

Periodontal treatment improves endothelial dysfunction in patients with severe periodontitis

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Background Because epidemiological studies provide evidence that periodontal infections are associated with an increased risk of progression of cardiovascular and cerebrovascular disease, we postulated that endothelial dysfunction, a critical element in the pathogenesis of atherosclerosis, would be present in patients with periodontal disease.

Methods We tested endothelial function in 30 patients with severe periodontitis and 31 control subjects using flow-mediated dilation (FMD) of the brachial artery. The groups were matched for age, sex, and cardiovascular risk factors. Three months after periodontal treatment, including both mechanical and pharmacological therapy, endothelial function was reassessed by brachial artery FMD. Markers of systemic inflammation were measured at baseline and at follow up.

Results Flow-mediated dilation was significantly lower in patients with periodontitis than in control subjects ($6.1\% \pm 4.4\%$ vs $8.5\% \pm 3.4\%$, $P = .002$). Successful periodontal treatment resulted in a significant improvement in FMD ($9.8\% \pm 5.7\%$; $P = .003$ compared to baseline) accompanied by a significant decrease in C-reactive protein concentrations (1.1 ± 0.9 vs 0.8 ± 0.8 at baseline, $P = .026$). Endothelium-independent nitro-induced vasodilation did not differ between the study groups at baseline or after periodontal therapy.

Conclusion These results indicate that treatment of severe periodontitis reverses endothelial dysfunction. Whether improved endothelial function will translate into a beneficial effect on atherogenesis and cardiovascular events needs further investigation. (*Am Heart J* 2005;149:1050-4.)

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Periodontal disease is a common chronic infection caused by gram-negative bacteria such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Tannerella forsythensis* that colonize the subgingival biofilms.¹ In severe periodontitis, gingival infection and inflammation destroy the attachment apparatus, leading to alveolar bone and tooth loss.

Epidemiological research provides strong evidence that severe periodontitis is a risk factor for cardiovascular disease. A number of studies have demonstrated an association between periodontal disease and the risk of myocardial infarction and stroke.²⁻⁴ However, the identified relationships between periodontal disease and cardiovascular disease by no means indicate a causal association.

Because endothelial dysfunction is a key step in the pathogenesis of atherosclerosis,⁵ we postulated that endothelial function would be impaired in patients with severe periodontitis. We further hypothesized that periodontal treatment would improve endothelial dysfunction, thereby supporting the concept that therapeutic strategies aimed at reducing cardiovascular risk factors translate into improved endothelial health.

Methods

Patients

Patients aged 25 to 50 years with severe periodontitis were eligible to participate in the study. Patients were excluded if they had a history of cardiovascular disease, diabetes mellitus, hypertension, or hypercholesterolemia, if they were suffering from any systemic illnesses or if they had been treated for periodontitis within 6 months of the study. None of them were currently taking cardiovascular or antiinflammatory medication, and none of them had been taking antibiotic medication or antioxidant agents within 3 months of the study. In addition, healthy volunteers matched for age and sex served as controls. Diabetes was defined in agreement with the American Diabetes Association.⁶ Hypercholesterolemia was defined according to the third report of the National Cholesterol Education Program.⁷ Hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≤ 90 mm Hg, according to the American Heart Association/American College of Cardiology guidelines.⁸ All

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Table I. Participants' baseline characteristics

	Controls (n = 31)	Periodontal disease (n = 30)	P
Age (y)	40.2 (4.7)	41.2 (7.4)	.59
Women, no. (%)	16 (51)	19 (63)	.44
Body mass index (kg/m ²)	24.8 (3.6)	24.2 (5.4)	.19
Current smoking, no. (%)	8 (26)	9 (30)	.29
Total cholesterol (mg/dL)	203 (39)	207 (35)	.67
LDL-C (mg/dL)	133 (33)	123 (33)	.27
HDL-C (mg/dL)	61 (13)	67 (19)	.18
Triglycerides (mg/dL)	88 (48)	84 (50)	.31
Hemoglobin A _{1c} (%)	5.16 (0.39)	5.14 (0.35)	.82
Systolic blood pressure (mm Hg)	115 (15)	119 (20)	.57
Diastolic blood pressure (mm Hg)	80 (10)	79 (10)	.73

Values are mean (SD) unless otherwise indicated.

subjects provided written informed consent as approved by the institutional review boards.

Periodontal examination

All clinical parameters were assessed by trained periodontists and a calibration exercise was performed to obtain acceptable intraexaminer reproducibility. The evaluation included the assessment of bleeding on probing as an indication of an existing periodontal inflammation and the measurement of probing depth and gingival recession on 6 sites on each tooth (with the exception of the third molars) using a pressure-calibrated digital recording device. Probing depth is defined as the distance from the gingival margin to the base of the probeable crevice. Gingival recession was measured as the distance from the cemento-enamel junction to the gingival margin. These 2 values were summed for clinical attachment levels, which is a valid measure of historical periodontal destruction.⁹ The presence of advanced periodontitis was determined using established criteria,¹⁰ which included involvement of at least 6 teeth with pocket depth >5 mm and loss of attachment of ≥3 mm in 3 aspects of each involved tooth. In control subjects, periodontal disease was excluded if they had no tooth pocket depth ≥2 mm and no attachment loss ≥3 mm. Subjects with intermediate-severity periodontitis were excluded from the study. Periodontal parameters were recorded at baseline and 3 months after end of treatment.

Periodontal therapy

All patients underwent conservative nonsurgical periodontal therapy.¹¹ This consisted of extensive explanation of the disease and oral hygiene instruction with several supragingival cleaning sessions. After a satisfactory hygiene index had been achieved, all clinical parameters were recorded and a careful subgingival instrumentation was performed. The scaling and root planing were carried out according to the individual needs of the patients, using a variety of hand instruments and, where appropriate, a piezoelectric ultrasonic scaler. The entire procedure was completed in 2 sessions. Usually, one jaw was treated per session, starting with the advanced sites. Local anesthesia was used only occasionally for isolated sites, upon

Table II. Brachial artery parameters and markers for systemic inflammation in control subjects and in patients before and after treatment of periodontal disease

	Controls	Periodontal disease	
		Before treatment	After treatment
Baseline brachial diameter (mm)	3.4 (0.6)	3.5 (0.8)	3.4 (0.8)
FMD (mm)	0.29 (0.1)	0.2 (0.14)*	0.3 (0.14)†
FMD (%)	8.5 (3.4)	6.1 (4.4)*	9.8 (5.7)†
Nitroglycerin-associated dilation (%)	22.5 (6.7)	23.7 (3.2)	23.5 (7.9)
Brachial artery IMT thickness (mm)	0.29 (0.1)	0.27 (0.07)	0.27 (0.06)
ESR (s)	4.9 (3.2)	7.8 (4.2)*	6.6 (3.4)
White blood cell count (×10 ³ /μL)	5.5 (1.4)	5.7 (1.3)	5.1 (1.4)
C-reactive protein (mg/L)	0.8 (0.8)	1.7 (1.6)‡	1.1 (0.9)§

Values are mean (SD).

*P < .01 versus controls.

†P < .01 versus before treatment.

‡P < .05 versus controls.

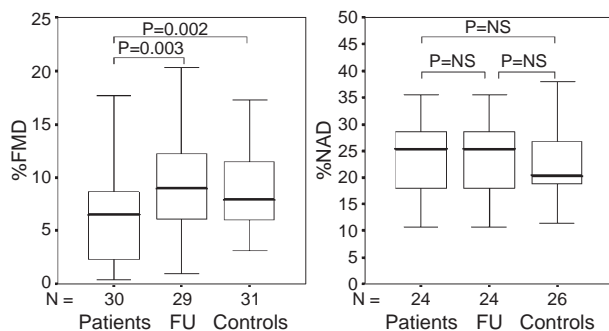
§P < .05 versus before treatment.

the patient's request. Chlorhexidine gluconate (0.1%) mouth washes were continued for 14 days. Systemic antimicrobial therapy was administered for 7 days and consisted of a combination of amoxicillin plus clavulanic acid and metronidazole as adjunctive therapy.¹² The patients were reevaluated 12 weeks after periodontal treatment. In the intervening period, their compliance was checked twice and a professional cleaning was performed. Residual pockets of more than 5 mm that bled after probing were rescaled. Treatment of periodontitis was considered successful when there was no bleeding on probing and no pocket depth >5 mm at follow-up.

Brachial artery measurements

Brachial artery reactivity was assessed within 1 week of the initial treatment and 3 months after end of treatment. Endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent nitroglycerin-associated dilation of the brachial artery were examined noninvasively according to a previously described method.¹³ In brief, patients were studied in a quiet, temperature controlled room in the morning after an overnight fast. Two-dimensional images from the right brachial artery were obtained above the antecubital crease at baseline and after 1 minute of hyperemia created by 5 minutes' forearm cuff occlusion with an Acuson Sequoia 512 ultrasound system equipped with a 13.0-MHz linear array transducer (Acuson, Mountain View, Calif). After at least 10 minutes of rest, to allow restoration of baseline conditions, we assessed endothelium-independent brachial artery dilation by recording 2-dimensional images before and 4 minutes after administration of sublingual nitroglycerin. Nitroglycerin was not administered to individuals with clinically significant bradycardia or hypotension or to individuals with a history of migraine headaches. Digitized end-diastolic images were analyzed off-line by a single investigator who was blinded to the individuals' clinical status and image sequence. The lumen-intima bound-

Figure 1



Relative flow-mediated (%FMD) and nitroglycerin-associated (%NAD) dilation of the brachial artery of patients with severe periodontitis before and after periodontal treatment and of control subjects. FU, follow-up.

aries were identified manually with electronic calipers and the diameter of a vessel segment was determined as an average derived from multiple diameter measurements.

Intima and media thickness (IMT) of the brachial artery was measured at the far wall with electronic calipers above the antecubital fossa at the peak of the vessel arch. Measurements were obtained at 2 sites per image in 2 different images per patient. The mean of 4 measurements was defined as brachial artery IMT.

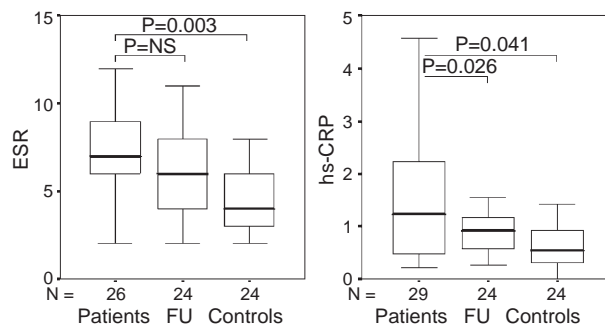
Biochemical analysis

Concentrations of total cholesterol, triglycerides, high-density lipoprotein, and hemoglobin A_{1c} were measured using automated analyzers (Modular Analytics, Roche/Hitachi, Basel, Switzerland; Adams A1c HA-8160, Menarini Diagnostics, Firenze, Italy). Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated with Friedewald formula. High-sensitivity C-reactive protein was measured with a nephelometric method (Dade-Behring Inc, Deerfield Ill).

Statistical analysis

Group differences in age, blood pressure, lipid profile, erythrocyte sedimentation rate (ESR), high-sensitive C-reactive protein, brachial artery diameter, flow-mediated and nitroglycerin-mediated dilation of the brachial artery, and brachial artery wall thickness were analyzed using unpaired *t* test. Analysis of normality was performed with the Kolmogorov-Smirnov test. Nonnormally distributed data were logarithmically transformed before comparative analysis. Categorical parameters were analyzed by χ^2 test or Fisher exact test when required. Univariate analysis of variance with FMD as a dependent variable and periodontal disease as a fixed factor was used to control for confounders identified by univariate analysis. The paired *t* test was used to assess changes in brachial artery diameters and biochemical markers. A *P* value <.05 was considered statistically significant. All analyses were completed with SPSS for Windows version 10.1 (SSCP Inc, Chicago, Ill). Data are presented as mean \pm SD.

Figure 2



Markers of systemic inflammation of patients with severe periodontitis before and after periodontal treatment and of control subjects. hs-CRP, high sensitive C-reactive protein.

Results

The clinical characteristics of 30 otherwise healthy patients with severe periodontitis and 31 healthy controls are summarized in Table I. As shown, the groups were well matched.

The structural and functional brachial artery parameters as well as markers of systemic inflammation are summarized in Table II. Baseline diameter of the brachial artery was comparable between the patient and the control groups and did not change after treatment of periodontitis. Flow-mediated dilation expressed as change in diameter and as percent change was significantly lower in patients with periodontitis before treatment than in healthy controls. Three months after treatment, FMD significantly improved and returned to values comparable to those of healthy controls (Figure 1). On regression analysis using age, sex, smoking status, body mass index, and high-density lipoprotein cholesterol (HDL-C) as covariates, brachial artery FMD was still significantly impaired in patients with periodontitis ($P < .0001$). Nitroglycerin-associated dilation did not differ significantly between controls and patients with periodontitis before and after treatment (Figure 1).

To investigate structural vessel wall alterations, we measured brachial artery IMT. As shown, there was no significant difference in brachial artery IMT between groups or with time (Table II).

Patients with periodontitis had significantly higher baseline levels of ESRs and concentrations of C-reactive protein (Table II) than did controls. After periodontal treatment, C-reactive protein concentrations significantly decreased and there was a trend toward a reduction in ESRs (Figure 2). However, differences in white blood cells did not reach statistical significance in controls and in patients at baseline and follow-up. There was a trend toward an inverse correlation between FMD and ESR

($r = -0.28$, $P = .05$) and between FMD and C-reactive protein concentration ($r = -0.24$, $P = .1$) that did not reach statistical significance.

There was no statistically significant change in total cholesterol ($P = .28$), LDL-C ($P = .37$), HDL-C ($P = .71$), triglycerides ($P = .06$), and systolic ($P = .68$) and diastolic ($P = .2$) blood pressure at follow-up.

Discussion

The results from this study showed that severe periodontitis generates an inflammatory reaction associated with endothelial dysfunction in the conduit brachial artery. These findings are in accordance with a recently published study by Amar et al¹⁴. They were able to demonstrate that patients with advanced, but not with mild, periodontal disease exhibited endothelial dysfunction and elevated C-reactive protein. A number of studies have demonstrated associations between the presence of infectious agents and endothelial dysfunction in a clinical and experimental setting.^{15,16} Interestingly, in a recent study, Prasad et al¹⁷ were able to demonstrate that the serologic response to multiple intracellular pathogens (cytomegalovirus, herpes simplex virus 1, hepatitis A virus, and bacteria such as *Chlamydia pneumoniae* and *Helicobacter pylori*) was an independent risk factor for the endothelial dysfunction and the presence and severity of coronary artery disease. The results from their study support the concept that cardiovascular risk is related to the aggregate number of potentially atherogenic pathogens to which an individual is exposed.

Endothelial dysfunction significantly improved after successful periodontal treatment and returned to values comparable to those of the healthy control group. Thus, our results indicate that there is a causal relationship between periodontal disease and endothelial dysfunction. The influence of infectious agents, especially *C pneumoniae*, as contributors to atherogenic inflammation has been extensively studied and reviewed.¹⁸ In a randomized, prospective, controlled trial, Parchure et al¹⁹ were able to show that azithromycin treatment for 5 weeks produced significant improvement in FMD in patients with coronary artery disease and serologic evidence of *C pneumoniae* infection. Another investigation has failed to demonstrate an association between treatment of *C pneumoniae* and endothelial function,²⁰ a disparity that may be caused by the short-term antibiotic treatment regimens used in that study. However, there is a lack of direct proof that therapeutic improvement in endothelial function due to reduction of pathogen burden translates into lower cardiovascular morbidity and mortality.

We demonstrated functional brachial artery impairment in patients with periodontal disease in the absence of structural vessel wall alterations in relatively young

patients. In the Atherosclerotic Risk in Communities study, the multivariable logistic regression model of cross-sectional data on 6017 persons aged 52 to 75 years indicates that severe periodontitis (OR 1.31, CI 1.03-1.66) is associated with carotid artery IMT ≥ 1 mm.²¹ Thus, endothelial dysfunction is an early event in atherogenesis before anatomic evidence of atherosclerosis appears.

The mechanism by which periodontal disease disrupts vascular homeostasis remains unclear. However, in our study, markers of systemic inflammation were significantly elevated in patients with periodontitis and decreased after treatment. This finding is consistent with the observation that a variety of therapeutic strategies to improve endothelial dysfunction are associated in parallel with a decrease of C-reactive protein levels.²²⁻²⁴ A possible pathway of systemic inflammation in oral infection is the release of exotoxins or endotoxins such as lipopolysaccharides into the blood stream.¹ Alternative explanations include direct invasion of the vessel wall by oral pathogens triggering, in part, an inflammatory response that translates into endothelial dysfunction. Indeed, topical oral inoculation with the major periodontal pathogen *P gingivalis* has recently been demonstrated to exacerbate early atherosclerosis in apolipoprotein E-null mice.²⁵ Furthermore, a number of recent studies have demonstrated that periodontal pathogens exist in atherosclerotic lesions and actively invade human and bovine endothelial cells.¹

A potential limitation to our study is that the association between severe periodontitis and endothelial dysfunction may reflect residual confounding by known or yet unknown cardiovascular risk factors despite our attempt to match the groups and check for confounding with regression analysis. Whether the effect on endothelial function and C-reactive protein actually was due to mechanical or pharmacological periodontal treatment remains unclear. It is, however, unlikely that an unspecific antiinflammatory effect of antibiotic therapy was responsible for improved endothelial function and inflammatory markers 12 weeks after the end of periodontal treatment.

Regardless of the mechanisms, we have shown that severe periodontitis is associated with significant endothelial dysfunction that is reversible after successful periodontal treatment. There is increasing evidence from numerous clinical trials that improvement of endothelial function translates into lower rates for cardiovascular events.⁵ It is therefore reasonable to conduct further research to determine whether improved endothelial health after successful periodontal treatment in high-risk patients prevents atherosclerotic complications. In the meanwhile, patients should be made aware of the possible relationship between periodontal disease and cardiovascular events.

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