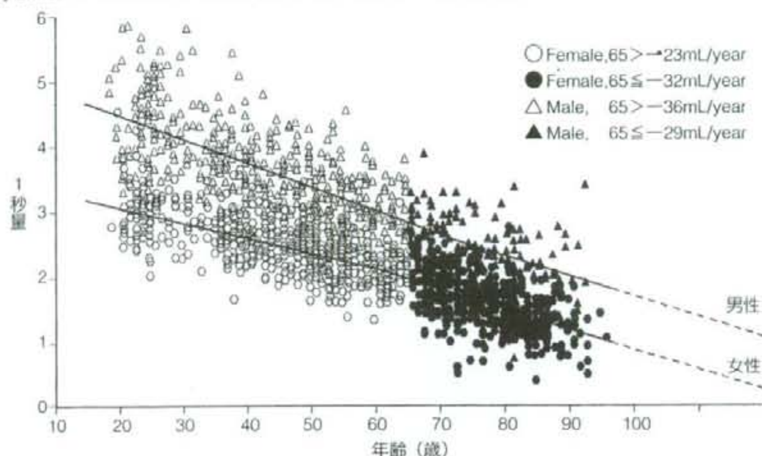
図1　加齢による呼吸機能低下

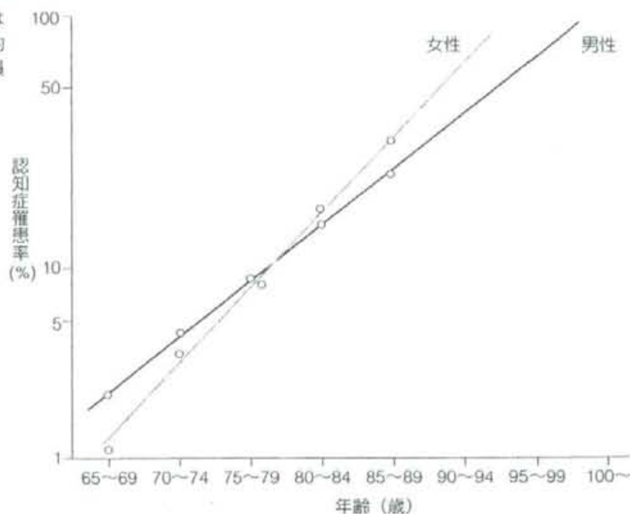
1秒量の横断的調査では，男女とも1秒量は加齢とともに直線的に低下し，女性では約100歳で1Lを切り，呼吸機能低下により死にいたると考えられる。閾値は非喫煙健常者。



(Nakamura M, Matsui T, Ohrui T, Kida K, Yamaya M, Sasaki H: Gender crossover of lung function. Geriatr Gerontol Int, 2: 127-130, 2002.)

図2　加齢と認知症罹患率

日本人の認知症罹患率は加齢とともに指数関数的に増え90〜100歳で全員が認知症になる。



(Ohrui T, Yamaya M, Arai H, Sasaki H: Review: Care for the older people. Intern Med, 42: 932-940, 2003.)

Annals of Medicine. 2008; 40: 29–38

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REVIEW ARTICLE

HDL biogenesis and cellular cholesterol homeostasis

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Abstract
Mammalian somatic cells do not catabolize cholesterol and therefore must export it to maintain sterol homeostasis at the levels of cells and whole body. This mechanism may reduce intracellular cholesterol accumulated in excess, and thereby contribute to prevention or cure of atherosclerotic vascular lesions. High-density lipoprotein (HDL) plays a central role in this reaction by removing cholesterol from cells and transporting it to the liver, the major cholesterol catabolic site to bile acids. Two independent mechanisms are identified for the cellular cholesterol release. One is non-specific diffusion-mediated 'efflux' of cell cholesterol that is trapped by various extracellular acceptors including lipoproteins. Cholesterol acyl esterification on HDL provides a driving force for net outflow of cell cholesterol in this pathway, and some cellular factors may also enhance this reaction. The other is apolipoprotein-mediated process to generate new HDL particles by removing cellular phospholipid and cholesterol. This reaction is mediated with a membrane protein, ATP binding cassette transporter (ABC) A1, and helical apolipoproteins recruit cellular phospholipid and cholesterol to assemble HDL particles. The reaction is composed of two elements: assembly of HDL particles with phospholipid by apolipoprotein, and cholesterol enrichment in this HDL. ABCA1 is essential for the former step, and apolipoproteins are dissociated from HDL or secreted from cells and interact with ABCA1 in their free form. The latter step requires other cellular factors, such that ABCA1 mediates production of cholesterol-rich and cholesterol-poor HDL while ABCA7 produces only cholesterol-poor HDL.

Key words: *ABC, apolipoprotein, caveolin, cholesterol efflux, HDL, membrane*

Introduction

Cholesterol constitutes a membrane domain 'raft' by forming a cluster with sphingolipid to provide an microenvironment for accumulation of specific membrane proteins related to intracellular signal transduction, and therefore plays essential key roles in the biological functions of the cell membrane especially for intercellular communication. Biosynthesis of cholesterol is therefore carried out in all the somatic cells in most animals requiring a complicated 37 steps in order to maintain such cellular functions. In contrast, catabolism of cholesterol is very limited in peripheral cells of vertebrates, and most of cholesterol molecules in the body are transported to the major organ for its catabolism, the liver, except for a small but important part in steroidogenic cells. In the liver, cholesterol is converted to bile acids that are heavily reused in an entero-hepatic circulation. It should be noted that cholesterol is never converted to energy. Bile acids still contain a sterol backbone, and it is biodegraded by bacteria mostly after excretion. Thus, we recognize it as an important and valuable molecule that should not be wasted at all. We are well prepared for crisis management of cholesterol shortage, but very poorly for its overload.

The regulation of cholesterol biosynthesis and receptor-mediated lipoprotein uptake have been extensively characterized for a long time (1), and the regulatory mechanism of cholesterol biosynthesis has been well established at the molecular levels such as sterol regulatory element binding protein system (2,3). On the other hand, release of cholesterol from somatic cells is equally important for cholesterol homeostasis both for cells and whole body, but understanding of this part has been substantially behind. However, knowledge has rapidly accumulated in this field in the last several years, and significant progress has been made for understanding the mechanism for cellular cholesterol release.

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Abbreviations

LDL	low-density lipoprotein
HDL	high-density lipoprotein
LCAT	lecithin: cholesterol acyltransferase
CETP	cholesteryl ester transfer protein
ACAT	acylCoA cholesterol acyltransferase
PLTP	phospholipid transfer protein
ABC	ATP-binding cassette transporter
HPLC	high-performance liquid chromatography

Key messages

- Cholesterol in extrahepatic cells, except for steroidogenic cells, must be released and transported to the liver for its conversion to bile acids mainly mediated by high-density lipoprotein (HDL), as its major catabolic pathway both for cellular and whole body levels.
- Cell cholesterol release is mediated by two independent mechanisms: a physicochemical diffusion-mediated pathway in which one of the driving forces for the net release is lecithin: cholesterol acyltransferase (LCAT) reaction on HDL, and an HDL biogenesis by the interaction of helical apolipoprotein and cellular lipid mediated by ATP-binding cassette transporter (ABC) A1.
- Helical apolipoprotein, represented by apoA-I, must be in a free form to interact with ABCA1-expressing cells to generate HDL, and it either dissociates from HDL or is secreted as a free form before the interaction for HDL biogenesis.
- Cholesterol enrichment of HDL in the ABCA1-mediated HDL biogenesis is independent of assembly of HDL particles with cellular phospholipid, and cholesterol-rich and cholesterol-poor HDL are generated by apoA-I in the presence of transfected-and-expressed ABCA1 and ABCA7, respectively.

Release of cellular cholesterol and its transport to the liver are both mediated by high-density lipoprotein (HDL). This pathway is under kinetic control and in a steady state with assembly and clearance of plasma lipoproteins and with extracellular cholesterol metabolism by lecithin: cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and other active molecules (4). However, the most critical step for this pathway is the release of cholesterol from the cells, and it is also one of the key components of cellular cholesterol homeostasis. This pathway is often referred to as the concept of 'reverse cholesterol transport' and an anti-atherosclerosis nature of HDL, based on the two lines of evidence that plasma HDL level is negatively correlated to the risk of atherosclerotic vascular disease (5) and that incubation of the cells with HDL results in reduction of cellular cholesterol *in vitro* (6). Two major mechanisms are proposed for the cellular cholesterol release step (7–9): non-specific diffusion-mediated cell cholesterol 'efflux', and apolipoprotein/ATP-binding cassette transporter (ABC) A1-mediated biogenesis of HDL particles from cellular lipids.

Non-specific release of cell cholesterol

Non-specific cholesterol efflux from the cellular surface by physicochemical cholesterol exchange between the cell membrane and extracellular 'acceptors' is perhaps mediated by its diffusion in an aqueous phase. Net release of cellular cholesterol is driven by extracellular acyl-esterification of cholesterol by LCAT in this pathway. This concept was first proposed by Glomest in 1968 (10) as HDL is a major cholesterol acceptor in this reaction because of its capacity for cholesterol accommodation and because it provides a major and optimum site for the LCAT reaction. This is under kinetic control and the net release of cell cholesterol is in fact demonstrated only when outflow diffusion of cell cholesterol is not a rate-limiting factor (11,12) (Figure 1). Scavenger receptor B1 seems to expedite cholesterol exchange rate between cell membrane and HDL, perhaps through a specific mode of binding to HDL (13–16). ABCG1/ABCG4 alters intracellular cholesterol distribution to the direction to increase its release by this pathway (17).

Apolipoprotein-mediated HDL assembly

The other important mechanism is an assembly of new HDL particles with cellular phospholipid and cholesterol upon the direct interaction of helical apolipoproteins of HDL with cells. Many specific cellular functions are required for this reaction, including a cellular interaction site for apolipoprotein and specific intracellular cholesterol trafficking for the HDL assembly. This reaction seems to be a major source of plasma HDL, and ABCA1 is a key cellular factor.

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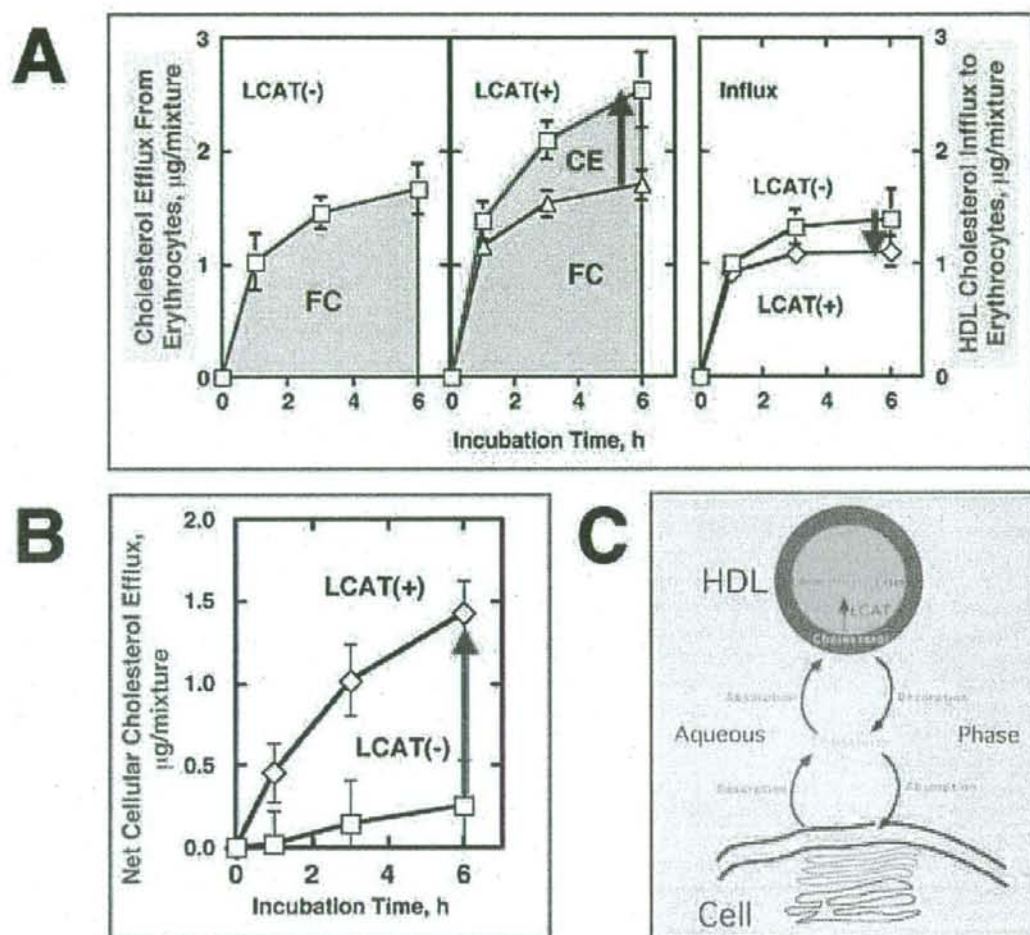

Figure 1. LCAT-mediated net cholesterol release from erythrocytes (12). Pig erythrocytes that lack apolipoprotein-mediated cell cholesterol release were used for increasing the cellular cholesterol pool in order to provide a high off-diffusion rate of cellular cholesterol and to make LCAT reaction a rate-limiting factor for net cholesterol efflux. Panel A shows cell cholesterol efflux to HDL in the medium in the absence and presence of LCAT measured by pre-labelling cell cholesterol. Cholesterol esterification by LCAT results in just as much increase of cell cholesterol efflux (an upward arrow). In contrast, influx of HDL cholesterol into erythrocytes measured by pre-labelling HDL cholesterol. Cholesterol influx was reduced in the presence of cholesterol esterification on HDL (a downward arrow). FC, free cholesterol; CE, cholesteryl ester. Panel B shows the net cholesterol efflux calculated from the results in the panel A. There is no net flux between erythrocytes and HDL without LCAT, and LCAT generates the net outflow of cell cholesterol to HDL (an upward arrow). Overall results indicated that acyl esterification of cholesterol on HDL is the driving force for its net release from cells by its diffusion between HDL and cell surface (Panel C).

The first finding of HDL assembly by cellular lipid and extracellular helical apolipoproteins was our observation that apolipoproteins of HDL, such as apoA-I, A-II, and E, remove phospholipid and cholesterol from mouse peritoneal macrophages and generate new HDL particles (18) (Figure 2). The lipoprotein thus generated meets the criteria of preβ-HDL with respect to physical and chemical properties (18) (Figure 2AB), morphological appearance (19,20), and biochemical characteristics such as reactivity to LCAT (11,21) (Figure 2C). Cholesterol in the cells reciprocally decreased mainly in the compartment accumulated as cholesteryl ester (18). The reaction can be carried out by various helical apolipoproteins having amphiphilic helices composed of some 20–22 amino acid residues, so that apoA-I, A-II, A-IV, E, and insect apoIII all generate HDL (18,22,23), and so do synthetic

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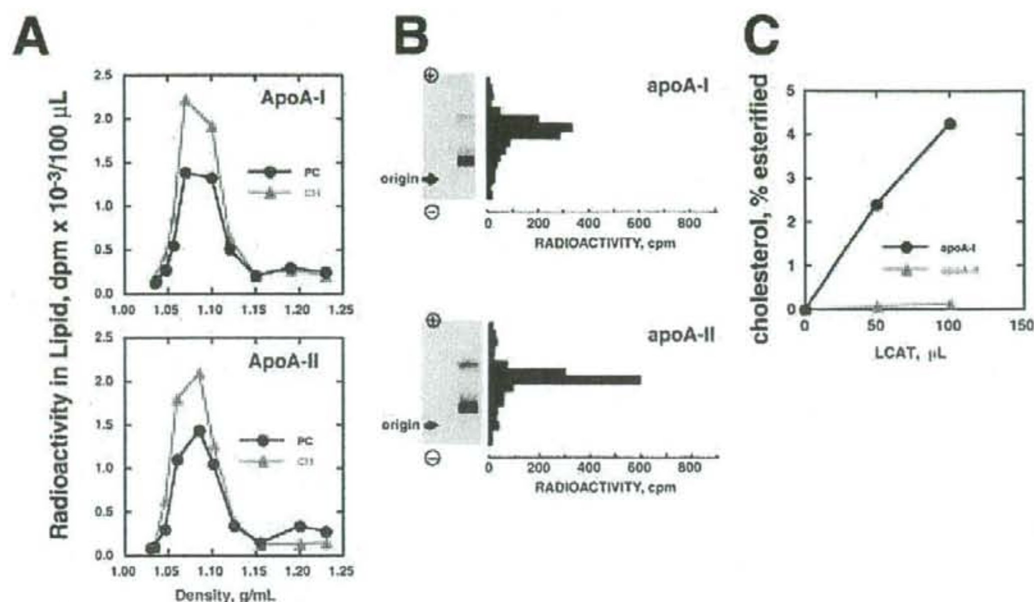

Figure 2. HDL biogenesis by apolipoproteins and cellular lipid. Panels A and B show the results of incubation of mouse peritoneal macrophages with apoA-I or apoA-II. The medium was analysed by ultracentrifugation (Panel A) and agarose gel electrophoresis (Panel B, bands of fast and slow mobility in each gel indicate HDL and LDL, respectively) (18). Panel C demonstrates the reactivity to LCAT (activity was standardized for plasma LCAT activity) of the HDL generated by human fibroblasts and apoA-I or apoA-II (11).

amphiphilic peptides as far as they meet such criteria (23,24). More recently the peptides were shown to be active whether composed of D- or L-amino acids (25). It seems that certain numbers of the helical segment are required to carry out the reaction.

The physiological relevance of this reaction became evident by the finding that the cells from patients with Tangier disease, familiar HDL deficiency, lack the interaction with apolipoprotein and the HDL assembly (26,27). Mutations were identified in the gene of ATP-binding cassette transporter A1 (ABCA1) in patients with this disease (28–33), and disruption of this gene resulted in the HDL deficiency in mice. Thus, ABCA1 was shown to be essential for production of plasma HDL (34,35). While apolipoproteins do not interact with the Tangier cells and generate no HDL (26,27), the cells are intact for the non-specific diffusion-based cholesterol release (26). This means that ABCA1 may act as or create a direct interaction site for apolipoproteins to generate HDL. To support this idea, induction of the HDL assembly reaction in RAW264 cells by cAMP is accompanied by induction of apoA-I binding and expression of ABCA1 (36,37). Thus, ABCA1 essentially functions as a mediator for apolipoprotein-cell binding and for subsequent assembly of nascent HDL particles from apolipoprotein and cellular phospholipid/cholesterol.

Helical apolipoproteins are in equilibrium between a lipid-bound form and a dissociated form from the lipid surface presumably free in solution. Although the dissociation constants of apolipoproteins are not known directly for the HDL surface, the constants measured for the LDL-size lipid particles are all in the order of 10^{-7} M, which may not be irrelevant to be extrapolated for the HDL surface (38,39). Assuming that the dissociation constant of apoA-I is in this range, and binding capacity of HDL is just enough to accommodate the total plasma apoA-I, a few percent of plasma apoA-I can be lipid-free in the aqueous phase in equilibrium. It should be noted that the K_m for the HDL assembly reaction is less than 1% of plasma apoA-I concentration (18) so that this protein in a free form can carry out the reaction at the V_{max}. Also, there are several reactions that reportedly liberate helical apolipoproteins from the HDL surface in plasma, such as CETP in the presence of free fatty acids (40–42). Phospholipid transfer protein (PLTP) (43) by itself also releases apolipoprotein from HDL, and transfer of cellular phospholipid and cholesterol to HDL was indeed enhanced by PLTP (44).

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Apolipoproteins can be transferred from HDL to the cell surface simply due to the higher affinity of free apolipoproteins for the cells than for lipid surface (45).

We investigated the ABCA1-dependent interaction of HDL particles with cells (46) (Figure 3). ABCA1 mediates the interaction only of the protein moiety of HDL but not its lipid (Figure 3AB). It was also shown that a monoclonal antibody specific for lipid-free apoA-I selectively inhibited the ABCA1-dependent part of cell cholesterol release to HDL (46) (Figure 3CD). These findings were magnified when apoA-I was displaced by apoA-II to increase lipid-free apoA-I. In that paper, kinetic analysis of the data indicated that apoA-I has an affinity for HDL as high as that for cellular surface, and apoA-I could still be transferred from HDL to cell surface. It is thus not irrelevant to speculate that apolipoproteins dissociate from HDL and interact with the cells in their lipid-free form to generate new HDL particles.

Major sites for synthesis of helical apolipoproteins, especially for the main apolipoprotein of HDL, apoA-I, are believed to be the liver and intestine. In contrast to apoB-containing lipoproteins, however, no HDL particles, not even premature HDL, have been identified in the secretory pathways such as the endoplasmic reticulum and the Golgi apparatus in the cells of these organs. Nevertheless, HDL particles are found in the culture media of the hepatocytes (47,48) or in the perfusate of the liver (49,50), mostly as a so-called nascent HDL that is composed mostly of surface lipids, phospholipid, and cholesterol, not containing much core lipid, and

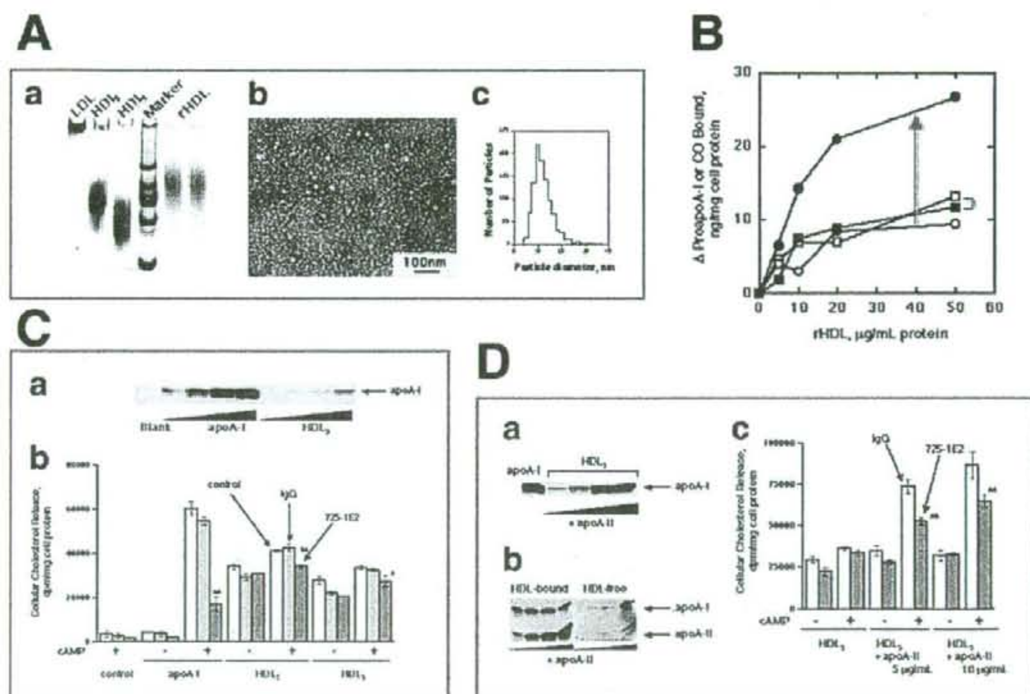

Figure 3. Binding of HDL components to RAW264 cells when ABCA1 expression is induced by cAMP (46). Panel A shows reconstituted HDL of apoA-I (proapoA-I), cholesteryl oleate and egg phospholipid. Panel B shows the results of binding of the particles labelled with uniformly labelled proapoA-I with ^{3}H and ^{14}C-cholesteryl oleate. An upward red arrow between open and closed circles indicates the increase of proapoA-I binding by induction of ABCA1 expression by cAMP. Red lines between open and closed squares indicate change of cholesteryl oleate (CO) binding by inducing ABCA1 expression. Binding takes places only with protein of HDL. Panels C and D demonstrate inhibition of the ABCA1/apoA-I-mediated cholesterol release by the monoclonal antibody specific for lipid-free apoA-I, 725-1E2. Panel C-a shows specificity of the antibody against lipid-free apoA-I, and Panel C-b shows inhibition by the antibody of the apoA-I- and HDL-mediated cell cholesterol release induced by cAMP. ApoA-I-mediated cholesterol release was inhibited by 75% of the cAMP-induced increment, and the increment of the HDL-mediated cholesterol release by cAMP was inhibited to the same extent as the apoA-I-mediated release was inhibited. Panel C shows the results of the similar experiments performed in the presence of apoA-II. ApoA-II displaces apoA-I from the HDL surface to make it a free form (Panel D-a and D-b), and therefore the increment of cell cholesterol release was larger in this condition (Panel D-c). The antibody inhibited so much as the apoA-I-mediated cholesterol release (Panel D-c).

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consequently in a disc-like shape. The question then becomes how and where these particles are formed. If the apolipoprotein-cell interaction is a major mechanism for production of HDL, it is possible that HDL is assembled by an autocrine mechanism, such that apoA-I or E are first secreted by the cells and then interact with the cell surface to generate HDL (51,52). This hypothesis has more directly been supported by using an ABCA1 inhibitor, probucol, and the above-mentioned antibody specific to lipid-free apoA-I to inhibit ABCA1-dependent HDL assembly by hepatocytes (53). When HepG2 cells were treated with probucol, apoA-I otherwise found associated with HDL was secreted all in a free form (Figure 4A). In the presence of the antibody, generation of HDL was completely inhibited (Figure 4BC) while it did not influence the pre-produced HDL in the medium.

Thus, lipid-free apolipoprotein is to be released whether from cells or from HDL particles to interact with cellular ABCA1 for assembly of HDL particles from cellular lipid. Alternatively, apolipoproteins may interact in part with the membrane already somewhere before the secretion through the same mechanism as extracellular apolipoprotein reacts (54,55). This view may be consistent with the finding of the abnormal Golgi structure in the hepatocytes of ABCA1 knock-out mice (34) and differential generation of HDL with endogenous apoE and exogenous apoA-I by rat astrocytes (56).

Assembly of HDL particles and cholesterol enrichment

Apolipoprotein recruits primarily phospholipid rather than cholesterol to form stable HDL particles in this

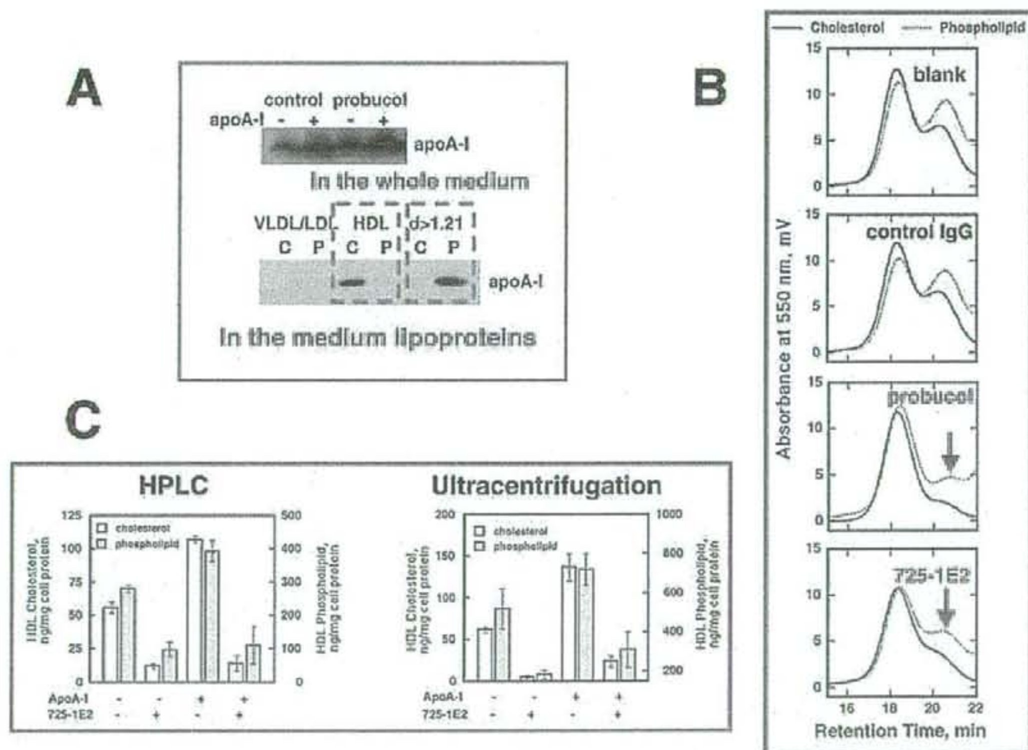

Figure 4. Biogenesis of HDL by HepG2 cells with endogenous apoA-I (53). Panel A shows the results of apoA-I secretion when HepG2 cells were treated with an ABCA1 inhibitor, probucol (P), in comparison to control (C). Secretion of apoA-I into the medium is unchanged by the treatment of the cells with probucol, but apoA-I was recovered all in a lipid-free form by the treatment while it was otherwise all bound to HDL. Panel B shows marked decrease of HDL production by HepG2 cells when ABCA1 was inactivated by probucol or the monoclonal antibody specific to lipid-free apoA-I, 725-1E2, was present in the medium, demonstrated as HPLC profiles of the media. Solid lines indicate cholesterol, and dotted lines indicate choline-phospholipid. Panel C demonstrates the same results shown as quantitative data by using the HPLC analysis data and the ultracentrifugation analysis data.

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HDL assembly pathway (57). HDL generated by this reaction contains largely phospholipid and unesterified cholesterol, and the LCAT-mediated cholesterol esterification on the generated HDL perhaps helps the maturation of this HDL as it generates core cholesteryl ester (11,21). However, unlike cholesterol release by non-specific diffusion-mediated reaction, cholesterol esterification does not result in further enhancement of cellular cholesterol release when the HDL generated is already cholesterol-rich (11).

HDL-like particles can be formed *in vitro* with helical apolipoproteins and phospholipid, with or without core lipid and cholesterol, without specific catalysts except for the requirement of energy for dispersion of the components to homogeneity (58). The reaction always yields the particles of certain sizes composed of at least a few hundreds of phospholipid molecules. Therefore, HDL-like particles are a thermodynamically stable molecular assembly for helical apolipoproteins and phospholipid. The physicochemical nature of apolipoprotein-phospholipid interaction is that 'lipidation' of apolipoprotein takes place primarily with phospholipid in a kind of snap-in manner rather than 'gradual growth'. On the other hand, apolipoprotein cannot form a complex with cholesterol alone. When apolipoprotein interacts with cells through ABCA1, the same type of reaction should take place to generate HDL. In fact, disc-like HDL particles are generated primarily with membrane phospholipid when apoA-I interacts with the cells in the presence of ABCA1 (Figure 5A). However, it has not yet been evident whether premature HDL particles found in plasma are produced by this reaction and are direct precursors of plasma HDL, such as preβ-HDL, γ-LpE, and LpA-IV (59). It should be noted that Miller and colleagues suggested that preβ-HDL in human peripheral tissue fluid should be considered substantially produced locally rather than filtered from blood plasma (60). This finding may support the view that at least apoA-I locally dissociates from HDL and produces new preβ-HDL by removing lipid from peripheral cells.

It was recently reported that ABCA7 also mediates the HDL assembly *in vitro* in a similar manner to ABCA1 when transfected and over-expressed in HEK293 cells (61–63). Analysis of the HDL products by size exclusion high-performance liquid chromatography (HPLC) revealed that ABCA1 generates two different types of HDL, large cholesterol-rich and small cholesterol-poor, while ABCA7 produces only small and cholesterol-poor HDL (Figure 5B) (63,64). Although this reaction may not significantly contribute to the regulation of plasma HDL concentration (65) and the expression of the ABCA7 gene is not regulated for the HDL biogenesis (66), it is still of interest to examine the difference between the two ABC proteins in order to elucidate the mechanism for ABCA1 to remove cellular cholesterol more efficiently in the HDL biogenesis.

Closing remarks

The finding of the mutation in ABCA1 opened a new gate for studying cellular cholesterol homeostasis with respect to its releasing mechanism. This

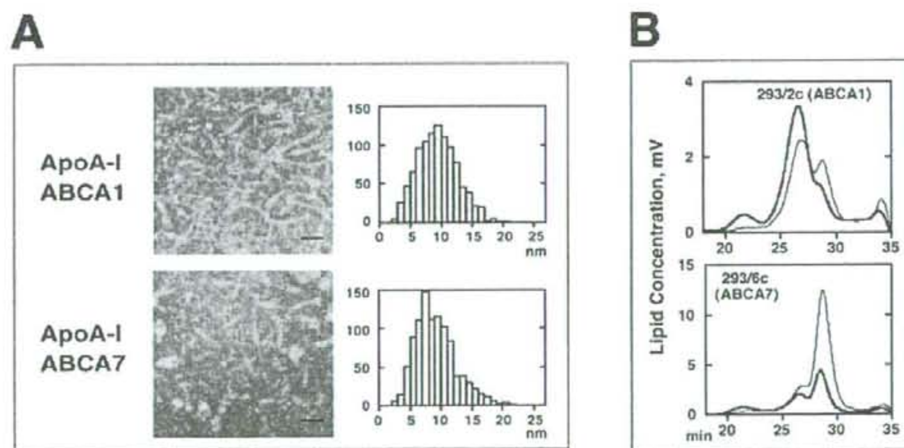

Figure 5. HDL particles generated by apoA-I when ABCA1 or ABCA7 are transfected and over-expressed in HEK293 cells (64). Panel A shows electron microgram of the particles isolated from the medium by ultracentrifugation. Scale bars indicate 100 nm. Histograms represent the results of the measurement of diameters. Panel B shows the results of the HPLC analysis. Thick solid lines represent cholesterol, and thin solid lines represent choline-phospholipid.

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protein undoubtedly plays an essential role in apolipoprotein-mediated assembly of HDL. It is, however, still unclear how ABCA1 functions to mediate the interaction of helical apolipoprotein with phospholipid in the cell membrane. In order to maintain cholesterol homeostasis, diffusion-mediated physicochemical cholesterol release functions as much as the apolipoprotein-mediated pathway both at the cellular level and for the whole body. Therefore, Tangier patients may not develop general and massive cholesterol accumulation since the diffusion-mediated system is preserved (67). This is the same in LCAT deficiency patients who lack a driving force for the net cholesterol release by the diffusion-mediated system but not the apolipoprotein-mediated reaction (68). Thus, the two systems back up each other to maintain cellular and body cholesterol homeostasis (69).

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Effects of Fibrate Drugs on Expression of ABCA1 and HDL Biogenesis in Hepatocytes

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Abstract: Fibric acid-shaped drugs raise high-density lipoprotein (HDL) cholesterol by upregulating the HDL-related genes through activating peroxisome proliferater activated receptor (PPAR)-α. We investigated the effects of fibrates to induce expression of adenosine triphosphate–binding cassette transporter A1 (ABCA1) and increase HDL biogenesis in hepatocytes. Fenofibrate, bezafibrate, gemfibrozil, and LY518674 were tested for HepG2 cells and primary-cultured mouse hepatocytes. All the compounds examined increased ABCA1 expression and HDL biogenesis dependent on PPARα in association with the liver X receptor α upregulation. While fenofibrate and LY518674 showed exclusive dependency on PPARα for these activities, bezafibrate and gemfibrozil exhibited dependency on PPARβ/δ and PPARγ as well. On the other hand, cholesterol-enrichment of HDL may involve PPARγ for fenofibrate and bezafibrate, and PPARβ/δ for the fibrates examined except for bezafibrate. We concluded that fibrates enhance expression of ABCA1 in hepatocytes to contribute to increase of the HDL biogenesis in a PPAR-dependent manner, whether exclusively or nonexclusively on PPARα.

Key Words: fibrates, PPAR, LXR, ABCA1, HDL, hepatocytes, cholesterol

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High-density lipoprotein (HDL) plays a central role in cholesterol transport from extrahepatic tissues to the liver for its conversion to bile acids, so that HDL is thought to be antiatherogenic by removing cholesterol from atheromatous vascular lesions.[1,2] The liver also provides the major source for HDL biogenesis mediated by adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1),[3–5] mainly as an autocrine reaction with endogenously synthesized apolipoprotein (apo) A-I.[6,7] Pharmacological increases of HDL is expected to be beneficial for prevention of atherosclerotic diseases predicted in animal models[8–12] and action of drugs on hepatic HDL biogenesis should be evaluated.

Fibrates-based compounds are widely used drugs that reduce plasma triglyceride (TG) level through activation of a nuclear receptor, peroxisome proliferator activated receptor (PPAR)-α.[13] These drugs are also known for raising HDL by two independent mechanisms.[14] Increase of TG-rich lipoprotein increases TG transfer to HDL in exchange for cholesteryl ester transfer from HDL by plasma cholesteryl ester transfer protein, leading to production of small cholesterol-poor HDL as TG in HDL is being hydrolyzed.[15] Reduction of TG by fibrates therefore increases HDL cholesterol by reversing this process. In addition, fibrates were also shown to increase transcription of the genes for HDL biogenesis; apoA-I gene in the liver,[16] and ABCA1 gene by at least Wy14643[17] and fenofibrate[18] through activation of the liver X receptor (LXR) in fibroblasts and/or macrophages.

PPARs are ligand-activated transcription factors that form an obligate heterodimer with the retinoid X receptor (RXR) to bind to defined PPAR regulatory elements in the promoter region of target genes.[19] PPARα was first identified in mice,[20] with PPARβ (currently termed more commonly as PPARβ/δ)[21] and PPARγ reported in frogs and mice.[22] PPARs are found in all mammalian species[23] and each subclass has unique tissue specificity and functions. PPARα is highly expressed in the liver and regulates peroxisomal and mitochondrial β-oxidation of fatty acids.[24] PPARγ is expressed in white and brown adipose tissues, the liver, kidney, and heart, and controls adipocyte differentiation, fat storage, and inflammation.[25] PPARβ/δ is ubiquitously expressed and serves as a key regulator of fat metabolism in peripheral tissues by coordinating fatty acid oxidation and energy uncoupling.[26]

Many studies suggested that fibrates exert their effects through a PPARα-mediated pathway.[13,27] However, some fibrates are known to activate other PPARs; bezafibrate can also be a ligand for PPARβ/δ and PPARγ.[28,29] Detail of PPAR subtype specificity still remains uncertain for the clinically used fibrates. It is also important to clarify whether their act in the liver, the major HDL-producing organ, to regulate the HDL-related genes. We thereby undertook examination of the effects on the HDL biogenesis in hepatocytes, HepG2 cells, and mouse primary-cultured hepatocytes of the clinically used or to-be-used fibrates; fenofibric acid (an active form of fenofibrate), bezafibrate, gemfibrozil, and LY518674.[30,31] All these drugs stimulated the ABCA1 gene, perhaps in an LXRα-dependent manner in these cells. However, some fibrates were also shown to act through PPARs other than the α-subtype.

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MATERIALS AND METHODS

Experimental Animals and Cell Culture

Hepatocytes were isolated according to the ethylenediamine tetraacetic acid collagenase two-steps perfusion method as previously described[6] from the liver of the C57BL/6 mice as well as of the *Abca1*-null mice obtained as ABCA1-deficient heterozygotes (DBA/1-*Abca1*$^{tm1Jdm/J}$) from Jackson Animal Laboratories (Stony Brook, NY) and bred for 8 generations to have the C57BL/6 genetic background, and of the PPARα null mice that had the same genetic background[32] as bred for 10 generations. Their genotypes were confirmed as described.[32,33] Heaptocytes were prepared from the animals at the age of 20 to 24 weeks fed with essential chaw diet (MEQ, Oriental Yeast Co. Ltd., Tokyo) and used in primary culture at a concentration of 0.2×10^6 cells/mL in collagen-coated plate in the presence of Dulbecco's Modified Eagle Medium containing 4.5 g/L glucose.[6] The experimental procedure was approved by Animal Welfare Committee of Nagoya City University Graduate School of Medical Sciences according to the institutional guidelines (approval number H17-15). HepG2 cells (American Type Culture Collection; ATCC HB8065) were cultured as 0.6×10^6 cells/mL in a modified Eagle's minimum essential medium (MEM; Sigma) containing 10% fetal bovine serum in 60-mm dish in a CO_2 incubator at 37°C.[6] These cells were incubated with fenofibric acid (Tyger Scientific, referred as finofibrate hereafter), bezafibrate (Sigma), gemfibrozil (Sigma), or LY518674 (synthesized in house)[31] in MEM-containing 0.02% (w/v) bovine serum albumin (BSA) for 16 to 18 hrs. Culture medium was used for lipoprotein analysis and the cells were used for mRNA analysis and cell proteins measurement.

Lipid and Lipoprotein Analysis

The culture media were collected at the designated time and analyzed for lipoproteins.[6] HDL and low density lipoprotein/very low density lipoprotein (LDL/VLDL) fractions were isolated as density fractions as 1.063 to 1.21 g/mL and below 1.063 g/mL, respectively, by ultracentrifugation in a himac CP80β by using a P50AT4 rotor at 49 k rpm for 16 hrs at 4°C. Lipid was extracted from each lipoprotein fraction with four volumes of chloroform: methanol (2:1, v/v) overnight and the organic layer was used for the determination of free cholesterol and choline-phospholipid by enzymatic assay systems (Kyowa Medics, Japan).[6]

Western Blotting Analysis of Protein

ABCA1 in the cellular membrane fraction was analyzed by Western blotting as previously described.[6] Briefly, cells were treated with a hypotonic 50 mM Tris-HCl buffer (pH 7.4) containing protease inhibitors. After removing cell debris and nuclei by centrifugation at 800 rpm, the supernatant was ultracentrifuged at 90,000 rpm for 1 h to recover the bulk membrane fraction as a pellet. The bulk membrane fraction, 60 μg protein, was dissolved in 9 M urea, 2% Triton X-100, and 1% dithiothreitol and analyzed by 6% polyacrylamide electrophoresis for Western blotting by using specific antibodies against ABCA1, Bip/GRP78, Integrin β1. The conditioned medium (10 μL/lane) was similarly analyzed in 12% SDS-PAGE for apoA-I, apoA-II, apoE, and albumin by using respective specific antibody.

RNA Extraction and Real-Time Quantitative Polymerase Chain Reaction (PCR)

Total RNA was extracted from cells by using RNA extraction reagent (Isogen, Nippon Gene). Single strand cDNA was synthesized by a SuperScript preamplification system (Invitrogen) from 5 μg of the total RNA. PCR was carried out for the cDNA by using primers (sense and antisense) of human ABCA1 (5′-GAA CTG GCT GTG TTC CAT GAT-3′ and 5′-GAT GAG CCA GAC TTC TGT TGC-3′), human apoAI (5′-AGA GAC TGC GAG AAG GAG GTG-3′ and 5′-CAG ATC CTT GCT CAT CTC CTG-3′), mouse ABCA1 (5′-CTC AGA GGT GGC TCT GAT GAC-3′ and 5′-CCC ATA CAG CAA GAG CAG AAG-3′), mouse apoAI (5′-ACG TAT GGC AGC AAG ATG AAC-3′ and 5′-AGA GCT CCA CAT CCT CTT TCC-3′), human LXRα (5′-TCT GGA GAC ATC TCG GAG GTA-3′ and 5′-GGC TCA CCA GTT TCA TTA GCA-3′), human LXRβ (5′-GCG AAG TTA CTT TTG AGG GTA-3′ and 5′-CTC CTT TAC AGT GGG TGA AGA-3′), human RARα (5′-AGC ATC CAG AAG AAC ATG GTG-3′ and 5′-AAT GAT GCA CTT GGT GGA GAG-3′), human RXRα (5′-CTA CTG CAA GCACAAGTA CCC-3′ and 5′-CTG GGC CAC AGA CAA GTA GTA-3′), human PPARα (5′-TCG GTG ACT TAT CCT GTG GTC-3′ and 5′-TTC TCA GAT CTT GGC ATT CGT-3′), human PPARδ (5′-TCT CTC TTC CCT TCT CCC TTG-3′ and 5′-GGC TCA AGT CTT TTG CTC TGA-3′), human PPARγ (5′-ATG GAG CCC AAG TTT GAG TTT-3′ and 5′-AAA CAG CTG TGA GGA CTC AGG-3′) and human β-actin (5′-CTG ACC CTG AAG TAC CCC ATT-3′ and 5′-TCT GCG CAA GTT AGG TTT TGT-3′), and mouse β-actin (5′-ATG GTG GGA ATG GGT CAG AAG -3′ and 5′-CAC GCA GCT CAT TGT AGA AGG-3′) (synthesized by Hokkaido System Science, Japan). Quantification of mRNA for these primers products were accomplished by using SYBR Green PCR master mix reagent in an ABI PRISM 7700 sequence detection system (Applied Biosystems, Japan).

RNA Interference

The expression of PPARα, PPARβ/δ, PPARγ were knocked down by using respective siRNAs. HepG2 cells, at a confluence of 30 % to 50 %, were transfected with designed siRNAs for PPARα (siRNA: UAU CAC UGU CAU CCA GUU CCA GUG C control siRNA: UAU GAC CAG UCU UAC CGA UUC CUG C), PPARδ (siRNA: CUC ACA UGC AUG AAC ACC GUA GUG G, control SiRNA: CUC GAA ACG UAC GUA AAC CCU GUG G), and PPARγ (siRNA: AAA UGU UGG CAG UGG CUC AGG ACU C, control siRNA: AAA GAG UGU UAC GGU GUC GGA CCU C) using oligofectamine (Invitrogen) in presence of OPTI-MEM I (Invitrogen Corporation) by incubating for 6 hrs. The cells were incubated in 1 mL of MEMα medium (Sigma) containing 10% (v/v) fetal bovine serum for 66 hrs, washed with phosphate buffered saline, and treated with 30 μM of each fibrate in the MEMα medium containing 0.02% BSA for 16 to 18 hrs. Lipid levels in the conditioned media were determined and ABCA1 in the bulk membrane fractions analyzed as

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described above. The expression of each gene was evaluated by analyzing specific mRNA by real-time PCR.

Other Methods

Protein content of each sample was determined with a bicinchoninic acid assay reagent (Pierce) using BSA as a standard. The statistical significance was evaluated using 2-tailed Student's *t* test and analysis of variance Scheffe's test.

RESULTS

Hepatic HDL Biogenesis

Figure 1A shows the effect of fibrates on lipoproteins secretion by HepG2 cells and mouse primary hepatocytes. Fenofibrate, bezafibrate, gemfibrozil, and LY518674, all increased HDL release from both types of cells. Release of LDL/VLDL was uninfluenced but their cholesterol content was reduced by fibrates in those from primary hepatocytes. HDL secretion by the *Abca1*-null hepatocytes was very low and unaffected by any of the fibrates. Increase of HDL release was dose-dependent with these fibrates for 5, 15, 20, 30, 45 μM as reaching maximal at 30 μM without showing apparent cytotoxicity in HepG2 cells, so that 30 μM was used hereafter. The fibrates increased mRNA of ABCA1 and apoA-I in both HepG2 cells and primary hepatocytes (Fig. 1B). Figure 1C shows apoB and apoA-I in the VLDL/LDL and HDL fractions in the HepG2 conditioned medium. Each apolipoprotein was found exclusively in the respective lipoprotein fraction, and apoA-I increased by the fibrates treatment. Decrease of apoB was noticed by the treatment. Fibrates increased ABCA1 protein in both types of cells shown by Western blotting, as well as secretion of apoA-I, A-II and E into the medium (Fig. 1D). In summary, the fibrates tested increased HDL biogenesis in hepatocytes accompanied by increase of expression of the HDL-related genes including ABCA1.

Transcription Factors

Since Wy14643 and fenofibrate were shown to increase ABCA1 expression via the LXR system,[17,18] the effects of fibrates were examined in HepG2 cells on LXRα, LXRβ, the retinoic acid receptor α (RARα) and the retinoid X receptor α (RXRα). The LXRα ligands, 22-*R*-hydroxy cholesterol (at 30 μM) and TO901317 (at 10 μM), increased HDL release by 52%, and bezafibrate further increased it by 21%. However, neither an RAR ligand, the all *trans* retinoic acid, nor an RXR ligand, 9-*cis* retinoic acid, induced the increase in HDL production by HepG2 cells. Increase of LXRα mRNA was shown with all the fibrates tested (Fig. 2). In contrast, none of the fibrates induced the increase of LXRβ, RARα, and RXRα mRNAs (Fig. 2).

Role of PPARα

PPAR target genes were examined in HepG2 cells by use of siRNA against PPARα mRNA. Expression of PPARα was suppressed by 68% with 200 nM of siRNA at the end of 72 hours incubation, and this level of suppression was maintained during the period of the HDL secretion measurement for another 16 hrs. Figure 3A shows HDL biogenesis under this condition. The siRNA treatment suppressed the increase of HDL biogenesis by all the fibrates tested indicating that these drugs achieve their effects through a PPARα-dependent pathway. In addition, bezafibrate and gemfibrozil increased HDL biogenesis by about 30%, even in the presence of the PPARα siRNA. Western blotting analysis showed that the increase of ABCA1 was reversed by the siRNA treatment, when fenofibrate and LY518674 were used but not bezafibrate or gemfibrozil (Fig. 3B). Thus, involvement of PPARα is not exclusive in the action of bezafibrate and gemfibrozil. When primary-cultured hepatocytes isolated from the *Pparα*-null mice were used, none of the fibrates enhanced HDL biogenesis (Fig. 4A). However, ABCA1 protein was increased by bezafibrate and gemfibrozil, consistent with the results of the PPARα siRNA treatment of HepG2 cells (Fig. 4A). Thus, among the fibrates tested, bezafibrate and gemfibrozil act through an extra-PPARα pathway as well. The reason why the increase of ABCA1 is not reflected in the HDL biogenesis in the *Pparα* null mouse hepatocytes is unknown.

Roles of PPARβ/δ and PPARγ

To examine the involvement of PPARβ/δ or PPARγ in the action of fibrates on HDL biogenesis, PPARβ/δ or PPARγ gene was knocked down by specific siRNAs in HepG2 cells. The levels of mRNA were reduced by 75% and 70% for PPARδ and PPARγ by respective siRNAs at 200 nM after the 72 hours of treatment. The reduced levels of expression were maintained for another 16 hours used for the HDL biogenesis experiments. When PPARβ/δ was knocked down, HDL-lipid release was decreased only with gemfibrozil and LY518674 for cholesterol, and with gemfibrozil for phospholipid (Fig. 5A). Fenofibrate only slightly reduced HDL-cholesterol release. When PPARγ was knocked down, the background HDL biogenesis decreased and the fibrate-induced reaction was decreased only for release of HDL-cholesterol, moderately by fenofibrate and remarkably by bezafibrate (Fig. 5B). ABCA1 expression was not influenced by knockdown of either PPARs except for slight decrease with gemfibrozil by knockdown of PPARβ/δ, being consistent with the findings that the siRNA treatment did not influence phospholipid release as a parameter of HDL biogenesis (Fig. 5C), presumably because ABCA1 expression is adequately stimulated by the PPARα pathway. The results also indicated that cholesterol content in HDL is influenced by some fibrates via PPAR β/δ or PPARγ.

Effects of Multiple Suppression of of PPARs

Finally, HDL biogenesis and ABCA1 expression were examined under conditions of multiple knockdown of PPAR genes in HepG2 cells. The left two panels of Figure 6A show the effects on HDL biogenesis of combined knock-down of PPARα and PPARβ/δ, PPARα, and PPARγ, and all three receptors, and the right panel shows mRNA expression of ABCA1 under the same conditions. Figure 6B demonstrates ABCA1 protein expression. When PPARα and PPARβ/δ were knocked down, ABCA1 was increased by bezafibrate and gemfibrozil, being reflected perhaps in the insignificant increase of HDL biogenesis by these two compounds. The data were consistent with those in Figure 5B where knockdown of PPARγ decreased cholesterol content in HDL released by bezafibrate and gemfibrogil. When PPARα and PPARγ were

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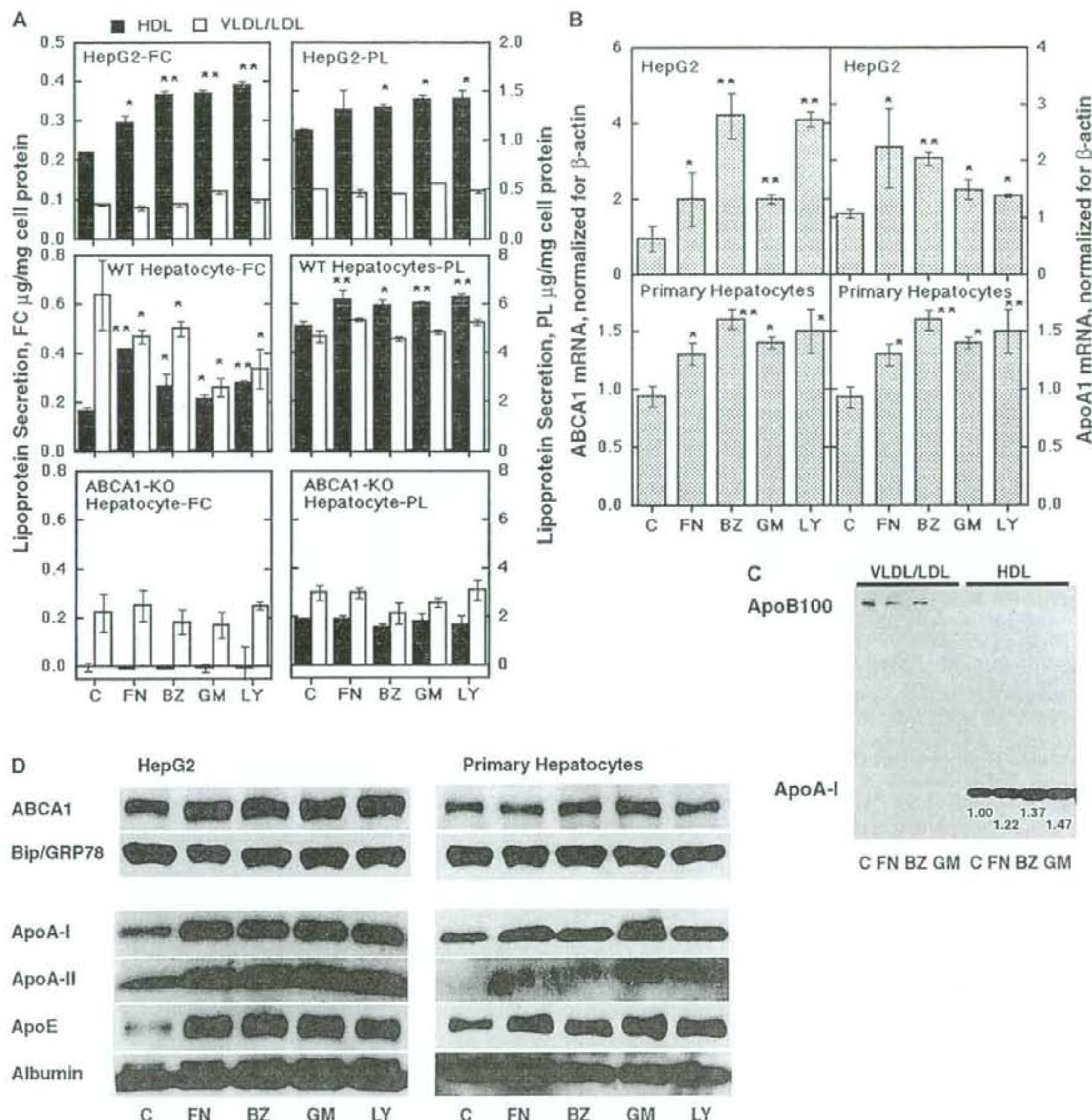

FIGURE 1. Effects of fibrates on HDL biogenesis by hepatocytes in vitro. A, Release of lipoproteins from HepG2 cells and primary hepatocytes isolated from C57BL/6 wild type and *Abca1*-null mice. The cells were exposed to four different fibrate drugs; fenofibrate (FN), bezafibrate (BZ), gemfibrozil (GM), and LY518674 (LY) at a concentration of 30 μM for 16 hrs. The conditioned medium was fractionated by ultracentrifugation as described and free cholesterol (FC) and choline-phopsholipid (PL) measured for HDL (solid column), VLDL/LDL (open column) fractions. B, Messenger RNA level of ABCA1. Total RNA was extracted from the cells after the fibrate treatment as shown in the panel A, and quantitative PCR was performed. The ABCA1 mRNA levels were normalized by those of β-actin mRNA. The values represent mean ± SD for three determinations [$^*P < 0.05$, $^{**}P < 0.01$ from control (C)] for panels A and B. C, Change in apoB and apoA-I in VLDL/LDL and HDL fractions by fibrates in the HepG2 conditioned media. The media lipoproteins were isolated by ultracentrifugation and analyzed by Western blotting by using the respective specific antibody. Numbers indicate fold increase of apoA-I from control based on digital scanning of bands. D, Western blotting analysis of ABCA1 of the cell membrane and the HDL-related proteins in the medium, after the fibrate treatment as shown in the panel A.

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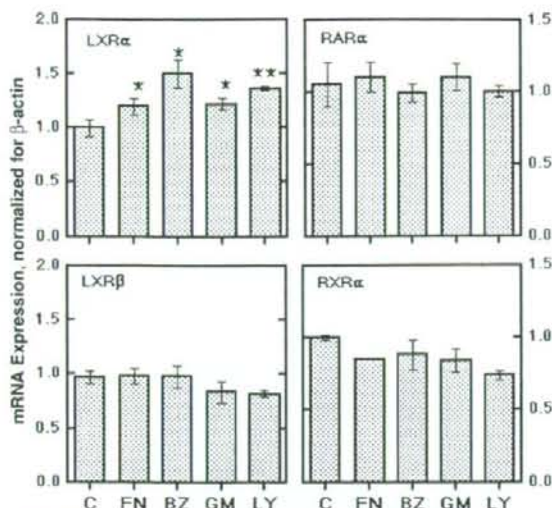

FIGURE 2. Effects of fibrates on the expression of the nuclear receptors. HepG2 cells were incubated with the fibrates [fenofibrate (FN), bezafibrate (BZ), gemfibrozil (GM), and LY518674 (LY)] as described in the legend for Figure 1. Expression of the mRNA for LXRα, LXRβ, RARα, and RXRα were determined by real-time quantitative PCR. The results were normalized by the mRNA levels of β-actin. The values represent mean ± SD for three determinations [$^*P < 0.05$, $^{**}P < 0.01$ from control (C)].

knocked down, ABCA1 was increased by bezafibrate and gemfibrozil, and HDL biogenesis was also increased by these compounds. The results were consistent with the decrease of the gemfibrozil-induced HDL biogenesis by knockdown of PPAR β/δ. Thus, fenofibrate and LY518674 act exclusively through PPARα in hepatocytes for increasing ABCA1 and the HDL biogenesis. On the other hand, bezafibrate and gemfibrozil act also through PPARβ/δ and PPARγ for these reactions. However, cholesterol-enrichment in HDL may be upregulated by extra-PPARα pathways, even by fenofibrate and LY518674.

DISCUSSION

Actions of clinically used fibrates for HDL biogenesis by hepatocytes were investigated by using HepG2 cells and mouse primary hepatocytes. Fenofibrate, bezafibrate, gemfibrozil, and LY518674 were all active in increasing ABCA1 expression and HDL biogenesis dependent on PPARα. Fenofibrate and LY518674 exclusively use PPARα for ABCA1 expression and HDL biogenesis. On the other hand, actions of bezafibrate and gemfibrozil apparently involve extra-PPARα pathways as some of their effects remain after knockdown of the PPARα expression in HepG2 cells and in the hepatocytes derived from *Pparα*-null mice. However, knockdown of the PPARβ/δ gene resulted in decrease of HDL cholesterol release by fenofibrate and LY518674 without changing HDL-phospholipid release and ABCA1 expression, while it reduced release of both HDL cholesterol and HDL phospholipid and ABCA1 expression by gemfibrozil. When PPARγ was knocked down, increase of HDL cholesterol release by bezafibrate was markedly reduced and that by fenofibrate was slightly suppressed without changing ABCA1 expression and release of HDL-phospholipid. Thus, there may be a specific mechanism(s) for increase of HDL cholesterol through β/δ or γ subtypes of PPAR used by some fibrates, being independent of the PPARα pathway. Multiple knockdown of the PPARs indicated that bezafibrate and gemfibrozil potentially mediate their effects through PPARβ/δ and PPARγ for the increase in ABCA1 expression and accordingly HDL biogenesis.

It should be noted that the increase in ABCA1 by bezafibrate and gemfibrozil was not reflected in HDL biogenesis in primary hepatocytes isolated from the *Pparα*-null mice for unknown reasons. Biogenesis of HDL is a complicated reaction involving many factors, so that inborn deficiency of the PPARα gene may cause some other alteration of these factors of the HDL assembly machinery. Indeed, the baseline secretion

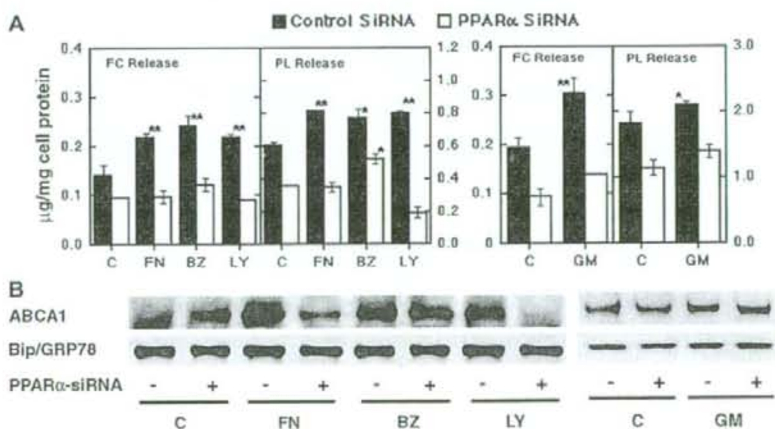

FIGURE 3. A role for PPARα in the effect of fibrates on HDL biogenesis in HepG2 cells. PPARα was down regulated by a specific siRNA and the effect of the fibrates on HDL biogenesis examined. A, Release of HDL from HepG2 cells during 16 hrs in the presence of the indicated fibrates [fenofibrate (FN), bezafibrate (BZ), LY518674 (LY) and gemfibrozil (GM)] at 30 μM after the 72-hour treatment with 200 nM PPARα-siRNA. The HDL fraction in the conditioned medium was analyzed for free cholesterol (FC) and choline-phospholipid (PL). The values represent mean ± SD for three determinations; $^*P < 0.05$ and $^{**}P < 0.01$ from control (C). B, Western blotting analysis of ABCA1. The experimental conditions are same as those for panel A.

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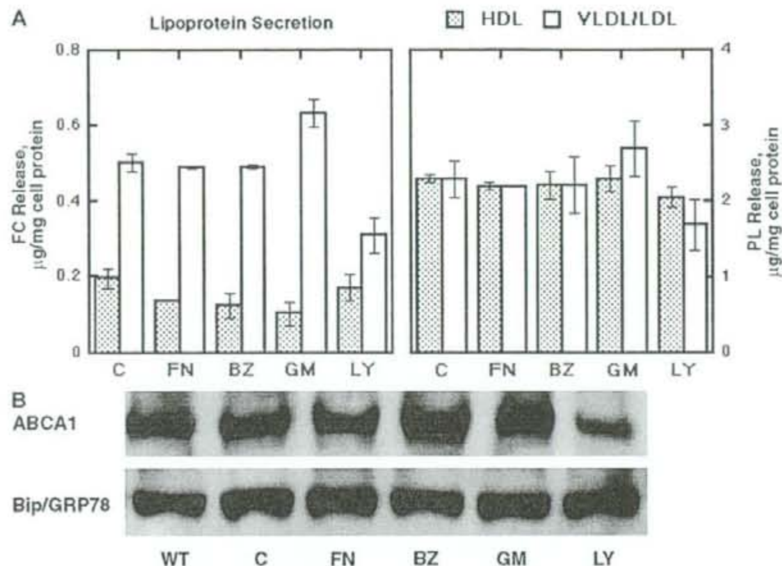

FIGURE 4. A role for PPARα in the effect of fibrates on HDL biogenesis in primary hepatocytes. Primary hepatocytes were prepared from *Pparα*-null mice (C57BL/6). The cells were incubated in the presence of the indicated fibrates [fenofibrate (FN), bezafibrate (BZ), gemfibrozil (GM), and LY518674 (LY)] at 30 μM. A, HDL (solid column) and VLDL/LDL (open column) in the conditioned medium were analyzed for free cholesterol (FC) and choline-phospholipid (PL). The values represent mean ± SD for three determinations. B, Western blotting analysis was carried out for ABCA1 by using 60 μg of bulk membrane protein of the mouse primary hepatocyte prepared as above. WT indicates the hepatocyte membrane of the wild-type C57BL/6 mouse without fibrate treatment.

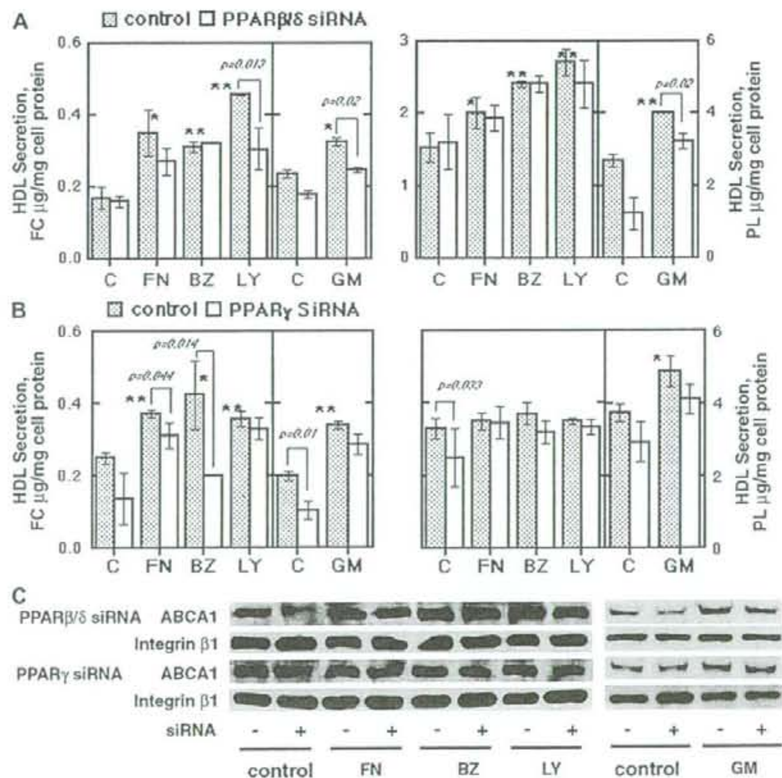

FIGURE 5. Effect of downregulation of the PPARβ/δ and PPARγ expression by specific SiRNA treatment on the HDL production in HepG2 cells. The effect of knocking down PPARβ/δ or PPARγ on the biogenesis of HDL was examined by using specific siRNA treatment in HepG2 cells. A and B, Effects of fibrates on the HDL biogenesis in HepG2 cells in the presence of the fibrates [fenofibrate (FN), bezafibrate (BZ) and LY518674 (LY)] at 30 μM after the pretreatment with 200 nM of PPARβ/δ siRNA (B) or PPARγ siRNA (C) (open column) and control siRNA (hatched column). The conditioned medium was analyzed for HDL-free cholesterol (FC) and HDL-choline phospholipid (PL). The values represent mean ± SD for three determinations ($^{*}P < 0.05$, $^{**}P < 0.01$ from control). C, Western blotting analysis for ABCA1 of the bulk membrane fraction (60 μg of protein) of HepG2 cells treated as above with the PPARβ/δ or PPARγ siRNA and subsequently with the fibrate [fenofibrate (FN), bezafibrate (BZ), and LY518674 (LY)].

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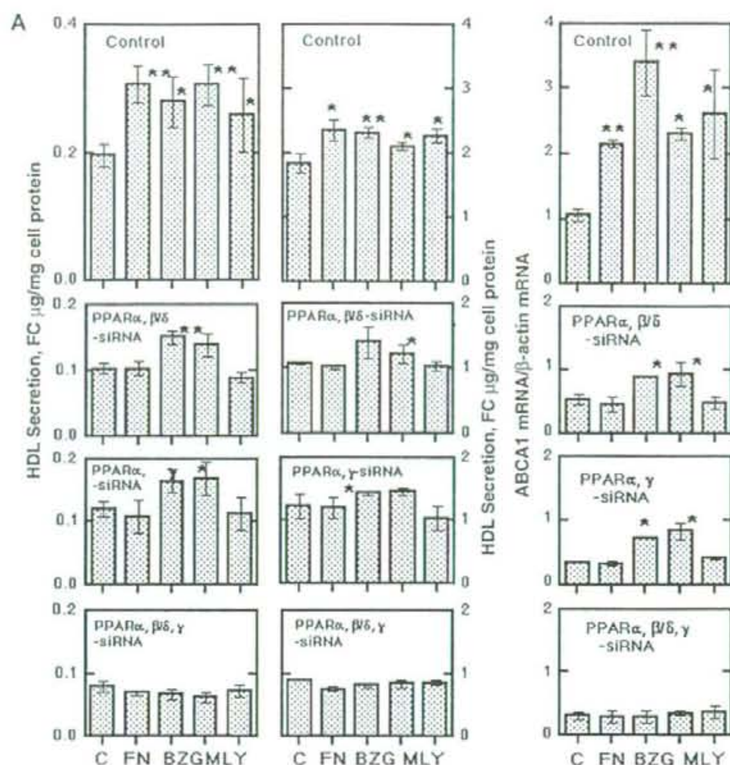

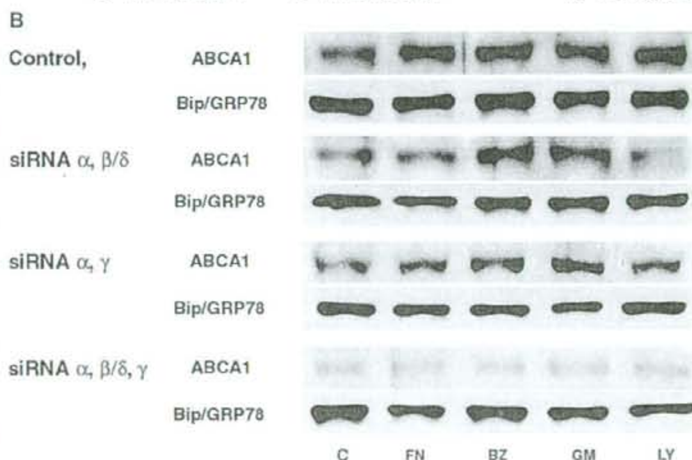

FIGURE 6. Dependency of fibrate actions on PPARβ/δ and PPARγ in HepG2 cells. A, The HDL biogenesis in HepG2 cells when knockdown of PPARα, PPARα + PPARβ/δ, PPARα + PPARγ, and PPARα + PPARβ/δ + PPARγ. The experimental condition for the treatment of HepG2 cells were the same as the experiments above with siRNA and fibrates, except for the double or triple knockdown of the PPARs. The effects were determined for the fibrates [fenofibrate (FN), bezafibrate (BZ), gemfibrozil (GM), and LY518674 (LY)] at 30 μM. The conditioned medium was analyzed for the HDL-cholesterol and -phospholipid. Expression of ABCA1 mRNA was determined by quantitative PCR. The results were normalized by the mRNA levels of β-actin. The values represent mean ± SD for three determinations (*$P < 0.05$, **$P < 0.01$ from control). B, Western blotting of ABCA1 of the bulk membrane of the HepG2 cells conditioned as above.

of HDL was much lower by these cells than that by the hepatocytes from the wild-type mice.

It was previously shown that LXRα expression is increased in extrahepatic cells by Wy14643[17] and fenofibrate[18] and that fenofibrate increases ABCA1 expression by an LXRα-dependent mechanism.[18] It should also be noted that fenofibrate ester, a pro-drug for fenofibrate, directly activates LXRα,[34] although this compound was not used in such a form in this study. All fibrates examined increased LXRα expression in HepG2 cells (Fig. 2), so that the LXRα pathway is likely used by these drugs to activate ABCA1 in the liver.

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Fibrates are widely used drugs for reducing plasma TG-rich lipoproteins and are expected to reduce the risk of atherosclerotic vascular disease as high plasma TG is one of the major risk factors for atherosclerosis including coronary heart diseases.[35] The reason for the increased risk by high TG is not exactly clear. At least two major factors seem to be involved. First, some of the TG-rich lipoproteins may be directly atherogenic, such as remnant lipoproteins and VLDL, especially apoE-rich particles in such categories.[35] Second, secondary remodeling of lipoprotein profiles caused by high TG and cholesteryl ester transfer protein (CETP) reaction such as generation of small and cholesterol-poor HDL and generation of "small-dense LDL."[36] Reduction of TG by fibrate reduces the atherogenic TG-rich lipoprotein and reverses undesirable change of lipoprotein profile caused by high TG. In addition, fibrates were also shown to increase HDL independently of the reduction of TG.[17,18] Large-scale clinical trials demonstrated that reduction of the elevated plasma TG prevented the coronary heart events, secondary or primary, by bezafibrate,[37] gemfibrozil,[38] and fenofibrate.[39]

It is not exactly clear if this is due to reduction of the TG-rich atherogenic lipoprotein or to improvement of other undesirable aspects of the lipoprotein profile. However, attempt at statistical analysis of the data of these trials may suggest that the increase in HDL-cholesterol is an independent factor contributing the risk reduction.[38]

Fibrates are known as PPARα agonists and induce genes related to fatty acid metabolism, in particular β-oxidation.[13] This is also a primary underlying mechanism for upregulation of the HDL-related genes by these drugs.[16–18,40] However, PPARβ/δ and PPARγ are also reported to be involved in the upregulation of HDL-related genes and increase HDL biogenesis.[11,29,41–43] Fibrate may have agonist activity for other PPARs than PPARα, so that a part of their direct effects on HDL biogenesis may be related to such a broader spectrum of agonist activity.

The present study showed that, in hepatocytes as the major source of plasma HDL, fenofibrate, and LY518674 are rather exclusive agonists of PPARα for expression of ABCA1 and may have a specific function to enrich the HDL with cholesterol in addition to stimulate the biogenesis of HDL particles. On the other hand, bezafibrate and gemfibrozil seem to have pathways in addition to PPARα activation to increase HDL biogenesis. Bezafibrate seems to use preferably PPARγ and gemfibrozil rather use PPARβ/δ, although such selectivity does not seem exclusive. These findings add new insight to understanding the underlying mechanism for the increase of HDL and the antiatherogenic effects of fibrates, especially for their differential evaluation for their clinical endpoints.

It was recently reported that ABCA1 is regulated by the dual mechanism in hepatocytes by LXRα and SREBP2, perhaps to prevent the returned cholesterol to the liver from reentry to the systemic circulation.[44] However, the present study showed that direct activation of the LXR system still increases ABCA1 expression and HDL biogenesis in hepatocytes.

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