Biliary and Non-Biliary Contributions to Reverse Cholesterol Transport

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Abstract

Purpose of Review—The process of reverse cholesterol transport (RCT) is critical for disposal of excess cholesterol from the body. Although it is generally accepted that RCT requires biliary secretion, recent studies show that RCT persists in genetic or surgical models of biliary insufficiency. Discovery of this non-biliary pathway has opened new possibilities of targeting the intestine as an inducible cholesterol excretory organ. In this review we highlight the relative contribution and therapeutic potential for both biliary and non-biliary components of reverse cholesterol transport (RCT).

Recent Findings—Recently, the proximal small intestine has gained attention for its underappreciated ability to secrete cholesterol in a process called transintestinal cholesterol efflux (TICE). Although this intestinal pathway for RCT is quantitatively smaller than the biliary route under normal physiological conditions, the TICE pathway is highly inducible, providing a novel therapeutic opportunity for treatment of atherosclerotic cardiovascular disease (ASCVD). In fact, recent studies show that intestine-specific activation of RCT protects against ASCVD in mice.

Summary—It is well known that the small intestine plays a gatekeeper role in the maintenance of cholesterol balance. Through integrated regulation of cholesterol absorption and TICE, the small intestine is a key target for new therapies against ASCVD.

Keywords
cholesterol; lipoprotein; bile; reverse cholesterol transport

Introduction

Cholesterol is a key component of membranes in all vertebrates, and plays important roles in membrane dynamics, signal transduction, bile acid synthesis, and steroid hormone production [1,2]. However, excessive cholesterol accumulation in the body can promote the development of several chronic diseases including ASCVD. Although statin drugs have modestly improved mortality due to ASCVD, one of every six deaths in the United States is still attributable to this cholesterol-driven disease [3]. Given this unmet need for effective therapies, there is increasing interest in targeting new pathways that either reduce low-density lipoprotein (LDL) cholesterol or increase high-density lipoprotein (HDL)-driven RCT. The purpose of this review is to discuss recent insights into a novel intestinal pathway for RCT that has the potential to address this unmet therapeutic need. Here we discuss the

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current state of knowledge surrounding RCT, with particular emphasis on the relative contribution and therapeutic potential for both biliary and non-biliary components of RCT.

A New Model for Reverse Cholesterol Transport

The process of RCT was originally proposed in the 1970s as a means for the body to rid itself of excess cholesterol [4], and experimental evidence has supported active RCT in all mammals studied [2,4,5,6]. Importantly the efflux of cholesterol from macrophages in the artery wall, and subsequent disposal of this cholesterol through the RCT pathway is highly predictive of ASCVD burden in humans and rodents [7,8]. Classically, RCT was thought to involve the movement of cholesterol from peripheral tissues to the liver for excretion into bile and subsequent loss through the feces [2,4–6]. In this classic model of RCT biliary secretion was obligatory for fecal disposal [4–6]. However, emerging evidence supports an unexpected role for the small intestine in actively excreting plasma-derived cholesterol in a process known as TICE [9–12]. Given that several comprehensive reviews have been recently written surrounding the historical evidence for TICE [9–12], this will not be discussed in detail here. Instead, we will highlight the most recent advances in our understanding of the TICE pathway. In agreement with previous studies in humans [13,14], dogs [15,16], rats [17–19], and mice [20–29] we recently established that TICE exists in novel genetic and surgical mouse models [29]. In these studies we demonstrated that the specific process of macrophage RCT as well as mass fecal neutral sterol loss persist in both genetic and surgical models of biliary cholesterol insufficiency [29]. Indeed, both biliary and non-biliary pathways are operative in delivering cholesterol to the lumen of the small intestine for fecal excretion [9–29]. However, our understanding surrounding the relative contributions of the biliary and non-biliary components to RCT in animal models is still in its infancy.

The Relative Contribution of Biliary and Non-Biliary Pathways for RCT

To design both safe and effective therapeutic strategies for targeting RCT, additional work is needed to quantify the relative contributions of the biliary and non-biliary pathways to RCT. Clearly targeting the non-biliary TICE pathway is a much safer option, given that increasing biliary cholesterol secretion promotes cholesterol gallstone disease [30–32], which is a major healthcare burden already affecting more than 20 million people in the United States [33,34]. To selectively target TICE we must attempt to understand the flux through each arm of the RCT pathway. In chow-fed C57BL/6J mice the non-biliary pathway accounts for approximately 33% of total fecal neutral sterol loss [23] and roughly 20% of total fecal sterol loss in FVB mice [20]. Although these TICE estimations in chow-fed mice are quite underwhelming, it is important to point out that TICE is a highly dynamic pathway that can be upregulated under certain conditions. In support of this, in three independent mouse models (ABCG5/G8<sup>−/−</sup>, Mdr2<sup>−/−</sup>, and NPC1L1<sup>LiverTg</sup>) with severely diminished biliary cholesterol secretion, fecal neutral sterol loss is only modestly decreased [35] or not altered at all [20,21,29]. Furthermore, in dogs and rats with complete biliary diversion, fecal neutral, but not acidic, sterol los is actually increased 2–7 fold [15–19]. This unequivocally indicates that the non-biliary TICE pathway can be stimulated under conditions of biliary insufficiency, and has the capacity to maintain [20,21,29] or even increase [15–19] fecal cholesterol loss. Non-biliary RCT is also quite sensitive to pharmacological intervention [20,23,24,27,29]. In fact, activation of the liver × receptor (LXR) can dramatically augment non-biliary macrophage RCT [29] and increase mass fecal neutral sterol loss [20,23,29]. The dynamic nature of TICE is best illustrated with LXR activation, where the contribution of TICE to fecal cholesterol loss increases from 33% in vehicle treated mice to 63% in LXR agonist-treated mice [23]. Furthermore, small molecule activation of the peroxisome proliferator activated receptor δ also stimulates TICE specifically without affecting biliary
cholesterol levels [24]. Most recently it was shown that the cholesterol absorption inhibitor ezetimibe specifically activates TICE [27]. The active search for novel pharmacologic activators of TICE is ongoing, but before testing such compounds in clinical trials we must first understand the capacity of TICE in humans.

Although there are studies in humans showing that the gut can contribute to the pool of cholesterol in the intestinal lumen [13,14], the relative contribution of the biliary and non-biliary pathways to RCT has not been firmly established. A data set that hints at the contribution of the TICE pathway comes from a study of three patients with complete obstruction of the common bile duct due to carcinoma of the head of the pancreas [14]. Because this illness totally removed the input of cholesterol from the biliary pathway, the amount of intestinal cholesterol secretion could be measured. In these patients it was determined the intestine secreted on average 350 mg cholesterol/day. If the average input of cholesterol into the gut from bile is 800–1,300 mg/day [36,37], then 20–30% of the endogenous cholesterol in the lumen of the human intestine could originate from the TICE pathway. Collectively, studies in both animal models and man suggest that TICE accounts for approximately 20–30% of fecal neutral sterols under normal conditions. However, TICE can be potently stimulated by either biliary insufficiency or by drug treatment, providing strong evidence that the TICE pathway is highly dynamic and amenable to pharmacologic intervention.

**Targeting the TICE Pathway for Prevention of ASCVD**

Within the last year there has been exciting new evidence that specifically targeting intestinal RCT can have profound effects on ASCVD progression [38,39]. These important studies have demonstrated that intestine-specific activation of LXR signaling can promote macrophage RCT as well as protect against ASCVD in mice [38,39]. Although providing evidence that selective pharmacological manipulation of the small intestine can protect against atherosclerosis, much additional work is needed to design novel therapeutic strategies specifically targeting TICE. The question now becomes how do we target non-biliary RCT without increasing biliary RCT and consequently the risk of gallstone formation? The answer will come from ongoing studies characterizing the following aspects of non-biliary RCT (Figure 1): (1) Plasma lipoproteins targeted to the intestine, (2) Intravascular metabolism of TICE-competent lipoproteins, (3) Intestinal lipoprotein receptors/transporters on the basolateral membrane, (4) Proteins involved in cholesterol trafficking within enterocytes, (5) Cholesterol transporter(s) on the brush border membrane, and (6) Acceptors of cholesterol in the intestinal lumen.

**The Role of Lipoproteins in TICE**

Non-biliary RCT almost certainly relies on plasma lipoproteins to move cholesterol from either peripheral tissues and/or the liver to the small intestine for secretion. Importantly, we believe that the liver plays a central role in producing lipoproteins necessary for TICE, and support for this concept has been recently reviewed [9]. Although historically HDL has been implicated in promoting RCT [4,5,7,8], there are several studies indicating that HDL is not rate limiting in biliary or non-biliary RCT. A key observation supporting this is that ABCA1−/− mice, which have virtually no circulating HDL cholesterol, have normal biliary and fecal cholesterol levels [40,41], and display similar LXR-induced increases in RCT as wild type mice [42]. Likewise, mice deficient in apolipoprotein A-I have normal biliary and fecal cholesterol levels [43]. Furthermore, intestinal uptake of HDL-cholesteryl ester (CE) was similar in vehicle-treated control and MDR2−/− mice, and was reduced to the same extent in these two genotypes following LXR agonist treatment [44]. In several mouse models with augmented TICE [21,45,46], apolipoprotein E (apoE)-rich HDL is significantly
increased implicating this lipoprotein class in TICE. However we have recently eliminated apoE expression in a mouse model where TICE predominates (NPC1L1− liverTg mice), and found no effect on biliary or non-biliary fecal cholesterol excretion (Temel RE, Brown JM, unpublished data).

In contrast to HDL, hepatic apoB-containing lipoproteins such as VLDL appear to play an important role in TICE. For instance, the concentration of VLDL cholesterol was significantly elevated in LXR agonist-treated ABCA1−/− mice, while the level of HDL cholesterol was unchanged [42]. Furthermore, we recently reported that antisense oligonucleotide (ASO)-mediated knockdown of acyl-CoA:cholesterol acyltransferase 2 (ACAT2) promotes TICE due to the apparent ability of nascent VLDL to deliver 2–3 fold more cholesterol to the proximal small intestine [22]. We have also recently discovered that knockdown of hepatic MTP, a protein essential for the lipidation and production of VLDL [47], caused a greater than 50% reduction in TICE (Temel RE, Brown JM, unpublished data). Based upon this experimental evidence, we believe that following lipolysis of the core TG, VLDL remnants or further catabolic products of VLDL (IDL, LDL) can deliver cholesterol to the small intestine for secretion. However, much additional work is needed to determine the mechanism by which apoB-containing lipoproteins can be specifically targeted to the proximal small intestine to promote TICE.

**Receptors Involved in Intestinal Lipoprotein Cholesterol Uptake**

Under the assumption that HDL is involved in delivering cholesterol to the small intestine, several studies have analyzed the role of the HDL receptor, SR-BI, in TICE. Using an intestinal perfusion system, TICE was found to be paradoxically increased, not decreased, in SR-BI deficient (SR-BI−/−) mice [25]. When treated with vehicle or LXR agonist, control and SR-BI−/− mice showed the same amount of intestinal uptake of HDL CE [44]. In addition, we (Temel RE, Brown JM, unpublished data) recently found that non-biliary RCT is unaffected in mice with intestine-specific overexpression of SR-BI [48]. These results strongly indicate that intestinal SR-BI is not involved in TICE and further support the conclusion that HDL does not participate in delivering cholesterol to the gut for secretion through the TICE pathway.

It would be logical to assume that the LDL receptor (LDLR) could be involved in TICE since apoB-containing lipoproteins appear to be the primary shuttle for moving cholesterol to the gut. However, the small intestine of LDLR deficient mice was able to accumulate cholesterol that originated from VLDL-containing liver perfusate [22]. In addition, LXR agonist treatment significantly increases TICE [20,23,28] in spite of increased ubiquitination and degradation of intestinal LDLR protein [49]. Other members in the LDL receptor family such as LRP, VLDL receptor (VLDLR), and apoER2 are also expressed in the gut [50,51], and could conceivably act as primary or secondary receptors for apoB-containing lipoproteins. However, LXR-driven upregulation of the E3 ubiquitin ligase Idol targets not only the LDLR but also VLDLR and apoER2 for proteasomal degradation [49,52]. Thus, the uptake of apoB-containing lipoproteins by the small intestine could involve a currently unidentified receptor.

**Trafficking of Cholesterol Across Enterocytes and Into the Intestinal Lumen**

The factors involved in trafficking cholesterol through the enterocytes for the TICE pathway remain poorly understood. If apoB-containing lipoproteins are involved in delivering cholesterol to the small intestine then it is likely that these lipoproteins are endocytosed and ultimately degraded in lysosomes. Therefore, TICE would require NPC1 and NPC2 to move the cholesterol out of the lysosomal compartment [53]. Interestingly, compared to control mice, mice deficient in NPC1 have double the amount of cholesterol in the small intestine.
Since NPC1 and NPC2 deficient mice do not have a defect in cholesterol absorption [54], the intestinal accumulation of cholesterol in the absence of NPC1 [54] is almost certainly the result of LDL cholesterol being trapped in the lysosomes [53]. Rab9 and LIMP2 could also be involved in intracellular trafficking of cholesterol derived from TICE, given that their intestinal expression was significantly elevated in mice treated with PPARδ agonists [24]. However, much additional work is obviously required to fully understand the subcellular trafficking itinerary of TICE-derived cholesterol within the enterocyte.

After being moved from the basolateral to apical membrane of the enterocytes, cholesterol is presumably actively effluxed into the lumen of the gut. Because of their ability to pump cholesterol into the intestinal lumen, the heterodimeric sterol transporters ABCG5 and ABCG8 would logically play an essential role in TICE. However, in a study using stable isotopes to measure the contribution of several pathways to fecal cholesterol excretion, it was found that although reduced by 40%, TICE was not eliminated in mice lacking ABCG5 [23]. Moreover, no difference in intestinal cholesterol secretion was observed in control and ABCG8 deficient mice subjected to intestinal perfusion [28]. Importantly, ABCG5 and ABCG8 are necessary for LXR-stimulated biliary and non-biliary RCT [23,56,57]. These results indicate that although ABCG5 and ABCG8 do contribute to LXR-stimulated RCT [23,56,57], other apical transporters are likely involved in the secretion of cholesterol from enterocytes into the intestinal lumen under normal physiological conditions.

The secretion of cholesterol into the intestinal lumen would presumably require an acceptor. Intestinal perfusion of mice showed that a mixture of bile acids and phospholipid could drive TICE [28]. However, the amount of phospholipid in the perfusate appeared to determine the level of intestinal cholesterol secretion [28]. The dependence on phospholipid for TICE is similar to what is observed for the movement of cholesterol into the bile [20]. Moreover, the highest levels of TICE have been reported to occur in the proximal small intestine, a region of the gut that should contain a high concentration of bile-derived phospholipid. Studies examining the luminal acceptors of TICE hold great promise for therapeutic interventions that would not require systemic distribution.

Conclusion

The process of RCT is strongly negatively correlated with ASCVD burden in humans and rodents [7,8]. Although RCT was historically believed to rely solely on biliary secretion [2,4–6], recent evidence unequivocally demonstrates that a non-biliary pathway for RCT exists [9–29]. Unfortunately, to date very little progress has been made to identify the molecular mechanisms that define TICE. In order to effectively target this pathway, the following challenges will need to be addressed: (1) establishing quantitative methods to measure nonbiliary fecal sterol loss in primates and man, (2) characterizing the hepatic and plasma lipoprotein metabolism that is requisite for nonbiliary fecal sterol loss, (3) identifying the intestinal transport proteins/receptors involved, (3) identifying the luminal acceptor molecules promoting TICE, and (4) identifying bona fide drug targets to specifically modulate the intestinal component of RCT. Advancement in these areas has strong potential for novel anti-ASCVD therapies.

Acknowledgments

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Abbreviations Used

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<th>Abbreviation</th>
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<tr>
<td>ABCG5/G8</td>
<td>ATP-binding cassette transporters G5 and G8</td>
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<td>ACAT2</td>
<td>Acyl-CoA:cholesterol acyltransferase 2</td>
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<td>ASO</td>
<td>antisense oligonucleotide</td>
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<td>ASCVD</td>
<td>atherosclerotic cardiovascular disease</td>
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<td>HDL</td>
<td>high density lipoprotein</td>
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<td>LDL</td>
<td>low density lipoprotein</td>
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<td>LXR</td>
<td>liver × receptor</td>
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<td>MDR2</td>
<td>P-glycoprotein</td>
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<td>NPC1L1</td>
<td>Niemann Pick-C1 Like-1</td>
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<tr>
<td>PPARδ</td>
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<td>RCT</td>
<td>reverse cholesterol transport</td>
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<tr>
<td>SR-BI</td>
<td>scavenger receptor class B type I</td>
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<td>TICE</td>
<td>transintestinal cholesterol efflux</td>
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References

   * This paper demonstrates that the measurement of cholesterol efflux capacity from macrophages, as a measure of HDL function, is inversely correlated with cardiovascular disease burden in humans.


29. Temel RE, Sawyer JK, Yu L, et al. Biliary sterol secretion is not required for macrophage reverse cholesterol transport. Cell Metab. 2010; 12:96–102. [PubMed: 20620999] **First study to show that the specific process of macrophage RCT persists in mice genetically or surgically lacking the ability to secrete cholesterol into bile.


Key Points

- TICE contributes ~30% of total RCT in normal conditions, but importantly TICE can be potently stimulated by either biliary insufficiency or by drug treatment, providing strong evidence that the TICE pathway is a highly dynamic pathway that is amenable to pharmacologic intervention.

- The TICE pathway is a much more attractive drug target since increased biliary secretion promotes gallstone formation.

- To effectively target TICE, we need to understand: hepatic cholesterol trafficking, plasma lipoprotein carriers and intravascular metabolism, basolateral transporters, trafficking pattern across the enterocyte, apical efflux mechanisms, and lumenal acceptors.
Figure 1.