Highly electronegative LDL from patients with ST-elevation myocardial infarction triggers platelet activation and aggregation

Running head: Thrombogenicity of electronegative LDL

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Supplemental Material

Detailed Methods

Clinical diagnosis

Patients with STEMI typically experienced persistent chest pain, and their EKGs showed ST elevation >0.1 mV in at least 2 contiguous precordial leads or at least 2 adjacent limb leads, with or without elevation of cardiac enzymes. Blood samples were collected after the insertion of an intravenous catheter, before administering 0.9% normal saline and primary percutaneous coronary intervention for acute STEMI patients. During the recovery stage, continual cardiac enzyme testing was required for monitoring heart muscle damage. For patients with STEMI, tests for complete blood count, platelet count, and hemostasis (PTp, PTc, PT/INR, PPTp, and PPTc) were performed. For the diagnosis of MI, cardiac enzyme levels were measured for cardiac troponin I, creatine kinase–MB (the heart isoenzyme of creatine kinase), and creatine phosphokinase. Brain natriuretic peptide was measured to reflect the severity of concomitant heart failure. Lipid parameters measured in the blood of patients with STEMI included total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), LDL cholesterol (LDL-C; also measured in control subjects), and fasting blood sugar.

LDL sample preparation

To prevent contamination and protein degradation, 1% penicillin/streptomycin and Complete Protease Inhibitor Cocktail (Roche Diagnostics, Indianapolis, IN; 1 tablet/100 mL plasma) were added into plasma samples. EDTA (0.5 mg/mL) and N_2 were also added to avoid sample oxidation during the entire process of preparation and preservation. The isolated subfractions were N_2-sealed and stored at 4°C during sample characterization.[16]

LDL analysis

Because L1 and L5 from STEMI patients may differ from those of previously studied subjects, we characterized the effects of all L1 and L5 subfractions on EC apoptosis as previously described[10] and examined the apoplipoprotein content of all L1 and L5 subfractions by performing sodium dodecyl sulfate

polyacrylamide gel electrophoresis (SDS-PAGE; 4-20%). We also compared platelet-activating factor (PAF) components in L1 and L5 from healthy subjects (n=2) and patients STEMI (n=7) by using electrospray ionization mass spectrometry.

Parallel plate flow chamber assay

We performed a parallel plate flow chamber assay similar to that described by Cruz and colleagues.[23] A fibrinogen-coated dish formed the lower surface of the parallel plate flow chamber, and a silicone rubber gasket determined the flow path height of 254 μm. The flow chamber was filled with phosphate-buffered saline (PBS). PRP was aspirated through the flow path by using a syringe pump (Harvard Apparatus Inc.). The flow rate was 0.6 mL/min and produced a wall shear rate of 1500 S^{-1}.

Western blot analysis

Protein concentrations were measured by using the Bradford assay (Biorad). To probe membrane proteins, we used goat anti-phosphorylated PKCα (Santa Cruz Biotechnology, Inc.), mouse anti-PKCα (EMD Millipore Corp.), rabbit anti-phosphorylated PI3K (Cell Signaling Technology), rabbit anti-PI3K (Santa Cruz Biotechnology, Inc.), rabbit anti-phosphorylated Akt (Santa Cruz Biotechnology, Inc.), and rabbit anti-Akt (Santa Cruz Biotechnology, Inc.) antibodies. Secondary anti-rabbit, -goat, and -mouse IgG antibodies were conjugated to horseradish peroxidase (GeneTex Inc.). Signals were amplified by using ECL Plus chemiluminescent reagents (Millipore) and were recorded and visualized with a G:Box iChemiXT image analyzer (Syngene).

Supplemental Table 1. Patient Characteristics and Biochemical Profiles

Baseline characteristic	Control (n=30)	STEMI (n=30)	*P*-value
Male: Female	14:16	25:5	0.003[c]
Age (yr)	37.2 ± 10.6	59.2 ± 13.3	< 0.001[d]
Patient history			
Hypertension	0.10 (3/30)	0.43 (13/30)	0.004[c]
Diabetes	0.13 (4/30)	0.47 (14/30)	0.005[c]
Medications[a]			
Antiplatelet drugs	NA	0.13 (4/30)	-
Statins	NA	0.07 (2/30)	-
Waist (cm)	77.5 ± 10.0	NA	-
Systolic BP (mm Hg)	112.1 ± 15.5	NA	-
Diastolic BP (mm Hg)	74.2 ± 12.3	NA	-
Glu AC (mg/dL)	95.3 ± 18.2	113.3 ± 23.4	0.002[e]
Lipid profile			
T-CHOL (mg/dL)	179.3 ± 32.9	179.1 ± 33.9	0.985[d]
TG (mg/dL)	78.6 ± 59.8	119.6 ± 65.6	0.001[e]
HDL-C (mg/dL)	55.6 ± 14.2	38.5 ± 8.6	< 0.001[d]
LDL-C (mg/dL)	108.1 ± 28.4	116.7 ± 32.4	0.281[d]
L5%	1.5 ± 1.1	15.4 ± 14.5	< 0.001[e]
[L5][b] (mg/dL)	1.7 ± 1.5	18.9 ± 21.0	< 0.001[e]
Cardiac enzymes			
TropI (ng/mL)	NA	69.2 ± 53.3	-
CK-MB (ng/mL)	NA	186.2 ± 133.8	-
CPK (IU/L)	NA	2011 ± 1340	-
CK-MB/CPK	NA	9.5 ± 3.7	-
BNP (pg/mL)	NA	616.6 ± 938.3	-

STEMI, ST-elevation myocardial infarction; BP, blood pressure; Glu AC, fasting blood sugar; T-CHOL, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; L5%, L5 percentage; TropI, cardiac troponin I; NA, not analyzed; CK-MB, the heart isoenzyme of creatine kinase; CPK, creatine phosphokinase; BNP, brain natriuretic peptide.

[a]Medications taken within the 3 months preceding study enrollment, including antiplatelet drugs such as aspirin and clopidogrel; statins such as atorvastatin and rosuvastatin; and high blood pressure treatment such as amlodipine and amlodine/benazepril.

[b][L5], the concentration of L5, calculated as L5% × LDL-C (mg/dL).

[c]*P*-value was determined by using the chi-square test.

[d]*P*-value was determined by using a *t* test.

[e]*P*-value was determined by using the Wilcoxon rank-sum test. Nonparametric statistical analysis was performed because sample values did not conform to a normal distribution (Shapiro–Wilk test for normality, $P<0.01$).

Supplemental Table 2. Stratified Analysis of L5% in Patients With STEMI and Control Subjects

Characteristics	Controls	STEMI patients	*P*-value[a]
Gender: Male	1.3 ± 0.4 (n=14)	13.9 ± 14.2 (n=25)	<0.0001
Gender: Female	1.7 ± 1.4 (n=16)	22.8 ± 15.0 (n=5)	0.001
Non-obese	1.4 ± 0.7 (n=29)	15.5 ± 14.7 (n=29)	<0.0001
Diabetic	1.7 ± 0.4 (n=4)	18.7 ± 12.1 (n=13)	0.007
Non-diabetic	1.5 ± 1.2 (n=26)	12.8 ± 16.0 (n=17)	<0.0001
Hypertensive	1.6 ± 0.4 (n=7)	13.6 ± 8.8 (n=16)	0.0005
Non-hypertensive	1.5 ± 1.2 (n=23)	17.9 ± 19.3 (n=14)	<0.0001
Dyslipidemic	1.6 ± 0.4 (n=7)	11.3 ± 9.2 (n=14)	0.002
Non-dyslipidemic	1.5 ± 1.2 (n=23)	18.9 ± 17.4 (n=16)	<0.0001
Not using clopidogrel	1.5 ± 1.1 (n=30)	15.5 ± 15.2 (n=27)	<0.0001
Not using statins	1.5 ± 1.1 (n=30)	15.9 ± 14.8 (n=28)	<0.0001

[a]*P*-values were determined by using a chi-squared test.