Evaluation of Osteoinductive and Osteoconductive Effect of the Amniotic Membrane in Bone Defects due to Open Fractures in Rabbits

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Received 2018 May 09; Revised 2018 May 29; Accepted 2018 June 01.

Abstract

Background: The repair of long bone segmental defects is one of the most challenging problems in orthopaedic surgery.

Objectives: The current researchers carried out animal experiments on the use of Human Amniotic Membrane (HAM) in bone defect to evaluate the osteoinductive and osteoconductive effects, and also to use it as a guide for regular production of bone without waiting for membrane production (MASQUELET method).

Methods: Twenty New Zealand white male adult rabbits were used in the study, and divided to four groups. The surgical site was prepared with the purpose of working on the left forearm diaphysis. In each radius, a bone defect of 15-mm in length was created. The fixation of the radius was not done because the support of the ulna was sufficient. The defect was not filled with anything in group 1; however, a tube-shaped HAM was inserted in group 2, a Tendon-shaped HAM was utilized for group 3 and a Tube-shaped HAM + bone graft (demineralized bone matrix, DBM) was used in group 4.

Results: Bone formation was radiographically observed in the defects, which had been implanted using tube-shaped HAM (group 2), which was complete in 60% and partially complete in 40% of the cases. No bone formation was seen at up to eight weeks after surgery in group 1 and 3. A small amount of bone formation was observed at both ends and the ulnar site of the defect in group 4.

Conclusions: The results of the study indicated that tube-shaped HAM could have an osteoconductive effect in large segmental bone defects, yet could not have an osteoinductive effect.

Keywords: Human Amniotic membrane (HAM), Rabbits, Osteoconductive, Osteoinductive

1. Background

One of the challenging problems in orthopaedic surgery is bone segmental defects repair (1). Segmental bone defects often result in complicated problems with significant long-term morbidity (2). Because of uncontrollable graft resorption of traditional bone graft, they are limited, even when the recipient site is well vascularized. More recently, the Masquelet technique has been identified as a potential treatment strategy (2).

Until the recent years, the Masquelet technique has rarely been studied or assessed among bone reconstruction techniques, in surgical literature (3). The Masquelet technique, which is the use of a temporary cement spacer followed by staged bone grafting, is a contemporary treatment for handling of a post traumatic bone defect (2). Any defect with a minimum length that cannot be spontaneously bridged leading to non-union is described in the surgical literature as critical size defect (CSD) (4).

Although several methods have been proposed for bone reconstruction, they have specific indications and limitations. Established methods are distraction osteogenesis and bone transport, or bone grafting, allografts, bone marrow aspirate, and growth factors. The concept of an induced-membrane represents another strategy for bone regeneration, especially in cases with large bone defects. This method includes a two-stage procedure, where a ‘biological’ membrane is induced after use of a cement spacer in the first stage as a foreign body response that acts as a ‘chamber’ for the insertion of autologous bone-graft in the second stage. The concept of guided bone regeneration (GBR) has also been used for bone reconstruction that uses a membrane to prevent soft-tissue invasion in the defect and forms a ‘chamber’ to ‘guide’ the bone production process (4). The GBR technique promotes the increase of
osteoblastic proliferation and bone matrix production (5). The clinical use of the human amniotic membrane (HAM) is also in line with the modern concept of biomechanical GBR (6).

Human amniotic membrane is a biomaterial, lacking any vascular tissue, which consists of three layers i.e. a single epithelial cell layer, intermediate basement membrane layer, and a mesenchymal cell layer. Certain characteristics of HAM make it a useful biomaterial for therapeutic purposes, including anti-inflammatory, anti-scarring, promotion of epithelialization (7-9), anti angiogenic effect, anti-pain properties (10, 11), mechanical properties (12), enhanced healing of wounds (13, 14), adhesive effect of the stromal layer (15, 16), anti-adhesive effect of the epithelial layer (17, 18), low immunogenicity property (19, 20), antibacterial and antiviral activity (21, 22), anticancer effects (23), and induction of apoptosis (24).

Furthermore, HAM has been used in clinics for the treatment of various problems, such as coverage of skin defects, anti-inflammation in burned skin or ulcers (25, 26), corneal surface reconstruction (9, 27), ocular surface reconstruction (17, 28), control of wound infection (29), treatment of oral facial defects (30), bladder augmentation (31), and reconstruction of long ureteral defects (32). Based on properties of HAM, in this study it was decided to work on animals to evaluate the osteoinductive and osteoconductive effects of HAM. Moreover, it was decided to use it as a guide for regular production of bone without invasion of new bone to soft tissue. In this way, with use of a prepared membrane, it is not needed to wait six weeks to produce a membrane (that was needed in the Masquelet method), and the second stage of surgery could be performed faster; which may enable the patients to return to work earlier.

2. Methods

This research was performed at Jundishapur University of Medical Sciences, Ahvaz, Iran. Human amniotic membrane was obtained via cesarean section from selected human subjects. It was separated from the remaining chorion by blunt dissection. After blood clot cleaning with sterile saline solution, it was incubated in 800,000 IU penicillin G. One week later, the paper adherent HAM was cut in 2 × 1.5 cm tapes.

Twenty New Zealand white male adult rabbits with an average weight of 3 kg were used and divided to four groups. A week before surgery, the animals entered the Bioterio of the Ahvaz Jundishapur University of Medical Sciences to become acclimated to the new environment. Water and food were suspended eight hours before surgery. The anesthesia was induced with administration of ketamine at dose of 30 mg/kg, intraperitoneally. For maintenance of anesthesia, Promazil was administered (1 mg/kg).

The surgical site was prepared to work in the left forearm diaphysis. A 2.5-cm incision was made on the middle part of the radius, extending to the periosteum with a scalpel blade No. 15. Then, the flap was lifted with a curette leaving the bone exposed. Progressive drilling was performed with surgical burs. In each radius, 15 mm in length, bone defects were created, which was the critical size of bone defect. The fixation of radius was not done because the support of ulna was enough. The defect was not filled in group 1 (control group), tube-shaped HAM was used in group 2 (evaluating the bone formation and osteoconductive and osteoinductive effects of the HAM), and tendon-shaped HAM in group 3 (the bone formation and osteogenic effect of the HAM), and a tube-shaped HAM + bone graft (evaluating the effect of BG in bone production when it is used with HAM) was applied in group 4. Demineralized Bone Matrix (DBM) was used as bone graft.

After repair of muscle, the skin was closed. After surgery, the rabbits were kept caged freely and given their usual regimen of food and water. After the surgical procedure, a single dose of antibiotic was administered (0.25 gr, Cefazolin IM). Radiographs were performed on the day of the surgery, then at four weeks and eight weeks after surgery. After eight weeks, the animals were killed by administering intravenous overdose of ketamine. To obtain bone samples, a longitudinal lateral approach was used, completely removing the forearm by disarticulation. Once extracted, the forearm was placed in 10% formaldehyde, and the site of the surgery was submitted to light microscopic analysis.

3. Results

In the current study no infection, mortality or morbidity was seen.

3.1. Radiographic evaluation

Bone formation was radiographically observed in the defects implanted using a tube-shaped HAM (group 2), that was complete in 60% of the subjects and partially complete in 40% of the subjects. No bone formation was seen at up to eight weeks after surgery in Group 1 and Group 3 (the control and tendon-shaped HAM). A small amount of bone formation was observed at both ends and the ulnar site of the defect in Group 4 (HAM + BG) (Figure 1-5).
3.2. Microscope findings

(+) is the amount of bone formation (mature bone trabeculae) in 10/HPF, and as there are 4 groups thus:
- 80% to 100% bone formation → 4+
- 50% to 80% bone formation → 3+

30% to 50% bone formation → 2+
< 30% bone formation → 1+

In the control group (Group I), no bone formation was seen. In Group 2 (tube shaped HAM), the surgical bone defect was filled by mature bone trabeculae, lamellar bone,
and wide medullary spaces filled with yellow and red marrow. Osteoprogenitor cells surrounding the bone was also observed. In addition, signs of HAM infiltrated by mononuclear cells adjacent to immature bone were found. The amount of bone formation was 4+ in 60% of the subjects (three cases) and 3+ in 40% of the cases (two cases). The evaluation of osteoconductive effect of HAM was performed using Fisher’s exact test that showed a significant difference (P = 0.008) (Figure 6–7).

In Group 3 (tendon shaped HAM), very small foci of calcification and tiny fragments of woven bone, intermingled with soft tissue were noted (Figure 8).

In Group 4 (HAM + BG), the surgical bone defect was filled with immature chondroid tissue with signs of early mineralization (Figure 9). Moreover, the presence of multinucleated foreign body type giant cells and foamy macrophages ingested in the DBM was seen (Figure 10). Scattered immature bone trabeculae surrounded by multinucleated foreign body type giant cells and also mononuclear cells with calcified foci was found. The amount of bone formation was 2+ in 60% of the subjects (three cases).
and 1+ in 40% of the cases (two cases). For the comparison of Group 4 and the control group, the Fisher’s exact test was applied and the results showed a significant difference (P = 0.008).

The mean bone formation in Group 2 was 3.6 ± 0.54 and in Group 4 was 1.6 ± 0.54, which indicated a significant difference based on the Mann-Whitney U test (P = 0.007).

The absence of bacterial colonies in the experimental groups was also seen.

### 4. Discussion

As indicated earlier, the repair of long bone segmental defects is one of the challenging problems in orthopaedic surgery, and in the recent years, Masquelet’s induced membrane technique, proposed in 1986, which focuses on the use of a temporary cement spacer followed by staged bone grafting complimented by an induced biomembrane, has been proposed as a potential treatment strategy.

The authors of the paper believe that the anti-inflammatory, anti-scarring, promotion of epithelialization (7, 8), anti-pain properties (10, 11), mechanical properties (12), enhanced healing of wounds (13, 14), adhesive effect of the stromal layer (15, 16), anti-adhesive effect of the epithelial layer (17, 18), low immunogenicity property (19, 20), antibacterial and antiviral activity (21, 22), and anticancer effects (23) and induction of apoptosis (24) of HAM, make it an extremely useful biomaterial for therapeutic purposes.

Furthermore, HAM has been used for guided bone regeneration in small bone defects in skulls and mandibles of rabbits alongside other materials (5). However, it has not been used in large bone defects (critical size) in long bones.

Masquelet et al. designed a method for large bone defects reconstruction. They produced a membrane and used it for guided bone regeneration. Six weeks was required for the membrane production. It was based on these results that the authors of this paper decided to use a prepared membrane instead of induced membrane to reduce the need for several complex surgeries and expedite the amount of time needed for the patient to return to work.

In this study, microscopic results showed that in the eight weeks period, a greater amount of bone tissue was formed in Group 2 (tube-shaped HAM) than in the other groups, which showed a significant difference (P < 0.05). This indicated that HAM has an osteoconductive effect. In Group 4, the presence of multinucleated foreign body type giant cells and foamy macrophages ingested the DBM (bone graft) was seen and bone formation was less than group 2, which in itself can show the immune response against DBM. However, the mean bone formation in Group 4 was less than Group 2, which was unexpected. These re-
sults were in contrast to studies that showed HAM + BG had more bone formation (5). It is believed that one possible reason is that such a result might be due to the use of human BG (xenograft) in the study. What is more, is that there was no bone formation in Group 3 (evaluate the osteogenic effect of HAM) and it was proposed that this could be due to use of a soft tissue in the filling of the bone defect. It was also suggested that the authors might have destroyed the multi potential effect of HAM cells during the preparation process of the membrane. In Group (2), HAM existence in the defect prevented the invasion of soft tissue to the space.

The absence of bacterial colonies in the experimental groups was also seen. This may have been the result of the antibacterial activity of HAM, in accordance with the findings of Monica Fernande Gomes et al. (5).

These results indicate that tube-shaped HAM could have an osteoconductive effect in large segmental bone defects, yet could not have an osteoinductive effect. This might increase the speed and amount of bone production using autograft or multi-potential cells of Wharton’s jelly from the umbilical cord in the space (bone defect) created; however, there is a need for further studies in this regard. One can use HAM as a prepared membrane and use in bone defect as a guide for bone production. Tube-shaped HAM can prevent soft tissue invasion to this space while six weeks is not needed for this membrane production (this time is needed in the Masquelet method).

The limitation of this study was the low sample size, because it was a pilot study.

Acknowledgments

The paper was issued from the thesis of Dr. Ahmad Abbassazadeh. This study was financially supported by Alvaz Jundishapur University of Medical Sciences.

References


