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Plasma Protein Lipofuscin-like Fluorophores in Men with Coronary Artery Disease Treated with Statins

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Background. Lipid oxidation products react with protein to produce lipofuscin-like fluorophores (P-LLF) and modified apolipoprotein B that is an important element of the atherogenic properties of oxidized low-density lipoprotein (oxLDL). The aim of this study was to compare plasma concentrations of P-LLF between men with coronary artery disease (CAD) treated with statin drugs and healthy controls and to identify determinants of P-LLF.

Methods. Plasma markers of protein modification including P-LLF and oxidized low-density lipoprotein-4E6 (oxLDL-4E6), low-density lipoprotein-conjugated dienes (LDL-CD), lipid peroxides, apolipoprotein B, and serum albumin were measured in 24 men with CAD who were receiving statin therapy and 20 healthy men in the same age range.

Results. Plasma P-LLF (+23%, $p = 0.001$) was significantly higher and plasma oxLDL-4E6 (−33%, $p < 0.001$) and apolipoprotein B (apoB) (−30%, $p < 0.001$) concentrations were significantly lower in men with CAD compared with controls. Plasma P-LLF concentration was correlated significantly with plasma apoB ($r = -0.596$, $p < 0.001$), serum albumin ($r = 0.518$, $p < 0.001$), and age ($r = 0.390$, $p = 0.009$) and these variables were independent predictors of P-LLF in the study population. Plasma P-LLF was no longer significantly higher in men with CAD when plasma apoB concentration was taken into account.

Conclusions. Plasma P-LLF concentration is abnormally high and appears to be closely associated with lower levels of apoB in men with CAD receiving statin therapy. ApoB may be a preferential target of reactive aldehydic lipid oxidation products and a decrease in apoB may increase the quantity of these products available for condensation with albumin. © 2007 IMSS. Published by Elsevier Inc.

Key Words: Lipofuscin-like fluorophores, Oxidized LDL, Apolipoprotein B, Albumin, Oxidative stress, Coronary artery disease, Statins.

Introduction

Protein lipofuscin-like fluorophores (P-LLF) are formed by the reaction of aldehydic lipid oxidation products with protein (1) and are found in aging cells, plasma (2), oxidized low-density lipoprotein (oxLDL) (3) and atherosclerotic lesions (3). The majority of P-LLF in plasma is located in the albumin fraction (2). Plasma P-LLF concentrations have been suggested as a marker of *in vivo* lipid peroxidation (2). In animals, administration of oxidized lipid or carbon tetrachloride enhances oxidative stress and increases plasma P-LLF concentration (2). Plasma P-LLF concentrations are abnormally high in patients with hypertension (2) and diabetes (2). Abnormally high levels of P-LLF may also be expected in patients with coronary artery disease (CAD) because there is evidence they have increased levels of oxidative stress (4–6). However, few studies have determined plasma P-LLF concentrations in patients with CAD.

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Reactive aldehydes from lipid oxidation modify the apolipoprotein B (apoB) moiety of low-density lipoproteins (LDL) during the formation of oxLDL (7). Thus, oxLDL is also a marker of protein oxidative modification by reactive aldehydic lipid oxidation products. OxLDL is thought to have an important role in the development, progression, and stability of human atherosclerotic lesions (7,8). Oxidation of LDL is believed to occur mainly in the artery wall. Prolonged residence of LDL in the arterial subendothelial space may provide the opportunity for oxidative modification of LDL, and the oxLDL formed may diffuse into the circulation (8). After sufficient oxidative modification of apoB, oxLDL is excessively internalized by macrophages via scavenger receptors, resulting in the formation of foam cells that are characteristic of early atherosclerotic lesions (7). High plasma oxLDL concentrations are a risk factor for CAD (9−12).

Virtually all patients who are diagnosed with CAD currently receive therapy with a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin) drug. These drugs are the primary therapy for decreasing LDL cholesterol levels and reducing risk of cardiovascular events. There is evidence that statins have antioxidant activity (13). They decrease superoxide production and attenuate the oxidative susceptibility of LDL (13). Furthermore, statins also decrease circulating levels of oxLDL (14−16). Because this decrease in oxLDL is in proportion to the concomitant decrease in plasma apoB (14), it is thought that statin therapy decreases oxLDL mainly by reducing the amount of substrate LDL available for oxidative modification in the artery wall (14).

Whether plasma P-LLF concentrations are abnormal in patients with CAD receiving statin therapy is unknown. The aim of the present study was to compare plasma P-LLF and oxLDL between men with CAD receiving statin therapy and healthy controls and to test for relationships between these variables.

Materials and Methods

Subjects

Twenty four men aged 49−68 years with angiographically proven CAD defined as one or more stenoses >50% arterial diameter were recruited from the Cardiovascular Clinic of the Cardiology Department, Dunedin Hospital. Exclusion criteria included myocardial infarction, presence of unstable angina, coronary artery bypass grafting, heart failure or other serious illnesses within the preceding 3 months, cigarette smoking, and use of antioxidant supplements. All men with CAD had been previously diagnosed with stable angina within 1−10 years prior to the study. Stable angina was defined according to the definitions of the AHA/ACC 2002 guidelines (17). The patients had symptoms for a median (range) of 2 (1−10) years and 75% had a previous percutaneous coronary intervention and three had cardiac bypass surgery at least 6 months prior to the study. The median (range) ejection fraction was 52% (38−72%). Twenty men aged 47−75 years and without a history of serious illness were recruited from advertisements and from the staff of the University of Otago. The presence of coronary disease was excluded by clinical history including absence of symptoms of angina or exertional dyspnea and a negative history of hypertension, previous cardiovascular events, or coronary revascularization. No subjects reported cigarette or antioxidant supplement use. The Otago Ethics Committee approved the study and all participants gave written informed consent before commencing the study.

Clinical Parameters

Clinical parameters including anthropometric data, health status, and drug treatments of the participants were obtained prior to entry into the study.

Blood Sampling

Venous blood was collected into tubes containing solid dipotassium EDTA or into plain tubes. Plasma and serum were prepared by centrifuging the tubes at 1500 × g for 15 min at 4°C. Aliquots of plasma and serum were immediately stored at −80°C.

Laboratory Methods

Lipofuscin-like fluorophores were measured at 350 nm excitation and 460 nm emission in an aqueous solution of plasma proteins after delipidation with ethanol/diethylether (3/1, v/v) (2). Values were expressed relative to fluorescence of a solution of quinine sulfate (0.05 μg/mL in 0.5 mol/L sulfuric acid). Plasma oxLDL-4E6 concentration was measured in duplicate by immunoassay using the mB-4E6 monoclonal antibody in a commercial kit (Mercodia, Uppsala, Sweden). Intra-assay coefficient of variation for this assay is 6%. Conjugated dienes were measured at 234 nm in the cyclohexane solution of lipids extracted from the plasma LDL fraction as described previously (18). Briefly, the LDL fraction was isolated from plasma using buffered heparin, extracted twice with chloroform-methanol (2/1, v/v), the chloroform was evaporated under oxygen-free nitrogen, and the residue was redissolved in cyclohexane. Cholesterol was measured using an enzymatic method (19) with Preciset standards (Boehringer Mannheim, Mannheim, Germany), in the extract after removal of solvent and redissolving the residue in propan-2-ol. Plasma peroxides were measured by an enzymatic method using horseradish peroxidase as described previously (20) with an incubation time of 45 min.

High-density lipoprotein cholesterol (HDL-C) was measured in the supernatant after precipitation of

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