Atherosclerosis 230 (2013) 322–329

Contents lists available at ScienceDirect

Atherosclerosis

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

Inhibition of intestinal cholesterol absorption with ezetimibe increases components of reverse cholesterol transport in humans

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ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form
3 August 2013
Accepted 5 August 2013
Available online 13 August 2013

Keywords:
Ezetimibe
Reverse cholesterol transport
Cholesterol efflux
Cholesterol absorption
Fecal sterols

ABSTRACT

Objective: Reverse cholesterol transport (RCT) can be defined as a pathway of flux of cholesterol from peripheral tissues to the liver for potential excretion into feces. This prospective, placebo-controlled, double-blind crossover study assessed the effect of ezetimibe on several RCT parameters in hyperlipidemic patients.

Methods: Following 7 weeks of treatment (ezetimibe 10 mg/day or placebo), 26 patients received 24-h continuous IV infusions of [3,4-$^{13}C_2$]-cholesterol, then took heavy water (^2H_2O) by mouth. Cholesterol excretion was measured by quantification of neutral/acid sterols in stool and blood samples during 7 days post-infusion with continued treatment. Plasma de novo cholesterol synthesis was assessed by ^2H-labeling from ^2H_2O.

Results: Ezetimibe significantly reduced levels of low-density lipoprotein cholesterol (22%, $P < 0.001$) without significant changes in triglycerides and high-density lipoprotein cholesterol and significantly increased the flux of plasma-derived cholesterol into fecal neutral sterols by 52% ($P = 0.04$) without change in flux into fecal bile acids. Total fecal neutral sterol output increased by 23% ($P = 0.02$). Plasma de novo cholesterol synthesis increased by 57% ($P < 0.001$). The fractional clearance rate (FCR) of plasma cholesteryl-ester trended higher (7%; $P = 0.055$) with a reduction in absolute cholesteryl-ester production rate (9%, $P < 0.01$). Whole-body free cholesterol efflux rate from extra-hepatic tissues into plasma was not measurably changed by ezetimibe.

Conclusion: Ezetimibe treatment approximately doubled the flux of plasma-derived cholesterol into fecal neutral sterols, in association with increases in total fecal neutral sterol excretion, FCR of plasma cholesterol ester, and plasma de novo cholesterol synthesis. These effects are consistent with increased cholesterol transport through the plasma compartment and excretion from the body, in response to ezetimibe treatment in hyperlipidemic humans.

Clintrials.gov: NCT00701727.

1. Introduction

Ezetimibe is an inhibitor of intestinal cholesterol absorption acting through inhibition of the Niemann-Pick C1-like1 (NPC1L1) transporter [1]. In humans, once-daily treatment with ezetimibe monotherapy (10 mg) inhibits cholesterol absorption on average by 54–65% and results in about a 20% reduction of plasma low-density lipoprotein cholesterol (LDL-C) [2,3]. A similar incremental

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http://dx.doi.org/10.1016/j.atherosclerosis.2013.08.006

reduction in LDL-C with ezetimibe treatment is observed when coadministered with other cholesterol-lowering therapies, including statins, bile acid sequestrants, and fenofibrate, but not dietary plant sterols [4]. The LDL-C lowering effect of ezetimibe treatment is usually attributed primarily to increased catabolism of LDL-C, presumably due to the up-regulation of the LDL-receptor, in response to reduced cholesterol delivered to the liver via the chylomicron pathway [3,5,6].

Studies in mice have suggested that ezetimibe may also augment parameters of reverse cholesterol transport (RCT) [7,8]. Although increased macrophage-to-feces cholesterol transport has been demonstrated with ezetimibe in animals [7–9], the role of this effect in contributing to a reduction in atherosclerosis as reported in mouse and rabbit models is uncertain [10]. In humans, ezetimibe alone and combined with simvastatin significantly decreased fractional cholesterol absorption compared with placebo ($P < 0.001$) [2,3] and also significantly increased fecal sterol excretion, while simvastatin had no effect on absorption rates and slightly decreased fecal sterol excretion [3]. Treatment of a sitosterolemic subject with ezetimibe is reported to have resulted in complete regression of xanthomatosis [11]. These findings raise the hypothesis that ezetimibe may reduce the cholesterol content of peripheral tissues (including the vascular wall) by increasing RCT as well as lowering plasma LDL-C levels.

Inhibition of NPC1L1 reduces the intestinal absorption of cholesterol, both endogenous and exogenous [1,12]. In humans, only about 25–35% of intestinal cholesterol is derived from diet, while the remaining ~2/3 originates from the liver in bile, from sloughed intestinal cells, and perhaps an additional direct transintestinal cholesterol efflux (TICE) route [13,14]. Biliary cholesterol can be derived from at least three distinct sources: hepatic de novo cholesterol synthesis, hepatic cholesterol stores, or cholesterol cleared from plasma lipoproteins. If only hepatic-derived biliary cholesterol were responsible for the increased excretion of cholesterol seen with ezetimibe treatment, it would be anticipated that this would have no impact on cholesterol flux from extra-hepatic tissues, including the vascular wall/atherosclerotic plaque [12]. On the other hand, if the source of the excreted cholesterol is at least partially derived from plasma lipoproteins, this may represent a component of the RCT pathway by which cholesterol that is effluxed from body tissues can exit the body, thereby reducing the cholesterol burden of extra-hepatic tissues, potentially including the vascular wall/atherosclerotic plaque [12,14].

The RCT pathway involves the net movement of cholesterol from peripheral tissues to the liver and can be conceptually viewed as comprising several major components: 1) the efflux of free cholesterol from tissues into plasma, 2) cholesterol transport with associated esterification in plasma, 3) hepatic uptake of plasma sterols, and 4) excretion into fecal sterols [12]. As the techniques used to measure macrophage-to-stool RCT in rodents [8,10] are not generally applicable to humans, alternative approaches to assessing RCT are needed. A method that measures several *in vivo* elements of RCT components in humans, namely efflux, plasma transport and excretion, using stable, non-radioactive isotopic tracers was recently developed and published by several authors of this study (Fig. 1) [15,16]. Here, we carried out a randomized, placebo-controlled, crossover study, investigating the effect of ezetimibe on RCT parameters in 26 hyperlipidemic human subjects using this approach. Thus, barring a specific effect of ezetimibe treatment on macrophage cholesterol metabolism without an effect on whole-body cholesterol fluxes, the methods applied here in humans would be anticipated to yield results similar to those observed in the RCT studies in rodents, assuming the effect of ezetimibe treatment is consistent across species.

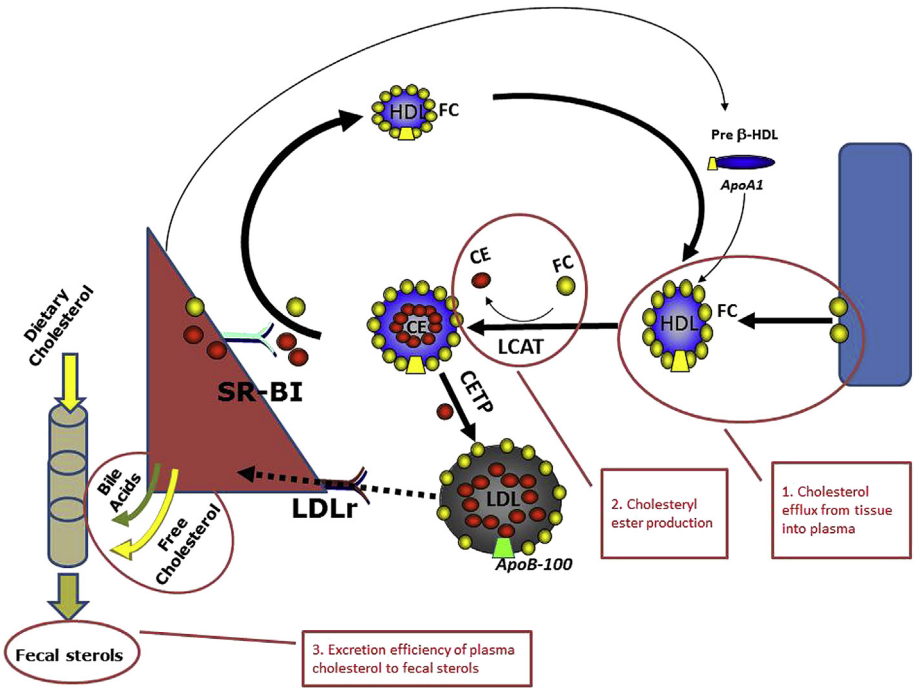

Fig. 1. Efflux, plasma transport and excretion components of reverse cholesterol transport measured in this study. FC is effluxed from peripheral tissues via transporter-dependent and -independent mechanisms. Once on the HDL-particle, FC can be esterified to CE and transferred to LDL-particles via CE-transfer protein. FC and CE are delivered to the liver for secretion in bile as FC or BA, and then to the intestine with the possibilities of absorption and "recycling" or excretion in the feces. Cholesterol flux is measured with an intravenous infusion of $^{13}C_2$-cholesterol. Sterol masses and enrichments in plasma FC, CE, and fecal neutral and acidic sterols are determined. Fluxes though each arm of RCT were calculated as described in Methods. FC = free cholesterol, CE = cholesterol ester, CETP = CE-transfer protein, NS = neutral sterols, BA = bile acids, LCAT = lecithin-cholesterol acyltransferase; SR-BI = Scavenger Receptor type B1; LDLr = LDL receptor. Adapted from Expert Review of Cardiovascular Therapy, April 2008, Vol. 6, No. 4, Pages 447-470 with permission of Expert Reviews Ltd [40].