IJMS
Vol 27, No.2, June 2002

Original Article

The Effect of Bone Marrow Graft on Bone Healing: A Radiological and Biomechanical Study

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Abstract

Background: There are multiple methods for acceleration of bone healing. Bone marrow has been shown to contain osteoprogenitor cells.

Objective: To determine the effect of percutaneous bone marrow injection on bone healing.

Methods: 15 mature, 2-kg-weighing white New Zealand rabbits from both sexes were randomized into 3 groups: Group I: Only osteotomy of right radius was performed and a 5 mm bony defect was made. Group II: Same as group I, with the defect filled by bone-graft, taken from the iliac crest. Group III: Same as group I plus percutaneous bone marrow injection 5 days after the operation. 45 days post-operation, the rabbits were sacrificed. The radii of both forelimbs were isolated and tested by tensile testing machine.

Results: There was a significant difference between groups I and III (p<0.001) for toleration of maximum load and bending stiffness. No significant difference was observed between groups II and III for these parameters.

Conclusion: Bone marrow injection has an effect on bone healing comparable to bone grafting. This technique is non-invasive and simple without any significant complication.

Iran J Med Sci 2002; 27(2):63-66

Keywords • Bone marrow injection • bone graft • delayed union • non-union

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Introduction

To stimulate the process of bone healing, several methods have been so far suggested. This modalities include use of ultrasound, electrical stimulation, exposure to electromagnetic field, bone graft, interporous hydroxyapatite (as a bone graft substitute). All of these methods are rather time-consuming and require special instruments and may need second incision.[1-10]

Several independent studies have demonstrated extensive osteogenic potential of bone marrow.[4,11-13] The ability of red marrow to form new bone was first suggested by Goujon,[2,12-14] as early as 1869.

Burwell placed bone marrow in a paravertebral muscle pouch and observed bone formation.[2,13] McGaw and Harbin successfully obtained healing of fibular defect in the dog.[8,10,13] Werntz *et al*, noted osseous union in 50% of rat femoral defects when filled with bone marrow alone [8,12,13] All these reports suggest that bone marrow might be a logical graft material and that autogenous bone marrow grafting could be used in the treatment of difficult situations of delayed union and nonunion.

This study was conducted to determine whether percutaneous bone marrow grafting leads to increased bone production and whether it has any effect on the early healing of fracture and bone defect.

Materials and Methods

Fifteen adolescent, 2-kg-weighing, white New Zealand rabbits from both sexes were used. They were randomized into three equal groups. Right forelimbs and left hip regions of the animals were shaved. Under ether anesthesia, each rabbit was positioned supine and under sterile condition an incision measuring 3 cm was made over the right radius. The radius was then exposed and a 5-mm bony defect was made using a small saw. All bone debris were meticulously washed and wiped away. Because the radius and ulna are adhered together by interosseous membrane, adequate stability was achieved by leaving the ulna intact without any fixation of the radius.

Five rabbits (group I) returned to their cages after operation. In group II (n=5), the bony defect was filled with bone graft taken from the iliac crest. In group III (n=5), five days after osteotomy, the rabbits were re-anesthetized and 2 ml bone marrow which was obtained through a small puncture wound over the greater trochanter from medullary cavity of the femur, was injected into the bony defect. The percutaneous technique was chosen to speed the operative procedure and to eliminate wound healing difficulties.[9,13,14] The reason for the delay was to let the skin and subcutaneous tissue heal enough to not get the liquid marrow leak out of the wound. Forty-five days post-operatively the rabbits were sacrificed. The radius and ulna were removed as a unit by disarticulating the radiocarpal and elbow joints. Simple radiographs were taken from all specimens.

To avoid damage to the callus, the surrounding soft tissues were dissected away carefully. To avoid its effect in biomechanical study, we osteotomized the attaching ulna by a high-speed saw at both ends.

A three-point bending test was done over the affected and contralateral sides with universal tensile testing machine (TT-CM-L-Instron Model-0.5 cm/min).

Changes in the length (ΔL) and toleration of maximum load were detected from the graph sketched by the machine. The bending stiffness was derived using the following equation:

$$\text{Stiffness} = \frac{L^3 \cdot F}{4\,W \cdot T^3 \cdot \Delta L}$$

where F is the applied load in kg, L is the distance between two mandibles of the machine which had a constant value of 40 mm, ΔL is the elongation in mm, and W and T are the great and small diameters of the bone in mm, respectively.

Results

Clinical

There was no superficial or deep infection during the post-operative course.

After 45 days, when we sacrificed the rabbits and dissected the radius bone in each limb, in group I, the 5-mm gap produced during the first surgery was still present in all 5 cases, and the fractures were not healed (Fig. 1).

In groups II and III, all fractures were healed by adequate callus formation (Figs. 2, 3). There was no significant difference between pre- and post-operative rabbit weights.

Radiological

There was 4- to 5-mm gap at the site of osteotomies in all cases of group I, less than 1-mm gap in four, and 2- to 3-mm gap in one of five rabbits in both groups II, and III (Fig. 4).

Figure 1: Right and left radii in group I

Figure 2: Right and left radii in group II

Figure 3: Right and left radii in group III

Biomechanical

In biomechanical evaluation, toleration of the maximum load, elongation and bending stiffness were measured in both radii of all cases.

The toleration of the maximum load, the bending stiffness and the elongation (ΔL) in the left radii (normal side) did not show any significant difference among the studied groups. In the right radii (osteotomized side), however, all the three measured parameters showed significant ($p<0.001$) difference between groups I (simple osteotomy) and III (bone marrow injection) ($p<0.001$). With regard to these parameters, no difference was observed between groups II (bone graft) and III (bone marrow injection).

Group

I

II

III

Figure 4: Roentgenogram of right and left radii

Discussion

Bone marrow contains the osteoprogenitor cells (OPC) that are important participants in bone formation and fracture healing.[2-5,11,15] Two types of osteoprogenitor cells have been demonstrated; one that is induced to produce bone (inductive OPC) and another that is determined to produce bone (determined OPC). The former exists in all connective tissues and is thought to be undifferentiated mesenchymal cells. The latter is found only in marrow and is already differentiated into a bone producing line. In this study, objective biomechanical and radiological differences were demonstrated between fractures and bony defects grafted with bone chips and bone marrow. In each case of bone marrow grafting, the osteotomy line appeared as more radiodense as bone grafting. There was a significant difference between groups I and III ($p<0.001$) showing that the bone-grafted limb has a better tolerance in biomechanical study than a simple osteotomy, but no significant difference was observed between bone grafted limb and bone marrow injected limb. From this experimental study and the review of the literature, it is apparent that percutaneous bone marrow grafting may have a potential therapeutic value in the management fractures.

Acknowledgment

This study was performed in Animal Laboratory, Shiraz University of Medical Sciences. The authors thank Dr. N. Tanideh for his kind cooperation, assistance and preparation of the materials.

References

1 Bucholz RW, Carlton A, Homes R: Interporous hydroxyapatite as a bone graft substitute in tibial plateau fractures. *Clinic Orthop 1989;* **240:** 53-62.

2 Christian CA: General principles of fracture treatment. In: *Campbell's Operative Orthopaedics,* 9th ed, Mosby,**1998**.

3 Goh JCH, Bose K, Kang YK, et al: Effects of electrical stimulation on the biomechanical properties of fracture healing in rabbits. *Clin Orthop 1988;***233:**268-73.

4 Harkess JW, Ramsey WC, Harkess JW: Principles of fracture and dislocation (Chapter 1). In: .*Rockwood and Green's Fractures in Adults.* 4th ed, Lippincott-Raven, **1996**.

5 Holmes R, Mooney V, Bucholz R, et al: A coralline hydroxyapatite bone graft substitute. *Clinic Orthop 1984;* **188:**252-62.

6 Hulth A: Basic science and pathology. Current concepts of fracture healing. *Clinic Orthop 1989;* **249:**265-84.

7 McLeod KJ, Rubin CT: The effect of low-frequency electrical fields on osteogenesis. *J Bone Joint Surg 1992;***74-A:**920-9.

8 Pienkowski D, Pollack SR, Brighton CT, et al: Low-power electromagnetic stimulation of osteotomized rabbit fibulae. *J Bone Joint Surg 1994* **76-A:** 489-501.

9 Sartoris DJ, Gershuni DH, Akeson WH, et al: Coralline hydroxyapatite bone graft substitutes. *Radiology 1986;***159:**133-7.

10 Wray JB: The influence of various hormones on the fracture-healing process. *Clinic Orthop 1967;***50:**324.

11 Buckwalter JA: The specialty of orthopaedics. In: *Turek's Orthopaedics Principles and Their Application.* 5th ed, *J B Lippincott,* **1994**.

12 Connolly J, Guse R, Lippiello, et al: Development of osteogenic bone marrow preparation. *J Bone Joint Surg (Am) 1989* **71-A:**684-91,

13 Healey JH, Zimmerman PA, Medonnell JM, et al: Percutaneous bone marrow grafting of delayed union and nonunion in cancer patients. *Clinic Orthop 1990;***256:**280-5.

14 White AA, Panjabi MM, Southwick WO: The four biomechanical stages of fracture repair. *J Bone Joint Surg 1977;***59-A:**188-92.

15 Bruder SP, Kraus KH, Goldberg VM, et al: The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects. *J Bone Joint Surg 1988;* **80- A:**985-96.