Original Article

Healing of extraction socket following local application of simvastatin: A split mouth prospective study

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ABSTRACT

Background: The role of simvastatin in lowering serum cholesterol level is well described. However, recent findings suggest they have a role in bone formation as well.

Aim and objectives: The present prospective study was conducted to evaluate the efficacy of simvastatin on bone formation in extraction sockets.

Materials and methods: 15 patients undergoing all four first premolar extraction were selected based on inclusion and exclusion criteria. Extraction sockets of left premolars (24 and 34) were considered as cases and right premolars (14 and 44) as controls. Overall 30 extraction sites were assigned to each group. Atraumatic extraction was done in all cases following which simvastatin mixed with gelatin sponge was placed in extraction socket of 24 and 34 while only gelatin sponge was placed in 14 and 44. All sockets were then closed with 3-0 vicryl. The patients were kept on follow-up and complications such as dry socket, pain, and swelling were recorded. Intra oral peri apical radiographs were taken immediately after extraction and at 2nd month and 4th month to record changes in the density of alveolar bone. The radiographic measurements were compared and the differences were statistically analyzed.

Result: Percent increase in bone density at the end of 8th week and 16th week was significantly high in case as compared to the control group.

Conclusion: Local application of simvastatin induces bone formation in extraction sockets. Application is very simple and provides a very cost effective way of faster bone regeneration following tooth extraction.

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1. Introduction

New bone formation is an event involving production of new bone matrix by osteoblasts, the bone forming cells, and its subsequent mineralization.¹ In the process of bone formation, various growth factors like bone morphogenetic proteins (BMPs) plays a critical role in the proliferation and differentiation of osteoblasts.^{1,2} BMP-2 causes differentiation of multipotent stem cell line into osteoblast-like cells.²

In a search for agents that activates BMP-2, Mundy et al.³ examined more than 30,000 compounds and found that statins can effectively enhance new bone formation in vitro and in rodents by specifically increasing the BMP-2 genes.

Statins are a group of medications used to treat hypercholesterolemia.^{4,5} They are specific inhibitors of 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase¹ which is involved in the conversion of HMG-CoA to mevalonate, an early rate-limiting step in the synthesis of cholesterol in the liver.⁴ Statins competitively inhibit this enzyme and reduces the hepatic synthesis of cholesterol.⁶

Statins are well tolerated and have an excellent safety record.⁵ However, extensive use of statins have led to an increase in number of other beneficial effects as well, so-called pleiotropic effects.^{7–11} With respect to these effects, statins also augment the expression of bone morphogenetic protein-2, a potent simulator of osteoblast differentiation and its activity, and promote mineralization by cultured osteoblasts, indicating that statins have an anabolic effect on bone.¹²

The statin family includes naturally existing lovastatin and chemically modified simvastatin and pravastatin.¹³ The use of simvastatin as an adjuvant to bone grafting procedures in oral and

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maxillofacial surgery has been increasing in popularity since its introduction in 1999 by Mundy et al.³ However statins are hepatoselective and mainly degraded in the liver.^{1,14,15} Their local concentration is too low to take effect by traditional delivery systems. Therefore an effective delivery system should be chosen in order to obtain full effect of simvastatin on bone.¹³

In the previous literature, different authors have used various substances like collagen matrix sponge,^{2,14,16} gelatin sponge,^{17,18} PLGA,¹³ methyl cellulose^{15,19} as a carrier for successful local delivery of simvastatin. In our study we had used gelatin sponge as a carrier for local delivery of simvastatin in fresh extraction sockets.

The aim of the present study was to evaluate the efficacy of simvastatin on bone formation, if applied locally on fresh extraction sockets along with gelatin sponge as a carrier.

2. Materials and methods

A prospective study was done on 15 patients requiring all four first premolar extractions in Department of Oral and Maxillofacial Surgery, Government Dental College and Research Institute, Bangalore. The research protocol was reviewed by the Institutional Ethical Committee and Review Board of the Government Dental College and Research Institute, Bangalore, Karnataka, India. Patients were selected based on inclusion and exclusion criteria.

Inclusion criteria include:

- (1) Patients requiring all four first premolar extractions.
- (2) Age group 18–35 years.
- (3) Teeth to be extracted should have minimum periapical changes radiographically and probing depth not more than 2–3 mm clinically.
- (4) Teeth which can be extracted by intra-alveolar (forceps method) under local anesthesia.

Exclusion criteria include:

- (1) Medically compromised patients (systemic diseases affecting bone metabolism).
- (2) Teeth having radiographically evident large periapical changes (abscess/granuloma/cyst).
- (3) Teeth requiring transalveolar/open extraction.

Patients who met the above inclusion and exclusion criteria were selected and consent was obtained. All patients were explained about the procedure. Case and control group were divided such that in each patient site 14 and 44 belongs to control group while site 24 and 34 belongs to case group. Overall, 30 extraction sites were assigned to each group.

Detailed case history was taken and routine hematological investigations were carried out.

Before initiating the procedure, a pre-operative IOPA using paralleling technique was taken for all four extraction sites.

The procedure was performed by the same surgeon under local anesthesia (lignocaine 2% with epinephrine 1:100,000) to avoid bias. All four first premolars were extracted carefully, with minimal soft tissue reflection and without causing any damage

to the underlying alveolar bone. The socket was then gently irrigated with normal saline and hemostasis was achieved.

Immediately after the extraction, gelatin sponge mixed with simvastatin (10 mg tablet crushed and mixed with normal saline) was placed in extraction sockets with respect to 24 and 34 while only gelatin sponge was placed in extraction sockets with respect to 14 and 44. Once it is placed, the sockets were closed with 3-0 vicryl to prevent gelatin sponge from getting displaced and immediate post-operative IOPA were taken for all four extraction sites.

Patients were instructed to apply gentle pressure on the gauze pack over the operated site for a period of 30 min. Chemical plaque control with chlorhexidine gluconate solution (0.2% 1 min, TID) was advised 24 h following the procedure for the first post-operative week. Antibiotics (Cap. amoxycillin 500 mg for five days) and analgesics (Tab. ibuprofen 400 mg + Paracetamol 325 mg TID for five days) were prescribed.

The patients were recalled for regular follow up. Complications such as dry socket, pain, swelling, pus discharge, if any, arising out at first post-operative week were recorded. IOPA were taken immediately after extraction and at 8th week and 16th week post-operatively. The radiographs were taken using the paralleling technique to eliminate radiographic elongation or foreshortening. Radiographs obtained were digitized and opened through *ImageJ software*. Outline of extraction socket was determined by using preoperative IOPA. The same outline was transferred to subsequent follow up radiographs to determine exact socket area and mean gray values were recorded for the selected area.

The obtained data was entered in SPSS v21 software for windows and was statistically analyzed using descriptive and analytical statistics. A *p* value of less than 0.05 was considered statistically significant.

3. Result

Out of 15 patients selected for the study, 6 patients (40%) were male and remaining 9 were female (60%). The mean age of the patients was 21.8 ± 2.86 years with a range of 18–27 years.

Mean gray value immediately after extraction for case was 65.26 ± 2.67 while it was 66.15 ± 2.54 for controls. There was no statistically significant difference present between the two groups (*p* value 0.094).

The mean gray value for case and control group increased to 79.09 ± 3.66 and 73.81 ± 3.38 respectively at the end of 8 weeks. It further increased to 91.65 ± 3.55 and 82.33 ± 4.01 for case and control group respectively at the end of 16 weeks.

Statistically significant difference was present between the two groups with regard to increase in mean gray value both at the end of 8 weeks (*p* value <0.00001) as well as 16 weeks (*p* value <0.00001).

Mean percent increase in gray values at the end of 8 weeks and 16 weeks for case and control group are given in Table 1

Comparison of different extraction sites within case and control group is given in Tables 2 and 3 respectively (Figs. 1–16).

Clinically, complications such as dry socket, pain, swelling, pus discharge were absent on all sites throughout the follow up period.

Table 1

Mean percent increase in gray values at the end of 8 weeks and 16 weeks for case and control group.

	Case	Control	<i>p</i> value
Mean percent increase in gray value at 8 weeks	$21 \pm 3.09\%$	$11.58 \pm 2.9\%$	<0.00001
Mean percent increase in gray value at 16 weeks	$40.5 \pm 4.14\%$	$24.46 \pm 3.67\%$	<0.00001

Table 2

Comparison of different extraction sites within case.

	Extraction site 24	Extraction site 34	p value
Mean gray value immediately after extraction	65.4 ± 2.8	65.12 ± 2.63	0.39
Mean gray value at 8 weeks	78.82 ± 3.72	79.36 ± 3.71	0.35
Mean gray value at 16 weeks	90.99 ± 3.56	92.3 ± 3.54	0.16
Mean percent increase in gray value at 8 weeks	20.52 ± 2.78%	21.87 ± 3.32%	0.12
Mean percent increase in gray value at 16 weeks	39.2 ± 3.99%	41.8 ± 4.0%	0.04

Table 3

Comparison of different extraction sites within control.

	Extraction site 14	Extraction site 44	p value
Mean gray value immediately after extraction	66.08 ± 2.74	66.23 ± 2.43	0.44
Mean gray value at 8 weeks	73.43 ± 3.27	74.20 ± 3.56	0.27
Mean gray value at 16 weeks	81.65 ± 3.72	83.02 ± 4.30	0.18
Mean percent increase in gray value at 8 weeks	11.15 ± 2.92%	12.01 ± 2.92%	0.21
Mean percent increase in gray value at 16 weeks	23.59 ± 3.26%	25.32 ± 3.96%	0.10

Fig. 1. Control (14) pre-operative IOPA. **Fig. 3.** Control (14) immediate post-operative IOPA. **Fig. 2.** Case (24) pre-operative IOPA. **Fig. 4.** Case (24) immediate post-operative IOPA.

Fig. 5. Control (14) 2 months post-operative IOPA.

Fig. 8. Case (24) 4 months post-operative IOPA.

Fig. 6. Case (24) 2 months post-operative IOPA.

Fig. 9. Control (44) pre-operative IOPA.

Fig. 7. Control (14) 4 months post-operative IOPA.

Fig. 10. Case (34) pre-operative IOPA.

Fig. 11. Control (44) immediate post-operative IOPA.

Fig. 14. Case (34) 2 months post-operative IOPA.

Fig. 12. Case (34) immediate post-operative IOPA.

Fig. 15. Control (44) 4 months post-operative IOPA.

Fig. 13. Control (44) 2 months post-operative IOPA.

Fig. 16. Case (34) 4 months post-operative IOPA.

4. Discussion

The removal of a tooth initiates a sequence of events including inflammation, epithelialization, and remodeling. Sockets heal by secondary intention, with remodeling continuing to 1 year after the extraction. During the course of remodeling, bone resorption can occur which may amount to 0.5 mm per year and can create difficulties in future implant placement. Therefore, one should aim to preserve and often create the necessary bone for implant stabilization.²⁰

Simvastatin belongs to a group of drugs known as statins. Statins are specific inhibitors of 3-hydroxy-3 methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in the cholesterol synthesis pathway. They are amongst the most widely used drugs in the treatment of hypercholesterolemia.^{1,4,5}

Besides the lipid lowering effect of statins, the results of many studies have demonstrated the therapeutic impact of the pleiotropic effects of statins.^{7–12} Mundy et al.³ first reported that simvastatin stimulates in vivo bone formation in rodents. Simvastatin stimulates the expression of bone anabolic factors such as vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2 (BMP-2),^{21,22} and promotes osteoblast differentiation and mineralization in non-transformed osteoblastic cells (MC3T3-E1 cells).²³ Simvastatin also markedly increased mRNA expression for alkaline phosphatase, type I collagen, bone sialoprotein, and osteocalcin (OCN) in non-transformed osteoblastic cells (MC3T3-E1), while suppressing gene expression for collagenase-1, and collagenase-3.²³

The effect of statins when administered systemically appears to be minimized by clearance by the liver¹³ and exceedingly high doses of systemically applied statins can raise the risk of liver failure, kidney disease, and rhabdomyolysis.^{14,15} This has led to the application of simvastatin locally for effective bone regeneration with virtually no side effects.

Gutierrez et al.²⁴ determined that topical application of statin was 50 times more active on bone formation in rats than oral administration. Therefore, various carriers like gelatin sponge,^{17,18} collagen sponge,^{2,14,16} PLGA,¹³ methyl cellulose^{15,19} were used in the past for successful local delivery of simvastatin. However in our study, we had used gelatin sponge as a carrier because it gets resorbed slowly and thus the simvastatin powder had a slow sustained release to have maximum effect on bone.¹⁷ Also it is biocompatible, bioresorbable and has a sponge-like form, so adapts easily to the shape of defects.¹⁸

A study conducted by Wong and Rabie²⁵ showed that there was 308% more bone in defects grafted with statin mixed with a carrier of collagen matrix than in those grafted with carrier alone after 14 days.

The present study showed that the case group in which extraction sockets were grafted with simvastatin and gelatin sponge had a significantly high mean gray values at the end of 8 as well as 16 weeks as compared to control group. Also, the percent increase in mean gray value at 8 as well as 16 weeks was significantly high in case group as compared to control. This clearly shows that simvastatin has a definitive positive role in bone formation. Our results were also in accordance with the previous studies conducted by Özeç et al.,¹⁸ and Wu et al.,¹³ in Wistar rats.

Within the case group, on comparing the extraction sites in maxilla with mandible, it was found that the mean percent increase in gray value was slightly more in mandibular sites as compared to maxillary sites both at 8 weeks as well as 16 weeks. However statistically significant difference was present only at 16 weeks.

Similar comparison was made in control group as well and similarly, it was found that the mean percent increase in gray value was more in mandibular site as compared to maxillary site.

However, the difference was statistically not significant at both 8 weeks as well as 16 weeks.

This shows the effect of simvastatin on mandibular sites is slightly more as compared to maxilla. The authors hypothesized this can be attributed to the fact that there was slightly greater release of growth factors in mandibular bone thereby enhancing production of bone matrix and their subsequent calcification.

To the best of author's knowledge, this study was the first to compare the difference in bone formation in mandibular and maxillary sites on local application of simvastatin.

No clinical complications like infection, pain, swelling, draining sinus were observed throughout the follow up period which suggests that simvastatin gelatin sponge is well tolerated by the patients and is safe for local application.

The main drawback of the study was the small sample size and follow up period of 16 weeks was short to comment on the final quality of bone formed. So a study with larger sample size and longer follow up period should be carried out. Also histologic analysis was not done to assess the quality of newly formed bone.

5. Conclusion

Local application of simvastatin induces bone formation in extraction sockets. Application is very simple and provides a very cost effective way of faster bone regeneration following tooth extraction. However, study with larger sample size and longer follow up is needed before drawing any definitive conclusion. Further research is also needed to make out the difference in bone formation on different anatomic sites on local application of simvastatin.

Conflicts of interest

The authors have none to declare.

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