ELSEVIER

Atherosclerosis 178 (2005) 365–370

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Plasma phospholipid transfer protein activity and subclinical inflammation in type 2 diabetes mellitus

K.C.B. Tan[a,*], S.W.M. Shiu[a], Y. Wong[a], S. Tam[b]

[a] *Department of Medicine, Queen Mary Hospital, University of Hong Kong, Pokfulam Road, Hong Kong, Hong Kong*
[b] *Department of Clinical Biochemistry Unit, Queen Mary Hospital, University of Hong Kong, Pokfulam Road, Hong Kong, Hong Kong*

Received 14 May 2004; received in revised form 18 August 2004; accepted 7 September 2004
Available online 20 October 2004

Abstract

Phospholipid transfer protein (PLTP) transfers phospholipids between lipoproteins, and plays an essential role in HDL metabolism. The regulation of PLTP is poorly understood and recent evidence suggests that PLTP activity increases during acute-phase response. Since type 2 diabetes is associated with chronic subclinical inflammation, the objective is to determine whether inflammation modulates PLTP activity in diabetes. Plasma PLTP activity was assayed by measuring the transfer of radiolabeled phosphatidylcholine from liposomes to HDL and high-sensitivity C-reactive protein (CRP) by immunoturbidimetric assay in 280 type 2 diabetic patients and 105 controls. Plasma PLTP activity (2364 ± 651 nmol/ml/h versus 1880 ± 586 nmol/ml/h in control, mean $\pm$ S.D., $P < 0.01$) and CRP (1.64 (0.89–3.23) mg/l versus 0.99 (0.53–2.33) mg/l, median (interquartile range), $P < 0.01$) were increased in diabetic subjects. PLTP activity correlated significantly with age, BMI, HbA1c, log(CRP) and apolipoprotein AI and B in diabetic subjects. General linear model analysis showed that only apolipoprotein AI, age, BMI and log(CRP) were independent determinants of PLTP activity. In conclusion, PLTP activity is increased in diabetes and apolipoprotein AI is a major determinant of PLTP activity. There is also an independent association between CRP and PLTP activity, suggesting that subclinical inflammation may influence PLTP activity in diabetes.

Keywords: Phospholipid transfer protein; C-reactive protein; Inflammation; Acute-phase response; Type 2 diabetes mellitus

1. Introduction

Phospholipid transfer protein (PLTP) is a multifunctional, HDL-associated lipid transfer protein [1]. Phospholipid transfer protein transfers phospholipids between different lipoprotein species, and plays an essential role in the metabolism of HDL. It transfers surface remnants from triglyceride-rich lipoproteins to HDL during lipolysis and is therefore important in the maintenance of HDL levels [2]. It also modulates the size and composition of HDL particles, generates pre-β-HDL particles and stimulates cellular cholesterol/phospholipid efflux, a function important for the reverse cholesterol transport process [3,4]. In addition, PLTP has been recently shown to play a role in the secretion of apolipoprotein (apo) B-containing lipoproteins [5]. Phospholipid transfer protein not only can transfer all subclasses of phospholipids between different lipoprotein species, it can also transfer and neutralize lipopolysaccharide, a mediator of the inflammatory response [6]. Recent evidence suggests that the acute-phase response is associated with changes in PLTP activity. In subjects with a systemic inflammatory response, PLTP activity is increased, and the decrease in HDL during the acute phase of inflammation is partly caused by reduced lecithin:cholesterol acyltransferase (LCAT) and increased PLTP activities [7].

Plasma PLTP activity is also known to be increased in patients with type 2 diabetes [8–10] but the mechanisms that underlie the increased PLTP activity are not yet clear. There are some data suggesting that the regulation of PLTP may be

* Corresponding author. Tel.: +852 2855 4769; fax: +852 2816 2187.
E-mail address: kcbtan@hkucc.hku.hk (K.C.B. Tan).

0021-9150/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2004.09.005

partly related to glucose and insulin metabolism [8,11]. It has been shown that type 2 diabetes is associated with chronic subclinical inflammation, with small but definite increases of serum or plasma concentrations of several acute-phase proteins including C-reactive protein (CRP), serum amyloid A, fibrinogen, α1-acid glycoprotein and plasminogen activator inhibitor-1 [12–16]. Since PLTP activity has been shown to increase during the acute-phase response, the aim of this study is to determine whether chronic subclinical inflammation contributes to the increase in PLTP activity in patients with type 2 diabetes independent of hyperglycaemia.

2. Methods

Two hundred and eighty type 2 diabetic patients with normal renal and liver function were recruited from the diabetes clinics. Patients on lipid lowering agents were eligible if lipid-lowering agents had been withdrawn for 8 weeks. Seventy-five percent of the patients were on oral hypoglycaemic agents (sulphonylurea and/or metformin and/or alpha-glucosidase inhibitor) and the rest were on insulin therapy. The duration of diabetes was 9.7 ± 6.7 years (mean ± standard deviation). Forty-six percent of the patients had hypertension and 4% had a history of cardiovascular disease. The majority of the hypertensive patients were on angiotensin-converting enzyme inhibitors and/or calcium antagonists, with only 12 and 8% of the patients on beta-blockers and low-dose thiazide diuretic, respectively. One hundred and five non-diabetic controls of similar age and body mass index (BMI) were recruited from the local community. Fasting blood was taken for the measurement of plasma lipids, apolipoproteins, PLTP activities and CRP. All subjects gave informed consent and the protocol was approved by the Ethics Committee of the University of Hong Kong.

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 Analyzer System (Bio-Rad Laboratories, Inc., California, USA). Plasma glucose was determined by enzyme-coupled spectrophotometric kinetic method using hexokinase on the Hitachi-717 analyzer (Boehringer Mannheim, GmbH, Germany). 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.

Plasma PLTP activity was measured with labeled liposome vesicles as the phospholipid donor and excess pooled human HDL as phospholipid acceptor. Phospholipid transfer activity was measured with [^{3}H]dipalmitoylphosphatidylcholine (DPPC; Amersham Biosciences, UK)-containing vesicles that were prepared as described [10]. Transfer of radiolabeled phospholipids was measured by incubating an aliquot of 10 μl of each sample with [^{3}H]DPPC vesicles (125 nmol of phosphatidylcholine) and HDL (250 μg of protein) in a final volume of 400 μl at 37 °C for 1 h. Vesicles were subsequently precipitated by the addition of 300 μl of a solution of 500 mM NaCl, 215 mM MnCl, 445 units/ml heparin, and the radioactivity of a 500-μl aliquot of the supernatant was measured. The inter-assay coefficient of variation was 5.2%.

Results in this study were expressed as the means and standard deviations, or as median and interquartile range if the distribution of the data were skewed. Triglyceride and CRP were logarithmically transformed before analyses were made because of the skewed distribution. Student's *t*-test was used to compare continuous variables from the diabetic patients and controls. Associations between different parameters were determined by Pearson correlation coefficients. General linear model univariate analysis was used to assess the relationships between plasma PLTP activity and various variables simultaneously. All analyses were performed using SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL, USA).

3. Results

The clinical characteristics of the diabetic patients and controls are shown in Table 1. The diabetic patients were older ($P < 0.05$) with significantly higher fasting triglyceride, apo B, and lower HDL and apo AI than the controls. Plasma CRP was increased in the diabetic patients and log(CRP) correlated with BMI ($r = 0.30$, $P < 0.001$), log(TG) ($r = 0.15$, $P = 0.01$) and apo B ($r = 0.21$, $P = 0.001$), whereas in the controls, log(CRP) correlated with BMI ($r = 0.25$, $P = 0.01$), HDL ($r = -0.25$, $P = 0.01$) and apo AI ($r = -0.20$, $P = 0.04$). Plasma PLTP activity was also significantly elevated in the diabetic patients compared to the controls and the differences remained significant even after adjusting for age, sex and BMI. Diabetic patients on oral hypoglycaemic agents had similar plasma PLTP activity, CRP and HbA1c levels as those on insulin therapy. In the diabetic patients, PLTP activity correlated with age ($r = 0.14$, $P = 0.02$), BMI ($r = 0.15$, $P = 0.01$), HbA1c (Fig. 1a), and log(CRP) (Fig. 1b). There were no associations with plasma lipids although there was a trend towards a weak correlation with log(TG) ($r = 0.11$, $P = 0.066$). Significant correlations with apo AI (Fig. 2a) and apo B (Fig. 2b) were found. In the controls, PLTP activity correlated only with apo AI ($r = -0.21$, $P = 0.03$).

To determine what were the important determinants of plasma PLTP activity in diabetes, general linear model univariate analysis was performed. Gender, smoking status, and continuous variables which showed an association with plasma PLTP activity (age, BMI, HbA1c, log(CRP), apo AI and B) were entered into the model and the results are shown