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Fenofibrate and extended-release niacin improve the endothelial protective effects of HDL in patients with metabolic syndrome

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ABSTRACT

Background: Fibrates and niacin are at present the most effective therapies to increase plasma levels of high density lipoprotein-cholesterol (HDL-C); to date, limited data are available on their effects on HDL protective functions.

Methods and results: Within a multicenter, randomized, open-label, cross-over study, 37 patients with metabolic syndrome received 6 weeks' treatment with fenofibrate or extended-release niacin (ER niacin), with a 4 weeks' wash-out period. HDL ability to preserve endothelial cell homeostasis was assessed by incubating cultured endothelial cells with HDL isolated from patients at baseline and after each treatment. HDL isolated from patients at baseline were as effective as control HDL in inhibiting vascular cell adhesion molecule-1 (VCAM-1) expression, but less efficient in promoting endothelial cell nitric oxide (NO) release. Both fenofibrate and ER niacin increased HDL ability to inhibit TNFα-induced VCAM-1 expression (+7% and +11%, respectively). Fenofibrate and ER niacin also improved the impaired HDL ability to induce the expression of endothelial nitric oxide synthase and NO production (+10% and +8%, respectively). Interestingly, HDL isolated after treatment showed an ability to promote endothelial NO release similar to HDL isolated from controls. No differences were observed between the two drugs. With both drugs, HDL function was improved irrespective of baseline HDL-C levels.

Conclusion: Treatment with fenofibrate or ER niacin in patients with metabolic syndrome not only increased HDL-C levels but also improved the endothelial protective effects of HDL.

1. Introduction

Several studies have demonstrated an inverse correlation between plasma levels of high density lipoprotein cholesterol (HDL-C) and the incidence of coronary heart disease (CHD), independent of low density lipoprotein cholesterol (LDL-C) levels [1,2]. HDL-mediated atheroprotection is generally ascribed to the key role of HDL in the reverse transport of cholesterol from peripheral tissues to the liver for its excretion into the bile (RCT) [3]. However, several evidences suggest that HDL are able to exert a series of RCT-unrelated effects that could well contribute to atheroprotection [4]. In particular, HDL are involved in the maintenance of endothelial cell function and prevention of endothelial dysfunction through several mechanisms, including their anti-inflammatory role and their ability to promote nitric oxide release [5,6].

The anti-atherosclerotic role of HDL prompted the development of HDL-raising therapy to reduce the residual CHD risk observed even with aggressive statin treatment [7]. Inhibitors of the cholesteryl ester transfer protein (CETP), that potently increase plasma HDL-C levels, are under development, although the first two molecules, torcetrapib and dalcetrabip, failed for different reasons [8]. At present, the most effective HDL-raising agents available in clinical practice are fibrates and niacin. Fibrates are agonists of the peroxisome proliferator activated receptor-α and can increase plasma HDL-C levels up to 15% [9,10]. Niacin is a GPR109a agonist that can increase plasma HDL-C levels up to 30% [9,10]. Despite increasing HDL-C levels, these agents did not show a beneficial effect in reducing clinical endpoints [11–14].

The plasma level of HDL-C is generally used as a measure of circulating HDL particles and thus of HDL-mediated atheroprotection. However, this assumption is presently questioned and the measure of different

Abbreviations: ABCA1, ATP-binding cassette A1; ABCG1, ATP-binding cassette G1; CEC, cholesterol efflux capacity; CER, cholesterol esterification rate; CETP, cholesteryl ester transfer protein; CETR, cholesteryl ester transfer rate; CHD, coronary heart disease; eNOS, endothelial nitric oxide synthase; ER niacin, extended release niacin; HDL, high density lipoproteins; HUVEC, human umbilical vein endothelial cell; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; NO, nitric oxide; RCT, reverse cholesterol transport; SR-BI, scavenger receptor BI; VCAM-1, vascular cell adhesion molecule-1.

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HDL subclasses, that have been shown to exert peculiar atheroprotective activities, and/or the direct assessment of HDL function are suggested by many researchers [15,16]. Few studies have evaluated the effects of fibrates and niacin on the HDL-mediated ability to promote macrophage cholesterol efflux, showing that both drugs improve the capacity of HDL to accept cell cholesterol via different pathways [10]. Fibrates, by increasing small HDL particles, promote the ABCA1-mediated efflux pathways, while niacin shows modest effects on the pathways involving large HDL, in agreement with the changes observed on HDL particles [9]. Much less is known on the effects of these drugs on other atheroprotective function of HDL. The present study was thus designed to explore the effects of fenofibrate and ER niacin, given to dyslipidemic patients with the features of the metabolic syndrome at equipotent HDL-C-raising doses (half of the maximum recommended dose for ER niacin), on the ability of plasma HDL to preserve endothelial cell functions.

2. Methods

2.1. Patients and trial design

The study was a multicenter, open-label, randomized cross-over study aimed at evaluating the effects of fenofibrate and ER niacin on HDL distribution and function, as previously described [17]. Patients with primary hypercholesterolemia or mixed dyslipidemia (LDL-C >130 to <190 mg/dl and triglycerides >135 to <440 mg/dl) and with either low (<40 mg/dl) or normal (≥40 mg/dl to <59 mg/dl) HDL-C levels at low CHD risk were enrolled (protocol BP20843) [17]. After a run-in period of 2-week for treatment-naïve or of 6-week for patients taking lipid-lowering therapies, during which any previous lipid-interfering therapy was discontinued, patients were randomized to one of the two treatment sequences: fenofibrate 160 mg/d for 6 weeks, or ER niacin 0.5 g/d for 3 weeks followed by 1 g/d for 3 weeks, with 4 weeks' wash-out before being crossed over to the alternative treatment. Fenofibrate and ER niacin doses were selected in order to obtain a comparable increase of HDL-C. Healthy volunteers with an HDL-C ≥59 mg/dl were recruited as untreated control subjects. Plasma levels of total cholesterol, LDL-C, triglycerides, and HDL-C were measured by certified enzymatic colorimetric tests, and apoA-I and apoA-II levels by nephelometry [18]. HDL size and HDL small, medium, large and total particle number were evaluated by nuclear magnetic resonance [19]. Plasma levels of LCAT and CETP were measured by competitive ELISA [20]; cholesterol esterification rate and cholesteryl ester transfer rate were measured as described [21,22]. Pathway-specific serum cholesterol efflux capacity (CEC) were evaluated using cell-based assays [23]. To confirm a return to baseline values after wash-out, lipid and functional parameters at the start of the first and second treatment periods were compared.

2.2. HDL purification

HDL (d = 1.063–1.21 g/ml) were purified by sequential ultracentrifugation from plasma samples of 37 patients (18 with low HDL-C and 19 with normal HDL-C) collected at the beginning and at the end of each treatment period, and of 18 controls. Purified lipoproteins were dialyzed against sterilized saline immediately before use. HDL concentrations are expressed as mg of protein/ml.

2.3. Cell studies

Experiments were performed on primary cultures of human umbilical vein endothelial cells (HUVEC) purchased from Clonetics (Lonza), in M199 with 0.75% BSA, 1% FCS. HDL were used at the protein concentration of 1.0 mg of protein/ml in all experiments.

The inhibition of vascular cell adhesion molecule-1 (VCAM-1) expression induced by TNFα was assessed as previously described [24]. Briefly, endothelial cells were incubated overnight with HDL, washed with PBS and then stimulated with TNFα (10 ng/ml) for 8 h; VCAM-1 concentration in conditioned medium was evaluated by a commercial matched antibody pairs ELISA kit (BioSource) and normalized by the protein concentration of the total cell lysate.

Nitric oxide (NO) production and endothelial nitric oxide synthase (eNOS) expression in endothelial cells were evaluated as previously described [25]. Expression of eNOS was evaluated by SDS-PAGE and immunoblotting after overnight incubation with HDL. Membranes were developed against total eNOS (BD Biosciences), stripped and reprobed with an antibody against β-actin (Sigma-Aldrich Chemie). Bands were visualized by enhanced chemiluminescence (GE Healthcare Biosciences) and analyzed with a GS-690 Imaging Densitometer and Multi-Analyst software (Bio-Rad Laboratories). NO production was evaluated after 30 min incubation with HDL by fluorescence using diacetate 4,5-diaminofluorescein (DAF-2 DA, Sigma-Aldrich Chemie). Fluorescence intensity was detected with a Synergy Multi-Mode microplate reader equipped with the GEN5 software (BioTek). For each sample, fluorescence was normalized by the protein concentration of the total cell lysate.

2.4. Statistical analysis

Data are expressed as mean ± SD, if not otherwise stated. Variations in the measured parameters after each treatment period were expressed as delta, calculated by subtracting the value at the end of the treatment period from the pre-treatment value. The effects of fenofibrate and ER niacin, and carry-over effects were evaluated according to methods proposed by Grizzle [26]. Baseline and post-treatment values in patients were compared with controls by two sample t-test. The association between delta of HDL function and delta of other biochemical parameters was tested by Spearman correlation. Tests were two-sided and the *P* values below 0.05 were considered as significant. All analyses were performed by using the SAS Statistical package v. 9.2 (SAS Institute Inc.).

3. Results

3.1. Plasma lipid profile of patients and controls

Baseline characteristics of patients and control subjects are reported in Table 1. Patients had a higher proportion of males and were older than control subjects and showed the typical phenotype of the metabolic syndrome, characterized by high body mass index, large waist circumference, low HDL-C, and elevated LDL-C and triglycerides. Plasma lipid changes observed with fenofibrate and ER-niacin in patients enrolled in the present study are reported in Tables 2 and 3; drug-induced changes were very similar to that observed in the entire cohort of patients [17]. Total and LDL cholesterol plasma levels were significantly lowered with fenofibrate and remained unchanged with ER-niacin. Plasma triglyceride levels were reduced by both drugs; fenofibrate was more effective than ER-niacin. HDL-C levels were increased at the same extent by the two agents, as expected at the used doses. Both drugs raised apoA-I levels, with no statistical difference between the two treatments, while apoA-II levels significantly increased only with fenofibrate.

3.2. Endothelial protective effects of HDL from patients with metabolic syndrome

HDL capacity to protect the endothelium was tested as (i) HDL ability to inhibit the cytokine-induced adhesion molecule expression, and (ii) HDL ability to stimulate eNOS expression and induce endothelial cell NO production. When compared at the same protein concentration, HDL isolated from patients at baseline were as effective as HDL isolated from healthy controls in inhibiting the cytokine-induced expression of