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New developments in selective cholesteryl ester uptake

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Abstract

Purpose of review—Selective lipid uptake (SLU) is known to be a major pathway of lipoprotein cholesterol metabolism in experimental animals and humans, but remains poorly understood. This review provides a brief overview of SLU mediated by the HDL receptor scavenger receptor B-type I (SR-BI), and highlights several surprising new findings related to the impact of SLU pathways in cholesterol homeostasis.

Recent findings—Under certain conditions, SR-BI-mediated SLU contributes to reverse cholesterol transport (RCT) independently of ABCG5/G8-mediated biliary cholesterol secretion, implying a novel trafficking mechanism. Hepatic SR-BI expression and RCT are decreased in diabetic mice. Farnesoid X receptor (FXR) and the microRNAs miR-185, miR-96 and miR-223 are emerging therapeutic targets for increasing SR-BI expression. SR-BI-independent selective cholesteryl ester uptake is a newly characterized pathway in macrophage foam cells.

Summary—New findings underscore the importance of SR-BI-mediated SLU in hepatic SLU and RCT, while indicating that further investigation is needed to define SLU pathways, including SR-BI-independent macrophage selective cholesteryl ester uptake. The intracellular trafficking of cholesterol in these pathways appears to be critical to their normal function and is a major subject of ongoing studies.

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Conflicts of interest

There are no conflicts of interest.

Keywords

HDL cholesterol; macrophage foam cell; reverse cholesterol transport; scavenger receptor B-type I; selective cholesteryl ester uptake

INTRODUCTION

Selective lipid uptake (SLU) is a process by which lipids are transferred from lipoproteins to cells without endocytosis and degradation of the lipoprotein particles. SLU was first described independently by two groups in 1983 in studies of the metabolism of dual-labeled HDL in rats [1,2]. Using a non-hydrolyzable [³H]cholesteryl ether label to follow cholesteryl ester uptake, both groups showed that HDL cholesteryl ester was cleared more rapidly from plasma than HDL protein and accumulated selectively in several tissues, including liver, adrenals and gonads. A series of elegant studies led by R.C. Pittman identified key features of SLU [3,4] (Table 1). Selective uptake occurs with LDL as well as HDL, and many lipids are transferred including not only cholesteryl ester, the major topic of this review, but also free cholesterol, triglyceride, phospholipid, vitamins and hormones. Krieger [5] later discovered that SLU of HDL cholesteryl ester is largely mediated by the canonical HDL receptor, scavenger receptor B-type I (SR-BI). SLU via SR-BI plays a key role in plasma HDL cholesteryl ester clearance in the liver and in delivery of cholesterol for steroid hormone synthesis in steroidogenic cells. However, despite more than 30 years of research, the mechanism and functional significance of SLU, both SR-BI-mediated and SR-BI-independent, remains incompletely understood. In this review, we focus on new findings related to SLU and cholesterol homeostasis.

SCAVENGER RECEPTOR B-TYPE I: AN HDL RECEPTOR THAT MEDIATES SELECTIVE LIPID UPTAKE

SR-BI is a member of the class B family of scavenger receptors. The role of SR-BI in hepatic SLU was established by a number of studies in genetically manipulated mice [5]. SR-BI is responsible for a major portion of HDL cholesteryl ester uptake in liver. Mice with a null mutation in one or both alleles of SR-BI have a dose-dependent increase in plasma cholesterol levels, whereas apolipoprotein A1 levels are unchanged, consistent with a SLU process [6]. Three loss-of-function mutations in SCARB1 (the human gene for SR-BI) were recently identified, and heterozygote carriers had a 32–37% increase in HDL cholesterol (HDL-C), indicating a similar role of SR-BI as a determinant of plasma HDL-C in humans [7,8]. These variants confer differing effects on SR-BI that include altered SR-BI expression, translation and protein stability [9*]. Beyond hepatic HDL uptake, SR-BI-mediated SLU also plays a major role in the delivery of cholesterol to the adrenal, ovary and testis for steroidogenesis, a topic that is beyond the scope of the current review [10–12].

SR-BI-mediated selective cholesteryl ester uptake is a two-step process requiring first, ‘productive’ binding of lipoprotein to the extracellular domain of SR-BI and second, selective delivery of cholesteryl ester into the cell mediated by its extracellular domain [12,13]. Studies *in vitro* showed that SR-BI is able to mediate SLU without requiring

additional proteins or specialized cell structures. On the basis of thermodynamic and kinetic data, SR-BI was proposed to form a lipophilic channel between the bound lipoprotein particle and the plasma membrane [14], consistent with the identification of essential hydrophobic residues of SR-BI [15] and with the ability of SR-BI to mediate SLU in the absence of cellular energy [16]. Indeed, the bulk of SR-BI selective cholesteryl ester uptake is generally considered to occur at the plasma membrane with the transfer of HDL cholesteryl ester into a reversible membrane pool that remains available for efflux back to HDL prior to internalization into the cell [17,18]. SR-BI can also promote HDL particle internalization, and intracellular selective transfer may make a small contribution to total selective cholesteryl ester uptake in hepatocytes [19]. Following its selective uptake, cholesteryl ester is hydrolyzed to unesterified cholesterol by neutral cholesterol esterase(s), the identities of which remain uncertain, although hormone sensitive lipase is responsible for the bulk of cholesteryl ester hydrolysis in steroidogenic cells [20]. It is also unclear whether hydrolysis takes place at the plasma membrane or possibly following the transfer of cholesteryl ester into the cytoplasmic compartment by an undefined mechanism.

Studies of the structure of SR-BI have provided some insight into the mechanism of SLU. SR-BI is an 82 kDa integral transmembrane glycoprotein that consists of a large extracellular domain or 'loop' that is anchored to the cell surface by two transmembrane sequences connecting the extracellular domain to relatively short N-terminal and C-terminal cytoplasmic tails [12]. SR-BI is heavily glycosylated and inhibition of glycosylation leads to altered intracellular transport as well as defective SLU [21]. SR-BI has been shown to oligomerize into dimers and possibly larger oligomers [22]. SR-BI dimerization requires a glycine dimerization motif in the N-terminal transmembrane domain of SR-BI and enhances selective cholesteryl ester uptake without affecting HDL binding [23]. The extracellular loop of SR-BI contains six highly conserved extracellular cysteine residues, and mutation of any one of four of these was shown to significantly reduce SR-BI activity [24–26]. The positions of the two corresponding disulfide bonds were recently determined [27[•]]. Interestingly, one of these bonds, linking cysteines 321 and 323, is not essential for receptor expression and selective cholesteryl ester uptake activity [27[•]]. Although SR-BI has been shown to interact with PDZK1 (NHERF3) in hepatocytes and certain other cell types [28], this interaction regulates SR-BI stability and levels but does not directly affect SLU activity [28,29]. Most recently, two other members of the same family of scaffolding proteins, NHERF1 and NHERF2, were shown to interact with SR-BI in steroidogenic cells and to downregulate SR-BI activity and steroid production by decreasing SR-BI protein levels through a novel translation/post-translation mechanism [30[•]]. Finally, studies of the higher-order structure of the SR-BI protein are notably absent in the literature but will be critical in understanding how this receptor mediates lipid transfer.

SR-BI carries out a number of important physiologic functions in addition to selective uptake, including efflux of free cholesterol out of cells, functioning as a sterol 'sensor' for plasma membrane cholesterol [31[•]], involvement in HDL-induced cell signaling in various cell types and acting as a coreceptor for hepatitis C virus [32]. These topics have been the focus of a number of recent reviews [33,34] and are not discussed further here.

SCAVENGER RECEPTOR B-TYPE I AND REVERSE CHOLESTEROL TRANSPORT

Reverse cholesterol transport (RCT) is the process by which excess cholesterol is transferred from peripheral tissues to HDL, delivered to the liver via SR-BI-mediated SLU and secreted into the bile for elimination (Fig. 1; RCT indicated in white and black arrows) [35,36]. A nonbiliary, transintestinal route for RCT has also been described, but SR-BI does not appear to play a major role in this pathway and, in fact may oppose it [37]. A number of studies have indicated that SR-BI-mediated hepatic SLU is normally coupled to biliary excretion (Fig. 1, white arrow) [38,39]. Coupling was postulated based on quantitative correlations between the rate of cholesteryl ester uptake and biliary cholesterol secretion, consistent with the concept of a preferential delivery to bile of cholesterol acquired by selective HDL cholesteryl ester uptake. As more than 75% of total biliary cholesterol secretion occurs via ABCG5/G8, it has often been assumed that cholesterol acquired via SR-BI is secreted into the bile largely by the action of this heterodimer. However, it was shown that enhancement of biliary cholesterol secretion by SR-BI overexpression was independent of ABCG5/G8, as a nearly identical increase was seen in ABCG5/G8-null mice [40]. The converse was shown in recent studies of SR-BI/ABCG5/G8 DKO mice in which SR-BI deficiency was associated with a decrease in both biliary cholesterol secretion and macrophage RCT similarly in wild type and ABCG5/G8-null backgrounds [41^{***}]. These findings suggest that under some conditions SR-BI-directed biliary cholesterol is eliminated by a distinct mechanism, perhaps via SR-BI-mediated efflux at the bile canicular membrane [41^{***},42]. However, it is possible that compensatory changes occurred with severe manipulation of SR-BI expression that influence the interpretation of the results.

It appears that the SR-BI-mediated SLU pathway for biliary cholesterol secretion is dysregulated in disease states such as the metabolic syndrome and diabetes mellitus, conditions that are known to be associated with accelerated atherosclerosis despite excessive biliary cholesterol secretion and cholesterol gallstone formation [43]. One possibility is that disruption of SR-BI-mediated selective uptake leads to ‘uncoupling’ of hepatic HDL cholesterol delivery and the enterohepatic cycle, a change that may be a key contributor to risk for atherosclerotic cardiovascular disease in these patients. In line with this concept, hyperglycemia was previously shown to decrease SR-BI expression in human HepG2 hepatocytes and sucrose-fed rat livers [44], and in obese and insulin-resistant leptin-deficient (ob/ob) mice [45]. More recently, hepatic SLU was found to be decreased in alloxan-induced diabetic mice despite unaltered SR-BI expression, a phenomenon attributed to glycation of HDL [46^{***}]. In these mice, RCT was decreased despite increased biliary cholesterol and bile acid secretion, supporting the uncoupling hypothesis. Additional studies are needed to determine whether SR-BI-mediated SLU is altered in diabetic patients.

Metabolic diseases may also impair RCT by altering the intracellular trafficking of cholesterol following SLU. For example, there is now evidence that HDL-associated apoE may oppose biliary delivery of HDL-derived cholesterol [47^{***}]. In this study, overexpression of apoE increased hepatic SR-BI-mediated selective HDL cholesteryl ester uptake but did not increase the rate of RCT, suggesting an uptake pathway that is unproductive with respect

to biliary cholesterol secretion. However, this effect was reversed with probucol treatment, a finding that was taken to indicate that apoE-stimulated selective cholesteryl ester uptake was directed into a cholesterol pool that was preferentially reincorporated into HDL by ABCA1 rather than secreted into the bile [47[•]]. This result is especially relevant in the context of dysfunctional, large apoE-containing HDL that are observed in dyslipidemia [6,48,49]. These large particles may be unable to deliver their cholesterol to bile due to altered cellular cholesteryl ester trafficking. The mechanism for altered trafficking of cholesterol from apoE-enriched HDL is not known.

As hepatic SR-BI has consistently been shown to be protective in mouse models of atherosclerosis, modulation of SR-BI expression in liver is an attractive therapeutic strategy. Farnesoid X receptor (FXR) was previously suggested to be a possible target for increasing SR-BI expression, as FXR-null mice had reduced SR-BI protein levels [50]. A recent study has confirmed direct regulation of SR-BI by FXR and identified three consensus sequences for FXR binding in the SR-BI promotor region [51[•]]. Interestingly, synthetic FXR agonists increased HDL-C delivery to the liver and reduced plasma cholesterol in C57/BL6 and CETP-tg mice and in nonhuman primates [52[•]]. This was associated with increased transhepatic cholesterol efflux and reduced atherosclerosis, supporting a protective role for hepatic HDL-C delivery [53,54]. One FXR agonist, obeticholic acid, has been demonstrated to be well tolerated for human use and is currently in a phase 3 clinical trial for the treatment of primary biliary cirrhosis. Other possible therapeutic targets include the recently identified miRNAs that regulate SR-BI expression [55[•]]. The human SR-BI 3' UTR contains target sites for miR-185, miR-96 and miR-223, and these miRNAs were shown to significantly decrease SR-BI mRNA and protein expression in HepG2 cells as well as SLU.

MACROPHAGE SELECTIVE CHOLESTERYL ESTER UPTAKE

Macrophages are a common cell type in atherosclerotic lesions and are thought to play an important role in the pathogenesis of the disease [56]. Much attention has been given to endocytic pathways that are involved in the uptake of various forms of modified LDL leading to macrophage foam cell generation. Native LDL can also induce foam cell formation at high concentrations by a process that involves nonsaturable uptake of LDL particles in fluid-filled pinosomes [57]. Interestingly, it was documented more than 20 years ago that macrophages take up cholesteryl ester selectively from both HDL and LDL [58,59], a phenomenon that was subsequently shown to be independent of SR-BI [60,61]. When we investigated the potential contribution of SLU to native LDL-induced foam cell formation, selective cholesteryl ester uptake was found to be the major mode of cholesterol uptake [62^{••}]. Macrophage selective cholesteryl ester uptake was high-capacity with no evidence of saturation at high LDL concentrations, a feature that may be unique to this cell type. At 1 mg/ml of LDL protein (equivalent to approximately 150 mg/dl of LDL-C), the rate of uptake was approximately equal to the total cholesterol content of the cells every 4 h, resulting in extensive deposition of Oil Red O-positive lipid droplets. The mechanism of macrophage selective uptake remains unclear, but it was shown to be independent of LDL particle uptake and scavenger receptors.

Initial results also suggested substantial cholesterol efflux during treatment with native LDL because the measured rate of cholesteryl ester uptake was high compared with the measured cholesterol mass in the cells [62^{***}]. This was confirmed by comparison of the rates of cholesteryl ester uptake and cholesterol efflux, which indicated that the bulk of LDL-derived cholesteryl ester was rapidly hydrolyzed and effluxed. This finding is especially striking in comparison to modified LDL: under conditions producing equal cholesterol loading with native LDL or acetylated LDL (the modified lipoprotein most commonly used to generate experimental macrophage foam cells), the rate of efflux observed was much lower with acetylated LDL (J.M. Meyer, unpublished observation). This finding was consistent with an earlier study [63]. Similar differences between native and oxidized LDL were observed (J.M. Meyer, unpublished observation). This suggests that native LDL-derived cholesterol is preferentially directed to be effluxed from cells, which may be expected to be a less pathogenic process compared with modified LDL uptake. The ability of macrophages to take up and subsequently hydrolyze cholesteryl ester to cholesterol via the SLU pathway suggests a possible mechanism by which macrophages might efficiently scavenge and hydrolyze cholesteryl ester at sites of necrosis or tissue damage for retrograde transport to the liver.

CONCLUSION

Recent findings provide new insights into the function of SLU in cholesterol homeostasis but also raise many questions. The mechanism by which SR-BI promotes lipid transfer into cellular membranes has been elusive for many years, and a combination of mutation analysis with potential studies on the higher-order structure of SR-BI, including X-ray crystallography and NMR spectroscopy, should be very informative. The trafficking of cholesterol and cholesteryl ester in cells is also a major challenge and may be aided by expanded use of fluorescent analogs including dehydroergosteryl ester (a cholesteryl ester analog) to visualize sub-cellular localization [64], and photoactive cross-linking cholesterol reagents and their esters for identifying specific proteins involved in cellular lipid transport [65]. The latter would be especially useful for identifying unknown proteins involved in the SR-BI-independent selective cholesteryl ester uptake pathway in macrophage foam cells. Finally, the importance of SR-BI and RCT in human disease remains to be tested, although at least one drug that could raise SR-BI expression is currently in clinical trials. In light of the great potential of SR-BI and other SLU pathways to affect human health, continued investigation into the mechanism and significance of SLU pathways is well justified.

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KEY POINTS

- Selective cholesteryl ester uptake is a major step in HDL metabolism and RCT.
- SR-BI-mediated hepatic SLU is normally coupled to biliary cholesterol secretion, and uncoupling may be a key contributor to ineffective RCT in metabolic diseases.
- FXR and the microRNAs miR-185, miR-96 and miR-223 are emerging therapeutic targets for increasing hepatic SR-BI expression.
- SR-BI-independent selective cholesteryl ester uptake is a newly characterized pathway in macrophage foam cells.

FIGURE 1.

Proposed model for selective cholesteryl ester uptake pathways in RCT. Gray and black arrows indicate forward cholesterol transport and RCT, respectively; white arrows highlight the specific steps involving SLU. Hepatic cholesterol originating from intestinal absorption (1) or de novo synthesis is secreted in VLDL particles (2) that are remodeled to LDL. LDL accumulates in atherosclerotic lesions and may be oxidized (3) and taken up by macrophage scavenger receptors (4). Native LDL is taken up by pinocytosis and LDL cholesteryl ester by SLU (5). Selective cholesteryl ester uptake leads to efficient hydrolysis and efflux to HDL. HDL cholesterol is in turn delivered to the liver by SR-BI-mediated SLU (6) that leads to preferential biliary delivery and excretion. ABCG5/G8 contributes the majority of biliary cholesterol secretion (7) but appears to do so in an SR-BI-independent manner. ‘Uncoupling’ in metabolic disease states may involve normal or increased ABCG5/G8 biliary secretion (7) but impaired HDL-coupled transport (6). RCT, reverse cholesterol transport; SLU, selective lipid uptake.

Table 1

Major features of the scavenger receptor B-type I-mediated selective cholesteryl ester uptake pathway

HDL or LDL as substrate
Rate of uptake: FC >CE >TG >PL
Requires apolipoproteins (apoA1, apoE, apoC)
Nonendocytic
Reversible plasma membrane CE pool
No particle-cell membrane fusion
CE hydrolysis by a neutral cholesterol esterase

CE, cholesteryl ester; FC, free cholesterol; PL, phospholipid; TG, triglyceride.