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A selective peroxisome proliferator-activated receptor δ agonist PYPEP suppresses atherosclerosis in association with improvement of the serum lipoprotein profiles in human apolipoprotein B100 and cholesteryl ester transfer protein double transgenic mice

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ABSTRACT

Objective. Although peroxisome proliferator-activated receptor (PPAR) δ agonists have been shown to improve the serum lipoprotein profiles in humans, the impact of the changes in these lipoprotein profiles on atherosclerosis remains to be elucidated. The aim of this study was to investigate the relationship between the selective PPARδ agonist-induced alterations of serum lipoprotein profiles and the development of atherosclerosis in human apolipoprotein B100 and cholesterol ester transfer protein double transgenic (hApoB100/hCETP-dTg) mice with human-like hypercholesterolemic dyslipidemia.

Methods. hApoB100/hCETP-dTg mice fed an atherogenic diet received a novel PPARδ agonist (PYPEP) or vehicle for 18 weeks, followed by evaluation of atherosclerosis. Serum samples were collected during the treatment period at least at 3-week intervals to determine the lipoprotein levels and the levels of an inflammatory marker, macrophage chemotactic protein-1 (MCP-1), and to analyze the lipoprotein profile by fast protein liquid

Abbreviations: PPAR, peroxisome proliferator-activated receptor; CETP, cholesteryl ester transfer protein; hApoB100/hCETP-dTg, human apolipoprotein B100 and human cholesteryl ester transfer protein double transgenic; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; ApoA-I, apolipoprotein A-I; MCP-1, macrophage chemoattractant protein-1; LXR, Liver X receptor; RXRα, retinoid X receptor; RARα, retinoic acid receptor alpha; TRβ, thyroid hormone receptor beta; FXR, farnesoid X receptor.

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chromatography. The cholesterol efflux capacity of high-density lipoprotein (HDL) was examined using [^{3}H]-cholesterol labeled macrophages.

Results. Compared with vehicle treatment, PYPEP treatment caused increases in the serum levels of HDL cholesterol and apolipoprotein A-I (ApoA-I), as well as reductions in the serum non-HDL cholesterol and MCP-1 levels. The HDL fraction from the PYPEP-treated group maintained its cholesterol efflux capacity and showed an increased population of smaller HDL particles. PYPEP substantially suppressed atherosclerotic lesion progression, and the lesion areas had significant correlations with non-HDL cholesterol, HDL cholesterol, ApoA-I and MCP-1 by Pearson's correlation analysis. A multiple regression analysis revealed that non-HDL cholesterol and ApoA-I were significantly associated with the atherosclerotic lesion area.

Conclusion. A novel PPARδ agonist, PYPEP, suppressed atherosclerotic lesion progression by improving the serum lipoprotein profiles, including increased levels of ApoA-I and functional HDL particles, as well as a reduced non-HDL cholesterol level, in hApoB100/hCETP-dTg mice with human-like hypercholesterolemic dyslipidemia.

1. Introduction

Hypercholesterolemic dyslipidemia has been implicated in the progression of atherosclerosis, which is associated with inflammatory responses within the vessel walls [1,2]. Although aggressive lowering of low-density lipoprotein (LDL) cholesterol levels reduces atherosclerotic vascular diseases, a substantial residual risk still remains after statin therapy [3]. Recently, high-density lipoprotein (HDL) or its major constituent, apolipoprotein A-I (ApoA-I), has been suggested to exert anti-atherogenic functions, including reverse cholesterol transport, which is promoting the efflux of cholesterol from macrophages within the arterial wall and returning it to the liver [4]. Several clinical studies have suggested that not only elevating the HDL cholesterol levels but also increasing the functional HDL particles is a promising strategy for reducing the remaining risk [5–7].

Peroxisome proliferator-activated receptor (PPAR) δ belongs to a family of lipid-activated nuclear receptor transcription factors including PPARα and PPARγ [8,9]. Several PPARδ agonists have been demonstrated to increase serum HDL cholesterol levels as well as reduce serum non-HDL cholesterol levels [10,11], but their therapeutic impact on atherosclerosis has not been established. In rodent studies with LDL receptor- or apolipoprotein E (ApoE)-deficient mice, PPARδ agonists have been shown to attenuate atherosclerosis by reducing serum non-HDL cholesterol levels and by exerting anti-inflammatory properties [12,13]. However, these mouse models are not always sufficient for examining the clinical significance of the relationship between the lipoprotein profile and atherosclerosis because the cholesterol metabolism in these mice is mechanistically different from that in humans due to the lack of cholesteryl ester transfer protein (CETP) and apolipoprotein B100 (ApoB100) [14]. Human ApoB100 and CETP double transgenic (hApoB100/hCETP-dTg) mice have been proposed as an alternative model of atherosclerosis with human-like hypercholesterolemic dyslipidemia [15].

In the present study, we investigated the effects of a novel PPARδ agonist, PYPEP [16], on the impaired serum levels of lipoprotein and a pro-inflammatory chemokine, macrophage chemoattractant protein-1 (MCP-1), and on atherosclerotic lesion progression in hApoB100/hCETP-dTg mice. Moreover, the relationship between the alterations of serum lipoprotein profiles and the development of atherosclerosis was determined.

2. Materials and Methods

2.1. Chemicals

PYPEP (tert-butyl-4-(2-hydroxyethyl)-4-(pyrrolidin-1-yl)-piperidine-1-carboxylate) was synthesized at Shionogi (Osaka, Japan) [16]. GW501516 was purchased from NARD Institute (Hyogo, Japan) [17].

2.2. Transactivation Assays

Transactivation assays using the ligand binding domains of human PPARs were conducted as described previously with minor modifications [18]. Briefly, a cDNA of the putative ligand binding domain encoding amino acids 167–468, 182–505 or 138–441 of PPARα, PPARγ or PPARδ, respectively, was inserted into the pBIND expression vector (Promega, WI) containing elements of GAL4 (amino acids 1–147) [19–21]. CHO-K1 cells were co-transfected with one of the human PPAR ligand binding domains and the GAL4 chimeric expression vector described above, together with a pG5luc GAL4-responsive reporter gene plasmid (Promega, WI) using the FuGENE6 transfection reagent (Roche Applied Science, Basel, Switzerland) according to the manufacturer's instructions. For the transactivation assay, the cells transiently expressing each PPAR were cultured in 96-well plates for 24 h and washed twice with phosphate buffered saline (PBS). The cells were then incubated in HEPES buffer in the presence or absence of relevant dilutions of test compound dissolved in dimethyl sulfoxide (DMSO) for 24 h before measurement of the luciferase activity. The luciferase activity was measured using Pikkagene reagent (Toyo Ink, Tokyo, Japan) according to the manufacturer's instructions. The half maximal effective concentration (EC_{50}) values were calculated from the curves fitted to the concentration–response data with a four