Detection of periodontal bacteria in thrombi of patients with acute myocardial infarction by polymerase chain reaction

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Backgrounds Numerous reports have demonstrated that periodontal bacteria are present in plaques from atherosclerotic arteries. Although periodontitis has recently been recognized as a risk factor for coronary artery disease, the direct relationship between periodontal bacteria and coronary artery disease has not yet been clarified. It has been suggested that these bacteria might contribute to inflammation and plaque instability. We assumed that if periodontal bacteria induce inflammation of plaque, the bacteria would be released into the bloodstream when vulnerable plaque ruptures. To determine whether periodontal bacteria are present in thrombi at the site of acute myocardial infarction, we tried to detect periodontal bacteria in thrombi of patients with acute myocardial infarction by polymerase chain reaction (PCR).

Methods We studied 81 consecutive adults with ST-segment elevation acute myocardial infarction who underwent primary percutaneous coronary intervention (PCI). All patients underwent removal of thrombus with aspiration catheters at the beginning of percutaneous coronary intervention, and a small sample of thrombus was obtained for PCR.

Results The detection rates of periodontal bacteria by PCR were 19.7% for *Aggregatibacter actinomycetemcomitans*, 3.4% for *Porphyromonas gingivalis*, and 2.3% for *Treponema denticola*.

Conclusions Three species of periodontal bacteria were detected in the thrombi of patients with acute myocardial infarction. This raises the possibility that such bacteria are latently present in plaque and also suggests that these bacteria might have a role in plaque inflammation and instability. (Am Heart J 2012;163:164-7.)

Rupture of coronary atherosclerotic plaque with consequent platelet aggregation and thrombus formation is the major cause of acute coronary syndrome.[1] Clinical and pathologic studies have indicated that rupture, erosion, and ulceration of plaque participate in thrombus formation and that vulnerable plaque consists of a lipid core covered by a thin fibrous cap.[2] Vulnerable plaque is also prone to local inflammation,[3,4] and chronic inflammation may cause plaque instability.[5]

Recently, many studies have shown a correlation between periodontal disease and coronary artery disease.[6,7] Periodontitis has been recognized as a new risk factor for coronary artery disease alongside hypertension, diabetes, hyperlipidemia, and smoking. However, the direct relationship between local inflammation of periodontitis and distant coronary arterial inflammation has not yet been clarified.[8] Chiu[9] performed a histologic analysis of human carotid endoatherectomy specimens and found *Porphyromonas gingivalis* (PG) and *Streptococcus sanguis* in unstable plaques. In addition, we have detected *Actinobacillus actinomycetemcomitans* (AA), PG, *Bacteroides forsythus*, *Treponema denticola* (TD), and *Campylobacter rectus* in samples of coronary artery plaques by polymerase chain reaction (PCR).[10] Furthermore, the presence of AA, *Fusobacterium nucleatum-periodonticum-simiae* group, PG, *Prevotella intermedia* (PI), *Prevotella nigrescens*, and *Tannerella forsythia* (TF) in atheromatous plaques from coronary arteries have been demonstrated by Gaetti-Jardim Jr et al using PCR.[11] Because periodontal bacteria have been detected in atherosclerotic plaques, it has been suggested that such bacteria might take part in causing inflammation and plaque instability. We assumed that if periodontal bacteria induced inflammation and disruption of plaques, the

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Table I. Species-specific PCR primers for 5 periodontal bacteria

Primer pairs (5′-3′)	Amplicon length (bp)
AA	
AAA CCC ATC TCT GAG TTC TTC TTC	557
ATG CCA ACT TGA CGT TAA AT	
PG	404
AGG CGA CTT GCC ATA CTG CG	
ACT GTT AGC AAC TAC CGA TGT	
PI	575
TTT GTT GGG GAG TAA AGC GGG	
TCA ACA TCT CTG TGG GCT GCG T	
TF	
GCG TAT GTA ACC TGC CCG CA	641
TGGC TTC AGT GTG AGT TAT ACC T	
TD	316
TAA TAC CGA AGC TCA TTT ACA T	
TCA AAG TCT CTG TGG GCT GCG A	

bacteria would be released into the bloodstream or into the thrombus when the vulnerable plaque ruptured. To the best of our knowledge, periodontal pathogens have never been found in the thrombus. Therefore, we tried to detect periodontal bacteria in thrombus obtained from the occluded site in patients with acute myocardial infarction.

Methods

Patient inclusion criteria

We enrolled 81 consecutive adult patients with ST-segment elevation acute myocardial infarction who underwent primary percutaneous coronary intervention (PCI) from July 2008 to October 2009 at the Ichikawa General Hospital. We excluded patients with non–ST-elevation myocardial infarction or unstable angina. Patients who arrived at the hospital ≥24 hours after the onset of symptoms were also excluded from the study even if they subsequently underwent primary PCI. The patients signed an informed consent that was approved by the ethics committee of Tokyo Dental College in June 2008.

Primary PCI and sample collection

Coronary angiography was done before primary PCI to identify the culprit lesion. After passing a PCI guidewire through this lesion, the thrombus at the occluded site was removed with an aspiration catheter. Continuous aspiration was repeated several times until the operator decided that no further aspiration is required. Then 2 mL of this blood, visually confirmed including white thrombus, was immediately cooled to −80°C for storage until analysis.

Sample extraction and analysis

DNA was extracted by using a Puregene kit (Gentra-Systems, Minneapolis, MN) as described previously with minor modifications.[12] Each blood sample was treated with red blood cell lysis solution, and cells were precipitated. After lysis of the red blood cells, further cell lysis solution was added to the supernatant. Lysates were incubated at 65°C for 60 minutes, after which RNase was added and incubation was performed for a further 30 minutes. After addition of a protein precipitation solution, lysates were centrifuged for 10 minutes at 2000*g*. DNA was concentrated by adding 6 mL of 100% isopropanol to the supernatant and further centrifugation. The DNA pellet thus obtained was processed for PCR. Standard precautions were taken when handling reagents and samples. Analyses were done in a double-blind manner. Detection of AA, PG, PI, TF, and TD was performed according to the method described by Ashimoto et al[8] using the primers listed in Table I.

Table II. Number of patients with periodontal bacteria, the detection rates for each species, and details of the detection

	AA	PG	PI	TF	TD
No.	**17 (21.0%)**	**3 (3.7%)**	**0 (0.0%)**	**0 (0.0%)**	**2 (2.5%)**
Case 1	Positive	Positive	–	–	Positive
Case 2	Positive	Positive	–	–	-
Case 3	Positive	Positive	–	–	–
Case 4	Positive	–	–	–	–
Case 5	Positive	–	–	–	–
Case 6	Positive	–	–	–	–
Case 7	Positive	–	–	–	–
Case 8	Positive	–	–	–	–
Case 9	Positive	–	–	–	–
Case 10	Positive	–	–	–	–
Case 11	Positive	–	–	–	–
Case 12	Positive	–	–	–	–
Case 13	Positive	–	–	–	–
Case 14	Positive	–	–	–	–
Case 15	Positive	–	–	–	–
Case 16	Positive	–	–	–	–
Case 17	Positive	–	–	–	–
Case 18	–	–	–	–	Positive

Patient characteristics

We examined whether various patient characteristics differed between the group with periodontal bacteria (DNA [+] group) and the group without such bacteria (DNA [−] group). The characteristics compared were major coronary risk factors and the severity of coronary artery disease.

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Results

Using blood samples from the 81 patients, analysis of DNA revealed periodontal bacteria in 18 patients (22.2%). Of the various periodontal bacteria, AA was most frequently detected. Multiple species of bacteria were detected from 3 of the 18 samples, 2 of which contained AA and PG. The detection rates of AA, PG, PI, TF, and TD by PCR were 21.0%, 3.7%, 0.0%, 0.0%, and 2.5%, respectively (Table II). Table III shows patient characteristics, including their coronary risk factors and the

Table III. Characteristics of the patients with periodontal bacteria

	Total	DNA (+)	DNA (–)	
	81	18 (22.2%)	63 (77.8%)	*P*
Age range (mean)	32-90 (64)	40-85 (63)	32-90 (65)	.30*
Male	67 (82.7%)	16 (88.9%)	51 (81.0%)	.43†
Coronary risk factors				
Hypertension	41 (50.6%)	9 (50.0%)	32 (50.8%)	.95†
Diabetes	31 (38.3%)	9 (50.0%)	22 (34.9%)	.25†
Hypercholesterolemia	41 (50.6%)	9 (50.0%)	32 (50.8%)	.95†
Cigarette smoking	44 (54.3%)	7 (38.9%)	37 (58.7%)	.14†
Prior MI	5 (6.3%)	0 (0.0%)	5 (7.8%)	.25†
Severity				
Single-vessel disease	41 (50.6%)	11 (61.1%)	30 (47.6%)	.31†
Multivessel disease	40 (49.4%)	7 (38.9%)	33 (52.4%)	

MI, Myocardial infarction.
*Unpaired Student *t* test.
† 2 × 2 χ^2 and Fisher exact probability tests.

Table IV. Background factors of patients with each species of periodontal bacteria

	AA	PG	TD
No. detected	17	3	2
Age range	40-85	40-65	40-48
Male (%)	15 (88.2)	3 (100.0)	2 (100.0)
Coronary risk factors			
Hypertension (%)	9 (52.9)	1 (33.3)	0 (0.0)
Diabetes (%)	9 (52.9)	2 (66.7)	0 (0.0)
Hypercholesterolemia (%)	9 (52.9)	1 (33.3)	1 (50.0)
Smoking (%)	7 (41.2)	2 (66.7)	0 (0.0)
Prior MI (%)	0 (0.0)	0 (0.0)	0 (0.0)
Severity			
Single-vessel disease (%)	6 (35.3)	2 (66.7)	0 (0.0)
Multivessel disease (%)	11 (64.7)	1 (33.3)	2 (100.0)
Culprit lesion			
RCA (%)	6 (35.3)	2 (66.7)	1 (50.0)
LAD (%)	10 (58.8)	1 (33.3)	0 (0.0)
LCX (%)	1 (5.9)	0 (0.0)	0 (0.0)
LMT (%)	0 (0.0)	0 (0.0)	1 (50.0)

RCA, Right coronary artery; *LAD*, left anterior descending artery; *LCX*, left circumflex artery; *LMT*, left main trunk.

severity of coronary artery disease. In the DNA (+) group, no patient had a history of myocardial infarction, and patients with single-vessel disease outnumbered those with multivessel disease. In the DNA (−) group, patients with multivessel disease outnumbered those with single-vessel disease. However, there were no significant differences of coronary risk factors or the severity of coronary artery disease between the DNA (+) and the DNA (−) groups (Tables III and IV).

Discussion

Chronic periodontitis is an inflammatory disease caused by anaerobic gram-negative rods and spirochetes,[13] and these microorganisms are present in significant numbers in human periodontal lesions.[14] Periodontal bacteria have also been detected in atherosclerotic lesions.[15,16] Furthermore, a relationship has been reported between acute myocardial infarction and colonization by periodontal bacteria[17-19] as well as between toothbrushing and the risk of cardiovascular disease.[20] In this study, we detected DNA of periodontal bacteria in 18 patients in coronary thrombi from the 81 patients associated with acute myocardial infarction. These bacteria were sited distant from the mouth, which is where such bacteria are usually detected. Because numerous studies have shown that patients with periodontitis are at risk for bacteremia,[21-23] the periodontal bacteria might reach the peripheral vessels while alive. It is possible that some bacteria may have reached the heart, such as infective endocarditis. It has been suggested that periodontal bacteria often entering the blood stream are usually killed by the immune system, and the report of Pussinen et al[16] that serum antibodies to PG and AA were associated with coronary artery disease[24] supports this hypothesis. Periodontal bacteria might actually colonize the coronary arterial walls because they are anaerobic. However, it is uncertain whether the periodontal bacteria that reached peripheral vessel or coronary artery affect the atherosclerotic lesion. In this study, we assumed that periodontal bacteria might have a role in the rupture of vulnerable plaque associated with acute coronary syndromes such as myocardial infarction. The question is whether bacteria residing in the coronary arteries could induce plaque instability. With regard to this question, PG has been reported to hydrolyze the fibrous cap[25] and induce platelet aggregation.[26] In this study, AA and TD were detected in addition to PG, but there have been no reports of AA or TD having similar properties to PG. If AA and TD were also found to hydrolyze the fibrous cap of plaques and induce platelet aggregation, a clinical hypothesis could be framed that periodontal bacteria

can cause persistent inflammation resulting in rupture of plaque and that some bacteria may then be released into the bloodstream and induce platelet aggregation. In addition, the bacteria would be detected from the platelet thrombus, such as that in this study. The finding of a persistent increase of cellular adhesion molecules[26] further supports the concept of sustained vascular inflammation after an acute coronary event. With current knowledge, we cannot answer the question of whether periodontal bacteria become attached to already existing atherosclerotic lesions or these bacteria promote the atherosclerosis and induce instability of plaque. Investigating these problems would require seeding bacteria into the arterial wall using some animal to ascertain whether this causes myocardial infarction.

This is not a control study but an observation research. If control study could be done with such as a healthy individual or patients with stable effort angina, further consideration would be obtained. However, there was a problem in ethics to extract blood from coronary arteries in the healthy individual or patients with effort angina because the aspiration catheter might have a considerable risk of coronary injury. It would also be important to investigate the presence of periodontitis in these patients and clarify differences of periodontal infection between the DNA (+) and DNA (−) groups. To do so, bacterial DNA detected from periodontitis should be compared with DNA detected from the coronary arteries, and we did not confirm our prediction that patients with diabetes would be significantly more common in the DNA (+) group than the DNA (−) group despite that patients with coronary artery disease often have diabetes as a risk factor and that diabetes also increases the risk of infection. The detection rate might be >22.2% (in this study) if much >2 mL of blood would be gathered as samples so that a different conclusion could be obtained. We would like to resolve these problems in a future study. The relation between the periodontal bacterium and the coronary artery disease will be clarified definitely, collecting a lot of samples and data concerning more cases.

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