Expression of Matrix Metalloproteinases and Tissue Inhibitor of Metalloproteinase-1 in Carotid Stenosis

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(Received January 21, 2014; Revised January 28, 2014; Accepted February 4, 2014)

Abstract

It is still controversial whether the specific subtypes of matrix metalloproteinases are related to clinical characteristics in carotid stenosis patients. Carotid atherosclerotic plaques were obtained from 34 patients, 21 males and 13 females with mean age of 71.1. Carotid endarterectomy was performed under the monitoring of EEG and carotid stump pressure. Expression of matrix metalloproteinase-2, 8, 9, and tissue inhibitor of metalloproteinase-1 in the atherosclerotic plaque was assessed by immunohistochemical slides stained with primary antibodies semi-quantitatively. Twenty patients (58.8%) were symptomatic stenosis and 15 (44.1%) had stable plaques without evidence of fibrous cap rupture. Strong stain (degree 3) of matrix metalloproteinase-2 and 9 was dominant in asymptomatic group (P= 0.026 and P= 0.0007, respectively). Strong stain of matrix metalloproteinase-2 was dominant in stable plaque group (P= 0.026). Specific subtypes of matrix metalloproteinases can be related to clinical characteristics such as symptoms or plaque morphology. (J Med Life Sci 2014;10(3):209-213)

Key Words : Atherosclerosis Carotid Arteries Matrix metalloproteinases

Introduction

Carotid stenosis is part of systemic atherosclerosis which causes various neurologic disabilities or deaths. Recent studies have presented that various subtypes of the matrix metalloproteinases (MMPs) family showed increased or decreased expression in plasma or atherosclerotic plaques[1] and they have suggested that MMPs might play divergent roles in atherosclerotic process such as remodeling of extracellular matrix or plaque rupture[2]. However, it still remains controversial whether the expression of specific subtypes of MMPs family is related to clinical characteristics or plaque morphology in carotid stenosis patients. This study is focused on analyzing the relationship between clinical characteristics and the degree of expression of MMP-2, MMP-8, MMP-9, and tissue inhibitor of metalloproteinase-1 (TIMP-1) in carotid stenosis.

Materials and Methods

Thirty-four patients who underwent endarterectomy for symptomatic or asymptomatic carotid stenosis from March 2010 to December 2012 were included. This study was approved by Institutional Review Board of Jeju National University Hospital. The patients' characteristics are presented in Table 1.

Table 1. Patients' characteristics (n=34)

Age (Mean, years)		71.1	
Sex	M	21	(61.7%)
	F	13	
Hypertension or diabetes		28	(82.4%)
Dyslipidemia		10	(29.4%)
Atherosclerotic disease*		9	(26.5%)
Current smoker		8	(23.5%)

* It means history of diagnosis or treatment for coronary atherosclerosis, atherosclerotic aneurysm of aorta, or any type of peripheral arterial atherosclerotic disease.

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This study is supported by grant from Department of Industry-University Cooperation in Jeju National University (2013).

Symptomatic carotid stenosis patients who did not have contraindications for general anesthesia based on the medical history, careful physical examination, and routine laboratory studies were treated by carotid endarterectomy (CEA). Asymptomatic patients who showed rapid progression of carotid stenosis on MRI or carotid duplex, and more than 70% of stenosis at initial evaluation also underwent CEA. CEA was performed under general anesthesia and monitoring of EEG. Temporary carotid shunt (Pruitt-Inahara outlying carotid shunt, LeMaitre Vascular, Burlington, USA) was selectively used based on the intraoperative EEG findings and carotid stump pressure. If carotid stump pressure was less than 40mmHg or changes in EEG after clamping of carotid artery, the shunt was used. Postoperative intensive care such as strict control of blood pressure and systemic neurologic examination was performed and follow-up diffusion MRI was used based on the mental status or neurologic symptoms.

Immunohistochemical analysis for the expression of the MMPs and TIMP-1 was performed as follows. Formalin-fixed paraffin-embedded tissue blocks of the endarterectomy specimens were cut at 4 mm slices, deparaffinized in xylene, and rehydrated with graded ethanol. A standard immunohistochemical technique was performed using a Ventana Benchmark XT immunostainer (Ventana Medical Systems Inc., AZ, USA). Heat epitope retrieval provided by the immunostainer was performed for 60 minutes. The enzymatic reactivity was visualized with 3, 3' - diaminobenzidine. The primary antibodies used were anti-MMP-2 (Diagnostic BioSystems, California, USA; clone mom 312; dilution 1:25), anti-MMP-8 (Santa Cruz Biotechnology Inc, TX, USA; clone H-45; dilution 1:400), anti-MMP-9 (Abnova, Taipei, Taiwan; clone SB15c, dilution 1:1000), and anti-TIMP-1 (Abnova, Taipai, Taiwan; clone 2A5; dilution 1:50). They were diluted with PBS buffer-based dilution solution and incubated for 32minutes at 37℃. For the negative controls, the slides were stained by omitting the primary antibody from the protocol and substituting it with commercially available non-immune mouse immunoglobulin G serum (DAKO, Carpinteria, CA, USA).

The immunohistochemical slides were evaluated and interpreted by one pathologist who was blinded to the patients' clinical findings. The protein expression was scored by staining intensity and positive areas; 0 (no signal), 1 (focal weak reaction), 2 (focal strong or diffuse weak reaction), or 3 (diffuse strong reaction).

A Pearson chi square test (SPSS, version 12.1 for Windows, Chicago, IL, USA) was used for determining the differences in expression of MMPs and TIMP-1 between various clinical characteristics; a p-value < 0.05 was considered statistically significant.

Results

Twenty (58.8%) patients had symptoms of stroke or transient ischemic attack within 6 weeks of surgery. Temporary shunt was used in nine (26.5%) patients during CEA. Although two of them showed initial stump pressure higher than 40mmHg, temporary shunt was used unexpectedly because they showed abrupt slowing and asymmetry in EEG after clamping of internal carotid artery. After temporary shunt was applied, asymmetric EEG disappeared immediately and they did not have any neurologic sequels postoperatively. Intracerebral hemorrhage was detected immediately after the operation in one patient and no significant postoperative neurologic complications were detected in other patients.

When we compare the asymptomatic group (n=14) to symptomatic group (n=20), the proportion of patients with hypertension or diabetes was significantly higher in symptomatic group (P=0.021) and there were no significant differences in other clinical characteristics. When we define the 'stable plaque' as plaque with intact fibrous cap (without evidence of plaque rupture) and 'vulnerable plaque' as plaque with ruptured fibrous cap and exposed red thrombi or lipid core debris, there was no difference of plaque morphology between asymptomatic and symptomatic groups. When the degree of expression '3' is considered to identify 'strong stain' and the degree of expression '2,1, and 0' are considered to identify 'moderate to weak stain' , MMP-2 and MMP-9 showed significant difference in expression between asymptomatic and symptomatic group (P= 0.026 and P= 0.0007, respectively) (Table 2) (Fig 1). There were a larger number of 'strong stain' of MMP-2 and MMP-9 in asymptomatic group. When we compare stable plaque group (n=15) to vulnerable plaque group (n=19), there were no significant difference in age, or proportion of symptomatic patients. When we use the above-mentioned classification according to the degree of expression, MMP-2 showed significant difference between stable and vulnerable plaque group. There was a larger number of 'strong stain' of MMP-2 in stable plaque (P= 0.026) (Table 3).

Table 2. Differences in clinical characteristics and MMPs expression between the asymptomatic and symptomatic group

	Asymptomatic group (n=14)	Symptomatic group (n=20)	
Age (mean, years)	68.6	72.8	
Sex	M:F 7:7	M:F 14:6	
Hypertension or diabetes	10 (71.4%)	18 (90%)	P=0.021
Current smoker	4 (28.6%)	4 (20%)	
Stable plaque (n=15)	6 (43%)	9 (45%)	
Ruptured plaque (n=19)	8 (57%)	11 (55%)	
MMP-2			
Strong stain	8	4	
Moderate to weak stain	6	16	P= 0.026
MMP-8			
Strong stain	4	8	
Moderate to weak stain	10	12	P=0.493
MMP-9			
Strong stain	11	4	
Moderate to weak stain	3	16	P=0.0007
TIMP-1			
Strong stain	4	8	
Moderate to weak stain	10	12	P=0.493

* It means history of diagnosis or treatment for coronary atherosclerosis, atherosclerotic aneurysm of aorta, or any type of peripheral arterial atherosclerotic disease.

Table 3. Differences in clinical characteristics and MMPs expression between stable plaque group and vulnerable plaque group

	Stable plaque (n=15)	Vulnerable plaque (n=19)	
Age (mean, years)	70.5	71.5	P= 0.727
Sex	M:F 9:6	M:F 12:7	
Hypertension or diabetes	10 (66.7%)	15 (78.9%)	P= 0.278
Current smoker	3	5	
Asymptomatic patients (n=14)	6 (40%)	8 (42.1%)	
Symptomatic patients (n=20)	9 (60%)	11 (57.9%)	
MMP-2			
Strong stain	12	8	
Moderate to weak stain	3	11	P= 0.026
MMP-8			
Strong stain	5	7	
Moderate to weak stain	10	12	P= 0.493
MMP-9			
Strong stain	8	13	
Moderate to weak stain	7	6	P= 0.371
TIMP-1			
Strong stain	8	4	
Moderate to weak stain	7	15	P= 0.050

* It means history of diagnosis or treatment for coronary atherosclerosis, atherosclerotic aneurysm of aorta, or any type of peripheral arterial atherosclerotic disease.

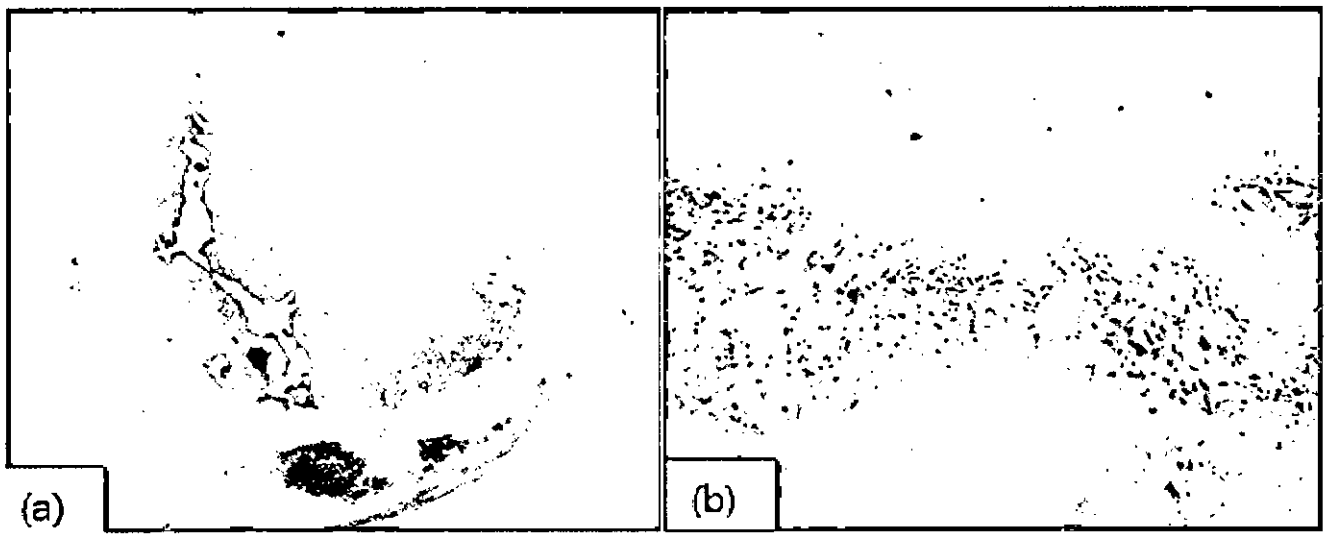

Figure 1. Photomicrographs of immunohistochemistry results: Stable plaque (fibrocalcified plaque without rupture of fibrous cap) (a) shows strong (degree 3) stain of MMP-2 (b).

Discussion

MMPs are a family of membrane-bound proteases which are structurally related and share a Zn^{2+}-based catalytic mechanism[3]. Because it has been suggested that overactivation of MMPs causes destruction of extracellular matrix rather than remodeling[2], it is considered that MMPs play a role in rupture of fibrous cap leading to neurologic ischemic events. Therefore, it was initially hypothesized that symptomatic carotid stenosis patients may have a larger number of vulnerable plaques and show stronger expression of various subtypes of MMPs as well as weak expression of TIMP because uncontrolled expression MMPs and subsequent thinning of fibrous cap by MMPs' degradation of extracellular matrix would finally lead to stroke. Shah et al reported that human monocyte-derived macrophages induces collagen breakdown in fibrous cap of atherosclerotic plaque and such activities are suppressed by MMP inhibitors in acute coronary syndromes[4]. It has also been shown that highly inflamed atheromatous plaques presented increased global MMP activities and rupture-prone human plaques had increased MMP-8, 11, 14, and 16[5].

. However, there were no significant differences in plaque morphology between asymptomatic and symptomatic patients. Shishkina et al reported that 77% of endarterectomy specimens removed from asymptomatic carotid stenosis patients showed ruptured plaques[6] and Jayasooriya et al suggested that silent cerebral infarction on brain CT and microemboli detected on transcranial doppler (TCD) are risk factors of future stroke in asymptomatic carotid stenosis[7]. Based on such studies, asymptomatic carotid stenosis patients can have silent ruptured plaques. Asymptomatic patients of this study were clinically silent at the time of operation and may have unrecognized infarction or microemboli. Although TCD was performed as a part of routine preoperative evaluation for the included patients, microemboli were not detected in any asymptomatic patients.

Although most of studies concerning the role of MMPs in atherosclerosis have suggested that they can promote macrophage invasion, plaque inflammation, and angiogenesis as well as thinning of fibrous cap, each subtype of MMPs may have different role in advances in atherosclerotic plaques. Sluijter et al reported that levels of MMP-2 are increased in fibrous rather than rupture-prone atheromatous human carotid plaques[8] and this is in accord with the result of this study. It was also suggested that MMP-2 and MMP-14 promote vascular smooth muscle cell migration and proliferation by increasing fibrous cap thickness, which lead to promoting plaque stability[9]. MMP may have dual role in plaque rupture and stability or vulnerability may be determined by expression of predominant subtype of MMP and their level of activities[10]. Further study concerning the role of MMPs in carotid stenosis should focus on relationship between the selective inhibition or activation of specific subtypes MMPs and clinical features and it seems to be used as therapeutic target. In conclusion, MMP-2 and MMP-9 showed increased expression in asymptomatic carotid stenosis and MMP-2 showed increased expression in stable plaque rather than vulnerable plaque. Further study should be performed to investigate the role of each subtype of MMPs in carotid atherosclerotic process.

Acknowledgement

This study is supported by the grant from Department of Industry-University Cooperation in Jeju National University.

References

1) Nilsson L, Jonasson L, Nijm J, Hamsten A, Eriksson P. Increased plasma concentration of matrix metalloproteinase-7 in patients with coronary artery disease. Clin Chem 2006;52:1522-7
2) Newby AC. Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol Rev 2005;85:1-31
3) Newby AC. Metalloproteinases and vulnerable atherosclerotic plaques Trends Cardiovasc Med 2007;17(8):253-8
4) Shah PK, Falk E, Badimon JJ et al. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. Circulation 1995;92:1565-9
5) Choudhary S, Higgins CL, Chen IY et al. Quantitation and localization of matrix metalloprotienases and their inhibitors in human carotid endarterectomy tissues. Arterioscler Thromb Vasc Biol 2006;26:2351-8
6) Shinshkina VS, Toklueva LR, Kashirina SV et al. Comparison of morphological features of carotid arteries atherosclerotic plaques with clinical-instrumental data in symptomatic and asymptomatic patients with severe carotid atherosclerosis. Kardiologiia 2013;53:25-31
7) Jayasooriya G, Thapar A, Shalhoub J, Davies AH. Silent cerebral events in asymptomatic carotid stenosis. J Vasc Surg 2011;54:227-36

8) Sluijter JP, Pulskens WP, Schoneveld AH et al. Matrix metalloproteinase 2 is associated with stable and matrix metalloproteinases 8 and 9 with vulnerable carotid atherosclerotic lesions - a study in human endarterectomy specimen pointing to a role for different extracellular matrix metalloproteinases inducer glycosylation forms. Stroke 2006;37:235-9
9) Newby AC. Matrix metalloproteinases regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates. Cardiovasc Res 2006;69:614-24
10) Hower CD, Dassow MS, Kajdacsy-Balla A, Seabrook GR, Jean-Claude J, Towne JB, Cambria RA. Metalloproteinases levels are decreased in symptomatic carotid plaques. J Surg Res 2000;88:155-9