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RESEARCH PAPER

Efficacy of Simvastatin in Bone Regeneration After Surgical Removal of Mandibular Third Molars: A Clinical Pilot Study

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Abstract
Introduction Simvastatin, a common cholesterol-lowering drug that inhibits hepatic hydroxymethylglutaryl coenzyme A reductase, the rate-limiting enzyme in the mevalonate pathway, increases expression of the BMP-2 gene and thus promotes bone regeneration.
Materials and methods A study was conducted in mandibular third molar sockets to study the efficacy of the drug by implanting it into sockets (experimental group) and observations were made over 3 months to compare the healing with the (control group).
Conclusion The results showed faster regeneration of the bone in the simvastatin site using the gray level histogram values.

Keywords Simvastatin · Bone regeneration · Third molar sockets

Introduction

Tissue repair following surgical or trauma-related injuries remains a challenge in maxillofacial reconstruction. The healing process initiates an orderly but complex sequence of events that re-establish the integrity of the damaged tissues. If the result of the repair process is tissue that is structurally and functionally the same as the original tissue, then regeneration is said to have taken place. However, if the tissue integrity is replaced with the formation of fibrous connective tissue or scar, then repair is said to have occurred. Whereas a fibrous scar may be normal for soft tissue healing, it is suboptimal in the case of bone healing. Multiple cytokines and growth factors contribute to the success of wound repair. Recent advances in wound biology characterized the molecular pathways governing wound repair and point toward novel therapeutic targets and suitable approach to promote faster tissue healing in vivo. In the past few decades with the emerging multidisciplinary approach embracing the clinical, biological, and engineering fields, fundamental biological discoveries have rapidly been translated from the laboratory bench top to the patient's bedside and vice versa. Albeit most current findings are still in the preclinical state for "proof-of-principle," the potentials for future clinical implications are promising [1].

Bony defects occurring after oral surgical procedures may cause severe aesthetic and functional problems. Bone grafting has continuously played an important role in the correction of craniofacial defects. Bone grafting is a dynamic phenomenon in which a successful graft is applied, heals, becomes incorporated, revascularizes and eventually assumes the desired form. Different bone grafts used for increasing the rate of bone formation and augmentation include autografts, allografts, xenografts or alloplastic bone substitutes.

The search for an ideal material for bone grafting remains a formidable challenge. Autogenous bone grafts are till date the "gold standard" for bone grafting as they alone offer the three necessary components for bone repair—osteoinduction, osteogenicity and an osteoconductive matrix. But they have their own disadvantages like, donor site morbidity, limited availability, post-operative discomfort for the patient and increased surgical time.

Bone morphogenetic proteins (BMPs) are important regulators of osteogenic differentiation during repair of fractures. Wang et al. [2] showed that BMP-2 causes differentiation of a multipotential stem cell line into osteoblast-like cells. To discover small molecules that induce BMP-2, Mundy et al. [3] examined more than 30,000 compounds from a collection of

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natural products and tested the effects of compounds on the expression of the BMP-2 gene. They identified a statin, a common cholesterol-lowering drug that inhibits hepatic hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the mevalonate pathway, as the only product in the collection that specifically increased expression of the BMP-2 gene. They also reported that statins stimulated formation of bone in animals. Studies have shown that statins are well tolerated by the surrounding tissues with no evidence of inflammation and are appropriate for application in humans [4–6]. The use of simvastatin as an adjuvant to bone grafting procedures in oral and maxillofacial surgery has been increasing in popularity since its introduction in 1999 by Mundy et al. [3]. The purpose of this prospective clinical study was to evaluate the osseous regeneration, clinically and radiographically, after surgical removal of mandibular third molars with local application of simvastatin.

Aims and Objectives

1. To assess the efficacy of Simvastatin in bone regeneration in the extraction sockets of mandibular third molars with visual assessment of a series of IOPA radiographs at different time intervals of healing.
2. To compare and study the rate of bone regeneration in the socket using a gray scale histogram at different time intervals of healing.
3. To compare the clinical outcome with a control group.

Methodology

The present study was undertaken at the Department of Oral and Maxillofacial Surgery with due permission of the institutional ethical committee. All patients were explained the procedure and an informed consent was signed by them.

A total of 30 patients; both male and female aged between 18 and 40 years, with bilateral similarly impacted mandibular third molars, with no active infection and who had no systemic problems nor any abusive habits were divided into into two groups irrespective of sex and age:

Group A (control group): 30 patients
Group B (Simvastatin group): 30 patients

Pre-operative Evaluation

Orthopantomograph (OPG) or an intraoral periapical radiograph (IOPA) with parallel technique was taken to assess third molar angulation to the long axis of second molar. The selected bilateral impacted teeth were of similar type on both the sides.

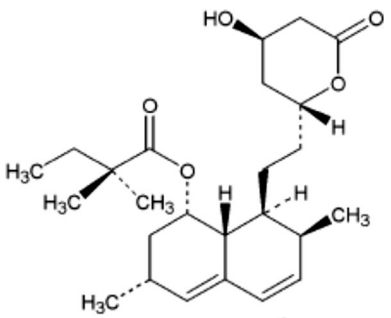
For all patients, following blood investigations were carried out: Complete Haemogram, Hb g %, Total leukocyte count (TLC), Differential Leukocyte count (DLC), Clotting time (CT), Bleeding Time (BT), Platelet count (PC) and Test for HIV and Australia antigen (HbsAg).

Material-Statins

Cholesterol is an essential component of cell membranes and is the immediate precursor of steroid hormones and bile acids. However, in excessive amounts, cholesterol becomes an important risk factor for cardiovascular disease. Although dietary cholesterol can contribute to changes in serum cholesterol levels, more than two-thirds of the body's cholesterol is synthesized in the liver. Therefore, inhibition of hepatic cholesterol biosynthesis has emerged as the target of choice for reducing serum cholesterol levels. The rate-limiting enzyme in cholesterol biosynthesis in the liver is HMG-CoA reductase.

Lovastatin, became the first of this class of cholesterol-lowering agents to be approved for clinical use in humans in 1979. Since then, several new statins have emerged as one of the most effective class of agents for reducing serum cholesterol levels. Statins work by reversibly inhibiting HMG-CoA reductase through side chains that bind to the enzyme's active site and block the substrate–product transition state of the enzyme.

Statins, both natural and chemically modified, are commercially available, including pravastatin, simvastatin, fluvastatin, atorvastatin, cerivastatin, and most recently, pitavastatin and rosuvastatin. These compounds are structural analogs of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A). They are most effective in reducing LDL. Other effects include decreased oxidative stress and vascular inflammation with increased stability of atherosclerotic lesions. It has become standard practice to initiate reductase inhibitor therapy immediately after myocardial infarction, irrespective of lipid levels. Simvastatin is butanoic acid, 2,2-dimethyl-,1,2,3,7,8,8a-hexahydro-3,7-dimethyl 8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl]-1-naphthalenyl ester, [1*S*-[1α,3α,7β,8β(2S*,4S*),-8aβ]]. The empirical formula of simvastatin is $C_{25}H_{38}O_5$ and its molecular weight is 418.57. Its structural formula is:



Simvastatin is a white to off-white, nonhygroscopic, crystalline powder that is practically insoluble in water, and freely soluble in chloroform, methanol and ethanol. Absorption of the ingested doses of the reductase inhibitors varies from 40 to 75 % with the exception of fluvastatin, which is almost completely absorbed. All have high first-pass extraction by the liver. Most of the absorbed dose is excreted in the bile; about 5–20 % is excreted in the urine. Plasma half-lives of these drugs range from 1 to 3 h except for atorvastatin, which has a half-life of 14 h, and rosuvastatin which has a half life of 19 h. Simvastatin is given orally in a dose of 20–40 mg daily. The toxic dose of simvastatin is 160 mg. Adverse effects are reported in less than 1 % of the patients, mostly as abdominal pain, feeling of weakness, very rarely joint pain and memory loss. Doses above 80 mg taken chronically may cause liver damage, type II Diabetes or myopathy [7].

Statins work by reversibly inhibiting HMG-CoA reductase through side chains that bind to the enzyme's active site and block the substrate–product transition state of the enzyme. Over the past decade, animal studies have shown their anabolic effect on bone. Simvastatin stimulates BMP-2 and nitric oxide formation and regional bone formation in rat mandible models. Simvastatin increases mRNA expression for BMP-2, vascular endothelial growth factor (VEGF), alkaline phosphatase, type 1 collagen, bone sialoprotien and osteocalcin in MC3T3-E1 cells [8].

About 10–15 min prior to surgery simvastatin tablet was crushed and dispensed on the sterile dappen dish and mixed with 2 ml of normal saline.

Methodology

The surgical procedures were performed by a single surgeon. Patients were put on an antibiotic course commencing 1 day before surgery to be continued post-operatively for 5 days. Under all aseptic precautions local anaesthesia was administered and the bilaterally impacted mandibular third molars were removed. After tooth removal, the socket was examined for any sharp bony margins. Sharp bony edges, tooth particles, dental follicles etc. were smoothened with bone file. Socket was irrigated with normal saline to remove bone debris and the study socket was prepared for the placement of Simvastatin (10 mg) powder along with gelfoam as carrier moistened with 2 ml normal saline solution. We chose 10 mg as a safe dose to insert in the socket with a carrier gelfoam surrounding it. The powder cannot be directly inserted as it will cause severe inflammation in the surrounding soft tissues. The idea was to have the statin protected by gel foam and as the gelfoam resorbed slowly, the powder would have a slow sustained release effect. We never got any adverse reaction to the drug in this way. The wound was closed primarily with 3–0 black braided silk. All the patients were given routine post-operative instructions. All the patients were given following prescription—Cap. Amoxicillin 500 mg thrice a day for 5 days, Tab. Diclofenac potassium thrice a day for 3 days, Tab. Metronidazole 400 mg thrice a day for 3 days.

The patients were followed up radiographically and clinically on days 1, 2, 3, 30, 60 and 90. They were asked to rate their pain, observed for local or facial swelling, any signs of infection, delayed healing or graft rejection.

Pain

The patients were asked to rate the pain intensity on a 5 point Visual Analogue Scale (VAS) as—0 = no pain, 1 = mild pain, 2 = moderate pain, 3 = severe pain, 4 = very severe.

If increased pain was found on the grafted site, then the inference was that there is increased local inflammatory reaction to the drug.

Swelling

Pre-operatively, the swelling was measured by taking a horizontal distance from the corner of the mouth to the lobe of the ear and a vertical distance between outer canthus of the eye and angle of lower jaw using silk suture following the natural convexity of the patient's face. The procedure was repeated on first, third and seventh post-operative days. The swelling was compared to the non graft side to gauge whether the Simvastatin created additional swelling in the soft tissues other than the expected surgical swelling.

Bone Density Measurement

Osseous regeneration was evaluated with the help of standardized intraoral periapical radiographs. On first post-operative day IOPA radiographs were taken and evaluated by the gray level histogram and the procedure was repeated at 1 month, and 3 months post-operatively.

All the IOPA images were digitalized with the help of a scanner ("DENTAMERICA\CAMREX\AviCap.exe").

The mean gray level histogram values of the scanned IOPA images of the extraction sockets were obtained in Adobe Photoshop 7.0 software [9].

Bone Density Analysis

This was done by digital software program "Adobe Photoshop version 7.0" that enlarges the standard intra-oral periapical radiograph with better resolution.

A particular area was selected on the intra-oral periapical film which included the defect. The mean value of that selected area was noted and compared.

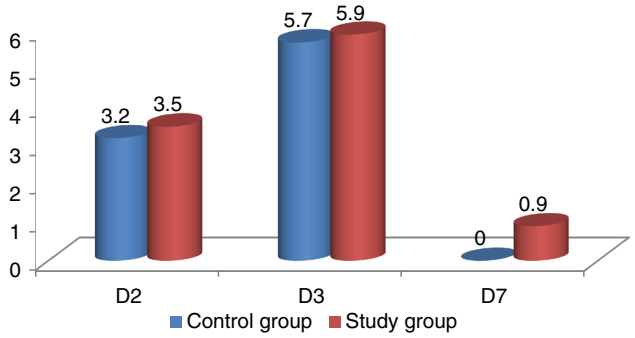


Fig. 1 Mean values for percentage of facial swelling between control and study group on different days

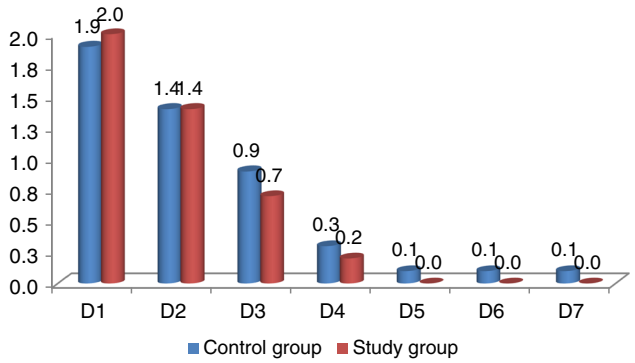


Fig. 2 Mean values on different days for VAS for pain between control and study groups

Steps

1. First the Adobe Photoshop was opened,
2. Image imported
3. Histogram option selected
4. Cursor placed at the centre of the socket
5. Radio-opacity of bone filling the socket at the centre recorded.

- Mean values of the selected area recorded and transferred to the excel sheet for ease of comparison.

Results

All results were calculated using the mean value and standard deviation for each of the parameters considered and checked for statistical significance using paired '*t*' test.

There was no significant difference in the facial swelling observed in both the control group and simvastatin group on days 2, 3 and day 7 (Fig. 1).

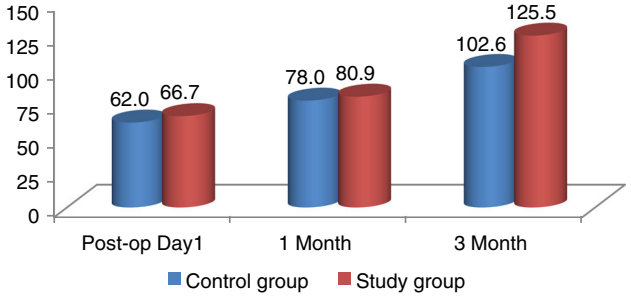


Fig. 3 Mean values on different days for gray level histogram values between control and study groups

After applying paired 't' test there was no significant difference between mean values of VAS for pain in control and study group from day 1 to day 7 (Fig. 2).

As per Fig. 3 the mean values of gray level histogram at post-operative day 1 was 78.0 with a minimum and maximum of 44.3 and 78.7, respectively in control group, whereas, 66.7 was the mean value with minimum and maximum of 47.1 and 80.8 in the study group. Significant differences were observed between the control and study groups, when analysed by paired '*t*' test, with a '*t*' value of 2.62 and probability of 0.036 (Figs. 4, 5).

After one month, the mean values of gray level histogram was 71.3 with a minimum and maximum of 61.3 and 91.5, respectively in control group, whereas, 80.9 was the mean value with minimum and maximum of 68.0 and 91.5 in the study group. Significant differences were observed between the control and study groups, when analysed by paired '*t*' test, with a '*t*' value of 2.06 and probability of 0.047.

After three months, the mean values of gray level histogram at post-operative stage was 102.6 with a minimum and maximum of 85.3 and 111.1, respectively in control group, whereas, 125.5 was the mean value with minimum and maximum of 102.6 and 152.0 in the study group. Highly significant differences were observed between the control and study groups, when analysed by paired '*t*' test, with a '*t*' value of 5.94 and probability of 0.000. At all the stages, significantly higher values were observed in the study group, compared to the control group.

Inference of the above mentioned results was that there was no difference in the pain and swelling on both sides indicating no adverse reaction to the drug. The gray scale comparisons indicated clearly that the bone formation was accelerated on the experimental site.

Discussion

Bone induction has a wide range of clinical applications; however, many bone induction techniques are still

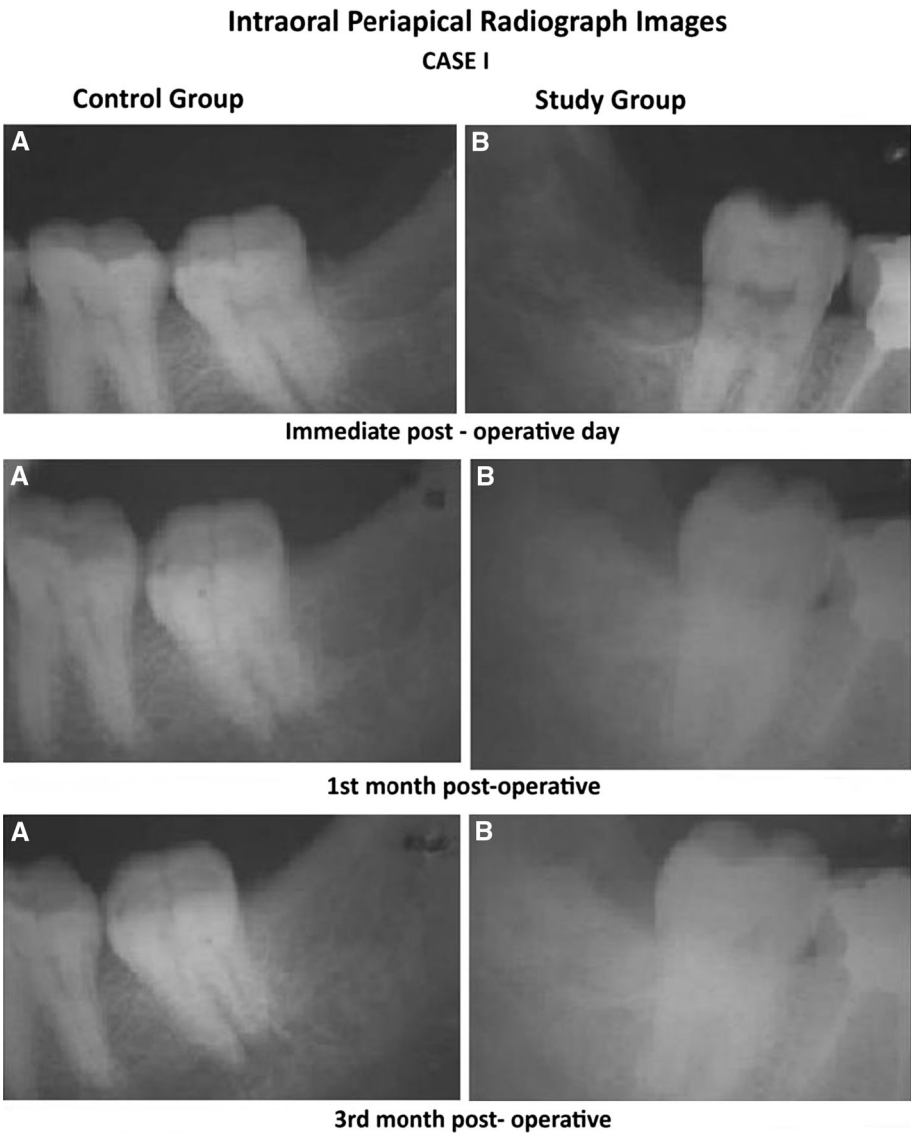


Fig. 4 Intra oral periapical radiographs: Case 1

undergoing active research and have their own shortcomings. In recent years, many researchers have investigated the utilization of statin, a drug that turns on the genes for bone formation, in bone grafting and found that this drug has tremendous osteoinductive effect and great promise in routine use in ridge augmentation and bone grafting in the craniofacial region [10–14]. Alternate materials and methods have been sought as effective bone graft substitutes because of various disadvantages of using autografts (the current gold standard) and allografts. Recently, the 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase inhibitors (statins) that have been safely administered orally for 10 years to reduce serum cholesterol and the subsequent risk of heart attack with little side effects were found to induce bone formation comparable to maximal doses of bone morphogenetic protein 2 [15–17]..

The positive effects of simvastatin as reported in literature are:

- 'Jump starts' the cascade of osteogenesis in bone graft [2, 16, 18].
- Improves trabecular bone density [9, 19]
- Provides earlier availability of growth factors and BMP [20–23].
- Promotes early consolidation of the graft [24–27]
- Hastens the mineralization of the graft [28, 29].
- Enhances bone regeneration [30, 31].
- Activator in wound healing [32–36].

Simvastatins were studied at experimental and histochemical levels by the medical researchers since many years. They are in use as anticholesterol drugs since the 80s. The focus was shifted to their osteoinductive properties by the initial research started by Mundy et al. [3]. All

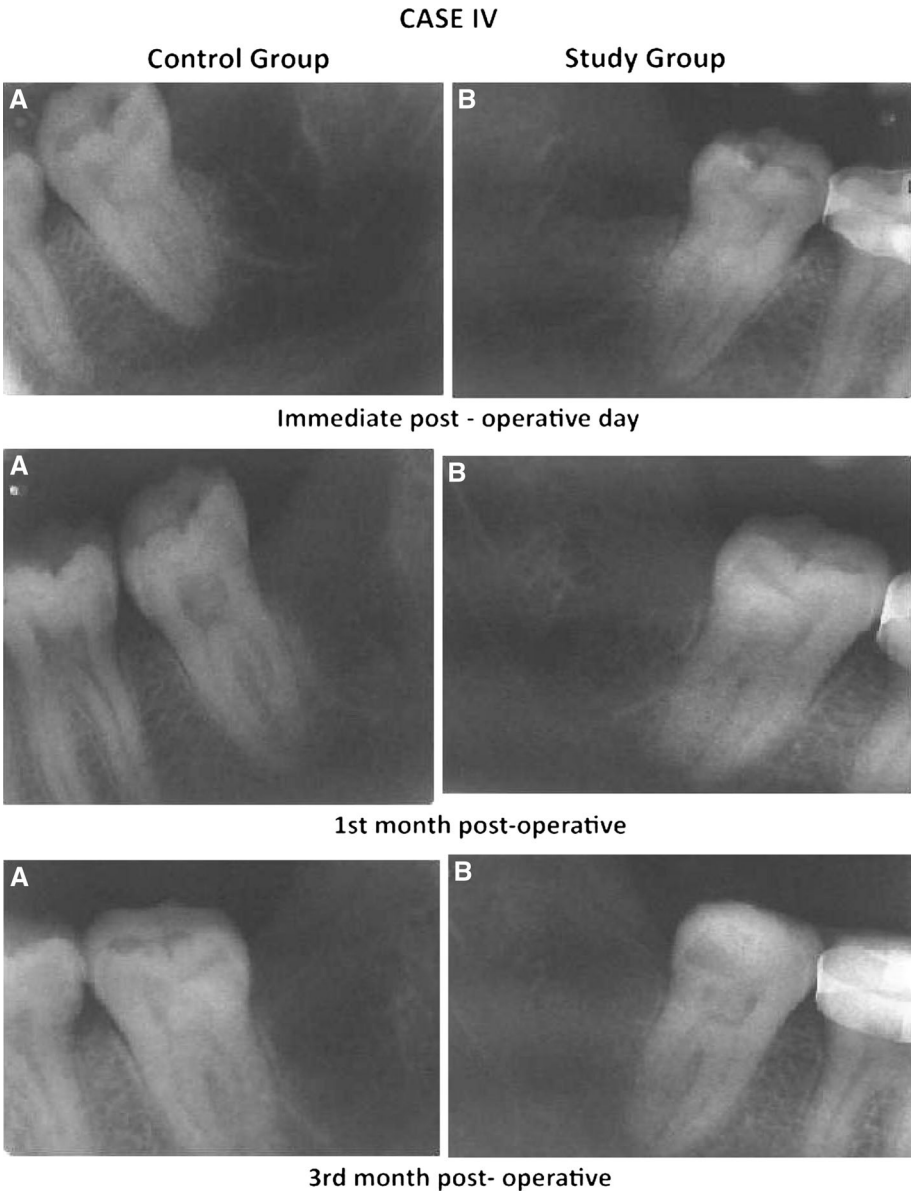


Fig. 5 Intra oral periapical radiographs: Case 2

the above mentioned osteoinductive and osteoconductive actions of simvastatins were similar to the maximal doses of BMP2; because they induce Heat Shock Protien 27, enhance mRNA expression for BMP2, Alkaline Phospahatase (ALP), Osteocalcin and Vascular Endothelial Growth Factor by inhibition of Rho-associated Kinase activity in osteoblasts, bone marrow cells and stem cells in vitro and in vivo in rats and in rabbits. Clinical reports suggest that statins may reduce the risk of fractures and osteoporosis in patients [8].

The statins are not susceptible to proteolytic digestion at the tissue site but have very short half lives and so are not very effective for bone regeneration through oral administration and can have side effects if systemically injected or applied locally at high doses. Simvastatin has been used as a 2.5 % topical gel in periodontal pockets with positive results [20]. 10^{-8} M Simvastatin promoted osteogenic activity in vitro [39]

Statins increase the number of circulating Endothelial Progenitor Cells (EPCs), accelerate re-endothelalization after carotid ballon myocardial surgery and improve post-ischemic cardiac function. Statins induce angiogenesis by promoting the proliferation, migration and survival of EPCs [40].

Hypercholesterolemia is associated with increase in platelet reactivity. Statins cause decrease in the thrombogenic potential of these cells due to mechanisms still not understood [8].

Studies indicate that Statins decrease systemic and vascular inflammation by decreasing the C-Reactive

Protien (Ls-CRP). Ls-CRP is produced by the liver in response to proinflammatory cytokines, such as Interleukin-6 (IL-6) and reflects low grade systemic inflammation [8].

In short Statins induce angioneogenesis, stimulate osteoblasts, decrease vascular inflammation, are anti thrombogenic and stimulate formation of BMP2 which has resulted in formation of bone in ectopic sites experimentally.

The density of the bone formed in the extraction socket was calculated by measuring the grey level histogram values of the digitalized IOPA radiographic images.

In the present study mean grey level histogram values of the digitalized IOPA images of the third molar extraction socket of both control and study groups were calculated at immediate post-operative day, 1 month and 3 month post-operative period by Adobe Photoshop 7.0 software. The digitalization of the IOPA radiographs were carried out with the help of a scanner ("DENTAMERICA\CAMREX\AviCap.exe"). Radio-opacity of the bone filling the socket was evaluated at the centre of the socket using grey level histogram. All the mean values of both groups were recorded and entered into a master sheet for ease of comparison. The results were better in the study group than in the control group. The mean values of the grey level histogram were highly significant at 3rd month post-operatively and was significant at immediate post-operative and first month post-operative period. Data suggested evidence of early bone formation and maturation radiographically in study group as compared to control group at 1st and 3rd month post-operatively.

We also assessed the percentage of facial swelling in both the study and the control groups. The percentage of facial swelling was numerically greater on the study side as compared to the control side at 2nd and 3rd post-operative day as reported by Ozec [23]. But it was not clinically significant. Nyan et al. concluded that simvastatin combined with calcium sulfate caused substantial bone regeneration in rat calvarial defects; however, with a considerable soft tissue inflammation and scabbing of the skin overlying the calvaria. Thus, the local effects of simvastatin could be dose- and carrier-dependent [26].

Pain was also assessed with VAS scale and it was found that the severity of pain was equal in both study and control groups and the results were not significant. However the intensity of pain was higher on the day of surgery and gradually reduced.

Our findings are supported by Maciel-Oliveira et al. [37] and Griffiths and Cartmell [38]. In their study, they suggest that simvastatin stimulates bone regeneration when it is locally administered into defects created in the rat alveolar process. As the ultrastructural and immunocytochemical observations have shown, bone formation started earlier in the simvastatin-treated rats than in the controls. Additionally, the laid down matrix presented a lamellar appearance.

Our study also is in accordance with Ayukawa et al. [28]. In their study they demonstrated the effect of the local administration of simvastatin on the healing of artificially created bone defects. In the histologic and histomorphometric study, local application of simvastatin successfully increased the bone regeneration.

Mouhamed et al. [5] in their human study concluded that both digital radiological examination and histological analysis prove that adding simvastatin in tricalcium phosphate improves bone formation. Hassan et al. [6] concluded that the use of simvastatin accelerates bone graft healing, maturation, maintains its volume to a great extent and decreases its resorption. It also increases the density of the graft compared to a native bone or autogenous bone graft in human bone graft remodeling after ridge reconstruction, which is again in accordance with our study.

Conclusion

The limitation of the present study was that 3 months post-operative follow up period was short to comment on the efficacy of Simvastatin in complete bone regeneration process but adequate enough to evaluate the effects of Simvastatin in initiating and enhancing hard tissue healing. Long term follow up along with histological study of the bone is required for assessment of the efficacy of Simvastatin. Further research is needed to determine the optimal therapeutic threshold, mode of application and the effectiveness in humans for bone regeneration.

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