Original Article

Bacteriostatic Effect of Simvastatin on Selected Oral Streptococci *in Vitro*

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Abstract

Context and Objective: Simvastatin is a widely used cholesterol-lowering drug, which has been found to have a number of pleiotropic effects. The aim of this study was to evaluate the antimicrobial effectiveness of simvastatin against selected oral streptococci as determined by the minimum inhibitory concentration (MIC). **Methods:** *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus anginosus*, and *Streptococcus salivarius* were the test microorganisms. The serial dilution method was used to determine the MIC of simvastatin against these organisms. The MIC was defined as the lowest concentration of simvastatin that completely inhibited growth of the test organisms. **Results:** The data indicate that simvastatin inhibits the growth of the test organisms, with MIC's ranging from 7.8 to 15.6 μg/ml. **Conclusions:** Simvastatin has MIC's against the selected bacteria that compare favorably with reported values for topical agents such as essential oil, chlorhexidine gluconate, and triclosan. The levels of simvastatin required to inhibit bacterial growth of oral bacteria exceed the reported levels of the drug found in plasma or crevicular fluid of patients who are treated with this cholesterol-lowering drug. However, clinical studies are warranted to investigate the potential use of simvastatin as a novel antiplaque agent that could be used in local drug delivery to the oral cavity of those patients who are prescribed this cholesterol-lowering drug.

Keywords: *Bacteriostatic, simvastatin, streptococci*

Introduction

Cardiovascular disease (CVD), including heart attack, angina, and stroke, is ranked as the number one cause of mortality worldwide.[1] High blood cholesterol is linked to CVD.[2] Statins, cholesterol-lowering drugs, are first choice drugs for reducing the chance of suffering a CVD event.

A number of unintended side effects of statins have been reported.[3] Although not thought of traditionally as antimicrobials, statins have been shown to have antimicrobial effects.[4] Hence, the aim of this study was to assess the *in vitro* efficacy of simvastatin against selected strains of oral streptococci as determined by the minimum inhibitory concentration (MIC).

Methods

Bacterial strains

Streptococcus mutans (25,175), *Streptococcus anginosus* (33,397), *Streptococcus sanguis* (10,556), and *Streptococcus salivarius* (2593) were purchased from the American Type Culture Collection (Manassas, VA, USA). All streptococci were inoculated in/on brain heart infusion (BHI) broth/agar and grown at 37°C in anaerobic jars in an atmosphere of carbon dioxide. The concentration of log phase cells that were used was between 10^8 and 10^{10} colony-forming unit (CFU)/ml as determined by serial plating.

Preparation of simvastatin

Simvastatin (5 mg, Sigma Chemical Co., St. Louis, Mo., USA) was solubilized in 100% dimethyl sulfoxide (DMSO) resulting in a 12 mM solution, and then diluted 1:2 in eight steps with DMSO to make stock solutions ranging from 6 mM to 24.7 nM.

Determination of minimum inhibitory concentration by broth dilution assay

For bacterial growth studies, 75 μl of each simvastatin/DMSO solution was used. To this, a fixed culture of bacteria (75 μl bacterial suspension, OD 600 nm = 1.5, 10^{10}–10^{12} CFU/ml) and 2.85 ml media were added to obtain a final volume of 3 ml. The final concentration of DMSO in each experimental tube was 2.5%. DMSO alone (75 μl), added to bacterial suspension (75 μl) and media (2.85 ml), was used as control. Growth curves were

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generated for each tube by removing 100 µl samples, adding 900 µl clear media, and measuring turbidity on a spectrophotometer at 600 nm. The MIC was considered to be the lowest concentration of simvastatin that prevented bacterial growth, i.e., a clear test tube.[5] Each clear experimental tube was subsequently subcultured onto agar plates and the plates were incubated for 24 h to determine minimum bactericidal concentration (MBC) of simvastatin. Experiments were repeated three times for each bacterial species.

Growth curve determination of bacteriostatic action of simvastatin

For each strain of bacteria, growth curves were started by adding a fixed culture of bacteria (150 µl bacterial suspension, OD 600 nm = 1.5) to 5.7 ml of BHI media. Growth was monitored by measuring the increase in turbidity on a spectrophotometer (OD = 600 nm). After 3 h, simvastatin was added at its MIC, and turbidity was measured for another 6 h. At the end of this time (9 h. total incubation), the cells were pelleted in sterile Eppendorf tubes, washed twice in sterile isotonic saline, and transferred back into sterile BHI (6 ml). The turbidity was measured for another 15 h (24 h total). Growth curves were generated without simvastatin to serve as control. Experiments were repeated three times for each bacterial species.

Results

Determination of minimum inhibitory concentration by broth dilution assay

Growth curves for *S. anginosus* in the presence of simvastatin are shown in Figure 1. Similar curves were generated for the other streptococci (data not shown). The MIC of simvastatin against the selected oral bacteria was determined to be 37.5 µM (15.6 µg/ml) for *S. mutans* and *S. sanguis* and 18.75 µM (7.8 µg/ml) for *S. anginsous* and *S. salivarius*. However, the minimum bactericidal activity was not determined by subculture since aliquots (100 µl) from clear culture tubes showed bacterial growth when streaked onto agar plates and incubated for 24 h. This measure of antibacterial activity indicates that simvastatin is a bacteriostatic antimicrobial agent against these bacteria.

Growth curve determination of bacteriostatic action of simvastatin

When MIC concentrations of simvastatin were added to growing bacterial cultures, slowed growth rates were observed as compared to control growth curves. However, when the simvastatin-treated bacteria were washed and transferred to growth medium lacking simvastatin, they resumed growth [Figure 2]. This measure of antibacterial activity confirms that simvastatin is a bacteriostatic antimicrobial agent against these strains of bacteria.

Discussion

Statins, cholesterol-lowering drugs, are first choice drugs for reducing the chance of suffering a CVD event. Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase leading to decreased synthesis of endogenous cholesterol.[6] HMG-CoA reductase is a rate-limiting enzyme in the human mevalonate pathway, an important cellular metabolic pathway present in all higher eukaryotes and many bacteria.[7] The bacteria used in this study possess the gene for HMG-CoA reductase.[8] Statins also have a range of cholesterol independent results, including anti-inflammatory functions[9,10] and antimicrobial activity.[11] Statins inhibit several clinical isolates of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, *Escherichia coli*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans in vitro*.[12,13] Likewise, there is some evidence that statins may aid in treating a range of other bacterial infections.[14]

Although the exact mechanism by which simvastatin inhibits the growth of these streptococci is unknown, it is possible that it inhibits bacterial HMG-CoA reductase. There are two distinct classes of HMG-CoA reductase enzymes, the human or eukaryotic Class I enzyme and the prokaryotic Class II enzyme.[15] Crystal structures of a representative of each class of the enzyme have been determined, the Class I human enzyme[7] and the Class II enzyme from *Pseudomonas mevalonii*.[16] The enzymes are not identical, having different crystal structure, and they react differently to statins.[17] The Class II enzymes are not as easily inhibited by statin drugs, requiring a thousand-fold higher concentration of statins than the Class I enzymes.[18]

These experiments demonstrate the *in vitro* bacteriostatic effect of simvastatin on *S. mutans*, *S. anginsosus*, *S. sanguis*, and *S. salivarius*.

Only data for *S. anginosus* were presented in the figures, but similar results were obtained for the other bacteria, all of which are potentially pathogenic. Oral streptococci of the *S. anginosus* group are frequently found in abscesses.[19] *S. mutans*, in addition to being the primary causal agent responsible for dental caries,[20] is commonly found in coronary plaque specimens.[21] Along with *S. mutans*, *S. salivarius* and *S. sanguis* belong to the viridans group streptococci, common etiologic agents of subacute bacterial endocarditis.[22]

Simvastatin has *in vitro* efficacy against the specific strains of bacteria used in this study at concentrations slightly less than the observed MIC's of 15.6–7.8 µg/ml, i.e., slowed growth curves were observed down to approximately 1.0 µg/ml. DMSO, a cryopreservative agent routinely used in microbiology, was used to solubilize simvastatin and had no effect on bacterial growth.[23] The MBC could not be determined because the simvastatin/DMSO combination

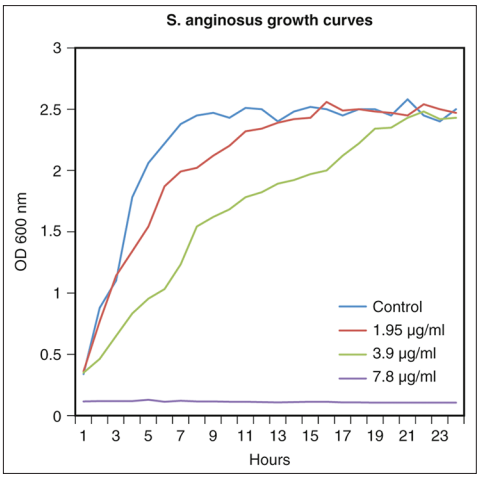

Figure 1: Growth curve of Streptococcus anginosus in the presence of simvastatin: An example of the interference caused by the minimum inhibitory concentration (7.8 µg/ml) and at two concentrations (3.95 and 1.98 µg/ml) below the minimum inhibitory concentration

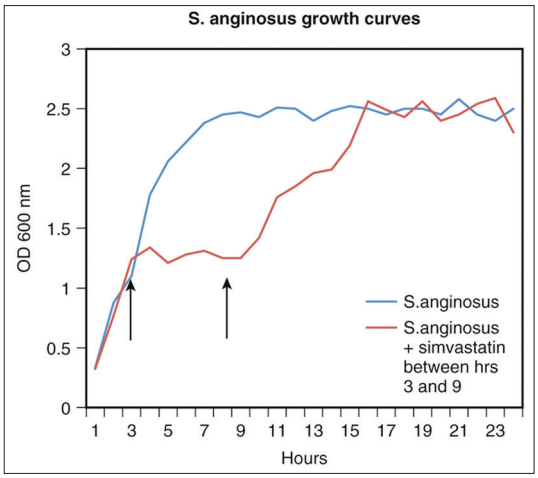

Figure 2: Effect of minimum inhibitory concentration of simvastatin on growth curve of *Streptococcus anginosus*. Simvastatin (minimum inhibitory concentration = 7.8 µg/ml) added to log phase (left arrow) inhibited growth of the bacteria; removal of simvastatin (right arrow) allowed bacteria to resume growth, thus confirming bacteriostatic effect of simvastatin

became insoluble at concentrations higher than 4–5 times the MIC. Routinely, the MBC of bacteriostatic agents is many-fold higher than their MIC.[24] At its MIC, simvastatin prevents the growth of the tested bacteria but does not kill them. As this study was conducted using *in vitro* treatment of planktonic cells, it is not clear whether similar effects would be seen *in vivo* insofar as the bacteria would be contained within a biofilm. Studies are underway to assess the effect of statins on bacteria in a biofilm.

Simvastatin is one of the mainstays of treatment for controlling hyperlipidemia. Other statins, for example, rosuvastatin, pravastatin, lovastatin, fluvastatin, and atorvastatin, are available commercially, each having different pharmacokinetic properties. Only simvastatin was used in this study. Simvastatin is an inactive lactone prodrug which is reversibly converted to a competitive inhibitor of HMG-CoA reductase, simvastatic acid, in the gut wall and other tissues.[25] The bioavailability of simvastatin is <5%, and its half-life is 2–5 h.[26] Thus, for the average adult who has 5 L of blood and ingests one single dose of 60 mg simvastatin, the active metabolite reaches its peak plasma concentration of 0.6 µg/ml several hours later. Thus, these experiments indicate that simvastatin concentrations needed for *in vitro* antimicrobial inhibition (1.95–15.6 µg/ml MIC) slightly exceed the concentration present in human blood or crevicular fluid during statin treatment (0.6 µg/ml). This would imply that there is no relevant antibacterial effect of statins at concentrations attained in plasma or crevicular fluid. However, the results of *in vitro* studies are difficult to translate directly into clinical practice. MIC values are laboratory measures of a fixed concentration of an antibacterial agent being tested against an initially fixed concentration of bacteria that does not necessarily correspond to bacterial densities at site of infection. Clinical studies are warranted. Nevertheless, since the MIC is a measure of the potency of an antibacterial drug, simvastatin is less potent than either penicillin or amoxicillin (MIC 0.03–0.06 µg/ml) against oral streptococci.[27,28]

Statins have the potential to benefit oral health when locally delivered. In a clinical trial, Pradeep and Thorat[29] reported greater increase in clinical attachment and greater decrease in gingival index and probing depth at chronic periodontitis sites treated nonsurgically with scaling and root planing and locally delivered simvastatin, compared to scaling and root planing plus placebo in humans. Likewise, another clinical study utilizing topical application of simvastatin in the treatment of chronic periodontitis, indicated that scaling and root planning in the presence of a simvastatin gel significantly inhibited pro-inflammatory cytokines in crevicular fluid.[30] To date, there are no clinical studies relating simvastatin to reduced dental caries. However, although it has been widely used for its systemic hypolipidemic effect, it has not been considered as a topical antimicrobial agent. It is well known that dental caries is associated with microbial biofilm, of which streptococci are important members in both health and disease.[31] Dentists recommend the regular removal of this film on the teeth as the best treatment for preventing both dental caries and periodontal disease. However, it is appreciated by practicing dentists that most people have difficulty in accomplishing effective oral hygiene. Thus, any agent that adds even temporary stasis of biofilm formation could be a complementary method of plaque control, thus altering the disease process. The practical implications of the present study are that it would be a great advantage, for those prescribed simvastatin, to have this drug simultaneously help both systemically and locally in the oral cavity. This could be accomplished by having statin users chew, swish, and swallow, which is feasible since the drug is suitable to mucous membrane, odorless, tasteless, and does not alter taste perception.[32]

As a bacteriostatic agent, simvastatin may act synergistically with other plaque control agents and thus work in localized adjunctive therapy. It has been used in this way to eradicate *Helicobacter pylori* in patients receiving triple therapy for the treatment of peptic ulcer.[33] Bacteriostatic agents are often as effective as bactericidal agents in the treatment of Gram-positive infections in patients with uncomplicated infections and noncompromised immune systems.[34] Thus, simvastatin may prove to be a good candidate for a therapeutic agent to be used in local drug delivery to target oral bacteria.[35] In this regard, the MIC of simvastatin against oral bacteria compares favorably with essential oil (MIC 512 μg/ml),[36] chlorhexidine gluconate (MIC 1–2 μg/ml),[36] and triclosan (MIC 7.8 μg/ml).[37] These *in vitro* findings add to the existing evidence[38] that simvastatin has potential use as a novel antiplaque agent.

Conclusions

This study demonstrates the *in vitro* antimicrobial effect of simvastatin on streptococci commonly found in the mouth. Simvastatin has efficacy against these specific strains of bacteria at concentrations slightly less than the observed MIC's of 15.6–7.8 μg/ml, which compares favorably with reported values for topical agents such as essential oil, chlorhexidine gluconate, and triclosan. For patients who are prescribed simvastatin, this drug may act synergistically with other plaque control agents and thus work in localized adjunctive therapy if chewed and swished orally before swallowing.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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