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Fenofibrate, HDL, and cardiovascular disease in Type-2 diabetes: The DAIS trial

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ABSTRACT

Background: There are conflicting reports on the role of fibrates in CVD-risk. Several studies indicate beneficial effects of fibrates on CVD risk in type-2 diabetic patients. We tested how fenofibrate changes lipoprotein subfractions and glucose homeostasis in type-2 diabetic patients.
Study design: Selected markers of lipid and glucose homeostasis and inflammation were measured in 204 diabetic patients who participated in the Diabetes Atherosclerosis Intervention Study (DAIS) and were randomly assigned to 200 mg fenofibrate or placebo. Percent changes from baseline until a minimum of 3 years (average 39.6 months) on therapy (end of study) were calculated for all study parameters.
Results: The concentrations of total LDL-C and small dense LDL-C (sdLDL-C) did not change on fenofibrate compared to placebo. Compared to placebo, fenofibrate significantly decreased concentrations of triglyceride and remnant-like particle cholesterol (RLP-C) and activity of lipoprotein-associated phospholipase A_2 (Lp-PLA_2), while significantly increased concentrations of HDL-C. In contrast to other lipid-modifying drugs (e.g. statins) which increase HDL-C by increasing large (α-1) HDL particles, fenofibrate increased HDL-C by increasing the smaller, less antiatherogenic HDL-C particles, α-3 and α-4. Furthermore, despite lowering TG levels by 20%, fenofibrate failed to decrease pre-β1 levels. On fenofibrate, glycated serum-protein levels increased moderately, while insulin and adiponectin levels did not change.
Conclusion: On fenofibrate, lipid homeostasis improved and Lp-PLA2 activity decreased while there was no improvement in glucose homeostasis. Despite increasing HDL-C and decreasing triglyceride levels, fenofibrate failed to improve the antiatherogenic properties of the HDL subpopulation profile.

1. Introduction

The major cause of death in patients with type-2 diabetes (T2DM) is cardiovascular disease (CVD) [1,2]. Diabetic patients, if not receiving insulin, often have decreased high-density lipoprotein cholesterol (HDL-C) and elevated triglyceride (TG) levels [3]. In the Helsinki Heart Study (HHS) and the Veterans Affairs HDL Intervention Trial (VA-HIT), administration of gemfibrozil, a PPAR-α agonist, caused a concomitant decrease in CVD risk by increasing HDL-C and decreasing TG levels [4,5]. However, in a post-hoc analysis of VA-HIT, Robins et al. concluded that the CVD risk-lowering effects of gemfibrozil could not be entirely explained by the modest increase in HDL-C observed in the treatment arm [6]. The reduction in CVD was greatest in those individuals who had at least some of the characteristics of the metabolic syndrome both in the HHS and the VA-HIT [4,5]. In the latter, Rubins et al. found that the beneficial effects of gemfibrozil on CVD events were greater in patients with either T2DM or pre-diabetes [7]. Moreover, measurement of HDL subpopulations in VA-HIT participants indicated that gemfibrozil decreased the levels of the large, antiatherogenic α-1 HDL particles; though baseline levels of α-1 HDL were inversely associated with future CVD events [8,9].

In previous lipid-lowering intervention trials, only post-hoc subgroup analyses on people with diabetes have been presented.

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The Diabetes Atherosclerosis Intervention Study (DAIS) was the first study specifically designed to investigate whether correcting dyslipidemia in type-2 diabetes mellitus with fenofibrate would reduce coronary artery disease (CAD) as determined by angiography [10]. We present an analysis on the effects of fenofibrate on LDL and HDL subpopulations and other emerging CVD risk factors on a subset of the DAIS study.

2. Methods

2.1. Study design and population

DAIS took place in 11 clinical centers in Canada, Finland, France, and Sweden between 1996 and 1999, as previously described [10]. Eligible participants were patients with dyslipidemia and T2DM aged 40–65 years, with or without previous coronary intervention. The lipid and diabetes eligibility characteristics were assessed during an 8-week baseline period during which participant were not receiving lipid-lowering medications of any kind but were following an American Heart Association/National Cholesterol Education Program Step 1 diet. The same diet was maintained throughout the treatment period. Lipid entry criteria were: total cholesterol to HDL-C ratio $\geq$4:1, plus either an LDL-C concentration of 3.5–4.5 mmol/L and TG concentration of $\leq$5.2 mmol/L, or a TG concentration of 1.7–5.2 mmol/L and $\leq$LDL-C 4.5 mmol/L. Diabetes entry criteria were: 1) T2DM as indicated by a fasting plasma glucose concentration without treatment of more than 7.8 mmol/L, or a plasma glucose concentration of 11.0 mmol/L or more 2 h after a 75 g oral glucose load, or on treatment with glucose-lowering drugs; 2) diagnosis after age 35 years; 3) no history of ketoacidosis; and 4) adequate glycemic control (hemoglobin A1c <170% of laboratory's upper normal limit). DAIS was not a trial of the effects of glycemic control; as such, participants' physicians were allowed to adjust the glucose-lowering drug regimen to optimize control in individual patients. Eligible patients were assigned to fenofibrate or placebo with stratification by sex, previous coronary intervention, and clinic center using a permuted blocks randomization procedure. The treatment period was at least 3 years. The protocol was reviewed and approved by each institution's ethics committee, and all participants gave informed consent to take part.

DAIS analyzed 207 subjects in the fenofibrate and 211 in the placebo arm. All subjects for whom plasma samples were available for further analyses were included: 108 subjects (51.2%) in the fenofibrate arm and 96 subjects (45.5%) in the placebo arm.

2.2. Laboratory measurements

Fasting plasma samples stored at −80 °C were used. Automated chemistries were measured on a Hitachi 911 analyzer. Total cholesterol, TG, and HDL-C were measured using kits from Roche. ApoA-I and highly-sensitive C-reactive protein (hsCRP) were measured using immunoturbidimetric assay kits from Wako Diagnostics (Richmond, VA). Small dense LDL-C (sdLDL-C) and LDL-C were measured using kits from Denka-Seiken (Japan). Remnant-like particle cholesterol (RLP-C) was measured using kits from Kyowa-Medex (Japan). Insulin was measured with kits from Kamiya Biomedical (Seattle, WA), glycated albumin was measured with kits from Asahi Kasei Pharma (Japan), adiponectin was measured with kits from Otsuka Pharmaceutical (Japan). Lipoprotein-associated phospholipase A2 (Lp-PLA2) concentration and activity were measured at DiaDexus (San Francisco, CA).

ApoA-I-containing HDL particles were determined by 2-dimensional, non-denaturing gel electrophoresis followed by immunodetection and image analysis as described earlier [11,12]. Briefly: in the first dimension, HDL was separated from 4 μl plasma on 0.7% agarose gel by charge into preβ-, α-, and preα-mobility particles. In the second dimension, each sample was further separated according to size by non-denaturing polyacrylamide gel electrophoresis (on 3–35% concave gradient gels). Gels were electro-transferred to nitrocellulose membranes. ApoA-I was immunolocalized by incubation with monospecific goat human apoA-I antibody for 6 h. After the unbound first antibody was washed off with PBST, membranes were incubated with ^{125}I-labeled secondary antibody. Signals were quantitatively determined by image analysis using a FluoroImager (Molecular Dynamics, Sunnyvale, CA). Ten apoA-I-containing HDL subpopulations were delineated; signals were measured in each area, and used for calculating the percent distribution. Concentration of each subpopulation was calculated by multiplying percentiles by total plasma apoA-I concentration. CVs for HDL subpopulation measurements are consistently <15% in our laboratory.

2.3. Data and statistical analysis

Percent changes from baseline until a minimum of 3 years (average 39.6 months) on therapy (end of study) were calculated for all study parameters. Assays which yielded data outside of the measureable level were imputed as the lower or upper limit of detection as appropriate. Missing data secondary to plasma volume insufficiency was imputed using multiple imputation by chained equations (MICE) utilizing all lipid parameters in the MICE model. A burn in of 10 iterations was used to reach converge to produce each of 20 multiple imputations for the final analytical data set. Therefore, all 204 participants for whom any plasma volume was available were included in the analysis. The normality of percent differences were assessed via a Shapiro–Wilk test, and means and standard deviations were calculated. Intra- and intergroup differences from baseline were analyzed with univariate and bivariate linear regression, respectively. The median and interquartile range of percent changes were reported for parameters which violated the normality assumption, and intra- and inter-group differences were analyzed with median quantile regression. All p values and confidence intervals are reported unadjusted, but the false discovery rate method was employed [13]. All analyses were performed using STATA version 12 (StataCorp, TX, USA).

3. Results

There were no differences at baseline between the two arms in any of the parameters investigated (result not shown). Table 1 shows plasma lipid, inflammatory, and metabolic parameters in the fenofibrate and placebo groups. LDL-C increased 10.1% ($p = 0.01$) in the placebo and 5.5% ($p = 0.43$) in the fenofibrate group resulting in no significant difference between the two treatment groups ($p = 0.57$). Concentration of sdLDL-C slightly increased in the placebo group (3.5%, $p = 0.48$) and slightly decreased (−11.8%, $p = 0.07$) in the fenofibrate group, but the difference between the two groups was not significant ($p = 0.60$). TG decreased more in the fenofibrate (−29.1%, $p < 0.001$) than in the placebo group (−9.4%, $p = 0.04$) resulting in a significant treatment difference ($p < 0.001$) even after taking into consideration multiple comparisons. Concomitantly, RLP-C decreased more in the fenofibrate (−31.9%, $p < 0.001$) than in the placebo (−7.2%, $p = 0.11$) group with a treatment difference of −24.6 ($p < 0.001$). Fenofibrate increased HDL-C more (9.9%, $p < 0.001$) than placebo treatment (2.0%, $p = 0.13$) resulting in a significant difference between the two groups of 7.9% ($p = 0.002$). ApoA-I increased slightly more in the fenofibrate than in the placebo group (5.1%, $p = 0.002$ vs. 1.2%, $p = 0.39$), but the difference between the two treatments was not significant ($p = 0.07$). Glycated albumin (GA), a marker of diabetes,