Osteoinductive and Osteoprotective Characteristics of Statins

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Abstract

Background: Statins are widely used agents for lowering cholesterol and reducing the risk for a heart attack. Recent data suggest that statins regulate bone metabolic activity by stimulating new bone formation both *in vitro* and *in vivo*. Statins were identified as a potent activator of bone morphogenic protein-2, an important promoter of osteoblastic differentiation. In addition, statins inhibit mevalonate production and isoprenoids required for osteoclastogenesis, which may contribute to their bone-sparing effect. These findings suggest that statins could be considered as potential agents for treating osteoporosis and possibly periodontal disease. **Objective:** The purpose of this article is to review existing literature on *in vitro* studies investigating bone morphogenic protein-2 promoting activity of statins and *in vivo* studies investigating effects of statins on bone formation and preservation in the oral cavity. **Materials and methods:** An electronic search of MEDLINE-PubMed was performed through December 2008 for *in vitro* studies addressing effects of statins on bone morphogenic protein-2 production and *in vivo* studies evaluating the effects of statins on bone formation and preservation. **Results:** Simvastatin was the agent in the majority of reviewed investigations. Most studies supported the hypothesis that simvastatin possessed osteoinductive properties mediated via bone morphogenic protein-2 and osteoprotective properties arising from the inhibition of osteoclast activation. **Conclusions:** Simvastatin shows potential as a therapeutic agent in the prevention and treatment of inflammation-induced bone destruction.

Key words: Statins, osteoblasts, BMP-2, periodontitis, resorption, regeneration

Introduction

Three-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) competitive inhibitors, also known as statins, are widely used agents for lowering cholesterol and reducing the risk for a heart attack. They have been on the market for over 10 years and appear to have relatively good safety profiles (Talbert, 2006). Mevalonate, the product of HGM-CoA reductase enzyme reaction, is the precursor not only of cholesterol, but also of many nonsteroidal isoprenoids vital for diverse cell functions. It appears that the inhibition of this enzyme is responsible for numerous pleiotropic properties of statins. *Table 1* gives a brief summary of these effects. Mevalonate is the precursor of farnesyl-pyrophosphate and geranyl-pyrophosphate, two agents necessary for activation of osteoclasts. By interfering with mevalonate production, statins block one pathway for bone resorption (*Figure 1*). Additionally, the ability of statins to induce bone morphogenic protein-2 (BMP-2), an important stimulator of osteoblastic differentiation, has gained the attention of dental researchers. Bone morphogenic protein-2 has been found to improve fracture healing and repair bony defects in animal and human studies (Bax *et al.*, 1999; Einhorn *et al.*, 2003; Schmidmaier *et al.*, 2002). The stimulatory effect of statins on bone formation was first discovered in animal models of osteoporosis in an attempt to identify new oral anabolic agents (Mundy *et al.*, 1999). The increased bone resorption, and consequently decreased bone quantity and quality, typically found in osteoporosis is also one of the principal characteristics of periodontal disease. As the search for an ideal periodontal therapeutic agent continues, it is only natural to try and translate the results from research in orthopedics into a periodontal model to regenerate osseous tissue. The purpose of this article is to review existing literature on *in vitro* studies investigating BMP-2-promoting activity of statins and *in vivo* studies investigating effects of statins on bone formation and preservation in the oral cavity.

Materials and methods

An electronic search of MEDLINE-PubMed was performed up to and including December 2008 for *in vitro* studies addressing effects of statins on BMP-2 production and *in vivo* studies evaluating the effects of statins on bone formation and preservation.

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Table 1. Pleiotropic properties of statins.

Study/Authors	Mechanism of action	Systemic effect
Schachinger *et al.*, 2000; Halcox *et al.*, 2002; Heeschen *et al.*, 2002; Wolfrum *et al.*, 2003	Modulate endothelium derived nitric oxide, increase expression of tissue-type plasminogen activator	Attenuation of endothelial dysfunction and atherosclerosis progression, prevention of acute coronary syndrome
Wenke *et al.*, 1997; Kwak *et al.*, 2000	Represses MHC classII-mediated T-cell activation	Immunomodulation, immunosuppression
Laufs *et al.*, 1997; Laufs *et al.*, 1999	Blocks hypoxia-mediated down-regulation of nitric oxide synthase.	Alleviation of hypoxia-mediated pulmonary hypertension
Ridker *et al.*, 1999; Ridker *et al.*, 2008	Reduce C-reactive protein	Anti-inflammatory, cardioprotective
Vaughan *et al.*, 2000; Crisby *et al.*, 2001	Suppress secretion of IL-1β and IL-6	Anti-inflammatory
Aikawa *et al.*, 1998; Bellosta *et al.*, 1998	Decrease the secretion of MMPs	Anti-inflammatory
Wolozin *et al.*, 2000; Fassbender *et al.*, 2001	Reduces Aβ polypeptide production	Prevention and treatment of dementia including Alzheimer's disease
Staal *et al.*, 2003; Gonyeau, 2005	Inhibition of HMG-CoA reductase	Inhibition of bone resorption (osteoprotective)
Mundy *et al.*, 1999; Gutierrez *et al.*, 2008; Seto *et al.*, 2008;	Stimulation of osteoblasts differentiation, enhancement of BMP-2 production	Enhanced bone formation (osteoinductive)

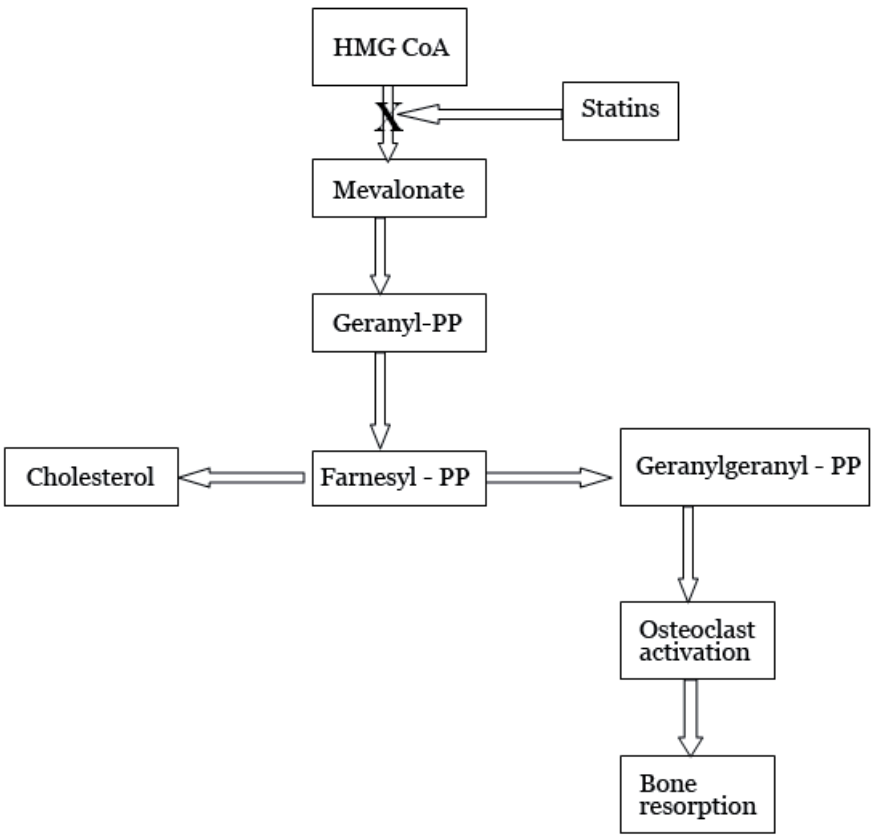

Figure 1. Inhibition of osteoclast activation by statins

In vitro studies

Mundy *et al.* (1999) first showed stimulation of bone formation by statins *in vitro* and also *in vivo* in rodents. Cultured murine (2T3) or human (MG-63) bone cells exposed to statins (simvastatin, fluvastatin, mevastatin and lovastatin) showed enhanced expression of BMP-2 mRNA, as assessed by Northern blot analysis. The effect appeared to be specific. The statins did not alter expression of the BMP-4 promoter. To further investigate the biological effects of statins on bone, the researchers added them to neonatal murine calvarial bones in organ cultures. Lovastatin, simvastatin, fluvastatin and mevastatin each increased new bone formation by approximately two- to three-fold. There was also a striking increase in new bone and in osteoblast cell numbers at all stages of differentiation.

Fluvastatin, simvastatin and pravastatin were the agents of choice for Sonobe *et al.* (2005) in a model utilizing bone marrow-derived mesenchymal cells. If statins stimulated bone formation in bone marrow-derived mesenchymal stem cells they might have beneficial effects in the treatment of bone fractures. However, the results did not support the hypothesis. Fluvastatin, simvastatin and pravastatin did not significantly enhance mineralization, alkaline phosphatase activity (ALP) or osteocalcin. To delineate the mechanism by which statins induced bone formation, Song *et al.* (2003) investigated the effect of simvastatin on osteoblastic and adipocytic differentiation in primary cultured mouse bone marrow stromal cells (BMSCs). Simvastatin treatment enhanced the expression of mRNA for osteocalcin, increased ALP activity and induced high expression of BMP-2 in BMSCs, while inhibiting adipocytic differentiation. Promotion of osteoblastic differentiation in non-transformed osteoblastic cells (MC3T3-E1) and rat bone marrow cells was reported by Maeda *et al.* (2001). Simvastatin enhanced ALP activity and mineralization in a dose- and time-dependent fashion. The results indicated that simvastatin had an anabolic effect on bone through the promotion of osteoblastic differentiation, suggesting that it could be used for the treatment of osteoporosis. Additionally, simvastatin was found to affect in similar manner the ALP activity of cultured rat calvaria cells in experiments by Seto *et al.* (2008). Simvastatin maintained high ALP activity and increased bone nodule formation in rat calvaria cells in a dose-dependent manner. The results indicated that simvastatin is capable of increasing and maintaining a high level of osteoblastic function. Another study on statin-induced osteoblast differentiation in mouse myoblast C2C12 cells addressed the effects of simvastatin on inflammatory mediators (Yamashita *et al.*, 2008). Simvastatin supported BMP-induced osteoblast differentiation via its antagonizing action on the TNF-alpha-to Ras/Rho/MAPK pathway and augmenting BMP-Smad signaling, suggesting a potential usage of this statin to ameliorate inflammatory bone destruction.

Chronic periodontitis is an inflammatory disease. Bone loss in periodontitis is the result of an imbalance between various stimulating and inhibiting cytokines in the inflamed tissues, produced by immune cells as well as by resident cells such as periodontal ligament (PDL) cells and gingival fibroblasts. Yazawa *et al.* (2005) analyzed the effects of simvastatin on cell proliferation and osteoblastic differentiation in PDL cells. ALP activity, osteopontin, BMP-2, osteocalcin and calcium contents were measured. The results suggested that at low concentration (10^{-8} M) simvastatin exhibited a positive effect on proliferation and osteoblastic differentiation of human PDL cells, and that these effects were caused by the inhibition of the mevalonate pathway. Thus, the majority of published studies to date have used simvastatin as a therapeutic agent. Simvastatin is the only statin available in generic form, making it very convenient to purchase for research. Therefore, the conclusions for *in vitro* and *in vivo* studies will be based mainly on research data generated from models utilizing simvastatin.

Conclusions for in vitro studies

Various concentrations of simvastatin were effective in promoting osteoblastic differentiation. This promotion is dose-dependent and time-dependent. In most studies, maximum effect was seen in the 10^{-6} to 10^{-8} M concentration range. The bone-promoting effect was observed in various human and animal bone cell lines (2T3, MG-63, BMSC, MC3T3-E1), as well as in myoblasts and PDL cells, but not in bone marrow-derived mesenchymal stem cells. The bone-sparing effect of simvastatin, and of statins in general, is derived from inhibition of the mevalonate pathway. The pathway is responsible not only for cholesterol synthesis, but also for the formation of compounds needed for osteoclast activation. Statins, by inhibiting the HMG-CoA reductase pathway, act to abate bone resorption.

In vivo studies

Thylin *et al.* (2002) tested if bone stimulation could be induced by two single-dose drug delivery systems appropriate to periodontal therapy. The investigators used five treatment groups: control; methylcellulose gel over the calvarium injection; gel with simvastatin 2.2 mg injection; implanted membrane containing gel only; or implanted membrane containing simvastatin. Both gel with simvastatin and membrane with simvastatin resulted in an increase in bone thickness. The authors concluded that a single, high dose of simvastatin could stimulate murine cranial bone apposition. The murine calvaria model was used also by Nyan *et al.* (2007) to examine whether simvastatin stimulates bone regeneration when combined with calcium sulfate as a carrier.

Eight-millimeter defects were created in rat calvaria and treated with calcium sulfate or a combination of 1 mg simvastatin and calcium sulfate. The combination appeared to promote bone formation in critical size rat calvaria defects after resolution of intense soft tissue inflammation at five weeks. Reducing the simvastatin dose from 2.2 to 0.5 mg reduced inflammation to a more clinically acceptable level without sacrificing bone growth potential, but cyclooxygenase (COX)-associated inflammation appears to be necessary for *in vivo* bone growth (Stein *et al.*, 2005). Topical administration of simvastatin for the recovery of alveolar bone loss in rats was investigated by Seto *et al.* (2008). Alveolar bone resorption in rats was induced by nylon ligature placed around maxillary molars. After ligature removal, simvastatin was topically injected into buccal gingiva for 70 days. The findings demonstrated that simvastatin had the potential to stimulate osteoblastic function and that topical administration of simvastatin might be effective for the recovery of alveolar bone loss in rats. The ligature-induced bone resorption model was applied to ovariectomized rats in order to assess any protective features of simvastatin (Vaziri *et al.*, 2007). Histologic analysis demonstrated that the simvastatin groups developed significantly less periodontal breakdown. It was concluded that simvastatin showed protective features against the effect of periodontitis on the attachment apparatus and alveolar bone.

Modes of preserving the residual ridge after tooth extraction are the focus of intense periodontal research. Wu *et al.* (2008) investigated the effect of simvastatin application on residual ridge resorption following tooth extraction in a rat model. A significantly greater bone mineral density and relative height of the residual alveolar ridge was reported for the experimental group. The findings indicate that local application of simvastatin would effectively preserve the residual alveolar bone by promoting bone formation in the extraction socket. Results reported by Nishimura (2008) regarding local application of simvastatin to the tooth sockets of Wistar rats were not totally corroborative. All groups treated with simvastatin showed higher bone mineral content and thickness of cortical bone; however, the statin group exhibited less bone formation in the tooth socket. The results support the role of simvastatin as a bone preservation agent. More definitive studies need to be performed to evaluate its ability to stimulate new bone formation within a defined environment such as an extraction socket.

The topical injection of simvastatin model was transferred from mouse calvaria to beagle dogs (Morris *et al.*, 2008). Human-sized 3-walled intrabony defects and class II furcation defects were created. Buccal edentulous ridge thickness was 29% greater with simvastatin, but the simvastatin groups had bone height loss in interproximal intrabony and furcation defects. The investigators concluded that multiple injections of simvastatin are not appropriate for the treatment of intrabony or furcation defects. However, this approach shows potential to augment bone thickness in closed alveolar environments. Finally, Ma *et al.* (2008) compared the effect of oral and local administration of simvastatin to a beta tricalcium phosphate-filled defect around an implant to the effect of recombinant human BMP-2 (rhBMP-2). Oral and local simvastatin were ineffective at promoting either ceramic resorption or bone formation due to the inefficient delivery system. Thus, further studies to identify the optimal delivery system are needed.

Conclusions for in vivo studies

Most studies suggest that local application of simvastatin enhances bone formation and helps preserve alveolar bone and may be considered for use as a bone graft agent. These effects were confirmed for various concentrations and various application systems (gel, sponge, polyglycolic/polylactic acid carrier). The type of carrier significantly influences the bioavailability of simvastatin and further trials are needed to elucidate the optimal delivery system. It should be noted that although simvastatin exhibited the ability to enhance bone formation and preserve bone in the calvaria and ligature model, these models are better suited as a means to measure bone loss and not necessarily models for periodontal disease. Clearly, more studies need to be performed that evaluate the therapeutic potential of statins in a well defined, clinical periodontal setting.

Discussion

Growth factors are naturally occurring polypeptides that regulate various aspects of cell growth and differentiation. Those with osteogenic potential have been used alone or in combination with various bone grafts in the treatment of intraoral and extraoral osseous defects. Among all growth factors, BMP-2 has shown immense potential for improving fracture healing and regeneration of osseous defects in various animal and human studies. However, BMP-2 is expensive to synthesize, which makes it less affordable for the majority of patients affected by periodontal disease. A less costly therapeutic approach that could safely stimulate endogenous BMP-2 production would be of enormous clinical and financial benefits. Statins as a group, and particularly simvastatin, have been shown to stimulate BMP-2 transcription, translation and bone formation in intraoral and extraoral defects when applied locally. The osteoinductive effect is augmented by their inhibitory action on the synthesis of mediators necessary for osteoclast activation.

In conclusion, statins show potential as pharmacological agents for bone preservation and periodontal regenerative therapy. Further investigations are needed to determine the ideal carrier system for local delivery, the optimal concentration of each statin for maximum stimulatory effect and the number of applications required. In addition, potential beneficial or adverse effects on other local cell populations present at the surgical site need to be evaluated before a new therapeutic agent emerges.

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