Bone 63 (2014) 53–60

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Effects of osteogenic medium on healing of the experimental critical bone defect in a rabbit model

Ahmad Oryan [a], Amin Bigham-Sadegh [b,*], Fatemeh Abbasi-Teshnizi [a]

[a] Department of Veterinary Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
[b] Faculty of Veterinary Medicine, Shahrekord, Iran

ARTICLE INFO

Article history:
Received 21 November 2013
Revised 13 February 2014
Accepted 18 February 2014
Available online 25 February 2014

Edited by: David Fyhrie

Keywords:
Osteogenic medium
Maintenance medium
Bone healing
Rabbit model

ABSTRACT

Today, finding an ideal biomaterial to treat the large bone defects, delayed unions and non-unions remains a challenge for orthopedic surgeons and researchers. Several studies have been carried out on the subject of bone regeneration, each having its own advantages. At the same time, a variety of disadvantages still remain. The present study has been designed in vivo to evaluate the effects of osteogenic medium on healing of experimental critical bone defect in a rabbit model. Twenty New Zealand albino rabbits, 12 months old, of both sexes, weighing 2.0 ± 0.5 kg were used in this study. An approximately 10 mm segmental defect was created in the mid portion of each radius as a critical size bone defect. In the osteogenic medium group (n = 5) 1 ml osteogenic medium, in the maintenance medium group (n = 5) 1 ml maintenance medium, and in the normal saline group (n = 5) 1 ml normal saline were injected in the defected area while the defects of the rabbits of the control group (n = 5) were left empty. Radiological evaluation was done on the 1st day and then at the 2nd, 4th, 6th and 8th weeks post injury. Biomechanical and histopathological evaluations were done 8 weeks post injury. The radiological, histological and biomechanical findings of the present study indicated a superior bone healing capability in the osteogenic and maintenance medium groups, by the end of 8 weeks post-surgery, in comparison to the normal saline and control groups. In conclusion, this study demonstrated that the osteogenic medium and maintenance medium could promote bone regeneration in long bone defects better than the control group in rabbit model.

Introduction

The number of patients suffering from bone tumor resections, fracture defects, or chronic infection is rapidly increasing and more than 1 million bone graft operations are performed in the United States every year [1]. Therefore, it is still a challenge for orthopedic surgeons and researchers to find an ideal biomaterial for treatment of the large bone defects, delayed unions and non-unions.

Following surgical procedures or trauma to the bone tissue the healing cascade starts with acute inflammation which is associated with polymorphonuclear cell infiltration, edema, fibrin and blood clot accumulation and hyperemia. This acute inflammatory phase usually lasts for about 4 days [2] and is then followed by the chronic stage of inflammation which is coincident with infiltration of macrophages, lymphocytes and plasma cells. At acute and chronic inflammatory phases, the metalloproteinases, growth factors and vasoactive substances gradually accumulate in the injured area, particularly in the blood clot, to participate in proliferation of the osteoblasts and endothelial cells and to control production of the bone matrix by osteoblasts in the next phase of fracture healing which is fibroplasia or proliferative phase of healing. Collagen type III, glycosaminoglycans, and proteoglycans are secreted by the osteoblasts at the fibroplasia stage of fracture healing. This phase is then followed by the remodeling or maturation phase which is a long standing stage and may even last for several years [2]. In treatment of nonunion and bone defects, autograft is the gold standard for bone repair. However, there are some disadvantages associated with the autografts, such as the limited abundance in supply, new nerve damage, persistent pain and new fractures. Allografts have been used successfully in the orthopedic operations owing to its excellent osteoconductivity and abundance in supply. However, allografts have the potential risk of infection, disease transmission and immune response. On the other hand, allografts are inferior in promoting bone regeneration compared to the autografts, because they require processing, sterilization steps, and preservation before they are used [3–5]. To date, several studies have been conducted to promote bone regeneration. Some of these studies include: application of bone marrow with static magnetic field [6], coral with human platelet rich plasma [7], hydroxyapatite with human platelet rich plasma [8], omentum with adipose tissue stem cells [9], demineralized bone matrix [10,11], nano-hydroxyapatite/collagen,

* Corresponding author at: Department of Veterinary Surgery and Radiology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
E-mail address: dr.bigham@gmail.com (A. Bigham-Sadegh).

http://dx.doi.org/10.1016/j.bone.2014.02.010
8756-3282/

synthetic poly (glycolic-co-lactic acid) polymer [12] and true bone ceramics or sintered bovine bone [13,14]. Each has its own advantages, at the same time various disadvantages still remain. For example, ceramic and polymer-based bone graft substitutes are mostly osteoconductive but are not potentially osteoinductive. Other problems may include unsuitable degradation rates and inferior mechanical properties. In addition, protein- or growth factor based bone graft substitutes usually require addition of an osteoconductive scaffold for structural support [15,16].

Thus far, bone morphogenic proteins (BMPs) have been used in clinical trials to enhance bone healing properties [17–19]. It has been stated that the BMPs are able to stimulate the local undifferentiated mesenchymal cells to transform into osteoblasts (osteoinduction), leading to early bone formation [20–23]. However, critical views on the use of BMPs have recently surfaced due to their short half-lives, high cost and ineffectiveness [24–26].

In a recent study, Bigham-Sadegh et al. [9] inadvertently showed that maintenance medium (MM) with omentum had superior osteogenic properties in comparison to the omentum alone and the control groups. Osteogenic medium (OM) supplemented with L-ascorbic acid 2-phosphate (AsA2-P), dexamethasone (Dex) and β-glycerophosphate (β-GP) has been commonly used for the osteogenic differentiation of the mesenchymal stem cells (MSCs) in vitro [26–28]. In other studies, osteogenic medium has been added to the adipose derived stem cells (ASC) to induce the differentiation of these cells to osteoblasts; the osteogenic medium induced-ASC was then used to evaluate the healing of the bone defect. These studies have shown a significant enhanced bone healing with the osteogenic medium-induced ASCs compared to the non-induced ASCs [29–31]. In addition, a more recent study showed that, osteogenic medium enhances differentiation of the human adipose derived stem cells (hASC) towards bone-forming cells significantly more than growth factors in a tri-dimensional (3D) culture [32]. Therefore, the present study has been designed to evaluate the effects of the osteogenic medium on healing of an experimental critical bone defect in a rabbit model.

Materials and methods

Animals and operative procedures

Twenty New Zealand albino rabbits, 12 months old, of both sexes, weighing 2.0 ± 0.5 kg, were kept in separate cages, fed a standard diet and allowed to move freely during the experimental period. The animals were randomly divided into four equal groups such as osteogenic medium (OM) group (n = 5) [osteogenic medium is a combination of the maintenance medium with L-ascorbic acid 2-phosphate, dexamethasone and β-glycerophosphate that has been used for the osteogenic differentiation of MSCs in vitro], maintenance medium [Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY), containing 10% fetal bovine serum (FBS, Gibco), 100 μg/ml streptomycin and 100 U/ml penicillin] (MM) group (n = 5), normal saline (NS) group (n = 5) and empty defect group (n = 5, control group). All the animals were anesthetized by intramuscular administration of 40 mg/kg ketamine hydrochloride and 5 mg/kg xylazine. The right forelimb of all the animals was prepared aseptically for operation. A 5 cm incision was made craniomedially in the skin of the fore limb and the radius was exposed by dissecting the surrounding muscles. An osteoperiosteal segmental defect was then created on the middle portion of each radius at least twice as long as the diameter of the diaphysis for creation of a nonunion model [33]. As the diameter of the radius of the adult New Zealand albino rabbits is about 5–6 mm, the radial defect was 10–12 mm long. Subcuticular and skin incisions were closed routinely. In the OM group 1 ml osteogenic medium, in the MM group 1 ml maintenance medium, and in the NS group 1 ml normal saline were injected in the defected area 4 days after operation, while the defects in the rabbits of the control group were left empty. The animals were housed in compliance with our institution's guiding principles "in the care and use of animals". The local Ethics Committee for Animal Experiments approved the design of the experiment.

Post operative evaluations

Radiological evaluation

To evaluate bone formation, union and remodeling of the defect, radiographs of each forelimb were taken postoperatively on the 1st day and then at the 2nd, 4th, 6th and 8th weeks post injury. The results were scored using the modified Lane and Sandhu scoring system [34] (Table 1).

Biomechanical evaluation

Eight weeks after operation the rabbits were euthanized for histopathological and biomechanical evaluation. All rabbits were euthanized and the radius attached to the ulna was harvested. The connective tissue was removed, and only the bony structure was kept. The radius and ulna were not separated. Biomechanical test was conducted on the fused radius and ulna in the defected area of each rabbit. The tests were performed using a universal tensile testing machine (Instron, London, UK). The three-point bending test was performed to determine the mechanical properties of bones. The bones were placed horizontally on two rounded supporting bars located at a distance of 30 mm, and were loaded at the midpoint of the diaphysis by lowering the third bar so that the defect was in the middle and had an equal distance from each grip. The force was first received by the ulna and then delivered to the healed defected area of radius. The bones were loaded at a rate of 10 mm/min until fracturing occurred. Deformation (delta w) and ultimate (maximum) load were detected from the graph sketched by the machine.

The bending stiffness was derived using the following equation: Bending stiffness (or bending rigidity) $S = EI$ in N mm^2 is the product of the Elastic modulus E and the axial second moment of inertia I. This is calculated by the formula: $S = EI = (L^3 / 48) \times (\text{delta } F / \text{delta } w)$, where L is the distance between the supporting bars, F is the force, and w is the deformation. Delta F / delta w is taken from the (most) linear part of the load–deformation curve [7]. The data derived from the load–deformation curves, such as ultimate load and bending stiffness, were expressed as Mean ± SD for each group.

Histopathological evaluation

Immediately after biomechanical testing, the specimens were referred for histopathological evaluation. Histopathological evaluation was carried out in the injured area of five rabbits of each group. Sagittal sections containing the defect were cut with a slow speed saw. Each slice was then fixed in 10% neutral buffered formalin. The formalin-

Table 1
Radiographical findings for healing of the bone defect.

Bone formation	
No evidence of bone formation	0
Bone formation occupying 25% of the defect	1
Bone formation occupying 50% of the defect	2
Bone formation occupying 75% of the defect	3
Bone formation occupying 100% of the defect	4
Union (proximal and distal ends were evaluated separately)	
No union	0
Possible union	1
Radiographic union	2
Remodeling	
No evidence of remodeling	0
Remodeling of medullary canal	1
Full remodeling of cortex	2
Total possible points per category	
Bone formation	4
Proximal union	2
Distal union	2
Remodeling	2
Maximum score	10