Purpose: Although vascular bundle implantation in the necrotic bone induces angiogenesis, it has not been an established treatment of avascular necrosis of the bone, because revascularization is slow and unreliable. Platelets contain many growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), Interleukin-1 (IL-1) and basic fibroblast growth factor (bFGF). These growth factors contribute to increase vascularity and bone regeneration. Therefore, we expected platelet-rich plasma (PRP) to accelerate angiogenesis of vascular bundle implantation. The aim of this study was to evaluate acceleration of angiogenesis in a necrotic bone combined with vascular bundle implantation and a single injection of platelet-rich plasma (PRP) and to search the possibility of a clinical application of PRP for osteogenesis in the necrotic bone.

Materials and Methods: Adult male Japanese white rabbits weighing from 3.0 to 3.5 kg were used in this study. Ten ml of autologous whole blood which was drawn off from each rabbit adding 1 ml of 3.8% citrate phosphate dextrose (CPD) was centrifuged at 1500 rpm for 10 minutes to separate the plasma containing platelets from the red cells and then centrifuged for an additional 5 minutes at 2500 rpm to separate 1ml PRP from the platelet poor plasma (PPP). A 2x2 cm segment of the bone was harvested as a free bone graft. The bone was frozen for 5 minutes in the liquid nitrogen to ensure complete cellular necrosis. A hole larger than the diameter of the saphenous bundle was made in the bone. Then, the saphenous artery and its venae comitantes were exposed and mobilized as a vascular bundle. The bundle was passed through the hole of the bone. In the experimental group, the bone was wrapped with a silicon sheet following injection of 1 ml of PRP into the hole and then transplanted subcutaneously in the thigh. Injection of 1ml of saline solution instead of PRP was used in the control group.

In both groups, neovascularization was evaluated at 1 and 2 weeks after surgery. Latex contrast medium was infused with manual pressure from femoral artery to analyze neovascularization, and then the bone was taken out and decalcificated by modified Spalteholz bone clearing technique.

An angiographic score was calculated as the ratio of circles crossed by opacified arteries divided by the total number of circles in the specimen. This angiographic score reflects vascular density in the specimens. Length of newly formed vessels was measured at 10 points perpendicular to the implanted vascular bundle at 2-week intervals. The Mann-Whitney U test was used for statistical evaluation. Values less than 0.05 were considered significant.

Result: Neovascularization was observed along the implanted vascular bundle in both experimental and control groups. At 1 week after surgery, invasion of newly formed vessels into the necrotic bone was clearly observed in both groups (Fig. 1A and B). At 2 weeks after surgery, neovascularization increased in both experimental and control groups (Fig. 2A and B).

At 1 week after surgery, the angiographic score of the experimental group (0.305±0.03: mean ± standard error) was significantly higher than that of the control group (0.176±0.08). At 2 weeks after surgery, the angiographic score of the experimental group (0.526±0.04) was significantly higher than that of the control group (0.270±0.045) (Fig. 3A).

The mean length of newly formed vessels at 1 week after surgery of the experimental group (2.070±0.65) was higher than that of the control group (0.976±0.32). At 2 weeks after surgery, the mean length of the experimental group (3.320±0.197) was significantly higher than that of the control group (1.804±0.259) (Fig. 3B).

Conclusion: Our results showed that a single injection of PRP was effective and accelerated angiogenesis in the vascular-implanted necrotic bone. A clinical application of PRP to osteogenesis of the necrotic bone may be feasible.