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The autoantibody expression against different source of oxidized low density lipoprotein in patients with acute myocardial infarction

Tze-Che Wang, Chung-Chi Hsu, Yi-Ping Chin, Yuh-Ling Lin*

Department of Medicine, Taipei Medical University, 250 Wu-Hsing St., Taipei, Taiwan

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Abstract

The aim of this study was to examine the expression of antibodies against two different sources of low density lipoprotein (LDL) that were oxidized by $CuSO_4$, in patients with early stage of acute myocardial infarction (AMI). When LDL purified from sera with high level of LDL was used as a modified antigen, the results indicated that the titers of antibodies against the oxidized LDL in 30 patients were increased by 135% compared to those in normal subjects; however, the titers of antibody against modified LDL purified from normal-range LDL in the same patients were only slightly increased by 52%. Comparing the levels of autoantibody expressed in the high LDL sera group, high triglyceride sera group, and AMI patients sera group (total of 41; in addition to 30 AMI patients, 11 more sera of AMI patients were collected), the amount of autoantibody against the oxLDL purified from high LDL sera in AMI patients sera group was significantly increased up to 195%. In contrast to AMI patients, the sera titers against the same antigen in two subject groups with either high LDL or high triglyceride are only 50% higher than normal subjects. Moreover, the ratio of thromboxane B_2 over 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PG $F_{1\alpha}$) in the acute myocardial infarction patients was 1.79, which is much lower than the normal subjects, 4.19. Concluding from the above observations, we suggest that the expression level of anti-oxidized LDL antibody may play a role on the pathogenesis of acute myocardial infarction disease, but is independent with the levels of thromboxane A_2 and prostacyclin in the examined sera.

Keywords: oxLDL; Autoantibody; Acute myocardial infarction; Thromboxane; Prostacyclin

1. Introduction

The increasing evidence have shown that the formation of atherosclerosis is an inflammatory process in arterial wall and characterized by the accumulation of macrophages and activation of T lymphocytes. Thus, it is not surprising that autoimmune factors were involved in this process [1,2]. Several epidemiological studies showed high correlation between increased levels of antibodies against oxidized low density lipoprotein (oxLDL), and the expression of the antibodies was also associated with atherosclerosis vascular disease [3–5], progression of carotid arteriosclerosis [6], and potential development of myocardial infarction [7]. OxLDL is heterogenous, both the protein and the lipid moieties of the molecule can be modified [8]. The native and certain chemically modified forms of LDL have the ability to bind to scavenger receptors in vitro, which can lead to unregulated accumulation of cholesterol in macrophages and the formation of foam cells [9,10]. OxLDL could be one of the physiological ligands for scavenger receptor since the presence of oxLDL has been reported in vivo. For example, oxLDL could be extracted from atherosclerotic lesions [8,11,12], and its epitopes recognized by autologous antibodies were found in atherosclerotic lesions in human and animal models [8,13,14]. Autoantibodies reacted with oxLDL are both presented in plasma and lesions of human and animals [8,14–17]. In addition to oxLDL, two factors known to be associated with arterial thrombosis are thromboxane A_2 (Tx A_2) and prostacyclin (PG I_2). Particularly, for PG I_2, its deficiency was reported to cause coronary thrombosis or vasospasm leading to acute myocardial infarction disease (AMI) [18]. An increased expression of PG I_2 could significantly improve the binding activity to LDL in liver cell [19].

Although prognosis or diagnosis of AMI patients by detecting the existence of anti-oxLDL antibodies in the sera still remains controversial, the aim of this study was to

* Corresponding author. Tel.: +886-2-27361661x3152; fax: +886-3-3979280.

E-mail address: yllin@tmu.edu.tw (Y.-L. Lin).

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further examine the relative levels of autoantibody against two different sources of oxLDL in patients with early stage AMI disease when compared to normal subjects. In addition, correlation of the antibody expression level with the clinical diagnosis of AMI patients was reestimated. The correlations of the levels of thromboxane B_2 (product of Tx A_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PG $F_{1\alpha}$; product of PG I_2) with atherosclerosis were also determined in this study.

2. Materials

Bovine serum albumin (BSA; from fraction V), diethanolamine, *p*-nitrophenyl phosphate and alkaline phosphatase-conjugated goat anti-human IgG (Fab specific) were purchased from Sigma (St Louis, MO). Sodium bicarbonate ($NaHCO_3$), EDTA (Titriplex II), sodium phosphate salt, potassium bromide (KBr), Tween 20, sodium hydroxide, and copper sulfate ($CuSO_4$) were purchased from E. Merck (Darmstadt, Germany). Enzyme-linked immunosorbent assay (ELISA) plate was purchased from Dynatech Laboratories (Chantilly, VA).

3. Subjects and methods

3.1. Patients

Sera were collected from the following four groups of subjects: Group 1, 26 normal subjects with no lipid disorder or cardiovascular disease; Group 2, 27 patients with high level of LDL (H-LDL; 150–220 mg/dl) sera; Group 3, 25 patients with high level of triglyceride (H-TG; 200–560 mg/dl) sera; Group 4, 41 patients (ages distributed from 29 to 91 years old; 33 males and 8 females) with early stage of acute myocardial infarction (AMI). The sera were collected within 36 h after chest pain. Electrocardiograms showed acute nontransmural or transmural infarction, and creatinine phosphokinase (CK) levels were all elevated (ranging from 500 to 3500 U) with high creatinine phosphokinase MB (ranging from 30 to 250 U). Thirty out of forty-one AMI patients and normal subjects were examined for immunogenicity of antibodies against two different sources of oxLDL that were purified from high LDL and normal-range LDL sera, respectively, and oxidized with $CuSO_4$. Forty-one AMI patients and other groups of patients were only tested for autoantibody expression against the modified oxLDL from high LDL sera as an antigen.

3.2. Preparation of LDL from human sera and LDL oxidized by $CuSO_4$

LDL was isolated and purified from two groups of sera by ultracentrifugation according to the method of Itabe et al. [20]. One group was with high LDL sera (hLDLa) (above 170 mg/dl) and the other group was in a normal-range LDL sera (nLDLa) (below 120 mg/dl). Briefly, the 250 μl phosphate-buffered saline with 0.25 mM EDTA (PBS–EDTA buffer, pH 7.4) was layered on top of 750 μl sera in microcentrifuge tube and centrifuged at $550{,}000 \times g$ force for 7 min (TLA 100, Beckman) to remove chylomicron. After the chylomicron was removed, 250 μl PBS–EDTA was again added to top layer of sera, and centrifuged at $550{,}000 \times g$ for 2.5 h to remove very low density lipoprotein (VLDL). The LDL was finally purified from sera by adding 50% KBr to adjust the density to $1.019 < d < 1.063$, then centrifugation in $550{,}000 \times g$ for 4.5 h. The purified LDL in Ham's F-10 medium was oxidized by 10 μM $CuSO_4$ for 18 h, and then stopped by addition of EDTA solution to final concentration of 0.25 mM. The lipid peroxide contents of oxLDL were determined by thiobarbituric acid reactive substances (TBARS) and expressed as malondialdehyde equivalents [21], and also separated by 1% agarose gel electrophoresis to check the mobility shift.

3.3. ELISA method of anti-oxidized LDL antibody assay

The three groups of low density lipoprotein including native LDL, oxidized nLDLa, and oxidized hLDLa were dissolved in a 0.15 M $NaHCO_3$ solution, then coated with 3 μg/well on ELISA plate, individually. After the blocking procedure with 3% BSA, the 50× diluted sera (diluted by 1% BSA in PBS buffer) from AMI patients and normal subjects were integrated against the three groups of lipoprotein, respectively, and the solution was incubated for 1 h at 37 °C. After incubation, the plates were washed by washing solution (PBS buffer with 0.5% Tween 20), and alkaline phosphatase-conjugated goat anti-human IgG was added. The solution was incubated at room temperature for

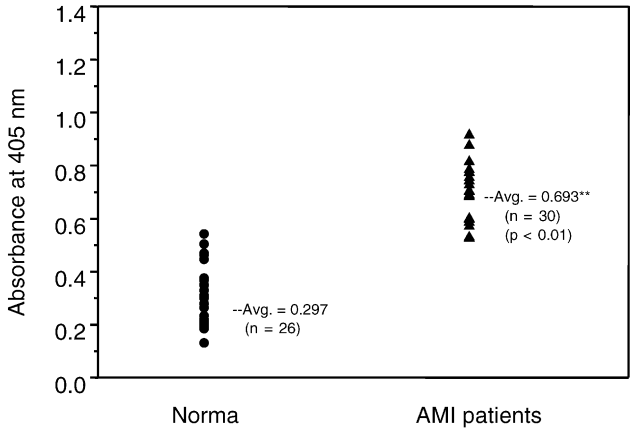

Fig. 1. Titer expression of autoantibody against oxidized LDL from high-LDL serum (hLDLa) differed in AMI patients and normal subjects. The titers of oxLDL antibody were 135% higher in AMI patients than in normal subjects.

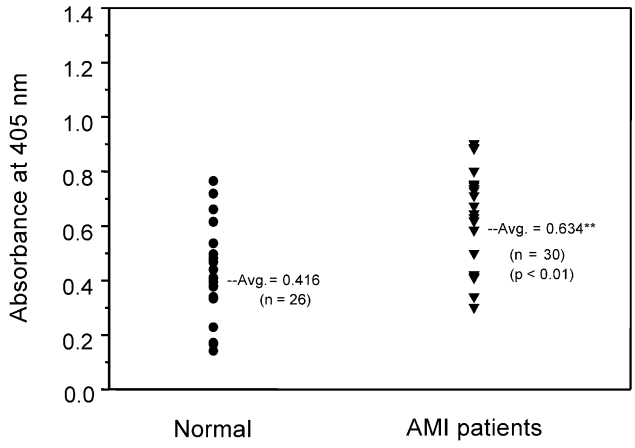

Fig. 2. Expression of autoantibody against oxLDL from normal LDL serum (nLDLa) differed in AMI patients and normal subjects. The titers of oxLDL antibody were 52% higher in AMI patients than in normal subjects.

1 h. Finally, the results were shown by absorbance at a 405-nm wavelength expressed as optical density (OD) under *p*-nitrophenyl phosphate substrate integration. The titer of antibodies is expressed by direct proportion of OD value in this study. Sera from high triglyceride (H-TG, 200–560 mg/dl) and high LDL (H-LDL, 150–220 mg/dl) subjects were also assayed against the oxLDL purified from hLDLa serum according to the above methods.

3.4. Assay of Tx B_2 and 6-keto-PG $F_{1\alpha}$ concentrations in sera

The measurement of Tx B_2 and 6-keto-PG $F_{1\alpha}$ concentrations in sera were made using an EIA kit according the procedure described by the manufacturer (Cayman, MI). The sera were diluted 100 × by dilution buffer.

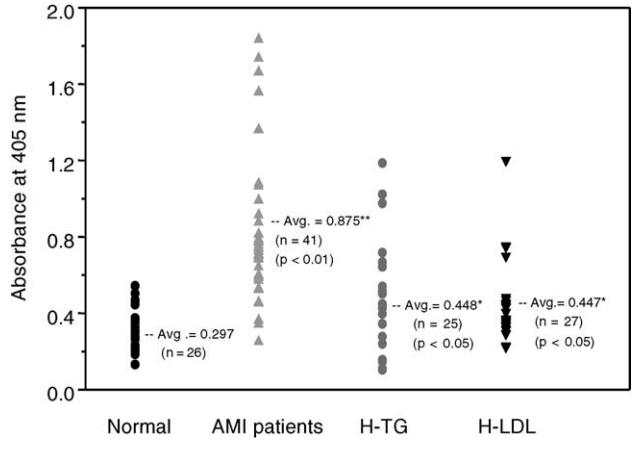

Fig. 3. Comparison of antibody against oxidized LDL from high-LDL serum (hLDLa) expression in four groups including AMI patients, H-TG, H-LDL and normal subjects. The antibody titers in AMI patients were 195% higher than in normal subjects, and the titers in H-TG subjects and in H-LDL subjects were also 50% and 50% higher, respectively, than in normal subjects.

Table 1
The autoantibody expression against three different LDL antigens in sera of normal subjects and AMI patients

	Native LDL	oxLDL (nLDLa)	oxLDL (hLDLa)
Normal subjects ($n=26$)	0.128 ± 0.046^{a}	0.416 ± 0.242^{c}	0.297 ± 0.155^{b}
AMI patients ($n=30$)	0.166 ± 0.037^{a}	0.634 ± 0.334^{de}	0.693 ± 0.334^{e}

Values shown for titer (expressed as absorbance at 405 nm; O.D.) are mean ± S.D. The results were analyzed by one-way ANOVA statistical method.

3.5. Statistical methods

Student's *t*-test and one-way ANOVA were used for statistical analyses. p values <0.05 and <0.01 were considered as statistically significant and very significant, respectively.

4. Results

The results showed that the titers of anti-oxLDL antibody against oxLDL purified from hLDLa modification in 30 patients with AMI (OD = 0.693) were 135% higher than those in normal subjects (OD = 0.297) (Fig. 1), but the titers of antibody in the same AMI patients (OD = 0.634) were only 52% higher than those in normal subjects (OD = 0.416) when modified nLDLa was used as an antigen (Fig. 2). Later on, 11 more AMI patients' sera were collected, with total of oxLDL antibody titers in 41 AMI patients; the titers of antibodies (average OD = 0.875) were 195% higher than those in normal subjects (average OD = 0.297), which is with statistically very significant difference ($p<0.01$). The autoantibody titers in H-TG subjects (average OD = 0.448) and H-LDL subjects (average OD = 0.447) were 50% and 50% higher, respectively, than those in normal control (OD = 0.297), which is also with statistically significant difference ($p<0.05$) (Fig. 3). Serum antibodies from AMI patients and normal subjects all had low binding affinity with native LDL antigen (average OD = 0.161 ± 0.046 and 0.128 ± 0.046, respectively) (Table 1). The immunoreactivity of the antibodies against two different sources of oxLDL

Table 2
The concentration of thromboxane B_2 (Tx B_2), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PG $F_{1\alpha}$), and Tx B_2/6-keto-PG $F_{1\alpha}$ ratio in normal subjects and AMI patients

Subject	Tx B_2 (ng/ml)	6-keto-PG $F_{1\alpha}$	Tx B_2/PG $F_{1\alpha}$
Normal ($n=26$)	10.32 ± 1.89	2.46 ± 0.74	4.19
AMI patients ($n=41$)	1.81 ± 0.71	1.01 ± 0.49	1.79

Values (mean ± S.D.) were taken from at least four experiments.

in normal sera was significantly different ($p < 0.05$). The titer of antibody for nLDLa-derived oxLDL was higher than the oxLDL purified from hLDLa; however, there was no significant difference between two different sources of antigen binding in 30 AMI patients (Table 1).

The means of Tx B_2 and 6-keto-PG $F_{1\alpha}$ levels in sera were 1.81 ± 0.71 and 1.01 ± 0.49 ng/ml in 41 AMI patients, and 10.32 ± 1.89 and 2.46 ± 0.74 ng/ml in normal control. The ratio of Tx B_2/6-keto-PG $F_{1\alpha}$ was 1.79 in AMI patients, and 4.19 in normal control (Table 2).

5. Discussion

Atherosclerosis has long been a medical problem in humans. Acute myocardial infarction is the leading cause of death in Western countries, and also in Asian countries in past decades. It has become apparent that coronary atherosclerotic lesions with less severe angiographic disease are associated with rapid progression to severe stenosis or total occlusion, and these lesions may account for acute myocardial infarction [22]. All these problems begin from the atherosclerotic plaques that become disrupted and cause a thrombosis. The atherosclerotic plaques that are prone to rupture characteristically have a thin fibrous cap, a large lipid core, activated smooth muscle cells, monocytes/macrophages, and T lymphocytes [2,23]. The cause that triggers the plaques to rupture still remains unclear up to date. Various reports provide evidence that oxidation of LDL plays an important role in the pathogenesis of atherosclerosis [4,17,24]. OxLDL has been the main issue in atherogensis and plague rupture, reported to be toxic and chemotactic for circulating monocytes [9].

LDL can be oxidized in vivo by several mechanisms including NO synthase, NADPH oxidase, cyclooxygenase I, II, etc. [25,26]. Autoantibodies to many epitopes of oxLDL are found in atherosclerotic lesion of human, and the atherosclerotic lesions contain large quantities of IgG [27]. Autoantibodies against oxLDL have been reported in normal subjects and in the patients with arteriosclerosis, but their possible pathogenic role is not yet well defined [28,29]. Mironova et al. [29] purified the anti-oxLDL autoantibodies from human sera by affinity chromatography with the use of oxLDL cross-linked to Sepharose, and the purified autoantibodies contained IgG (of subclasses 1 and 3) as the predominant isotype and were primarily specific for oxLDL. The predominance to IgG1 and IgG3 antibodies is significant from the standpoint of potential pathogenicity, since these two subclasses activate the classic complement pathway system and have the highest binding affinities for F_c gamma receptors on phagocytic cells [30]. Ryan et al. [31] also reported increased autoantibody titers in patients with acute myocardial infraction.

In our study, we found that the level of autoantibody expression against oxLDL from two different sera groups had the different results in normal subjects. The oxLDL antigen from sera with high-range LDL sera, the autoantibody against oxLDL was moderately higher in patients with H-LDL and H-TG, respectively, and remarkably higher in patients with the early stage of acute myocardial infarction; therefore, the H-LDL and H-TG seemed only minor relative with this antibody expression. In contrast, the antibodies against oxLDL as an antigen from sera with normal-range LDL sera had the higher binding activity (Table 1, $OD = 0.416 \pm 0.242$) as compared with oxLDL from high-range LDL sera (Table 1, $OD = 0.297 \pm 0.155$) in normal subjects. According to the above results, the expression levels of autoantibodies in AMI patients were obviously higher than normal subjects by using oxLDL from hLDL as a modified antigen. We assume that some parts of this binding motif in oxLDL have some differences between nLDLa and hLDLa and could be recognized by normal subject only but not AMI patient. This different motif of oxLDL antigen including part of lipid and protein might not be caused by $CuSO_4$ modification in vitro, but through some in vivo pathways to create the epitopes recognized by LDL autoantibodies. However, it is possible that the epitopes of oxLDL from hLDLa recognized by LDL autoantibodies are either caused by spontaneously modified LDL already existing in circulation, or because of their LDL unique characteristics that lead to generation of epitopes better recognized by the autoantibodies. It could show that the binding affinity of the autoantibody against oxidatively modified nLDLa and hLDLa was different in normal subjects group but not in AMI patients; therefore, it may be important to choose the source of oxLDL as an antigen in the assay of autoantibody expression against oxLDL. The antibodies may be the cause of the disease rather than simply a diagnostic marker. More detail studies on autoantibodies causing cardiovascular disease will be conducted in this lab.

The level of Tx B_2 was almost decreased more than fivefold in AMI patients compared with normal subjects, and the level of 6-keto PG $F_{1\alpha}$ only decreased twofold. This could be caused by the aspirin or related medicine usually to be taken by AMI patients. However, the low ratio of Tx B_2/6-keto PG $F_{1\alpha}$ in AMI patients seems to be independent with the high level of the autoantibodies expression.

Acknowledgements

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