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Dose-dependent effect of rosuvastatin on apolipoprotein B-100 kinetics in the metabolic syndrome☆

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Abstract

In a randomized, double-blind, crossover trial of 5-week treatment period with placebo or rosuvastatin (10 or 40 mg/day) with 2-week placebo wash-outs between treatments, the dose-dependent effect of rosuvastatin on apolipoprotein (apo) B-100 kinetics in metabolic syndrome subjects were studied. Compared with placebo, there was a significant dose-dependent decrease with rosuvastatin in plasma cholesterol, triglycerides, LDL cholesterol, apoB and apoC-III concentrations and in the apoB/apoA-I ratio, lathosterol:cholesterol ratio, HDL cholesterol concentration and campesterol:cholesterol ratio also increased significantly. Rosuvastatin significantly increased the fractional catabolic rates (FCR) of very-low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and LDL-apoB and decreased the corresponding pool sizes, with evidence of a dose-related effect. LDL apoB production rate (PR) fell significantly with rosuvastatin 40 mg/day with no change in VLDL and IDL-apoB PR. Changes in triglycerides were significantly correlated with changes in VLDL apoB FCR and apoC-III concentration, and changes in lathosterol:cholesterol ratio were correlated with changes in LDL apoB FCR, the associations being more significant with the higher dose of rosuvastatin. In the metabolic syndrome, rosuvastatin decreases the plasma concentration of apoB-containing lipoproteins by a dose-dependent mechanism that increases their rates of catabolism. Higher dose rosuvastatin may also decrease LDL apoB production. The findings provide a dose-related mechanism for the benefits of rosuvastatin on cardiovascular disease in the metabolic syndrome.

Keywords: Metabolic syndrome; Kinetics; Apolipoprotein B-100; Statin

1. Introduction

Dyslipoproteinemia is a well-recognized risk factor for cardiovascular disease (CVD) in insulin resistance and the metabolic syndrome. This type of dyslipidemia is associated with a high rate of hepatic cholesterol synthesis, which contributes to overproduction of VLDL apoB [1] and impaired catabolism of LDL apoB and remnant lipoproteins [2]. Insulin resistance is also associated with enhanced hepatic triglyceride synthesis and delayed catabolism of triglyceride-rich (TRL) and apoB-containing lipoproteins [3]. The delayed catabolism of TRL is in part a consequence of depressed activity of lipoprotein lipase (LPL) and decreased hepatic clearance receptors, and increased apoC-III concentration, as well as competition of exogenous and endogenously derived lipoproteins for common removal pathways [4–7]. Increased plasma triglycerides and delayed catabolism of LDL contributes to the accumulation in plasma of small dense LDL particles [8]. Furthermore, elevated cholesteryl ester transfer protein (CETP) activity increases the cholesterol content of triglyceride-rich apoB-containing

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lipoproteins, and contributes to generation of small dense LDL particles [9].

Statins inhibit hydroxymethylglutaryl (HMG) CoA reductase and thereby reduce cholesterol biosynthesis and up-regulate hepatic LDL receptor [10]. Atorvastatin, simvastatin, and pravastatin have been shown to increase the catabolism of apoB-containing lipoproteins, in insulin resistance, type 2 diabetes and mixed hyperlipidemia alone [11–13]. However, none of these statins have been shown to inhibit hepatic secretion of apoB in these subjects.

Rosuvastatin is a highly effective hydrophilic and hepatospecific HMG CoA reductase inhibitor (statin) in clinical use [14]. It has been shown to yield greater reduction in LDL cholesterol than that of some other statins at an equivalent dose [15], to improve triglyceride and LDL abnormalities in mixed hyperlipidemia [16], and to reduce CETP mass and activity in subjects with hypertriglyceridemia [16]. These changes in CETP may contribute significantly to the remodeling of LDL particles sufficient to enhance their clearance from plasma. Recent animal studies also suggest that rosuvastatin may inhibit hepatic VLDL production and triglyceride synthesis and enhance hepatobiliary lipid excretion [17,18]. These observations provide a rationale for the specific benefits of rosuvastatin in improving lipoprotein transport in the metabolic syndrome.

In this present study, we aim to assess the effects of two doses of rosuvastatin on apoB transport in subjects with the metabolic syndrome. We hypothesized that the higher dose would have a more favorable effect on apoB transport by increasing clearance of lipoproteins and possibly by reducing their production rate.

2. Materials and methods

2.1. Subjects

Twelve men with the metabolic syndrome by the NCEP ATP III definition [19] and HDL cholesterol $\leq$1.2 mmol/L were recruited. Subjects with LDL cholesterol >6 mmol/L, triglycerides >4.5 mmol/L, diabetes mellitus (fasting glucose >7 mmol/L), cardiovascular disease, renal dysfunction (macroproteinuria and/or serum creatinine >150 μmol/L), hypothyroidism, abnormal liver or muscle enzymes, alcohol consumption >30 g alcohol/day, use of lipid modifying agents and apolipoprotein E2/E2 genotype were excluded. All were non-smokers and were consuming ad libitum, weight maintenance diets. Participants provided informed written consent, and the study was approved by the Ethics Committee of Royal Perth Hospital.

2.2. Study design and clinical protocols

This was a randomized, double-blind, placebo-controlled, three-way cross-over trial. Eligible subjects entered a 4-week weight maintenance, placebo run-in period followed by randomization to a 5-week treatment period of either 10 or 40 mg rosuvastatin or matching placebo taken orally at night, with cross-over to two further 5-week treatment periods interspersed by 2-week wash-outs. Advice was given to continue on isocaloric diets and maintain physical activity constant during the study. Compliance with rosuvastatin or placebo was assessed by tablet count.

All subjects were admitted to the metabolic ward in the morning after a 12 h fast. They were studied semi-recumbent and allowed water only. Venous blood was collected for biochemical measurements. Body weight and height were measured and arterial blood pressure recorded using a Dinamap1846 SX/P monitor (Critikon, Tampa, FL). Dietary intake was assessed using 24 h dietary diaries and DIET 4 Nutrient Calculation Software (Xyris Software, Qld, Australia).

A single bolus of D_3-leucine (5 mg/kg) was administered intravenously within a 2 min period into an antecubital vein via a Teflon cannula. Blood samples were taken at baseline and at 5, 10, 20, 30, 40 min, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10 h after isotope injection, with additional fasting blood samples (24, 48, 72 and 96 h) collected in the morning on the following 4 days. All procedures were repeated at the end of each treatment period.

2.3. Isolation and measurement of isotopic enrichment of VLDL, IDL and LDL apoB-100

The isolation of VLDL, IDL and LDL apoB was performed as previously described [20]. In brief, VLDL, IDL and LDL fractions were isolated from 2 mL plasma by sequential ultracentrifugation (Optima XL-100K; Beckman Coulter, Fullerton, Australia) at densities of 1.006, 1.019 and 1.063 g/mL, respectively; precipitated by isopropanol, delipidated, hydrolyzed and derivatized using the oxazolinone derivative. Plasma-free leucine was isolated by cation-exchange chromatography using AG 50 W-X8 resin (Biorad, Richmond, CA) following removal of plasma protein with 60% perchloric acid. The isotopic enrichment was determined using gas chromatography–mass spectrometry (GCMS) with selected ion monitoring of samples at a mass-to-charge ratio (m/z) of 212 and 209 and negative ion chemical ionization. Tracer-to-tracee ratios were derived from isotopic ratios of each sample. Intra-assay CVs for plasma leucine, VLDL, IDL and LDL-apoB were <8.0%

2.4. Quantification of apoB and other analyses

Following isotope administration, four pooled plasma samples were collected at different time points during the study. ApoB in VLDL, IDL and LDL fractions was quantified by the Lowry method [21]. Inter-assay CV for this procedure was <5.0%.

Plasma total cholesterol, triglycerides and HDL cholesterol concentrations were determined by enzymatic methods (Hitachi, Tokyo, Japan; Roche Diagnostic GmbH, Mann-