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EVALUATION THE EFFECT OF HYALURONIC ACID ON BONE HEALING PROCESS: AN EXPERIMENTAL STUDY IN THE RABBITS

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ABSTRACT

This study was aimed to assess the effect of hyaluronic acid HA on bone healing in mandibular bone defect in the rabbits. Ten adult White male rabbits with a mean weight of 2.3 ± 0.42 kg were used as the animal model. Two cavities of 4mm diameter have been created in the lateral surface of the mandible. One of the cavities was filled with 1% HA gel soaked onto a pre-cut absorbable collagen sponge (test one), while the other hole was left for normal healing (control one). On the 4, 8 weeks, the rabbits have been sacrificed in equal numbers and defective regions have been extracted. At 8 weeks, histological analysis of specimens extracted from the cavities with HA showed large areas of woven bone contained osteocytes and covered by lining of osteoblasts .On the other hand small areas of woven bone and areas of osteoid bone were observed in the control cavities. Hyaluronic acid enhances the bone healing regeneration.

KEYWORDS: Bone grafts, bone healing, hyaluronic acid.

INTRODUCTION

Bone graft materials that are presently used in bone defects are autogenous bones, allogeneic bones, xenogeneic bones, and alloplastic materials.[1] Ideally, the graft material is required to have the ability to facilitate osteogenesis, stability when implanted with the graft, low risk of infection, ready availability, low antigenicity, and a high level of reliability.[2] Autograft is considered the gold standard for its biocompatibility, capability of osteogenesis, osteoinduction, and osteoconduction. However, the use of autografts has shown increased treatment time, surgical complications, pain and dysfunction at the harvested site, high cost, and limited bone availability for the graft.[3] On the other hand, allogenic and xenogenic bone graft may lead to immunologic reactions, infections, and improper fibrous healing.[4] Other studies used growth factors like recombinant human bone morphogenetic protein (rhBMP), or platelet rich plasma (PRP) with various types of bone grafts to enhance and accelerate bone regeneration.[5-7] Hyaluronic acid is a high-molecular-weight non-sulfated glycosaminoglycan present within the extracellular matrix. Hyaluronic acid (HA) can promote cell migration and differentiation during tissue formation and plays an important role in wound healing.[8-10] The purpose of this study is to evaluate the effect of hyaluronic acid on bone healing in mandibular bone defect in the rabbits.

MATERIALS AND METHODS

Experimental animals

Ten adult White male rabbits with a mean weight of 2.3 ± 0.42 kg were used as the animal model. Experimental protocols were approved by University of Al Andalus university Committee of Animal Research.

Surgical procedures

All surgical procedures were performed under general anesthesia with a combination of 35 mg/kg intramuscular ketamine and 5 mg/kg subcutaneous xylazine. Local anesthesia, consisting of 2% lidocaine with 1:100,000 epinephrine was infiltrated into the lateral surface of the mandibular body. The surgical site was shaved, prepared with 10% povidone-iodine solution, and draped to maintain aseptic conditions. A 1.5 dissection was performed through the subcutaneous and muscle layers. The periosteum was carefully elevated to expose the lateral aspect of the mandibular body. Two intra bony cavities were made under a constant irrigation of sterile saline for each rabbits. The diameter of each cavity was 4 mm . One of the cavities was filled with 1% HA gel soaked onto a pre-cut absorbable collagen sponge (experimental one).While the other cavity was left for normal healing (control one) fig(1). The wound was closed in layers, using 4-0 Vicryl sutures. Postoperative analgesic included buprenorphine (0.3 mg intramuscular).

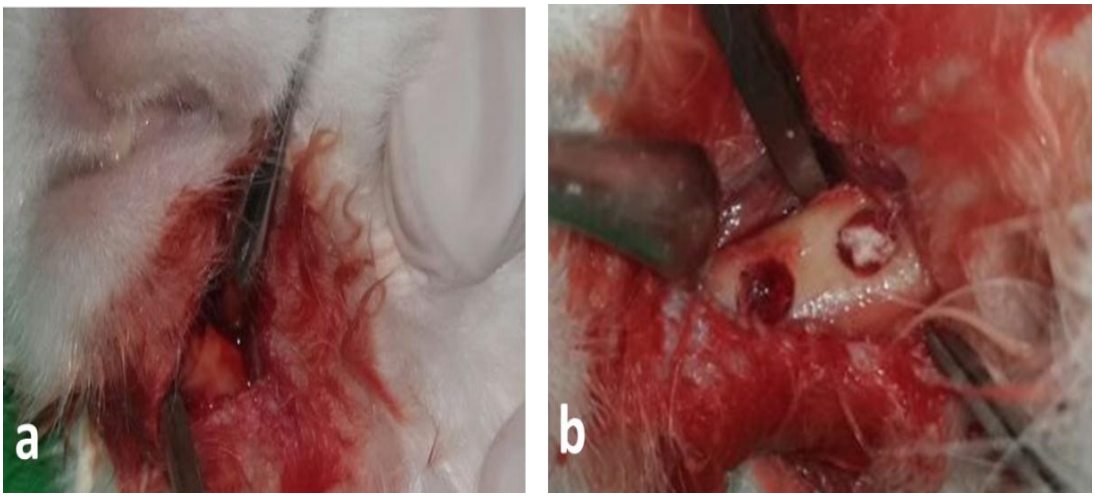

Figure 1: intraoperative photograph, a: submandibular dissection, b: test and control cavities.

Specimen preparation

On 4, 8 weeks, the rabbits were grouped into equal numbers sacrificed by an intravenous over dose of pentobarbital sodium. Bone segments on which experiment has been done were extracted and kept in a 10% neutral buffered formalin solution for at least 3 days. The specimens were then decalcified in the formic acid solution. When sufficiently soft, tissue samples were processed and embedded in paraffin for histological examination. Standard 4–5-mm sections were prepared and transferred onto slides for each block of tissue. All slides were stained with haematoxylin and eosin, and evaluated using a light microscope.

RESULTS

After 4 weeks

Histological analysis of specimens extracted from cavities treated with HA showed an areas of early bone formation in form of osteoid bone trabeculae surrounded by osteoblasts separated by marrow spaces, Additionally, high vascularity areas and collagen fibers were detected. On the other hand, the control cavities showed a formation of dens connective tissues and new blood vessels and small areas of osteoid bone trabeculae (fig.2).

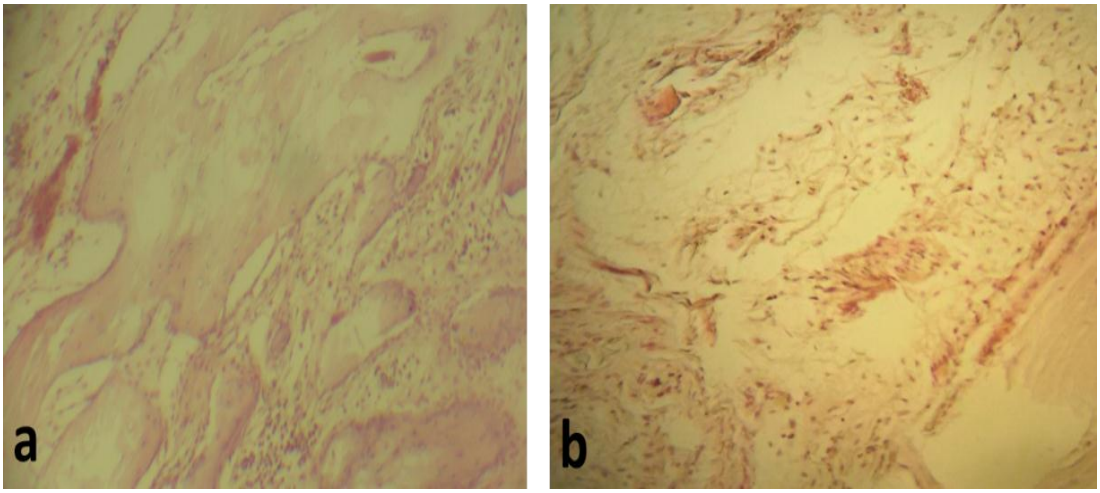

Figure 2: histologic analyses of 4 weeks biopsy sample, a:test group, b:control group: (h&e staining, x 100).

After 8 weeks

Histological analysis of specimens extracted from cavities with HA showed al large areas of woven bone contained osteocytes and covered by lining of osteoblasts and surrounding by a dense connective tissue. On the other hand, small areas of woven bone and areas of osteoid bone surrounded by connective tissue were observed in control cavities (fig.3).

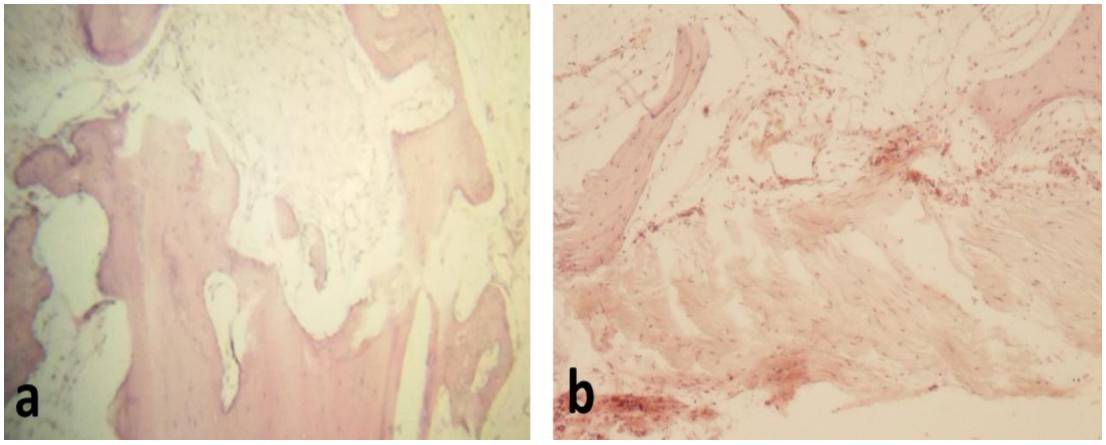

Figure 3: histologic analyses of 8 weeks biopsy sample, a: test group, b:control group: (h&e staining, x 100).

DISCUSSION

This study was aimed to assess the effect of hyaluronic acid on bone healing in mandibular bone defect in the rabbits. Hyaluronic acid (HA) is one of the essential components of extracellular matrix, which plays a predominant role in tissue morphogenesis, cell migration, differentiation, and adhesion.[11,12] The results of Previous studies showed the ability of HA in enhancing bone healing experimentally and cynically.[13-16] In addition, HA has been used as a carrier for demineralized bone allograft without reducing its effectiveness for sinus lift augmentation.[17] Aslan et al.[14] noted that HA stimulated bone healing through accelerating the three phases of healing; inflammation, proliferation and migration of mesenchymal cells and they confirmed that HA needs an osteoconductive scaffold to be effective, as their findings showed that associating HA with bone grafts improved the rate of bone formation in each evaluation period. In the present study the Hyaluronic acid is loaded in absorbable collagen sponge. Collagen sponges are well-characterized carrier systems that provide a sustained release of biomolecules with a putative role in bone regeneration.[18,19] The histological analysis after 8 weeks in present study showed an increasing in new bone formation compared to untreated bone cavities. This is in agreement with previous studies which recorded the capacity of HA in supporting the significant bone formation when combined with bone marrow stromal cell and basic fibroblast growth factors,[12] with recombinant human bone morphogenic protein _2.[13] and with spongiosal bone graft.[14] In addition, it was shown that HA with a collagen sponge could stimulate improvement of bone formation in bone defects.[20] Mendes et al. revealed that HA could enhance healing in tooth sockets by promoting the expression of bone morphogenetic protein-2 and osteopontin.[21] Kim et al.[22] demonstrated that the use of HA that can promote wound healing, it may be beneficial and indicated when treating infected sockets. Sasaki & Watanabe[9] showed that HA is capable of accelerating new bone formation through mesenchymal cell differentiation, in a bone marrow ablation model in rat femurs. They were able to demonstrate that bone formation had already been induced at day 4 after the application of HA. On the other, histomorphometric measurements in the study of Segari et al[23] revealed that, there was no influence of adding HA to CP as adjunctive to osseous tissue healing. However, it was suggested that HA has a molecular weight-specific and dose-specific mode of action that may enhance the osteogenic and osteoinductive properties of bone graft materials.[12]

CONCLUSIONS

Under the conditions of this study, hyaluronic acid could enhance the bone healing regeneration.

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