Experimental study of the effect of platelet-rich plasma on osteogenesis in rabbit

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Platelet-rich plasma (PRP) is produced from a patient’s own blood by centrifugation, and PRP contains several kinds of growth factors in high concentration such as platelet derived growth factor (PDGF), transforming growth factorβ (TGFβ), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), and so on. These growth factors have proved to offer an improved quality and speed of healing for both hard and soft tissue. In this study, PRP compounded with porous bioceramic was used to repair a bone defect in rabbit radius. The radiographic and histological qualitative and quantitative observations were performed to evaluate osteogenesis.

METHODS

Platelet-rich plasma preparation
The whole process of making PRP requires strictly sterile. After anaesthesia, 5 ml blood were drawn from rabbit’s central ear artery by a 10 ml injection syringe with 1 ml citrate phosphate dextrose (CPD), agitated for 15 seconds, then transferred into a centrifugal chamber and centrifuged at 3650 r/min (1500 × g) for 10 minutes. All supernatant and the upper 1 - 2 mm red blood cells were pipetted into another centrifugal tube, which was centrifuged at 3000 r/min (1000 × g) for 10 minutes. After discarding about three-quarter of supernatant, the remainder, about 0.8 ml, agitated for several seconds, was PRP. Then a sterile 2 ml syringe was required, drawing 0.8 ml of PRP, 0.2 ml of the coagulate (mixture of 1 ml of 10% calcium chloride mixed with 1000 units of coagulase), and 0.2 ml of air to act as a mixing bubble. The syringe was agitated for about 10 seconds to initiate clotting and formed platelet gel.

Animal experiment
Twenty-four New Zealand white rabbits, weighing 2.5 - 3.0 kg were used in this study. Left or right foreleg was randomly chosen as the experimental side, the other side was the control. After sterilization, a 10 mm length of bone defect was created in the mid-upper part of radius. The bioceramic associated PRP was fit in the defect for the experimental side. Control limbs received only bioceramic. After operation, animals have normal diet, neither antibiotic nor leg fixation. Rabbits were sacrificed at 2, 4, 8 and 12 weeks. At each interval, two rabbits were chosen for radiographic observation, two for light microscope and image analysis, and two for electron microscope.

Platelet counts and growth factor content measurement
Platelet counts and growth factor content in each sample, including whole blood and PRP, were measured. A standard hemocytometer was used to measure platelet counts and commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D System, Inc., USA) were used to quantify the concentrations of PDGF-AB, TGF-β1, according to the manufacturer’s instructions.

Observation for osteogenesis
Radiography, histology including light microscopy and transmission electron microscopy, image analysis were performed on all operated forelegs for all intervals.

Statistical analysis
SPSS V. 10.0 software was performed for statistical analysis. Data are reported as mean ± standard deviation at a significance level of \( P < 0.05 \), and \( t \) test was used to compare data between the experimental and control sides.

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for all intervals.

RESULTS

Platelet count, concentration of PDGF-AB and TGFβ
Platelet concentration in PRP is $9.3 \times 10^{11}/L$, which is 3.2 times of the platelet concentration ($2.9 \times 10^{11}/L$) in the whole blood. Concentration of PDGF-AB and TGFβ was higher in PRP than that in the whole blood (Table).

<table>
<thead>
<tr>
<th>Growth factor (ng/ml)</th>
<th>Whole blood ($n=24$)</th>
<th>PRP ($n=24$)</th>
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<tbody>
<tr>
<td>TGFβ</td>
<td>$42.14 \pm 12.47$</td>
<td>$142.85 \pm 32.26^*$</td>
</tr>
<tr>
<td>PDGF-AB</td>
<td>$29.36 \pm 9.31$</td>
<td>$92.25 \pm 18.21^*$</td>
</tr>
</tbody>
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* $P=0.000$ versus whole blood.

Radiographs
After 2 weeks, radiographs showed that the cut ends were evident in both operated limbs. After 4 and 8 weeks, in the experimental side, a thicker layer of callus, which connected with host cortical bone, packed the bioceramic. In the control side, callus formation was observed mainly in the double ends of bioceramic, flocculent and obscured radiodensity shadow was showed in the defect (Fig.). After 12 weeks, cortical bone and medullary cavity were observed in the experimental side, while the callus partially covered the defect in the control side.

Electron microscopy
After 2 weeks, in comparison to the control side, the amount of mitochondria and rough endoplasmic reticulum (RER) was much more, collagen around osteoblasts arranged more closely, and in better order in the experimental side. After 4 weeks, the quantity and maturity of osteoblasts in the experimental side were better than in the control side. After 8 weeks, the lamellar bone was formed with numerous and some local deposition of calcium salt was observed in the experimental side. After 12 weeks, numerous osteoblasts and a high amount of lamellar bone in the experimental side were observed with a few of bone lacuna, but immature bone tissue in the control side.

Histomorphometry
The new bone formation increased significantly with time in both sides and was higher in the experimental side than that in the control side. At the 2nd week, the percentage of new bone formation inside bone defects in the experimental groups was $(12.47 \pm 6.32)\%$, and $(8.25 \pm 3.17)\%$ in the control groups ($P=0.012$). At the 12th week, the percentage of new bone formation inside bone defects in the experimental groups was $(68.23 \pm 11.65)\%$, in the control groups it was $(50.19 \pm 14.46)\%$ ($P=0.001$).

DISCUSSION

In the experimental side, new fibrous tissue and bone formation were observed in the areas adjacent to the cut ends of the host bone after 2 weeks. After 4 and 8 weeks, part of bioceramic was degraded, and the experimental cavities were filled by formed new bone. A large number of osteoblast and new bone were observed in the pores of bioceramic. On the surface of defect, continuous bony callus was formed, which bridged host bone and bioceramic. After 12 weeks, defects were covered completely by cortical bone, which connected the host cortex. In the control side, compared to the contemporary experimental, formed new bone was immature, and the quantity of osteoblast and new bone in the center of bioceramic was low.

Studies have demonstrated that growth factors, such as PDGF, TGFβ, IGF, VEGF, etc., can stimulate bone formation and bone healing and these results have made them candidates for use in orthopedic surgery. In bone regeneration, PDGF and TGFβ are the most mediators. PDGF induces proliferation of undifferentiated mesenchymal cells (increase of the population of healing cells), angiogenesis (endothelial mitosis into functioning
capillaries, and macrophage activation (removing factors of the wound site and a second-phase source of growth factors to continue repair and bone regeneration). It also enhances bone regeneration in conjunction with other growth factors, such as IGF-1, TGFβ. TGFβ, can enhance the chemotaxis and mitogenesis of osteoblast precursors, and inhibit bone resorption. Marx and colleagues reported that PRP accelerated bone repair due to high concentration of PDGF, TGFβ and other growth factors.

In 1995, Slater et al. used human platelet concentrate as a supplement of basic medium to support the proliferative and functional activity of human fetal osteoblast-like cells, and found that platelet-supplemented medium stimulates proliferation and maintains the differentiated function of human osteoblast-like cells. They reported that platelets may play an important role in healing of fracture and also may be useful as a cheap autologous source of multiple growth factors to enhance osteoblast proliferation in vivo and in vitro. Autologous platelet-rich plasma gel was used first with apparent clinical success in the Department of Oral and Maxillofacial Surgery by Whitman et al., and they concluded that platelet gel is a good autologous grant, and overcomes the disadvantages of fibrin glue and recombinant growth factors. Recently it has undergone a significant increase in use as adhesive with cancellous bone particles in oral and maxillofacial surgery bone grafting procedures.

In this study, we use porous bioceramic compounded with PRP to repair long bone defect, and the results show that PRP addition accelerates new bone formation and bone healing at least in 12 weeks in rabbit. At the 4th week, under light microscopic observation, a large number of osteocytes and neoformed bone were seen in the pores of implant in the experimental side, while there were mainly fibrous tissues formed in the pores in the control side. At the 8th week, we can see on X-ray radiographs that the bioceramics were packed by a thick layer of dense material (new bony callus) in the PRP side, in the control side, there was only flocculent shadow around the implants. At the 12th week, implants were covered completely with cortical bone in the PRP side, while cortical bone was not continuous in the control side.

As a result, it was suggested that PRP would be a good candidate for use to repair long bone defect in orthopedics. The mechanism of PRP’s repairing bone defect may be, as Marx and colleagues reported, that the initiation of bone regeneration starts with the release of PDGF and TGFβ from the degradation of platelets in the graft. PDGF stimulates mitogenesis of marrow stem cells and transfer endosteal osteoblasts into the graft to increase their number. It also starts an angiogenesis of capillary budding into the graft by inducing endothelial cell mitosis. TGFβ initially activates fibroblasts and preosteoblasts to mitoses and increase their number, as well as promoting their differentiation towards mature functioning osteoblasts. Continued TGFβ secretion influences the osteoblasts to lay down bone matrix and the fibroblast to lay down collagen matrix to support capillary in growth.

PRP is a concentration of autologous platelets, and it poses no risk for transmissible disease in human being. And growth factors of platelet are in their normal ratios, which result in most effective interworks. That distinguishes PRP from recombinant growth factors such as recombinant human PDGF or the recombinant human BMPs, which are single growth factors often in super physiologic doses. In addition, PRP can be produced easily and rapidly.

However, at present there are some doubts in bone repair PRP. Some questions yet remain: how and when growth factors are secreted by platelets, what is their viability, what is the correlation between their concentration and the number of platelets, and so on. Further investigations are necessary to clarify these points.

REFERENCES


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