

Simvastatin: Its potential new role in periodontal regeneration

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Abstract

Periodontal therapy is aimed at the restoration of tissues destroyed by disease. However, achieving greater predictability with regenerative therapy requires the introduction of an agent which not only hampers tissue destruction but also enhances the regenerative capabilities of the periodontal tissues. Pharmacologic agents offer great promise in this direction. Simvastatin, which is used for the treatment of hypercholesterolaemia, is a universally accepted and relatively inexpensive drug. Its long-term systemic administration in humans has been shown to result in increased bone mineral density. Local application of simvastatin has been shown to stimulate bone formation in rodents both in vitro and in vivo and in human periodontal ligament cells in vitro. These effects seem to be associated with an increased expression of BMP-2 and reduced formation of metabolites of the mevalonate pathway, which it blocks. Many other mechanisms of action have also been proposed and studied. This article reviews the effects of simvastatin and examines its potential role in periodontal regenerative therapy.

Keywords: Simvastatin; Periodontitis; Periodontal regeneration; BMP-2.

Introduction

Periodontal disease is a major oral health problem. Over the years, various treatment modalities have been tried with varying success to correct periodontal attachment and alveolar bone loss resulting from this disease. The most desirable outcome of such procedures 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. Topical delivery of biological molecules like Bone Morphogenetic Protein-2 (BMP-2) (Cochran et al., 1999) and Fibroblast Growth Factor (Kimoto et al., 1998) 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 (Garrett et al., 2001).

Pharmacologic compounds, which have been shown to affect bone growth, could offer a safe and cost effective alternative to this problem. 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 (Fisher et al., 1999). However, bisphosphonates do not stimulate new bone formation. Another widely used group of drugs is that of statins like simvastatin, atorvastatin, cerivastatin, etc. They also act on the

mevalonate pathway, albeit at a different level. They are competitive inhibitors of the rate limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) (Garrett et al., 2001). Since cholesterol is the main product of the mevalonate pathway statins are used orally to treat hypercholesterolaemia and hyperlipidaemia. The safety profile of statins is well documented (Guthrie, 2006).

Simvastatin, a synthetic statin has a number of pleiotropic effects as well. In addition to its anti-resorptive actions, it has been found to exert anabolic effects on bone. These effects have been elucidated in the form of increased bone mineral density in diabetes mellitus patients who were administered statins systemically for the correction of increased cholesterol levels (Chung et al., 2000). Similarly, a significantly decreased risk of hip fractures has been observed in elderly individuals after being given statins orally for a period of three months to three years (Wang et al., 2000). 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 (Cunha-Cruz et al., 2006). The anabolic effects on bone have been attributed mainly to an upregulation of BMP-2 by simvastatin and other members of the statin family (Mundy et al., 1999). The biologically significant antiinflammatory and antioxidant properties of simvastatin are other pleiotropic effects of interest from a periodontal

therapeutic standpoint (Davignon and Laaksonen, 1999).

However, periodontal therapy necessitates a focussed effect in specific defects, suggesting the importance of local application of this drug. A number of studies have concentrated on the effects of locally administered simvastatin on bone formation (Jeon et al., 2008; Morris et al., 2008; Vaziri et al., 2007). It has also been observed that application of this agent to a culture of human periodontal ligament cells enhances their proliferation and metabolism (Yazawa et al.,

2005). Therefore, simvastatin could play a significant role as a therapeutic agent in the treatment of periodontal disease.

Molecular structure of simvastatin and its effect on mevalonate pathway

Alberts and co-workers (Alberts et al., 1980) isolated lovastatin (formerly called mevinolin), the first statin for use in humans, from a mould *Aspergillus terreus*. Simvastatin is a chemically modified derivative of lovastatin. It is a butanoic acid with the empirical formula $C_{25}H_{38}O_5$.

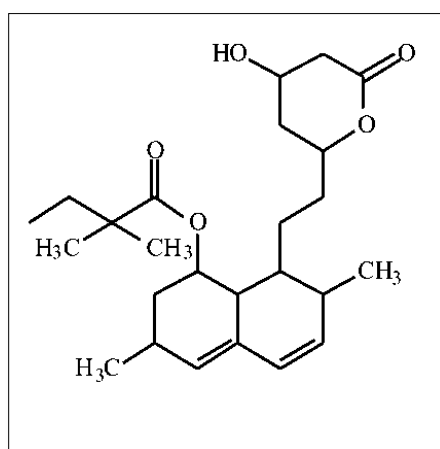


Fig. 1: Molecular Structure of Simvastatin.

It contains a hexahydronaphthalene ring with two major side chains, viz. dimethylbutyrate ester and a second one, which contains a hydroxyacid (Figure 1). 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, simvastatin 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 (Figure 2) and consequently cholesterol synthesis (Garrett et al., 2001; Brunton et al., 2007).

Long-term systemic use in humans

A number of studies reveal that long-term systemic administration of simvastatin for decreasing plasma cholesterol levels in humans has beneficial effects on the skeleton. Chung and co-workers (Chung et al., 2000) carried out a retrospective review of the medical records of sixty-nine patients suffering from diabetes mellitus. Thirty-six of these patients (treatment group) took HMG-CoA reductase inhibitors like simvastatin, lovastatin, and pravastatin for hypercholesterolaemia. The remaining thirty-three patients (control group) did not take these drugs and had normal cholesterol levels. Bone mineral density or BMD of the spine, neck, femoral neck, femoral trochanter, and total hip were measured. The findings suggested that simvastatin and other HMG-CoA reductase inhibitors increased BMD in patients with type 2 diabetes mellitus. Chan and colleagues

(Chan et al., 2000) carried out a case-control study of women aged 60 years or older and found that regular statin use among them was associated with a more than 50% reduction in

the risk of pathologic fracture. Similarly, the findings of Wang et al. (2000) supported an association between statin use and a reduction in the risk of hip fracture in elderly patients.

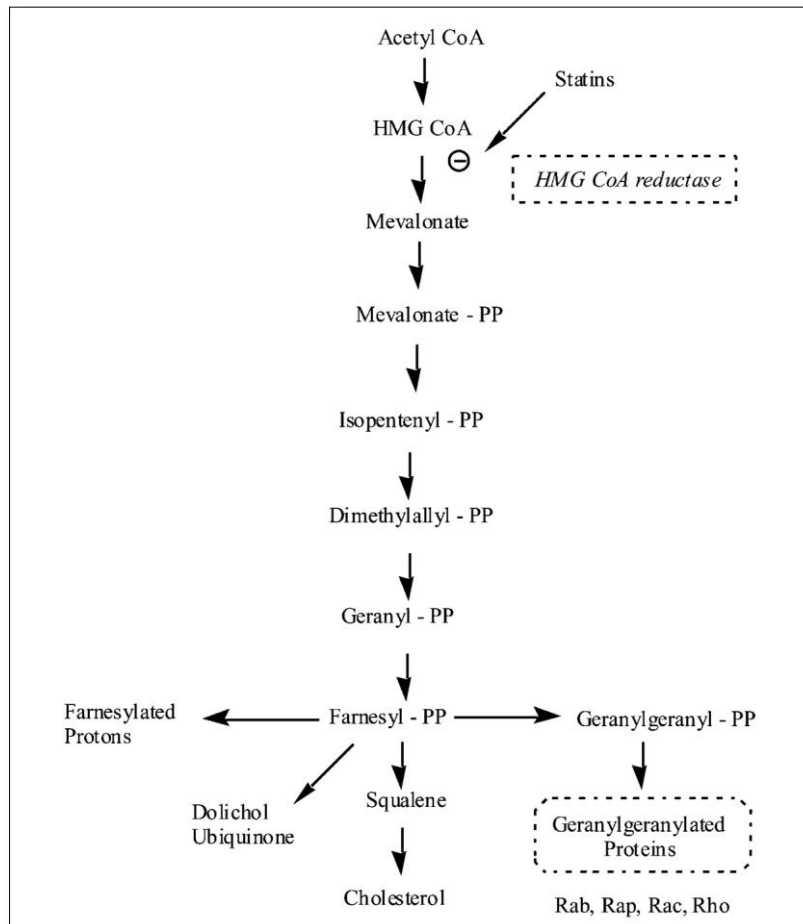


Fig. 2: Mevalonate Pathway and the Role of Statins in its Inhibition (Garrett et al., 2001).

Findings of animal model studies

Mundy et al. (1999) tested the effects of more than 30,000 compounds on bone formation. They found that the addition of statins, including simvastatin, to neonatal murine calvarial bone in organ culture increased new bone formation by 2- to 3- fold. Also, subcutaneous injections of lovastatin and simvastatin over the murine calvaria in vivo resulted in an almost 50% increase in new bone formation after five days of treatment. A number of other workers have since reported increased bone formation after local and systemic application of simvastatin in various animal models (Jeon et al., 2008; Morris et al., 2008; Vaziri et al., 2007; Skoglund et al., 2002; Thylin et al., 2002; Ayukawa et al., 2004; Stein et al., 2005; Skoglund and Aspenberg, 2007;

Seto et al., 2008; Lee et al., 2008). The findings of these studies are encouraging from a periodontal perspective because they demonstrate a direct effect of locally applied simvastatin on bone formation.

Effects on bone metabolism

Architecture of the alveolar bone is the net result of bone resorption and formation. As mentioned earlier, successful management of periodontal bone loss with a pharmacologic compound should preferably involve inhibition of resorption as well as an upregulation of formation of bone.

Inhibition of bone resorption: 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 simvastatin. Apart from cholesterol, there are a number of other products of this pathway. These include compounds called isoprenoids (Goldstein and Brown, 1990), which are primarily responsible for the prenylation of GTP-binding proteins and involved in cytoskeletal function and vesicular trafficking. 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. As a result, osteoclast inactivation occurs and bone resorption is inhibited (Fisher et al., 1999). 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 (Garrett et al., 2001).

Upregulation of bone formation: Local stimulation of 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. 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.

In another study, it was found that compactin, a known inhibitor of HMG-CoA reductase induced BMP-2 promoter activity in a concentration dependent manner in transfected human osteosarcoma cells. The induction by compactin seemed to be specific for BMP-2 gene. Simvastatin was also found to induce this promoter activity and appeared to be more potent than compactin (Sugiyama et al., 2000). BMP-2 and BMP-3 have been shown to enhance collagen synthesis by 60-70%. In addition, only BMP-2 induces a significant increase in cellular alkaline phosphatase activity at doses ranging between 20 and 200 ng/ml (Takuwa et al., 1991). These findings suggest that stimulation of bone formation by simvastatin is mediated by BMP-2. This association is further substantiated by the observation that noggin, a natural antagonist of BMP-2, inhibits bone formation stimulated by simvastatin (Garrett et al., 2001).

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 is involved in the process of endochondral bone

formation and stimulates osteoblastic differentiation leading to new bone formation (Maeda et al., 2003).

Further, the effect of simvastatin on the Akt pathway in endothelial cells was investigated by Kureishi et al. (2000). Akt acts downstream of VEGF to ensure proper blood vessel development. Secondly, it acts as an activator of endothelial cell nitric oxide production, thereby controlling vasomotor activity. Akt is also essential for endothelial cell migration towards VEGF. The data collected showed that simvastatin rapidly induced the phosphorylation of Akt, which increased its protein kinase activity. It also induced the Akt-mediated phosphorylation of endothelial nitric oxide synthase (eNOS), leading to nitric oxide production, and promoted endothelial cell survival. Production of nitric oxide by viable endothelium is known to serve several protective functions, including inhibition of apoptosis. It was also observed that like VEGF, simvastatin treatment of the cultured cells promoted the formation of capillary-like tubes. Thus it was concluded that simvastatin induced the formation of vascular structure in vitro by activating the protein kinase Akt in endothelial cells.

Antioxidant and anti-inflammatory properties

Simvastatin has been shown to inhibit the ability of macrophages to oxidise low-density lipoproteins (LDL) (Giroux et al., 1993). Various studies have shown that statins reduce the plasma levels of inflammatory markers like C-reactive protein (Davignon and Laaksonen, 1999). Ikeda and Shimada (1999) studied the effects of statins on the production of interleukin-6 (IL-6) by cultured human monocytes and smooth-muscle cells. The addition of statins significantly decreased IL-6 production by these cells. It has also been suggested that the statin mediated decrease in CRP concentrations could be due to an inhibition of IL-6 in the vascular tissues (Ikeda et al., 1999). Thus statins, including simvastatin, are believed to have biologically significant antioxidant and anti-inflammatory effects, which could prove beneficial in the treatment of periodontitis.

Effects on the periodontium

The periodontium comprises of four types of tissue, namely the gingiva, periodontal ligament, alveolar bone, and cementum. Cells derived from the periodontal ligament are believed to play an important role in the healing of alveolar bone. In vitro studies have demonstrated that they exhibit osteoblast-like

properties (Arceo et al., 1991) and are responsible not only for osteogenesis and osteoclasts, but also for fibrogenesis and fibroclasis, and cementogenesis and cementoclasts (Melcher, 1976).

Yazawa et al. (2005) carried out an in vitro study using periodontal ligament cells obtained from human teeth. To assess cell differentiation and proliferation these cells were cultured in monolayer with simvastatin for 24 to 72 hours. To analyse osteoblastic differentiation, they were grown in organoid culture for 7, 14, and 21 days.

It was observed that simvastatin enhanced cell proliferation and metabolism dose-dependently after 24 hours. It also promoted cell proliferation significantly. The maximum effect was seen at simvastatin concentrations of 10^{-8} and 10^{-7} M.

After 7 days, alkaline phosphatase activity was promoted dose dependently and the maximum effect was seen at a concentration of 10^{-8} M. The same concentration caused a significant increase in the levels of osteopontin, an osteoblast differentiation marker. A 10^{-8} M concentration of simvastatin also resulted in a significant increase in the calcium content of cultures after 21 days.

The addition of mevalonate, an intermediate of the mevalonate pathway, to the culture caused a decrease in alkaline phosphatase activity to control level. This showed that the effect of simvastatin on human periodontal ligament cells was mediated by the mevalonate pathway.

The authors concluded that relatively low concentrations of simvastatin promoted cell proliferation and osteoblastic differentiation. They also observed that the optimal concentration of simvastatin corresponded to the administration of a 20 mg tablet, which leads to a plasma concentration of the order of 10^{-8} M.

In their retrospective cohort study of 1,021 individuals suffering from chronic periodontal disease, Cunha-Cruz et al. (2006) combined dental records with drug dispensing data. They observed that any statin dispensed in the first three years after the initial periodontal exam was significantly associated with reduced tooth loss risk. Pradeep and Thorat (2010) recently reported a greater decrease in gingival index and probing depth at sites treated with scaling and root planing (SRP) and locally delivered simvastatin as compared to SRP plus placebo in human subjects with chronic periodontitis. In addition, more clinical attachment level gain as well as

significant infrabony defect fill was seen in the simvastatin treated individuals.

Applications in periodontal therapy

Periodontitis is characterised by an inflammatory breakdown of the tooth supporting structures. Periodontal therapy aims at arresting this breakdown and restoring periodontal tissues to their original structure and function. Simvastatin has been shown to inhibit bone resorption. However, this effect appears minor in comparison to its anabolic action on new bone formation and osteoblast maturation (Mundy et al., 1999). It also possesses antiinflammatory and antioxidant properties (Davignon and Laaksonen, 1999). It could therefore have a potential role in regenerative therapy.

It is 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 (Garrett et al., 2001). 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 (Yazawa et al., 2005) could be combined with bone grafts to enhance their regenerative potential. The low cost and impressive long-term safety profile (Guthrie, 2006) of this compound make it a suitable agent in periodontal therapy.

Conclusion

Simvastatin, a competitive inhibitor of the enzyme HMG-CoA reductase, is a widely used cholesterol-lowering drug. It has been found to have a number of pleiotropic effects. The findings of studies involving simvastatin have been encouraging and its effects on bone metabolism favour its use in the treatment of periodontal defects. The antioxidant and antiinflammatory properties of this compound could further facilitate healing of such defects. However, long-term clinical studies in human subjects are required to evaluate the potential benefits of simvastatin in periodontal regenerative therapy.

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