European Journal of Pharmacology 740 (2014) 135–143

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

Anacetrapib and dalcetrapib differentially alters HDL metabolism and macrophage-to-feces reverse cholesterol transport at similar levels of CETP inhibition in hamsters

François Briand [a], Quentin Thieblemont [a], Elodie Muzotte [a], Noémie Burr [a], Isabelle Urbain [a], Thierry Sulpice [a], Douglas G. Johns [b,*]

[a] Physiogenex SAS, Prologue Biotech, Labège, France
[b] Department of Cardiovascular Diseases, Atherosclerosis, Merck Research Laboratories, Rahway, NJ, USA

ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form
10 June 2014
Accepted 17 June 2014
Available online 5 July 2014

Keywords:
Cholesteryl ester transfer protein inhibition
Reverse cholesterol transport
High density lipoprotein
Hamster

ABSTRACT

Cholesteryl ester transfer protein (CETP) inhibitors dalcetrapib and anacetrapib differentially alter LDL- and HDL-cholesterol levels, which might be related to the potency of each drug to inhibit CETP activity. We evaluated the effects of both drugs at similar levels of CETP inhibition on macrophage-to-feces reverse cholesterol transport (RCT) in hamsters. In normolipidemic hamsters, both anacetrapib 30 mg/kg QD and dalcetrapib 200 mg/kg BID inhibited CETP activity by ~60%. After injection of ^{3}H-cholesteryl oleate labeled HDL, anacetrapib and dalcetrapib reduced HDL-cholesteryl esters fractional catabolic rate (FCR) by 30% and 26% (both $P<0.001$ vs. vehicle) respectively, but only dalcetrapib increased HDL-derived ^{3}H-tracer fecal excretion by 30% ($P<0.05$ vs. vehicle). After ^{3}H-cholesterol labeled macrophage intraperitoneal injection, anacetrapib stimulated ^{3}H-tracer appearance in HDL, but both drugs did not promote macrophage-derived ^{3}H-tracer fecal excretion. In dyslipidemic hamsters, both anacetrapib 1 mg/kg QD and dalcetrapib 200 mg/kg BID inhibited CETP activity by ~65% and reduced HDL-cholesteryl ester FCR by 36% (both $P<0.001$ vs. vehicle), but only anacetrapib increased HDL-derived ^{3}H-tracer fecal excretion significantly by 39%. After ^{3}H-cholesterol labeled macrophage injection, only anacetrapib 1 mg/kg QD stimulated macrophage-derived ^{3}H-tracer appearance in HDL. These effects remained weaker than those observed with anacetrapib 60 mg/kg QD, which induced a maximal inhibition of CETP and stimulation of macrophage-derived ^{3}H-tracer fecal excretion. In contrast, dalcetrapib 200 mg/kg BID reduced macrophage-derived ^{3}H-tracer fecal excretion by 23% ($P<0.05$ vs. vehicle). In conclusion, anacetrapib and dalcetrapib differentially alter HDL metabolism and RCT in hamsters. A stronger inhibition of CETP may be required to promote macrophage-to-feces reverse cholesterol transport in dyslipidemic hamsters.

1. Introduction

Despite the intensive use of LDL-lowering statin therapies, cardiovascular events rates remain high and largely contribute to mortality worldwide (Baigent et al., 2005). Given the inverse correlation between HDL-cholesterol levels and cardiovascular risk (Miller et al., 1977), therapies raising HDL-cholesterol levels represent an alternative strategy to further reduce cardiovascular events rate.

Among these therapies, cholesteryl ester transfer protein (CETP) inhibition is an attractive target to efficiently increasing HDL-cholesterol and reducing LDL-cholesterol levels (Barter and Rye, 2012). By mediating the transfer of cholesteryl esters and triglycerides between HDL and VLDL/LDL lipoproteins, CETP plays a major role in the macrophage-to-feces reverse cholesterol transport (RCT) pathway. This anti-atherogenic process mediates the return of cholesterol from peripheral tissues (e.g. macrophages in the arterial wall) by HDL to the liver for further biliary and fecal excretion (Rosenson et al., 2012). Therefore, the impact of CETP inhibition on the RCT pathway requires investigation to ensure that treatment with CETP inhibitors would be beneficial against atherosclerosis, since HDL functionality is thought to be more relevant than HDL-cholesterol level itself (Arsenault and Després, 2012).

While measurement of macrophage-to-feces RCT in human is not available yet, the use of animal models, like the hamster (Briand, 2010), has been helpful in evaluating the effects of CETP

* Corresponding author.
E-mail address: Douglas_Johns@merck.com (D.G. Johns).

http://dx.doi.org/10.1016/j.ejphar.2014.06.022
0014-2999/

inhibitors on this anti-atherogenic pathway (Tchoua et al., 2008; Niesor et al., 2010; Castro-Perez et al., 2011; Briand et al., 2013). Anacetrapib is currently tested in the clinic as a potent CETP inhibitor increasing HDL-cholesterol by ~130% and reducing LDL-cholesterol by ~30%, without adverse effect (Cannon et al. 2010). We have recently demonstrated that maximal CETP inhibition with anacetrapib raises HDL-cholesterol levels by reducing HDL-cholesteryl esters catabolism and promotes macrophage-to-feces RCT in dyslipidemic hamsters (Castro-Perez et al., 2011). Another CETP inhibitor, dalcetrapib, modestly raised HDL-cholesterol levels by ~30% in humans, and had its clinical development recently halted due to futility (Schwartz et al., 2012). A hamster study demonstrated that dalcetrapib, but not anacetrapib, stimulated RCT and macrophage-derived cholesterol fecal excretion in normolipidemic hamsters (Niesor et al., 2010). However, the effects of dalcetrapib on macrophage-to-feces RCT in a dyslipidemic hamster model have never been investigated. How dalcetrapib alter HDL-cholesteryl esters catabolism in both normolipidemic and dyslipidemic hamsters is also unknown.

Based on the available preclinical and clinical studies, dalcetrapib and anacetrapib have different effect on lipoprotein profile, and this could be due to a weaker potency of dalcetrapib in inhibiting CETP as compared with anacetrapib (Barter and Rye, 2012). To clearly evaluate the effects of both drugs on macrophage-to-feces RCT and HDL metabolism, we therefore compared both CETP inhibitors at similar levels of CETP inhibition in both normolipidemic and dyslipidemic hamsters.

2. Materials and methods

2.1. Animals

All animal protocols were reviewed and approved by the Merck Research Laboratories Institutional Animal Care and Use Committee (Rahway, NJ) and were in agreement with US National Institute of Health and European Commission guidelines.

Male Syrian golden hamsters (Elevage Janvier, Le Genest Saint Isle, France, 6-week old, weight 91–100 g at beginning of study) were given free access to food and water.

After a 1-week acclimation period, hamsters were placed on a high-fat diet (45% kcal from fat (lard), 35% kcal from carbohydrate, 20% kcal from protein, 0.12% cholesterol; Research Diets, New Brunswick, NJ) for 4 weeks to induce dyslipidemia, as described previously (Castro-Perez et al., 2011).

After 4 weeks of high fat diet, blood samples were taken in fed conditions to randomize hamsters into homogenous treatment groups according to HDL-cholesterol, total cholesterol and triglycerides levels. Hamsters were then maintained on the high-fat diet and were treated orally with vehicle (methyl cellulose 0.5%), anacetrapib 1, 30 or 60 mg/kg QD or dalcetrapib 200 mg/kg BID for 2 weeks. The doses of 1 and 30 mg/kg anacetrapib were determined in pilot dose-ranging studies that indicated these doses produced similar inhibition of CETP as compared with dalcetrapib 200 mg/kg QD in dyslipidemic (1 mg/kg anacetrapib QD) or normolipidemic (30 mg/kg anacetrapib QD) hamsters. The dose of 60 mg/kg anacetrapib QD was used to get a maximal CETP inhibition, as described previously (Castro-Perez et al., 2011). The dose of dalcetrapib 200 mg/kg BID was also determined from pilot studies as the highest dose to get a maximal CETP inhibition with this compound. After 2 weeks of treatment, blood samples were taken in fed conditions to measure biochemical parameters prior to in vivo RCT and HDL kinetics experiments.

2.2. Biochemical analysis

Total cholesterol and triglycerides were assayed using commercial kits (Biomerieux,Marcy l'Etoile, France).

HDL-cholesterol was determined using the phosphotungstate/$MgCl_2$ precipitation method (Austin et al. 1984).

Plasma CETP activity was measured by fluorescence using commercial kits (Roarbiomedical, New York, NY).

Fast Protein Liquid Chromatography (FPLC) lipoprotein profiles analysis (total cholesterol) using pooled plasma was performed as previously described (Briand et al., 2010).

2.3. in vivo macrophage-to-feces reverse cholesterol transport

in vivo macrophage-to-feces reverse cholesterol transport (macrophage-RCT) was performed as described previously (Castro-Perez et al., 2011). J774 cells from the American Type Culture Collection (ATCC, Manassas, VA), grown in RPMI/HEPES with 10% FBS and 0.5% gentamycin, were radiolabelled with 5 μCi/ml ^{3}H-cholesterol and cholesterol loaded with 50 μg/ml oxidized LDL over 48 h.Radiolabelled cells were then washed with RPMI/HEPES and equilibrated for 4 h in RPMI/HEPES supplemented with 0.2% BSA and gentamycin. Cells were pelleted by low speed centrifugation and resuspended in MEM/HEPES prior intra-peritoneal injection into individually caged hamsters (~2.5×10^6 cells containing ~10×10^6 dpm in 500 μl MEM/HEPES). Hamsters had free access to food and water and were kept treated until the end of the 72 h or 240 h experiment. Blood was collected at indicated time points to measure ^{3}H-tracer recovery in 50 μl plasma or HDL after phosphotungstate/$MgCl_2$ precipitation. At the end of the experiment, hamsters were euthanized by cervical dislocation under isoflurane anesthesia, exsanguinated and liver was then harvested and weighted. A ~50 mg liver sample was homogenized in 500 μl distilled water with an ultrasound probe to measure ^{3}H-tracer hepatic recovery. ^{3}H-tracer recovery in feces continuously collected during the experiment was performed as described previously (Castro-Perez et al., 2011). Briefly, homogenized fecal samples in ethanol/water (v/v) were saponified prior ^{3}H-cholesterol and ^{3}H-bile acids chemical extraction with hexane and ethyl acetate. After radioactivity counting, both remaining extracts were used to determine fecal total cholesterol and total bile acids mass, as described (Briand et al., 2012).

Results were expressed as a percentage of the radioactivity injected and recovered in plasma, HDL, liver and feces. Plasma volume was estimated as 3.5% of the body weight (Briand et al., 2010).

2.4. in vivo ^{3}H-cholesteryl oleate-HDL kinetics

in vivo HDL kinetics were performed as described previously (Castro-Perez et al., 2011), with minor modifications. HDL (d = 1.07–1.21) from a fresh normolipidemic hamsters plasma pool were isolated by ultracentrifugation. After dialysis, HDL particles were radiolabelled with ^{3}H-cholesteryl oleate with human lipoprotein deficient serum over 24 h. Radiolabelled HDL particles were then re-isolated by ultracentrifugation and extensively dialyzed prior intravenous injection. The day before the HDL kinetics, a catheter was inserted into the jugular vein under isoflurane anesthesia for both tracer injection and blood collection. The day of the experiment, hamsters were weighed and placed into individual cages with free access to food and water. ^{3}H-cholesteryl oleate labeled HDL (4–5 million dpm) were injected intravenously through the jugular vein then blood was collected at time 5 min, 1 h, 3 h, 6 h, 24 h and 48 h after injection. Plasma was immediately isolated for radioactivity measurement in HDL after phosphotungstate/$MgCl_2$ precipitation. Feces were collected over 72 h after injection to enable sufficient amount of feces collected for radioactivity counting, as described in the in vivo macrophage-RCT experiment above. At the end of the 72 h experiment, liver was harvested and weighed and a ~50 mg liver sample was used to determine ^{3}H-tracer hepatic recovery, as described above. Results were expressed as a percentage of the injected dose in liver and feces. Because the