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Topical Impact of Nanosimvastatin on Bone Fracture

Luay H. Jalil

College of Dentistry, Al-Farahidi University, Baghdad, Iraq

Abstract

Bone healing is a vert complex process. Fracture healing is a major challenge in orthopaedic practice and research. Simvastatin is an encouraging novel bone regeneration inducer due to its safety, low cost, and clinical convenience. However, the specific mechanism of simvastatin's effect on bone growth, as well as the best dose, are unknown. Simvastatin is a biodegradable polymer that promotes bone growth. A total number of 16 adult albino rabbits were divided into two groups (8 rabbits /each). A defect was done in bone and filled with gel only for controls and treatment specimens were filled with nanoliposomal simvastatin. The results showed that the treatment group with nanosimvastatin gel had a significant improvement in bone healing compared to the control group.

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Email: Luay.h.Jalil@@uofarahidi.edu.iq

Introduction

Bone healing is a very complex process that relies on the coordinated action of numerous cell lineages on a series of biological processes. Fracture healing assessment is a major challenge in orthopedic practice and research [1]. Bone healing requires the regeneration of a functional bone structure [2]. Traditional therapies, such as bone grafts, and different types of synthetic bone substitutes are still challenging either because of their plain side effects or because they are financially. Tissue-engineering technique, in which osteogenic stem cells and/or bone growth factors are multiplied or loaded in extracellular matrices in vitro before being transplanted in vivo to repair bone defects, is the most recent approach. The growth factors stimulate bone formation, while the matrices operate as a scaffold that incrementally dissolves as bone ingrowth progresses. This should give the new bone sufficient structure and functionality. However, due to the absence of a solid technique for amplifying and guided differentiation, the use of stem cells is still in the experimental stage [3]. Simvastatin is a white, crystalline powder with a low water solubility. It possesses antihypercholesterolemia properties. It works by blocking the reductase of 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A). This enzyme catalysis cholesterol production. Pravastatin was originally reported to prevent heart transplant rejection in 1990, indicating that statins had an immunosuppressive impact [4]. Simvastatin, a cholesterol-lowering medicine, was found to improve bone production by boosting the expression of the bone morphogenetic protein-2 (BMP-2) gene in bone cells in a previous investigation of osteoporosis. Simvastatin has also been linked to enhanced bone density and a lower risk of osteoporotic fracture in more recent clinical investigations. Simvastatin has been gaining traction as a promising novel bone regeneration inducer due to its safety, low cost, and clinical availability. However, the specific mechanism of simvastatin's effect on bone growth, as well as the best dose, are unknown. The success of bone repair is aided by a variety of cytokines and growth factors [5]. The purpose of this study was to determine a better bone regeneration inducer.

Materials and Methods

We applied the prepared gel to a surgical damage to the left rabbit femur with follow-up of the healing process.

We used simvastatin in a vehicle gel that included cholesterol and lecithin and simvastatin phosphate buffer (pH 7.4).

The chloroform film approach was used to standardize a variety of liposomal solutions. Cholesterol and lecithin were combined and

dissolved in a 9 ml organic combination of chloroform and methanol (2:1), respectively. A thin coating of lipids was deposited inside the round bottom flask of the device after the organic solvent was evaporated using a rotary evaporator. With vigorous shaking, sixty ml of 0.2 M phosphate saline buffer pH 7.4 containing 1mg s simvastatin [6] and 1 mM[1.] EDTA was added to the deposited film, and liposome suspension formed instantly [7].

A total of sixteen adult albino rabbits were used in current study (2.2-3 Kg). Animals were kept in individual cages and were kept in similar conditions (22-24 C0) with light and dark cycle, as well as unlimited pellet meal and water ad libitum. Before the surgical procedure, a veterinary professional performed an assessment to examine the animal's overall health and condition [8].

Food was stopped 9-10 hours before anesthesia was administered. Animal hair was shaved off the left femur, and the region was disinfected with a povidone iodide solution [9]. Food was not eaten for eight to ten hours before anaesthesia was administered. Each animal was given 0.2 mg of vagal tonus to reduce intramuscular atropine sulphate dose of mg/kg injection. Anesthesia was used to put the animals to sleep. Ketamine (50 mg/kg) was injected intramuscularly.

intramuscular injection of (weight in kilograms) and intramuscular injection of (weight in kilograms) diazepam (5.0–10.0 mg/kg body weight). Antimicrobial prophylaxes (preoperative antimicrobial prophylaxes) consisting of ceftrioxone was injected at a rate of 50 mg/kg. The defect that done in bone was filled with nanoliposomal simvastatin for treatment groups and filled with gel only for control groups; sectioned femurs were fixed in 10% formalin in phosphate buffer saline PBS 0.1 M for 48 hrs. Two concentrations were excluded from the study (2% and 0.5%). sectioned femurs were fixed in 10% formalin in phosphate buffer saline PBS 0.1 M for 48 hrs.: At the end of the experimental periods after 6th weeks. The areas of connective tissue and bone were measured in relation to the overall measurement area in histomorphometric evaluations. To avoid any potential bias, the middle section of each core was chosen. I used the image J analysis tool to investigate.

$$\text{Percentage of change \%} = \frac{\text{defect of bone(A)} - \text{contrast area(B)}}{\text{mean of intercorticalbone area (C)} - \text{contrast area(B)}} * 100$$

Volume percentages (percent) of new bone were estimated using histomorphometric measurements of the samples. After identifying the external and internal surfaces of the original femur, the total area was calculated, and the cavity and soft tissue (non-bone region) were circled and defined(10)determined the following histomorphometric parameter.

Area new bone %= [(1 – (non-bone area / total area)] × 100 (%)

Results

Figure 1 shows surgical procedure. At the end of the study, no wound infection was observed. (Figure 2). Nano simvastatin showed mineralization early and complete bridging of defect was seen at end 6th week after treatment group, in contrast to control group that reported less evidence of healing with significant differences (p-value 0.03, Table 1).

In Figure 3, an example of the image J analysis taken for rabbit femur. The area of the defect was replacement with low density bone of granulation tissue in the controls (Figure 4).

For the treated animals, there was significant increase of cellular component of the newly formed soft callus at the site of defect compared with 6th week sections appeared as clusters of osteoblasts surrounded by variable amount of eosinophilic pink osteoid partially mineralized to appear slightly grayish pink at some foci, generally osteoid precipitation is more diffused compared with 6th week sections (control) (Figures 5 and 6).

At the end of 6th week of treatment, bone formation in the treatment group was significantly higher than control group (p= 0.000) (Table 2).

Discussion

Numerous experimental research has investigated the use of statins in bone tissues in combination with various vehicle systems [10]. Simvastatin works by increasing the activity of osteoblasts while inhibiting the activity of osteoclasts. This is accomplished by bone morphogenetic proteins (BMPs) driving osteoblastic cell development (through TNF-a-to-Ras/Rho/mitogen-activated protein kinase competition) [11]. It was reported that the outcome of radiological investigation in all treatment periods revealed an increase in radiopacity and has come to agreement with following gel production and application in the rabbit femur, after taking the right dose [12]. Biological complexity of bone healing of fractures is still an open field for research [13]. Tumors, bone infection, atrophy, and surgical procedures can all cause bone defects in the maxillofacial region. Rabbits were used as models because they acquire skeletal maturity at around [6] months of age. In comparison to other animals, the rabbit has a rapid bone turnover [14]. When compared to rats and mice, the rabbit model has the benefit of being easier to manage and set aside for greater bone abnormalities. The usage of males was limited to avoid possible hormonal changes in females, which could have influenced the results. The cycle of rabbit bone repair takes approximately 42 days, according to consensus. Allows for the investigation of the beginning, intermediate, and final stages of bone repair evaluations conducted over 7, 21, and 42 days . Because it contains red bone marrow and a considerable number of spongy bone and osteoprogenitor cells, the femoral epiphysis is a good candidate for cavity preparation. The femoral epiphysis also allows for the creation of a defect [14]. The goal of this study is to see how simvastatin affects different pathways of bone growth. The effect of simvastatin on entochondrostosis of long tubular bones and intra-membranous ossification of flat bones was investigated using a rabbit radial defect and a monkey calvaria defect model, respectively. The rabbit radial defect is the traditional model for studying bone formation, with a 20 mm long defect regarded the upper limit for self-repair [15]. Simvastatin's ability to increase bone formation and decrease bone loss was first demonstrated in rats after stomach administration and subcutaneous tissue injection [15].

Conclusion

The current study showed that nano-simvastatin gel as a treatment for fractures and bone deformities accelerated their recovery.

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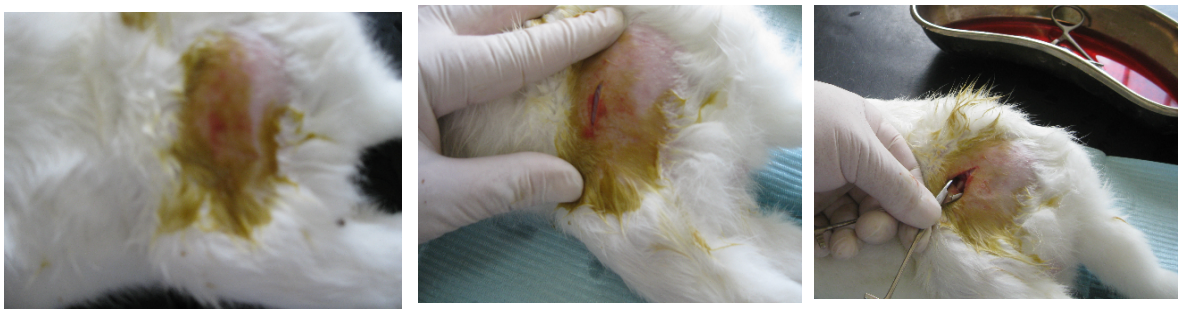


Figure 1. Surgical procedure.

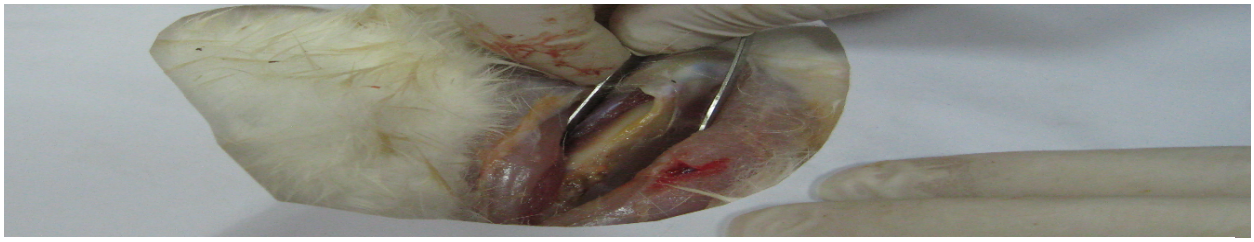


Figure 2. No infection was observed.

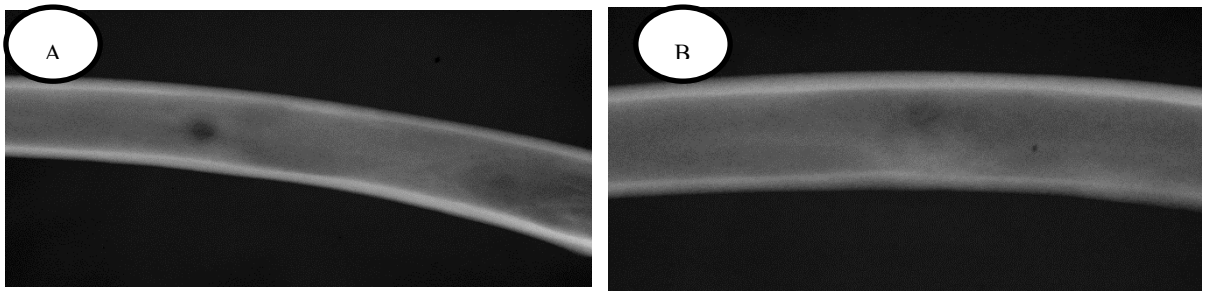


Figure 3. Rabbit femur for test (A) and control animal (B).

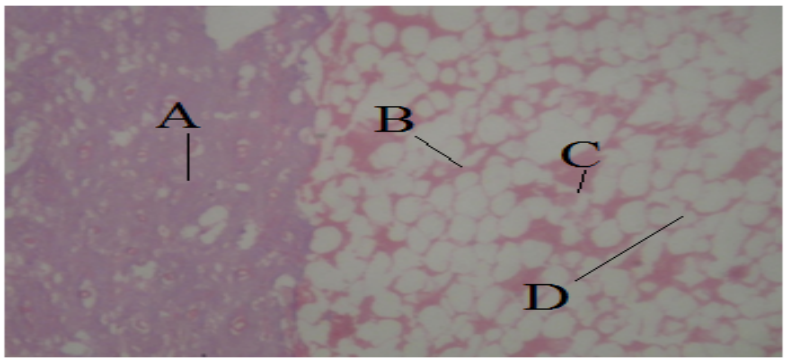
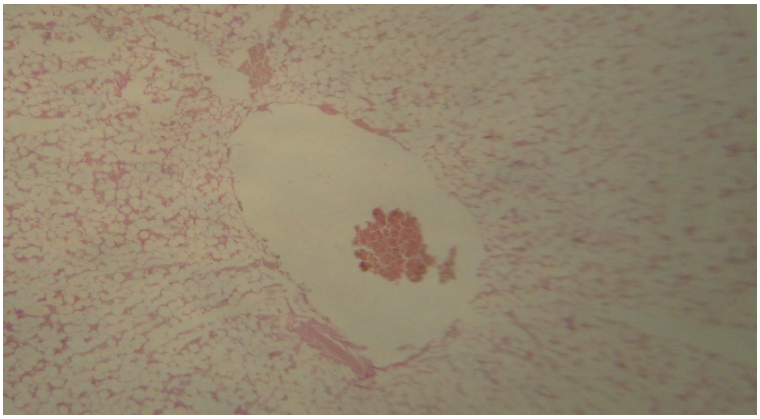
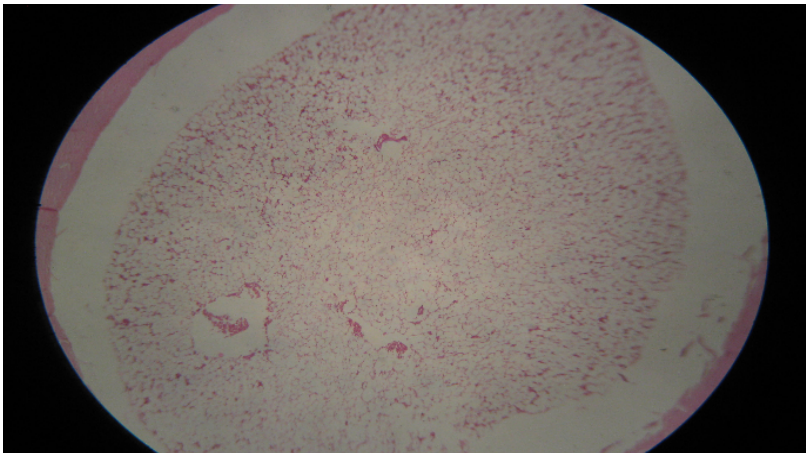


Figure 4. (A) Photomicrograph of rabbit left femoral bone at site of defect at control group after 2 weeks of defect induction showing the original mature bone (A) and the soft callus tissue formed at site of defect composed of mesh like collagen bundles (B), a nidus or foci of osteoid precipitations (C), and newly formed capillaries (D). Magnification 165 X, Staining H&E.



(B) The process if ossification amount of maturation and mineralization of osteiod is much more progressed at peripherally than it is in center of newly formed callus.



(C) Few remnants of fibrin casts still apparent, no sign of inflammatory reaction, few newly formed capillaries can be noted, few multinucleated osteoclasts observed at noticed sections.

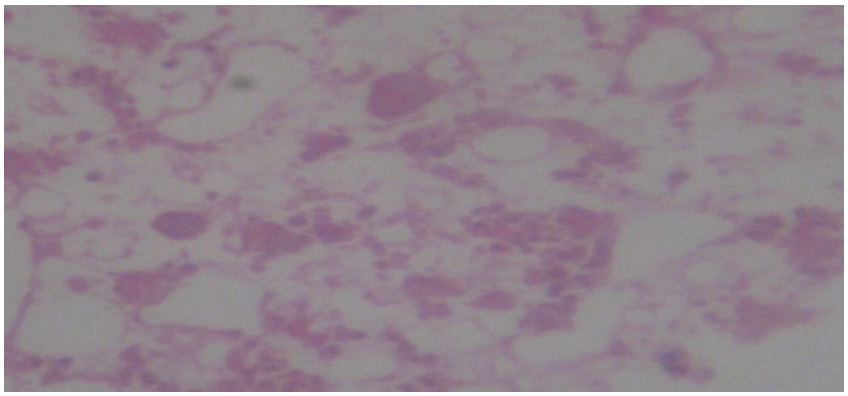


Figure 5. The areolar tissue bridges between those partially ossified segments still recognizable, vascularization and capillaries are more prominent. Photomicrograph of rabbit, femoral bone at site of defect at treated group after 6 weeks of defect induction showing areolar tissue at the center of the callus demonstrating mature capillaries and foci of proliferating osteoblasts. Staining H&E.

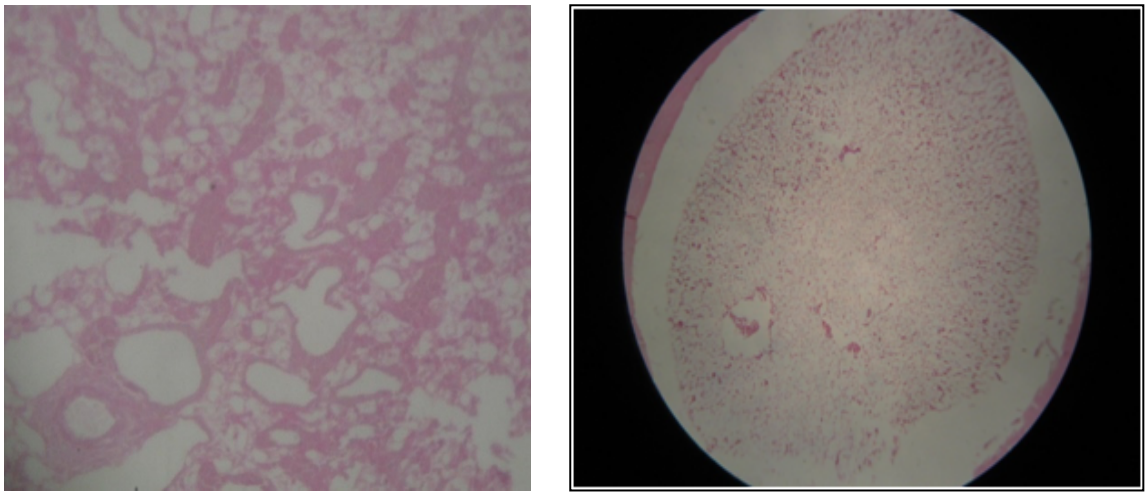


Figure 6. Photomicrograph of rabbit femoral bone at site of defect at treated group after 6 weeks of defect induction showing a magnified view of previous H&E staining.

Group Statistics					
Group	N	Mean	Standard Deviation	Standard.Error Mean	p-value
Control group	8	46.0000	10.74709	4.80625	0.030*
Treatment group	8	64.4000	11.37102	5.08527	

Independent T Test significant difference-*p* value ≤0.05*

Table 2. New bone after created defects in rabbit femur (n=8).

Group Statistics					
Group	N	Mean	Standard Deviation	Standard Error Mean	p-value
Control group	8	0.5720	0.04970	0.02223	0.000
Treatment group	8	0.9020	0.07190	0.03216	

Independent T Test significant difference-*p* value ≤0.05