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PCSK9 inhibition alters the lipidome of plasma and lipoprotein fractions

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ABSTRACT

Background and aims: While inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9) is known to result in dramatic lowering of LDL-cholesterol (LDL-C), it is poorly understood how it affects other lipid species and their metabolism. The aim of this study was to characterize the alterations in the lipidome of plasma and lipoprotein particles after administration of PCSK9 inhibiting antibody to patients with established coronary heart disease.

Methods: Plasma samples were obtained from patients undergoing a randomized placebo-controlled phase II trial (EQUATOR) for the safe and effective use of RG7652, a fully human monoclonal antibody inhibiting PCSK9 function. Lipoprotein fractions were isolated by sequential density ultracentrifugation, and both plasma and major lipoprotein classes (VLDL-IDL, LDL, HDL) were subjected to mass spectrometric lipidomic profiling.

Results: PCSK9 inhibition significantly decreased plasma levels of several lipid classes, including sphingolipids (dihydroceramides, glucosylceramides, sphingomyelins, ceramides), cholesteryl esters and free cholesterol. Previously established ceramide ratios predicting cardiovascular mortality, or inflammation related eicosanoid lipids, were not altered. RG7652 treatment also affected the overall and relative distribution of lipids in lipoprotein classes. An overall decrease of total lipid species was observed in LDL and VLDL + IDL particles, while HDL-associated phospholipids increased. Following the treatment, LDL displayed reduced lipid cargo, whereas relative lipid proportions of the VLDL + IDL particles were mostly unchanged, and there were relatively more lipids carried in the HDL particles.

Conclusions: Administration of PCSK9 antibody significantly alters the lipid composition of plasma and lipoprotein particles. These changes further shed light on the link between anti-PCSK9 therapies and cardiovascular risk.

Abbreviations: CAD, coronary artery disease; CE, cholesteryl ester; Cer, ceramide; DAG, diacylglycerol; Gb3, globotriasoylceramide; Glc/GalCer, glucosyl/galactosylceramide; GOF, gain-of-function; LacCer, lactosylceramide; LDL-C, LDL-cholesterol; LPC, lysophosphatidylcholine; LOF, loss-of-function; HDL-C, HDL-cholesterol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; TAG, triacylglycerol; TC, total cholesterol; TG, triglyceride.

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1. Introduction

Atherosclerosis and coronary artery disease (CAD) are associated with increased plasma low-density lipoprotein cholesterol (LDL-C) concentration and inflammatory processes within the atherosclerotic lipid-laden lesions [1]. Statins are widely used drugs to reduce cholesterol levels and they also possess anti-inflammatory properties. However, in some patients statin treatment does not result in sufficient LDL-C reduction and the use of statins is quite commonly restricted due to side effects, such as muscle pain, fatigue and weakness [2]. Moreover, statins are associated with development of diabetes [3], and therefore new drugs are being developed that would further inhibit the atherosclerotic processes with fewer side effects.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) has gained interest as a new therapeutic target for coronary heart disease after it was discovered that gain-of-function (GOF) mutations in the *PCSK9* gene lead to hypercholesterolemia [4] whereas loss-of-function (LOF) mutations are associated with hypocholesterolemia and reduced risk of developing coronary heart disease [5]. PCSK9 is highly expressed in liver and regulates LDL-C levels by enhancing lysosomal degradation of the hepatic low-density lipoprotein receptors (LDLRs) [6]. Therapeutic approaches are based on the inhibition of PCSK9 function, and the major strategies for inhibition include anti-PCSK9 antibodies, small molecule inhibitors and antisense oligonucleotide gene silencing approaches [7]. Recently, the first outcome trial with an anti-PCSK9 antibody showed reduction in non-fatal cardiovascular events [8].

While the effect of PCSK9 function on LDL-C and cholesterol metabolism has been intensively investigated, its role in metabolism of other lipid species has been underexplored, despite the evidence suggesting that inhibition of PCSK9 may cause profound changes in lipid metabolism. For instance, previous findings from our laboratory from a transgenic mouse model showed that several sphingolipid species were affected by PCSK9 deficiency [9]. Notably, in human serum several lipid species were decreased non-proportionally to LDL-C reduction [9,10]. This raises the possibility that PCSK9 deficiency may reduce atherosclerosis via reduction of LDL-C concentration as well as by changes in levels of other lipid classes.

To further investigate the role of PCSK9 in regulating lipid metabolism, we performed lipidomic analysis in human subjects enrolled in Phase II trial for RG7652, a fully human IgG1 monoclonal antibody that prevents the binding of PCSK9 to hepatic LDLR, thereby increasing the cell surface expression of the LDLR and enhancing LDL-C clearance. To better understand the observed changes in response to treatment with RG7652, whole plasma as well as major plasma lipoprotein classes, VLDL + IDL, LDL or HDL, were profiled in this study.

2. Materials and methods

2.1. Patients and samples

Fasting plasma samples at baseline and 29 days after treatment were obtained from 40 non-diabetic patients (Table 1) with established coronary heart disease (CHD) who were participating in a randomized, double-blind, phase II trial (EQUATOR) investigating safe and effective use of RG7652, a monoclonal antibody against PCSK9 [11]. Early time point of day 29 was chosen, as the aim was to investigate acute changes of the lipidome, after single administration of RG7652 that still reflected robust and stable LDL-lowering effect [11]. The EQUATOR trial (clinicaltrials.gov: NCT01609140) was performed in accordance with the International Conference and Harmonization guidelines and the Declaration of Helsinki, and all patients provided written informed consent by local institutional review boards/ethics committees. The patients in this subpopulation of the EQUATOR trial were receiving four different dose-regimens of RG7652 administration (Table 1) that were equally effective in lowering LDL-C [11], and for the statistical analyses all samples from treated groups were pooled into a single set of patients. Altogether 25 patients were receiving RG7652 and 15 placebo (Table 1). The criteria for patient selection was as follows: no diabetes, chronic kidney disease, or familiar hypercholesterolemia and all were Caucasians. RG7652 and placebo groups for the study were well matched for cholesterol levels, however, a slight imbalance was evident in triglyceride (TG) levels (Table 1).

Table 1
Baseline characteristics of the study cohort (N = 40).

	RG7652	Placebo	*p*-value
No of subjects	25	15	–
Age	64.0 (56.0–70.0)	67.0 (61.5–69.5)	0.393
BMI	29.1 (24.8–31.2)	29.1 (26.9–31.1)	0.489
Gender			
Male	15 (60%)	8 (53%)	–
Female	10 (40%)	7 (47%)	–
Statin			
Yes	15 (60%)	11 (73%)	–
No	10 (40%)	4 (27%)	–
Treatment			
400 mg/4 W	5 (20%)	0 (0%)	–
400 mg/8 W	6 (24%)	0 (0%)	–
800 mg/8 W	11 (44%)	0 (0%)	–
800 mg/12 W	3 (12%)	0 (0%)	–
Placebo	0 (0%)	15 (100%)	–
TC (mg/dL)	211 (188–241)	208 (178–215)	0.295
LDL-C (mg/dL)	126 (108–159)	132 (112–153)	0.989
HDL-C (mg/dL)	45 (36–57)	54 (46–61)	0.281
TG (mg/dL)	159 (113–182)	113 (85–132)	0.046
ApoA1 (mg/dL)	136 (122–162)	145 (130–169)	0.364

Values for continuous variables represent median and interquartile range.

2.2. Lipoprotein isolation from human plasma

Major lipoprotein classes were isolated from plasma samples by sequential ultracentrifugation using table-top ultracentrifuge (Beckmann Optima TL, USA) and KBr for density adjustment [12]. Serum sample (0.18–0.3 mL) was first adjusted to the density (d) of 1.019 g/mL and the centrifuge tube filled with a d = 1.019 g/mL KBr solution to the total volume of 3 mL. The samples were centrifuged at 5 °C for 2 h at the speed 100,000 rpm (corresponding to relative centrifugal force of 500,000×*g*). After centrifugation, very low and intermediate density lipoproteins (VLDL + IDL) were recovered in the top 1 mL fraction and the infranatant fraction was adjusted to the density of 1.063 g/mL using solid KBr, filled again to final volume of 3 mL with d = 1.063 g/mL KBr solution and centrifuged (5 °C, 3 h, 100,000 rpm). The top 1 mL fraction contained low density lipoprotein (LDL) particles. To get the total HDL fraction the infranatant fraction was adjusted with KBr to the density of 1.21 g/mL, the vials filled with KBr density (1.21 g/mL) solution and then centrifuged (5 °C, 18 h, 100,000 rpm). Total HDL was obtained in top 1 mL fraction. The isolated fractions were stored at −80 °C before analysis.

2.3. Lipidomic analysis of plasma and lipofractions

Lipids from total plasma and VLDL + IDL, LDL and HDL particles were extracted using a modified Folch lipid extraction [13,14] performed on a Hamilton Microlab Star robot. Samples were spiked with known amounts of non-endogenous synthetic internal standards. After lipid extraction, lipids were resolubilized in