PERCUTANEOUS BONE MARROW GRAFTING OF FRACTURE (AN EXPERIMENTAL STUDY IN RABBITS)

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Abstract- Since bone marrow has been shown to contain osteoprogenitor cells, an experiment was devised to test its effects when injected percutaneously into osteotomies sites in rabbit radii. In this experimental study, the osteogenicity and it's effect on early bone repair of bone marrow grafts were investigated. The purpose of this study was to determine whether bone marrow grafted percutaneously led to increased bone production or had any effect on the early healing of fractures. The parameters tested included, cross-sectional area of callus (XS), breaking load (BL), tensile strengh (TS) and callus volume (CV) at the fracture site. At two weeks postgrafting four parameters, specially callus volume, were significantly higher (0.001< P < 0.005) in grafted radii than in the contralateral saline controls. By four weeks all four parameters were significantly greater in the bone marrow grafted radii than in the contralateral saline controls. Serial radiographs and histology confirm this advanced fracture healing in the grafted bones. There were no differences between the external callus of BM (Bone marrow) and SC (Saline control) radii but the internal callus between the end of the cortical bone did show a difference between the two sides. The earlier and more abundant callus at the bone marrow grafted sites, was felt to provide earlier and greater stability, resulting in decreased early healing time when contrasted with the saline controls. Percutaneous bone marrow grafting is a simple semi-invasive technique that may have potential clinical application.
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Key Words: Bone marrow, fracture healing, histopathology, osteotomy, callus formaion radius of rabbit

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 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 (1). Recently, both the bone marrow (BM) alone and bone marrow in conjunction with a preserved xenograft (Kielbone) have been used successfully for treatment of nonunions and bony defects.

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

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MATERIALS AND METHODS

Thirty adolescent, one years old, 4 kg, Newzealand, male white rabbits were used. Twenty eight had bilateral radial osteotomies and two of them died.

OSTEOTOMIES

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 2 cm from the radiocarpal joint, bilateral transverse radial osteotomies were made using hand-held nasal septum saw of 2 mm 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 were closed over as a deep layer using 4-0 vicryl sutures.

To obtain the marrow, a small puncture wound was made over the right greater trochanter. A # 18 gauge

spinal needle was used to aspirate 2 ml of marrow from the medullary cavity of the femur.

The marrow was heparinized. The amount of marrow used was determined by volume alone. Left upper extremity was chosen and 2 ml of marrow was injected into the osteotomy site percutaneously. Two ml of normal saline solution was injected into the contralateral osteotomy site. 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 was affected with wound infection and another with pulmonary edema.

RADIOGRAPHY

After operation, weekly anteroposterior and lateral radiographs were taken of the rabbits' forelimbs. This gave high resolution radiographs of identical magnification of both sides. Comparable views were obtained. 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 in two groups : at two or four 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 wraps and put in a 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 once 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 for tensile testing.

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: Load cell 500 kg, chart speed 10 cm per minute, crosshead speed 0.2 cm per minute).

The cross-sectional area at the osteotomy line was traced out and then measured accurately with fine collis. The tensile strenght (TS) was calculated as the ratio of breaking load to cross-sectional area at the osteotomy site (TS=BLW).

CALLUS VOLUME

In order to measure its volume, the callus was surgically removed from the cortex under a dissecting microscope. The volume of the callus fragments was then measured accurately by volume displacement in a 1 ml pipet .

Since this could only be done on bones after the rabbit had been sacrificed, callus volume of bones was measured with only volumetrically.

HISTOLOGY

All bones were fixed in 10% buffered formaline solution then decalcified in %5 nitric acid and 7 micron paraffin sections were stained with hematoxyline and eosin.

STATISTICAL ANALYSIS

Because of the relatively small sample size, both the paired t-test were used to evaluate the result. At the $P<0.01$ level both the parametric and the nonparametric statistics gave the same results. The data using the paired t-test are presented. These data are presented as a series of graphs. Since the data were examined in a paired fashion, the mean of the paired difference plus or minus the standard deviation is portrayed graphically to more accurately represent the standard deviation in a paired study. This also explains why the range is large between bones but the standard deviations are low, since all results are paired.

RESULTS

OSTEOTOMILES

The gross specimens retrieved after sacrificing the animals appeared similar on both saline control (SC) and bone marrow (BM)-grafted sides at two weeks. At four weeks there was a noticeable more bulky callus on the BM-grafted side. The soft tissues were somewhat more adherent 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.

A summary of the measured results are tabulated . 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.

Table 1. Mesured results- osteotomies

				range		mean				
parameter	groups	weeks	n	BM	SC	BM	SC	Mean of paired differnce±SD	p	% BM > SC
XS	A1	2	1	30.2	29.1	40.1	34.45	5.8±3.1	0.005<P<0.001	100%
			2	36.8	32.9					
			3	53	45.3					
			4	46.7	42.2					
			5	44	35.2					
			6	29.8	21					
XS	B1	4	1	36.95	29.3	46.4	36.8	9.6±2.2	P< 0.001	100%
			2	48.25	36					
			3	51.05	43.1					
			4	55.3	48					
			5	43.15	31.5					
			6	43.65	33.1					
BL (kg)	A1	2	1	3.46	1.2	4.6	2.7	1.9±0.5	P< 0.001	100%
			2	3.5	1.3					
			3	8.4	6					
			4	5.8	4.1					
			5	4.8	3.7					
			6	1.75	0.07					
BL (kg)	B1	4	1	20	8	29	13.7	15.4±5.02	P< 0.005	100%
			2	31.5	13.9					
			3	33.5	15.6					
			4	44	21					
			5	21.5	11.7					
			6	24.5	12.4					
TS (kg/mm) * 0.01)	A1	2	1	11.45	3.85	10.99	6.98	4.0±2.7	0.01<p<0.05	100%
			2	9.5	3.95					
			3	15.84	13.54					
			4	12.4	9.7					
			5	10.9	10.51					
			6	5.87	0.33					
TS *0.01= BL/XS	B1	4	1	54.12	27.3	61.75	36.76	25.0±8.2	0.001<p<0.005	100%
			2	65.28	38.61					
			3	65.62	36.19					
			4	79.56	43.75					
			5	49.82	37.14					
			6	56.12	37.46					
CV (mm^3)	A1	2	1	144.9	56.3	160.32	99.03	46.2±2.8	0.001<p<0.005	100%
			2	164.5	76.3					
			3	233	194					
			4	200.9	136.3					
			5	180.9	101.3					
			6	55	30					
CV (mm^3)	B1	4	1	203	108	288.16	197.5	967±17.5	P< 0.001	83%
			2	293.5	221.4					
			3	323.5	200.3					
			4	271	285					
			5	254.5	158					
			6	283.5	176.4					

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

At four weeks, however, CV, XS, BL, and TS were all significantly higher (P<0.001) BM-grafted radii versus their paired SC (Fig. 1, 2, 3, 4).

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 on the BM-grafted side at two weeks. But at four weeks the difference was only 83% (Table 1).

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 great increase in the radiographic optical density of the osteotomy line of BM-grafted radii versus SC radii.

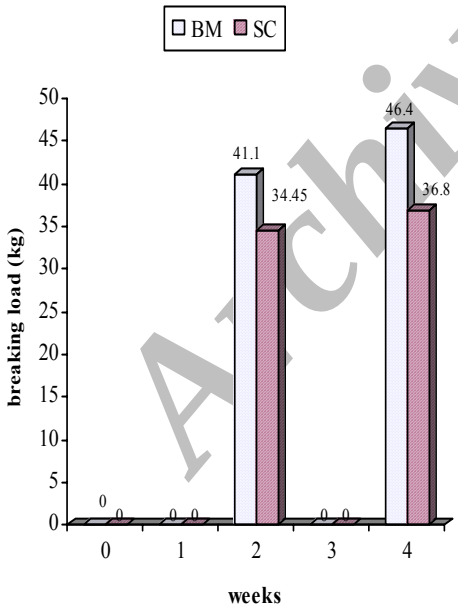


Fig. 1. Cross-section area at fracture site (BM= Bone Marrow, SC= Saline Control)

HISTOLOGY

The external callus 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. The BM-grafted radii showed a bridgine trabecular bony callus between the cortical ends (Fig. 5A, 5B).

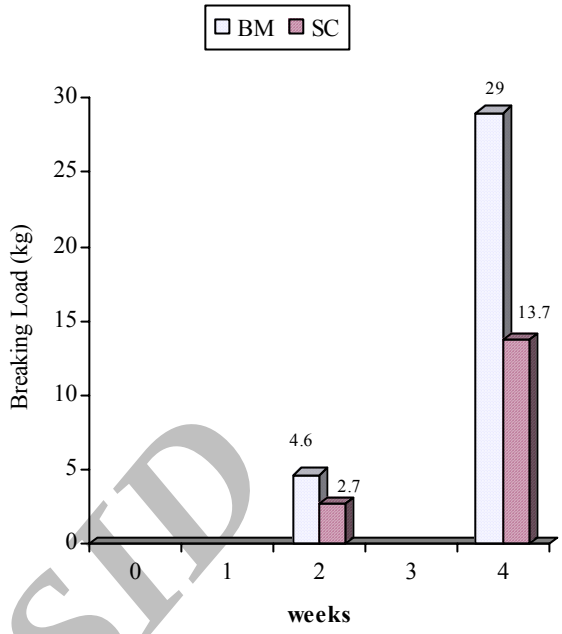


Fig. 2. Breaking load at fracture site (BM=Bone Marrow, SC= Saline Control)

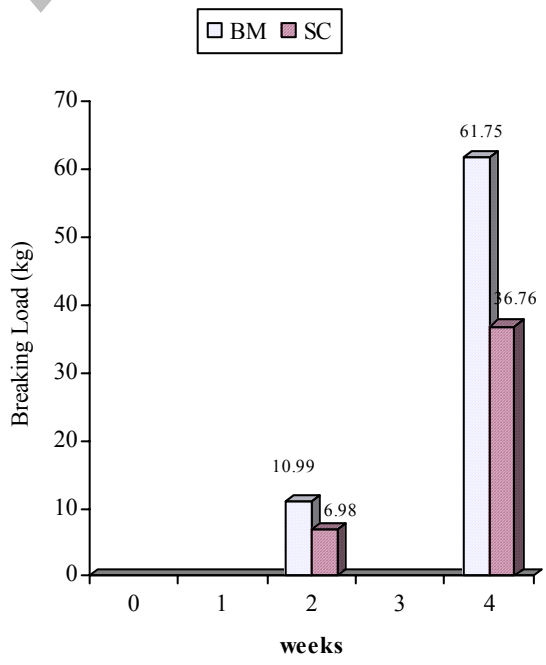


Fig. 3. Tensile strength at fracture site (BM=Bone Marrow, SC= Saline Control)

On the SC side there was only an immature fibrous tissue filling this gap.

An example of the histology of bilateral osteotomies is shown. The bone formation occurred from either end of the SC side with a central gap. A bony bridge is shown on the BM-grafted side laid down onto the adherent ulna (Fig. 6A, 6B).

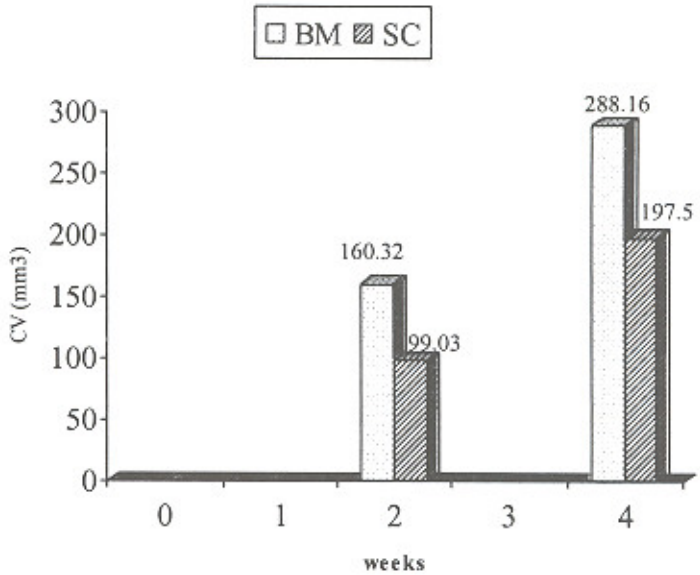


Fig. 4. Callus volume at fracture site (BM=Bone Marrow, SC= Saline Control)

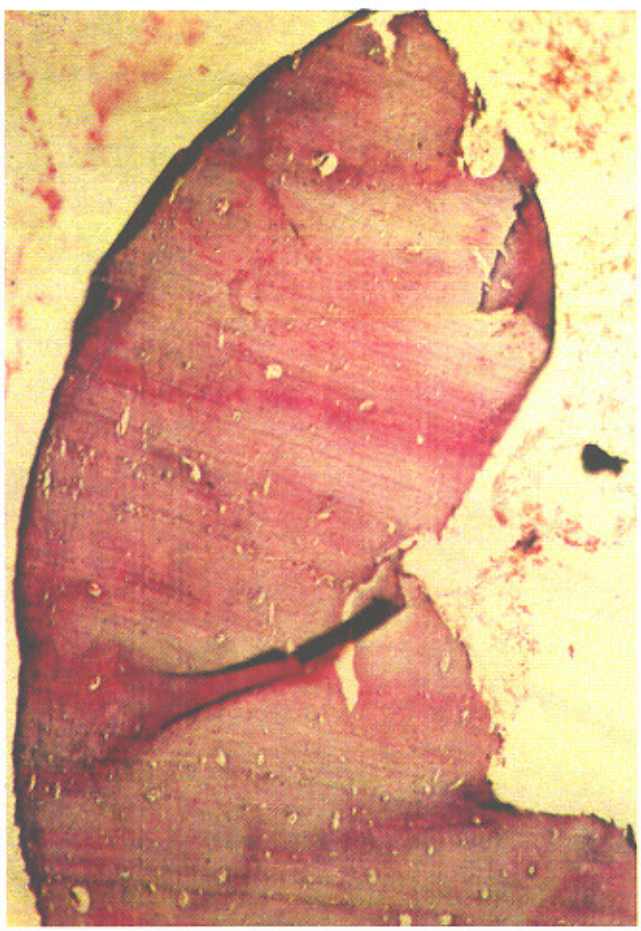
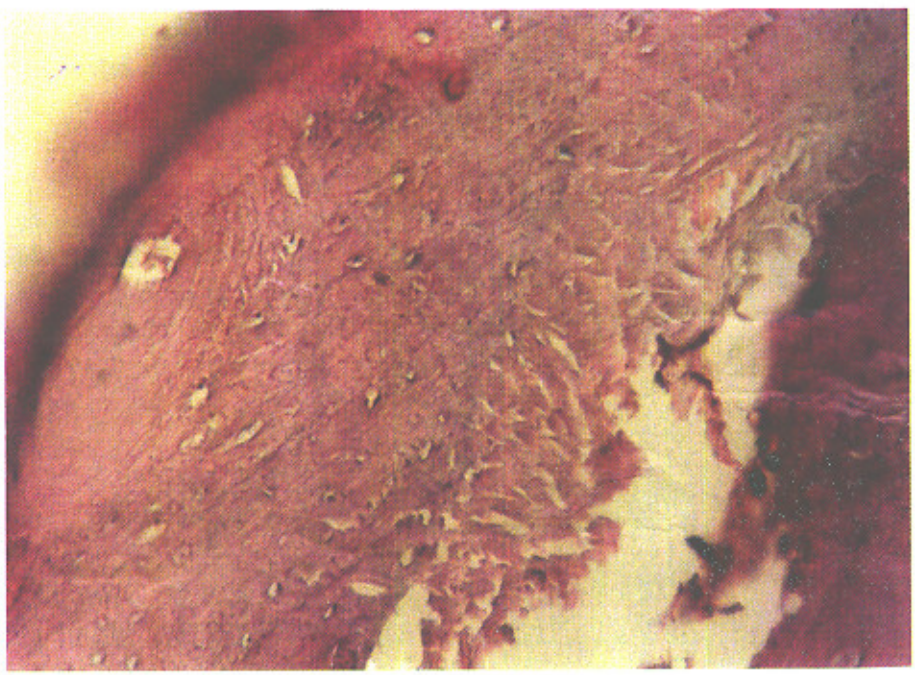
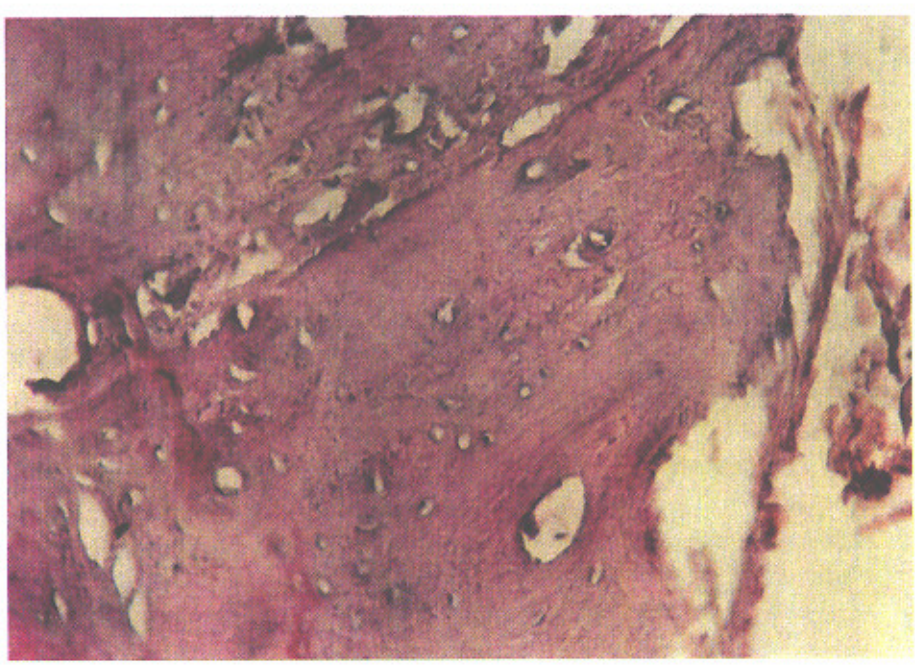


Fig. 5A. Section of osteotomy in bone marrow grafted radius eight weeks after grafting. Note the complete osseous bridging between the cortical ends (decalcified sections) (H & E, ×100)

Fig. 5B. 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 and axial the cortical ends (decalcified sections) (H & E ×400)



A



B

Fig. 6A. A and 6B. (7A) Section of osteotomy in contralateral saline control radius eigth weeks after grafting. Note the spongy bone (cancellous bone) with the thin intersecting lamellae and fibrous between the cortical ends. These were shown that the bone healing was delayed

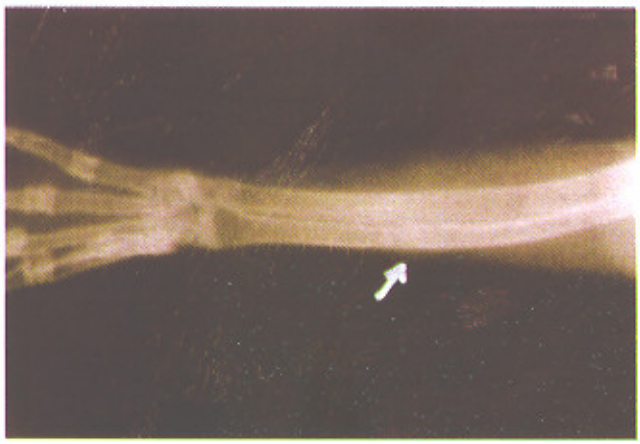


Fig. 7. Anteroposterior radiograph of radius osteotomies at eight weeks after bone marrow grafting. Note the healing and remudling of fracture site complete

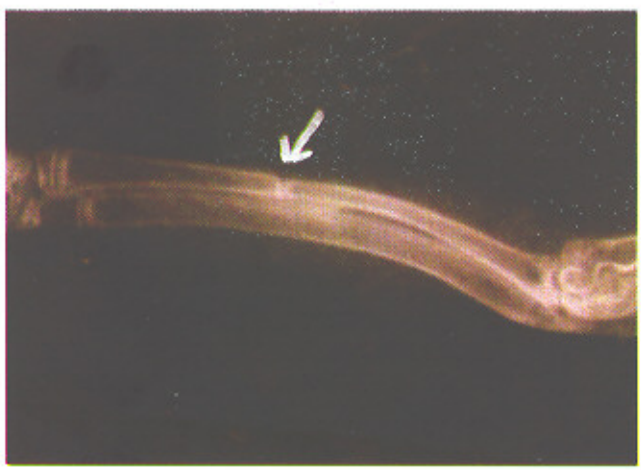


Fig. 8. Lateral radiograph of radius osteotomies at eight weeks after saline injected on the other (right) fore arm. Note the healing and remudling of fracture site was not completed

DISCUSSION

In this study biomechanical, histologic, and radiologic objective differences were demonstrated between fractures and bony defects grafted with bone marrow and contralaterally paired saline control bones. The only initial difference between the two sides was the material injected. This controls any reaction one might see from a "second iniury phenomenon." The question then is whether the differences measured are due to augmentation of bony healing by bone marrow grafts of inhibition of healing by saline. Two facts point towards the former argument. First, the animals used as unilateral saline controls failed to show any noticeable differences between saline or no saline. Secondly, rabbits are notorious for their rapid bony healing. It is hard to imagine how inhibitory 2 ml of saline could be to a young rabbit fracture since it would be almost immediately absorbed. Little difference would be expected to be measured. Since significant differences were measured between the two sides, the authors believe that the differences are attributable to the bone marrow graft.

Bone marrow is known to be osteogenic immediately after bone grafting (1,2). Any differences attributable to grafted marrow should be maximal during the initial phase of bone grafting of fractures and bony defects do lead to a difference in initial osteogenicity and fracture healing. 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 bone marrow graft is maximal in the first two to three weeks after grafting.

This is evident by the callus volume results. At two weeks the difference in callus volume was 100%. By four weeks, however, a significant drop down to 83% occurred. This suggests that callus production on the BM side is slowing down relative to the increase in callus production on the SC side at four weeks. Both the qualitative and quantitative radiologic results support this hypothesis as well.

Radiographic changes beginning from two weeks postgrafting in both the osteotomies 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. Since tensile strength is proportional to healing time, this again demonstrates a difference in the stage of early fracture healing to bone marrow grafting.

A probable explanation for the more mature stage of bone healing in BM-grafted osteotomies lies in the larger callus being laid down at an earlier stage. This provides better scaffolding, leading to earlier and improved stabilization at the osteotomy line. Better fracture immobilization advances the tensile healing in time, further promoting and maturing the healing process. If this pattern continues there would be an overall decrease in healing time (1).

The increase in callus volume is thought to arise from the osteogenicity of the bone marrow graft. Bone marrow contains osteogenic cells, which, when grafted orthotopically, respond to the osteoinductive stimuli present by producing bone. A further control using blood instead of saline could have been used to study the effect of marrow plus blood versus blood on its own. This difference of cells alone would more clearly prove whether the grafted marrow cells contributed directly to the increase in new bone formation.

The osteogenicity of bone marrow has been traced to the stromal and endosteal cells of the marrow. Two types of osteoprogenitor cells (OPC) have been demonstrated: one that is induced to produce bone (IOPC), and one that is determined to produce bone (DOPC).

The former (IOPC) exists in all connective tissues and is thought to be an undifferentiated mesenchymal cell. The latter (DOPC) is found only in marrow and is already differentiated into a bone-producing line. Inducible osteoprogenitor cells respond by producing bone to local stimuli (e.g., fracture, bone graft, etc.) osteoinductive stimuli. By fractionation of rabbit bone marrow, subpopulations of cells enriched with determined or inducible properties have been isolated. Because bone marrow is the only tissue that contains an abundance of both determined and inducible osteoprogenitor cells, it is a logical graft choice. McGaw and Harbin in 1934 were among the first to demonstrate the osteogenic activity of bone marrow. They grafted bony defects in dog fibulae with bone marrow alone and compared this with contralateral ungrafted defects.

Only the BM-grafted defects filled the gap with bone. Since then, there has been much active interest in the osteogenicity of bone marrow. Burwell demonstrated that bone grafts containing bone marrow were more osteogenic than grafts without added bone marrow (2).

Quantification of bone-graft-derived bone by Gray and Elves showed that greater than 60% of the bone produced by a corticocancellous bone graft originated from the grafts endosteal and bone marrow cells (30% from perosteum, 10% from osteoinduction of local tissue by the bone matrix) (3). Numerous other studies have confirmed these findings (2,3). Bone is a property of the matrix of the bone graft and not a property of the endosteal or bone marrow cells (4,5).

Recent experiments have combined bone marrow with Kiel bone (6). This composite xeno-autograft was then implanted in the paravertebral muscles in rats (6). The impregnated (fertilized) xenograft was highly osteogenic. Plenk et al (6). applied this technique to graft bony defects in rats and confirmed the marked osteogenicity of the composite xenoautograft. Recently Salama reported excellent clinical results using this technique to graft bony defects, nonunions, spinal fusions arthrodesis, and tibial plateau fractures in 98 patients (6). Graham reported ten nonunions grafted successfully using this composite of bone marrow and Kiel bone(7) . Since Kiel bone is poorly osteinductive and acts mainly as a scaffold, this composite graft is really a form of bone marrow grafting (6). Shapoff et al (8) have shown that the size of the particle that is mixed with the bone marrow affects its osteogenicity. Using a smaller particle size (100-300 microns) is more osteogenic. Thus, percutaneous bone marrow grafting might be optimized by mixing it with fine particulate bank bone such that its injectability would not be compromised (9).

Bone marrow grafting on its own, without added bone matrix, has been used in oral surgery. Jackson et al reported five palatal defects successfully healed by grafting autogenous iliac-crest bone marrow(10). In orthopedics, bone marrow alone has never been used clinically instead of bone grafting. It is recognized, however, that slush grafts that are mostly bone marrow are very osteogenic when used in closed intramedullary nailing (11).

The concept of bone grafting percutaneously was introduced by Herzog in 1951. He used a large bore needle and small cancellous chips to graft a nonunion (12). Since bone marrow has a liquid texture, combining the percutaneous grafting technique introduced by Herzog and the bone marrow graft introduced by McGraw seem a logical next step. A percutaneous technique minimizes the risk from anesthesia, infection, and surgery. An abundant source of autogeneic bone marrow is generally available and can be obtained under local or general anesthesia. Injection of the graft into the host site can be facilitated by image, intensification for accurate graft placement. The graft itself utilizes the most osteogenic cells of a bone graft and does not introduce devitalized tissue (dead bone). This could be advantageous in certain clinical situations (e.g., infection). A theoretical risk of heterotopic bone may exist if injection into soft tissues occurs (13).

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 bone marrow grafting (14,15).

From this experimental study and from the review of the literature, it is apparent that percutaneous bone

marrow grafting may have a potential as a therapeutic modality of clinical importance. Clearly further animal and clinical studies are necessary.

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