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Probucol normalizes cholesteryl ester transfer in type 2 diabetes

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ABSTRACT

Aims: Accelerated cholesteryl ester transfer (CET) protein (CETP) activity is believed to promote macrovascular disease in patients with type 2 diabetes (T2D) by increasing the cholesterol burden of the apoB – containing triglyceride-rich lipoprotein (TGRLP) CE acceptors and promoting small dense LDL formation. While previous studies have shown that this same abnormality is present in patients with type 1 diabetes (T1D) and was normalized by the anti-oxidant drug probucol, its effects on CET in T2D are unknown.

Patients and methods: The net mass transfer of CE from HDL to the apoB lipoproteins (VLDL + LDL) was studied in intact plasma from seven T2D patients before and two months after treatment with probucol (1 g/day).

Results: Before treatment, CET was significantly greater than controls at 1 and 2 h ($p < .005$). Recombination studies showed that this disturbance was attributable to dysfunction of VLDL and not due to altered behavior of HDL or CETP. Probucol treatment normalized CET in all subjects and significantly lowered plasma cholesterol (pre-Rx: 197 ± 4.5 vs post-Rx: 162 ± 27.1 mg/dL; mean ± S.D.; $p < .025$) and HDL-C (pre-Rx: 46.4 ± 7.5 vs post-Rx: 39.1 ± 4.0; $p < .025$) without changing glycemic control.

Conclusions: By normalizing CET in T2D, probucol likely reduces the formation of atherogenic lipoproteins. This effect on CET is achieved through qualitative alterations in CETP's lipoprotein substrates and not through changes in CETP or HDL. Since probucol also has potent anti-oxidative and anti-inflammatory properties, it may have a new role to play in lipoprotein remodeling that reduce cardiovascular risk in T2D.

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Abbreviations: CET, cholesteryl ester transfer; CETP, cholesteryl ester transfer protein; TGRLP, triglyceride-rich lipoproteins; T2D, type 2 diabetes; T1D, type 1 diabetes; VLDL, very low density lipoprotein; LDL, low density lipoprotein; PLTP, phospholipid transfer protein; LCAT, plasma lecithin–cholesterol acyltransferase

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1. Introduction

In their role of intravascular remodeling the apoB-(VLDL, LDL) and apoA-(HDL) containing lipoproteins, the lipid transfer proteins CETP, PLTP, and lipases act collectively to not only influence their composition, metabolism, and function, but also their potential atherogenicity [1]. Earlier studies in animals showing that species with high CETP activity like the rabbit are prone to dietary-induced atherosclerosis, whereas the dog and rat that naturally lack CETP are resistant [2] implied that in the presence of dyslipidemia, CETP promoted atherogenesis. This association was strengthened when Okamoto et al. [3] showed that CETP in human plasma promoted the formation of atherogenic CE-rich remnant particles which are now known to penetrate the artery wall and initiate or aggravate atherogenesis [4].

In accord with earlier evidence suggesting that increased CET was proatherogenic, we have reported that CET is accelerated in groups of atherosclerosis-prone subjects with hypercholesterolemia [5] and in T1D [6,7] and T2D [8,9]. More recently de Vries et al's finding that CET is a determinant of intimal medial thickness in T2D patients further supports the concept that high rates of transfer of CE's from HDL to the apoB-containing lipoproteins is involved in atherogenesis [10].

When CET is accelerated in the dyslipidemias of metabolic diseases involving insulin resistance such as T2D, increases occur in the reciprocal net transfer of CE and TG between HDL and TGRLP respectively and concomitantly in the conversion of selected TGRLP to LDL and catabolism of apoA-I [11]. These events lead to a CETP-related reduction in HDL-C and to the phenotype of increased plasma TG, reduced HDL-C, and increased small dense LDL that typically is found in the metabolic syndrome and in T2D populations [12].

Probucol is a phenolic antioxidant compound that was introduced in 1977 in the pre-statin era as a lipid-lowering agent when the role played by inflammation and oxidation in atherogenesis was not fully understood. Although its anti-inflammatory and anti-oxidant properties have been shown subsequently to have beneficial vasculoprotective clinical effects, it was withdrawn from the market in western countries in 1995 because of its limited efficacy as an LDL-lowering agent and anxiety about its HDL-lowering effect and possible prolonged QT interval on ECG [13]. Despite this alarm, however, probucol continued to be prescribed without adverse effects in several Asian countries (China, Korea, Japan) and found to promote regression of tendinous xanthoma in patients with familial hypercholesterolemia [14,15] and to decrease carotid intimal thickness [16] and the rate of restenosis [17] and repeat revascularization after angioplasty [18] in patients with CHD.

Efforts to develop probucol congeners that retain its anti-inflammatory and antioxidant actions without lowering HDL have been focused on their impact on HDL metabolism and function [19]. As a result, little attention has been directed to their effects on the transport of the TGRLP or on CET in atherogenic dyslipidemia. We have previously shown that probucol treatment normalized the function of the TGRLP and CET in T1D [20] and in nondiabetic subjects with hypercholesterolemia [21]. In the present study, we sought to determine whether probucol had similar salutary effects in patients with T2D.

2. Materials and methods

2.1. Subjects

Seven subjects (five postmenopausal women receiving no hormonal replacement and two men; ages 57 ± 12 years) with T2D (duration 5 ± 7 years who were otherwise healthy were studied as outpatients. The diagnosis of T2D had been made on the basis of fasting hyperglycemia (>140 mg/dL) on at least two occasions and lack of history of ketoacidosis. All subjects were treated with sulfonylureas alone and followed weight-maintaining American Diabetes Association diets. Seven healthy normolipidemic non-obese hospital and laboratory employees (five females, ages 30–34; two males ages 28–55 years; triglyceride 80 ± 2.1; cholesterol 178.5 ± 28.8; HDL-C 64.3 ± 4.2 mg/dL) served as controls. Informed consent was obtained. The experimental protocol was approved by the Human Investigation Committees of Cook County Hospital and Rush-Presbyterian-St. Luke's Medical Center. No participant smoked cigarettes, took drugs that affected lipid metabolism, drank more than three ounces of alcohol per day, or had evidence of renal or liver disease.

2.2. Study design

Blood samples were obtained after an overnight fast (12–14 h) from T2D and control subjects at baseline and two months after probucol treatment (Lorelco, 500 mg twice daily). A 6 ml aliquot of plasma was used immediately for the measurement of cholesteryl ester transfer activity; the remaining plasma was frozen for lipid analyses later. Probucol was well tolerated and no significant side effects were observed. ECG's were not obtained pre- and post-treatment to assess possible probucol-related QT-interval effects. Compliance determined by pill counts was 90 ± 9%. Body weights remained stable and three-day dietary menus obtained by a registered dietitian at the time of each blood sampling were compared to document that there were no significant changes in composition and caloric content.

2.3. Analyses

Enzymatic kit methods were employed to measure cholesterol, plasma glucose (Boerhringer Mannheim Diagnostics Inc., Indianapolis, IN) and triglycerides (Sigma Chemical, St. Louis, MO). Free (unesterified) cholesterol was determined with a kit in which cholesterol ester hydrolase was omitted. LDL was calculated by the Friedewald formula and glycated hemoglobin levels measured by an immunoaffinity column method as previously described [6].

2.4. Cholesteryl ester transfer

Cholesteryl ester transfer activity (CET) of intact plasma was determined in each T2D subject before and after probucol