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Effects of simvastatin, ezetimibe and simvastatin/ezetimibe on mitochondrial function and leukocyte/endothelial cell interactions in patients with hypercholesterolemia

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ABSTRACT

Background: Cholesterol-lowering therapy has been related with several beneficial effects; however, its influence on oxidative stress and endothelial function is not fully elucidated.
Aims: To investigate the effect of simvastatin and ezetimibe on mitochondrial function and leukocyte −endothelium interactions in polymorphonuclear cells of hyperlipidemic patients.
Methods: Thirty-nine hyperlipidemic patients were randomly assigned to one of two groups: one received simvastatin (40 mg/day) and the other received ezetimibe (10 mg/day) for 4 weeks, after which both groups were administered combined therapy for an additional 4-week period. Lipid profile, mitochondrial parameters (oxygen consumption, reactive oxygen species (ROS) and membrane potential), glutathione levels, superoxide dismutase activity, catalase activity and leukocyte/endothelial cell interactions and adhesion molecules -VCAM-1, ICAM-1, E-selectin, were evaluated.
Results: An improvement in lipid profile was observed after administration of simvastatin or ezetimibe alone (LDLc: −40.2 vs −19.6%, respectively), though this effect was stronger with the former ($p < 0.001$), and a further reduction was registered when the two were combined (LDLc: −50.7% vs −56.8%, respectively). In addition to this, simvastatin, ezetimibe and simvastatin + ezetimibe significantly increased oxygen consumption, membrane potential and glutathione content, and decreased levels of ROS, thereby improving mitochondrial function. Furthermore, simvastatin + ezetimibe increased catalase activity. In addition, simvastatin and simvastatin/ezetimibe improved leukocyte/endothelium interactions by decreasing leukocyte rolling and adhesion and increasing leukocyte rolling velocity. Finally, simvastatin, ezetimibe and simvastatin + ezetimibe reduced levels of the adhesion molecule ICAM-1, and ezetimibe + simvastatin significantly decreased levels of E-selectin.
Conclusion: Co-administration of simvastatin and ezetimibe has an additive cholesterol-lowering effect and beneficial consequences for mitochondrial function and leukocyte/endothelium interactions in leukocytes of hypercholesterolemic patients.

1. Introduction

Oxidative stress, mitochondrial dysfunction and inflammation are related to cardiovascular diseases (CVD) and atherogenesis [1]. One of the key phases in the development of atherogenesis is the

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oxidative modification of low-density lipoprotein (LDL) particles in the endothelium. Oxidative stress has also been implicated in leukocytes from patients with insulin resistance and/or high levels of lipids, and an increase in the number of leukocytes has been highlighted as a putative marker of low-grade chronic inflammation and early cardiovascular risk in these subjects [2]. In addition, proinflammatory cytokines and reactive oxygen species (ROS) are released by activated leukocytes within the inflammatory atherosclerotic plaque. In fact, the oxidation of LDL particles by ROS can activate the immune response, thus inducing the inflammatory cascade [3]. The overproduction of ROS under pathophysiologic conditions is a general feature of the development of CVD, in particular atherosclerosis. These ROS can be released from different sources, such as xanthine oxidase, myeloperoxidase, lipoxygenase, nicotinamide adenine dinucleotide phosphate oxidase, the uncoupling of nitric oxide synthase and, especially, mitochondria [4].

Leukocytes can adhere to the endothelium surface and migrate in order to kill the pathogens by generating ROS production during the phagocytic process. Under some situations of hypercholesterolemia there is an increased recruitment of leukocytes [5], which is related to endothelial dysfunction, and cardiovascular events are enhanced when blood pressure is high [6].

Statins are used to lower cardiovascular risk by reducing LDL production in the liver and by increasing their removal from the blood [7]. This beneficial action is attributed mainly to their lipid-lowering properties, but also to other effects by which they improve endothelial function, thereby decreasing inflammation and oxidative stress, and reduce thrombus formation [8]. However, some statin-treated patients continue to be at risk due to high levels of apolipoprotein (Apo) B [9]. Ezetimibe in combination with statin treatment reduces lipoprotein levels [10–12]. Today, there is controversy about whether or not ezetimibe exerts pleiotropic effects alone or only in combination with statin, and whether or not statin on its own or a combination of statin and ezetimibe have comparable antioxidant and antiinflammatory effects. In this sense, it has been published that simvastatin 40 mg, simvastatine/ezetimibe 10/10 mg and rosuvastatin 10 mg reduce the levels of inflammatory parameters such as oxidized LDL, 8-Epi prostaglandin F2 alpha and phospholipase A2, thus exerting an anti-inflammatory effect [13].

In light of this controversy, the present study was carried out to evaluate the effects of simvastatin, ezetimibe and simvastatin + ezetimibe on the lipid profile of patients with hypercholesterolemia, as well as on mitochondrial function, antioxidant content and leukocyte/endothelial interactions.

2. Materials and methods

2.1. Subjects

Patients with hyperlipidemia were recruited from the Service of Endocrinology and Nutrition of University Hospital Dr. Peset (Valencia, Spain). The inclusion criteria were selected following ACC/AHA guidelines for the treatment of blood cholesterol to reduce atherosclerotic cardiovascular disease risk in adults [14]. Exclusion criteria were diabetes mellitus, thyroid, neoplastic, renal, liver or chronic inflammatory disease and a triglyceride concentration >400 mg/dL.

2.2. Study design

The study consisted of a randomised, parallel trial and took place over a period of 2 months. Patients with hyperlipidemia were randomized into two groups: one group was administered simvastatin (40 mg/day) for 4 weeks, after which ezetimibe (10 mg/day) was added for the following 4 weeks (SIMV/SIMV + EZET Group), and the other group began taking ezetimibe (10 mg/day) for the first 4 weeks, after which simvastatin (40 mg/day) was added for the following 4 weeks (EZET/EZET + SIMV Group). Patients were encouraged to maintain their dietary habits and their normal pattern of activity.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by our hospital's Ethics Committee. Written informed consent was obtained from all patients. On the basis of these criteria, 42 patients were enrolled in the study, 39 patients completed the study and 3 dropped out because of intolerance to the treatment. The trial was registered in clinicaltrials.gov with study number NCT02304926.

2.3. Clinical and analytical parameters

In all subjects, an anthropometric and analytical evaluation was performed at baseline, 4 weeks and 8 weeks. Weight (kg), height (m) and waist (cm) were measured in all patients and their body mass index (BMI = weight (kg)/height $(m)^2$) then calculated. Blood was collected from the antecubital vein at 8–10 a.m, after 12 h of fasting. LDLc concentration was calculated using the Friedewald method. Total cholesterol and triglycerides were measured by means of enzymatic assays, and high-density lipoprotein cholesterol (HDLc) concentrations were recorded with a Beckman LX-20 autoanalyser (Beckman Coulter, La Brea, CA, USA) using a direct method. The intraserial variation coefficient was <3.5% for all determinations. Apo AI and B were determined by immunonephelometry (Dade Behring BNII, Marburg, Germany) with an intra-assay variation coefficient of <5.5%.

2.4. Cells

Human polymorphonuclear leukocytes (PMNs) were obtained from citrated blood samples and incubated for 45 min with dextran (3%). The supernatant was centrifuged at 250 g for 25 min over Fycoll-Hypaque. Lysis buffer was added to the pellet and centrifuged at room temperature (100 g, 5 min). PMNs were counted in a Scepter device (Millipore, MA, USA), washed in HBSS medium and stored in complete RPMI media.

2.5. Measurement of mitochondrial oxygen consumption, ROS production, membrane potential and glutathione content

PMNs were resuspended (5×10^6 cells/mL) in HBSS medium and placed in a gas-tight chamber. A Clark-type O_2 electrode (Rank Brothers, Bottisham, UK) was employed to evaluate mitochondrial O_2 consumption [15]. Sodium cyanide (10^{-3} mol/l), a mitochondrial complex IV inhibitor, was used as a negative control. Measurements were recorded using the Duo.18 data-device (WPI, Stevenage, UK). Rate of O_2 consumption (V_{O2max}) was calculated with the Graph Pad programme.

Total ROS production was evaluated in PMNs with the fluorescent probe (5×10^{-6} mol/L, 30 min) 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) by using a fluorimetry Synergy Mx plate reader (BioTek Instruments, Winooski, VT). The fluorescent dye tetramethylrhodamine methyl ester (TMRM, 5×10^{-6} mol/L) was used to assess mitochondrial membrane potential ($\Delta\Psi_m$). GSH content was estimated following incubation (30 min) with the fluorochrome 5-Chloromethylfluorescein Diacetate (CMFDA, 2.5×10^{-6} mol/L; excitation 492/emission 517 nm). In short, cells were seeded on 96-well plates, washed with phosphate-buffered saline and incubated with the corresponding fluorochrome diluted in phosphate-buffered saline. After 15 min at 37 °C,