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Statin Intake Is Associated with MMP-1 Level in Gingival Crevicular Fluid of Patients with Periodontitis

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Abstract

BACKGROUND—This study was conducted to assess if statin intake is associated with clinical parameters of periodontitis and matrix metalloproteinase (MMP) levels in gingival crevicular fluid (GCF) of nondiabetic and diabetic patients.

METHODS—We first determined the effect of simvastatin on MMP expression in mononuclear cells. We then recruited 117 nondiabetic and diabetic patients, who all had periodontitis and took or did not take statin, and measured periodontal probing depth (PPD) and clinical attachment level (CAL), and collected gingival crevicular fluid (GCF) to quantify MMPs.

RESULTS—The *in vitro* studies showed that simvastatin potently inhibited the expression of MMP-1, -8, and -9 upregulated by lipopolysaccharide (LPS) and high glucose in mononuclear cells. The patient study showed that, after adjusting for age and smoking status, PPD in diabetic patients on statin was significantly less than that in diabetic patients not on statin. MMP-1 level in GCF of nondiabetic and diabetic patients on statin was lower than that of nondiabetic and diabetic patients not on statin, respectively. No difference was found for MMP-8 and -9 levels in GCF.

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These authors contributed equally to the study.

Competing interests

We declare that we have no conflict of interest.

Ethical approval

Approved by the University Institutional Review Board, Medical University of South Carolina, Charleston, South Carolina, USA.

Author contributions

The study was designed by Y Huang and RS Leite. Patient recruitment and clinical work was done by CJ Poston and TC Pierce. Laboratory work was done by Y Li, CW Brinson and Z Lu. Statistical analysis was performed by AW Lauer and Y Huang. Y Huang, CJ Poston, TC Pierce and RS Leite drafted and edited the manuscript. Manuscript was reviewed by CJ Poston, TC Pierce, Y Li, CA White, Z Lu, AW Lauer, RS Leite and Y Huang.

CONCLUSION—Statin intake is associated with reduced PPD in diabetic patients and MMP-1 level in GCF in either nondiabetic or diabetic patients.

Keywords

Statin; Periodontitis; Diabetes mellitus; Matrix metalloproteinase-1

Introduction

Periodontitis is a primarily bacterial infection of the supporting structures of teeth, characterized by tissue inflammation and destruction that eventually lead to tooth loss (Beck et al., 1996, Offenbacher, 1996). Studies have established a crucial role of host inflammatory response to bacteria-derived virulence factors such as lipopolysaccharide (LPS) in the development of periodontitis (Madianos et al., 2005, Taubman et al., 2005). LPS activates Toll-like receptor (TLR)4 in macrophages and other types of cells in periodontal tissue and upregulates the expression and secretion of collagen-degrading matrix metalloproteinases (MMPs) that contribute to periodontal tissue degradation and alveolar bone loss (Gu et al., 2011). To treat periodontitis, in addition to deep cleaning (scaling and root planning) and periodontal surgeries, pharmacological treatment using drugs such as doxycycline that targets MMPs is also a useful approach (Gapski et al., 2009, Gorska & Nedzi-Gora, 2006). In recent years, the potential application of statins as a pharmacological treatment of periodontitis has been assessed by many preclinical and clinical studies (Ting et al., 2015, de Mones et al., 2015).

Statins, the inhibitors of HMG-CoA reductase, are prescribed widely for the treatment of hypercholesterolemia and atherosclerotic cardiovascular diseases (Zhou & Liao, 2009). Besides cholesterol lowering, statins also exert a variety of so-called “pleiotropic” effects including anti-inflammatory activity (Soran & Durrington, 2008, Kostapanos et al., 2008). The statin use is more common in diabetic patients than that in nondiabetic patients since diabetic patients have increased risk of cardiovascular events (Wierzbicki, 2003). Due to the effectiveness of statins in the prevention and treatment of cardiovascular diseases and low risk of statin-associated adverse effects, statins have been taken by significantly increased nondiabetic and diabetic patients in recent years (Mason, 2009, Paraskevas, 2008, Wenger, 2014).

Studies have shown that periodontitis and diabetes have a two-way interaction, i.e., diabetes increases the risk of developing periodontitis and worsens existing periodontitis (Mealey, 1999), and periodontitis confers attributable risk factors to diabetes and cardiovascular disease (Offenbacher et al., 2012). Therefore, it is important to determine the effect of statins on periodontitis in diabetic patients. However, the study reporting the impact of statins on diabetes-associated periodontitis is scarce, although several studies have narrated the beneficial effect of statins on periodontal health in nondiabetic patients (Estanislau et al., 2014, Sangwan et al., 2013, Subramanian et al., 2013).

In this study, we assessed if statin intake is associated with clinical parameters of periodontitis and MMP levels in gingival crevicular fluid (GCF) of both nondiabetic and diabetic patients.

Methods

Cell Culture

Human U937 mononuclear phagocytes (Sundstrom & Nilsson, 1976) were purchased from American Type Culture Collection (Manassas, VA). The cells were cultured in a 5% CO₂ atmosphere in RPMI 1640 medium (GIBCO, Invitrogen Cop. Carlsbad, CA) containing normal glucose (5 mM) or high glucose (25 mM), 10% fetal calf serum, 1% MEM non-essential amino acid solution, and 0.6 g/100 ml of HEPES. Glucose concentrations at 5 and 25 mM have been used commonly as normal and high glucose, respectively, for cell treatment *in vitro* (Nareika et al., 2008, Nareika et al., 2007, Sundararaj et al., 2009). U937 mononuclear cells were seeded at the density of 1×10^5 per well of 12-well plate and cultured with medium containing either normal glucose (5 mM) as control or high glucose (25 mM) for 3 days. After changing the medium, the cells were treated with 100 ng/ml of LPS isolated from *A. actinomycetemcomitans* (strain Y4, serotype B) (Yu et al., 2011), in the absence or presence of 10 μ M of simvastatin for 24 h. After the treatment, culture medium was collected for quantification of MMPs.

RNA Extraction and Reverse Transcription

U937 mononuclear cells cultured in normal glucose (5 mM)-containing medium were treated without or with 100 ng/ml of LPS in the absence or presence of 10 μ M of simvastatin for 24 h. Our previous study has shown that 10 μ M of simvastatin inhibited LPS-stimulated MMP-1 secretion by 80% (Sundararaj et al., 2008). Control cells and cells for each treatment were cultured in duplicate. After the treatment, total RNA was isolated from cells using RNeasy minikit (Qiagen, Santa Clarita, CA). First-strand complementary DNA (cDNA) was synthesized with the iScript™ cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA) using 20 μ l of reaction mixture containing 0.5 μ g of total RNA, 4 μ l of 5x iScript reaction mixture, and 1 μ l of iScript reverse transcriptase. The complete reaction was cycled for 5 minutes at 25°C, 30 minutes at 42°C and 5 minutes at 85°C using a PTC-200 DNA Engine (MJ Research, Waltham, MA).

PCR Arrays

The reverse transcription reaction mixture was then diluted 1:10 with nuclease-free water and used for PCR amplification in the presence of the primers. Duplicate complementary DNA was combined and subjected to PCR array. The human extracellular matrix & adhesion molecules RT² profiler PCR array (SuperArray Bioscience Corp.) was performed using 2X SuperArray RT² qPCR master mix and the first strand cDNA by following the instruction from the manufacturer. All the gene expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a housekeeping gene. To compare the gene expression in cells treated with LPS or/and simvastatin, the difference of cycle threshold (*Ct*) was first calculated by the following formula: $Ct = Ct_{\text{in cells treated with simvastatin or/and LPS}} - Ct_{\text{in control cells}}$. The gene expression in cells treated with LPS or/and simvastatin was presented as the fold of the control gene expression and calculated as 2^{-Ct} .

Patients

All patients, who received periodontal treatments at our University Dental Clinic, were selected by our periodontal faculty and residents based on the inclusion and exclusion criteria. In addition to the statuses of diabetes and statin usage, our inclusion criteria also included male or female patients aged 35–70 and clinical attachment loss (CAL) > 5 mm in 2 or more teeth, which met the diagnostic criteria for chronic periodontitis according to the classification of 1999 (Flemmig, 1999). The exclusion criteria included serum creatinine 1.6 mg/dl, abnormal hepatic function, hemoglobinopathy, unwillingness to sign the informed consent form or enter the study, aggressive periodontitis, platelet and coagulation disorders. One hundred seventeen patients with periodontitis who met the inclusion and exclusion criteria were recruited for this study, including 53 nondiabetic patients not taking statin (Group 1), 28 nondiabetic patients taking statin (Group 2), 11 diabetic patients not taking statin (Group 3), and 25 diabetic patients taking statin (Group 4). All patients had two clinical visits for the study. During the first visit, the patients received a comprehensive periodontal evaluation including periodontal probing depth (PPD) and CAL measurements using the cement-enamel junction (CEJ) as a reference point as described previously (Salvi et al., 1997). The teeth with periodontal disease were identified and GCF was collected from the deepest pockets (at least 6 mm) prior to non-surgical periodontal therapy. During the second visit scheduled 6 weeks after the first visit, GCF was collected again from the same sites. All diabetic patients were diagnosed with type 2 diabetes prior to the first visit. All patients had hemoglobin A1c (HbA1c) test during the first visit and also provide information about statin treatment including duration, dose, name of statin drugs. All patients provided informed consent for the GCF collection. The study protocol and consent form were approved by the University Institutional Review Board (approval number: 20244; approval date: July 6, 2010) and the study was performed in accordance with the Declaration of Helsinki.

GCF Sampling

The teeth with periodontitis were identified before GCF sampling. Two sites per patient with maximum PPD and CAL were selected as the sampling sites. For GCF sampling, selected sites for GCF collection were cleaned with cotton rolls and a saliva ejector. The teeth surfaces were dried gently with air spray. A Periopaper strip (Oraflow Inc, Smithtown, NY) was inserted into the gingival sulcus until slight resistance was encountered. The strip was left the gingival sulcus for 1 minute. For each patient, the same sites were sampled at the first and second visit. During GCF collection, samples contaminated with blood and/or saliva were excluded from the study. The strips were placed in Eppendorf tubes and stored in –80°C. On the day of the assays, 300 µl of PBS (pH 7.2) was added to the tubes containing the strips. The strips were gently shaken for 1 min and then centrifuged at 2000 *g* for 5 min to elute GCF proteins. After strip removal, the supernatant was subjected to quantification of MMPs.

Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA kits to quantify MMP-1 in culture medium and MMP-1, MMP-8, and MMP-9 in GCF were purchased from R&D System (Minneapolis, MN). The assays were performed

according to the protocol provided by the manufacturers. According to the manufacturer, the detection range for MMP-1 kit is from 0.2 to 10 ng/mL and the sensitivity is 0.095 ng/mL; the detection range for MMP-8 kit is from 0.2 to 10 ng/mL and the sensitivity is 0.06 ng/mL; the detection range for MMP-9 kit is from 0.3 to 20 ng/mL and the sensitivity is 0.156 ng/mL.

Statistical Analysis

Differences in demographics were assessed using Fisher's Exact test and Wilcoxon Signed Rank tests. A multivariate regression model was fitted for each continuous outcome and adjusting for confounder variables such as age and smoking status. A random intercept term was used in the model to account for repeated measures for CAL and PPD. Pairwise comparisons were made using least squares means. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Results

The Effect of Simvastatin on the Expression of MMPs and TIMPs in Mononuclear Phagocytes

We first used PCR array to profile the expression of MMPs and tissue inhibitors of metalloproteinase (TIMPs) in U937 mononuclear cells in response to LPS and/or simvastatin treatment. Results in Table 1 showed that while LPS stimulated the expression of MMP-1, -8 and -9 by 43.08-, 10.48-, 46.96-fold, respectively, simvastatin potently inhibited the stimulatory effect of LPS on MMP-1, -8 and -9 expression by 81%, 77% and 81%, respectively. Simvastatin also inhibited the expression of MMP-10 and -13, but the LPS-stimulated expression of MMP-10 and -13 was much lower than LPS-stimulated MMP-1 expression (about 0.1% and 0.4%, respectively, of MMP-1) according to the difference of cycle threshold (*C_t*) between MMP-10 or -13 and MMP-1. Our PCR array also showed that LPS did not stimulate MMP-2, -7, -11 and -15, as well as TIMP-1 and -2. Although LPS increased MMP-14 expression by 2.52-fold, simvastatin did not inhibit it.

Simvastatin Is A Potent Inhibitor of MMP-1 Secretion from Mononuclear Cells in Vitro

To confirm the above findings from PCR array, we treated U937 mononuclear phagocytes with LPS in the absence or presence of simvastatin for 24 h and then quantified MMP-1 protein released into culture medium. To further determine the impact of hyperglycemia on MMP-1 expression, we also cultured the cells in high glucose-containing medium (25 mM). Results showed that LPS remarkably stimulated MMP-1 secretion and high glucose further increased LPS-stimulated MMP-1 secretion by 2 folds, but simvastatin inhibited the stimulatory effect of LPS on MMP-1 secretion by cells exposed to normal glucose (5 mM)- or high glucose (25 mM)-cultured cells by 88% (Fig. 1), which is similar to the results from the PCR array regarding the inhibitory effect of simvastatin on LPS-stimulated MMP-1 mRNA expression.

Patient Study

We then conducted a patient study to validate our *in vitro* finding that statin inhibits the expression of MMP-1, MMP-8 and MMP-9 in mononuclear phagocytes. We recruited 117

patients for 4 groups as described in Materials and Methods, and quantified MMP-1, MMP-8 and MMP-9 in GCF of these patients. The demographic data were presented in Table 2.

Age—The ages of subjects in Groups 1, 2, 3 and 4 ranged from 38 to 67, 48 to 72, 48 to 68, and 45 to 68 with a mean $\pm$ standard deviation of 52.6 ± 7.8 , 61.4 ± 6.8 , 52.5 ± 9.8 , and 59.8 ± 6.1 , respectively. A statistically significant difference of average age was found between Groups 1 and 2 and between Groups 3 and 4, indicating that patients on statin were older than those not on it.

Smoking status—The smokers in Groups 1, 2, 3 and 4 were 21 (39.6%), 3 (10.7%), 1 (9.1%), and 6 (24%), respectively. A statistically significant difference was found between Group 1 and 2 and between Group 1 and 3.

Gender—No significant difference between patients on statin and those not on statin was found.

Race—No significant difference between patients on statin and those not on statin was found.

Diabetes—The fasting blood HbA1c values in Groups 1, 2, 3 and 4 were 5.73, 6.00, 7.10 and 7.64, respectively (Table 3). According to American Diabetes Association, HbA1c at 6.5% or higher is considered to be diabetic. Therefore, the HbA1c data are consistent with the patients' diabetic status: Groups 1 and 2 were nondiabetic and Groups 3 and 4 were diabetic. Among diabetic patients, 24 patients took Glucophage (metformin), 4 took Glucotrol (glipizide) and the rest of the patients took Actos (pioglitazone) or Humulin (insulin).

Statins—Among patients on statin treatment, 22 took Simvastatin, 13 took Atrovastatin, 12 took Pravastatin, 5 took Rosuvastatin and 1 took Lovastatin. The average number of years for statin intake was 7 years ranging from 6 months to 22 years.

The Relationship between Statin Intake and Clinical Parameters for Periodontitis

As shown in Table 4, CAL in nondiabetic patients taking statin (Group 2) is significantly less than that in nondiabetic patients not taking statin (Group 1) and PPD in diabetic patients taking statin (Group 4) is significantly less than that in diabetic patients not taking statin (Group 3). After adjusting for age and smokers, the difference of PPD between Groups 3 and 4 remained statistically significant.

The Relationship between Statin Intake and MMP Levels in GCF

To determine if statin intake is associated with the levels of MMPs in GCF, we quantified MMP-1, MMP-8, and MMP-9 proteins in GCF. As shown in Table 5, MMP-1 level in GCF of diabetic subjects (Group 3) during the first visit was significantly higher than that in GCF of nondiabetic subjects (Group 1), indicating that diabetes is associated with an increased MMP-1 level in patients not on statin. Furthermore, results showed that MMP-1 level in

nondiabetic patients on statin (Group 2) was lower than that of nondiabetic patients not on statin (Group 1) during the first visit. Similarly, MMP-1 level in diabetic patients on statin (Groups 4) was lower than that of diabetic patients not on statin (Group 3) during both visits. These findings indicate that statin intake is associated with a reduced MMP-1 level in both nondiabetic and diabetic patients. After adjusting for age and smoking status, the differences of MMP-1 between Groups 1 and 2 and between Groups 3 and 4 are still statistically significant. In contrast, statin intake was not associated with the levels of MMP-8 and MMP-9 in both nondiabetic and diabetic patients.

Discussion

In this study, we first demonstrated the inhibitory effect of simvastatin on MMP expression upregulated by LPS and high glucose in mononuclear cells *in vitro*. We then conducted a clinical study to validate our *in vitro* findings in patients who took statins and demonstrated that statin intake is associated with a reduced MMP-1 level in GCF of nondiabetic and diabetic patients with periodontitis. The findings from this clinical study are consistent with the results from our animal studies showing that simvastatin inhibited LPS-induced periodontal inflammation and alveolar bone loss in Sprague-Dawley rats (Jin et al., 2014b) and Zucker fat rats (Jin et al., 2014a). While the current study is in line with the clinical studies by Sangwan et al. (Sangwan et al., 2013) and Lindy et al. (Lindy et al., 2008) on nondiabetic patients, it reported for the first time that statin intake is also associated with improved clinical parameter of periodontitis in diabetic patients.

To elucidate the underlying mechanisms involved in statin-associated improvement of periodontitis, we focused on MMPs in GCF since it is well known that MMPs play a crucial role in periodontal tissue degradation. Interestingly, among the assayed MMPs, MMP-1 was the only one found to be associated with diabetes and statin intake. Results showed that MMP-1 level in GCF of diabetic patients not on statin was higher than that of nondiabetic patients not on statin, indicating that diabetes is associated with an increase in MMP-1 in GCF. This finding is consistent with our *in vitro* study showing that high glucose increased LPS-stimulated MMP-1 secretion from mononuclear cells by 2-fold (Fig. 1). Furthermore, results also showed that MMP-1 level in patients on statin was lower than that in patients not on statin, indicating that statin intake is associated with a reduced MMP-1 level in GCF from both nondiabetic and diabetic patients.

MMP-1 is considered the prototype for all the interstitial collagenases and plays a key role in both physiological and pathological tissue remodeling (Pardo & Selman, 2005, Vincenti & Brinckerhoff, 2002). MMP-1 has been implicated in periodontitis as recent studies showed that patients with periodontitis had a substantial elevation of MMP-1 in GCF as compared to periodontally healthy subjects (R et al., 2014) and treatment of periodontitis with scaling and root planning was associated with a reduced MMP-1 in GCF (Ghodpage et al., 2014). Furthermore, the importance of MMP-1 in periodontitis was underscored by a recent meta-analysis on the association between MMP-1 polymorphism and periodontitis susceptibility (Li et al., 2013). This analysis included 11 case-control studies with 1,580 patients with periodontitis and 1,386 control subjects, and showed a significant association between a single nuclear polymorphism of MMP-1 and periodontitis susceptibility.

In our previous studies, we have investigated the signaling and molecular mechanisms involved in the synergistic effect of LPS and high glucose on MMP-1 upregulation in mononuclear cells and gingival fibroblasts (Li et al., 2010, Lu et al., 2013, Maldonado et al., 2004, Nareika et al., 2009). We found that the extracellular signal-regulated kinase (ERK) pathway and transcription factor AP-1 plays a key role in the upregulation. Furthermore, we have also investigated the signaling and molecular mechanisms involved in the inhibition by simvastatin of MMP-1 expression in mononuclear cells. We found that simvastatin potentially inhibited MMP-1 upregulation by LPS and high glucose by targeting ERK pathway and subsequent AP-1 transcriptional activity, which is crucial for MMP-1 expression (Sundararaj et al., 2008).

In our current study, it is surprising to find that statin intake was not associated with MMP-8 and -9 levels in GCF of patients with periodontitis. Although the causes remain unknown and require further investigation, we proposed two possible mechanisms. First, it is known that multiple signaling pathways including ERK, c-Jun N-terminal kinase, p38 mitogen-activated protein kinase, protein kinase C, and nuclear factor kappa B cascades are involved in the regulation of MMP-8 and -9 expression (Chakraborti et al., 2003, Ito & Ikeda, 2003), but statin specifically targeting ERK pathway by inhibiting protein isoprenylation (Sundararaj et al., 2008). Therefore, if regulatory pathways other than ERK pathway are activated by periodontal pathogens and play a major role in the upregulation of MMP-8 and -9 expression, it is likely that no relationship between statin intake and the expression of MMP-8 and -9 could be observed. Second, studies have shown that GCF contains several proteinases such as cathepsin B and tryptase, and proteins in GCF are subjected to the degradation by proteinases (Gazi et al., 1996, McCrudden et al., 2013). The amount of a particular protein evading protease degradation in GCF depends on the sensitivity of this protein to the proteinases. If MMP-8 and -9 are more sensitive to the proteases than MMP-1, it is likely that no relationship between statin intake and the expression of MMP-8 and -9 could be observed.

In conclusion, this study demonstrated that statin intake was associated with a reduced MMP-1 level in GCF in both nondiabetic and diabetic patients. The findings from the current study suggest that statins may have beneficial effects on periodontitis in both nondiabetic and diabetic patients.

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References

- Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. *J Periodontol.* 1996; 67:1123–1137. [PubMed: 8910831]
- Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem.* 2003; 253:269–285. [PubMed: 14619979]

- de Mones E, Schlaubitz S, Catros S, Fricain JC. Statins and alveolar bone resorption: a narrative review of preclinical and clinical studies. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2015; 119:65–73. [PubMed: 25482549]
- Estanislau IM, Terceiro IR, Lisboa MR, Teles PD, Carvalho RD, Martins RS, Moreira MM. Pleiotropic effects of statins on the treatment of chronic periodontitis - a systematic review. *Br J Clin Pharmacol.* 2014; 79:877–885. [PubMed: 25444240]
- Flemmig TF. Periodontitis. *Ann Periodontol.* 1999; 4:32–38. [PubMed: 10863373]
- Gapski R, Hasturk H, Van Dyke TE, Oringer RJ, Wang S, Braun TM, Giannobile WV. Systemic MMP inhibition for periodontal wound repair: results of a multi-centre randomized-controlled clinical trial. *J Clin Periodontol.* 2009; 36:149–156. [PubMed: 19207891]
- Gazi MI, Cox SW, Clark DT, Eley BM. A comparison of cysteine and serine proteinases in human gingival crevicular fluid with tissue, saliva and bacterial enzymes by analytical isoelectric focusing. *Arch Oral Biol.* 1996; 41:393–400. [PubMed: 8809301]
- Ghodpage PS, Kolte RA, Kolte AP, Gupta M. Influence of phase I periodontal therapy on levels of matrix metalloproteinase 1 and tissue inhibitor of metalloproteinase 1. *Saudi Dent J.* 2014; 26:171–175. [PubMed: 25382950]
- Gorska R, Nedzi-Gora M. The effects of the initial treatment phase and of adjunctive low-dose doxycycline therapy on clinical parameters and MMP-8, MMP-9, and TIMP-1 levels in the saliva and peripheral blood of patients with chronic periodontitis. *Arch Immunol Ther Exp (Warsz).* 2006; 54:419–426. [PubMed: 17122880]
- Gu Y, Lee HM, Sorsa T, Salminen A, Ryan ME, Slepian MJ, Golub LM. Non-antibacterial tetracyclines modulate mediators of periodontitis and atherosclerotic cardiovascular disease: a mechanistic link between local and systemic inflammation. *Pharmacol Res.* 2011; 64:573–579. [PubMed: 21771657]
- Ito T, Ikeda U. Inflammatory cytokines and cardiovascular disease. *Curr Drug Targets Inflamm Allergy.* 2003; 2:257–265. [PubMed: 14561160]
- Jin J, Machado ER, Yu H, Zhang X, Lu Z, Li Y, Lopes-Virella MF, Kirkwood KL, Huang Y. Simvastatin inhibits LPS-induced alveolar bone loss during metabolic syndrome. *J Dent Res.* 2014a; 93:294–299. [PubMed: 24352501]
- Jin J, Zhang X, Lu Z, Li Y, Lopes-Virella MF, Yu H, Haycraft CJ, Li Q, Kirkwood KL, Huang Y. Simvastatin inhibits lipopolysaccharide-induced osteoclastogenesis and reduces alveolar bone loss in experimental periodontal disease. *J Periodontal Res.* 2014b; 49:518–526. [PubMed: 24117880]
- Kostapanos MS, Milionis HJ, Elisaf MS. An overview of the extra-lipid effects of rosuvastatin. *J Cardiovasc Pharmacol Ther.* 2008; 13:157–174. [PubMed: 18460672]
- Li D, Cai Q, Ma L, Wang M, Ma J, Zhang W, Pan Y, Wang L. Association between MMP-1 g.-1607dupG polymorphism and periodontitis susceptibility: a meta-analysis. *PloS one.* 2013; 8:e59513. [PubMed: 23527212]
- Li Y, Samuvel DJ, Sundararaj KP, Lopes-Virella MF, Huang Y. IL-6 and high glucose synergistically upregulate MMP-1 expression by U937 mononuclear phagocytes via ERK1/2 and JNK pathways and c-Jun. *J Cell Biochem.* 2010; 110:248–259. [PubMed: 20225236]
- Lindy O, Suomalainen K, Makela M, Lindy S. Statin use is associated with fewer periodontal lesions: A retrospective study. *BMC oral health.* 2008; 8:16. [PubMed: 18482446]
- Lu Z, Li Y, Samuvel DJ, Jin J, Zhang X, Lopes-Virella MF, Huang Y. MD-2 is involved in the stimulation of matrix metalloproteinase-1 expression by interferon-gamma and high glucose in mononuclear cells - a potential role of MD-2 in Toll-like receptor 4-independent signalling. *Immunology.* 2013; 140:301–313. [PubMed: 23800176]
- Madianos PN, Bobetsis YA, Kinane DF. Generation of inflammatory stimuli: how bacteria set up inflammatory responses in the gingiva. *J Clin Periodontol.* 2005; 32(Suppl 6):57–71. [PubMed: 16128830]
- Maldonado A, He L, Game BA, Nareika A, Sanders JJ, London SD, Lopes-Virella MF, Huang Y. Pre-exposure to high glucose augments lipopolysaccharide-stimulated matrix metalloproteinase-1 expression by human U937 histiocytes. *J Periodontal Res.* 2004; 39:415–423. [PubMed: 15491346]

- Mason CM. Preventing coronary heart disease and stroke with aggressive statin therapy in older adults using a team management model. *J Am Acad Nurse Pract.* 2009; 21:47–53. [PubMed: 19125895]
- McCrudden MT, Orr DF, Yu Y, Coulter WA, Manning G, Irwin CR, Lundy FT. LL-37 in periodontal health and disease and its susceptibility to degradation by proteinases present in gingival crevicular fluid. *J Clin Periodontol.* 2013; 40:933–941. [PubMed: 23952216]
- Mealey B. Diabetes and periodontal diseases. *J Periodontol.* 1999; 70:935–949. [PubMed: 10476904]
- Nareika A, Im YB, Game BA, Slate EH, Sanders JJ, London SD, Lopes-Virella MF, Huang Y. High glucose enhances lipopolysaccharide-stimulated CD14 expression in U937 mononuclear cells by increasing nuclear factor kappaB and AP-1 activities. *J Endocrinol.* 2008; 196:45–55. [PubMed: 18180316]
- Nareika A, Maldonado A, He L, Game BA, Slate EH, Sanders JJ, London SD, Lopes-Virella MF, Huang Y. High glucose-boosted inflammatory responses to lipopolysaccharide are suppressed by statin. *J Periodontal Res.* 2007; 42:31–38. [PubMed: 17214637]
- Nareika A, Sundararaj KP, Im YB, Game BA, Lopes-Virella MF, Huang Y. High glucose and interferon gamma synergistically stimulate MMP-1 expression in U937 macrophages by increasing transcription factor STAT1 activity. *Atherosclerosis.* 2009; 202:363–371. [PubMed: 18586252]
- Offenbacher S. Periodontal diseases: pathogenesis. *Ann Periodontol.* 1996; 1:821–878. [PubMed: 9118282]
- Offenbacher S, Barros SP, Altarawneh S, Beck JD, Loewy ZG. Impact of tooth loss on oral and systemic health. *Gen Dent.* 2012; 60:494–500. [PubMed: 23220304]
- Paraskevas KI. Statin treatment for rheumatoid arthritis: a promising novel indication. *Clin Rheumatol.* 2008; 27:281–287. [PubMed: 18066611]
- Pardo A, Selman M. MMP-1: the elder of the family. *Int J Biochem Cell Biol.* 2005; 37:283–288. [PubMed: 15474975]
- R PP, Bhavsar NV, Popat PR. Gingival crevicular fluid levels of Matrix Metalloproteinase-1 (MMP-1) and Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) in periodontal health and disease. *Singapore Dent J.* 2014; 35:59–64. [PubMed: 25496587]
- Salvi GE, Yalda B, Collins JG, Jones BH, Smith FW, Arnold RR, Offenbacher S. Inflammatory mediator response as a potential risk marker for periodontal diseases in insulin-dependent diabetes mellitus patients. *J Periodontol.* 1997; 68:127–135. [PubMed: 9058329]
- Sangwan A, Tewari S, Singh H, Sharma RK, Narula SC. Periodontal status and hyperlipidemia: statin users versus non-users. *J Periodontol.* 2013; 84:3–12. [PubMed: 22468682]
- Soran H, Durrington P. Rosuvastatin: efficacy, safety and clinical effectiveness. *Expert Opin Pharmacother.* 2008; 9:2145–2160. [PubMed: 18671469]
- Subramanian S, Emami H, Vucic E, Singh P, Vijayakumar J, Fifer KM, Alon A, Shankar SS, Farkouh M, Rudd JH, Fayad ZA, Van Dyke TE, Tawakol A. High-dose atorvastatin reduces periodontal inflammation: a novel pleiotropic effect of statins. *J Am Coll Cardiol.* 2013; 62:2382–2391. [PubMed: 24070911]
- Sundararaj KP, Samuvel DJ, Li Y, Nareika A, Slate EH, Sanders JJ, Lopes-Virella MF, Huang Y. Simvastatin suppresses LPS-induced MMP-1 expression in U937 mononuclear cells by inhibiting protein isoprenylation-mediated ERK activation. *J Leukoc Biol.* 2008; 84:1120–1129. [PubMed: 18625914]
- Sundararaj KP, Samuvel DJ, Li Y, Sanders JJ, Lopes-Virella MF, Huang Y. Interleukin-6 released from fibroblasts is essential for up-regulation of matrix metalloproteinase-1 expression by U937 macrophages in coculture: cross-talking between fibroblasts and U937 macrophages exposed to high glucose. *J Biol Chem.* 2009; 284:13714–13724. [PubMed: 19307187]
- Sundstrom C, Nilsson K. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). *Int J Cancer.* 1976; 17:565–577. [PubMed: 178611]
- Taubman MA, Valverde P, Han X, Kawai T. Immune response: the key to bone resorption in periodontal disease. *J Periodontol.* 2005; 76:2033–2041. [PubMed: 16277573]
- Ting M, Whitaker EJ, Albandar JM. Systematic review of the in vitro effects of statins on oral and perioral microorganisms. *Eur J Oral Sci.* 2015; 124:4–10. [PubMed: 26718458]

- Vincenti MP, Brinckerhoff CE. Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Res.* 2002; 4:157–164. [PubMed: 12010565]
- Wenger NK. Prevention of cardiovascular disease: highlights for the clinician of the 2013 American College of Cardiology/American Heart Association guidelines. *Clin Cardiol.* 2014; 37:239–251. [PubMed: 24633723]
- Wierzbicki AS. Statin therapy in people with diabetes and high-risk patients. *Hosp Med.* 2003; 64:16–19. [PubMed: 12572329]
- Yu H, Li Q, Herbert B, Zinna R, Martin K, Junior CR, Kirkwood KL. Anti-inflammatory effect of MAPK phosphatase-1 local gene transfer in inflammatory bone loss. *Gene Ther.* 2011; 18:344–353. [PubMed: 21068780]
- Zhou Q, Liao JK. Statins and cardiovascular diseases: from cholesterol lowering to pleiotropy. *Curr Pharm Des.* 2009; 15:467–478. [PubMed: 19199975]

Figure 1.

The effect of simvastatin on MMP-1 secretion by U937 mononuclear cells stimulated by high glucose and LPS. Human U937 mononuclear cells cultured with medium containing either normal glucose (5 mM) as control or high glucose (25 mM) for 3 days and then treated with 100 ng/ml of LPS in the absence or presence of 50 μ M of simvastatin (statin) for 24 h. After the treatment, culture medium was collected for quantification of MMP-1 using ELISA as described in Methods. The data presented are mean $\pm$ SD of one of three independent experiments with similar results. ** p <0.01 vs *; + p <0.01 vs *; ++ p <0.01 vs +; ## p <0.01 vs #.

Table 1

The Effect of Simvastatin on the Expression of MMP and TIMP Genes in Cells Treated with LPS

MMP genes	Cycle threshold (<i>Ct</i>)				Fold changes as compared to the control cells			Inhibition of LPS effect by simvastatin (% of LPS-increased expression)
	Control cells	Simvastatin-treated cells	LPS-treated cells	LPS and simvastatin-treated cells	Simvastatin-treated cells	LPS-treated cells	LPS and simvastatin-treated cells	
<i>MMP1</i>	29.00	31.57	23.57	25.82	0.17	43.08	9.05	81%
<i>MMP8</i>	30.03	31.32	26.64	28.35	0.41	10.48	3.20	77%
<i>MMP9</i>	29.84	30.41	24.29	26.54	0.67	46.96	9.82	81%
<i>MMP10</i>	36.59	34.69	33.32	33.87	3.72	9.59	6.58	35%
<i>MMP13</i>	36.10	34.45	31.72	34.08	3.14	20.88	4.06	85%
<i>MMP2</i>	25.79	25.43	25.22	24.98	1.28	1.48	1.75	-
<i>MMP7</i>	35.50	35.49	35.97	36.16	1.01	0.72	0.63	-
<i>MMP11</i>	34.53	33.92	33.95	34.22	1.52	1.50	1.24	-
<i>MMP14</i>	33.63	33.29	32.29	32.30	1.26	2.52	2.50	-
<i>MMP15</i>	33.51	32.23	33.29	32.06	2.42	1.17	2.74	-
<i>TIMP1</i>	22.39	21.75	21.72	21.56	1.56	1.59	1.77	-
<i>TIMP2</i>	25.80	25.39	25.94	25.31	1.33	0.91	1.41	-

U937 mononuclear cells cultured in normal glucose (5 mM)-containing medium were treated without or with 100 ng/ml of LPS in the absence or presence of 10 μ M of simvastatin for 24 h. Control cells and cells for each treatment were cultured in duplicate. After the treatment, RNA isolated from cells in the duplicates was combined and subjected to PCR array study as described in Methods. All the gene expression was normalized to GAPDH, a housekeeping gene. To compare the gene expression in cells treated with LPS or/and simvastatin, Ct was first calculated by the following formula: $Ct = Ct$ in cells treated with simvastatin or/and LPS - Ct in control cells. The gene expression in cells treated with LPS or/and simvastatin was presented as the fold of the control gene expression and calculated as $2^{-(Ct)}$.

Table 2

Demographic Data for the Study Population

	Group 1: Non-diabetic, non-statin user	Group 2: Non-diabetic, statin user	Group 3: Diabetic, non-statin user	Group 4: Diabetic, statin user
Total	53	28	11	25
Age	52.6 ± 7.8 ^a	61.4 ± 6.8 ^b	52.5 ± 9.8 ^c	59.8 ± 6.1 ^d
Smoker	21 (39.6%) ^e	3 (10.7%) ^f	1 (9.1%) ^g	6 (24%)
Gender				
Male	24 (45.3%)	19 (67.9%)	6 (54.6%)	18 (72%)
Female	29 (54.7%)	9 (32.1%)	5 (45.5%)	7 (28%)
Race/Ethnicity				
White (Non-Hispanic)	41 (77.4%)	21 (75%)	5 (45.5%)	11 (44%)
Black (Non-Hispanic)	12 (22.7%)	6 (21.4%)	6 (54.6%)	14 (56%)
Hispanic	0 (0%)	1 (3.6%)	0 (0%)	0 (0%)

The age was presented as mean ± SD. Other parameters were presented as number (percentile).

p<0.05, a vs. b; *p*<0.05, c vs. d; *p*<0.05, e vs. f; *p*<0.05, e vs. g.

Table 3

The Blood HbA1c Levels of Patients in the Study Population

	Group 1: Non-diabetic, non-statin user	Group 2: Non-diabetic, statin user	Group 3: Diabetic, non-statin user	Group 4: Diabetic, statin user
HbA1c (%)	5.73 ± 0.87 ^a	6.00 ± 0.52 ^a	7.10 ± 0.47 ^b	7.64 ± 1.53 ^b

Data presented are mean ± SD. $p < 0.0001$, a vs. b.

Table 4

The Periodontal Probing Depth (PPD) and Clinical Attachment Loss (CAL) in Patients with or without Statin Treatment

	Group 1: Non-diabetic, non-statin user	Group 2: Non-diabetic, statin user	Group 3: Diabetic, non-statin user	Group 4: Diabetic, statin user
PPD (mm)	6.90 ± 0.83	6.73 ± 0.80	7.22 ± 0.61 ^a	6.82 ± 0.72 ^b
CAL (mm)	7.45 ± 1.11 ^c	6.77 ± 1.11 ^d	7.41 ± 1.01	7.52 ± 1.33

Data presented are mean ± SD. $p = 0.0238$, a vs. b; $p = 0.0116$, c vs. d.

Table 5

Levels of MMPs in GCF Collected from Nondiabetic and Diabetic Patients with or without Statin Intake

		Group 1: Non-diabetic, non-statin user	Group 2: Non-diabetic, statin user	Group 3: Diabetic, non-statin user	Group 4: Diabetic, statin user
MMP-1 (pg/ml)	Visit 1	103 (38, 2535) ^a	66 (38, 1742) ^b	347 (310, 768) ^c	260 (190, 344) ^d
	Visit 2	102 (43, 2244)	80 (41, 515)	336 (315, 355) ^e	254 (189, 376) ^f
MMP-8 (ng/ml)	Visit 1	74 (2, 135) ^g	42 (9, 229)	15 (3, 105) ^h	33 (1, 139)
	Visit 2	93 (1, 148) ⁱ	47 (3, 14)	16 (5, 22) ^j	20 (3, 17)
MMP-9 (ng/ml)	Visit 1	135 (9, 416)	192 (21, 470)	196 (6, 415)	259 (2, 507)
	Visit 2	33 (7, 483)	108 (11, 631)	128 (13, 240)	212 (31, 480)

Data presented are medians (minimum, maximum).

$p=0.0023$, a vs. b; $p<0.0001$, c vs. d; $p=0.0003$, e vs. f; $p=0.0098$, g vs. h; $p=0.0023$, i vs. j.