Periodontal Infection Is Associated With Endothelial Dysfunction in Healthy Subjects and Hypertensive Patients

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Abstract—The purpose of this study was to evaluate endothelial function in patients with periodontitis. We evaluated forearm blood flow responses to acetylcholine and sodium nitroprusside in patients with periodontitis who had no other cardiovascular risk factors (32 men; 25±3 years of age), in a normal control group (20 men; 26±3 years of age), and in hypertensive patients with periodontitis (28 men and 10 women; 56±12 years of age) and without periodontitis (control group; 18 men and 6 women; 54±13 years of age). Forearm blood flow was measured using strain-gauge plethysmography. Circulating levels of C-reactive protein and interleukin-6 were significantly higher in the periodontitis group than in the control group. Both in healthy and hypertensive subjects, forearm blood flow responses to acetylcholine were significantly smaller in the periodontitis group than in the control group. Sodium nitroprusside–stimulated vasodilation was similar in the 2 groups. Periodontal therapy reduced serum concentrations of C-reactive protein and interleukin-6 and augmented acetylcholine-induced vasodilation in periodontitis patients with and without hypertension. After administration of N\(^4\)-monomethyl-L-arginine, an NO synthase inhibitor, forearm blood flow response to acetylcholine was similar before and after treatment. These findings suggest that periodontitis is associated with endothelial dysfunction in subjects without cardiovascular risk factors, as well as hypertensive patients, through a decrease in NO bioavailability and that systemic inflammation may be, at least in part, a cause of endothelial dysfunction, leading to cardiovascular diseases. (Hypertension. 2008;51[part 2]:446-453.)

Key Words: periodontitis • endothelial function • inflammation

Clinical and epidemiological studies have shown that periodontitis, an infection of the oral cavity caused by Gram-negative bacteria, is a risk factor for cardiovascular diseases (CVDs).\(^1\)–\(^3\) Although the mechanisms by which periodontal disease is related to CVD are unclear, it is thought that periodontitis-induced systemic inflammation contributes to the development and maintenance of atherosclerosis through activation of a biochemical reaction cascade, initiation and development of plaque formation, and injury of the endothelium. Endothelial dysfunction is the initial step in the development of atherosclerosis, leading to CVD.\(^4\) Several lines of evidence have shown that CVDs are associated with endothelial dysfunction.\(^5\)–\(^8\) It is well known that there is an association between inflammation and endothelial dysfunction.\(^9\) Recently, we have reported that chronic infection with Helicobacter pylori impaired endothelium-dependent vasodilation in healthy male subjects.\(^9\) Patients with periodontitis are ideal models for determining how endothelium-dependent vasodilation is affected by inflammation. Indeed, patients with periodontitis have impaired endothelial function.\(^10\)–\(^12\) Periodontal therapy improves endothelium-dependent vasodilation in these patients.\(^10\)–\(^12\) However, there is little information regarding the effects of periodontitis, per se, under conditions without confounding factors, including aging, hypercholesterolemia, diabetes mellitus, smoking, obesity, and menstrual cycle, that affect endothelial function on endothelium-dependent vasodilation and regarding the effects of periodontitis on endothelium-dependent vasodilation in patients with hypertension.

The purpose of this study was to evaluate endothelial function in patients with periodontitis, in subjects without cardiovascular risk factors, and in patients with hypertension. We also evaluated the effects of periodontal therapy on endothelial function in these subjects.

Methods

Protocol 1: Endothelial Function in Periodontitis Patients Without Cardiovascular Risk Factors

Patients with periodontitis who had no other cardiovascular risk factors, such as elevated levels of blood pressure, cholesterol, and glucose (32 men; mean age: 25±3 years) and normal healthy

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subjects as a control group (20 men; mean age: 26±3 years) were enrolled in this study. The 32 patients with periodontitis were randomly divided into a periodontitis treatment group (16 men; mean age: 25±3 years) and an untreated group (16 men; mean age: 25±4 years). None of the subjects had a history of serious medical problems. No patients in either group currently smoked or had a history of smoking. The ethical committee of Hiroshima University Graduate School of Biomedical Sciences approved the study protocol. Written informed consent for participation in the study was obtained from all of the subjects.

We measured the forearm blood flow (FBF) responses to intra-arterial infusion of acetylcholine (ACh) and to sodium nitroprusside (SNP) before periodontal therapy in 32 patients with periodontitis and 20 normal control subjects and in 16 patients who were treated with periodontitis and 16 untreated patients before and after the 24-week follow-up period. Subjects fasted the previous night for ≥12 hours. The study began at 8:30 AM. They were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature: 22°C to 25°C) throughout the study. A 23-gauge polyethylene catheter (Hakkow Co) was inserted into the left brachial artery for the infusion of vasoactive agents and to record arterial pressure with an AP-641G pressure transducer (Nihon Kohden Co) under local anesthesia (1% lidocaine). Another catheter was inserted into the left deep antecubital vein to obtain blood samples. Thirty minutes after maintaining the supine position, basal FBF was measured. Then, FBF responses to ACh (Daiichi Pharmaceutical Co), an endothelium-dependent vasodilator, and SNP (Mallushi Pharmaceutical Co), an endothelium-independent vasodilator, were measured. ACh infusion was administered at a dose of 3.75, 7.50, and 15.00 μg/min for 5 minutes, and SNP infusion was administered at a dose of 0.75, 1.50, and 3.00 μg/min for 5 minutes. These studies were carried out in a randomized fashion. Each study procedure proceeded after FBF had returned to baseline.

To examine the effect of periodontal therapy on the release of NO, we measured FBF in the presence of the NO synthase inhibitor Nω-monomethyl-L-arginine (L-NMMA; CLINALFA Co) in all of the subjects. The responses of forearm vasculature to ACh after intra-arterial infusion of L-NMMA (8 μmol/min for 5 minutes) were evaluated.

Baseline fasting serum concentrations of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, insulin, electrolytes, interleukin (IL)-6, and high-sensitivity (hs) C-reactive protein (CRP) were obtained after a 30-minute rest period before the study.

Protocol 2: Endothelial Function in Periodontitis Patients With Hypertension
Twenty-six hypertensive patients with periodontitis (18 men and 8 women; mean age: 54±13 years) and 38 hypertensive patients without periodontitis (28 men and 10 women; mean age: 56±12 years) were enrolled in this study. All of the patients had been treated for hypertension with calcium antagonists (n = 42), renin-angiotensin system inhibitors (n = 16), β-blockers (n = 9), and diuretics (n = 8) for ≥6 months, and none of the patients had diabetes mellitus, hyperlipidemia, or CVD or had been receiving any drugs other than antihypertensive drugs. Conventional therapy was continued throughout the study. Written informed consent for participation in the study was obtained from all of the subjects.

The 26 hypertensive patients with periodontitis were divided into a periodontitis treatment group (n = 17; 11 men and 6 women; mean age: 53±14 years) and an untreated group (n = 9; 7 men and 2 women; mean age: 55±11 years). Vasodilative responses to ACh and SNP were evaluated in a manner identical to that of the protocol in all of the patients before and after 24 weeks of treatment.

Definition of Periodontitis
Periodontal status was measured by a self-reported questionnaire that asked subjects about periodontal symptoms including gingival swelling and bleeding, purulent discharge, and tooth mobility, as described previously. In addition to a self-reported periodontal status, the dentists performed a routine oral examination for the diagnosis of periodontitis and confirmed the presence of the disease.

Treatment of Periodontitis
Patients received nonsurgical periodontal therapy that included oral hygiene instructions and subgingival scaling and root planning under local anesthesia as needed. Antibiotics were used for 4 to 7 days after intensive therapy. Then the patients performed mouth washes and teeth and subgingival brushing every day for 24 weeks. Data for subjects in whom periodontitis was confirmed after 24 weeks of periodontal therapy were excluded from the primary analysis.

Measurements of FBF
FBF was measured using a mercury-filled Silastic strain-gauge plethysmography (EC-5R, Hokanson, Inc) as described previously.

Analytical Methods
Samples of venous blood were placed in tubes containing sodium EDTA (1 mg/mL) and in polystyrene tubes. The EDTA-containing tubes were chilled promptly in an ice bath. Samples were stored at −80°C until the time of the assay. Serum concentrations of total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glucose, insulin, and electrolytes were determined by routine chemical methods. Serum concentration of hs-CRP was measured by a high-sensitivity nephelometry assay using a CRP kit (Dade Behring). Serum concentration of IL-6 was measured by a high-sensitivity ELISA (R&D System).

Statistical Analysis
Values are expressed as mean±SD. Values of P<0.05 were considered significant. The Mann-Whitney U test was used to evaluate differences between before and after periodontal treatment with respect to baseline parameters. Two-tailed Student’s paired t test was used to evaluate differences before and after treatment. Comparisons of dose-response curves of parameters during the infusion of the drug were analyzed by ANOVA for repeated measures. The data were processed using the software packages Stat View IV (Brainpower) or Super ANOVA (Abacus Concepts).

Results
Protocol 1: Endothelial Function in Periodontitis Patients Without Cardiovascular Risk Factors
The baseline clinical characteristics of the healthy subjects group and periodontitis group and the periodontitis treated group and untreated group are summarized in Table 1. Serum concentrations of IL-6 and hs-CRP, indices of systemic inflammation, were significantly higher in patients with periodontitis than in healthy subjects. There was no significant difference in the other parameters between the 2 groups. There was no significant difference in baseline clinical characteristics between the treated and untreated groups at 0 weeks of follow-up. The 24 weeks of treatment significantly decreased serum concentrations of IL-6 and hs-CRP. Periodontitis therapy did not alter other parameters. In the untreated group, the baseline clinical characteristics were similar at 0 weeks and 24 weeks of follow-up.

Intra-arterial infusion of ACh and SNP increased FBF in a dose-dependent manner in all of the subjects. The response of FBF to ACh was significantly less in patients with periodontitis than in healthy control subjects (Figure 1, left). Vasodilatory responses to SNP were similar in the 2 groups (Figure 1, centre). Intra-arterial infusion of L-NMMA significantly decreased basal FBF from 5.2±1.3 to 3.9±1.2 mL/min per 100 mL of tissue (P<0.05) in patients with periodontitis and...
from 5.1±1.2 to 4.4±1.1 mL/min per 100 mL of tissue (P<0.05) in healthy control subjects. After L-NMMA infusion, there was no significant difference between FBF responses to Ach in the 2 groups (Figure 1, right). The response of FBF to Ach was increased significantly by 24 weeks of treatment, whereas there was no significant difference between the FBF responses to Ach in the untreated group and from 5.1±1.4 to 3.9±1.2 mL/min per 100 mL of tissue (P=0.05) in the untreated group at 0 weeks and from 5.4±1.4 to 4.0±1.3 mL/min per 100 mL of tissue (P<0.05) in the treated group and from 5.2±1.3 to 3.8±1.0 mL/min per 100 mL of tissue (P<0.05) in the untreated group at 24 weeks of follow-up. L-NMMA completely abolished the periodontal therapy-induced augmentation of FBF response to Ach (Figure 2, bottom). No significant change was observed in arterial blood pressure or heart rate after intra-arterial infusion of either Ach or SNP in the presence and absence of L-NMMA in all of the subjects. There was a significant correlation between IL-6 levels and hs-CRP levels (r=0.61; P<0.01). After periodontal therapy, changes in IL-6 and hs-CRP were parallel. There was no significant relationship among the vascular responses to Ach and SNP and serum concentration of IL-6 or hs-CRP or among the increase in FBF responses to Ach and SNP and change in hs-CRP or IL-6.

**Protocol 2: Endothelial Function in Periodontitis Patients With Hypertension**

The baseline clinical characteristics of the hypertensive control group, periodontitis group, periodontitis-treated group,
and untreated group are summarized in Table 2. Serum concentrations of IL-6 and hs-CRP were significantly higher in hypertensive patients with periodontitis than in hypertensive patients without periodontitis. There was no significant difference in other parameters between the 2 groups. There was no significant difference in baseline clinical characteristics between the treated and untreated groups at 0 weeks of follow-up. The 24 weeks of treatment significantly decreased serum concentrations of IL-6 and hs-CRP. Periodontal therapy did not alter other parameters. In the untreated group, the baseline clinical characteristics were similar at 0 weeks and 24 weeks of follow-up.

The response of FBF to ACh was significantly less in patients with periodontitis than in control patients (Figure 3, left). Vasodilatory responses to SNP were similar in the 2 groups (Figure 3, centre). Intra-arterial infusion of L-NMMA significantly decreased basal FBF from 4.6±1.4 to 3.8±1.2 mL/min per 100 mL of tissue (P<0.05) in patients with periodontitis and from 4.5±1.6 to 3.7±1.2 mL/min per 100 mL of tissue (P<0.05) in control patients. After L-NMMA infusion, there was no significant difference in FBF response to ACh between the 2 groups (Figure 3, right). The response of FBF to ACh was increased significantly by 24 weeks of treatment, whereas there was no significant difference in the FBF response to ACh in the untreated group before and after the 24-week study period (Figure 4, top). The increases in FBF during the infusion of SNP were similar at the beginning and the end of the 24-week study period in both groups (Figure 4, middle). Intra-arterial infusion of l-NMMA significantly decreased basal FBF from 4.6±1.5 to 3.7±1.2 mL/min per 100 mL of tissue (P<0.05) in the treated group and from 4.5±1.6 to 3.7±1.2 mL/min per 100 mL of tissue (P<0.05) in the untreated group at 0 weeks and from 4.7±1.5 to 3.7±1.3 mL/min per 100 mL of tissue (P<0.05) in the treated group and from 4.5±1.5 to 3.9±1.2 mL/min per 100 mL of tissue (P<0.05) in the untreated group at 24 weeks of follow-up. L-NMMA completely abolished the periodontal therapy-induced augmentation of FBF response to ACh (Figure 4, bottom). No significant change was observed in arterial blood pressure or heart rate after intra-arterial infusion of either ACh or SNP in the presence and absence of l-NMMA in all of the subjects. There was a significant correlation between IL-6 levels and hs-CRP levels (r=0.57; P<0.01). After periodontal therapy, changes in IL-6 and
hs-CRP were parallel. There was no significant relationship among the vascular responses to ACh and SNP and serum concentration of IL-6 or hs-CRP or among the increase in FBF responses to ACh and SNP and change in hs-CRP or IL-6.

Discussion

In the present study, we demonstrated that periodontitis impaired endothelium-dependent vasodilation but not endothelium-independent vasodilation in healthy young men, as well as in patients with hypertension. Periodontitis therapy augmented ACh-induced vasodilation in forearm circulation through an increase in NO production, whereas the vasodilator responses to SNP did not change after periodontitis treatment. These findings suggest that periodontitis therapy has a beneficial effect on endothelial cell function but not on smooth muscle cell function.

The first purpose of this study was to determine the effects of periodontitis, per se, on endothelial function in humans. Therefore, we selected healthy young men to avoid the possibility of alteration in endothelial function caused by confounding factors, including hypertension, heart failure, atherosclerosis, hypercholesterolemia, diabetes mellitus, smoking, aging, and menstrual cycle. Periodontitis impaired endothelium-dependent vasodilation in healthy young men, suggesting that periodontitis is a predictor of endothelial dysfunction. Although we did not confirm the natural course of healthy young men who have periodontitis, it is postulated that periodontitis-induced inflammation is an initial step of endothelial dysfunction, leading to atherosclerosis.

In patients with hypertension who have impaired endothelial function, complication of periodontitis greatly increased the magnitude of endothelial dysfunction. In addition, appropriate periodontal therapy improved endothelium-dependent
vasodilation in hypertensive patients with periodontitis. These findings suggest that we should pay attention to the existence of periodontitis in patients with hypertension and vigorously treat periodontitis when we follow up the patients with hypertension.

There are several possible explanations for the periodontitis-induced impairment of forearm vascular response to ACh in humans. In the present study, after L-NMMA infusion, ACh-induced vasodilation was similar in subjects with periodontitis and normal control subjects, as well as patients with hypertension. In addition, enhanced response of forearm vasculature to ACh in the periodontitis treatment group was substantially inhibited by the NO synthase inhibitor L-NMMA. These findings suggest that periodontitis decreases the production of NO and that increase in NO production is involved in periodontal therapy-enhanced endothelium-dependent vasodilation.

It is likely that chronic inflammation caused by periodontitis is involved in endothelial dysfunction through a decrease in NO bioavailability, a decrease in NO production, and/or an increase in NO inactivation. In turn, endothelial dysfunction promotes inflammation of the vascular wall, leading to a vicious circle between endothelial dysfunction and inflammation. In a state of chronic inflammation, production of proinflammatory cytokines results in the activation of endothelial cells, leading to the induction of adhesion molecules, cytokines, growth factors, and vasoconstrictors. In addition, it has been shown that proinflammatory cytokines, such as tumor necrotic factor-alpha and IL-6, downregulate the expression of endothelial NO synthase (eNOS) and that tumor necrosis factor-α alone decreased the half-life of eNOS mRNA in human endothelial cells. Administration of these cytokines attenuates endothelium-dependent vasodilation in vivo. Interestingly, CRP also directly decreased eNOS mRNA and protein levels and enzymatic activity in human aortic endothelial cells. Several studies using atherosclerotic animal models and patients with atherosclerosis have shown that endothelial dysfunction is associated with an increase in reactive oxygen species. The activation of endothelial cells induced by
proinflammatory cytokines generates reactive oxygen species that inactivate NO. Amounts of antioxidant scavengers, such as superoxide dismutase, glutathione peroxidase, and catalase, are decreased in periodontitis. Therefore, an increase in NO inactivation by excess production of reactive oxygen species and an attenuated antioxidant system may contribute to endothelial dysfunction in patients with periodontitis.

A possible mechanism by which periodontal therapy improves endothelial function in patients with periodontitis is activation of the eNOS/NO pathway. In addition, chronic inhibition of inflammation may lead to functional and histological alterations of the vascular endothelium, resulting in enhanced vascular structure and function. This beneficial change in the endothelium after periodontal therapy may also contribute to the augmentation of endothelial function in patients with periodontitis.

Measurements of various biomarkers, including CRP and IL-6, have been proposed as a means for assessing the magnitude of inflammation and for predicting the risk of CVD. CRP, a marker of general inflammation, is established as an independent predictor of CVD risk. American Heart Association and Centers for Disease Control and Prevention recommend the clinical use of this marker to assess the risk of CVD. Several investigators have demonstrated that periodontitis is associated with high CRP levels and that periodontal therapy reduces CRP levels. In our study, periodontal therapy also significantly decreased CRP levels, but the improvement in endothelium-dependent vasodilation did not correlate with changes in CRP, suggesting that the effects of periodontal therapy on endothelial function are at least partly independent of this inflammatory marker. In addition, there was no significant relationship between basal CRP levels and endothelium-dependent vasodilation. However, we cannot rule out the possibility that reduction in inflammation contributes to the improvement in endothelium-dependent vasodilation in patients with periodontitis. Treatment of periodontitis would reduce the risk of mortality and morbidity of CVD through improvement in endothelial function. Discovery or rigid validation of potential biomarkers of inflammatory-related endothelial dysfunction is needed.

Limitations

Study design limitations should be considered in assessing the results of this study. We confirmed the beneficial effect of periodontal therapy on endothelial function from results for healthy young men using a randomized control study. Thus, hypertensive patients with periodontitis were not randomly divided into treated and untreated groups because of ethical considerations. After obtaining informed consent from patients for whom the procedure and the effects of periodontal therapy had been explained, each patient selected periodontal therapy or conventional therapy. The subjects enrolled in this study had mild-to-moderate periodontitis. Evaluation of severe periodontitis that requires surgical intervention may enable more specific conclusions concerning the role of inflammation, especially inflammatory markers, in endothelial function after periodontal therapy to be drawn.

Perspectives

Periodontitis is associated with endothelial dysfunction in individuals without cardiovascular risk factors, as well as hypertensive patients. Both periodontitis and endothelial dysfunction independently or concomitantly lead to atherosclerosis, resulting in cardiovascular complications. From a clinical perspective, it is important to select an appropriate intervention that is effective in improving endothelial dysfunction. Periodontal therapy improves endothelial function through an increase in NO bioavailability. Therefore, we should carefully check the existence of periodontitis. If periodontitis is present, it must be treated vigorously. Periodontal therapy, per se, is also a good therapeutic approach for improving endothelial dysfunction. Future large-scale clinical studies are needed to determine the long-term effects of periodontal therapy on the mortality and morbidity of CVD.

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Disclosures

None.

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