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Activation of PPARγ does not contribute to macrophage ABCA1 expression and ABCA1-mediated cholesterol efflux to apoAI

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ABSTRACT

Activation of macrophage ABCA1/G1 expression and cholesterol efflux is believed one of the mechanisms by which PPARγ inhibits atherosclerosis. PPARγ can also activate CD36 expression, a receptor for oxLDL, which may supply LXR ligands to activate LXR-ABCA1/G1 pathways. However, the controversial effects of PPARγ on ABCA1 expression have been reported. In this study, we used peritoneal macrophages isolated from wild type and CD36 deficient ($CD36^{-/-}$) mice to clarify if PPARγ ligands can influence ABCA1 expression by CD36 function. We found that CD36 deficiency had no effect on cholesterol efflux and ABCA1/ABCG1 expression at basal levels. In both cell types, PPARγ ligands (15d-PGJ2, troglitazone and pioglitazone) reduced ABCA1 expression and ABCA1-mediated cholesterol efflux to apoAI, with most by troglitazone. LXR ligand-induced ABCA1 expression and cholesterol efflux was attenuated by PPARγ ligands. Associated with decreased ABCA1 protein levels, ABCA1 mRNA and promoter activity were reduced by PPARγ ligands. Furthermore, high expressing PPARγ reduced ABCA1 expression and LXR-activated ABCA1 promoter in a CD36-independent manner. In contrast, ABCG1 expression was induced by PPARγ ligands while inhibited by PPARγ inactivation. Taken together, our study suggests that enhancement of macrophage cholesterol metabolism by PPARγ is not contributed by activating ABCA1 expression and ABCA1-mediated cholesterol efflux to apoAI, which is not involved by CD36 expression either.

1. Introduction

Formation of lipid-laden macrophage/foam cells is the initial and critical step in atherosclerosis, and it is inhibited by cholesterol efflux [1]. ATP-binding cassette transporter A1 (ABCA1) and ABCG1 enhance cholesterol efflux to apolipoprotein AI (apoAI) and high-density lipoprotein (HDL), respectively, by using the energy from ATP hydrolysis [2,3]. The ABCA1-mediated cholesterol efflux also enhances production of nascent HDL [4].

The inhibitory effects of ABCA1 on atherosclerosis have been well investigated. Mutations of ABCA1 expression result in Tangier disease in humans which is characterized by reduction of cholesterol efflux from peripheral tissues leading to a severe accumulation of cholesteryl esters, absence of plasma HDL and onset of premature coronary heart disease in patients [5]. Mice lacking ABCA1 expression display similar phenotypes [6]. In contrast, activation of ABCA1 expression enhances cholesterol efflux and inhibits atherosclerosis [7,8].

Peroxisome proliferator-activated receptor γ (PPARγ) is a transcription factor [9]. After activation by a ligand, PPARγ forms a heterodimer with retinoid X receptor α (RXRα). The complex of PPARγ/RXRα binds to the PPAR-responsive element (PPRE) in promoter to initiate transcription of the target genes. PPARγ plays an important role in different biological processes, such as lipid metabolism, homeostasis of glucose and energy, and inflammation. PPARγ synthetic ligands, thiazolidinediones (TZDs), enhance insulin sensitivity and improve glycemic control, which leads to a few TZDs are used to treat type 2 diabetic patients.

High PPARγ expression in macrophage/foam cells in lesion areas indicates its implication in the development of atherosclerosis [10]. PPARγ induces CD36 expression, a receptor for oxidized LDL (oxLDL) facilitating macrophage/foam cells formation and development of atherosclerosis [11]. However, several studies indicate

Abbreviations: ABCA1/G1, ATP-binding cassette transporter A1/G1; HDL, high-density lipoprotein; LXR, liver X receptor; PPARγ, peroxisome proliferator-activated receptor γ; TZDs, thiazolidinediones.

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that TZD inhibits atherosclerosis in animal models [12,13]. The TZD-induced CD36 expression may activate cholesterol efflux since the oxysterol contained in oxLDL can function as ligands to activate liver X receptors α and β (LXR), the transcription factors activating ABCA1/ABCG1 expression [14,15]. However, the controversial effects of PPARγ on macrophage ABCA1 expression and ABCA1-mediated cholesterol efflux to apoAI have been reported [16–21]. For instance, it has been reported that pioglitazone can't reverse atherosclerosis in LDL receptor knockout ($LDLR^{-/-}$) mice which is associated with inhibition of macrophage ABCA1 expression *in vivo* [22]. Therefore, it appears that inhibition of atherosclerosis by PPARγ is not contributed by ABCA1 expression. Herein, we used peritoneal macrophages isolated from wild type and CD36 knockout ($CD36^{-/-}$) mice to clarify the effects of PPARγ on ABCA1 expression and cholesterol efflux to apoAI, and if the PPARγ-induced CD36 is involved.

2. Methods

2.1. Cells

C57BL/6 wild type and $CD36^{-/-}$ (C57BL/6 background) mice were used to isolate peritoneal macrophages as described [23]. Cells were cultured (300×10^3 cells/cm^2) in complete RPMI medium and received treatment in serum-free medium.

2.2. Isolation of LDL and HDL, and preparation of apoAI, AcLDL and oxLDL

LDL (1.019–1.063 g/ml) and HDL (1.063–1.21 g/ml) were isolated from normal human plasma followed by preparation of acetylated LDL (AcLDL), oxidized LDL (oxLDL) and apoAI as described [23].

2.3. Determination of cholesterol efflux

Macrophages in 12-well plates were labeled in serum-free medium containing 50 μg/ml AcLDL (as a carrier of cholesterol labeling) and 150 nCi/ml ^{3}H-cholesterol for 24 h. After treatment, cells were used to determine cholesterol efflux as described [23].

2.4. Determination of mRNA and protein expression

Wild type peritoneal macrophages were used to determine ABCA1 mRNA expression (8.1 kb), which was normalized by GAPDH mRNA expression (1.3 kb), by Northern blot as described [23]. ABCA1 probe was generated by RT-PCR with the following primers: forward, 5′-TGGACATCCTGAAGCCAG-3′, backward, 5′-TTCTTCCCACATGCCCT-3′. Protein expression was determined by Western blot with polyclonal antibodies against ABCA1 (MW, 220 K), ABCG1 (MW, 65 K) (Novus Biologicals, Littleton, CO) and PPARγ (MW, 55 K) protein (CalBiochem, San Diego, CA), respectively. The loading of each sample was confirmed by determination of GAPDH protein (MW, 36 K).

2.5. Plasmid DNA preparation and transfection

Mouse ABCA1 promoter (pABCA1, from −179 to +227) or CD36 promoter (pCD36, from −2000 to +50) was constructed by PCR using mouse genomic DNA and the following primers. pABCA1: forward, 5′-TAGCCTCGAGGTCGCCGGTTTAAGGGGCG-3′, backward, 5′-TGCCAAGCTTCCTCTTACCTGTTTTCCACTTTG-3′; pCD36: forward, 5′-TAGCCTCGAGAGTATAGGGAAATGTCAGGGCCAGG-3′, backward, 5′-TGCCAAGCTTGAAAGGCTAGGAAACCATCCACCAG-3′. ABCA1 promoter with the LXRE or DR4 deletion (pABCA1/DR4del) was constructed using the Phusion Site-Directed Mutagenesis Kit from New England Biolabs (Ipswich, MA) with pABCA1 and the DR4-deleted primers. Promoter activity was determined using the Dual-Luciferase® Reporter Assay System (Promega, Madison, WI) as described [23].

Mouse PPARγ expression vector (pC2-PPARγ) was constructed by RT with total RNA isolated from adipocytes and oligo$(dT)_{18}$ primer, and PCR with the following primers: forward, 5′-TCTCGAGCTCAATGGGTGAAACTCTGGGAG-3′; backward, 5′-CCGCGGTACCCTAATACAAGTCCTTGTAGATCTCCT-3′. The PCR product with sequence confirmed was digested with *Sac*I and *Kpn*I, then subcloned into pEGFP-C2 vector.

2.6. Data analysis

All experiments were repeated more than 3 times, and representative results are presented. Data were presented as mean ± standard errors, and analyzed by Student's *t*-test using Prism 5 (GraphPad Software) with significant difference at $P < 0.05$.

3. Results

3.1. PPARγ ligands reduce cholesterol efflux to apoAI and ABCA1 expression

CD36 has been proposed to facilitate cholesterol efflux since it uptakes oxLDL which contains ligands for LXR to induce ABCA1 expression. To obtain the direct evidence demonstrating the role of CD36 in ABCA1 expression and cholesterol efflux, peritoneal macrophages isolated from wild type and $CD36^{-/-}$ mice were used to compare cholesterol efflux to apoAI or HDL, respectively. Fig. 1A indicates that cholesterol efflux either to apoAI or HDL was close between wild type and $CD36^{-/-}$ macrophages. ABCA1 and ABCG1 are molecules responsible for cholesterol efflux to apoAI and HDL, respectively. Consistently, ABCA1 and ABCG1 levels were also close between two cell types (Fig. 1B). Thus, at the basal levels, CD36 is not involved in macrophage cholesterol efflux and ABCA1 or ABCG1 expression.

Some PPARγ ligands have been reported to induce macrophage cholesterol efflux [14,15] which might be due to, 1) LXRα can be activated by PPARγ since a PPRE exists in the LXRα promoter; 2) PPARγ induces CD36 expression to uptake oxLDL, and the oxysterols in oxLDL can function as LXR ligands. However, the different effects have been reported [16–21]. To determine if PPARγ ligands can affect ABCA1 expression and ABCA1-mediated cholesterol efflux to apoAI in a CD36-dependent manner, [^{3}H]cholesterol-labeled wild type and $CD36^{-/-}$ macrophages were treated with 15d-PGJ2, troglitazone and pioglitazone followed by determination of cholesterol efflux and ABCA1 expression. 15d-PGJ2 and pioglitazone slightly while troglitazone substantially decreased wild type macrophage cholesterol efflux to apoAI in a concentration dependent manner (Fig. 1C, left panel). Interestingly, the similar inhibitory effects were observed in $CD36^{-/-}$ macrophage (Fig. 1C, right panel). For instance, troglitazone at 1 μM decreased cholesterol efflux to 52 and 50% of controls in wild type and $CD36^{-/-}$ macrophages, respectively (Fig. 1C).

We next determined the effects of PPARγ ligands on ABCA1 protein levels. Consistent with cholesterol efflux to apoAI, PPARγ ligands reduced ABCA1 protein expression in an order of troglitazone>15d-PGJ2≧pioglitazone, and the reduction of ABCA1 levels was close between wild type and $CD36^{-/-}$ macrophages (Fig. 1D). In contrast, PPARγ antagonist, GW9662, had a moderate inductive effect on ABCA1 expression in both cell types (Fig. 1E). More specifically, inhibition of PPARγ expression by siRNA (top row, Fig. 1F) increased ABCA1 expression and

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