Atherosclerosis 236 (2014) 448–455

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Periodontal bacteria in human carotid atherothrombosis as a potential trigger for neutrophil activation

Hélène Rangé [a, b, c], Julien Labreuche [a, 1], Liliane Louedec [a], Philippe Rondeau [d], Cynthia Planesse [d], Uriel Sebbag [e], Emmanuel Bourdon [d], Jean-Baptiste Michel [a], Philippe Bouchard [b, c], Olivier Meilhac [a, f, *]

[a] Inserm U1148, CHU X. Bichat, APHP, 46 Rue Henri Huchard, 75018 Paris, France
[b] Département de Parodontologie, Hôpital Rothschild, APHP, 5 Rue Santerre, 75012 Paris, France
[c] Université Paris Diderot, UFR d'Odontologie, 5 Rue Garancière, 75006 Paris, France
[d] Université de La Réunion, 15, Avenue René Cassin, 97744 Saint-Denis, Ile de La Réunion, France
[e] Centre Cardiologique du Nord, 36 Rue des Moulins Gémeaux, 93200 Saint-Denis, France
[f] CHU de La Réunion, Saint Denis, Ile de La Réunion, France

ARTICLE INFO

Article history:
Received 16 February 2014
Received in revised form
30 July 2014
Accepted 30 July 2014
Available online 11 August 2014

Keywords:
Atherosclerosis
Atherothrombosis
Intraplaque hemorrhage
Periodontitis
Periodontal disease
Periodontal bacteria
Tannerella forsythia

ABSTRACT

Objective: Epidemiological, biological and clinical links between periodontal and cardiovascular diseases are now well established. Several human studies have detected bacterial DNA corresponding to periodontal pathogens in cardiovascular samples. Intraplaque hemorrhage has been associated with a higher risk of atherosclerotic plaque rupture, potentially mediated by neutrophil activation. In this study, we hypothesized that plaque composition may be related to periodontal pathogens.
Methods: Carotid culprit plaque samples were collected from 157 patients. Macroscopic characterization was performed at the time of collection: presence of blood, lipid core, calcification and fibrosis. Markers of neutrophil activation released by carotid samples were quantified (myeloperoxidase or MPO, cell-free DNA and DNA-MPO complexes). PCR analysis using specific primers for *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcommitans*, *Treponema denticola*, *Prevotella intermedia* and *Tannerella forsythia* was used to detect DNA from periodontal pathogens in carotid tissues. In addition, bacterial lipopolysaccharide (LPS) and Immunoglobulins G against *T. forsythia* were quantified in atherosclerotic carotid conditioned medium.
Results: Intraplaque hemorrhage was present in 73/157 carotid samples and was associated with neutrophil activation, reflected by the release of MPO, cell-free DNA and MPO-DNA complexes. LPS levels were also linked to intraplaque hemorrhage but not with the neutrophil activation markers. Seventy-three percent of the carotid samples were positive for periodontal bacterial DNA. Furthermore, hemoglobin levels were associated with the detection of *T. forsythia* and neutrophil activation/inflammation markers.
Conclusion: This study suggests a potential role of periodontal microorganisms, especially *T. forsythia*, in neutrophil activation within hemorrhagic atherosclerotic carotid plaques.

1. Introduction

For over 20 years, epidemiologic data supporting the idea that periodontal disease may impact the progression and, potentially, the onset of cardiovascular disease has been the subject of numerous reports [1,2]. Furthermore, meta-analyses have suggested an association between periodontal and cardiovascular diseases [3–7]. The strength of this association, may, however, be confounded by the fact that the two groups share risk factors such as aging, diabetes, obesity and smoking. Biological evidence suggests a potential causal role for periodontal bacteria that could be

* Corresponding author. Inserm U1148: Laboratory for Vascular Translational Research, Hôpital Bichat, 46 Rue Henri Huchard, 75018 Paris, France.
Tel.: +33 1 40 25 86 00; fax: +33 1 40 25 86 02.
E-mail addresses: olivier.meilhac@inserm.fr, omeilhac@hotmail.com (O. Meilhac).
[1] Present address: Département de Biostatistiques, EA2694, UDSL, Université Lille Nord de France, CHU Lille, France.

http://dx.doi.org/10.1016/j.atherosclerosis.2014.07.034
0021-9150/

involved directly in atherogenesis [8,9] or indirectly by increasing circulating cytokines and inflammatory mediators. Periodontal bacteria could migrate from the gingival/dental sites to the vascular wall via the bloodstream and act directly via their virulence factors, such as gingipains, proteinases, fimbriae, and/or lipopolysaccharides (LPS). In addition, the capacity of periodontal bacteria to induce leukocyte recruitment may contribute to this biological process.

Numerous clinical studies have shown the presence of DNA from periodontal pathogens in atherosclerotic plaques (for review [10]) and others have recovered viable, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* from plaque samples [11–13]. The biological evidence underlying the bacterial hypothesis chiefly relies on reports of *in vitro* activation of endothelial cells by periodontal pathogens and LPS (via Toll-like receptors 4 and 2 [14,15]) and the subsequent induction of apoptosis [16]. Animal studies using models of atheroma (apoE−/− mice, rabbits and pigs [10]) or abdominal aortic aneurysm in rats [17] suggest that *P. gingivalis* may promote atherogenesis and thrombus formation. Leukocytes may also directly or indirectly stimulate endothelial cells which produce pro-atherogenic molecules, such as monocyte chemoattractant protein-1, intercellular adhesion molecule-1 or vascular cell adhesion protein-1 (for review [18]).

In cerebrovascular diseases, which represent 16.4% of the global cardiovascular deaths [19] and lead to major sequelae for patients, the role of intraplaque hemorrhage in favoring plaque vulnerability to rupture via neutrophil protease release has been clearly shown [20–23]. A recent study has underlined the biological role of the thrombus for trapping pathogens in order to prevent their dissemination [24].

We thus hypothesize that intraplaque hemorrhage may promote periodontopathogen translocation into atherosclerotic plaques that could in turn trigger neutrophil activation, leading to clinical complications. One hundred and fifty seven human carotid plaque samples were macroscopically and biochemically classified into hemorrhagic versus non-hemorrhagic groups. Markers of neutrophil activation and death (myeloperoxidase −MPO- and neutrophil extracellular traps −NETs-), oxidative stress and inflammation (carbonyl groups and NFκB activity) were quantified using the conditioned medium [20] obtained from each carotid sample. MPO is an enzyme released by neutrophils to kill bacteria and other pathogens whereas the formation of NETs is induced by infection. NETs consist of extracellular, highly decondensed chromatin, including histones and DNA associated with neutrophil granule proteins [25]. DNA extraction and specific amplification of five major periodontopathogens were performed. The biological data obtained for carotid plaque samples were analysed in view of the corresponding clinical characteristics of each patient.

2. Materials and methods

2.1. Tissue sampling

Human carotid endarterectomy samples ($n = 157$) and non-atherosclerotic mammary endarteries ($n = 10$) were collected from patients undergoing surgery at the Centre Cardiologique du Nord (Saint Denis, France). All patients underwent an interview before surgery to collect medical information (Table 1) and absence of objection to using their carotid samples, considered as surgical waste in accordance with French ethical laws (L.1211-3 to L.1211-9) and the INSERM Ethics Committee. Tissue samples were collected in cold RPMI (4 °C) containing antibiotics plus an antimycotic, and processed within 2 h after surgery.

Table 1
Patients, carotid plaque and biological characteristics according to hemoglobin quartiles.

	Hemoglobin, μg/mL				*P* for trend
	<52 *n* = 29	52–94 *n* = 30	95–196 *n* = 29	>196 *n* = 29	
Demographics					
Age, years, mean ± SD	70.8 ± 8.8	71.5 ± 12.8	73.8 ± 8.4	71.6 ± 9.3	0.58[a]
Men, *n*(%)	21 (72.4)	19 (63.3)	19 (65.5)	24 (82.8)	0.38[b]
BMI, kg/m^2, mean ± SD	26.5 ± 3.4	26.1 ± 3.8	26.4 ± 4.5	26.5 ± 4.2	0.97[a]
Medical history					
Diabetes, *n*(%)	9 (31.0)	11 (36.7)	9 (31.0)	8 (27.6)	0.68[b]
Hypertension, *n*(%)	20 (69.0)	22 (73.3)	22 (75.9)	22 (75.9)	0.53[b]
Hypercholesterolemia, *n*(%)	21 (72.4)	20 (66.7)	22 (75.9)	23 (79.3)	0.41[b]
Current or former smoker, *n*(%)	15 (51.7)	14 (46.7)	17 (58.6)	18 (62.1)	0.30[b]
Carotid data					
Symptomatic Carotid Plaque, *n*(%)	9 (31.0)	5 (17.2)	6 (20.7)	9 (31.0)	0.93[b]
Intraplaque hemorrhage, *n*(%)	11 (37.9)	12 (40.0)	10 (34.5)	24 (82.8)	0.002[b]
Intimal fibrin deposition, *n*(%)	8 (27.6)	10 (33.3)	6 (20.7)	7 (24.1)	0.53[b]
Lipid rich plaque, *n*(%)	11 (37.9)	15 (50.0)	10 (34.5)	8 (27.6)	0.25[b]
Calcified plaque, *n*(%)	20 (69.0)	20 (66.7)	21 (72.4)	13 (44.8)	0.10[b]
Biological data					
Carbonyl, nmol, median (IQR)	8.1 (6.6–9.5)	8.1 (6.1–9.3)	9.0 (5.3–9.7)	5.5 (4.8–9.8)	0.84[c]
DNA, ng/mL, median (IQR)	1100 (631–1505)	1308 (654–2771)	1360 (808–2379)	2194 (1258–2631)	0.005[c]
Myeloperoxidase, μg/mL, median (IQR)	61 (33–118)	145 (44–214)	87 (55–186)	230 (127–386)	<0.001[c]
Lipopolysaccharides, EU/mL, median (IQR)	0.30 (0.15–0.84)	0.73 (0.41–1.07)	0.42 (0.28–1.00)	1.50 (0.73–2.29)	<0.001[c]
DNA-MPO complexes, median (IQR)	1.04 (0.49–1.58)	2.19 (0.67–3.37)	1.43 (0.81–3.17)	3.53 (2.69–4.03)	<0.001[c]
NFκB activity, median (IQR)	0.26 (0.20–0.31)	0.28 (0.21–0.31)	0.29 (0.24–0.57)	0.34 (0.27–0.42)	0.035[c]
Presence of ≥1 bacterial species, *n*(%)	19 (65.5)	21 (70.0)	20 (69.0)	24 (82.8)	0.18[b]
Presence of Pg DNA, *n*(%)	11 (37.9)	15 (50.0)	11 (37.9)	10 (34.5)	0.58[b]
Presence of Tf DNA, *n*(%)	8 (27.6)	6 (20.0)	13 (44.8)	14 (48.3)	0.03[b]
Presence of Pi DNA, *n*(%)	9 (31.0)	9 (30.0)	8 (27.6)	9 (31.0)	0.95[b]
Anti-Tf IgG (EU)	0.021 (0.004–0.078)	0.053 (0.023–0.1)	0.080 (0.044–0.106)	0.119 (0.075–0.182)	<0.001[c]

[a] Analysis of variance trend test.
[b] Mantel-Haenszel trend test.
[c] Non-parametric analysis of variance trend test.