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The clinical implications of increased OX40 ligand expression in patients with acute coronary syndrome

Dong-Ming Liu, Jin-Chuan Yan *, Cui-Ping Wang, Guang-Hua Chen, Shu Ding, Pei-Jin Liu, Rong-Zeng Du

Department of Cardiology, Affiliated Hospital of Jiangsu University, Zhenjiang 212001, China

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ABSTRACT

Background: Increasing evidence show that OX40 ligand (OX40L), also known as tumor necrosis factor superfamily member 4 (TNFSF4), plays an important role in the pathogenesis of atherosclerosis. We investigated whether expression levels of soluble OX40L in serum and of membrane OX40L on platelets were related to serum concentrations of matrix metalloproteinases (MMPs) and stability of coronary atherosclerotic plaque in patients with acute coronary syndrome (ACS).

Methods: We included healthy controls ($n=30$), patients with stable angina (SA) ($n=40$) and patients with ACS, including unstable angina (UA) ($n=70$) and acute myocardial infarction (AMI) ($n=40$). The expression of OX40L on platelets (pOX40L) was analyzed with flow cytometry whereas serum concentrations of soluble OX40L (sOX40L), MMP-9 and MMP-3 were determined with ELISA. All coronary stenoses with ≥30% diameter reduction were assessed by angiographic coronary stenosis morphology.

Results: The expression of OX40L on platelets were significantly higher in patients with ACS (61.5±11.5) compared with healthy controls (28.9±7.4) or with the group of patients with SA (31.2±8.1) (mean fluorescence intensity±SD) ($p<0.001$). Similarly, we observed higher sOX40L concentrations in patients with ACS (34.6±9.3) compared with controls (10.2±4.7) or patients with SA (11.4±5.8) (ng/ml±SD) ($p<0.001$). Serum MMP-3 and MMP-9 levels in patients were two times greater than those in the control group. A positive correlation was observed between OX40L expression on platelets and MMP-9 and MMP-3 serum concentrations. OX40L expression on platelets were furthermore correlated with soluble OX40L in serum and with complex coronary stenoses ($r1=0.61$, $r2=0.57$, $p<0.001$).

Conclusion: Patients with ACS show increased OX40L system (pOX40L and sOX40L) expression which may create a proinflammatory milieu for aggravating the development of atherosclerosis, and may be a valuable marker for predicting the severity of ACS.

1. Introduction

Atherosclerosis, the predominant process underlying cardiovascular, is now considered to be a chronic (auto)-inflammatory disease [1–3]. Disruption of vulnerable atheromatous plaque is the most common pathogenic mechanism in acute coronary syndromes (including non-Q wave AMI, Q wave AMI and unstable angina). Integrity of the extracellular matrix constitutes a critical determinant in the stability of coronary atheromata. In particular, degradation of fibrillar collagen may decrease the ability of the fibrous cap to withstand mechanical stress. Several members of the MMP family contribute to collagen degradation: interstitial collagenase (MMP-1), stromelysin (MMP-3), and gelatinase B (MMP-9). It has been reported that MMPs play a pathogenic role in the development of ACS. Several receptor–ligand pairs are known to play a role in ACS. One of them is OX40/OX40 ligand. Studies have supported the emerging role of OX40/OX40L interaction in atherosclerosis. Recently, some researchers found that genetic variants in the OX40L locus were associated with myocardial infarction (MI) and severity of coronary artery disease in humans [4,5].

The presence of complex coronary plaques is known to be associated with rapid disease progression and adverse prognosis in patients with coronary artery disease. Complex lesions are thought to reflect a tendency toward thrombogenesis or further plaque disruption [6].

Until now, little information has addressed the potential relationship between OX40L expression and coronary complex stenosis morphology in patients with coronary artery disease (SA, UA and MI). Therefore, the present study was designed to investigate OX40L system expression (including pOX40L and sOX40L) and assess the correlation between them and the number of complex lesions in the patients. Furthermore, statistical correlation between MMPs (MMP3 and MMP9) and OX40L system was also evaluated.

2. Materials and methods

2.1. Reagents

Mouse–anti-human-OX40L, CD61-FITC and mouse IgG-PE-conjugates were from PharMingen. sOX40L ELISA kit was from Bender Medsystems (detection limit, 5 ng/ml).

* Corresponding author. Tel./fax: +86 511 85026169.
E-mail address: yanjinchuan@hotmail.com (J.-C. Yan).

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Table 1
Characteristics of the study groups

Groups	Control (n=30)	SA (n=40)	UA (n=70)	AMI (n=40)	p value[a]
Age, years	52±10	62±8	59±9	64±11	0.03
Sex, M/F	19/11	27/13	46/24	25/15	NS
Total cholesterol, mmol/l	4.7±0.7	4.9±0.8	5.1±0.9	5.0±1.1	0.12
HDL, cholesterol mmol/l	1.3±0.7	1.1±0.5	1.2±0.6	0.9±0.4	0.08
Triglycerides, mmol/l	1.6±0.3	1.5±0.4	1.7±0.8	1.8±0.9	NS
Medication, %					
Calcium antagonist	0	9	11	13	0.001
Nitroglycerin	0	13	21	19	0.001
ACEI	0	9	8	12	0.001
β-blockers	0	82	78	80	0.001
HMG-COA reductase inhibitors	0	61	64	67	0.001
Aspirin	0	95	98	98	0.001

[a] p-value patients with ACS and SA compared to control.

MMP-3 ELISA kit was obtained from Chemicon International Inc. (systems detection limit, 0.25 μg/l) and MMP-9 kit was from R&D systems (detection limit, 0.156 μg/l). Intra- and inter-assay CV was ≤6% for all tests.

2.2. Patients and control subjects

Patients undergoing clinically indicated diagnostic coronary angiography in our coronary care unit were consecutively registered. Seventy patients with unstable angina (Braunwald class III) had experienced ischemic chest pain at rest within the preceding 48 h, but without evidence of myocardial necrosis by enzymatic criteria. Forty patients with stable angina underwent coronary angiography because of signs and symptoms of clinically stable angina. Forty patients with AMI <6 h after the onset of symptoms. Inclusion criteria were typical chest pain and ST segment elevation in at least two contiguous electrocardiographic leads. For comparison, 30 sex- and age-matched volunteers served as a control group. Patients with infection, tumor, liver or kidney diseases were excluded (Table 1). All patients gave written informed consent.

2.3. Blood sampling protocol

Peripheral venous blood was drawn into blood collection tubes with or without sodium citrate. Citrated blood samples were centrifuged (200×g for 10 min at room temperature) to obtain platelet rich-plasma. Noncitrated blood samples were immersed in melting ice and allowed to clot for 1 h before centrifugation (1500 ×g for 10 min at 4 °C). The supernatant was stored at −80 °C until analysis. Samples were thawed only once.

2.4. Detection of OX40L on platelets by flow cytometry

Platelet immunostaining was performed as previously described [7]. Briefly, platelet rich-plasma was diluted 1:100 with PBS and incubated with the primary antibody (30 min, 4 °C). Then platelets were incubated with PE-conjugated second antibody (30 min, 4 °C) and analyzed using CELLQUEST software. For each treatment, the mean fluorescence intensity (MFI) value for the control stained population was subtracted from the MFI value of the positive-stained sample. Platelets were identified by gating on CD61-FITC positivity and their characteristic light scatter. The platelet population evaluated was ≥98% positive for CD61.

2.5. Measurements of MMP-9 and MMP-3

Levels of serum MMP-3 and MMP-9 were determined by ELISA according to the manufacturer's instructions.

2.6. Detection of sOX40L by ELISA

Levels of sOX40L were determined by ELISA (sOX40L detection limit, 5 ng/ml; with intra- and inter-assay CVs of <10% at different levels of sOX40L, Bender Medsystems) according to the manufacturer's instructions.

2.7. Angiographic coronary stenosis morphology

All coronary stenoses with ≥30% diameter reduction were assessed by two experienced cardiologists who had no knowledge of the results of serum soluble OX40L or the identity and clinical characteristics of the patients. Stenosis morphology was assessed as reported previously in several studies [8]. Briefly, stenoses were considered to be complex or smooth. Complex lesions were defined by the following features: 1) irregular morphology or scalloped borders, or both; 2) overhanging or abrupt edges perpendicular to the vessel wall; 3) ulceration; and/or 4) the presence of filling defects consistent with intracoronary thrombus. Coronary stenoses without complex features were classified as smooth lesions.

2.8. Statistical analysis

Statistical evaluation was performed with Graph pad software (Prism3.0) and SPSS11.5 software. Data were expressed as mean±SD and compared by unpaired t-test

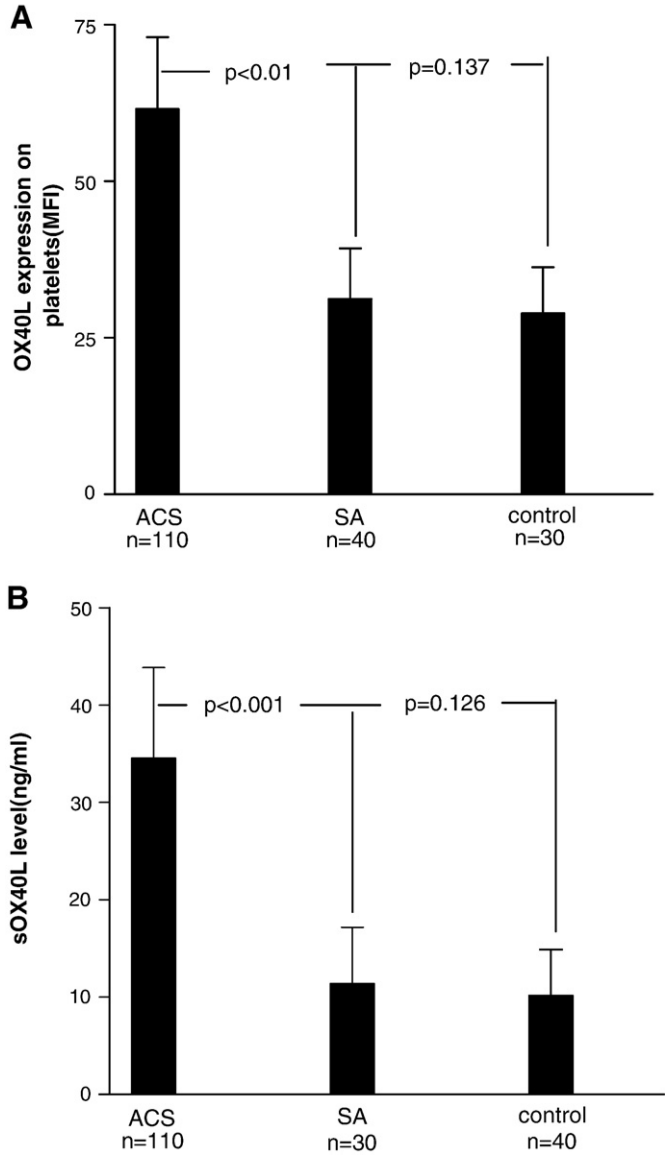

Fig. 1. OX40L expression on platelets and sOX40L in patients with ACS.

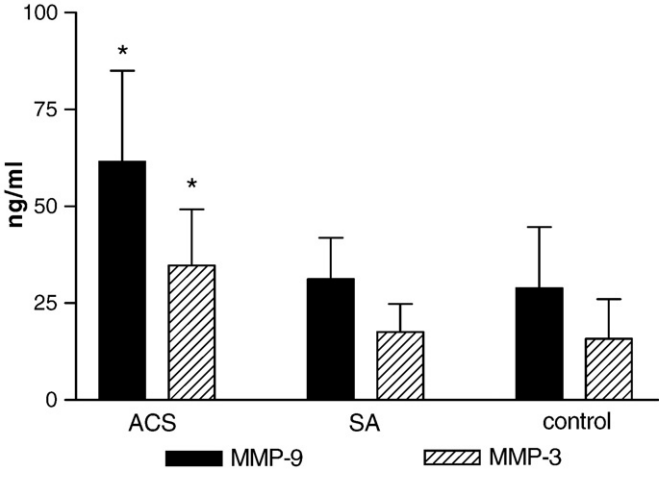

Fig. 2. Comparison of MMP-3 and MMP-9 serum levels between patients with ACS and controls (*p<0.01 vs SA group and controls).