Experimental Research

ANNALS OF MEDICINE & SURGERY

OPEN

The local application of a 1% simvastatin gel improves bone regeneration in osteoporotic mandibular defects: a rat model study

Karam Ahmad, DDS, OMFS, PhD[a], Mhammad Ali, DDS[b,*], Suliman Muhammad, DDS[c], Ali Daoud, MD, Path, PhD[d], Abdulkarim Khalil, DDS, OMFS, PhD[a]

Background: The purpose of this study is to evaluate the effectiveness of simvastatin in the bone formation of osteoporotic mandible in the rat model.

Methods and materials: Eight Wistar male rats at the age of 6 months, with a weighted average of 250-300 grams, were purchased from the Atomic Energy Authority in Damascus (Syria). Osteoporosis was induced through bilateral orchiectomy, a procedure that involves the removal of the testes, under anesthesia with a combination of ketamine and xylazine. The lateral mandibular portion was selected to create a physiologically critical bone defect. In each rat, two adjacent defects were created in the mandible, and simvastatin gel 1% was applied to one side of them. The rats were euthanized over the first 3 months, and the defective portions of the mandible were processed for histological analysis of formed bone.

Results: The study used Allen's scoring system to measure the amount of new bone formed in the defects treated with simvastatin compared to untreated defects. During the first and second months, the simvastatin-treated defects showed significantly higher bone formation than the untreated defects ($P < 0.05$), indicating that simvastatin had a positive impact on bone healing in the early stages. However, by the third month, this difference disappeared ($P > 0.05$), suggesting that simvastatin's effect on bone formation was strongest early on and did not provide additional benefits over time.

Conclusions: Our findings suggest that local application of simvastatin (1% gel) has favorable effects on bone formation in osteoporotic mandibular rats.

Keywords: bone regeneration, mandible defect, osteoporosis, rat study, simvastatin

Introduction

Osteoporosis is a common systemic metabolic bone disease and is considered a serious health problem, with rates steadily increasing in recent decades. By 2050, the global incidence of osteoporosis is expected to rise by around 310% in men and 240% in women because of the aging society occurring worldwide[1]. Osteoporosis is characterized by lowered bone mass and decreased bone strength, which increases the risk of fractures[2]. Mandibular osteoporosis can result from systemic hormonal changes, aging, long-term corticosteroid use, nutritional deficiencies (e.g., calcium and vitamin D), and hypogonadism. These factors collectively contribute to reduced bone mineral density and altered microarchitecture in the mandible, making it more susceptible to defects and delayed healing[3,4].

The majority of osteoporosis and osteopenia cases go undiagnosed, leading to fractures that can result in a high risk of complications and mortality[5,6]. Osteoporosis can be classified into two categories: primary and secondary osteoporosis. Primary osteoporosis is related to aging and/or a reduction in sex hormones. On the other hand, secondary osteoporosis is caused by factors such as the use of glucocorticoids, systemic diseases that affect bone turnover, or insufficient calcium intake[7]. Morphological changes due to osteoporosis can occur in both cortical and trabecular bone. Because of its larger surface area, trabecular bone is more significantly impacted by

HIGHLIGHTS

- All clinical and radiographical examination should be early performed to make the proper diagnosis of osteoporosis and to avoid the high probability of complications and mortality related to undiagnosed cases.
- It is recommended to use the local application of simvastatin in case of treatment bone defect due to its positive effects on bone formation.
- Human trials are needed for further evaluation with additional data for the simvastatin applications as a preventative treatment for osteoporosis.

[a]Department of Oral and Maxillofacial Surgery, Tishreen University Hospital, Lattakia, Syria, [b]Faculty of Dentistry, Al-Andalus University for Medical Sciences, Tartus, Syria, [c]Faculty of Dentistry, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu Mureș, Romania and [d]Department of Pathology, Tishreen University Hospital, Lattakia, Syria

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

*Corresponding author. Address: Faculty of Dentistry, Al-Andalus University for Medical Sciences, Tartus, Syria. Tel.: +963930759779.
E-mail: mhammad3li3li@gmail.com (M. Ali).

Annals of Medicine & Surgery (2025) 87:5503–5511

Received 19 March 2025; Accepted 1 July 2025

Published online 14 July 2025

http://dx.doi.org/10.1097/MS9.0000000000003581

osteoporosis than cortical bone[8]. As a result, other properties of the defective bone, such as the density, stiffness, or strength, are decreased due to the previous changes[9].

The use of bone grafts for repairing maxillofacial deformities is a relatively common procedure, and autologous bone is widely regarded to be the gold standard as a grafting material[10]. This technique exhibits considerable limitations and drawbacks. Consequently, numerous substitute materials have been developed to replace autologous bone grafts[11]. Statins, including Simvastatin, are hepatoselective and mainly degraded in the liver[12–14]. The conventional delivery systems fail to achieve a sufficient local concentration of statins to exert their therapeutic effects. Thus, an appropriate delivery system is necessary to ensure the effectiveness of simvastatin in promoting bone healing and regeneration[15].

The promotion of localized bone formation is essential for the repair of isolated bone defects. Additionally, administering treatment locally helps mitigate the risk of systemic side effects associated with medications. In the adult human skeleton, the processes of bone formation and bone resorption are closely linked both temporally and spatially, occurring in a sequence of activation, resorption, and formation. This cycle, known as remodeling, exhibits distinctive morphological characteristics that can be identified through histomorphometric analysis[16–19].

Remodeling is a coupled process where bone resorption and formation occur sequentially at the same site, maintaining bone homeostasis and repair. In contrast, modeling refers to bone formation and resorption occurring independently at different locations, allowing for changes in bone shape, size, and structure without necessarily maintaining the same balance as remodeling. The morphological characteristics of modeling differ from those of remodeling, and these differences can be distinguished through histomorphometric analysis[16,17,19].

Previously, numerous scientists believed that the primary process occurring in the rat skeleton was modeling, thus deeming rats unsuitable as models for osteoporosis studies. However, it has been observed that the rat skeleton undergoes a gradual shift from modeling to remodeling with age, affecting both cancellous and cortical bone[20]. This transition makes Wistar rats a suitable model for studying bone regeneration under osteoporotic conditions, as they progressively exhibit remodeling behavior analogous to that in aging humans[21].

The use of animal models, such as the bilateral orchiectomy rat model, is crucial in osteoporosis research because it simulates the hormonal deficiencies associated with the disease, allowing for the assessment of bone regeneration in a compromised environment. This model is widely used to study the effects of various therapeutic agents, including simvastatin, on bone healing, as it closely mimics the pathological conditions found in osteoporotic patients[22,23].

Osteoporosis is more prevalent in women, particularly postmenopausal women, due to estrogen deficiency. However, men are often underdiagnosed, and their fractures carry a higher mortality risk. Ethnic variations also exist, with higher fracture rates reported in Caucasian and Asian populations compared to African ancestry groups[24]. This study contributes to the literature by exploring a localized delivery method of simvastatin in mandibular defects, offering a promising therapeutic approach for enhancing bone healing in osteoporotic conditions. We aimed to evaluate the effectiveness of 1% simvastatin gel in promoting bone regeneration in osteoporotic mandibles of Wistar rats. Simvastatin was selected due to its dual benefits, its established role in improving bone health, and its cost-effectiveness compared to other adjuncts like stem cells and growth factors. By focusing on simvastatin, we sought to explore a promising and accessible option for enhancing bone regeneration in osteoporotic conditions.

While the osteogenic effects of simvastatin have been demonstrated in various models, our study is novel in applying a 1% simvastatin gel locally to mandibular defects in a validated bilateral orchiectomy-induced osteoporotic rat model. This provides insight into localized mandibular healing, a less-explored region compared to long bones. We hypothesize that local application of simvastatin gel significantly enhances bone regeneration in osteoporotic mandibular defects compared to untreated controls.

This study complies with the TITAN 2025 Guidelines for the transparent reporting of artificial intelligence in health research[25].

Methods and materials

Experimental animals

Eight Wistar rats at the age of 6 months, with a weighted average of 250-300 grams, were housed in ventilated cages at room temperature (25 ± 3 C) and humidity (65 ± 10%) with a 12:12 h light-dark cycle. The selection of eight animals was based on ethical considerations, aiming to minimize animal use while ensuring statistical significance, as guided by the principles of the 3Rs (Replacement, Reduction, and Refinement)[26]. Additionally, previous studies on similar models have shown that this sample size is adequate for detecting differences in bone regeneration outcomes[27]. While no power calculation was performed, this number allowed ethical reduction of animal use while still producing meaningful comparative results. The 90-day observation window captures the early-to-mid phase of bone remodeling in the mandible. Although complete remodeling can extend beyond this, previous research supports that substantial osteogenesis and trabecular changes are detectable by this time point[28]. This prospective randomized study, conducted from May to November 2022, included provisions for food and water access. Prior to the study's commencement, the rats underwent a 1-week acclimatization period. All animal trials adhered to the ARRIVE guidelines and were conducted in accordance with the U.K. Animals (Scientific Procedures) Act of 1986 and its associated regulations, as well as the guidelines outlined in EU Directive 2010/63/EU pertaining to animal experimentation[29]. The experiment was conducted and all procedures were approved and overseen by the university's Ethics Committee (Approval No. 657 on 23/11/2021).

Osteoporosis induction

The procedure was made under anesthesia with intraperitoneal administration of 50% ketamine and 50% xylazine at a dose of 0.1 ml/100 g body weight. All surgical procedures have been performed in aseptic conditions with sterile surgical materials. The general anesthesia was placed following local anesthesia using lidocaine hydrochloride 2% with 1:80 000 epinephrine in order to achieve local vasoconstriction and reduce surgical bleeding.

Bilateral orchiectomy was made using a scrotal approach. Initially, we used Povidone Iodine for sterilization after shaving the scrotal hair of incision site. A 1 cm median incision was made through the skin at the tip of the scrotum along with the cremaster muscles. After identifying the local anatomical structures, the testis and epididymis were excised, and the surrounding tissues were closed and sutured layer by layer. The same procedure was made on the other side, and all the wound areas were cleaned with an antiseptic solution after excision. Postoperative pain control was provided by using Diclonate 10%. After orchiectomy, rats were monitored for postoperative recovery for 1 week, followed by a 65-day period to allow for osteoporosis development prior to defect creation and simvastatin application.

Preparation of simvastatin gel

The formulation was adapted from previously described gel preparation methods using Carbopol 940P dissolved in 100 mL of distilled water, and then the solution was covered for 24 h in a dark place[30,31]. In all, 1 g of simvastatin was dissolved in 20 ml of 95% ethanol. The pharmaceutical solution was added to carbopol gel with continuous stirring, and 1.35 g of TEA (triethanolamine) was gradually added with stirring until reaching the appropriate consistency for topical application (Fig. 1). The gel was stored away from light, high-temperature, and humidity.

Local application of simvastatin

The procedure was made after 65 days of orchiectomy under anesthesia with intraperitoneal administration of 50% ketamine and 50% xylazine at a dose of 0.1 ml/100 g body weight. All surgical procedures have been performed in aseptic conditions with sterile surgical materials. The general anesthesia was placed following with local anesthesia using lidocaine hydrochloride 2% with 1:80 000 epinephrine in order to achieve local vasoconstriction and reduce surgical bleeding.

Figure 1. The proper shape of simvastatin gel for topical application.

A 15 mm long incision was made at the left lower border of the mandible (submandibular approach). Raising a flap was necessary to ensure adequate visibility and access to the surgical area. However, additional incisions were made in the muscles and periosteum to expose the body of the mandible. Two circular defects (2 mm high, 4 mm wide) were created using a trephine drill (Fig. 2A), and simvastatin gel 1% was applied one of the defected sites that created in all animals (Fig. 2B). Furthermore, we applied non-absorbable membrane to offer excellent structural integrity, space maintenance, and wound protection, with predictable bone formation (Fig. 2C).

Following, the tissues were closed using 4/0 absorbable sutures for the periosteum and muscles, and silk 4/0 for the skin. All surgical wound sites were treated with an antiseptic solution for cleansing postoperatively. Diclonate 10% provided as a postoperative pain control.

Histological analysis

All the experimental rats were euthanized over the first 3 months of application of simvastatin and were processed for histological analysis of bone union.

The biopsies of mandibles (n = 8) were fixed in a 10% neutral-buffered formalin solution for 48 h at room temperature. The fixed specimens were washed in distilled water and immersed in a decalcification solution of nitric acid for 48 h. Following paraffin embedding, 4-thick sagittal sections were taken of each specimen. Hematoxylin and Eosin (H&E) were used to reveal cellular details. The histological sections were evaluated according to the Allen Scoring, which is widely recognized for its validity and reliability in assessing bone healing[32]. This system provides a standardized and reproducible method, allowing for consistent scoring across different observers (Table 1). The structured criteria of the Allen scoring system ensure accurate evaluation of bone regeneration quality and quantity, enhancing the objectivity of the histological analysis.

Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS, version 17.0) software program. Independent Samples T-Test (n = 8) was employed to compare the bone healing outcomes between the control and study groups. A significance level of $P \leq 0.05$ was considered statistically significant.

Results

The study involved eight rats that were euthanized and evaluated histologically at three different time points: 30 days, 60 days, and 90 days post-surgery. The histological appearance and healing stages were documented using Allen's scoring system and supported by representative micrographs. The sequence of sacrifices was as follows: three rats were euthanized at 30 days, two rats at 60 days, and three rats at 90 days. This distribution allowed for a longitudinal assessment of bone regeneration at different stages, providing a comprehensive evaluation of the effects of simvastatin on bone healing over time.

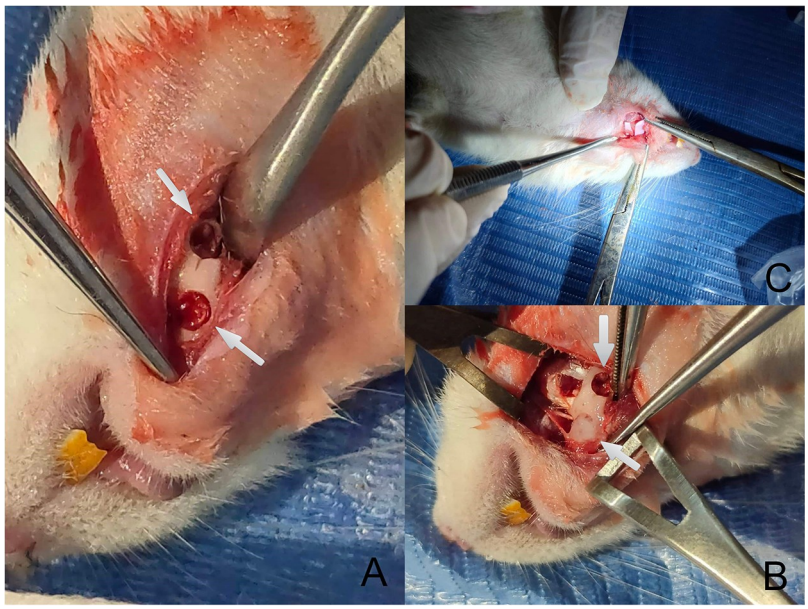

Figure 2. (A). Created-defects on the lateral side rat mandible. (B). Topical application of simvastatin gel 1% in the mesial bone defect. (C). Application of the non-absorbable membrane.

According to Allen's scoring system, on the 30th postoperative day, the histological findings demonstrated inflammatory granulation tissues with fibrous proliferation. Additionally, the beginning of incomplete formation cartilage tissues was noticed (Fig. 3). However, the values of effectiveness of simvastatin in treating bone defects were significantly higher than the control defects ($P < 0.05$) (Table 2).

On the 60th postoperative day, the histological sections showed chondrocytes with abundant, homogeneous, translucent matrix (Fig. 4A,B) but fibrous tissues in surrounding areas were noticed in (Fig. 4B). Beginning of banded and focal ossification was noticed on the cartilaginous floor (Fig. 4C), but complete cartilage union is clearly seen in Figure 4D. The values of effectiveness of simvastatin in treating bone defects were also significantly higher than in the control group ($P < 0.05$) (Table 2).

On the 90th postoperative day, the histological sections showed bone formation in the form of squares, especially in the surrounding areas of the defective bone (Fig. 5A and B), but the microscopical findings in (Fig. 5B-D) pretend to be more clear. An increase in bone formation was noticed in (Fig. 5C). There is no significant difference between the simvastatin-treated defects and the control defects on the 90th postoperative day ($P > 0.05$) (Table 2).

Discussion

The utilization of biomaterials and tissue engineering techniques for addressing bone defects has been extensively researched within the field of dental practice. In our study, we investigated the influence of local application of simvastatin gel 1% on bone formation in the osteoporotic male rat model. Osteoporosis has emerged as a significantly important factor affecting the quality of life in elderly individuals and is currently among the most extensively researched diseases. Research on osteoporosis has predominantly concentrated on treatments for women. Nevertheless, osteoporosis in men is also linked to conditions such as hypogonadism and hormonal changes related to aging[33,34]. Osteoporosis in males remains a significantly underdiagnosed and inadequately treated condition, leading to severe consequences[24,35,36].

Regarding the animal model, such a study must represent anatomical, physiological, and physiopathological analogous

Table 1

Fracture healing scoring system (Allen's fracture healing scoring system)

score	Healing staging	Measurement
0	Non union	**Fibrous tissues**
1	Incomplete cartilage union	**Cartilage with some fibrous tissues**
2	Complete cartilage union	**Entire cartilage**
3	Incomplete bone union with phase of ossification	**Predominantly cartilage with some trabecular bone**
4	Incomplete bone union with intermediate phase of ossification	**Equal amounts of cartilage and trabecualar bone**
5	Incomplete bone union with late phase of ossification	**Predominantly trabecular bone with some cartilage**
6	Complete bone union	**Entire bone**

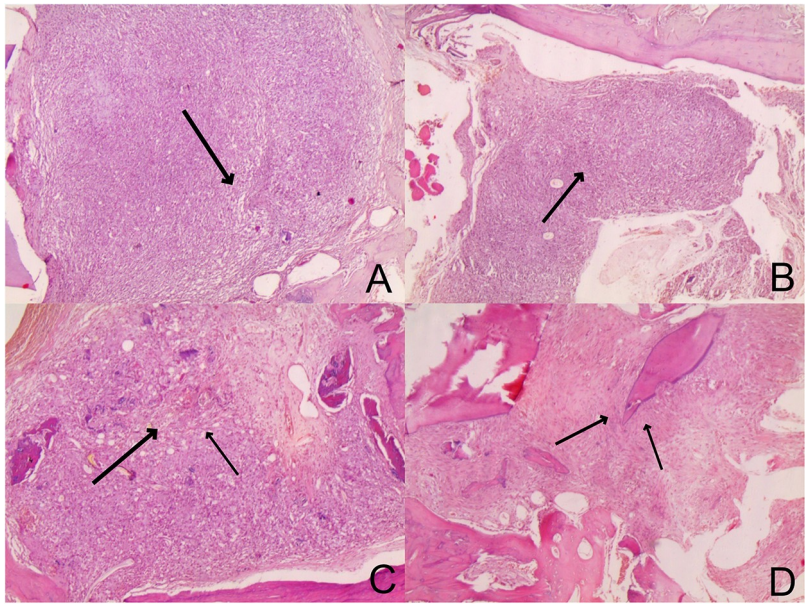

Figure 3. (A-C). Macroscopical features of the defected site on the 30th day after application of simvastatin gel (H&E × 10). The images display early bone healing process, indicating initial bone regeneration stages in the treated group. (B-D). Macroscopical features of the defected site on the 30th day without application of simvastatin gel (H&E × 10). The control group shows minimal bone healing with larger marrow spaces, suggesting slower bone regeneration compared to the simvastatin-treated group.

characteristics related to the human body[37]. The literature review published by O'Loughlin et al in 2008, on the most commonly used species in experimental research about bone regeneration, revealed that rats were the most commonly used in the reviewed articles[38]. Rat models are advantageous for bone regeneration research due to their high bioavailability, cost-effectiveness, ease of handling in laboratory environments, low incidence of biomaterial rejection, and chemical bone composition that closely resembles that of human bone[39]. We used orchiectomized male rats in our study, which is the standard animal model for osteoporosis studies[21,40]. Many studies aimed to describe methods for inducing osteoporosis in rats through hypogonadism by gonadal tissue removal[41]. It was found that hypogonadism, induced by orchiectomy in male rats, led to significant morphological changes in trabecular bone, particularly in femoral condyles, characterizing the male rat model for osteoporosis[24,42,43]. Studies suggest that androgen deficiency from orchiectomy affects osteoblastic cells with androgen receptors, reducing bone formation[44,45]. Additionally, estrogen deficiency in males increases bone resorption and osteoclast activity, leading to significant bone loss[46–48].

The gel was prepared in a modified form from the traditional method of preparation[30,31]. We have used the gel as a pharmacological form of simvastatin due to its wide advantages, such as the ease of spreading, adhesion strength, long retention period, easy to clean, and has no side effects to the skin. Unlike most water-white gels, the simvastatin gel was a milky white, opaque, cream-like material. The dosage of simvastatin for dental osteogenic purposes varied from as low as 1.2 mg applied locally to 10 mg/kg/day administered systemically[49]. It was estimated that the dose of the systemic circulation is less than 5% of the orally consumed dose. Accordingly, to test its possible boosting effect on

Table 2

Independent Samples T-Test comparing of bone healing according to Allen's fracture healing scoring system

Group	N	Mean	Std. Deviation	Std. Error Mean	Sig. (2-tailed)
30th postoperative day					
Simvastatin	3	.6667	.57735	.33333	**.000**
Control	3	.3333	.57735	.33333	
60th postoperative day					
Simvastatin	2	2.5000	.70711	.50000	**.003**
Control	2	1.5000	.70711	.50000	
90th postoperative day					
Simvastatin	3	5.6667	.57735	.33333	**.519**
Control	3	5.3333	.57735	.33333	

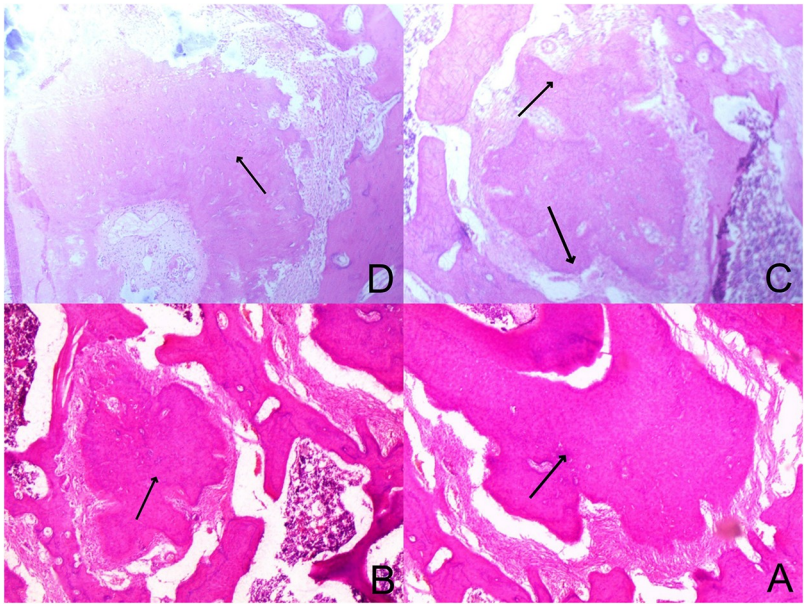

Figure 4. (A-C). Macroscopical features of the defected site on the 60th day after application of simvastatin gel (H&E × 10). These images demonstrate advanced bone healing, showing improved bone healing in the treated group. (B-D). Macroscopical features of the defected site on the 60th day without application of simvastatin gel (H&E × 10). The images show complete cartilage union, indicating slower healing progress.

osteoconductive biomaterials of critical bone defects, it is usually applied topically[50,51]. Depending on previous studies, 0.5–1 mg of simvastatin would be the optimal dose for stimulating maximum bone regeneration without inducing inflammation and within safe limits for intraoral topical applications[52].

Regarding the location of bone defects, studies on biomaterials for jaw bone regeneration frequently utilize extraoral sites to create critical bone defects, with a specific emphasis on calvarial bone[53] and femur bone[54,55]. During investigations assessing the efficacy of biomaterials in bone regeneration, the jaw emerged as

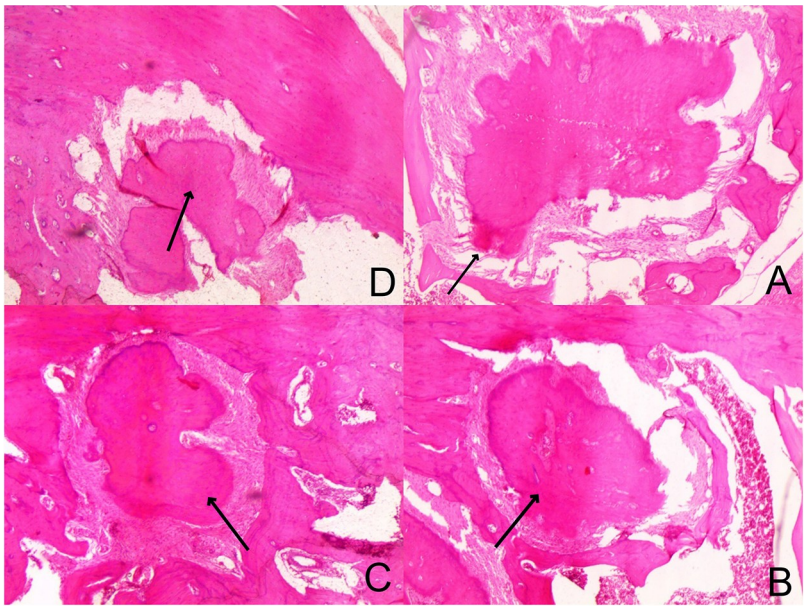

Figure 5. (A-C). Macroscopical features of the defected site on the 90th day after application of simvastatin gel (H&E × 10). These images display mature lamellar bone and well-organized trabeculae. (B-D). Macroscopical features of the defected site on the 90th day without application of simvastatin gel (H&E × 10). The images also show mature lamellar bone formation similar to the treated group, with no significant differences in bone regeneration observed at this time point.

the optimal site due to its ability to accurately replicate the anatomical features of surrounding bone in conjunction with adjacent soft tissues, vascular structures, as well as the mechanical stresses incurred during mastication, thereby offering a comprehensive model for evaluating regenerative outcomes[56,57].

Our study showed that the bone defects treated with simvastatin gel have better results in bone union. The rates of bone healing were higher on the simvastatin-treated defects than the control defects on the 30th and 60th postoperative days. On the 90th postoperative day, bone healing and regeneration rates were higher on the simvastatin-treated defects, but there was no significant difference revealed. Our results are in agreement with many previous studies that demonstrate the role of local statin therapy in the case of jaw treatment defects. Stein et al. illustrated that rats treated with 0.5 mg of simvastatin on the lateral side of the mandible were found to have a 45% increase in bone area compared to a control gel[58]. The local application of simvastatin gel against rat mandibles revealed significant stimulation to the regional bone formation rate in a study by Bradley *et al*[59]. Another study showed that a single topical application of simvastatin gel improved the quality of the regenerated bone postoperatively in defects created on the buccal aspect of the first molar roots in rats[28].

In the study we present, the osteogenesis process lasts for 3 months with no trace of the drug. Delayed bone ossification could be related to the higher osteoclastic activity in osteoporotic models, and limited bone regeneration was observed only at the margins defects. Notably, it is unusual to see the periosteum regeneration and pretends to regenerate from the margins[60].

Simvastatin, commonly used for managing hypercholesterolemia, has shown potential in enhancing bone healing by stimulating osteoblast activity and increasing bone mineral density (BMD), as observed in various clinical studies, including those involving postmenopausal women with osteoporosis[61,62]. The findings of the present study align with these observations, demonstrating that simvastatin can significantly promote bone regeneration in osteoporotic conditions, as shown in the rat model. These implications highlight the potential of simvastatin as a novel adjunctive therapy for bone healing in osteoporotic patients, warranting more research to fully validate its clinical benefits.

Although our study did not elucidate the precise mechanism by which simvastatin aids in bone healing, we have determined that a 1% simvastatin gel significantly enhances guided bone regeneration in the mandibles of orchiectomized Wistar rats. These promising outcomes across various medical and dental applications suggest new potential uses for statins in promoting bone formation.

The main limitation of our study was the small sample size, so studies in large sample sizes and radiographic evaluation (such as Micro CT) are required for more definitive and conclusive results. Another limitation was the use of a single dose of simvastatin, as the literature presents a great discrepancy about the most effective dose. Additionally, the use of only male rats with bilateral orchiectomy, while relevant for modeling androgen-deficient osteoporosis, limits extrapolation to postmenopausal female osteoporosis. Future studies should consider including ovariectomized female rats to capture sex-specific differences in bone remodeling and should take into account the limitations identified in this study. Nevertheless, this research has provided valuable insights into the direction and key considerations that should be addressed in subsequent studies.

Conclusions

In conclusion, the present study demonstrates that local application of simvastatin (1% gel) positively influences bone regeneration in osteoporotic conditions, as evidenced by our rat model with induced osteoporosis through orchiectomy. While these findings are promising, they are based on preclinical animal data and should be interpreted with caution when considering clinical applications. Further investigations using multiple doses of simvastatin and their effects on other CATABOLIC bone diseases are needed to confirm positive outcomes.

Ethical approval

The ethical approval have been performed by the Ethics Committee of Tishreen University (No. 657 in 23/11/2021).

Consent

Written informed consent was not obtained because this study does not include any human experiments.

Sources of funding

No source of funding.

Author contributions

KA: Data curation, Investigation, Writing the article. MA: Data Curation, Writing and drafting the article, Critical revision. SM: Data curation. AD, AKh: Supervision.

Conflicts of interest disclosure

The authors declare no conflict of interest.

Guarantor

Abdulkarim Khalil (DDS, OMFS, PhD).

Research registration unique identifying number (UIN)

This study is an animal experimental model, and according to the registration categories outlined by the Research Registry, registration is not applicable for this type of preclinical research.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Data availability statement

The data are available for sharing.

Acknowledgements

The authors express their gratitude to Dr Ali Zouebi and Dr Hadi Shamout for their valuable contributions to the surgical and experimental procedures conducted in the study being presented.

References

[1] Gullberg B, Johnell O, Kanis JA. World-wide projections for hip fracture. Osteoporos Int 1997;7:407–13.
[2] Lupsa BC, Insogna K. Bone health and osteoporosis. Endocrinol Metab Clin North Am 2015;44:517–30.
[3] Devlin H, Horner K. Mandibular radiomorphometric indices in the diagnosis of reduced skeletal bone mineral density. Osteoporos Int 2002;13:373–78.
[4] Riggs BL, Melton LJ, Robb RA, *et al*. Population-based study of age and sex differences in bone volumetric density, size, geometry, and structure at different skeletal sites. J Bone Miner Res 2004;19:1945–54.
[5] Liow MHL, Ganesan G, Chen JDY, *et al*. Excess mortality after hip fracture: fracture or pre-fall comorbidity? Osteoporos Int 2021;32: 2485–92.
[6] Brown C. Osteoporosis: staying strong. Nature 2017;550:S15–S17.
[7] Downey PA, Siegel MI. Bone biology and the clinical implications for osteoporosis. Phys Ther 2006;86:77–91.
[8] Lane JM, Osteoporosis. Medical prevention and treatment. Spine (Phila Pa 1976) 1997;22:32S–37S.
[9] Kostenuik PJ, Capparelli C, Morony S, *et al*. OPG and PTH-(1-34) have additive effects on bone density and mechanical strength in osteopenic ovariectomized rats. Endocrinology 2001;142:4295–304.
[10] Baba S, Inoue T, Hashimoto Y, *et al*. Effectiveness of scaffolds with pre-seeded mesenchymal stem cells in bone regeneration–assessment of osteogenic ability of scaffolds implanted under the periosteum of the cranial bone of rats. Dent Mater J 2010;29:673–81.
[11] Arima Y, Uemura N, Hashimoto Y, *et al*. Evaluation of bone regeneration by porous alpha-tricalcium phosphate/atelocollagen sponge composite in rat calvarial defects. Orthod Waves 2013;72:23–29.
[12] Lee Y, Schmid MJ, Marx DB, *et al*. The effect of local simvastatin delivery strategies on mandibular bone formation in vivo. Biomaterials 2008;29:1940–49.
[13] Calixto JC, Villaboim De Castro Lima CE, Frederico L, *et al*. The influence of local administration of simvastatin in calvarial bone healing in rats. J Craniomaxillofac Surg 2011;39:215–20.
[14] Jadhav SB, Jain GK. Statins and osteoporosis: new role for old drugs. J Pharm Pharmacol 2006;58:3–18.
[15] Wu Z, Liu C, Zang G, *et al*. The effect of simvastatin on remodelling of the alveolar bone following tooth extraction. Int J Oral Maxillofac Surg 2008;37:170–76.
[16] Parfitt AM. The cellular basis of bone remodeling: the quantum concept reexamined in light of recent advances in the cell biology of bone. Calcif Tissue Int 1984;36:S37–S45.
[17] Frost HM, Intermediary organization of the skeleton. 1986. Accessed March 21, 2024. https://search.worldcat.org/title/12262137.
[18] Dalle Carbonare L, Valenti MT, Bertoldo F, *et al*. Bone microarchitecture evaluated by histomorphometry. Micron 2005;36:609–16.
[19] Jee WSS, Harold M, Frost MD. D.S.c. (hon) - One man's association. J Musculoskelet Neuronal Interact 2006;6:113–21.
[20] Dennison E, Cole Z, Cooper C. Diagnosis and epidemiology of osteoporosis. Curr Opin Rheumatol 2005;17:456–61.
[21] Komrakova M, Krischek C, Wicke M, *et al*. Influence of intermittent administration of parathyroid hormone on muscle tissue and bone healing in orchiectomized rats or controls. J Endocrinol 2011;209:9–19.
[22] Na Zhou B, Zhang Q, Yun Lin X, *et al*. The roles of sclerostin and irisin on bone and muscle of orchiectomized rats. BMC Musculoskelet Disord 2022;23:1–11.
[23] Yu Y, Lee S, Bock M, *et al*. Promotion of bone formation in a rat osteoporotic vertebral body defect model via suppression of osteoclastogenesis by ectopic embryonic calvaria derived mesenchymal stem cells. Int J Mol Sci 2024;25.
[24] Khosla S. Update in male osteoporosis. J Clin Endocrinol Metab 2010;95:3–10.
[25] Agha R, Mathew G, Rashid R, *et al*. Transparency In The reporting of Artificial INtelligence – the TITAN guideline. Prem J Sci 2025.
[26] Hubrecht RC, Carter E. The 3Rs and humane experimental technique: implementing change. Anim an Open Access J from MDPI 2019;9.
[27] Xing D, Chen J, Yang J, *et al*. Perspectives on animal models utilized for the research and development of regenerative therapies for articular cartilage. Curr Mol Biol Reports 2016;2:90–100.
[28] MacIel-Oliveira N, Bradaschia-Correa V, Arana-Chavez VE. Early alveolar bone regeneration in rats after topical administration of simvastatin. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol 2011;112:170–79.
[29] Percie Du Sert N, Hurst V, Ahluwalia A, *et al*. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. BMC Vet Res 2020;16:1–7.
[30] Bregni C, Chiappetta D, Faiden N, *et al*. Release study of diclofenac from new carbomer gels - PubMed. Pak J Pharm Sci 2008;21:6–12. https://pubmed.ncbi.nlm.nih.gov/18166512/.
[31] Amnuaikit T, Ingkatawornwong S, Maneeruna D, *et al*. Caffeine topical gel formulation. Isan J Pharm Sci 2008;4:16–24.
[32] Allen HL, Wase A, Bear WT. Indomethacin and aspirin: effect of nonsteroidal anti-inflammatory agents on the rate of fracture repair in the rat. Acta Orthop 1980;51:595–600.
[33] Harman SM, Metter EJ, Tobin JD, *et al*. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore longitudinal study of aging. J Clin Endocrinol Metab 2001;86:724–31.
[34] Feldman HA, Longcope C, Derby CA, *et al*. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. J Clin Endocrinol Metab 2002;87:589–98.
[35] Misiorowski W. Osteoporosis in men. Prz Menopauzalny = Menopause Rev 2017;16:70–73.
[36] Stuermer EK, Sehmisch S, Tezval M, *et al*. Effect of testosterone, raloxifene and estrogen replacement on the microstructure and biomechanics of metaphyseal osteoporotic bones in orchiectomized male rats. World J Urol 2009;27:547–55.
[37] Gothard D, Smith EL, Kanczler JM, *et al*. Tissue engineered bone using select growth factors: a comprehensive review of animal studies and clinical translation studies in man. Eur Cell Mater 2014;28:166–208.
[38] O'Loughlin PF, Morr S, Bogunovic L, *et al*. Selection and development of preclinical models in fracture-healing research. J Bone Joint Surg Am 2008;90:79–84.
[39] Pinheiro FAL, de almeida barros Mourão CF, Diniz VS, *et al*. In-vivo bone response to titanium screw implants anodized in sodium sulfate. Acta Cir Bras 2014;29:376–82.
[40] Iwamoto J, Takeda T, Ichimura S. Differential effect of short-term etidronate treatment on three cancellous bone sites in orchidectomized adult rats. Keio J Med 2004;53:12–17.
[41] Alghamdi HS, Van Den Beucken JJJP, Jansen JA. Osteoporotic rat models for evaluation of osseointegration of bone implants. Tissue Eng Part C Methods 2014;20:493–505.
[42] Wink CS, Felts WJL. Effects of castration on the bone structure of male rats: a model of osteoporosis. Calcif Tissue Int 1980;32:77–82.
[43] Gürkan L, Ekeland A, Gautvik KM, *et al*. Bone changes after castration in rats. A model for osteoporosis. Acta Orthop Scand 1986;57:67–70.
[44] Kasperk CH, Wakley GK, Hierl T, *et al*. Gonadal and adrenal androgens are potent regulators of human bone cell metabolism in vitro. J Bone Miner Res 1997;12:464–71.
[45] Anderson FH, Francis RM, Selby PL, *et al*. Sex hormones and osteoporosis in men. Calcif Tissue Int 1998;62:185–88.
[46] Turner RT, Wakley GK, Hannon KS. Differential effects of androgens on cortical bone histomorphometry in gonadectomized male and female rats. J Orthop Res 1990;8:612–17.
[47] Riggs BL, Khosla S, Melton LJ. A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res 1998;13:763–73.
[48] Vidal O, Lindberg MK, Hollberg K, *et al*. Estrogen receptor specificity in the regulation of skeletal growth and maturation in male mice. Proc Natl Acad Sci U S A 2000;97:5474–79.
[49] Wang PS, Solomon DH, Mogun H, *et al*. HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. JAMA 2000;283:3211–16.
[50] Gutierrez GE, Lalka D, Garrett IR, *et al*. Transdermal application of lovastatin to rats causes profound increases in bone formation and plasma concentrations. Osteoporos Int 2006;17:1033–42.

[51] Hao J, Chou J, Kuroda S, *et al*. Injectable simvastatin gel for minimally invasive periosteal distraction: in vitro and in vivo studies in rat. Clin Oral Implants Res 2018;29:227–34.
[52] Chen S, Yang J-Y, Zhang S-Y, *et al*. Effects of simvastatin gel on bone regeneration in alveolar defects in miniature pigs - PubMed. Chin Med J 2011;124:3953–8.
[53] da Silva Barbirato D, Fogacci MF, Gusman H, *et al*. Hydroxyapatite calvaria graft repair in experimental diabetes mellitus in rats. J Craniomaxillofac Surg 2018;46:1576–85.
[54] van Houdt CIA, Ulrich DJO, Jansen JA, *et al*. The performance of CPC/PLGA and Bio-Oss® for bone regeneration in healthy and osteoporotic rats. J Biomed Mater Res B Appl Biomater 2018;106:131–42.
[55] Zhang Q, Jing D, Zhang Y, *et al*. Histomorphometric study of new bone formation comparing defect healing with three bone grafting materials: the effect of osteoporosis on graft consolidation. Int J Oral Maxillofac Implants 2018;33:645–52.
[56] Schortinghuis J, Ruben JL, Raghoebar GM, *et al*. Ultrasound to stimulate mandibular bone defect healing: a placebo-controlled single-blind study in rats. J Oral Maxillofac Surg 2004;62:194–201.
[57] Liu X, Bao C, Xu HHK, *et al*. Osteoprotegerin gene-modified BMSCs with hydroxyapatite scaffold for treating critical-sized mandibular defects in ovariectomized osteoporotic rats. Acta Biomater 2016;42:378–88.
[58] Stein D, Lee Y, Schmid MJ, *et al*. Local simvastatin effects on mandibular bone growth and inflammation. J Periodontol 2005;76:1861.
[59] Bradley JD, Cleverly DG, Burns AM, *et al*. Cyclooxygenase-2 inhibitor reduces simvastatin-induced bone morphogenetic protein-2 and bone formation in vivo. J Periodontal Res 2007;42:267.
[60] Junqueira JC, Mancini MNG, Carvalho YR, *et al*. Effects of simvastatin on bone regeneration in the mandibles of ovariectomized rats and on blood cholesterol levels. J Oral Sci 2002;44:117–24.
[61] Rejnmark L, Buus NH, Vestergaard P, *et al*. Effects of simvastatin on bone turnover and BMD: a 1-year randomized controlled trial in postmenopausal osteopenic women. J Bone Miner Res 2004;19:737–44.
[62] An T, Hao J, Sun S, *et al*. Efficacy of statins for osteoporosis: a systematic review and meta-analysis. Osteoporos Int 2017;28:47–57.