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Type 2 diabetes mellitus and endothelial lipase

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Abstract

Objective: Endothelial lipase (EL), a new member of the triacylglycerol lipase family, modulates the metabolism of high-density lipoproteins and is upregulated by inflammatory cytokines. Since type 2 diabetes is associated with chronic subclinical inflammation, we have determined whether serum EL concentration is increased in type 2 diabetes and investigated the effect of insulin on EL.
Methods: 237 type 2 diabetic patients on oral anti-diabetic agents, 111 type 2 diabetic patients on insulin therapy and 226 non-diabetic controls were recruited. Serum EL was measured by ELISA. To investigate the effect of insulin on EL production by endothelial cells, human aortic endothelial cells were incubated with insulin and EL mRNA and protein in cell medium was measured. Serum EL was also measured in 16 diabetic subjects before and after starting insulin therapy.
Results: Serum EL level was highest in patients on oral anti-diabetic agents whereas those on insulin had similar EL level as controls (oral: 26.7 ± 16.1 ng/ml; insulin: 23.3 ± 11.6, controls: 23.9 ± 12.0; ANOVA $p = 0.04$). In both controls and patients on oral anti-diabetic agents, EL correlated with log(CRP) ($r = 0.20$, $p = 0.003$; $r = 0.23$, $p < 0.001$, respectively) but no correlation was seen in patients on insulin. In vitro experiments showed that insulin significantly reduced EL mRNA and protein in human aortic endothelial cells in a dose-dependent manner. Serum EL concentration also significantly decreased in diabetic patients after starting insulin therapy ($p < 0.03$).
Conclusion: Serum EL concentration was increased in type 2 diabetic patients and was associated with the degree of subclinical inflammation and exogenous insulin therapy lowered serum EL concentration.

Keywords: Type 2 diabetes mellitus; Endothelial lipase; HDL; Insulin

1. Introduction

Endothelial lipase (EL) is a new member of the triacylglycerol lipase family and the sequence of EL reveals substantial homology with lipoprotein lipase (LPL) and hepatic lipase (HL) [1,2]. Although EL is 44% identical to LPL and 41% identical to HL, there are differences in the lipid substrate specificity among the three lipases. In contrast to LPL and HL, EL acts primarily as a phospholipase [3]. In addition to the differences in lipid substrate specificity, EL has distinct lipoprotein preferences in vitro compared with LPL and HL. Lipoprotein lipase and HL preferentially hydrolyze triglyceride-rich lipoproteins whereas EL is more active against high-density lipoprotein (HDL) [3]. However, EL is not HDL specific and is also capable of hydrolyzing apolipoprotein (apo) B-containing lipoproteins in vitro.

There is increasing evidence to suggest that EL plays a significant role in the metabolism of HDL. In animal studies, hepatic overexpression of EL after adenoviral gene transfer resulted in markedly decreased HDL cholesterol and phospholipid levels [1], and transgenic overexpression of EL under the control of the endogenous promoter resulted in moderately reduced HDL cholesterol levels [4]. On the other hand, antibody inhibition of mouse EL activity in wild-type, apoAI-transgenic and HL-deficient mice resulted in significantly increased HDL cholesterol and phospholipid levels [5]. HDL cholesterol levels were also significantly increased in the EL knockout mouse [4,6]. Hence, EL has been shown to be an important determinant of HDL metabolism and levels in murine models. However, the role of EL in human HDL metabolism is less clear. Most studies to date have

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focused on the effects of genetic polymorphisms in the EL gene on plasma lipids and lipoproteins. Genetic studies have demonstrated association between genetic variants in EL and HDL cholesterol and apoAI concentrations [7–10]. There are, however, very little data on the relation between plasma EL levels, lipid levels, and atherosclerosis in humans. Badellino et al. recently reported that human plasma EL concentrations, in both post-heparin and pre-heparin plasma, were significantly associated with features of the metabolic syndrome and with subclinical atherosclerosis in healthy individuals with a family history of premature coronary heart disease. Based on their results, they suggested that EL might be a pro-atherogenic factor in humans [11]. Paradis et al. demonstrated that EL was associated with visceral obesity and with inflammation in sedentary men [12,13]. Since type 2 diabetes is associated with low grade subclinical inflammation [14,15], we tested the hypothesis that serum EL concentration might be increased in patients with type 2 diabetes mellitus and investigated the effect of insulin on serum EL level.

2. Methods

Consecutive patients with type 2 diabetes mellitus attending the diabetes clinics at Queen Mary Hospital were invited to participate. Patients on insulin therapy were eligible if they had been previously managed with diet and an oral agent at some point and had no known history of diabetic ketoacidosis. A total of 348 type 2 diabetic patients were recruited, 237 were on oral anti-diabetic agents (10.1% on sulphonylurea, 13.6% on metformin and 76.3% on combination of sulphonylurea and metformin) and 111 were on insulin therapy (64% on insulin and metformin and the rest on insulin alone). Fasting blood samples and clinical data were collected. Two hundred and twenty-six non-diabetic controls of similar age were recruited from the local community. To investigate the effect of insulin therapy on serum EL concentration, 16 patients with secondary oral anti-diabetic drug failure were recruited and serum EL was determined before and after the institution of insulin therapy. The study was approved by the Ethics Committee of the University of Hong Kong and informed consent was obtained from all subjects.

Plasma total cholesterol and triglyceride were determined enzymatically (Boehringer Mannheim, Mannheim, Germany) on a Hitachi 717 analyzer (Boehringer Mannheim, GmbH, Germany). HDL-cholesterol (HDL-C) was measured by the same method after precipitation of apo B-containing lipoproteins with PEG 6000. LDL-cholesterol (LDL-C) was calculated by the Friedewald equation. Apolipoproteins AI and B were measured by rate nephelometry using the Beckman Array System (Beckman Instruments). HbA1c was measured in whole blood using ion-exchange high performance liquid chromatography by Bio-Rad Variant Analyser System (Bio-Rad Laboratories Inc., California, USA). Plasma high sensitivity CRP was measured by a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, GmbH, Mannheim, Germany) using anti-CRP mouse monoclonal antibodies coupled to latex microparticles. Serum fasting insulin was determined by commercial enzyme-linked immunosorbent assay (ELISA) kits (Mercodia, Uppsala, Sweden).

A competitive ELISA was set up for measuring human EL. Western blot analysis was first performed to prove that EL was detectable in human serum, EDTA-plasma and post-heparin plasma. 5 μg of total protein of EDTA-plasma, serum and post-heparin plasma (blood sample taken 15 min after 100 units/kg of heparin) from the same human subject were first electrophoretically separated on 12% denaturing sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gels and then transferred onto nitrocellulose membranes. Non-specific binding was blocked overnight with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 (TBST). The blocked membrane was incubated with a rabbit polycloncal antibody specific to human EL (Novus Biologicals, Littleton, CO) (1:500 dilutions with 1% nonfat milk in TBST) for 3 h at room temperature. The immunogen used for raising the antibody was an N-terminal synthetic peptide made to the human EL protein sequence. After washing the membrane with TBST a few times, the secondary horseradish peroxidase-conjugated goat-anti-rabbit IgG (Sigma Chemical Co., St. Louis, MO) (1:10,000) was added and incubated for another hour at room temperature. Antigen EL detection was performed by the ECL plus protocol (Amersham) according to the manufacturer's instructions. Fig. 1 showed that EL was present in serum, EDTA plasma and post-heparin plasma. EL mass in serum, plasma and post-heparin plasma was determined in eight subjects and plasma EL mass was approximately 80% of that in serum. EL mass in post-heparin plasma was 2-fold higher than that of serum or plasma, in keeping with the findings of Badellino et al. who have shown that EL can be released by heparin injection in humans [11].

For the ELISA, 96-well EIA microtiter plate (Costar, NY, USA) was coated with antigen, the N-terminal synthetic peptide of the human EL (Novus Biologicals, Littleton, CO)

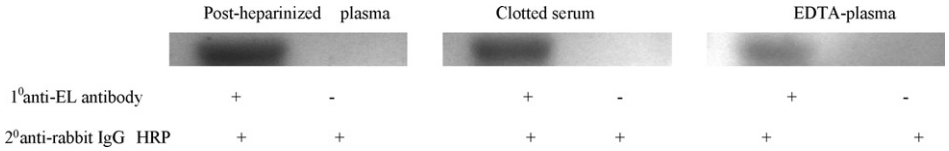

Fig. 1. Western blot analyses on EL from same subject's post-heparin plasma, clotted serum and EDTA-plasma. Protein samples were either probed with anti-EL antibody or IgG (5 μg total protein per lane loading). A 68 kDa band corresponding to EL was detected.