Nutrition, Metabolism & Cardiovascular Diseases (2017) 27, 768–774

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

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

Carotid plaque instability is not related to quantity but to elemental composition of calcification

S. Bischetti [a,1], M. Scimeca [a,b,c,1], E. Bonanno [a], M. Federici [e,f], L. Anemona [a], R. Menghini [e,f], S. Casella [a], M. Cardellini [e,f], A. Ippoliti [d], A. Mauriello [a,*]

[a] Department of Experimental Medicine and Surgery, University of Rome "Tor Vergata", Rome, Italy
[b] OrchideaLab S.r.l., Via del Grecale 6, Morlupo, Rome, Italy
[c] IRCCS San Raffaele Pisana, 00166 Rome, Italy
[d] Vascular Surgery, Department of Biomedicine and Prevention, University of Rome "Tor Vergata", Rome, Italy
[e] Department of Systems Medicine, University of Rome Tor Vergata, 00133 Rome, Italy
[f] Center for Atherosclerosis, Department of Medicine, Policlinico Tor Vergata, 00133 Rome, Italy

Received 22 December 2016; received in revised form 10 May 2017; accepted 22 May 2017
Available online 31 May 2017

KEYWORDS
Cardiovascular disease;
Atherosclerosis;
Plaque calcification;
Hydroxyapatite;
Energy dispersive X-ray microanalysis

Abstract *Background and aims:* Recent studies highlighted the role of calcification processes in the clinical progression of chronic cardiovascular diseases. In this study we investigated the relationship between the chemical composition of calcification and atherosclerotic plaque stability in carotid arteries.
Methods and results: To this end, we characterized the calcification on 229 carotid plaques, by morphology, immunohistochemistry, transmission electron microscopy and energy dispersive X-ray microanalysis. Plaques were classified into two categories: unstable and stable. No significant differences were found in the incidence of the various risk factors between patients with and without carotid calcification, with the exception of diabetes. The energy dispersive X-ray microanalysis allowed us to identify two types of calcium salts in the atheromatous plaques, hydroxyapatite (HA) and calcium oxalate (CO). Our results showed that calcification is a common finding in carotid plaques, being present in 77.3% of cases, and the amount of calcium is not a factor of vulnerability. Noteworthy, we observed an association between HA calcification and unstable plaques. On the contrary, CO calcifications were mainly detected in stable plaques.
Conclusions: The presence of different types of calcification in atheromatous plaques may open new perspectives in understanding the molecular mechanisms of atheroma formation and plaque instability.

Introduction

A pathological deposition of minerals or organic compounds was found in different tissues and was linked with different diseases, such as cancer and cardiovascular abnormalities [1]. In fact, calcification plays a critical role in the formation of important lesions in several diseases, such as atherosclerosis [2].

For long time, plaque calcification process was considered as an unregulated and dystrophic event, unrelated to physiological mechanisms. Conversely, in the last few

* Corresponding author. University of Rome Tor Vergata, Via Montpellier, 1, 00133 Rome, Italy.
E-mail addresses: simone.bischetti@gmail.com (S. Bischetti), manuel.scimeca@uniroma2.it (M. Scimeca), elena.bonanno@uniroma2.it (E. Bonanno), federicm@uniroma2.it (M. Federici), anemona@uniroma2.it (L. Anemona), menghini@med.uniroma2.it (R. Menghini), sara.casella.1987@gmail.com (S. Casella), cardellitti@med.uniroma2.it (M. Cardellini), ippoliti@med.uniroma2.it (A. Ippoliti), Alessandro.mauriello@uniroma2.it (A. Mauriello).
[1] Simone Bischetti and Manuel Scimeca equally contributed to the work.

http://dx.doi.org/10.1016/j.numecd.2017.05.006

years the presence of tissue calcification was reassessed and linked to regulated processes involving signals and pathways similar to the bone calcification process [3].

In particular, in the atherosclerosis lesions, the calcification process is strictly connected to areas of inflammatory activity where lipid oxidation and endothelial damage are observed, often concomitantly to microcalcifications in lipid deposition sites [4]. The formation of these microcalcifications can be mediated by cell death and release of apoptotic bodies in these inflamed sites. Apoptotic bodies are similar to vesicles found in the bone matrix, which contain the components required for the deposition of calcium crystals (including calcium and inorganic phosphate ions) and which facilitate the formation of needle-shaped crystals of hydroxyapatite (HA). Once larger hydroxyapatite crystals are formed, they cause the perforation of the outer membrane of vesicles and trigger the deposition of new calcium crystals via interaction with the extracellular environment [5]. Conversely, deposits of calcium oxalate (CO) arise when the solubility of this insoluble salt is exceeded. Often CO crystals were observed in atherosclerotic plaques in the coronary arteries [6].

The role of calcification on plaque vulnerability has been mainly studied in the coronary arteries.

Several studies using cardiac computed tomography (CT) have reported that the total coronary artery calcium (CAC) score is a well-established marker of coronary plaque burden and is associated with a high risk of adverse cardiovascular outcomes [7–9]. In a recent study, we demonstrated that coronary calcification has not itself a plaque vulnerability factor, because CAC do not predict the segment that will undergo rupture. Data on the role of calcium deposition on carotid plaques are still controversial [10,11]. Whether calcification has a stabilizing effect on the carotid plaque and makes it less prone to failure [12–15] or increases the risk of rupture [16–19] is still a matter of debate. It is tempting to speculated that different types of calcification may determine different effects on the stability of atherosclerotic plaque. Therefore, the main aim of this study was to investigate the relationship between presence and type of calcification and plaque phenotype in carotid arteries.

Methods

Cases selection

Our population comprised a total of 229 specimens from The Interinstitutional Carotid Tissue Bank (ICTB) [20] and comprised symptomatic (major stroke or transient ischemic attack) and asymptomatic patients submitted to surgical CEA at the University of Tor Vergata (Rome, Italy). All asymptomatic patients showed a carotid stenosis >60%. Informed consent was obtained from the subjects for data collection. For each patient, we collected one carotid sample.

Clinical data including risk factors and symptoms were recorded. The risk factors were defined utilizing the following criteria. Patient was considered as hypertensive if systolic blood pressure was >140 mm Hg and/or a diastolic blood pressure >90 mm Hg, or receiving antihypertensive treatment at the time of carotid endarterectomy. Diabetes mellitus was diagnosed in patients with fasting blood glucose >126 mg/dL and/or on oral treatment or insulin therapy. Patients with tobacco dependence were divided into smokers and former smokers. Former smokers who had stopped smoking less than five years before were considered as smokers and patients who had not smoked for >5 years were considered as non-smokers. Hypercholesterolemia was diagnosed in patients with total cholesterol level >200 mg/dL (>5.18 mmol/L), regardless of statin therapy and hypertriglyceridemia. Similar to hypercholesterolemia, patients were considered as hypertriglyceridemic when serum triglycerides levels were >150 mg/dL (>1.70 mmol/L) regardless of statin therapy. Abdominal obesity was diagnosed in patients with a waist circumference >102 cm in men or >88 cm in women.

Histology

The sampling collection and analysis methods have been previously reported [21,22]. Intraoperatively, carotid plaques were removed in bloc and fixed immediately upon removal in 10% buffered formalin. Specimens were cut transversely every 5 mm, embedded in paraffin and stained with hematoxylin-eosin. For each plaque, three to ten sections were examined according to the extension of the plaque (mean 5 sections per artery). In this way, the entire plaque was evaluated for the presence of calcification. In addition to the presence of calcifications, the following histological components of the plaques were evaluated: presence of acute or organized thrombosis, cap rupture or erosion, cap thickness, extension of necrotic core, intraplaque hemorrhage and inflammation. The latter was evaluated at a magnification of >400 using a test grid with an area of 0.22 mm^2 and 10 fields per section were counted, 5 in the cap and 5 in the shoulder. A cut-off of 25 inflammatory cells (CD68 positive macrophages and CD3 positive T-lymphocytes) high magnification field was used to define the presence of a mild or heavy inflammation.

Plaque classification

Plaques were classified according to the modified American Heart Association atherosclerosis classification [23] into two categories: unstable and stable ones. Unstable plaques included the thrombotic plaques with cap rupture or erosion and the vulnerable plaque or thin-cap fibroatheroma (TCFA), characterized by a fibrous cap less than 165 μm thick heavily infiltrated by macrophages, CD68 positive (>25 per high magnification field), without plaque rupture. Stable plaques were divided in fibroatheromatous and healed plaques. Fibroatheromatous plaques showed a thick fibrous cap (>165 μm) associated to the presence of calcification and a variable necrotic core. Healed plaques were defined as those showing multilayers of fibrous tissue and lipid rich necrotic core.