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Increased serum levels of β_2-GPI-Lp(a) complexes and their association with premature atherosclerosis in patients with rheumatoid arthritis

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ABSTRACT

Background: Our recent study found the existence of complexes of β_2-glycoprotein I (β_2-GPI) with lipoprotein (a)[Lp(a)] in circulation and the complex concentrations were increased in sera of systemic lupus erythematosus patients. The concentration of β_2-GPI-Lp(a) and its relationship with premature atherosclerosis were evaluated in rheumatoid arthritis (RA) patients.

Methods: Serum concentrations of β_2-GPI-Lp(a) were measured in 53 active RA patients and 40 healthy controls by a "sandwich" ELISA. β_2-GPI-ox-LDL, ox-Lp(a), ox-LDL and anti-β_2-GPI were also measured by ELISAs. In addition, inflammatory markers were examined.

Results: Serum β_2-GPI-Lp(a) (1.12 ± 0.25 U/ml vs. 0.87 ± 0.19 U/ml, $P<0.0001$) and β_2-GPI-ox-LDL (1.01 ± 0.20 U/ml vs. 0.80 ± 0.08 U/ml, $P<0.0001$) concentrations in RA were both significantly higher than those of controls. Ox-Lp(a) (8.38 ± 6.69 mg/l vs. 5.49 ± 4.31 mg/l, $P<0.05$) and ox-LDL (0.68 ± 0.65 mg/l vs. 0.37 ± 0.13 mg/l, $P=0.001$) were also higher in RA than in controls. The area under the ROC curve (AUC) for β_2-GPI-Lp(a) (0.787) was larger than for ox-Lp(a) (0.731). AUC of β_2-GPI-ox-LDL (0.858) was also larger than for ox-LDL (0.785). β_2-GPI-Lp(a) and β_2-GPI-ox-LDL were positively correlated with ox-Lp(a), ox-LDL and CRP, respectively.

Conclusions: β_2-GPI-Lp(a) complex concentrations increased in active RA. Inflammation and oxidative stress in RA contribute to the increase of ox-Lp(a) and subsequently the formation of β_2-GPI-Lp(a).

1. Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease associated with painful joints that affects approximately 1% of the population worldwide [1]. It presents not only involvement of joints but also endothelial dysfunction, dyslipidemia, and premature atherosclerosis. The death rate in RA is known to be higher than in the general population, and clinical cardiovascular events secondary to atherosclerosis are responsible for the excessive death rate [2–4]. But many questions remain unanswered about the pathogenesis of premature atherosclerosis in RA patients. Besides inflammation, some studies have suggested the role of autoimmune and oxidative stresses in the premature atherosclerosis in RA [5–7].

Oxidized lipoproteins are thought to contribute to the development of atherosclerosis. Several lines of evidence showed that oxidized low density lipoprotein (ox-LDL) and oxidized lipoprotein (a) [Lp(a)] induces intracellular accumulation of cholesteryl esters (CEs) in macrophages, which leads to their transformation into foam cells after being taken up by the scavenger receptor pathway [8,9]. Moreover, both of the oxidized lipoproteins induce adhesion molecular expression on monocytes, promoting their recruitment and adhesion to the endothelium [10,11]. Additionally, the oxidized lipoproteins have also been demonstrated to be involved in autoimmune mediated atherosclerosis. Recently, it was observed that ox-LDL interacts with β_2-GPI, a major autoantigen for anticardiolipin antibodies found in the sera of some autoimmune diseases, to form stable and possible pathogenic β_2-GPI-ox-LDL complexes in the arterial intima of atherosclerotic lesions, and the complexes were taken up avidly by macrophages via anti-β_2-GPI autoantibody-mediated phagocytosis [12–14]. Increased β_2-GPI-ox-LDL complex concentrations have been observed in the bloodstream of patients with autoimmune, such as systemic lupus erythematosus (SLE) and antiphospholipid syndrome [5,15–17]. Interestingly, our recent study demonstrated that Lp(a), mainly ox-Lp(a), also formed complexes with β_2-GPI *in vivo* and the concentrations of β_2-GPI-Lp(a) complexes increased in sera from SLE patients [18]. These results suggest that the β_2-GPI-Lp(a), like β_2-GPI-ox-LDL complexes, might act as putative autoantigen in autoimmune-mediated atherosclerotic vascular

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disease and the measurement of the circulating complexes may help to predict the development of atherosclerosis. In general, patients with RA are thought to expose to greater amount of oxidative stress [19,20]. Our previous studies together with others' have showed that ox-Lp(a) and ox-LDL concentrations were increased in RA patients with excessive cardiovascular events [21–23].

2. Materials and methods

2.1. Subjects and blood collection

Fifty-three patients with RA in this study were randomly selected from the Jinling Hospital. The diagnosis of RA was based on criteria proposed by the American College of Rheumatology [24]. The mean age of the patients was 54.5 ± 14.6 years (male 13, female 40). The mean disease duration was 104.7 months (range 1.2–396). All patients had subjective symptoms or objective tender/swollen joints and were being treated with a disease modifying antirheumatic drug (DMAD) (85% took methotrexate and 15% took leflunomide). Tender and swollen joint counts were performed on a 28 joint count. All the patients had no history of cerebral or myocardial infarction, or diabetes mellitus, liver or kidney disease, and known familial hyperlipidaemia, or malignant disease. Forty age and sex matched, healthy volunteers with no clinical or laboratory evidence of disease were selected as normal controls. The blood was sampled at least 12 h after fasting and serum was separated immediately and stored at −70 °C until analysis. This study was approved by the Ethics Committee of Jinling Hospital and all the subjects had given their informed consent.

2.2. Sandwich ELISAs for β_2-GPI-Lp(a) and β_2-GPI-LDL

β_2-GPI-Lp(a) was measured by a sandwich ELISA as previously described [18]. Briefly, five hundred microliters of serum was firstly incubated with $MgCl_2$ (final concentration 10 μmol/l) at 37 °C for 2 h, and then polyethyleneglycol (PEG)-6000 (Sigma) was added to isolate β_2-GPI-Lp(a) from endogenous free form of β_2-GPI. Anti-human β_2-GPI antibody was adsorbed onto microtiter plates by incubating at 8 μg/ml (dissolved in 0.05 mol/ml, pH 9.6, sodium carbonate/bicarbonate buffer, 100 μl/well) for 2 h at 37 °C and then overnight at 4 °C. After blocking with 1% gelatin in 0.01 mol/l PBS, samples diluted 1:6 (resuspended with gelatin in PBST) or serial reference sera were added to the wells and incubated for 2 h. The wells were then incubated with HRP-labeled polyclonal antibody against apolipoprotein (a) [apo(a)]. Color was developed with *o*-phenylenediamine and H_2O_2. The reaction was terminated, and optical density at 450 nm was measured.

β_2-GPI-ox-LDL was detected by a similar sandwich ELISA assay for β_2-GPI-Lp(a), with the exception that HRP-labeled quantitating polyclonal antibody against apo(a) was replaced by polyclonal anti-apoB. A pooled fresh-frozen serum sample (from 50 healthy subjects) was used as reference serum of β_2-GPI-Lp(a) and β_2-GPI-ox-LDL. Reference serum was also precipitated every time as serum sample. The two SD above the mean optical density of our studied 40 samples from healthy blood donors was arbitrarily expressed as 1 U/ml.

2.3. Sandwich ELISAs for ox-Lp(a) and ox-LDL

Ox-Lp(a) was measured by a sandwich ELISA using polyclonal antibody against ox-LDL as the capture antibody and detected with monoclonal anti-apo(a) enzyme conjugate as previously described [25]. Antibodies to ox-LDL were obtained by immunization of New Zealand white female rabbit with ox-LDL as described by Virella et al. [26]. The resulted rabbit antisera were first fractionated by affinity chromatography with immobilized protein G, and the IgG fractions were then absorbed in a column of immobilized native LDL. The washout from the column of immobilized native LDL, containing antibodies to ox-LDL and irrelevant IgG, was used to capture ox-LDL, which had no reactivity with native LDL [25]. Calculation of the concentration of ox-Lp(a) was based on the concentration of ox-Lp(a) as the standard [25]. Ox-LDL was determined by a sandwich ELISA, using monoclonal antibodies against Cu^{2+} oxidized LDL as the capture antibody and quantitating with anti-apo B enzyme conjugate [27]. Copper-oxidized LDL was used as ox-LDL standard.

Table 1
Serum β_2-GPI-Lp(a), β_2-GPI-ox-LDL, oxidized lipoproteins, anti-β_2-GPI and inflammatory marker concentrations in patients with RA.

Variables	RA (n = 53)	Control (n = 40)	*P*
β_2-GPI-Lp(a) (U/ml)	1.12 ± 0.25	0.87 ± 0.19	0.000
β_2-GPI-ox-LDL (U/ml)	1.01 ± 0.20	0.80 ± 0.08	0.000
Ox-Lp(a) (mg/l)	8.38 ± 6.69	5.49 ± 4.31	0.013
Ox-LDL (mg/l)	0.68 ± 0.65	0.37 ± 0.13	0.001
Anti-β_2-GPI (RU/ml)	8.97 ± 15.98	4.82 ± 4.64	NS
CRP (mg/l)	38.62 ± 48.34	<1.0	0.000
ESR (mm/h)	57.09 ± 27.91		
RF (IU/ml)	347.5 ± 331.6		
Tender joint count	17.6 ± 8.7		
Swollen joint count	19.5 ± 6.4		

Data are indicated as the mean ± SD.

2.4. Determinations of anti-β_2-GPI and inflammatory markers

Serum concentration of anti-β_2-GPI (IgAGM) was detected by an ELISA assay kit (EUROIMMUN, Germany). Cutoff value was <20 RU/ml. Erythrocyte sedimentation rate (ESR) was measured by the Westergren method. C-reactive protein (CRP) was measured by turbidometry, and rheumatoid factors (RF) were determined by nephelometry.

2.5. Statistical analyses

Statistical analyses were performed with SPSS 11.5 (Chicago, IL). The values were expressed as mean ± SD. Nonparametric Mann–

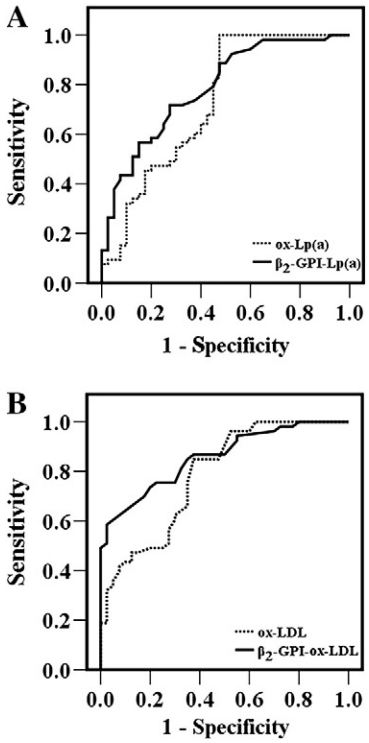

Fig. 1. ROC curves of β_2-GPI-Lp(a) and ox-Lp(a) (A), β_2-GPI-ox-LDL and ox-LDL (B) in RA patients and controls (n = 93).