New Approaches to Raising the HDL Cholesterol Level

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Abstract: Not only a high level of low-density lipoprotein (LDL) cholesterol, but also a low level of high-density lipoprotein (HDL) cholesterol, is a critical risk factor for atherosclerosis and coronary heart disease. Although fibrates and niacin can be used to improve low HDL cholesterol levels, their effect is not wholly satisfactory, so better drugs for the elevation of HDL cholesterol are desired. Among the many methods that may be used to raise HDL cholesterol levels, this review focuses on inhibitors of cholesteryl ester transfer protein (CETP) and on nuclear orphan receptor agonists that mediate the expression of ATP-binding cassette transporter 1 (ABC1).

Key words: High-density lipoprotein (HDL), Reverse cholesterol transport, Cholesteryl ester transfer protein (CETP), ATP-binding cassette transporter 1 (ABC1), Peroxisome proliferator-activated receptor δ (PPARδ), Retinoid X receptor (RXR), Liver X receptor (LXR), Apolipoprotein A-I (apoA-I).

INTRODUCTION

3-Hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors (statins) can significantly reduce the incidence of coronary heart disease by decreasing high levels of low-density lipoprotein (LDL) cholesterol [1], but the cardiovascular outcome of many treated patients is still unsatisfactory [2]. Not only a high LDL cholesterol level, but also a low high-density lipoprotein (HDL) cholesterol level, is a risk factor for atherosclerosis and coronary heart disease [3, 4]. Since HDL plays a role in the transfer of excess cholesterol from the peripheral tissues to the liver (reverse cholesterol transport) [5, 6] and in the inhibition of lipoprotein oxidation [7, 8], HDL is protective against atherosclerosis. However, current therapies for increasing a low plasma HDL cholesterol level are not wholly satisfactory. Fibrates, such as clofibrate and fenofibrate, can be used to achieve an increase of HDL cholesterol, but these drugs are only slightly effective. Nicotinic acid (niacin) increases HDL cholesterol by 26%, but its side effects limit patient compliance [9]. Therefore, safer and more effective drugs that can significantly increase the HDL cholesterol level are desired. There are many possible approaches to the elevation of HDL cholesterol, such as increasing the activity of lecithyl cholesterol acyl tranferase (LCAT) [10] or lipoprotein lipase [11], as well as increasing the level of phospholipid transfer protein [12], apoprotein A-I (apoA-I) [13], or ATP-binding cassette transporter 1 (ABC1) [14], modulating the expression of hepatic HDL scavenger receptor (SR-B1) [15], and inhibiting the activity of hepatic triglyceride lipase [11] or cholesteryl ester transfer protein (CETP). Among these targets, this review focuses on small molecule inhibitors of CETP and on orphan nuclear hormone receptor agonists that increase ABC1 expression and apoA-I-specific cholesterol efflux, because there have been some recent advances in the studies on these agents.

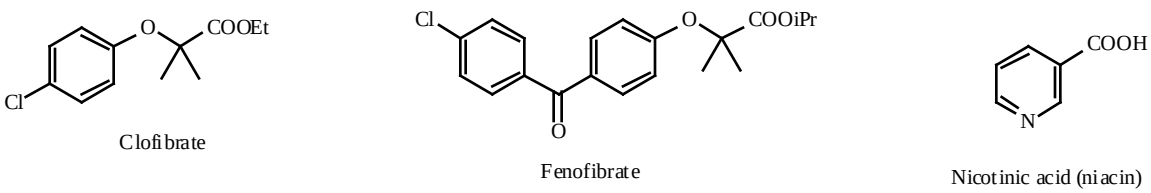

Fig. (1). Structures of fibrates and niacin.

CETP INHIBITORS

CETP is a plasma glycoprotein that mediates the exchange of cholesteryl ester (CE) in HDL for triglyceride (TG) in very low-density lipoprotein (VLDL) [16, 17]. This process decreases anti-atherogenic HDL cholesterol and increases pro-atherogenic VLDL cholesterol and LDL cholesterol (Fig. (**2**)), so CETP is a potentially atherogenic protein. Although a possible anti-atherogenic role of CETP has also been suggested because of its participation in reverse cholesterol transport [18-21], many studies have supported its atherogenicity [22-26, 38]. Therefore, an effective and safe CETP inhibitor may have potential as a

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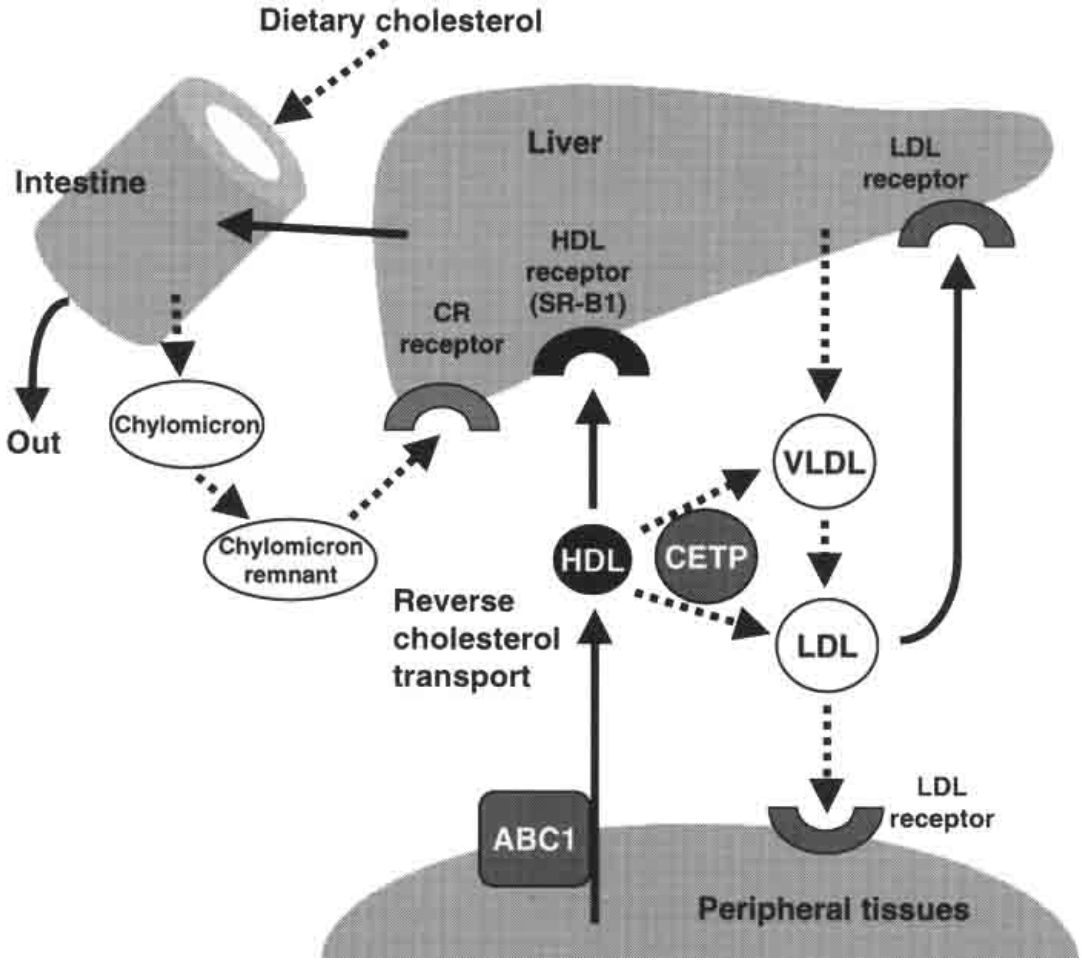

Fig. (2). Cholesterol transport.

novel anti-atherogenic drug and may be beneficial for patients with atherosclerosis and coronary heart disease. Human CETP consists of 476 amino acids and contains seven cysteines. As CETP is a monomer, it must possess at least one unpaired cysteine [27], and certain cysteine-modifying reagents are reported to be CETP inhibitors. Parke-Davis reported that a triazole (PD140195) could selectively inhibit CE transfer in a non-competitive manner (IC_{50} = 30 μM), while it did not inhibit TG transfer [28]. *In vitro* inhibitory activity of this triazole was markedly reduced in the presence of bovine serum albumin, suggesting that the low activity of PD140195 in plasma probably results from nonspecific binding to other plasma proteins. The anti-atherogenic effect of a small molecule CETP inhibitor was first reported by JT Inc [29, 30]. A 2-(acylamino) benzenethiol derivative (JTT-705) was found to inhibit CETP activity in human plasma (IC_{50} = 9 μM), and also produced 95% inhibition of CETP activity at an oral dose of 30 mg/kg in normal rabbits. Moreover, administration of JTT-705 (225 mg/kg/day) for six months caused a 90% increase of HDL cholesterol and decreased non-HDL cholesterol by 40-50% compared with the baseline values. Elevation of HDL cholesterol and reduction of non-HDL cholesterol by inhibition of CETP have also been demonstrated using an anti-CETP monoclonal antibody [31], a CETP vaccine [32], and a CETP antisense oligonucleotide [33]. The inhibitory mechanism of JTT-705 has been investigated using point mutations of recombinant human CETP. A C13S mutant, in which the cysteine at residue 13 was replaced by serine, was not inhibited by JTT-705, suggesting that the inhibitory mechanism involves the formation of a disulfide bond between the thiol form of JTT-705 and the cysteine residue at position 13 of CETP. JTT-705 was found to decrease atherosclerotic lesions by 70% in cholesterol-fed rabbits, which is the first evidence that a small molecule CETP inhibitor can retard the progression of atherosclerosis.

A trifluoro-3-amino-2-propanol (SC-795) was also reported by Pharmacia, and this agent inhibits CETP-mediated transfer of [^{3}H]CE from HDL to LDL in buffer (IC_{50} = 0.02 μM) and in human plasma (IC_{50} = 0.6 μM) [34, 35]. SC-795 is the (R)-(+)-enantiomer, which is 40 times more potent than the (S)-(-)-enantiomer, and it shows marked inhibition of CETP *in vitro*, although *in vivo* data have not been disclosed.

ORPHAN NUCLEAR HORMONE RECEPTOR AGONISTS

Administration of the rexinoid LG268 to mice was reported to dose-dependently inhibit cholesterol absorption and prevent the accumulation of cholesterol in the liver.

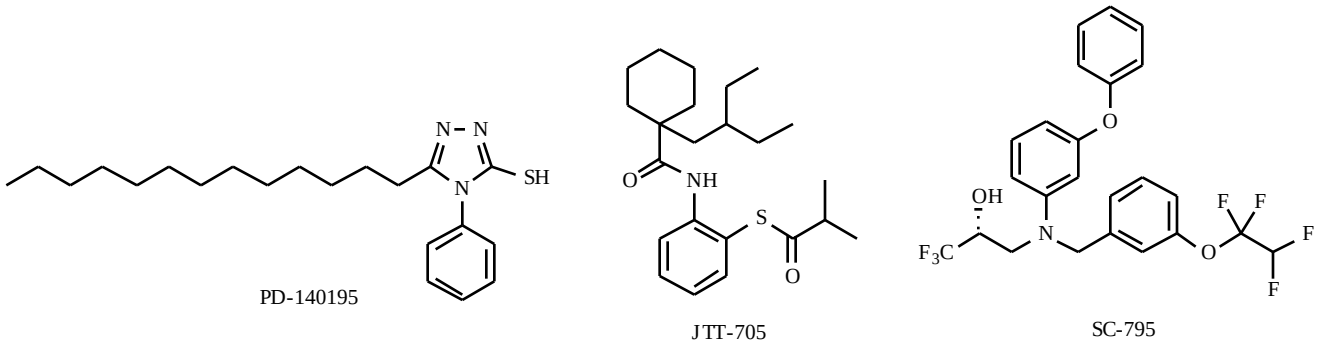

Fig. (3). Structures of CETP inhibitors.

Although total serum cholesterol levels were not changed, HDL cholesterol showed a relative increase. LG268 acts as a retinoid X receptor (RXR) agonist that activates both RXR/farnesoid X receptor (FXR) and RXR/liver X receptor (LXR) heterodimers. LG268 suppresses the expression of CYP7A1 and CYP7B1 (the two rate-limiting enzymes of bile acid synthesis) by activating the RXR/FXR heterodimer, and increases ABC1 expression by activating the RXR/LXR hetrodimer. A decrease in the bile acid pool is considered to be one mechanism by which rexinoids inhibit cholesterol absorption. Induction of ABC1 expression is considered to inhibit cholesterol absorption by exporting free cholesterol from enterocytes back into the intestinal lumen. ABC1 has been recently identified as a transporter that mediates the active efflux of cholesterol from cells to apoA-I [36], and its activity is considered to be rate limiting for the formation of HDL [14]. In fact, patients with Tangier disease have a defect of ABC1 protein and show low HDL cholesterol and high triglyceride levels in association with severe atherosclerosis [37-39]. Therefore, up-regulation of ABC1 expression may increase HDL cholesterol and promote reverse cholesterol transport [40]. Not only an RXR agonist, but also an LXR agonist (T0901317), has been reported to stimulate ABC1 production by specifically increasing the activity of RXR-LXR dimers [41, 42] (Fig. (**5**)). Moreover, exposure to both 20(S)-hydroxycholesterol (LXR ligand) and 9-cis retinoic acid (RXR ligand) achieves synergistic induction of ABC1 transcription and up-regulation of apoA-I mediated cholesterol efflux in cultured RAW cells [14]. Thus, regulation of ABC1 expression by RXR/LXR may be an important therapeutic target for prevention of atherogenesis.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors and that form heterodimers with RXR. PPARs have various effects on the metabolism of lipoproteins and fatty aicds. Fibrates reduce triglyceride levels and modestly raise HDL cholesterol by activating the nuclear transcription factor PPARα [43, 44]. In turn, PPARα activates lipoprotein lipase by inhibiting the expression of apolipoprotein C-III, an endogenous lipoprotein lipase inhibitor, and stimulates the synthesis of apoA-I to promote an increase of plasma HDL cholesterol [45-47]. However, fibrates only modestly raise the HDL cholesterol level. A selective PPARδ agonist (GW501516), which binds to RXR, induced a 2-fold increase of cholesterol efflux from cells to apoA-I by increasing the expression of the ABC1 reverse cholesterol transporter (Fig. (**5**)). In cultured human cell lines, such as THP1 (macrophage), 1BR3N (fibroblast), and FHS74 (intestinal),

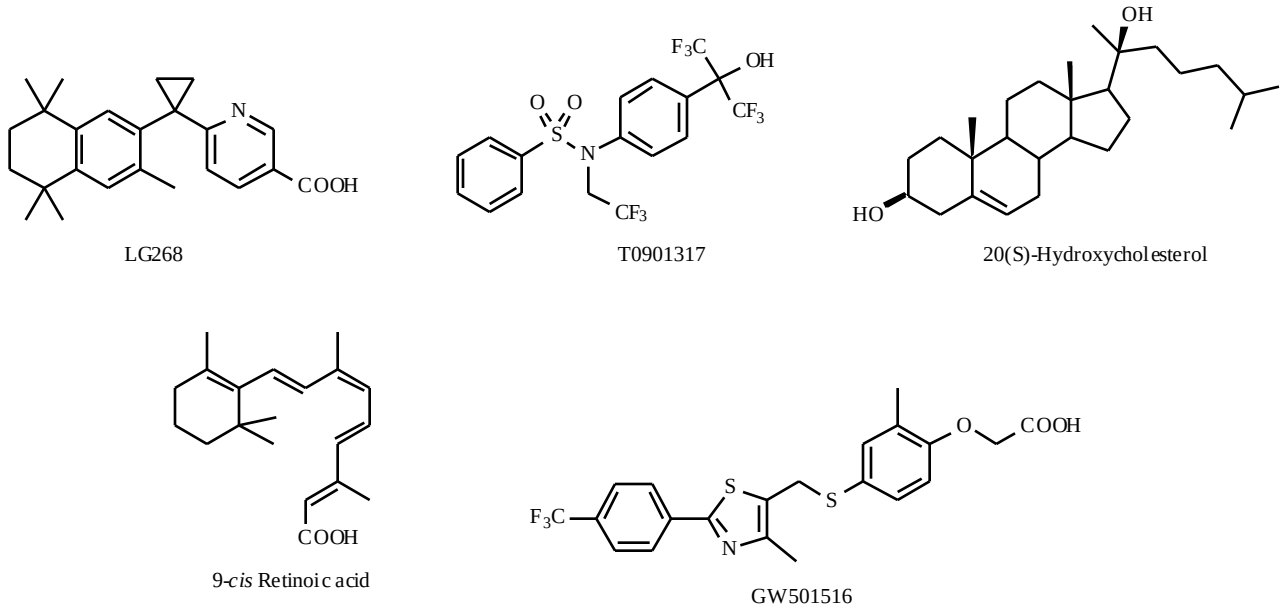

Fig. (4). Structures of orphan nuclear hormone receptor agonists.

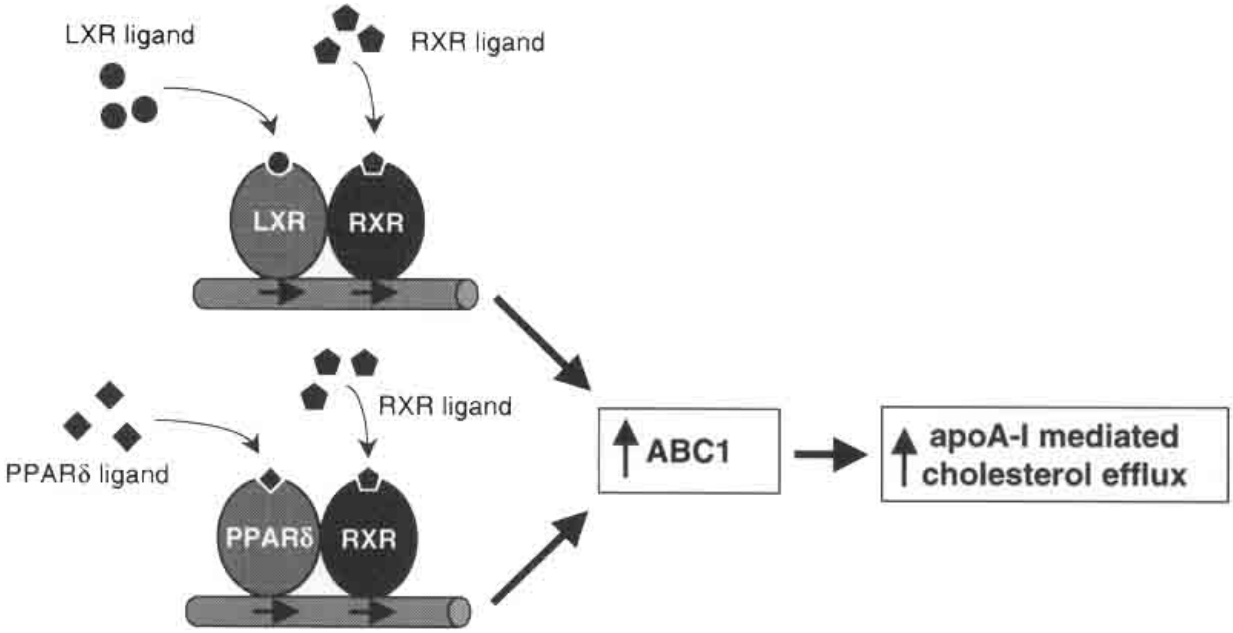

Fig. (5). Activation of ABC1 through activating of LXR-RXR heterodimer or PPARδ-RXR heterodimer.

GW501516 produced a several-fold increase of both ABC1 expression and apoA-I-specific cholesterol efflux. GW501516 also produced a dose-dependent increase of HDL cholesterol and a dose-dependent reduction of fasting triglycerides (by 79% and 56%, respectively, at 3.0 mg/kg) in primates. Thus, PPARδ agonists may represent a new approach to the treatment of cardiovascular disease by promoting reverse cholesterol transport [48]. The effect of fibrates on ABC1 and apo A-I expression may be mediated through PPARδ rather than PPARα, because most fibrates only have a weak influence on human PPARα and show low selectivity for PPARα relative to PPARδ and PPARγ [49].

CONCLUSIONS

HMG-CoA reductase inhibitors show a marked effect on the plasma cholesterol level, and are obviously beneficial for atherosclerosis and coronary heart disease. However, 58% of patients with coronary heart disease do not have an elevated LDL cholesterol levels [50]. So, reduction of cholesterol alone is not sufficient to treat all cases of coronary heart disease. Hypercholesterolaemia is not the only risk factor for the development of atherosclerosis and increasing the HDL cholesterol level may be the next target for the treatment of atherosclerosis and coronary heart disease.

Inhibition of CETP is a potential method of increasing the HDL cholesterol level [51], but it has been unclear whether CETP was pro-atherogenic or anti-atherogenic because its physiological role in reverse cholesterol transport has not been defined. Recently, a small molecule CETP inhibitor (JTT-705) has been shown to reduce atherosclerotic lesions in rabbits. This finding suggests that inhibition of endogenous CETP activity can retard the progression of atherosclerosis and thus confirms the atherogeicity of endogenous CETP. Further development of some of the compounds described in this review or newer compounds may lead to the clinical application of CETP inhibitors to increase HDL cholesterol, thus creating a new class of anti-atherogenic drugs.

Several orphan nuclear hormone receptors regulate cholesterol homeostasis by modulating the expression of various genes. In particular, RXR/LXR and RXR/PPARδ heterodimers up-regulate ABC1 expression. Thus, RXR, LXR, and PPARδ agonists increase the HDL level and increase apoA-I mediated reverse cholesterol transport from peripheral cells to the liver by elevating ABC1 expression. These orphan nuclear hormone receptor agonists may provide a new approach to the treatment of cardiovascular disease by promoting HDL-mediated reverse cholesterol transport.

ABBREVIATIONS

HDL	=	High-density lipoprotein
CETP	=	Cholesteryl ester transfer protein
ABC1	=	ATP-binding cassette transporter 1
PPAR	=	Peroxisome proliferatro-activated receptor
RXR	=	Retinoid X receptor
LXR	=	Liver X receptor
FXR	=	Farnesoid X receptor
LDL	=	Low-density lipoprotein
HMG-CoA	=	3-Hydroxy-3-methylglutaryl co-enzyme A

LCAT = Lecithyl cholesterol acyl tranferase

SR-B1 = Hepatic HDL scavenger receptor

TG = Triglyceride

VLDL = Very low-density lipoprotein

CE = Holesteryl ester

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