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Research Article

Effect of platelet-rich plasma on bone regenerate consolidation in distraction osteogenesis: An experimental study in rabbits

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ABSTRACT

Objective: The aim of this study was to determine the effect of platelet-rich plasma (PRP) on bone regenerate consolidation in a rabbit model of distraction osteogenesis.

Methods: In this study, 12 male New Zealand rabbits weighing 1600 to 2000 g were used. All the rabbits were randomly divided into two groups (n = 6 per group): PRP group and control group. A two-ring, circular external fixator was applied to the right tibia of each rabbit in both groups. After corticotomy, all the tibiae were distracted at a rate of 0.5 mm/day for 20 days. PRP was injected to the osteotomy sites on the 7th, 14th, and 21st days postoperatively. Mineral density of the new bone tissue formed in the distraction zone was measured using quantitative computed tomography in the 3rd, 4th, and 5th weeks. At the end of the 6th week, the animals were sacrificed, and the specimens were evaluated biomechanically and histologically.

Results: Microcomputed tomography assessment showed significant bone mineral density increase from the 3rd to the 6th week (62.3% and 43.7% for the PRP and control groups, respectively). In the PRP group, the measurements on the 3rd, 4th, and 5th weeks were 416 ± 29, 487 ± 9.9, and 675 ± 37.8 HU (Hounsfield units), respectively, whereas in the control group were 313, 374, and 450 HU, respectively. In the comparison of weekly measurements of the two groups, the increase in bone density in the PRP group was higher than that in the control group ($P < 0.001$). During the mechanical tests, in the PRP group, the mean torsion was 46.50°and the mean torque 0.53 Nm, while in the control group, the mean torsion was 19.33°and the mean torque 0.65 Nm. The mechanical analysis of the groups revealed significant differences in the mean maximum torsion angles ($P = 0.024$). The histological examination showed that both groups had external and internal periosteal calli. Callus tissue in four rabbits in the PRP group and two rabbits in the control group was remodeled; normal bone formation occurred and distracted bone ends were completely healed.

Conclusion: The results of the present study have indicated that PRP injection can enhance bone regenerate consolidation and increase bone mineral density during the healing process of distraction osteogenesis.

Introduction

Distraction osteogenesis is still one of the most common surgical procedures in the treatment of segmental bone defects. After tumor resection, osteomyelitis or pseudarthrosis surgery for limb lengthening is needed, and external fixators are the first-line treatment method.[1-4] This technique is advantageous in that large bone defects can be treated simultaneously with weight-bearing. However, it has some disadvantages, including infection, long consolidation time, and high economic and psychological burden due to longer hospitalization periods.[5]

Based on current knowledge, there are several studies and treatment options to reduce the bone consolidation time of distraction osteogenesis, including dynamization, ultrasound, extracorporeal shock waves, alternating electrical stimulation, and injection of various drugs.[6-9]

The current study aimed to evaluate the effect of platelet-rich plasma (PRP) injection on distraction osteogenesis radiologically, biomechanically, and histologically using a rabbit model. We hypothesized that PRP injection enhances bone consolidation and shortens the healing process.

Materials and Methods

Experimental groups

The present study used 12 right legs of 12 male New Zealand rabbits; the rabbits had a mean weight of 1795 ± 127 g (1600-2000 g). The experimental design and all procedures were approved by the Uludağ University Animal Care and Use Committee (27.03.2004/18). All animals were housed in standard cages, one animal per cage, and a standard 12-h light/12-h dark cycle was used postoperatively. The animals were fed rabbit chow and provided water ad libitum.

The animals were randomly divided into two groups (six rabbits in the PRP group and six rabbits in the control group). The rabbits were randomly numbered from 1 to 12. Those with odd numbers were included in group 1 and those with even numbers were included in group 2. All corticotomies were performed by a surgeon with at least 15 years of experience who

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had previous experience with distraction osteogenesis. The exploration and removal of the tibiae and histologic and mechanical evaluation were performed in a blinded manner.

Surgical procedure

All rabbits were anesthetized by intramuscular injection of ketamine (80 mg/kg) and xylazine (12 mg/kg). The lower extremity was shaved and sterilized, and the rabbits were placed under sterile drapes. A two-ring, circular external fixator was applied to the right tibia of each rabbit. Each ring was connected with one Kirchner wire (1 mm in diameter). Both rings were connected to each other with two rods. A small anteromedial longitudinal incision (4 mm in length) was made in the proximal tibia, and transverse corticotomy was performed. The periosteum and skin were sutured properly. Postoperatively, each rabbit had a 6-day latent period and then entered the distraction period at a target distraction rate of 0.5 mm/day.

PRP preparation

PRP was prepared using SmartPReP 2 APC + kit (Harvest, Germany). Approximately,20 mL of venous blood was obtained using a catheter, and 1 mL of citrate was required for each 20 mL. Afterward, the container was centrifuged at high speed for 14 min. At the end of this process, two fractions were formed, a red fraction on the bottom and a white fraction on the top. The remainder middle fraction, the 3-mL PRP fraction, was drawn with a special syringe and kept ready for administration (Figure 1a-b).

Radiological evaluation

The bone mineral density of the regenerate was measured by quantitative method using computed tomography (CT; Siemens Osteo Ct Somatom HiQ, Germany) in the department of radiology on the 3rd, 4th, and 5th weeks following the beginning of distraction. HU were used as the unit of measurement.[10] In the first measurement, the density of the cortical bones of the intact tibiae of the rabbits was measured and recorded as the reference value. Axial scanning was performed with 2-mm intervals from the proximal part of the distraction area to the distal cortical structure. Density measurements were performed at three different points each time, and the values were recorded. The level at which the measurements were made was marked on the external fixator to make subsequent measurements in the same area. Union evaluated by serial radiography for all rabbits (Figure 2).

Biomechanical evaluation

Biomechanical evaluation was conducted in the mechanical laboratory with a torsional tester (SMI MKII Torsion Testing Machine, TecQuipment Ltd., Nottingham, NG10 2AN, United Kingdom). For the tibia to be applied to the torsion tester, the tips of the tibiae were molded using liquid polyester. The tibiae were tested by torsional loading after complete solidification of the polyester. At the beginning of the mechanical loading with the torsion device, after the first moment of change in the digitally measured torque value, the angular values and devices measuring torque resistance were reset, and the test data started being recorded. The procedure was continued until bone fracture occurred, and the maximum moment and maximum torsional angulation of the bone were determined. The torque value indicating torque resistance is given in newton meter (Nm), and the angle unit in degrees.[11]

Histologic examination

Extracted tibiae were fixed in neutral buffered formaldehyde and processed for paraffin embedding. Paraffin sections were cut into 4-mm-thick slices using a microtome (Leica RM 2145; Leica Biosystems, Buffalo Grove, IL, USA) and stained with hematoxylin-eosin.

Regarding histological evaluation, the formation of external and internal calli, presence of endochondral and intramembranous ossification, state of vascularization in the healing area, and characteristics of the newly formed bone trabeculae were examined.

Statistical analyses

Variable distribution was measured with the Shapiro–Wilk test. In the analysis of quantitative independent data, Kruskal–Wallis and Mann–Whitney *U*-tests were used. The paired sample *t*- and Wilcoxon tests were used to analyze dependent quantitative data. Significance was set at a $P < 0.05$. A sample of five animals per group was calculated to be necessary to detect difference with statistical power of 0.80. Statistical Package for Social Sciences (SPSS) for Windows (version 12.0; IBM SPSS Corp, Armonk, NY, USA) was used in the data analysis.

Results

Bone density measurements were significantly increased during the experimental period compared to the initial values in both groups (62.3% and 43.7%, respectively) (Figure 3). In the PRP group, the values in the 3rd, 4th, and 5th weeks were 416, 487, and 675 HU, respectively, and in the control group 313, 374, and 450 HU, respectively. In the comparison of weekly measurements of the two groups, the increase in the density in the PRP group was higher than that in the control group ($P = 0.000$) (Table 1).

The tibiae of the rabbits broke in spiral oblique type at the point where the maximum torque resistance of the bone was exceeded during torsional testing. In the mechanical tests of the tibiae with distraction osteogenesis, the maximum torsion angles of the PRP group were greater than those of the control group ($P = 0.024$). There was no significant difference between the groups in terms of maximum torque values ($P = 0.589$) (Table 2).

In the control group, scattered chondrocytes, parallel to the distraction direction, were lined up in columns and appeared hypertrophied. More rarely, areas of intramembranous ossification where new bone trabeculae were formed from osteoblasts were frequently observed in the focal areas. In two rabbits, the callus tissue acquired mature bone structure, and the newly formed bone trabeculae and distracted bone ends were connected to each other. It was observed that the newly formed bone was less compact than the original bone, and the new cortex was also thinner (Figure 4a-b).

In the PRP group, similar to the control group, external and internal periosteal new bone formation was observed. Callus tissue was formed in four rabbits and gained normal bone structure, and the distracted bone ends were connected to each other. Irregular, unorganized callus tissue was observed in the internal area of the regenerate in two rabbits (Figure 5a-b).

HIGHLIGHTS

- PRP increases bone consolidation.
- PRP shortens healing process during distraction osteogenesis.
- In segmental bone defect surgery, PRP can be used to achieve good results.

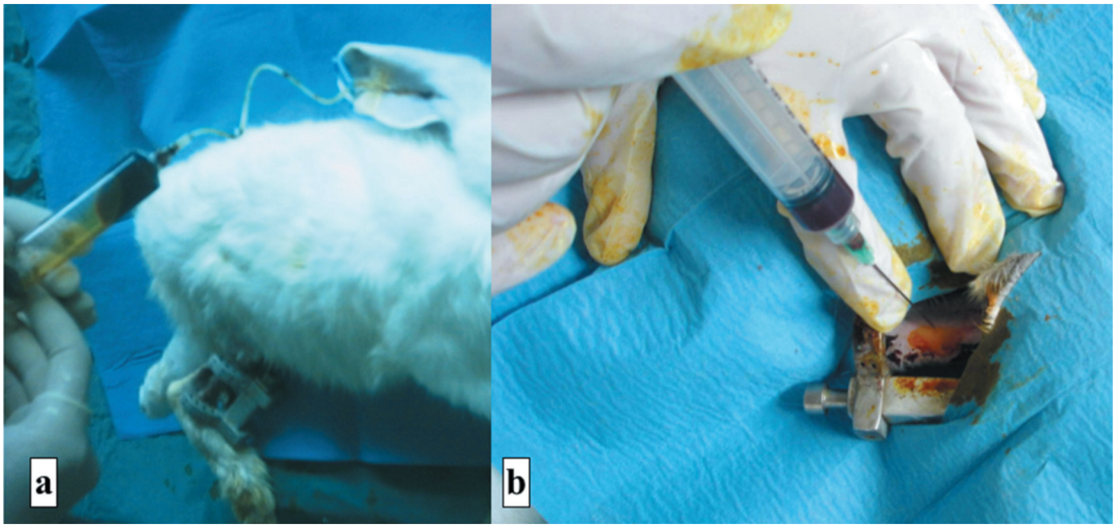

Figure 1. a, b. PRP preparation and administration.

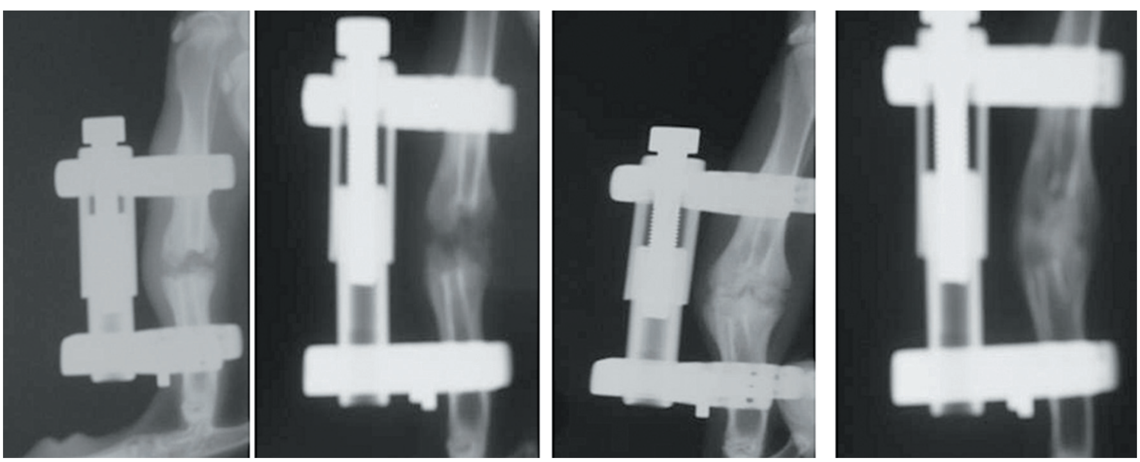

Figure 2. Serial radiographics shows the union of the bone.

Discussion

The most important finding of this study was that PRP injection enhances bone regenerate consolidation in distraction osteogenesis. As we expected, CT assessments show that PRP-injected rabbits have significantly higher bone mineral density. Histological and biomechanical test results also confirmed that PRP increases bone remodeling and newly formed bone durability during consolidation.

Several experimental studies have been reported on PRP injection into the distraction osteogenesis site to reduce consolidation phase and accelerate the healing process.[12] However, the results are still controversial, and PRP injection is not routinely used in limb lengthening surgery.[13,14] The effect of the PRP on bone healing may not be derived from only one factor, and these multiple growth factors derived from platelets have a synergistic effect.[15] Ali et al.[12] concluded that PRP injection has beneficial effects on bone regeneration, but evaluated the consolidation phase with radiography with only one investigator. These are subjective measurements; therefore, we evaluated consolidation with micro-CT measurements. Our results also show that PRP has a positive effect on bone mineralization and consolidation phase.

In the distraction osteogenesis model, intramembranous ossification has been observed during the distraction and consolidation phases.[16,17] Transchondroid bone formation has been described as a histological event.[18] During transchondroid ossification, the chondroid bone is formed by chondrocyte-like cells, with a gradual transition from fibrous tissue to chondroid bone.[18,19] Then, the bone regenerative process continues gradually during consolidation. The histological results of the current study reveal that PRP injection improves bone remodeling and enhances regenerate consolidation. A mature lamellar bone was dominant in four rabbits in the PRP group. The callus tissue was remodeled and gained normal bone structure. The bone ends were connected to each other in four rabbits in the PRP group. However,

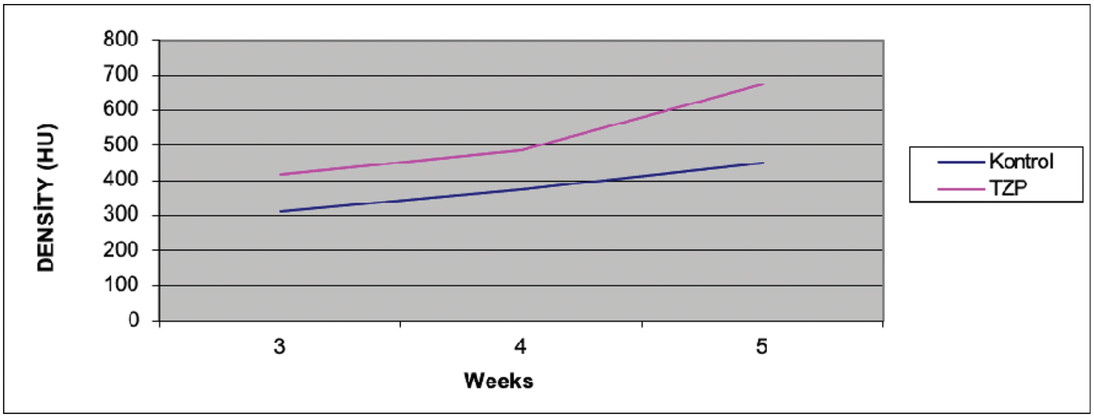

Figure 3. Change in bone regenerate density of the groups according to weeks (computed tomography measurements). HU, Hounsfield units.

Table 1. Comparison of Quantitative CT Measurements on the 3rd, 4th, and 5th Weeks between the PRP and Control Groups

CT evaluation	PRP ejected group		Control group		*P*
	Mean ± SD		Mean ± SD/n, %		
3th week (HU)	416.1	±29.1	313.3	±9.2	***0.000***[t]
4th week (HU)	487.6	±9.9	374.1	±21.6	**= *0.000***[t]
5th week (HU)	675.3	±37.8	450.0	±28.2	**= *0.000***[t]

SD, standard deviation; HU, Hounsfield Units; CT, computed tomography; PRP, platelet rich plasma
[t]t test;

Table 2. Comparison of the Biomechanical Test Results between the PRP and Control Groups

Biomechanic evaluation	PRP ejected group		Control group		*P*
	Mean ± SD		Mean ± SD/n, %		
Maximum torsion angle (degree)	46.5	±5.3	19.3	±2.8	**= *0.024***[t]
Torque (Nm)	0.53	±0.23	0.65	±0.33	= 0.0589[t]

SD, standard deviation; PRP, platelet rich plasma; Nm, newtonmetre.
[t]t test;

in the control group, the woven bone was observed with some predominant chondrocytes. In the control group, it was observed that the callus tissue gained mature bone structure and the newly formed bone trabeculae, and the distracted bone ends were connected to each other in two rabbits. Bone ends were connected to each other in four rabbits in the PRP group.

Limb lengthening surgery still has many complications and it has long-term healing process.[20] The treatment period can be reduced by increasing the distraction rate or shortening the consolidation phase.[21] However, increasing the distraction rate has potential risks and also increases the complication rates.[22,23] Thus, studies are focused on shortening of the consolidation phase with drug injection or other modalities to increase hardness or durability of the bone regenerate.[6-9] The durability of the regenerate is measured using biomechanical tests. In the period during which the mechanical test is applied, this region stretches and collagen fibers take a spiral form due to the fact that the regenerate is not yet mature and has an elastic structure. Thus, the bone can tolerate more torsion angles before reaching the torque strain that causes fracture. In our study, the mechanical test showed that the durability of the lengthened rabbit tibia was increased following PRP injection.

Bou Assi et al.[24] previously reported effect of PRP on bone healing after mandibular distraction in rabbits. They concluded that PRP has significant clinical implications on reducing the period of consolidation of the mandibles. Hernandez-Fernandez et al.[25] could not found any radiological or histological evidence that the administration of PRP in the early phases of distraction osteogenesis enhances bone formation. However they reported that increased diaphyseal thickness in the PRP enjected sheep femurs. According to human study, Latalski et al.[26] studied with 19 patient tibia and concluded that PRP enjection in distraction osteogenesis lead to better functional outcomes and improved patient satisfaction. Based on the current knowledge, PRP enjection enhances healing during distraction osteogenesis and our results confirms these studies.

The present study has several limitations. First, the sample size of the experimental group was small. Second, even if PRP was prepared using a standard method, we did not determine whether the concentration of platelet-derived growth factor is similar to that in the injected fluid. This is a pilot study and there is a need of prospective studies that evaluate bone regenerate consolidation in humans with a large number of patients.

In conclusion, PRP injection enhances bone regenerate consolidation and increases bone mineral density during the healing process of distraction osteogenesis.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Uludağ University, School of Medicine (27.03.2004/18).

Informed Consent: N/A.

Author Contributions: Concept - B.S.; Design - B.S.; Supervision - B.S.; Data Collection and/or Processing - M.K.; Analysis and/or Interpretation - B.S.; Writing - Y.A.

Conflict of Interest: The authors have no conflicts of interest to declare.

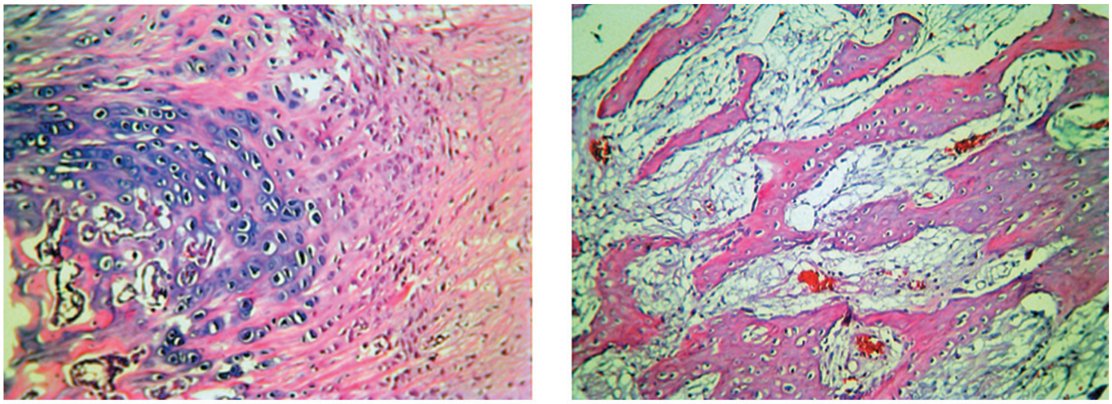

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Figure 4. a, b. Histological appearances of the bone regenerate in the control group (endochondral ossification [100×] and newly formed bone trabeculae in the area of intramembranous ossification [200×]).

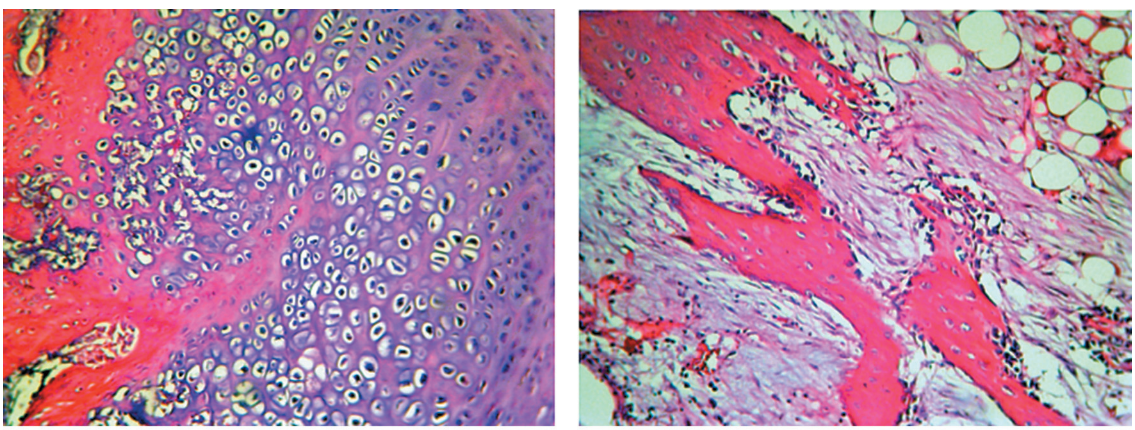

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Figure 5. a, b. Histological appearance of the bone regenerate in the Platelet-Rich Plasma group (endochondral ossification [100×] and intramembranous ossification [200×]).

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