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RESEARCH ARTICLE

Evaluation of Healing Potential of Autogenous, Macroscopic Fat Deposited or Fat Free, Omental Graft in Experimental Radius Bone Defect in Rabbit: Radiological Study

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ABSTRACT

This study was designed for evaluation of the difference between the ability of greater omentum graft with or without macroscopic fat deposition in acceleration of bone healing process. Adult female New Zealand white rabbits (n=15) were randomly divided into three equal groups. In groups A and B, the drilled hole on the left radius was filled by the omentum without and with macroscopic fat deposition, respectively while drilled hole on the right radius left intact for consideration as control. In group C, the drilled hole on the left and right radius was filled by the omentum sample with and without macroscopic fat deposition, respectively. Experimental bone defects on the radiuses were secured by the pieces of greater omentum, with or without macroscopic fat deposition, which obtained as an autogenous graft from each rabbit in accompany with control samples. Standardized serial radiography for evaluation of bone healing was performed and the difference in bone healing process in three groups of study was determined. According to the obtained data, the radius bones which filled by omentum without macroscopic fat deposition showed faster healing process than the radius bones which filled by omentum with macroscopic fat deposition (P<0.05).

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INTRODUCTION

Several phases have been explained for the process of fracture healing (Kealy and McAllister, 2000). The mainly determined subject in fracture healing is the periosteum. The periosteum is one source of precursor cells which develop into chondroblasts and osteoblasts that are essential to the healing of bone. The other sources for presentation of precursor cells are the bone marrow (when present), endosteum, small blood vessels, and fibroblasts (Brighton and Hunt, 1997). The ability of the autogenous omentum graft in acceleration of the bone healing by augmentation of vasculogenesis has been well documented and evaluation of the role of free greater omentum in experimental bone healing in rabbits has already been documented (Oloumi *et al.*, 2006). Impairing potential of fat cell in bone marrow transplantation (Tehran Times, 2009) and the efficacy of autogenous fat deposition in postponing bone healing procedure after spine laminectomy have been reported (Trout, 2010). In this study the difference between the greater omentum grafts with or without macroscopic fat deposition has been noticed which according to our knowledge is a new aspect of using different part of greater omentum for acceleration of bone healing.

MATERIALS AND METHODS

Fifteen female adult New Zealand white rabbits, weighing 1.5±0.3kg were randomly divided into three equal groups, i.e., A, B and C. General anesthesia was performed by intramuscular injection of Xylazine-Ketamine cocktail (Xylazine: 5mg/kg and Ketamine: 35mg/kg). The skin coat of antebrachium region of both forearms was prepared with surgical spirit.

A 3cm incision was induced on the lateral surface of the mid region of each antebrachium and after retraction of skin and soft tissues; a 1mm diameter hole was drilled on the cranial surface of the radius through the mid-shaft. For obtaining 3mm circular omentum samples (Fig. 1), approaching to the abdominal cavity was performed by a 2cm incision between the pelvic inlet and the umbilicus on the midline of ventral abdomen.

For group A, the omentum sample without macroscopic fat deposition was obtained. In this group, the drilled hole on the left radius was filled by the omentum sample without macroscopic fat deposition and the drilled hole on the right radius left intact for consideration as control and the skins were closed routinely. For group B the omentum sample with macroscopic fat deposition was obtained. In this group, the drilled hole on the left radius was filled by the omentum sample with macroscopic fat deposition and the drilled hole on the right radius left intact for consideration as control and the skins were closed routinely. For group C two samples of omentum with or without macroscopic fat deposition were obtained. In this group, the drilled hole on the left radius was filled by the omentum sample with macroscopic fat deposition and the drilled hole on the right radius was filled by the omentum sample without macroscopic fat deposition for comparing to each other and the skins were closed routinely.

Antibiotic therapy with Oxytetracycline 10% (2mg/kg BW IM for 5d) and routine antiseptic dressing, after applying Povidone-Iodine 10% solution in the location of sutures, were performed. We had two reasons for using Oxytetracycline with above protocol, first, the efficacy of this broad-spectrum antibiotic on both bone and soft tissue and next, the potential of high dose usage of this drug in postponing bone healing process (Plumb, 2002).

Orthogonal radiography with mammography films and cassette combination according to the technique chart specification were done by Sedecal X-ray Machine for both antibrachid, before and at days 1, 15, 30, 45 and 60 after the surgery and film processing was done by Protec automatic processor and routine film-reading procedure was carried out. Subjective evaluations of decreasing size of the drilled holes on lateral views were performed by giving one point for each 25% decreasing size. In grade I no change was observed, whereas grade II, III, IV and V showed 1-25, 26-50, 51-75 and 76 to 99% decreasing trend in the drill holes made, respectively followed by grade VI that showed 100% healing according to the obtained radiographic data (Table 1). The data analyses by Wilcoxon Signed Ranks Test with SPSS software were done and significances for the changes were calculated.

RESULTS

Unfortunately case A1 was dead on day 12 and case B5 had a fracture in right forelimb on the first day after surgery. Subjective evaluation data were collected on days 1, 15, 30, 45 and 60 according to the decreasing size of the drilled holes on lateral views (Table 1).

Fig. 2 shows the lateral view radiograph of rabbit normal forelimb. Fig. 3 to 8 show subjective evaluation grades I to VI, respectively. In group A, the obtained results of the study on bone healing process of the experimental defects on the left and right radius bones did not differ significantly (P=0.059). Similarly, in group B, the obtained results of the study on bone healing process of the experimental defects on the left and right radius bones did not show a significant difference (P=0.096). Difference in sum of ranks of bone healing grades of experimental bone defects in right and left forelimbs of group A and B has been shown (Table 2) but the difference was not significant (Table 3).

In group C, the obtained results of the study on bone healing process of the experimental defects on the left and right radius bones showed a significant difference (P=0.007). Sum of ranks of bone healing grades of experimental bone defects in right and left forelimbs of group C showed significant ($P<0.05$) difference (Table 3).

DISCUSSION

Studies in animal models have demonstrated that new blood vessels are intimately linked with healing fracture tissues and when the periosteum is activated, the process of bone formation from cells already present in the periosteum is augmented by proliferation and

Table 1: Subjective evaluation of decreasing size of the drilled holes on lateral views of forelimb on days 1, 15, 30, 45 and 60 of each sample

Cases	Left (L) or Right (R) of Forelimb									
	R	L	R	L	R	L	R	L	R	L
	Experimental Days									
	1		15		30		45		60	
A1	I	I	D	D	D	D	D	D	D	D
A2	I	I	II	III	IV	V	VI	VI	VI	VI
A3	I	I	III	II	V	IV	V	V	VI	V
A4	I	I	II	I	IV	III	VI	VI	VI	VI
A5	I	I	II	I	V	IV	V	V	VI	VI
B1	I	I	III	II	V	IV	VI	VI	VI	VI
B2	I	I	II	I	V	IV	VI	V	VI	VI
B3	I	I	II	III	VI	VI	VI	VI	VI	VI
B4	I	I	III	II	VI	VI	VI	VI	VI	VI
B5	F	I	F	I	F	IV	F	V	F	V
C1	I	I	V	II	V	IV	VI	V	VI	V
C2	I	I	III	II	VI	V	VI	VI	VI	VI
C3	I	I	II	I	III	II	VI	V	VI	VI
C4	I	I	I	I	IV	III	V	IV	VI	V
C5	I	I	I	I	V	IV	V	V	V	V

Table 2: Evaluation of left - right hand of bone healing grades of experimental bone defects in right and left forelimbs of each group.

Groups	Ranks	N	Mean Rank	Sum of Ranks
A	Negative Ranks	7[a]	5.00	35.00
	Positive Ranks	2[b]	5.00	10.00
	Ties	12[c]		
	Total	21		
B	Negative Ranks	6[a]	4.00	24.00
	Positive Ranks	1[b]	4.00	4.00
	Ties	13[c]		
	Total	20		
C	Negative Ranks	8[a]	4.50	36.00
	Positive Ranks	0[b]	0.00	0.00
	Ties	17[c]		
	Total	25		

a= Evaluation of left hand < evaluation of right hand; b=Evaluation of left hand > evaluation of right hand and c=Evaluation of left hand = evaluation of right hand.

Table 3: In group A and B the difference was not significant while in group C the difference was significant (P<0.05).

Test Statistics [b]		
Groups		evaluation of left hand-evaluation of right hand
A	Z	-1.667[a]
	Asymp. Sig. (2-tailed)	0.096
B	Z	-1.890[a]
	Asymp. Sig. (2-tailed)	0.059
C	Z	-2.714[a]
	Asymp. Sig. (2-tailed)	0.007

a=Based on positive ranks; b=Wilcoxon Signed Ranks Test;

differentiation of pericytes, which contribute a supplementary population of osteoprogenitor cells (Diaz-Flores *et al.,* 1992). Omentalisation of the fracture is a novel approach in an attempt to introduce a blood supply and encourage the formation of callus at the fracture site and to aid resolution of the infection (McAlinden *et al.,* 2009). Mikami (1981) reported that autogenous omental graft with microvascular anastomoses can be used to eliminate the dead space of bone. Surgical technique is important in acceleration of bone healing, on the other hand, putting a piece of omentum into the bone defect has a different result with attaching the piece of omentum over the bone defect by suturing it to the periosteum.

In groups A and B, we focused on the study of the potential of omentum sample without and with macroscopic fat deposition in acceleration of the bone healing for experimental defect, respectively. Difference in bone healing procedures of the experimental defects on the left and right radius bones in both the groups was non significant. In group C, we focused on comparing the potential of acceleration of bone healing between the omentum with macroscopic fat deposition (left radius) and without macroscopic fat deposition (right radius). In this group, the processes of healing in the right radius bone were faster than the left radius bone. From these results it can be interpreted that usage of the omentum samples with macroscopic fat deposition has a potential in postponing the bone healing processes. This postponing procedure likes the work of fat piece from the layer below the skin which will protect the bone defect as it heals in laminectomy procedure (Trout, 2010). According to our

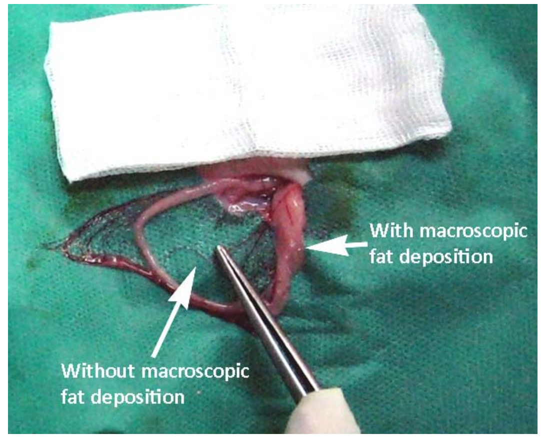

Fig. 1: Greater omentum with and without macroscopic fat deposition

Fig. 2: The lateral view radiograph of rabbit's normal forelimb

Fig. 3: Subjective evaluation grade I (decreasing size of the drilled holes is 0%).

Fig. 4: Subjective evaluation grade II (decreasing size of the drilled hole is 1-25%).

Fig. 5: Subjective evaluation grade III (decreasing size of the drilled hole is 26-50%).

Fig. 6: Subjective evaluation grade IV (decreasing size of the drilled hole is 51-75%).

Fig. 7: Subjective evaluation grade V (decreasing size of the drilled hole is 76-99%).

Fig. 8: Subjective evaluation grade VI (decreasing size of the drilled hole is 100%).

study, we will have some challenge with results of another study. Nottbaert *et al.* (1989) declared that lipid material extracted from the omentum contained a potent angiogenetic activator. Oloumi *et al.* (2006) showed the acceleration of bone healing by increasing of angiogenic potency due to the presence of angiogenetic activator. According to the results of our study we think that different part of omentum, depends on the amount of macroscopic fat deposition, can have different potential in omentalisation for the acceleration of bone healing. We recommend that using the part of omentum with no macroscopic fat deposition (if it is possible according to the patient body condition) has more efficiency in acceleration of bone healing process.

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