechanisms [19]. Beneficial effects of PRP on spinal fusion were reported by Hee et al. [20] in a prospective study comparing transforaminal lumbar interbody fusion (TLIF) with autograft. They demonstrated faster fusion but no increase in fusion rates. They included both 1-level and 2-level fusions. Fusion was assessed on X-ray. Therefore, we did not include plain radiographs in the diagnosis of fusion. The interpretation of fusion on the basis of static plain radiographs is subject to controversy [21, 22]. We preferred to use thin-slice CT-scan with reconstruction images in the coronal and sagittal planes, allowing for the determination of a degree of fusion rather than distinguishing between fusion and nonfusion, and to establish an eventual positive effect of PRP. We therefore developed a classification for optimal determination of the presence of bridging trabecular bone between the vertebral bodies. Our results indicate that the addition of PRP to a bone graft in experimental instrumented interbody spinal fusion provides additional benefit leading to solid bone union.

Conclusion

This article confirms the role of growth factors in clinical bone grafting and elucidates the mechanism of action and the points of influence that the fundamental growth factors PDGF and TGF-b exert on bone regeneration. The amplification of PDGF and TGF-b through the technique of platelet sequestration and concentration into a PRP is seen as an useful and practical tool for enhancing the rate of bone formation and the final quantity of bone formed. Our study presented evidence that these growth factor additions to bone grafts produce a quantifiably enhanced result in comparison to grafts performed without them.

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