Statins in Periodontal Regeneration - The current Scenario

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ABSTRACT:
Statins are the group of lipid lowering drugs commonly used to control cardiovascular and cerebrovascular diseases. Statins have structure similar to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and competitively inhibits HMG-CoA reductase, which is the rate-limiting step in the mevalonate pathway, thereby reducing blood cholesterol levels. Statins have potential anti-inflammatory effect. Statins in systemic and local application enhance osteoblastic differentiation and bone formation by upregulating bone morphogenetic proteins and by blocking the intermediate metabolites of the mevalonate pathway. Because of these effects statins can be used in treating periodontal disease and promoting periodontal regeneration.

INTRODUCTION
The discovery of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, called statins, was a breakthrough in the prevention of hypercholesterolemia and related diseases. Hypercholesterolemia is considered to be one of the major risk factors for atherosclerosis which often leads to cardiovascular, cerebrovascular and peripheral vascular diseases. The statins inhibit cholesterol synthesis in the body and that leads to reduction in blood cholesterol levels, which is thought to reduce the risk of atherosclerosis and diseases caused by it.

In the 1950s the Framingham heart study led by Dawber revealed the correlation between high blood cholesterol levels and coronary heart diseases. The primary goal was to inhibit the enzyme 3-hydroxy-3-
methylglutaryl-CoA reductase (HMGR) responsible for cholesterol biosynthesis in the body. In 1970s the Japanese microbiologist Akira Endo first discovered natural products with a powerful inhibitory effect on HMGR in a fermentation broth of Penicillium citrinum, which was named as compactin (mevastatin). Animal trials showed very good inhibitory effect, however in dogs it produced toxic effects at higher doses. In 1978, Alfred Alberts and colleagues at Merck Research Laboratories discovered a new natural product in a fermentation broth of Aspergillus terreus, their product showed good HMGR inhibition and they named the product mevastatin, which later became known as simvastatin.2 The mostly used statins in day to day use are simvastatin (20/30 mg) and atorvastatin (40/80 mg).

Periodontal diseases result in destruction of tooth supporting structures. The primary goal of periodontal therapy is the regeneration of lost attachment apparatus. Search for the cost effective and efficient agents to promote periodontal regeneration continues. Statins have pleiotropic effects like anti inflammatory, antioxidant and anabolic effects on bone apart from lipid lowering action. The statin family reportedly increased bone mineral density in humans and decreases the risk of fractures in osteoporotic and elderly patients. Horiuchi and Maidea pointed out that statins may be useful for treating periodontal disease in patients with osteoporosis.3 Furthermore, systemic administration of simvastatin is found to be associated with a reduced risk of tooth loss in patients diagnosed with chronic periodontitis as observed by a retrospective analysis over a seven-year period.4 Many studies have been carried out by the use of locally delivered statins in chronic periodontitis patients and showed good results. The present review focuses on the role of statins in bone metabolism and their applications in periodontal regeneration.

**Methods for Data Collection**

PubMed and Google Scholar were used to search original research articles, case reports, and other reviews about Statins and periodontal regeneration, from 2005 to 2012, from peer-reviewed journals. The keywords used were statins, periodontal regeneration, mevalonate pathway, HMG CoA reductase inhibitors. For further refinement, the following exclusion criteria were defined: Publications were limited to those of English language and from the scientific, peer-reviewed literature. From these searches, a total of 25 publications were selected for this review.

**Classification of Statins**

The statins differ with respect to their ring structure and these differences in structure affect the pharmacological properties of the statins, such as the affinity for the active site of the HMGR, rates of entry into hepatic and non-hepatic tissues, availability in the systemic circulation for uptake into non-hepatic tissues and routes and modes of metabolic transformation and elimination.

Statins have been grouped into two groups of statins according to their structure. Type 1 statins - Statins that have substituted decalin-ring structure that resemble the first statin ever discovered, mevastatin have often been classified as type 1 statins due to their structural relationship. Statins that belong to this group are Lovastatin, Pravastatin, Simvastatin.

Type 2 statins - Statins that are fully synthetic and have larger groups linked to the HMG-like moiety are often referred to as type 2 statins. One of the main differences between the type 1 and type 2 statins is the replacement of the butyryl group of type 1 statins by the fluorophenyl group of type 2 statins. This group is responsible for additional polar interactions that causes tighter binding to the HMGR enzyme. Statins that belong to this group are Fluvastatin, Cerivastatin, Atorvastatin and Rosuvastatin.

Lovastatin is derived from a fungus source and simvastatin and pravastatin are chemical modifications of lovastatin and as a result do not differ much in structure from lovastatin. All three are partially reduced napthylene ring structures. Simvastatin and lovastatin are inactive lactones which must be metabolized to their active hydroxy-acid forms in order to inhibit HMGR. Type 2 statins all exist in their active hydroxy-acid forms. Fluvastatin has indole ring structure, while atorvastatin and rosuvastatin have pyrrole and pyrimidine based ring structure respectively. The lipophilic cerivastatin has a pyridine-based ring structure.
Molecular Structure Of Statins

Statin contains a hexahydronaphthalene ring with two major side chains, viz. dimethylbutyrate ester and a second one, which contains a hydroxyacid (Figure 1).

![Molecular Structure of Statins](image1)

The hydroxyacid of the second chain forms a six membered analogue of the intermediate compound in the HMG-CoA reductase reaction, which is the rate-limiting step in the mevalonate pathway. As a result of its similarity to the compound HMG-CoA, statin is a reversible competitive inhibitor of the enzyme HMG-CoA reductase. The reaction catalysed by HMG-CoA reductase and inhibited by simvastatin is the conversion of HMG-CoA to a compound called mevalonate via an intermediate. Simvastatin like the other statins, is thus an inhibitor of the mevalonate pathway and consequently cholesterol synthesis.5

Effect On Bone Metabolism

The statin and bone story began when Wang et al. (1995) reported that lovastatin (Mevacor) reduced steroid-induced bone loss in New Zealand rabbits6,8. Further studies showed that atorvastatin, cerivastatin, fluvastatin, lovastatin and simvastatin stimulated cultured bone cells to make the osteogenic bone-morphogenic protein (BMP)-2.4,7 Lovastatin and simvastatin stimulated bone formation in cultured mouse calvariae and orally gavaged simvastatin (5 mg/kg / body weight) nearly doubled trabecular bone volume and increased bone formation by 50% in ovariectomized (OVX) rats.7

![Statin action on bone](image2)

Inhibition of the enzyme HMG-CoA reductase and the subsequent blockade of the mevalonate pathway is probably the most important mechanism of inhibition of bone resorption by statins. The reduction in mevalonate pathway intermediates by statins also prevent the synthesis of isoprenoid intermediates, farnesyl pyrophosphate(FPP) and geranyl geranyl pyrophosphate(GGPP). Isoprenoids are lipids attached by post translational modification to some small G-proteins including Ras and Ras like proteins (Rho,Rap,Rab,Ral). These proteins play important roles in cellular proliferation and differentiation, and, therefore, any perturbation of their activity influences cellular activity. Thus interference with the generation of isoprenoids leads to disruption of vesicular fusion and ruffled border formation of osteoclasts, which are essential for their bone resorbing activity(Figure 2).

As a result, osteoclast inactivation occurs and bone resorption is inhibited. The role of inhibition of mevalonate pathway is further elucidated by the finding that the effects of statins on bone are inhibited or even reversed by products of this pathway.5

Local stimulation of Bone Morphogenic Protein (BMP-2), a major bone growth regulatory factor, can lead to new bone formation. Mundy et al. (1999) identified that lovastatin, and simvastatin, mevastatin, and fluvastatin increased gene expression for BMP-2 in osteoblasts.7 The findings of their study were comparable to those seen in similar conditions after direct application of BMP-2 and Fibroblast Growth Factor-1 (FGF-1). There was also a striking increase in osteoblast cell numbers after statin application. Additionally, it has been observed that statins like simvastatin, atorvastatin,
and cerivastatin markedly enhance gene expression for vascular endothelial growth factor (VEGF) in MC3T3-E1 cells (preosteoblastic murine cells). VEGF, a bone anabolic factor, in osteoblasts regulate osteoblast function by increasing the expression of bone sialoprotein (BSP), osteocalcin (OCN), and type I collagen, as well as suppressing the gene expression of collagenases such as MMP-1 and MMP-13. 

Another study evaluated effect of atorvastatin on osteoblastic production of osteoprotegerin (OPG) and receptor activator of the nuclear factor ëB ligand (RANKL), essential cytokines for osteoclast cell biology. Whereas RANKL promotes osteoclast formation and activation, thus promoting bone resorption, OPG acts as soluble decoy receptor that antagonized the effects of RANKL. Mentioned study pointed out, atorvastatin increased OPG mRNA levels and protein secretion in human osteoblasts, and enhanced expression of osteoblastic differentiation markers, osteocalcin and alkaline phosphatase. Human osteoblasts treated with substrates of cholesterol biosynthesis, which are downstream of HMG CoA reductase reaction (mevalonate, and geranylgeranyl pyrophosphate), reversed atorvastatin-induced enhancement of OPG production.

Anti-inflammatory Effects of Statins

In periodontal disease, tissue destruction results from the interaction of the host’s immune responses with microorganisms in dental plaque. Statins has been suggested to have several anti-inflammatory effects which may also be important in treating periodontal disease. Statins are able to inhibit leucocyte function associated antigen (LFA-1) -intercellular adhesion molecule-1 interaction in vitro by binding to LFA-1. This binding inhibition might prevent leucocyte adhesion and extravasation to sites of inflammation and antigen presentation. In addition Weitz-Schmidt et al. found that inhibition of LFA-1 resulted in impaired T-cell costimulation. Statins also act in vitro as direct inhibitors of Major histocompatibility complex class II (MHC II) thereby suppressing T-cell function.

Statins also decreases the production of many proinflammatory cytokines.

Matrix metalloproteinases (MMPs) are responsible for degradation of extracellular matrix molecules in periodontal disease. Statins have been found to decrease the secretion of MMP-1, MMP-2, MMP-3 and MMP-9 in vitro.

Applications in periodontal therapy

Periodontitis is characterized by an inflammatory breakdown of the tooth supporting structures. The most desirable outcome of periodontal treatment is regeneration of the periodontal tissues lost as a consequence of disease. The need to achieve greater regeneration warrants the use of an agent, which not only inhibits resorption of the alveolar bone but also stimulates new bone formation. Bisphosphonates like alendronate are a commonly used group of drugs which inhibit bone resorption by blocking the mevalonate pathway. Some of the products of this pathway are involved in osteoclast maturation and activation and thus its blockade leads to inhibition of bone resorption. However, bisphosphonates do not stimulate new bone formation. Another widely used group of drugs is that of statins like simvastatin, atorvastatin, has been shown to inhibit bone resorption. Statins upregulate the expression of bone morphogenic protein-2 (BMP-2) by osteoblasts. BMP’s effect is to activate the differentiation pathway of osteoblasts. BMP’s act through the activation of SMAD pathway after which the cbfa1transcription factor is upregulated. Cbfa1 activation results in activation of osteocalcin gene, col I and alkaline phosphatase genes which form the matrix and favour mineralization. Topical delivery of biological molecules like Bone Morphogenetic Protein-2 (BMP-2) and Fibroblast Growth Factor has been shown to enhance bone growth. However, the use of these molecules seems to be associated with some drawbacks like degradation at the site of application and activation of a host immune response. Lovastatin and simvastatin may stimulate the osteoblastic differentiation of periodontal ligament cells via the ERK1/2 pathway. This suggests that the statins may be useful for regenerating periodontal hard tissue.

Studies on Statins Use in Periodontal Disease

Simvastatin are administered in the prodrug form, which is much more lipophilic than the active beta-hydroxyacid form. Because of this property, the simvastatin molecule can effectively cross cellular membrane barriers by passive diffusion. It also implies that it can be incorporated into hydrophobic delivery vehicles for local sustained release to achieve bone formation in periodontal defects.
Additionally, solutions of simvastatin in optimal concentrations could be combined with bone grafts to enhance their regenerative potential. The low cost and impressive long-term safety profile of this compound make it a suitable agent in periodontal therapy.

**Animal Studies**

Mundy et al. first reported that statins stimulate in vivo bone formation in rodents and increase new bone volume in mouse calvaria cell cultures. He identified that simvastatin may help in periodontal regeneration by inducing BMP-2 and TGF beta in osteoblasts. The findings of their study were comparable to those seen in similar conditions after direct application of BMP-2 and Fibroblast Growth Factor-1 (FGF-1).

Goes et al. found that Atrovastatin (ATV) reduced alveolar bone loss by over 47% (p<0.05), when compared to the group of untreated rats and concluded that ATV was able to prevent alveolar bone loss seen on a ligature-induced periodontitis model.

**Human Studies-Systemic Administration Of Statins**

Systemic administration of simvastatin is found to be associated with a reduced risk of tooth loss in patients diagnosed with chronic periodontitis as observed by a retrospective analysis over a seven-year period.

Lindy et al. examined the association of statin use and clinical markers of chronic periodontitis and concluded that patients on statin medication exhibit fewer signs of periodontal inflammatory injury than subjects without the statin regimen.

Saxlin et al. reported that statin medication appears to have an effect on the periodontium that is dependent on the inflammatory condition of the periodontium. The study was based on a subpopulation of the Health 2000 Survey, which included dentate non-diabetic, non-rheumatic subjects who did not smoke, aged 40-69 years (n=2032).

Fajardo et al. studied the effect of Atrovastatin (ATV) treatment on bone loss prevention in subjects with chronic periodontitis and reported that ATV have beneficial effects on alveolar bone loss and tooth mobility in subjects with periodontal disease.

Sangwan et al. reported that relative to the general population, hyperlipidemic subjects are more prone to periodontal disease and also stated that statins have a positive impact on periodontal health.

**Animal Studies- on Locally Delivered Statins**

In this study devices for sustained or intermittent release of simvastatin hydroxyacid were formed using a blend of cellulose acetate phthalate and a poly(ethylene oxide) and poly(propylene oxide) block copolymer, and they were implanted directly over the calvarium of young male rats. Drug-free devices were used as controls. After 9, 18, or 28 days, specimens were histologically evaluated for new bone formation. Intermittent delivery of simvastatin hydroxyacid in rats calvarium resulted in localized osteogenesis.

Local application of statins in healing sites or defects has been shown effective in new bone formation. Statin/collagen matrix grafts applied to the rabbit's calvaria caused expression of BMP-2, vascular endothelial growth factor and core binding factor 1 in healing bone within 5 days, and 308% more bone than collagen matrix controls.

**Human Studies- on Locally Delivered Statins**

Currently human studies using locally delivered simovastatin gel in periodontal defects have been reported.

Pradeep et al. investigated the effectiveness of Simvastatin (SMV), 1.2 mg, in an locally delivered SMV provides a comfortable and flexible method not only to improve clinical parameters but also enhances bone formation. Indigenously prepared biodegradable controlled-release gel as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis and reported that there was a greater decrease in gingival index and probing depth and more CAL gain with significant bone fill at sites treated with SRP plus locally delivered SMV in patients with chronic periodontitis.

Kinra et al. in his study showed that combination of allograft with a solution of simvastatin leads to significantly greater reduction in probing depth, gain in clinical attachment level, and linear defect fill than when the graft is used alone in the treatment of human periodontal infrabony defects.

Pradeep et al showed the effectiveness of simvastatin (SMV),1.2% on indigenouly prepared biodegradable controlled release gel as an adjunct
to scaling and root planing (SRP) in the treatment of chronic periodontitis in type II diabetes patients and reported that there was more clinical attachment gain with significant intrabony defect fill at sites treated with SRP and locally delivered simvastatin.

**Conclusion**

Statins have been suggested to have anti-inflammatory and bone anabolic effects. Thus, statins have a broad therapeutic effect beyond that of cardioprotection and potentially show great promise in regenerative therapies. Many studies have suggested that statin medication may also have beneficial effects on the periodontium. Statins may have therapeutic effect in the management of periodontal disease due to their effect on bone metabolism. This may lead to the identification of other potential molecular targets for drug discovery as well as other novel therapeutic approaches to enhance periodontal regeneration, if confirmed by prospective consecutive studies.

**REFERENCES**


