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Cyclic phosphatidic acid inhibits the secretion of vascular endothelial growth factor from diabetic human coronary artery endothelial cells through peroxisome proliferator-activated receptor gamma

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ABSTRACT

Atherosclerosis is a disease characterized by building up plaques formation and leads to a potentially serious condition in which arteries are clogged by fatty substances such as cholesterol. Increasing evidence suggests that atherosclerosis is accelerated in type 2 diabetes. Recent study reported that high level of alkyl glycerophosphate (AGP) was accumulated in atherosclerotic lesions. The presence of this phospholipid in mildly oxidized low-density lipoprotein (LDL) is likely to be involved in atherogenesis. It has been reported that the activation of peroxisome proliferator-activated receptor gamma plays a key role in developing atherosclerosis. Our previous result indicates that cyclic phosphatidic acid (cPA), one of bioactive lipids, potently suppresses neointima formation by inhibiting the activation of peroxisome proliferator-activated receptor gamma (PPARγ). However, the detailed mechanism is still unclear. In this study, to elucidate the mechanism of the cPA-PPARγ axis in the coronary artery endothelium, especially in patients with type 2 diabetes, we investigated the proliferation, migration, and secretion of VEGF in human coronary artery endothelial cells from diabetes patients (D-HCAECs). AGP induced cell growth and migration; however, cPA suppressed the AGP-elicited growth and migration in D-HCAECs. Moreover, AGP increased VEGF secretion from D-HCAECs, and this event was attenuated by cPA. Taken together, these results suggest that cPA suppresses VEGF-stimulated growth and migration in D-HCAECs. These findings could be important for regulatory roles of PPARγ and VEGF in the vascular processes associated with diabetes and atherosclerosis.

1. Introduction

Atherosclerosis is an inflammatory disease in which lesions or plaques accumulate in arteries (Hansson et al., 2006; Libby, 2006). The first step in the development of atherosclerotic lesions is the activation of cells in blood vessel walls, such as endothelial cells (Davignon and Ganz, 2004; Sumpio et al., 2002). It is well known that the endothelium is directly involved in vascular disease, stroke, heart disease, and diabetes (Rajendran et al., 2013). In particular, diabetes has been reported to increase the risk of cardiovascular disease by accelerating the formation of atherosclerosis (Kannel and McGee, 1979). Diabetes-associated atherosclerosis and vascular diseases are the principal causes of morbidity and mortality in patients with diabetes (Cade, 2008).

Lysophosphatidic acid (LPA) is a bioactive lipid that is produced in serum after the activation of multiple biochemical pathways (Moolenaar, 1995; Tigyi and Parrill, 2003). Some clinical studies have reported that the plasma LPA level is correlated with vascular diseases and that some species of LPA induce neointima formation, which is a characteristic feature of common vascular pathologies such as atherosclerosis (Shibata and Glass, 2009). However, previous reports indicated that neointima formation induced by unsaturated LPA is not mediated by LPA_1 and LPA_2 receptors, which are the major LPA-receptor subtypes of the G-protein coupled receptors (GPCRs) expressed in blood vessel walls (Zhang et al., 2004). Thus, numerous factors contribute to atherosclerosis, which is a complex disease that is accelerated by diabetes and metabolic syndrome. Moreover, metabolic syndrome occurs commonly in people

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with type 2 diabetes, thus increasing the incidence of cardiovascular diseases (Grundy et al., 1999; Lau et al., 2006).

We previously reported that unsaturated LPA species induced arterial wall remodeling in rat and mouse non-injury models, and this response required peroxisome proliferator-activated receptor gamma (PPARγ) (Tsukahara et al., 2010). The function of PPARγ has been studied extensively, and various synthetic and physiological agonists of this receptor have been identified (Tsukahara, 2012), including thiazolidinediones (TZDs) and numerous natural ligands containing fatty acids and phospholipids (Tsukahara, 2012). Recently, selected phospholipids such as LPA and alkyl-glycerophosphate (AGP, also known as alkyl-LPA) were identified as agonists of PPARγ (Fig. 1) (McIntyre et al., 2003; Tsukahara et al., 2006). Later, we reported that AGP is a specific agonist of PPARγ with higher affinity and potency than LPA. Fujiwara and colleagues reported that AGP, an analog of LPA, is a weak agonist of LPA receptors except for the LPA_5 receptor; however, only the LPA_5 receptor shows a preference for AGP over LPA (Williams et al., 2009). AGP has a higher potency than LPA for PPARγ activation (Tsukahara et al., 2006). Binding studies using the PPARγ ligand-binding domain showed that binding of the AGP was similar to that of the TZD rosiglitazone (Tsukahara et al., 2006). We recently identified cyclic phosphatidic acid (cPA) as an endogenous PPARγ antagonist generated by phospholipase D2 (PLD2) (Tsukahara et al., 2010). These observations suggest that activation of PPARγ is likely to lead to a complex cellular response.

Earlier studies have shown that PPARγ, which plays a key role in the cardiovascular system, is expressed in all cell types of the blood-vessel wall and in monocytes and macrophages (Bishop-Bailey, 2000). Coronary artery disease is the most common type of heart disease, and the leading cause of death from cardiovascular diseases typically involves atherosclerosis (Roger et al., 2012). Our results thus raise the possibility of using cPA as a lead compound to develop new treatments that act on PPARγ. However, the critical genes affected by the cPA-mediated inhibition in the endothelial cells of the coronary artery remain unknown. The migration of endothelial cells contributes to diverse aspects of vascular physiology such as the development of atherosclerosis. Currently, cPA is considered to play a role in the anti-neointima activity of carotid arteries (Tsukahara et al., 2010); therefore, it is important to understand the mechanisms that determine cPA sensitivity.

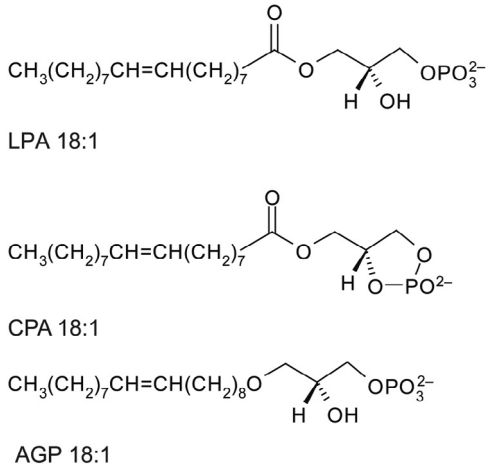

Fig. 1. Chemical structures of LPA, AGP, and cPA. It is made up of a glycerol backbone with a hydroxyl group, a phosphate group, and a long-chain saturated or unsaturated fatty acid. AGP is an alkyl-ether analog of LPA. AGP shows a higher potency than LPA for the intracellular LPA receptor PPARγ. cPA is a naturally occurring acyl analog of LPA. cPA is a weak agonist of plasma membrane LPA receptors, whereas cPA is an inhibitor of PPARγ.

Here, we focused on the regulation of vascular endothelial growth factor (VEGF) by cPA. VEGF is a growth factor that was first identified to be specific to the vascular endothelium (Ferrara et al., 2003). In mammals, the VEGF family consists of five members, VEGF-A, VEGF-B, VEGF-C, VEGF-D and the placenta growth factor (PLGF) (Olsson et al., 2006). VEGF is a potent mitogen and angiogenic growth factor that is the most critical regulator of vascular development (Ferrara et al., 2003). Previously, VEGF was shown to play a role in endothelial cell proliferation, migration, and atherosclerosis (Inoue et al., 1998). Several studies have demonstrated that PPARγ agonists regulate the expression of various genes including VEGF and its receptors (Yoshizaki et al., 2010). Rosiglitazone, a TZD and a synthetic agonist of PPARγ, has been reported to suppress VEGF expression through a PPARγ-responsive element in the promoter of the VEGF gene, and pioglitazone, another synthetic agonist of PPARγ, was reported to decrease VEGF expression (Peeters et al., 2005). On the contrary, other studies have reported that TZD drugs increase VEGF expression (Yoshizaki et al., 2010). The discrepancies in these results may have arisen from the use of distinct cell types, but the exact mechanisms remain unknown. Moreover, although cPA can arrest the growth of D-HCAECs, the relationship between the anti-cell growth effect of cPA and the regulation of VEGF expression is unclear. In this study, we determined that the PPARγ antagonist cPA potently inhibited the release of VEGF protein into the media of D-HCAECs that express high levels of PPARγ. The reduction in VEGF expression and secretion was associated with a decrease in mRNA expression and transcriptional inactivation of the VEGF promoter. We conclude that inhibition of PPARγ downregulates VEGF and that this mechanism may be involved in the regulation of D-HCAEC proliferation and migration.

2. Experimental procedures

2.1. Materials

A rabbit polyclonal anti-PPARγ antibody (sc-7196), a mouse monoclonal anti-β-actin antibody (sc-47778), a PPARγ small interfering RNA (siRNA) (sc-38304), and a control siRNA (sc-37007) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). A mouse monoclonal VEGF antibody (11081) was purchased from Immuno-Biological Laboratories (Fujioka, Gunma, Japan). We obtained cPA (18:1) as a gift from Dr. Kimiko Murakami-Murofushi (Ochanomizu University, Tokyo, Japan). AGP (18:1) and LPA (18:1) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA).

2.2. Cell cultures

Primary human coronary artery endothelial cells (HCAECs) and HCAECs from donors with type 2 diabetes (D-HCAECs) were purchased from Lonza (Walkersville, MD, USA) and propagated in endothelial cell growth medium (EBM™-2, Lonza) containing 5% FBS and manufacturer-recommended supplemental growth factors (EGM-2™ Bullekit™), antibiotics, and antimycotics. All assays were performed on cells between passages 3 and 12 and were repeated at least 3 times in duplicate or triplicate.

2.3. Reporter gene assays

PPARγ activation was measured in HCAECs and D-HCAECs transfected with 125 ng of the pGL3-PPRE-acyl-CoA oxidase luciferase vector, 62.5 ng of the pcDNA3.1-PPARγ vector, and 12.5 ng of the pSV-β-galactosidase (Promega) vector, which were constructed as previously reported (Tsukahara et al., 2006, 2010). At 24 h after transfection, cells were treated with Opti-MEM (Invitrogen) containing the test compounds dissolved in DMSO (up to 0.1%) and cultured for an additional 20 h. Luciferase activity was measured using the ONE-Glo Luciferase Assay System (Promega) and a LuMate microplate luminometer