Atherosclerosis 233 (2014) 359–362

Contents lists available at ScienceDirect

Atherosclerosis

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

Raising HDL with CETP inhibitor torcetrapib improves glucose homeostasis in dyslipidemic and insulin resistant hamsters☆

François Briand*, Bénédicte Prunet-Marcassus, Quentin Thieblemont, Clément Costard, Elodie Muzotte, Sylvie Sordello, Thierry Sulpice

Physiogenex SAS, Prologue Biotech, 516 Rue Pierre et Marie Curie, 31670 Labège, France

ARTICLE INFO

Article history:
Received 5 December 2013
Received in revised form
10 January 2014
Accepted 12 January 2014
Available online 23 January 2014

Keywords:
Cholesteryl ester transfer protein inhibition
Reverse cholesterol transport
High density lipoprotein
Glucose metabolism
Insulin resistance

ABSTRACT

We investigated whether raising HDL-cholesterol levels with cholesteryl ester transfer protein (CETP) inhibition improves glucose homeostasis in dyslipidemic and insulin resistant hamsters. Compared with vehicle, torcetrapib 30 mg/kg/day (TOR) administered for 10 days significantly increased by ~40% both HDL-cholesterol levels and ^{3}H-tracer appearance in HDL after ^{3}H-cholesterol labeled macrophages i.p. injection.

TOR significantly reduced fasting plasma triglycerides, glycerol and free fatty acids levels by 65%, 31% and 23%, respectively. TOR also reduced blood glucose levels and plasma insulin by 20% and 49% respectively, which led to a 60% reduction in HOMA-IR index (all $p < 0.01$). After ^{3}H-2-deoxyglucose and insulin injection, TOR significantly increased glucose uptake in oxidative soleus muscle, liver and heart by 26, 33 and 70%, respectively.

Raising HDL levels with the CETP inhibitor torcetrapib improves glucose homeostasis in dyslipidemic and insulin resistant hamsters. Whether similar effect would be observed with other CETP inhibitors should be investigated.

1. Introduction

Diabetic dyslipidemia is characterized by hypertriglyceridemia and low high density lipoprotein (HDL) cholesterol levels, which are known to be inversely correlated with cardiovascular risk [1]. Cholesteryl ester transfer protein (CETP) inhibition represents a novel therapeutic strategy to raise HDL-cholesterol levels and further reduces the risk of cardiovascular disease [2]. The benefit of HDL is thought to be related to its key role in reverse cholesterol transport (RCT), a process promoting the return of cholesterol from macrophage in the arterial wall to the liver for further biliary and fecal excretion [3]. Recent preclinical and clinical studies indicate that HDL may also affect glucose homeostasis through insulin secretion, AMP-activated protein kinase (AMPK) dependent glucose uptake in muscle and improvement of whole body insulin sensitivity [4,5]. Importantly, a post-hoc analysis of the ILLUMINATE trial, suggested that raising HDL-cholesterol levels with the CETP inhibitor torcetrapib improves glycemic control in type 2 diabetic patients [6]. We therefore tested the hypothesis that raising HDL with the CETP inhibitor torcetrapib would alter both RCT and glucose homeostasis in dyslipidemic insulin resistant hamsters [7]. This rodent model was selected as it does express CETP (unlike mouse or rat) and more closely reflects human lipoprotein metabolism [3].

2. Methods

Male Golden Syrian hamsters (91–100 g, 6 week-old, Elevage Janvier, Le Genest Saint Isle, France) were fed *ad libitum* over 4 weeks with a high fat/high cholesterol diet (HFHC, 0.5% cholesterol, 0.25% deoxycholate, 11.5% coconut oil, 11.5% corn oil) with 10% fructose in the drinking water, as described [7]. A total of 42 hamsters were used to perform the 3 *in vivo* experiments described below (macrophage-to-feces RCT, HDL-cholesteryl esters kinetics and insulin-stimulated glucose uptake). After 2 weeks of diet, the 42 hamsters were randomized according to their HDL-cholesterol and total cholesterol levels and were then treated orally over 10 days with vehicle ($n = 21$) or torcetrapib 30 mg/kg ($n = 21$) once daily.

After 10 days of treatment, hamsters were fasted overnight and blood was collected by retro-orbital bleeding to perform Fast Protein Liquid Chromatography (FPLC) and biochemical analysis, as described [7]. Plasma HDL particles were separated by precipitation

☆ This work has been funded by Physiogenex SAS.
* Corresponding author. Tel.: +33 561 287 048; fax: +33 561 287 043.
E-mail address: f.briand@physiogenex.com (F. Briand).

0021-9150/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.atherosclerosis.2014.01.028

of apolipoprotein B containing lipoprotein with phosphotungstate/$MgCl_2$. For each treatment group, a pool of plasma HDL (1 pool per group) was extensively dialyzed in saline. Protein concentration was then assayed with a commercial kit prior incubation with soleus muscle *ex vivo*, as described below.

After a 1-day recovery period, a first set of 14 hamsters was injected with ^{3}H-cholesterol labeled/oxidized LDL loaded macrophages ($n = 7$ per group) to measure macrophage-to-feces RCT, and a second set of 14 hamsters was injected with ^{3}H-cholesteryl oleate labeled HDL ($n = 7$ per group) to measure HDL-cholesteryl esters kinetics, as described [7,8].

To evaluate insulin-stimulated glucose uptake *in vivo*, a third set of 14 overnight fasted hamsters ($n = 7$ per group) was injected intravenously (jugular vein) under isoflurane anesthesia with 10 μCi of ^{3}H-2-deoxyglucose (^{3}H-2-DOG) and insulin (0.4U/kg) in fatty acids free-bovine serum albumin 0.1%. At time 30 min after ^{3}H-tracer injection, subcutaneous (inguinal) and visceral (epididymal) white adipose tissues, vastus lateralis (oxidative/glycolytic), extensor digitorum longus (glycolytic) and soleus (oxidative) muscles, liver and heart were collected and weighed prior to tissue homogenization to measure ^{3}H-radioactivity.

To test whether HDL particles stimulate AMPK activation in skeletal muscle, soleus muscles were dissected from 12 hamsters made dyslipidemic insulin resistant after 2 weeks of HFHC diet. After dissection, soleus muscles were incubated at 37 °C, 95% O_2:5% CO_2, in 1 mL Krebs–Ringer bicarbonate buffer (pH 7.3, 1% bovine serum albumin, 2 mM sodium pyruvate) for 30 min then kept in the same buffer, without (basal) or with HDL from hamsters treated for 10 days with vehicle or torcetrapib (800 μg protein/mL buffer), or the AMPK activator 5-aminoimidazole-4-carboxamide riboside (AICAR; positive control) at 2 mM, for an additional 60 min ($n = 6$ per condition). Muscles were then washed in PBS 1× and flash frozen prior to western blotting to evaluate phosphorylated-AMPK (antibody from Cell Signaling, ref#2535S) and AMPK (Cell Signaling, ref#2532S) expression by densitometry analysis (Image J software).

Data are expressed as mean ± SEM. Unpaired Student *t*-test or 1-way ANOVA + Dunnett post-test was used for statistical analysis. A $p < 0.05$ was considered significant.

3. Results

Compared with vehicle, torcetrapib treatment significantly reduced plasma CETP activity by 31% and increased both total cholesterol and HDL-cholesterol levels by 33 and 38% respectively (Table 1). No effect was observed regarding fecal cholesterol and bile acids mass excretion. Fasting plasma triglycerides, glycerol and free fatty acids levels were respectively reduced by 65, 31 and 23% in torcetrapib treated hamsters (all $p < 0.01$ vs. vehicle). As compared with vehicle, torcetrapib treatment significantly reduced blood glucose and plasma insulin levels by 20 and 49% respectively (both $p < 0.01$). Accordingly, HOMA-IR index was reduced by 60% with torcetrapib treatment ($p < 0.01$ vs. vehicle).

Table 1
Biochemical parameters in insulin resistant and dyslipidemic hamsters treated with vehicle or torcetrapib 30 mg/kg QD for 10 days.

	Vehicle	Torcetrapib 30 mg/kg QD
CETP activity (pmol/μL/h)	56.4 ± 2.8	38.8 ± 1.6***
Total cholesterol (g/L)	3.89 ± 0.13	5.19 ± 0.26***
HDL-cholesterol (g/L)	1.95 ± 0.12	2.70 ± 0.15***
Fecal cholesterol (μg/day)	380 ± 34	399 ± 51
Fecal total bile acids (μmol/day)	24 ± 3	23 ± 6
Triglycerides (g/L)	3.19 ± 0.21	1.11 ± 0.09***
Glycerol (mg/dL)	4.97 ± 0.48	3.44 ± 0.30**
Free fatty acids (mM)	0.634 ± 0.015	0.490 ± 0.017***
Blood glucose (mM)	6.1 ± 0.4	4.9 ± 0.1**
Insulin (μU/mL)	14.4 ± 2.5	7.3 ± 1.1**
HOMA-IR ([mM × μU/mL]/22.5)	4.0 ± 0.8	1.6 ± 0.3**

Plasma samples were collected from overnight fasted, insulin resistant and dyslipidemic hamsters, after 10 days of treatment with vehicle or torcetrapib 30 mg/kg QD ($n = 7$ per group, **$p < 0.01$, ***$p < 0.001$ vs. vehicle).

As shown by FPLC analysis (Fig. 1A), torcetrapib induced the appearance of apolipoprotein E-rich HDL particles, which also contained higher levels of apolipoprotein A-I (fractions #24-31), as compared with vehicle. The increase in HDL-cholesterol levels with torcetrapib was related to a significant 29% reduction in HDL-cholesteryl ester fractional catabolic rate (Fig. 1B) after ^{3}H-cholesteryl oleate labeled HDL i.v. injection. HDL-derived ^{3}H-tracer fecal excretion was not changed with torcetrapib treatment (data not shown). Hamsters treated with torcetrapib showed significantly higher ^{3}H-tracer appearance in both plasma and HDL (Fig. 1C), but not in feces (Fig. 1D), after ^{3}H-cholesterol labeled macrophage i.p. injection. After ^{3}H-2-DOG and insulin i.v. injection (Fig. 1E), torcetrapib treatment resulted in a 24 and 27% reduction in ^{3}H-2-DOG uptake by inguinal (IWAT) and epididymal (EWAT) adipose tissues, respectively (both $p < 0.05$ vs. vehicle). While torcetrapib did not change ^{3}H-2-DOG uptake in vastus lateralis (VL; oxidative and glycolytic fibers) and extensor digitorum longus (EDL; glycolytic) muscles, a 26% increase was observed in the oxidative soleus muscle ($p < 0.01$ vs. vehicle). As well, ^{3}H-2-DOG uptake was significantly increased by 33 and 70% in liver and heart, respectively.

To test whether the increase in HDL levels with torcetrapib treatment stimulates AMPK activation, HDL particles from vehicle-treated or torcetrapib-treated hamsters were incubated *ex vivo* with soleus muscles isolated from hamsters fed the HFHC diet for 2 weeks. As observed after FPLC analysis (Fig. 1A), HDL from torcetrapib-treated hamsters showed higher levels of apolipoprotein E and A-I (Fig. 1F). While HDL from vehicle-treated hamsters showed no effect, HDL from torcetrapib-treated hamsters significantly increased AMPK-phosphorylation by 1.5-fold, as compared with basal conditions.

4. Discussion

The present study demonstrates that raising HDL-cholesterol levels with the CETP inhibitor torcetrapib concomitantly alters both macrophage-to-feces RCT and insulin resistance in a hamster model.

In insulin-stimulated state, hamsters treated with torcetrapib showed higher glucose uptake in the oxidative soleus muscle, but not in vastus lateralis (oxidative/glycolytic) and extensor digitorum longus (glycolytic) muscles. This finding is consistent with the fact that, in rodents, oxidative muscles have higher amount of glucose transporters GLUT4 than glycolytic muscles [9]. However, we observed a trend towards lower glucose uptake in both visceral and subcutaneous adipose tissue *in vivo*, which contrasts with another *in vitro* study suggesting that HDL and apolipoprotein A-I increase glucose uptake in 3T3-L1 adipocytes culture [10]. While this discrepancy may come from experimental conditions and species differences, the significant reduction in fasting plasma free fatty acids and glycerol suggests that raising HDL-cholesterol and apolipoprotein A-I levels may be beneficial in preventing adipose tissue lipolysis, an effect already described in humans [11]. Our data also indicate that raising HDL levels show benefits at both the hepatic and cardiac levels. This observation is in line with other studies suggesting a protective effect of apolipoprotein A-I against non alcoholic steato-hepatitis [12] and heart failure [13].