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Effect of dalcetrapib, a CETP modulator, on non-cholesterol sterol markers of cholesterol homeostasis in healthy subjects

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ABSTRACT

Objective: Subjects with high HDL-C show elevated plasma markers of cholesterol absorption and reduced markers of cholesterol synthesis. We evaluated the effect of dalcetrapib, a cholesteryl ester transfer protein modulator, on markers of cholesterol homeostasis in healthy subjects.
Methods: Dalcetrapib was administered daily with or without ezetimibe in a randomized, open-label, crossover study in 22 healthy subjects over three 7-day periods: dalcetrapib 900 mg, ezetimibe 10 mg, dalcetrapib 900 mg plus ezetimibe 10 mg. Plasma non-cholesterol sterols lathosterol and desmosterol (cholesterol synthesis markers) and campesterol, β-sitosterol and cholestanol (intestinal cholesterol absorption markers) were measured. A hamster model was used to compare the effect of dalcetrapib and torcetrapib with or without ezetimibe on these markers and determine the effect of dalcetrapib on cholesterol absorption.
Results: Dalcetrapib increased campesterol, β-sitosterol, and cholestanol by 27% ($p = 0.001$), 32% ($p < 0.001$), and 12% ($p = 0.03$), respectively, in man (non-cholesterol sterol/cholesterol ratio). Dalcetrapib + ezetimibe reduced campesterol by 11% ($p = 0.02$); β-sitosterol and cholestanol were unaffected. Lathosterol and desmosterol were unchanged with dalcetrapib, but both increased with ezetimibe alone (56–148%, $p < 0.001$) and with dalcetrapib + ezetimibe (32–38%, $p < 0.001$). In hamsters, dalcetrapib and torcetrapib increased HDL-C by 49% ($p = 0.04$) and 72% ($p = 0.003$), respectively. Unlike torcetrapib, dalcetrapib altered cholesterol homeostasis towards increased markers of cholesterol absorption; cholesterol synthesis markers were unaffected by either treatment. Dalcetrapib did not change plasma ^{3}H-cholesterol level but increased ^{3}H-cholesterol in plasma HDL vs non-HDL, after oral dosing of labeled cholesterol.
Conclusion: Dalcetrapib specifically increased markers of cholesterol absorption, most likely reflecting nascent HDL lipidation by intestinal ABCA1, without affecting markers of synthesis.

1. Introduction

Cholesterol homeostasis can be assessed in various species by the simultaneous measurement of plasma levels of non-cholesterol sterols, i.e., lathosterol and desmosterol as markers of cholesterol synthesis and the plant sterols campesterol and β-sitosterol as markers of intestinal cholesterol absorption [1]. Predictably, statin-treated patients manifest a decrease in markers of cholesterol synthesis and an increase in markers of cholesterol absorption [2,3]. Similarly, administration of the cholesterol absorption inhibitor ezetimibe induces a decrease in plasma markers of cholesterol absorption and a reciprocal increase in markers of cholesterol synthesis [4,5]; these investigations established an inverse relationship between cholesterol synthesis and absorption [1,6].

A characteristic feature of subjects with high HDL-C is elevated levels of plasma markers of cholesterol absorption (β-sitosterol and campesterol) and correspondingly reduced markers of cholesterol synthesis. Although the mechanisms linking HDL-C levels to markers of cholesterol absorption are not completely understood, this relationship has been consistently observed and correlated with HDL-C levels in subjects across a range of plasma cholesterol levels and clinical conditions [4,6,7–9].

New approaches such as inhibition and modulation of cholesteryl ester transfer protein (CETP) are under consideration as means to increase HDL-C levels [10]. Clinical investigations with torcetrapib, the first CETP inhibitor to enter a large phase III outcomes study, raised concerns not only over the off-target effect of torcetrapib [11–13] but also about the functionality

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of the HDL raised by CETP inhibitors (CETPi). Consistent with previous studies indicating that CETP plays a role in delivery of HDL-CE to the liver via the SRB1 receptor [14], a process partially inhibited by torcetrapib [15], a recent preclinical report [16] suggested that HDL raised following complete CETP inhibition may not be efficient in macrophage-to-feces reverse cholesterol transport (RCT). This is in contrast to the CETP modulator (CETPm) dalcetrapib, which increases RCT and maintains pre-β-HDL, the preferred acceptor of cholesterol and β-sitosterol effluxed by the enterocyte ABCA1 transporter [17]. We therefore hypothesized that dalcetrapib would increase markers of cholesterol absorption without an increase in cholesterol synthesis.

In a first study, dalcetrapib was administered to healthy volunteers with or without the cholesterol absorption inhibitor ezetimibe [18]; ezetimibe provided a positive control for the model system [5]. We then compared the effects of dalcetrapib (CETPm) and torcetrapib, a CETP inhibitor (CETPi) on non-cholesterol sterol markers in the validated hamster model and measured the effect of dalcetrapib on orally administered ^{3}H-cholesterol absorption and distribution in plasma HDL and non-HDL fractions.

2. Methods

2.1. Materials

Dalcetrapib and torcetrapib were synthesized at Hoffmann-La Roche AG, Basel, Switzerland using standard procedures. Ezetimibe 10 mg was purchased as Ezetrol® from a commercial source. Epicholesterol, cholesterol, desmosterol, lathosterol and campesterol were purchased from Steraloids (Newport, RI, USA) and cholestanol and β-sitosterol were purchased from Fluka (Sigma–Aldrich, Buchs, Switzerland).

2.2. Clinical study methods

A detailed protocol is described in [18]. In brief, the study was performed in 27 healthy male subjects and conducted in compliance with the principles of the Declaration of Helsinki and performed according to Good Clinical Practice Guidelines. All subjects provided written informed consent. The protocol was reviewed by an independent ethics committee, Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale d'Alsace, Strasbourg, France. Dalcetrapib (300 mg tablets) was provided by Clinical Trial Supplies, F. Hoffmann-La Roche Ltd (Basel, Switzerland) in accordance with Roche standards and local regulations.

The study was a randomized, open label, crossover study with three 7-day treatment periods: dalcetrapib 900 mg alone, dalcetrapib 900 mg plus ezetimibe 10 mg and ezetimibe 10 mg alone, with 10–14 days washout between treatments. Pharmacodynamic parameters including fasting lipid profile (HDL-C, LDL-C, total cholesterol (TC), triglycerides (TG), and very-low-density lipoprotein cholesterol (VLDL-C)) and CETP mass and activity were determined pre-dose on Days 1 and 7 as described [18]. Lipid profile analysis was performed by Eurofins Medinet BV (Breda, The Netherlands) using a Roche Modular system, as follows: TC using cholesterol esterase, cholesterol oxidase; TG using Roche enzymatic method (lipase, glycerol kinase); HDL with WAKO direct homogeneous enzymatic method; LDL with Roche homogeneous enzymatic method. Non-cholesterol sterols were measured by Roche as described below. Samples from the 22 subjects who completed all three treatment periods were included in the analyses reported here.

2.3. Hamster study protocol

Male Golden Syrian hamsters (Charles River, Sulzfeld, Germany) of 110–130 g body weight were housed individually in a temperature controlled environment with a 12 h/12 h light/dark cycle. Animals received a high-fat diet (Provimi Kliba, Kaiseraugst, Switzerland, #2453) 10 g total daily containing 14% fat and 0.05% cholesterol. After 2 weeks of feeding, animals were assigned to treatment groups according to their blood HDL-C, LDL-C, and TG levels and body weight. Subsequently, hamsters received the same diet supplemented with 0.0014% ezetimibe (wt:wt; equivalent to approximately 1 mg/kg.day), 0.014% torcetrapib (wt:wt, equivalent to approximately 10 mg/kg.day) or 0.42% dalcetrapib (wt:wt; equivalent to approximately 300 mg/kg.day) for 7 days.

Blood samples were collected 2 h after food removal pre-treatment on Day 14 and post-treatment on Day 21. Pre-treatment plasma TC, TG, HDL-C and LDL-C levels were quantified by enzymatic methods on an automated analyzer (Hitachi 912, Roche Diagnostics AG system). Plasma lipoprotein cholesterol levels at the end of treatment were determined using the PEG precipitation technique. Briefly, 50 μl plasma was mixed with 50 μl of a 14% PEG 6000 (Fluka, #81253) solution freshly prepared in distilled water, vortexed for 20 s, and centrifuged at room temperature. The supernatant was then collected for cholesterol measurement as above.

2.4. Cholesterol absorption study

Hamsters were dosed orally twice daily with dalcetrapib (150 mg/kg in 0.5% hydroxypropyl methyl cellulose [Sigma]), ezetimibe (1 mg/kg) or vehicle for 7 days. Two hours following the final dose, hamsters were gavaged with ^{3}H-cholesterol (cholesterol [1,2-^{3}H (N)]), Perkin Elmer (specific activity: 47.9 Ci/mmol; 1.772 TBq/mmol) in olive oil and injected with the lipase inhibitor Poloxamer-407 (1 g/kg, intraperitoneally). ^{3}H-tracer appearance in plasma was monitored 3 h after label administration. The HDL fraction was isolated by PEG precipitation as above and radioactivity counted in a liquid scintillation analyzer (Perkin Elmer TriCarb 2800TR).

2.5. Analysis of plasma neutral sterols

Plasma samples were saponified in ethanolic potassium hydroxide after addition of epicholesterol as internal standard. Neutral sterols were converted to trimethylsilyl derivatives [19]. Quantification of cholestanol, desmosterol, lathosterol, campesterol and β-sitosterol including cholesterol for comparison was performed by GC–MS on a narrow-bore fused silica column (15 m × 0.25 mm i.d. × 0.25 μm film thickness) using a 6890N GC coupled to a MSD 5973 inert mass-selective detector (Agilent Technologies, Switzerland). Electron impact ionization was employed at 70 eV ionization energy. Selected ion monitoring was performed for highest sensitivity and selectivity: target and qualifier ions were chosen according to their intensities and uniqueness in the full mass spectrum. Target ion (first), qualifier ion (second) and relative retention times were as follows: epicholesterol (458.4, 443.4, and 1.0), cholesterol (443.4, 458.4, and 1.046); cholestanol (306.3, 403.3, and 1.050), desmosterol (343.3, 441.3, and 1.058); lathosterol (458.4, 255.2, and 1.069); campesterol (382.4, 343.3, and 1.092) and sitosterol (396.4, 486.4, and 1.135).

The concentration of cholestanol, desmosterol, lathosterol, campesterol and β-sitosterol was calculated from standard curves with epicholesterol as internal standard and expressed as concentrations (mg/dl) as well as the non-cholesterol sterol/cholesterol ratio.