RESEARCH ARTICLE

Biochemical Markers for Determining Vulnerable Atherosclerotic Plaque in Stenotic Patient

A Biochemical Markers Study of Myeloperoxidase (MPO), Matrix Metallo-Proteinase-9 (MMP-9), Secretory Phospholipase A2 (SPLA2) and CD40 Ligand

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BACKGROUND: Thrombus is a main cause of cardiac death. Therefore identifying which coronary artery plaque is vulnerable to rupture is a critical step for cardiac intervention to prevent future cardiac events. Systemic biochemical markers are used for predicting rupture of coronary plaque or identifying stenotic coronary artery plaque(s) vulnerable to rupture.

METHODS: Blood samples of 2 x 24 locations (2 x 10 controls, 2 x 12 stable plaques and 2 x 2 unstable plaques) of 13 patients to undergo stent placement were taken from an artery which showed no stenosis (control), 70% or more stenosis of stable plaques and unstable plaques, respectively. The blood samples were taken by using micro-catheter distally and proximally. Concentrations of MPO, MMP-9, SPLA2 and CD40L of each sample were assayed.

RESULTS: Concentration of MMP-9 in unstable coronary artery plaque (94.7 + 14.4 ng/ml) significantly increased compared with that of stable coronary artery plaque (71.0 + 67.8 ng/ml, p=0.024). SPLA2 concentration significantly decreased in unstable coronary artery plaque (45.9 + 14.0 pg/ml) compared with that of stable coronary artery plaque (80.9 + 39.3 pg/ml, p=0.015). Nine of ten studied subjects showed an average of 14.5% (range: 0.0 – 28.8%) decrease of the SPLA2 concentration in stable plaques compared with that of the non-stenotic coronary artery.

CONCLUSION: MMP-9 increased in unstable coronary artery plaque compared with that of stable coronary plaque. Unstable coronary artery plaques absorbed SPLA2 from the vasculars more than the stable plaques and control plaques. MMP-9 and SPLA2 may be used as markers of stability of a plaque in coronary artery in relation to its rupture potential.

KEYWORDS: stable and unstable plaque, Myeloperoxidase, Matrix Metallo-Proteinase-9, Secretory Phospholipase A2, CD40 Ligand.

Introduction

Of 241 cardiac deaths, 125 or 52% are caused by acute thrombi related to ruptures, erosions, and calcified nodules (1). Vulnerable plaques have large lipid cores and thin fibrous caps, and are infiltrated by macrophages (2). Angiographic definition of unstable plaque underlines the existence of 50% or more stenosis accompanied by at least 2 of the followings: intra luminal filling defect, ulcer in the plaque, irregular surface, and impaired blood flow (3).

The association between Myeloperoxidase (MPO) and vulnerable plaque has been investigated recently (4). Libby et al. showed co-localization of macrophages that expressed MPO and HOCl modified protein in plaque of a person who died of cardiac event (5). MPO can be used as a predictor of plaque rupture (6), even in Troponin T negative patients (7).

Matrix Metalloproteinase-9 (MMP-9) can be used as predictor of cardiovascular mortality (8). Detectable level of MMP-9 in the circulation is associated with left ventricular dimension and thickness of the wall in men (9). Serum MMP-9 of stenotic patients is significantly

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higher than that of controls (10). Plasma level of MMP-9 increases in infarction and unstable angina (11).

Inwald et al. explain the role of CD40 mediator in activating platelet in thrombosis, inflammation and atherosclerosis (12). Monocytes/macrophages in plaque stimulated by CD40L or T-cell membrane of CD4+ express pro-coagulant tissue factor and matrix degradation proteinase (13). Using MRI, Blake has shown that CD40L is a marker of lipid accumulation in carotid atheroma (14). CD40L in blood reflects platelet activation and instability of plaque (15).

Circulating sPLA2-II in blood has been demonstrated to be able to predict coronary events in initially healthy subjects and in patients with frank coronary heart disease including acute coronary syndromes (16). Lipoprotein that contains Apo-B and SPLA2-IIA can facilitate enzymatic hydrolysis of phospholipid lipoproteins through interaction with proteoglycan in the artery. Further modification of lipoprotein causes aggregation and lipoprotein fusion within the plaque (17).

Methods

PATIENTS

Thirteen patients scheduled to undergo stent placement in the coronary artery were involved in the study. Blood samples were taken from 12 x 2 (distal and proximal of the plaque) coronary artery stable plaques from 11 patients, and 2 x 2 (distal and proximal of the plaque) coronary unstable plaques from another two patients. Control blood samples were taken from each of the same patients from coronary artery that showed no stenosis (0% stenosis).

METHODS

Blood samples from coronary artery were taken using a micro-catheter. Unstable plaque is defined according to the criteria of Goldstein. The level of CD40L and MMP-9 in the blood samples were measured using ELISA kits from R&D Systems. MPO and SPLA2 levels were measured using ELISA kits from Oxis Research and Cayman Chemicals, respectively.

STATISTICAL ANAL□SIS

□ruskal-□allis and Mann-□hitney tests were used to compare means between groups.

Results

Table 1. Characteristics of the study subjects

Variable	Stable Plaque	Unstable Plaque	Total
n	2x12	2x2	14
Male	11/12 (92%)	2/2 (100%)	13/14 (93%)
Smoking	6/11 (55%)	2/2 (100%)	8/13 (62%)
Diabetes mellitus	3/12 (25%)	0/2 (0%)	3/14 (21%)
Dyslipidemia	7/12 (58%)	1/2 (50%)	8/14 (57%)
History of stent placement	2/12 (17%)	0/2 (0%)	2/14 (14%)
History of cardiac by pass	0/12 (0%)	0/2 (0%)	0/14 (0%)
Family history of CAD	5/11 (45%)	2/2 (100%)	7/13 (54%)
History of heart attack	8/12 (67%)	2/2 (100%)	10/14 (71%)
Hypertension	6/12 (50%)	2/2 (100%)	8/14 (57%)
BMI ≥ 25 kg/m2	7/12 (58%)	2/2 (100%)	9/14 (64%)
Stenosis > 1 artery	10/12 (83%)	2/2 (100%)	12/14 (86%)
Average age	58,0 years	46,0 years	56,3 years

Characteristics of the study subjects are shown in Table 1. Characteristics of the coronary artery plaques are shown in Table 2.

Table 2. Characteristics of the coronary artery plaques

	Control	Stable Plaque	Unstable Plaque
n	10	12	2
Average stenosis (range)	0%	85% (70-95%)	93% (90-95%)
Plaque position	RC: 1 (10%) CX: 4 (40%) LM: 5 (50%)	RC: 4 (33%) CX: 3 (25%) LM: 5 (42%)	RC: 0 (0%) CX: 1 (50%) LM: 1 (50%)

RC = right coronary artery, CX = circumflex, LM = left main.

Levels of MMP-9, MPO, SPLA2 and CD40L of the distal and proximal coronary artery plaques are shown in Table 3.

Table 3. MMP-9, MPO, SPLA2 and CD40L levels (mean + SD) in the distal and proximal coronary artery plaques

Biomarker	Control S	table Plaque U	nstable Plaque p	[1)]
MMP-9 (ng/ml)	77.5 ± 74.4	71.0 ± 67.8	94.7 ± 14.4	0.024*
MPO (ng/ml)	16.6 ± 6.7	15.2 ± 6.3	21.0 ± 4.7	0.082
SPLA2 (pg/ml)	99.7 ± 48.4	80.9 ± 39.3	45.9 ± 14.0	0.015*
CD40L (ng/ml) 1	.07 ± 0.69 1	.11 ± 1.09 0	.79 ± 0.38 0	.681

[1)] between stable and unstable plaque
* significance (p<0.05)

Among the biomarkers used, MMP-9 showed a significant increase in unstable coronary artery plaque as compared with stable coronary artery plaque (p=0.024). On the other hand, SPLA2 showed a significant decrease in unstable coronary artery plaque compared with that in stable coronary artery plaque (p=0.015).

Assessment of each individual subject showed the average SPLA2 level of distal and proximal artery plaque decreased in 9 of 10 subjects with stable plaques as compared with the control, with an average decrease of 14.52% and in the range of 0.05 – -28.82%.

Discussion

MMP-9 level increased, but SPLA2 level decreased in unstable coronary artery plaques. MMP-9 is a proteinase that is able to degrade extracellular matrix to make the fibrous cap become thinner and thus prone to rupture. SPLA2 becomes atherogenic when associated with Apo-B. In other words, decrease levels of soluble free SPLA2 indicate increase of associated forms, as illustrated below:

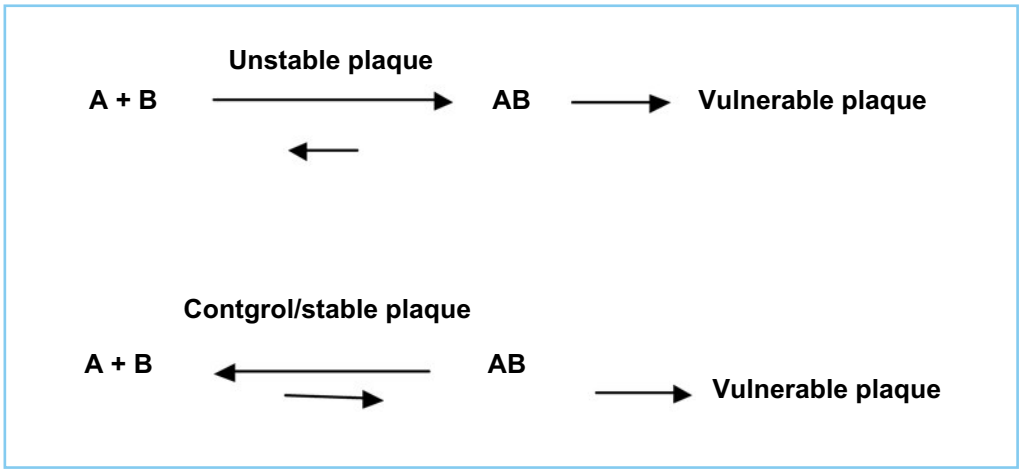

Figure 1. Schematic model of SPLA2 in associated and dissociated forms.
In vulnerable to rupture plaque SPLA2 is associated with LDL-cholesterol, but in stable or control plaques SPLA2 are in free form.
A=SPLA2, B=LDL-cholesterol.

MPO did not increase significantly in unstable plaque as compared with that in stable plaque. In plaque, MPO causes apoptosis of endothelial cells and erosion. According to Virmani, et al. (1), erosion occurs rarely in men, but the male subjects involved in this study comprised 92% of all study subjects.

CD40L di not increase or decrease significantly in unstable plaques as compared with that in stable plaques. It is possible that CD40L is a platelet activation marker, which plays role in thrombus generation after rupture of the plaque.

Conclusion

MMP-9 increased in unstable coronary artery plaques as compared with that in stable coronary plaques. □nstable coronary artery plaques absorbed SPLA2 from the vasculars in a greater amount than in stable plaques and non- plaques (control). In conclusion, MMP-9 and SPLA2 can be used as markers of stability of a plaque in coronary artery towards rupture vulnerability.

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