Scavenger receptor class B type I in high-density lipoprotein metabolism, atherosclerosis and heart disease: lessons from gene-targeted mice

B. Trigatti1, S. Covey and A. Rizvi
Department of Biochemistry, McMaster University, 1200 Main St West, Hamilton, ON, Canada L8N 3Z5

Abstract

The scavenger receptor class B type I (SR-BI) is a multi-ligand receptor that can mediate the binding and bi-directional lipid transfer between high-density lipoproteins (HDLs) and cells. It is expressed in a variety of tissues, including the liver, and in macrophages in atherosclerotic plaques. The physiological role of SR-BI has been tested in vivo by the genetic manipulation of SR-BI levels in mice. Mice lacking SR-BI exhibit impaired hepatic-selective HDL cholesterol uptake and increased atherosclerosis, suggesting that SR-BI is required for hepatic reverse cholesterol transport and normally protects against atherosclerosis. Surprisingly, elimination of SR-BI in apolipoprotein E knockout mice results in rapid development of occlusive coronary artery disease, accompanied by spontaneous myocardial infarction, reduced heart function and early death, which points to a role for SR-BI in protection against coronary heart disease. The in vivo role of macrophage SR-BI has been less clear. We have used bone-marrow transplantation to demonstrate that bone-marrow-derived SR-BI also normally protects against atherosclerosis in low-density lipoprotein receptor knockout mice. These results suggest that SR-BI may have multiple protective effects against atherosclerosis in different tissues.

Introduction

Atherosclerosis is a major cause of heart disease, stroke and peripheral vascular disease [1]. Plasma lipid (cholesterol and triacylglycerol) levels are major risk factors [2,3]. Low-density lipoprotein (LDL) cholesterol levels are directly correlated and high-density lipoprotein (HDL) cholesterol levels inversely correlated with risk, suggesting that LDL promotes, whereas HDL protects against, atherosclerosis [4]. The importance of HDL is underscored by the increased incidence of atherosclerosis in patients with Tangier’s disease or familial HDL deficiency, both of which are characterized by low HDL levels in plasma resulting from inactivation of the cholesterol efflux regulatory protein, ATP-binding cassette transporter (ABC)-A1 (reviewed in [5]). HDL may mediate atheroprotection via multiple mechanisms, which include (i) reverse cholesterol transport (see below), (ii) protection against oxidative damage and (iii) modulation of endothelial signalling events, e.g. stimulation of endothelial nitric oxide synthase activity (see [6] and references therein).

Reverse cholesterol transport is the HDL-mediated transport of excess cholesterol from peripheral tissues (e.g. macrophages in atherosclerotic plaques) to the liver for excretion or recycling ([7] and references therein). It involves multiple steps (Figure 1), beginning with the efflux of unesterified cholesterol from macrophages to lipid-free apo-

Key words: atherosclerosis, cholesterol, high-density lipoprotein (HDL), lipoprotein, scavenger receptor class B type I (SR-BI), transgenic.
Abbreviations used: ABC, ATP-binding cassette transporters; Apo, apolipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SR-BI, scavenger receptor class B type I; VLDL, very-low-density lipoprotein.

1To whom correspondence should be addressed (e-mail trigatti@mcmaster.ca).
SR-BI is expressed in liver, steroidogenic tissues, macrophage foam cells in atherosclerotic plaques and a variety of other tissues and cell types. In macrophage foam cells in the artery wall, it has been proposed that it participates in free cholesterol (FC) efflux to lipoprotein acceptors, including HDL. HDL-associated FC is converted into cholesteryl ester (CE) by the enzyme lecithin:cholesterol acetyltransferase and can either be transferred to other lipoproteins by cholesteryl ester transfer protein or remain associated with HDL. HDL-associated CE is taken up via selective lipid uptake by liver and by steroidogenic tissues. HDL CE taken up via selective uptake is preferentially secreted into the bile. SR-BI appears to participate in both early and late steps in selective uptake.


development of reverse cholesterol transport (Figure 1) to HDL and selective lipid uptake from HDLs suggested that it may play an important role in HDL-dependent reverse cholesterol transport from peripheral tissues (e.g. macrophages in the artery wall) to the liver (Figure 1). SR-BI is expressed in a variety of cell/tissue types, including liver hepatocytes and steroidogenic cells (which exhibit the highest selective uptake activity in vivo) (reviewed in [7]), as well as macrophage foam cells in atherosclerotic plaques, Kupffer cells in the liver, endothelial cells, and a variety of other cell types ([6,7,11] and references therein). The expression of SR-BI in both macrophages in atherosclerotic plaques and in liver hepatocytes raises the possibility that it could participate in both early (i.e. cholesterol efflux from macrophages) and late (hepatic-selective uptake) events in reverse cholesterol transport (Figure 1).

In vitro cell culture studies also suggested a role for SR-BI in mediating other potentially anti-atherogenic effects of HDLs; for example, SR-BI overexpression in cultured cells increases selective uptake of α-tocopherol from HDLs [16]. Furthermore, elimination of SR-BI in mice results in increased levels of α-tocopherol in plasma and decreased levels in a variety of tissues, including ovaries, testes, lung and brain [16]. Normal levels of α-tocopherol are present in liver, but levels in hepatic bile are decreased [16]. Thus, SR-BI may be important for tissue uptake of α-tocopherol and protection of cells against oxidative damage [17]. SR-BI can also mediate HDL-dependent signalling, inducing endothelial nitric oxide synthase (eNOS) and involving mitogen-activated protein kinase and Akt kinase, and other signalling pathways [18,19], and endothelial SR-BI mediates HDL-stimulated relaxation of blood vessels in mice [19,20].

**Genetic manipulation of SR-BI in mice**

The activities of SR-BI in vitro, revealed by studies with transfected cells in culture, led to the hypothesis that SR-BI may play an important role in mediating the anti-atherogenic effects of HDL. This has been tested directly by the manipulation of SR-BI gene expression in mice [21–25].

A variety of different strategies have been used to manipulate SR-BI expression in mice, and a number of different lines of mice with altered SR-BI expression have been generated. SR-BI knockout mice are null-mutants in which SR-BI is not expressed as a result of the replacement of a portion of the first coding exon and part of the subsequent intron with a neomycin resistance gene cassette [24]. Homozygous SR-BI knockout mice express no SR-BI, whereas heterozygous SR-BI knockout mice express approx. 50% of normal levels of SR-BI in livers and adrenal glands [24]. SR-Blatt mice contain an inserted neomycin resistance gene cassette in the promoter region of the SR-BI gene [25]. This mutation results in attenuated expression (approx. 50% of wild-type levels) in the liver with no apparent effect in steroidogenic tissues, such as the adrenal gland [25].

Mice that overexpress SR-BI have also been generated using different approaches. In the first study to manipulate SR-BI levels in mice, Kozarsky et al. [21] used a recombinant adenovirus to transiently overexpress SR-BI in livers of mice in vivo. Tall and co-workers [22] generated liver-specific SR-BI-overexpressing transgenic mice using an SR-BI cDNA construct driven by a liver-specific ApoE promoter and ApoE/CI control region. Similarly, Rubin and co-workers [23] generated SR-BI transgenic mice with different levels of liver-specific overexpression using an ApoA-1 gene promoter to drive expression of the recombinant SR-BI cDNA.

**HDL cholesterol metabolism**

Analysis of the consequences of genetic manipulation of SR-BI levels in these mice provided the first direct evidence that SR-BI plays an important physiological role in HDL cholesterol metabolism in vivo. Ablation of SR-BI expression in SR-BI knockout mice resulted in an approx. 2-fold increase in plasma cholesterol associated with abnormally large ApoE-enriched HDL particles [24] that contained high unesterified/esterified cholesterol ratios (approx. 1 in SR-BI knockout mice versus approx. 0.3–0.5 in wild-type control mice) [26,27]. The reason for the increased unesterified/esterified cholesterol ratios in HDL from SR-BI knockout mice is unclear, although it is consistent with the idea that SR-BI may normally play an important role in the clearance of HDL-associated unesterified cholesterol [28].

Decreased lipid storage was also observed in the adrenal cortex and ovarian corpora lutea from SR-BI knockout mice [24,29]. These steroidogenic tissues normally express high
levels of SR-BI ([7] and references therein), which are required for selective uptake and subsequent storage of HDL cholesterol [30]. These results suggested that SR-BI is normally required in vivo for selective uptake of HDL cholesterol by liver and steroidogenic tissues [24,29].

Varban et al. [25] demonstrated that SR-BIatt mice (approx. 50% of normal hepatic levels of SR-BI expression) exhibited more moderate increases in plasma HDL cholesterol and particle size, similar to those seen in heterozygous SR-BI knockout mice (also approx. 50% of normal levels of SR-BI expression in liver) [24]. They demonstrated that the selective clearance of HDL cholesterol from plasma and hepatic HDL cholesterol uptake was reduced by approx. 50% in SR-BIatt mice compared with normal mice [25]. Consistent with this, hepatic uptake of HDL cholesteryl ester was reduced by 90% in SR-BI knockout mice [27]. These studies provided direct evidence for SR-BI's requirement for hepatic selective HDL cholesterol uptake in vivo.

Hepatic overexpression of SR-BI either using recombinant adeno virus-mediated SR-BI gene transfer [21] or in transgenic mice [22,23] resulted in substantial decreases in plasma HDL [21–23] and non-HDL [22,23] cholesterol levels, and increased biliary cholesterol levels. Similarly, SR-BI knockout mice also exhibited reduced biliary cholesterol secretion and had less cholesterol in bile [29,31]. No effects on synthesis or biliary secretion of bile acids were observed, nor were overall liver cholesterol levels affected in these mice [31]. It is not clear if this lack of an effect on hepatic cholesterol levels was the result of induction of compensatory pathways in the liver to maintain overall cholesterol levels (such as decreased biliary cholesterol secretion or increased LDL-receptor-mediated lipid uptake [31]). The precise role of SR-BI in hepatic biliary cholesterol secretion is also not clear. SR-BI is expressed in hepatocyte sinusoidal membranes and has been reported on canalicular membranes, although this remains controversial (reviewed in [6]). Whether it plays a direct role in canalicular secretion of cholesterol in to bile or an indirect role (through altered HDL cholesterol uptake) remains to be determined.

SR-BI is also expressed in gall bladder mucosal epithelial cells and its levels there are regulated by bile cholesterol content in the gall bladder and by feeding a lithogenic diet [32]. No differences were seen, however, in gall-bladder-wall cholesterol content between wild-type and SR-BI knockout mice [32]. Also, despite the decreased gall bladder biliary cholesterol content in SR-BI knockout relative to control mice [29,31], a lack of SR-BI did not affect the formation of gall stones in response to a lithogenic diet [32,33].

**ApoB-containing lipoproteins**

SR-BI has also been implicated in clearance of ApoB-containing lipoproteins ([11] and references therein; [22,23]). Overexpression of SR-BI in transgenic mice has been reported to result in reduced levels of ApoB and cholesterol associated with non-HDL lipoproteins [22,23]. Overexpression of SR-BI in fat-fed heterozygous LDL receptor knockout mice resulted in decreased VLDL, LDL and HDL cholesterol [34]. In hemizygous human ApoB transgenic mice, overexpression of SR-BI resulted in decreased HDL cholesterol and non-HDL cholesterol on a normal chow diet; in contrast, when mice were maintained on a high-fat diet, increasing SR-BI levels affected only HDL cholesterol levels [35]. In fat-fed LDL receptor knockout mice with attenuated expression of SR-BI (SR-BIatt/LDL receptor knockout), a reduction in SR-BI levels was accompanied by increased LDL cholesterol and ApoB levels, suggesting that SR-BI played an important role in LDL cholesterol and ApoB clearance [36]. In contrast, in chow-fed SR-BI/ApoE double-knockout mice and fat-fed SR-BI/LDL receptor double-knockout mice, complete elimination of SR-BI was accompanied by increased cholesterol associated with large HDL particles, and reduced ApoB content [29,37] and non-HDL cholesterol [37] in plasma. The mechanisms underlying the reduction of plasma ApoB levels in SR-BI knockout mice are not clear. One possibility may involve altered ApoB-containing lipoprotein secretion due to altered hepatic uptake of HDL cholesterol [38].

**Atherosclerosis and coronary artery disease**

Both transgenic and adeno virus-mediated hepatic overexpression of SR-BI has been shown to suppress atherosclerosis in LDL receptor knockout mice that were maintained on a high-fat diet [34,35,39]. In human ApoB transgenic mice maintained on a high-fat diet, moderate (2-fold) overexpression of hepatic SR-BI reduced atherosclerosis by half, whereas high-level (10-fold) overexpression did not reduce atherosclerosis relative to non-SR-BI transgenic mice [35], and actually increased the level of atherosclerosis relative to mice that express moderate levels of SR-BI. This may have been the result of alterations in LDL structure or composition [35]. Decreased atherosclerosis in hepatic SR-BI-overexpressing mice correlated with either decreased levels of ApoB-containing lipoproteins [34] or with decreased levels of HDL cholesterol [35,39]. Therefore, under different circumstances overexpression of SR-BI may result in either decreased levels of atherogenic lipoproteins or increased HDL reverse cholesterol transport. This suggests that the consequences of overexpression of SR-BI for atherosclerosis may be more complicated than originally anticipated.

A variety of studies of the consequences of decreasing or eliminating SR-BI on atherosclerosis have been reported. In the first of these, the consequences of eliminating SR-BI in ApoE knockout mice were tested by generating SR-BI/ApoE double-knockout mice. These mice, which were fed a normal chow diet, developed substantial atherosclerotic plaques in their aortic sinuses by 5–7 weeks of age, at which time virtually no plaque had developed in the aortic sinuses of either ApoE or SR-BI single-knockout mice [29]. Similarly, elimination of SR-BI in LDL receptor knockout mice resulted in an approx. 6-fold increase in diet-induced aortic atherosclerosis [37]. Diet-induced atherosclerosis was also increased in mice deficient in SR-BI alone relative to wild-type controls [27] and in LDL receptor knockout mice with attenuated
expression of SR-BI [36]. In SR-BI knockout mice on wild-type, ApoE knockout or LDL receptor knockout backgrounds, increased atherosclerosis was accompanied by increased cholesterol associated with large HDL particles [27,29,37], suggesting that elimination of SR-BI primarily affected reverse HDL cholesterol transport.

Surprisingly, SR-BI/ApoE double-knockout mice developed severe coronary artery atherosclerosis which resulted in complete coronary artery occlusion and was accompanied by myocardial infarctions, reduced heart function, cardiac conductance defects and early death (between 5 and 8 weeks of age) [40]. This was not observed in SR-BI-heterozygous mutant/ApoE knockout mice (B. Trigatti, A. Braun and M. Krieger, unpublished work), nor were similar phenotypes reported in SR-BIatt/ApoE knockout mice [41]. Furthermore, myocardial infarction was not observed in 5-month-old SR-BI/LDL receptor double-knockout mice that had been maintained on a high-fat diet for 2 months [37]. These features of coronary heart disease in SR-BI/ApoE double-knockout mice could be rescued with probucol [42], an antioxidant drug that has been reported to increase selective cholesterol uptake in vitro [43], exhibit pro- and anti-atherosclerotic properties in animal models [44] and normalize plasma lipoproteins in SR-BI knockout mice [42,45].

**Tissue-specific manipulation of SR-BI in non-hepatic tissues: bone-marrow transplantation**

Tissue-specific manipulation of SR-BI has been used to investigate if SR-BI expression in bone-marrow-derived cells (including macrophages) may contribute to SR-BI-mediated atheroprotection. For these studies, bone-marrow transplantation (using either SR-BI knockout or wild-type mice as donors) was performed on lethally irradiated LDL receptor knockout mice to generate chimaeras with a SR-BI/LDL receptor double-knockout mice that had been maintained on a high-fat diet for 2 months [37]. These features of coronary heart disease in SR-BI/ApoE double-knockout mice could be rescued with probucol [42], an antioxidant drug that has been reported to increase selective cholesterol uptake in vitro [43], exhibit pro- and anti-atherosclerotic properties in animal models [44] and normalize plasma lipoproteins in SR-BI knockout mice [42,45].

**Concluding remarks**

Genetic manipulation of SR-BI in mice has revealed the key role that SR-BI plays in lipoprotein metabolism, and in protection against atherosclerosis and coronary heart disease. Bone-marrow transplantation studies have demonstrated that SR-BI expression in blood cells (probably macrophages) normally protects against atherosclerosis. The recent identification of mutations/polymorphisms in human SR-BI and their association with altered HDL and non-HDL cholesterol levels [48,49], suggests that variations in SR-BI expression/activity may influence atherosclerotic cardiovascular disease in humans as well.

Research in the laboratory of B.T. is supported by the following grants: MOP49608 from the Canadian Institutes of Health Research, NA4874 from the Heart and Stroke Foundation of Ontario; and a grant from the Natural Sciences and Engineering Research Council of Canada. B.T. is a Heart and Stroke Foundation of Canada New Investigator.

**References**


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**Figure 2 | Transplantation of SR-BI-deficient bone marrow increases atherosclerosis in LDL receptor knockout mice**

Representative micrographs of aortic arches from LDL receptor knockout mice, which have been transplanted with bone marrow from either an SR-BI+/+ (control, left panel) or an SR-BI−/− (right panel) donor. Atherosclerosis was induced by feeding mice a high-fat diet for 4 months. Aortas were stained for lipid (red) with Sudan IV. See [37].