Percutaneous Bone Marrow Grafting in Radial Fracture in Rabbits

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Summary

The osteogenicity and effect on early bone repair of bone marrow grafts were investigated. The purpose of this experimental study was to determine whether bone marrow grafted percutaneously leads to increased bone production or has any effect on the early healing of fractures. Thirty adolescent, male white rabbits were used. All of them had bilateral radial osteotomies. Rabbits were divided to equal five groups. The cross-sectional area of callus, breaking load, tensile strength and callus volume parameters at the fracture site were tested. At two weeks postgrafting callus volume were significantly higher ($0.001<P<0.005$) in grafted radii than in the contralateral saline control. By four weeks all parameters were significantly greater in the bone marrow grafted radii than in the control. Serial radiographs and histology confirm this advanced fracture healing in the grafted bones. There were no differences between the external callus of treated and control radii but the internal callus between the end of the cortical bone did show a difference between the two sides. Percutaneous bone marrow grafting is a simple semi-invasive technique that may have potential clinical application.

Key words: bone marrow, osteotomy, rabbit, percutaneous

Introduction

Osteogenic precursor cells, which are capable of producing bone, have been demonstrated among the stromal and endosteal cells of bone marrow. In fact, in

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autogenous corticocancellous bone grafts, bone marrow and endosteal cells produce greater that 60% of the graft-derived bone. Bone marrow has been used both clinically and experimentally in conjunction with cortical and/or cancellous bone to enhance the osteogenicity of these grafts. (Berggren *et al* 1981, Ito *et al* 1998) Recently, both the bone marrow (BM) alone and bone marrow in conjunction with a preserved xenograft (Kielbone) have been used successfully for treatment of nonunion and bony defects (Ito *et al* 1998, Jasty *et al* 1997).

In this experimental study, the osteogenicity and effect on early bone repair of bone marrow grafts was investigated. The purpose of this study was to determine whether bone marrow grafted percutaneously leads to increased bone production or has any effect on the early healing of fractures.

Materials and Methods

Animal. Thirty adolescent, 4-kg, male white rabbits were used. 28 had bilateral radial osteotomies and two of them dead. Rabbits were divided to five groups (A1, A2, B1, B2 and C) each of 6. Osteotomy site of left upper extremity in all groups was injected percutaneously with 2ml bone marrow (BM). Normal saline solution was injected into the right upper extremity with the same manner as a control (SC). Bone marrow was obtained from the right iliac crest of the same rabbit.

Osteotomy. The upper limbs and right hip regions of the rabbits were shaved. Under general anesthesia, the rabbits were positioned supine, prepped, and draped. Under sterile conditions identical bilateral incisions were made over both radii. A pin was placed in the radiocarpal joints. Measuring 2cm from the radiocarpal joint, bilateral transverse radial osteotomies were made using hand-held nasal septum saw of 2mm width. The osteotomy was stopped short of cutting into the very adherent ulna. All bone dust was meticulously washed and wiped away. Because the radius and ulna are very adherent one to the other, by their interosseous membrane, adequate stability was achieved by leaving the ulna intact without any fixation of the radius. The muscle and subcutaneous tissue was closed over as a deep layers using 4-0 vicryl sutures. A #18-gauge spinal needle was used to aspirate 2ml of marrow from the medullary cavity of the femur. The marrow was heparinized. The amount of marrow used was determined by volume alone. This raised a small fusiform swelling at the osteotomy site but no leakage through the now-healed incisions occurred. The rabbits were then returned to their cage and did not show any signs of pain. Two animals were excluded from the study because, one of them affected with wound infection and another with pulmonary edema.

Radiology. Lateral radiographs were taken from the rabbit forelimb. The first roentgenogram was taken on day 1, the second on day 6 and weekly thereafter until the rabbits were sacrificed.

Tensile testing. The rabbits were sacrificed at two (A1, A2), four (B1, B2) and eight (C) weeks postgrafting. The radius and ulna were removed as a unit by disarticulating the radiocarpal and humeroulnar joints. The bones were then wrapped in sterile wrap and put in freezer. Four parameters were measured to assess bony healing: breaking load (BL), cross sectional area at osteotomy site (XS), tensile strength (TS) and callus volume (CV). The soft tissues were dissected away carefully, avoiding loss of any callus. The bones were X-rayed one last time. The radius was removed from the ulna surgically without stressing the osteotomy line. Each end of the radius was embedded in methyl methacrylate. The bones were stored in saline overnight to ensure isohydration and then tensile tested using an Instron machine to measure the breaking load (parameters for tensile testing were load cell 500kg, chart speed 10cm/min, crosshead speed 0.2cm/min). The bones all broke through the osteotomy line in the osteotomy group. The cross- sectional area at the osteotomy line was traced out and then measured accurately with fine collis The TS was calculated as the ratio of breaking load to cross-sectional area at the osteotomy site.

Callus volume. The callus was surgically removed from the cortex under a dissecting microscope. The volume of the callus fragments was measured accurately by volume displacement in a $1 cm^3$ pipet. Callus volume of bones was measured with volumetrically.

Histology. All bones were fixed in 10% buffered formalin solution then decalcified in %5 Nitric acid, and 7μ paraffin sections were stained with Hematoxyline and Eosin.

Statistical analysis. Because of the relatively small sample size, the paired *t*-test was used to evaluate the results.

Results

Osteotomy. The gross specimens retrieved after sacrificing the animals appeared similar on both SC and BM-grafted sides at two weeks (Table 1). At four weeks there was a noticeable bulkier callus on the BM-grafted side. The soft tissues were somewhat more adhesive to the BM-grafted radii in the area of the osteotomy than on the SC side. This difference was subtle, and the soft tissue could still be dissected off easily. No soft tissue calcification or ossification could be seen or felt on the BM-grafted side.

Table 1. *Measured results of osteotomies*

Parameter	Groups	Weeks	N	Range		Mean		Mean of paired difference ±SD	P	%BM>SC
				BM	SC	BM	SC			
XS	A1	2	6	29.8-53	21-45.3	40.1	34.45	5.8±3.1	0.005<p<0.001	%100
	B1	4	6	36.9-55.3	29.3-48	46.4	36.8	9.6± 2.2	P<0.001	%100
BL (kg)	A1	2	6	1.75-8.4	0.07-6	4.6	2.7	1.9±0.5	P<0.001	%100
	B1	4	6	20-44	8-21	29	13.7	15.4±5.02	P<0.005	%100
TS (kg/mm)	A1	2	6	5.87-15.84	0.33-13.54	10.99	6.98	4.0±2.7	0.01<p<0.05	%100
	B1	4	6	54.12-79.5	27.3-43.75	61.75	36.76	25.0±8.2	0.001<p<0.005	%100
CV (mm^3)	A2	2	6	55-233	30-194	16.32	99.03	46.2± 2.8	0.001<p<0.005	%100
	B2	4	6	203-371	108-285	288.16	197.5	96.7±17.5	P<0.001	%83

XS=cross-sectional area at osteotomy site, SC=saline control, BL=breaking load, TS=tensile strength, BM=bone marrow, CV=callus volume, SD=standard deviation

At two weeks CV was significantly higher in BM-grafted radii than in the SC radii ($0.001<P<0.005$). There was no significant difference between the XS, BL, and TS of the BM-grafted radii at two weeks versus their paired SC however, they were all significantly higher ($P<0.001$) at four weeks. The first parameter to show a measurable difference was the CV. In both SC and BM-grafted radii there was a weekly increase in the CV seen in this early phase of fracture healing; however, this increase was taking place at different rates. Initially, there was a very rapid rise in the CV on the BM-grafted side, as compared with the SC side with a 100% greater callus at two weeks but at four weeks the difference was only 83%.

Radiography. In the blinded qualitative analysis of the radiographs a difference was felt to exist between the roentgenographic appearance of paired bones in each of the cases at four weeks. In every case, the osteotomy line on the BM-grafted side appeared more radiodense, on the lateral view in particular. More abundant callus formation was present in four-week grafted radii. No difference was detected in the optical density of the osteotomy line between the BM versus the SC radii at two weeks. At four weeks, however, there was a highly increase in the radiographic optical density of the osteotomy line of BM-grafted radii (Figure 1a, b).

a b

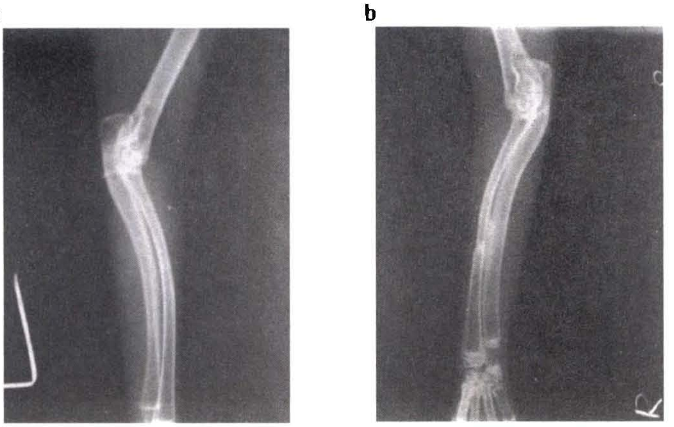
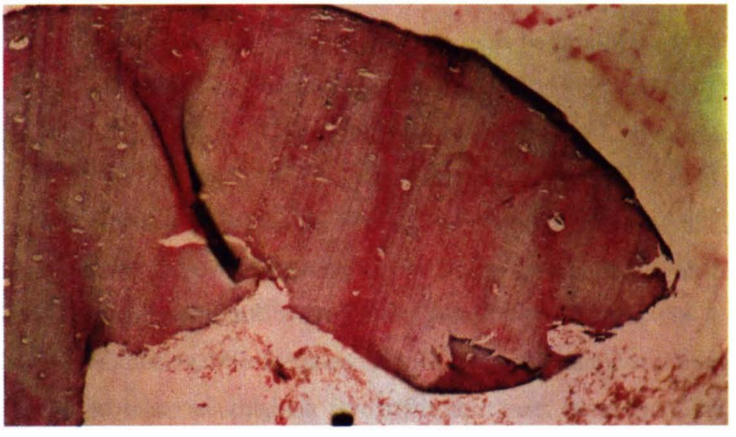


Figure 1. a) *Anteroposterior radiograph of radial osteotomies at 8 weeks after bone marrow grafting. Note the healing and remudling of fracture site completed. b) Lateral radiograph of radial osteotomies at eight weeks after saline injected on the other (right) fore arm. Note. The healing and remudling of fracture site was not completed*

Histology. The external calluses of BM and SC radii were identical in the appearance of the trabecular bone. The internal callus between the ends of the cortical bone did show a difference between the two sides. In the BM-grafted radii showed a bridgine trabecular bony callus between the cortical ends. On the SC side there was only an immature fibrous tissue filling this gap (Figures 2, 3).

a



b

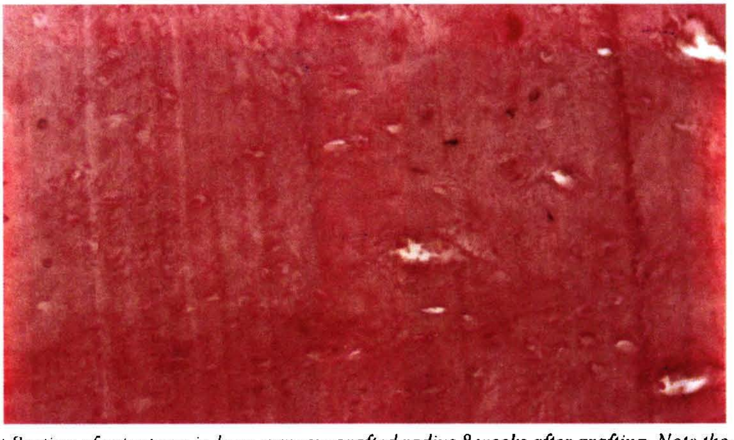
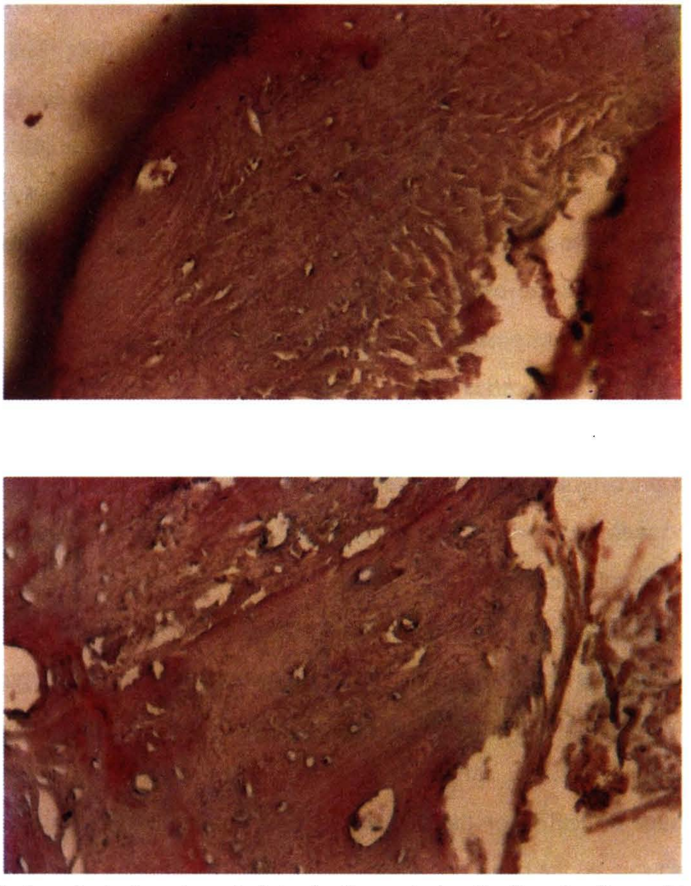


Figure 2. *a) Section of osteotomy in bone marrow grafted radius 8 weeks after grafting. Note the complete osseous bridging between the cortical ends. (decalcified sections) H&E × 100. b) Section of osteotomy in bone marrow grafted radius eight weeks after grafting. Note compact bone formed between the cortical ends. The basic unit of this bone consisting of concentrically arranged cylindrical lamellae around an axial the cortical ends. (decalcified sections) H&E × 400*

a



b

Figure 3. *a) Section of osteotomy in contralateral saline control radius 8 weeks after grafting. Note the spongy bone (Concellous b.) with the thin intersecting lamellae and fibrous between the cortical ends. These were shown that the bone healing was delayed. H&E ×100. b) Continued H&E ×250*

Discussion

BM is known to be osteogenic immediately after bone grafting. Any differences attributable to grafted marrow should be maximal during the initial phase of bone grafting of fractures and bony defects does lead to a difference in initial osteogenicity and fracture healing. The concept of bone grafting percutaneously was introduced by Herzog (1951). A percutaneous technique minimizes the risk from anesthesia, infection and surgery. Certain clinical situations that would not be strong indications for open bone grafting, such as delayed unions or fractures prone to delayed union, might be considered for percutaneous BM grafting. The purpose of this experiment was to develop a model in which the presence or absence of a biologic effect, and in particular increased bone formation, attributable to the grafted marrow could be demonstrated. Thus the bony healing following fractures was only examined during the first eight weeks (grafting on day 1 plus eight weeks). This study confirmed that the osteogenic competence of a BM graft is maximal in the first two to three weeks after grafting. This is evidenced by the callus volume results. The increase in callus volume is thought to arise from the osteogenicity of the BM graft. BM contains osteogenic cells, which when grafted orthotopically, respond to the osteoinductive stimuli present by producing bone. This supports the BM-grafted radii hypothesis. Both the qualitative and quantitative radiologic results support this hypothesis as well.

Radiographic changes beginning from two weeks postgrafting were consistent with a more mature stage of fracture healing on the BM side. Histologically, while the appearance of the external callus trabecular bone was the same, the consistent delay in bridging internal callus between the cortical ends again suggests a more advanced state of healing on the BM side. Therefore, while there is no difference in the healing process, the two sides are at different phases of bone healing BM slightly advanced versus SC. Biomechanically, early differences between BM and SC were found as well. The parameters measured were significantly higher on the BM side.

Quantification of bone-graft-derived bone showed that greater than 60% of the bone produced by a corticocancellous bone graft originated from the grafti's endosteal and BM cells (Gray and Elves 1979, Chapman 1980). Bone to be a property of the matrix of the bone graft and not a property of the endosteal or BM cells (Jasty *et al* 1997). Recent experiments have combined BM with Kiel bone. This composite xeno-autograft was then implanted in the paravertebral muscles in rate. The impregnated (fertilized) xenograft was highly osteogenic. Plenk *et al* (1972) applied this technique to graft bony defects in rats and confirmed the marked osteogenicity of the composite xenoautograft. Salama (1983) reported excellent clinical results using this technique to graft bony defects, nonunion, spinal fusion arthrodeses, and tibial plateau fractures in 98 patients. Graham (1982) reported ten nonunion grafted successfully using this composite of BM and Kiel bone. Shapoff *et al* (1980) has shown that the size of the particle that is mixed with the BM affects its osteogenicity. Using a smaller particle size (100-300μ) is more osteogenic. Thus, percutaneous BM grafting might be optimized by mixing it with fine particulate bank bone such that its injectability would not be compromise (Goldstein *et al* 1998). BM grafting on its own, without added bone matrix has been used in oral surgery. Jackson et al (1981) reported five palatal defects successfully healed by grafting autogenous iliac-crest BM. In orthopedics, BM alone has never been used clinically instead of bone grafting.

The results of this experimental study apparent that percutaneous bone marrow grafting may have potential as a therapeutic modality of clinical importance. Clearly further animal and clinical studies are necessary.

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