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Favorable effects of ezetimibe alone or in association with simvastatin on the removal from plasma of chylomicrons in coronary heart disease subjects

Otavio Celeste Mangili [a], Ana C. Moron Gagliardi [a], Leonardo Celeste Mangili [a], Carlos H. Mesquita [b], Luiz A. Machado Cesar [a], Akira Tanaka [c], Ernst J. Schaefer [d], Raul C. Maranhão [a], Raul D. Santos [a,*]

[a] Heart Institute (InCor) University of Sao Paulo Medical Scholl Hospital, Sao Paulo, Brazil
[b] Instituto de Pesquisas Energéticas e Nucleares, IPEN, Sao Paulo, Brazil
[c] Kagawa Nutrition University, Tokyo, Japan
[d] Lipid Metabolism Laboratory, Human Nutrition Research Center on Aging at Tufts University and Tufts University School of Medicine, Boston, MA, USA

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ABSTRACT

Objective: Reductions on the clearance from plasma of chylomicrons are associated with atherosclerosis. Statins improve the removal from plasma of chylomicrons in a dose dependent manner. There is controversy whether ezetimibe modifies the plasma clearance of chylomicrons. Effects of ezetimibe alone or in combination with simvastatin were compared with low and high dose of the latter, upon the kinetics of a chylomicron-like emulsion in coronary heart disease (CHD) patients.

Methods: 25 CHD patients were randomized for treatment with ezetimibe 10 mg (group 1) or simvastatin 20 mg (group 2) with progression to ezetimibe + simvastatin 10/20 mg or simvastatin 80 mg, respectively. Kinetic studies were performed at baseline and after each treatment period of 6 weeks. The fractional catabolic rates (FCR) of the emulsion labeled with ^{14}C-CE and ^{3}H-TG, that represent respectively chylomicron remnant and triglyceride removal, were calculated. Comparisons were made by ANOVA.

Results: The ^{14}CE-FCR in group 1 were 0.005 ± 0.004, 0.011 ± 0.008 and 0.018 ± 0.005 min^{-1} and in group 2 were 0.004 ± 0.003, 0.011 ± 0.008 and 0.019 ± 0.007 min^{-1} respectively at baseline, after 6 and 12 weeks ($p < 0.05$ vs. baseline, and 6 vs. 12 weeks). The ^{3}H-TG-FCR in group 1 were 0.017 ± 0.011, 0.024 ± 0.011 and 0.042 ± 0.013 min^{-1} and in group 2 were 0.016 ± 0.009, 0.022 ± 0.009 and 0.037 ± 0.012 min^{-1} at baseline, after 6 and 12 weeks ($p < 0.05$ vs. baseline, and 6 vs. 12 weeks). There were no differences between groups in time.

Conclusion: Both treatments increased similarly the removal from plasma of chylomicron and remnants in CHD patients.

1. Introduction

The impaired plasma removal of chylomicrons and remnants has been associated with the incidence and progression of coronary atherosclerosis even when fasting plasma lipids are within normal values [1–5]. These particles rapidly penetrate and accumulate in the subendothelial space of the arterial wall, leading to macrophage uptake and foam cell formation [6]. Changes in chylomicron metabolism are also implicated with high-density lipoprotein cholesterol (HDL-C) reduction and impaired reverse cholesterol transport [3]. In addition to being removed from plasma by their specific receptor [7] LRP, by heparan-sulphate proteoglycans (HSPG) [8] and by very low density lipoprotein (VLDL) receptors [9], chylomicron remnants are also removed by the LDL receptor (LDLR) [7].

Statins reduce plasma low-density lipoprotein cholesterol (LDL-C) levels by inhibiting hydroxy-methyl-glutaryl coenzyme A reductase (HMGCo-A reductase), which leads to decreased intracellular hepatic cholesterol pool and consequently up-regulation of the LDLR expression of in liver [10]. In addition to increasing the plasma clearance of LDL particles, statins also enhance the removal

* Corresponding author. Unidade Clínica de Lípides InCor HCFMUSP, Av Dr Eneas C Aguiar 44 Segundo Andar Sala 4, CEP-05403-900 Sao Paulo SP, Brazil. Tel.: +55 11 26615320; fax: +55 11 2661517.
E-mail addresses: raul.santos@incor.usp.br, rdsf@uol.com.br (R.D. Santos).

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of chylomicrons and its remnants from the blood, an effect proportional to the potency of the statin [11,12].

Ezetimibe inhibits intestinal cholesterol absorption acting on the Niemman-Pick C1 Like 1 receptor located at the enterocyte brush border [13]. When used alone in subjects, ezetimibe reduces LDL-C by only 12–14% [13]. However, in combination with low dose statins there is a clear-cut synergic effect, and LDL-C lowering reaches 50–60% reduction, which is the range attained only by the most potent statins at the maximally approved dosage [14,15]. This effect has been ascribed to increased expression of LDLR consequent to the incremental reduction in intrahepatic cholesterol pool, which leads to greater removal from plasma of the apoB-100 containing lipoproteins [13,16].

Triglyceride-rich emulsions similar to small-sized chylomicrons have been extensively used to study chylomicron and remnant metabolism in different clinical sets. Reductions in the clearance from plasma of emulsion and remnants have been clearly shown in subjects with atherogenic dyslipidemia [17], familial hypercholesterolemia [18] and in subjects with stable coronary heart disease (CHD) under or not statin treatment [5,11,19,20]. In CHD patients reduction in emulsion clearance and lipolysis was associated with presence and progression of the atherosclerotic plaque, as well as with clinical cardiovascular events [20,21]. Procedures that change the expression of the LDLR, like cholesterol feeding [22] or the use of statins [11,12], have respectively reduced and increased the removal from plasma of the emulsion and remnants.

There is limited evidence from the literature that ezetimibe decreases the concentration of chylomicrons and remnants [23,24]. This is clearer when ezetimibe is added to statins in normolipidemic subjects without previous manifestation of CHD [24]. Furthermore, the mechanisms behind these findings are not fully understood.

This study was aimed to investigate the effects of ezetimibe alone or in combination with low-dose simvastatin as compared to intermediate and maximum simvastatin doses upon the removal from plasma of a chylomicron-like emulsion in CHD patients. The results show that ezetimibe alone or in association with simvastatin improved the chylomicron metabolism pathways.

2. Methods

2.1. Study patients

Twenty-five stable CHD patients from the outpatient clinic of the Heart Institute (InCor) of the University of Sao Paulo Medical School Hospital were studied. None had an acute coronary or cerebrovascular event or revascularization in the last 6 months. The mean age was 60 years-old; 20 (80%) were of the male gender. Inclusion criteria were LDL-C >100 mg/dl and plasma triglycerides <500 mg/dl after a lipid lowering drug wash-out of 6 weeks, a safe period for wash-out in CHD patients [25]. All studied women were post-menopausal and were not in use of hormone replacement therapy. Exclusion criteria included heart, kidney and hepatic failure as well as type 2 diabetes and thyroid disease. This was a randomized, non-blinded study, with two arms (Groups 1 and 2):

Group 1: 13 subjects randomized to ezetimibe 10 mg/day for 6 weeks, followed by ezetimibe 10 mg plus simvastatin 20 mg/day for additional 6 weeks.
Group 2: 12 subjects randomized to simvastatin 20 mg/day for 6 weeks and after that simvastatin was up-titrated to 80 mg/day for additional 6 weeks.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Ethics Committee of the Hospital das Clinicas of the University of São Paulo Medical School (CAPEPesq, protocol number 1068/06), and a written informed consent was obtained from all patients. This trial was registered at Clinicaltrials.gov with the number NCT00481351. Both simvastatin (Zocor®) and ezetimibe (Ezetrol®) were donated by MSD (São Paulo, Brazil). The design, development and analysis of this study were totally done by the investigators.

2.2. Plasma biochemical and apolipoprotein analysis

Fasting blood collection was performed at baseline, 6 weeks (second evaluation) and 12 weeks (third evaluation) after the start of the experiment. Total cholesterol (TC), HDL-C, and triglycerides (TG) were determined by enzymatic methods (commercial kits–Roche, Somerville, NJ, USA) and LDL-C was calculated by Friedewald formula (LDL = T C-HDL-TG/5) for values up to 400 mg/dL. When TG values were between 400 and 500 mg/dl, direct determination of LDL-C was carried out using and enzymatic homogeneous LDL-C kit (Roche, Somerville, NJ, USA). The quantification of apolipoproteins (apo) A-I and apoB-100 were done using commercial kits (Roche, Mannhein, Germany). Serum apoB-48 was quantified at fasting states, from serum samples frozen at −80 °C using an ELISA kit manufactured by Shibayagi Co (Gunma, Japan) at Boston Heart Diagnostics (Framingham, USA). The assay uses a monoclonal antibody that only recognizes apoB-48 and not apoB-100. Glucose, creatine kinase (CK) and alanine aminotransferase (ALT) levels were determined by standardized automated laboratory methods (Roche, Mannhein, Germany).

2.3. Chylomicron-like emulsion kinetic study

The chylomicron-like emulsions were prepared as previously described [5] by ultrasonic irradiation of lipid mixtures containing 2% cholesterol, 23% lecithin, 6% cholesteryl oleate (^{14}C-CE) and 69% triolein (^{3}H-TG) with 20 μCi of ^{14}C-CE and 40 μCi of ^{3}H-TG. Emulsions were purified by ultracentrifugation in density gradients as described previously [5] and sterilized by passage through a 0.2 μm filter. All kinetic studies were performed after a 12-h fast. One vein from each arm was cannulated and maintained with a saline flush. The chylomicron-like emulsion was injected in a *bolus* (volume of 200–300 μl), containing 148 kBq (4 μCi) of ^{3}H-TG and 74 (2 μCi) of ^{14}C-CE, followed by a 5 ml saline flush. Blood samples were collected from the contralateral arm vein at pre-established intervals during 60 min (2, 4, 6, 10, 15, 20, 30, 45 and 60 min after emulsion injection). Blood was collected into tubes containing 50 μl of sodium heparin and centrifuged at 2700 rpm for 10 min. An aliquot of 1 ml of plasma was transferred to counting vials and 5 ml of scintillation solution PPO: DM-POPOP: triton-100/toluene (5 g: 0.5 g: 333 ml:/667 ml) added to the vials. Radioactivity in the samples was determined using a Packard 1660 TR spectrometer (Packard Meridien). The calculated inter-assay coefficient of variation for those kinetic analyses was <3%. As previously described [5], the radiation dose injected in each experiment was much bellow the 50 mSV limit for radioactive intake, as determined by the International Commission on Radiological Protection [26]. For ^{14}C-CE, the dose was 0.04 mSV and for ^{3}H-TG, 0.0025 mSV. Patients were submitted to kinetic studies at baseline, 6 weeks and at 12 weeks of follow-up.

2.4. Kinetic analysis

Fig. 1 shows the kinetic model used in this study. After entering the plasmatic compartment emulsions adsorb apolipoproteins like apoE, apoC-II and apoC-III [27] and are quickly incorporated into the plasma lipoprotein pool. The plasma decay curves of the