Distribution of Inflammatory Mediators in Carotid and Femoral Plaques

Wei Zhou, MD, Hong Chai, MD, PhD, Richard Ding, PhD, Ho Yin Colin Lam

BACKGROUND: Although atherosclerosis is a known systemic process, carotid and femoral atherosclerotic plaques are associated with distinctive complications and therapeutic outcomes. The purpose of this study was to evaluate inflammatory markers associated with carotid and femoral plaques.

STUDY DESIGN: Carotid and femoral endarterectomy specimens were harvested from surgical patients. The carotid specimens were further sectioned into central, peripheral, and relatively normal regions. Expressions of matrix metalloproteinase (MMP)-2 and MMP-9 in carotid and femoral specimens were compared and the distributions of MMP-2, MMP-9, and CD40 within the carotid specimens were further analyzed. Messenger RNA (mRNA) levels were measured with real-time polymerase chain reaction and proteins with ELISA assay and Western blot.

RESULTS: Despite no significant difference in the MMP-2 mRNA levels between carotid and femoral specimens, the common femoral specimens had significantly lower MMP-2 protein production and higher MMP-9 mRNA and protein expressions than those of the carotid specimens ($p = 0.015$, $p = 0.03$, and $p = 0.034$, respectively). Among carotid specimens, MMP-2 mRNA level was significantly lower in the central region ($p < 0.01$) and consistent with the distribution pattern of MMP-2 proteins. Interestingly, despite significantly lower MMP-9 mRNA expression in the central region of carotid plaques, active MMP-9 protein level was significantly higher. CD40 mRNA and protein levels were also higher in the central region. Immunohistochemistry analyses showed substantially increased macrophages and CD40 in the central region of the carotid plaques.

CONCLUSIONS: This study emphasizes that femoral and carotid plaques are distinct entities and that distribution of inflammatory molecular markers varied within these advanced atherosclerotic plaques. Additional analyses are warranted. (J Am Coll Surg 2010;211:92–98. © 2010 by the American College of Surgeons)

Atherosclerosis is a known systemic disease that affects multiple vascular beds and is the leading cause of death in the United States. Epidemiologic data suggest that the prevalence of peripheral arterial diseases (PAD) is 12.2% for American adults older than 60 years, increasing to 23.2% for those older than 80 years. The 10-year risk of death after a diagnosis of PAD is 40%.[1] Consequently, tremendous efforts have been made to investigate the pathophysiology and treatment strategies of PAD. The most common forms of PAD are carotid atherosclerotic diseases and lower-extremity obstructive diseases. Atherosclerotic lesions of carotid arteries are often associated with cerebrovascular accidents secondary to dislodge of embolic debris from the lesions. Advanced atherosclerotic lesions of femoral arteries are often obstructive, leading to lower-extremity ischemia, with symptoms ranging from mild claudication to major limb loss. Both forms of PAD frequently lead to symptoms that severely limit a patient's ability to perform daily routine activities and invariably create an enormous burden on health care costs.

Inflammatory mediators, including matrix metalloproteinase (MMP) and CD40 ligand (CD40L), are shown to play essential roles in development of atherosclerotic lesions.[2] Olson and colleagues showed that plasma MMP-9 levels were associated with atherosclerosis in the femoral artery and total MMP-9 concentration was higher in men with echolucent femoral plaques.[3] Orbe and colleagues concluded that MMP-9 was detected mainly in carotid as

Disclosure Information: Nothing to disclose.

This work is partially supported by the American College of Surgeon Faculty Research Fellowship (Zhou).

Abstract presented at the American College of Surgeons 95th Annual Clinical Congress, Surgical Forum, Chicago, IL, October 2009.

Received December 29, 2009; Revised February 9, 2010; Accepted February 26, 2010.

From the Division of Vascular and Endovascular Surgery, Department of Surgery, Stanford University, Stanford, CA (Zhou, Chai, Ding) and Cupertino High School, Cupertino, CA (Lam).

Correspondence address: Wei Zhou, MD, Division of Vascular and Endovascular Surgery, Department of Surgery, Stanford University, 300 Pasture Dr, H3640, Stanford, CA 94305. Email: weizhou@stanford.edu

ISSN 1072-7515/10/$36.00
doi:10.1016/j.jamcollsurg.2010.02.054

Abbreviations and Acronyms

CD40L = CD40 ligand
CEA = carotid artery endarterectomy
CFA = common femoral artery
MMP = matrix metalloproteinase
mRNA = messenger RNA
PAD = peripheral vascular disease
PARIS = Protein and RNA Isolation System
PCR = polymerase chain reaction.

compared with femoral arteries, and that the differences in the MMPs/tissue inhibitor of metalloproteinase−1 expression might determine the evolution of advanced atherosclerotic plaques and contribute to its vulnerability.[4] Others have shown that the presence of echolucent femoral plaques is associated with a greater prevalence of echolucent carotid plaques, probably as a consequence of a more pronounced inflammatory profile; and that CD40−CD40L interaction plays an important role in atheroma formation and MMP activation.[5,6]

Recognizing the roles of inflammatory mediators within the carotid and femoral plaques will help us to understand the pathogenesis of atherosclerotic lesions in these 2 distinct vascular beds, which, in turn, might enable us to provide lesion-directed target therapy. The purpose of this study was to evaluate inflammatory markers associated with carotid and femoral plaques and to analyze distributions of MMP-2, MMP-9, CD40, and macrophages within carotid atherosclerotic plaques.

METHODS

Chemicals and reagents

Recombinant human soluble CD40L was obtained from PeproTech. Endotoxin level in CD40L is <0.1 ng/μg (1EU/μg). Protein and RNA Isolation System (PARIS) and RNAlater were obtained from Ambion. Antibodies against active human MMP-9 were obtained from Thermo Scientific. ELISA kits were obtained from CalBiochem. High-capacity RNA-to-DNA kit and Power SYBR Green PCR Master Mix were obtained from Applied Biosystems. Trypsin/ethylenediamine tetraacetic acid, rabbit immunoglobulin G1 (isotype control immunoglobulin), and other chemicals were obtained from Sigma Chemical Co., unless otherwise specified.

Tissue collection

Patients who underwent routine carotid artery endarterectomy (CEA) or common femoral artery (CFA) endarterectomy were included in the study. All study subjects signed the informed consent before operations and the study was approved by the local Institutional Review Board and the hospital R&D committee. The atherosclerotic plaque from CEA and CFA that would otherwise be discarded were harvested and immediately stored in RNAlater at 4°C in a refrigerator before analyses. For carotid specimens, samples were divided into central and peripheral regions. A 3-mm transverse segment was cut from the center of each plaque or the peripheral region of the plaque. Then, a 3-mm segment that was obtained from the very proximal edge of a CEA specimen resembling less diseased common carotid artery was used as the normal control (Fig. 1). No peripheral or normal control area in the CFA specimen was identified.

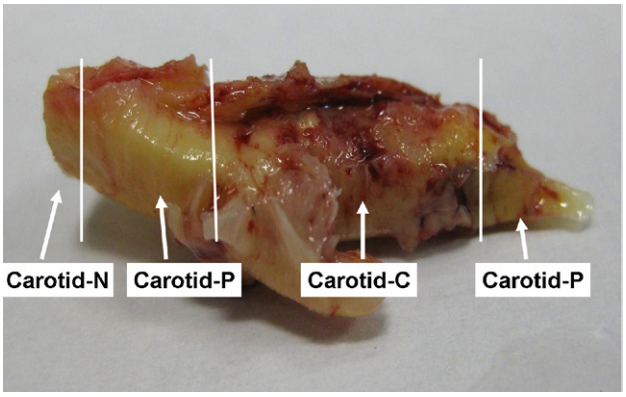

Figure 1. A carotid plaque being divided into 3 sections, including central (C), peripheral (P), and relatively normal (N) regions.

Patient clinical information was collected from the hospital electronic medical records system. Cardiovascular risk factors analyzed included history of smoking; diabetes mellitus; documented hypertension; hyperlipidemia; documented coronary artery disease on persantine-thallium nuclear stress test or a transthoracic echocardiogram; and chronic renal insufficiency, which was defined by serum creatinine >1.5 mg/dL. Additionally, medications including statin and aspirin were documented and preoperative symptoms were evaluated.

Real-time polymerase chain reaction

Specimens were placed into a standard extraction buffer (PARIS kit) and homogenized using an electrical homogenizer (Tissuemiser; Fisher Scientific). Homogenates were then centrifuged at 15,000 rpm for 20 minutes (4°C). Subsequent supernatants were collected for analyses. Total RNA from the specimen segments was isolated with the PARIS kit according to manufacturer's directions. Complementary DNA was generated by reverse transcription from messenger RNA (mRNA) with the use of the High Capacity RNA-to-DNA kit. The Power SYBR Green PCR Master Mix was used for real-time polymerase chain reac-