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15-Deoxy-prostaglandin J_2 inhibits PDGF-A and -B chain expression in human vascular endothelial cells independent of PPARγ

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Abstract

15-Deoxy-prostaglandin J_2 (15d-PGJ_2) is an endogenous ligand of peroxisome proliferator-activated receptor γ (PPARγ) and plays an important role in the regulation of endothelial cell growth and apoptosis. However, the detailed mechanisms are poorly understood. We hypothesized that 15d-PGJ_2 might affect PDGF expression in endothelial cells through activating PPARγ. Here we documented that 15d-PGJ_2 dose-dependently inhibited phorbol-12-myristate-13-acetate (PMA)-stimulated expression of the PDGF-A and PDGF-B chain in human umbilical vein endothelial cells (HUVEC) by Northern blot and Western blot analyses. In contrast, the synthetic and high-affinity PPARγ agonists, including ciglitazone and GW7845, did not affect PMA-induced PDGF expression. In addition, we found that the PPARγ antagonist GW9662 did not block the effects of 15d-PGJ_2 on PDGF expression. Furthermore, Northern blot analysis showed that 15d-PGJ_2 inhibited the expression of Sp1, which is a well-known positive regulator of PDGF transcription. Taken together, our results demonstrate that the inhibition of PDGF expression by 15d-PGJ_2 in HUVEC is independent of PPARγ, but may be through the downregulation of Sp1.

Keywords: 15d-PGJ_2; PDGF; PPARγ; Endothelial cell; Gene expression

The prostaglandins (PGs) are a family of structurally related molecules that are produced by cells in response to a variety of extrinsic stimuli and regulate cell growth, differentiation, and apoptosis [1,2]. PGD_2 is a major cyclooxygenase product in a variety of tissues and cells and has marked effects on a number of biological processes, including platelet aggregation, relaxation of vascular and nonvascular smooth muscles and nerve cells. PGD_2 undergoes dehydration in vivo and in vitro to yield additional, biologically active PGs of the J_2 series, PGJ_2, Δ^{12}-PGJ_2 and 15d-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2).

15d-PGJ_2 is abundant in endothelial cells and has been identified as an endogenous ligand of peroxisome proliferator-activated receptors γ (PPARγ), which belongs to the nuclear receptor superfamily and regulates target genes by binding to PPAR response elements [3,4]. It has been well documented that PPARγ is expressed in vascular smooth muscle cells (VSMC) and endothelial cells and plays an important role in the regulation of vascular cell proliferation, migration, and apoptosis [5–7]. Activation of PPARγ significantly inhibits VSMC proliferation and neointima formation after balloon injury [8,9]. PPARγ is regulated by a variety of growth factors and cytokines, including platelet-derived growth factor (PDGF), which is a critical mitogen to VSMC and endothelial cells in vasculature [10].

In the present study, we tested the 15d-PGJ_2 effect on PDGF expression in endothelial cells. Our results demonstrated that the inhibition of PDGF expression by 15d-PGJ_2 in endothelial cells is independent of PPARγ, but may be through the downregulation of Sp1.

Materials and methods

Materials. 15-Deoxy-prostaglandin J_2 (15d-PGJ_2), WY14643, and ciglitazone were purchased from BioMol Research Laboratories (Plymouth Meeting, PA). GW7845 and GW9662 were obtained from

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GlaxoSmithKline [11]. Anti-PDGF-B polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). [α-^{32}P]dCTP was purchased from Perkin–Elmer/NEN. Hybridization solution (MiracleHyb) was obtained from Stratagene (San Diego, CA).

Cell culture. Human umbilical vein endothelial cells (HUVEC) were purchased from BioWhittaker (San Diego, CA). The cells were cultured in endothelial cell growth medium-2 (EGM-2, BioWhittaker) containing 5% FBS, human basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF), human epithelial growth factor (EGF), vascular endothelial growth factor (VEGF), 50 μg/ml gentamicin, 50 ng/ml amphotericin-B, hydrocortisone, and ascorbic acid. For all experiments, early passage [3–5] HUVEC were grown to 85–95% confluence and made quiescent by serum starvation (0.4% FBS and exclude the growth factors) for 24 h. 15d-PGJ_2 and other PPARγ ligands were added 1 h before administration of PMA.

RNA isolation and Northern blot analysis. Twenty μg total RNA isolated from each condition by using acid-guanidinium thiocyanate was subjected to electrophoresis through 1% formaldehyde–agarose gels. After transferring to nylon membranes (Perkin–Elmer), the RNA was cross-linked to the membrane by a UV cross-linker (Bio-Rad). ^{32}P-labeled cDNA probes were generated by using the random primer labeling system (Invitrogen, CA). Blots were pre-hybridized, hybridized, and then washed once with 1× SSC at 65 °C for 15 min and once with 0.1× SSC, 1% SDS (w/v) at 65 °C for 30 min. The RNA sample loading differences were normalized by hybridization with a GAPDH cDNA probe.

Western blot analysis. The cell culture medium was collected 24 h after treating the cells with 15d-PGJ_2, cell debris was removed with centrifugation, and the medium was concentrated in the order of 10–20-fold with Biomax Column (Millipore). Forty μl concentrated cell medium was subjected to SDS–PAGE and electrotransferred to the PVDF membrane (Bio-Rad). After blocking in TBST buffer (20 mM Tris–HCl, 150 mM NaCl, and 0.1% Tween 20) containing 5% (w/v) nonfat dried milk, blots were incubated with specific antibodies against PDGF-B for 2 h at room temperature. The blots were then incubated with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology). The immunoactivity was visualized by the enhanced chemiluminescence detection system (ECL, Amersham Pharmacia Biotech) according to manufacturer's instruction.

Results

15d-PGJ_2 inhibits PMA-induced PDGF-A and PDGF-B chain expression in HUVEC

PDGF is an important regulator that mediates the aberrant behavior of vascular cells in the pathogenesis of vascular diseases such as atherosclerosis and restenosis [12,13]. Prostaglandins and its metabolites also play an important role in the regulation of vascular cell growth and apoptosis [14–16]. To understand the biological relevance of prostaglandins and PDGF in vasculature, we investigated whether 15d-PGJ_2 affects PDGF mRNA expression in HUVEC with or without the treatment of PMA, which is a potent inducer of PDGF-A and PDGF-B expression. As shown in Fig. 1, PMA treatment induced about 3.2- and 10-fold mRNA increases of PDGF-A and PDGF-B, respectively. In addition, 15d-PGJ_2 (1, 5, and 10 μmol/L) inhibited PMA-stimulated PDGF-A and PDGF-B mRNA expression in a dose-dependent manner. Moreover,

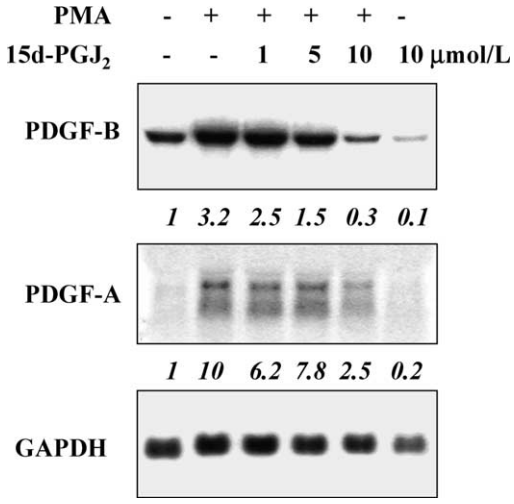

Fig. 1. 15d-PGJ_2 inhibits PMA-induced PDGF-A and PDGF-B mRNA expression in HUVEC. The quiescent cells were treated with increasing amounts (0, 1, 5, and 10 μmol/L) of 15d-PGJ_2 for 1 h prior to the administration of PMA (100 ng/ml) for 24 h. The mRNA expression levels of PDGF-A and PDGF-B in HUVEC were examined by Northern blot analyses. The relative values normalized by GAPDH of three independent experiments are shown under the images.

15d-PGJ_2 also reduced the steady-state mRNA levels of PDGF-A and PDGF-B.

We next examined the effects of 15d-PGJ_2 on PDGF protein levels in the culture medium of HUVEC. 15d-PGJ_2 (5 μmol/L) inhibited PMA-stimulated PDGF-B protein production and secretion (Fig. 2). Unfortunately, we could not detect any PDGF-A protein levels due to the quality of the anti-PDGF-A antibody. In addition, the lower expression level of PDGF-A in

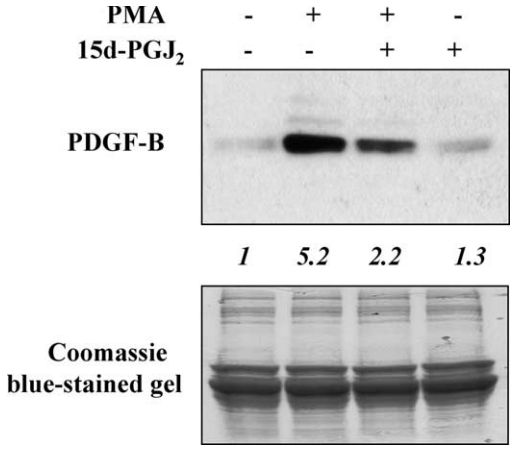

Fig. 2. 15d-PGJ_2 inhibits PMA-induced PDGF-B protein secretion in HUVEC. The quiescent cells were treated with or without 15d-PGJ_2 (5 μmol/L) for 1 h prior to the addition of PMA (100 ng/ml). After 24 h, the cell culture medium was collected and concentrated with a Biomax Column. PDBF-B protein levels in HUVEC culture medium were analyzed by Western blot analyses. The protein gel stained with Coomassie blue (bottom panel) confirmed the equal protein loading. Three independent experiments showed similar results.

HUVEC created another difficulty for Western blot analysis.

Taken together, our data indicated that 15d-PGJ_2 could inhibit PDGF-A and PDGF-B expression in HUVEC.

Suppression of PDGF expression by 15d-PGJ_2 is independent of PPARγ

It has been well documented that 15d-PGJ_2 functions as an endogenous ligand of PPARγ [4]. Therefore, we hypothesized that 15d-PGJ_2 might inhibit PDGF expression via PPARγ activation. However, recent emerging data suggest that 15d-PGJ_2 has various PPARγ-independent effects in many cell types [16–19]. To determine whether PPARγ activation was involved in the 15d-PGJ_2-induced PDGF suppression in HUVEC, the cells were treated with other high-affinity PPARγ-specific ligands, GW7845 (1 μmol/L) and ciglitazone (5 μmol/L). As shown in Fig. 3, both GW7845 and ciglitazone did not affect the expression of PDGF-A and PDGF-B, suggesting that PPARγ activation could not mimic the effect of 15d-PGJ_2-induced PDGF suppression in HUVEC.

It has also been documented that 15d-PGJ_2 can activate PPARα [4]. Thus, 15d-PGJ_2-induced PDGF suppression in HUVEC may be through PPARα activation. To address this hypothesis, the cells were treated with PPARα ligands, WY14643 (100 μmol/L), prior to the addition of 15d-PGJ_2. As shown in Fig. 3, WY14643 had no effect on PDGF mRNA expression in HUVEC.

To further confirm that 15d-PGJ_2-induced PDGF suppression is independent of PPARγ, GW9662, a specific antagonist of PPARγ was used. As shown in Fig. 4, GW9662 (1 μmol/L) failed to prevent the inhibitory effect of 15d-PGJ_2 on the PDGF expression.

15d-PGJ_2 downregulates the expression of Sp1

Sp1 and early growth response factor 1 (Egr-1) are the transcriptional factors that are responsible for the expression of PDGF in vascular calls [12,13]. They bind to the GC-rich region in the PDGF promoter driving the PDGF expression in both endothelial and vascular smooth muscle cells. To test whether 15d-PGJ_2-induced PDGF suppression was via the Sp1 and/or Egr-1 in HUVEC, Egr-1 and Sp1 expression levels were examined by Northern blot analyses. As shown in Fig. 5, Sp1 expression was significantly downregulated by 15d-PGJ_2

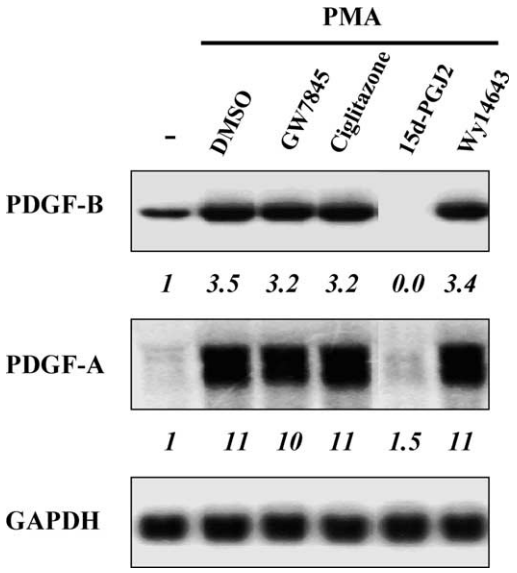

Fig. 3. Synthetic PPARγ and PPARα ligands do not affect the mRNA expression levels of both PDGF-A and PDGF-B in HUVEC. The quiescent cells were treated with 15d-PGJ_2, or other synthetic PPARγ ligands GW7845 (1 μmol/L), ciglitazone (5 μmol/L), or PPARα ligand WY14643 (100 μmol/L) for 1 h prior to the addition of PMA (100 ng/ml). After 24 h, total RNA was isolated and the levels of PDGF-A, PDGF-B, and GAPDH mRNA were determined by Northern blot analyses. The data shown are representative of three independent experiments. Average values for PDGF mRNA normalized by GAPDH level are shown under the image.

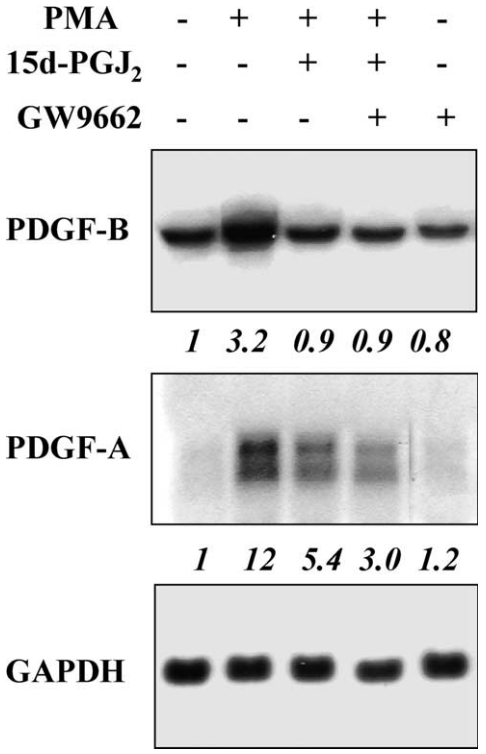

Fig. 4. PPARγ antagonist cannot reverse the suppression of PMA-induced PDGF-A and PDGF-B expression by 15d-PGJ_2 in HUVEC. The quiescent cells were pretreated with or without GW9662 (1 μmol/L) for 30 min prior to the addition of 15d-PGJ_2 (5 μmol/L). After 1 h, the cells were treated with PMA (100 ng/ml) for 24 h, and then total RNA was isolated for Northern blot analysis. Equal loading was confirmed by hybridization with GAPDH probe. The relative PDGF-A and PDGF-B mRNA levels normalized by GAPDH are shown on the bottom of each panel. Results are representative of three independent experiments.

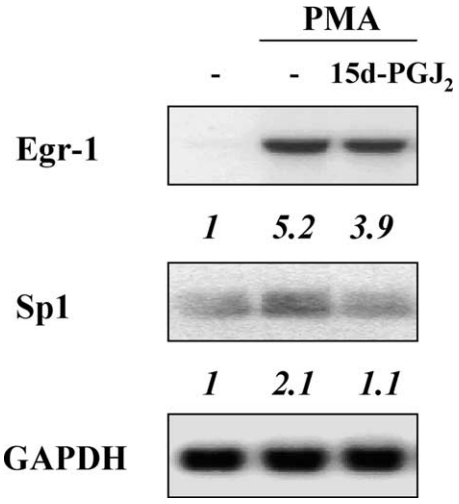

Fig. 5. 15d-PGJ_2 inhibits PMA-induced Sp1 expression in HUVEC. The quiescent cells were treated with or without 15d-PGJ_2 for 1 h prior to the addition of PMA (100 ng/ml). After 24 h, total RNA was isolated and the levels of Egr-1 and Sp1 mRNA were determined by Northern blot analyses. The data shown are representative of three independent experiments performed. The relative values for Egr-1 and Sp1 mRNA normalized by GAPDH level are shown under the images.

stimulation. However, there was no effect of 15d-PGJ_2 on Egr-1 expression in HUVEC. Taken together, these results indicated that the suppression of PDGF expression by 15d-PGJ_2 might be mediated by the downregulation of Sp1.

Discussion

PDGF is a potent mitogen and chemoattractant that functions as an important mediator in the pathogenesis of vascular disease. In many pathological conditions, PDGF expression within vasculature is increased by local production from endothelial cells, vascular smooth muscle cells (VSMC), and platelets. Stimulation of the PDGF receptors on VSMC can activate several signaling pathways, including p38-, MEK1/MAPK-, and PI3-kinase-mediated signal pathways, which transfer the signal into the nucleus and stimulate the proliferation and migration of VSMC. PPARγ is highly expressed in cells within the atherosclerotic plaque and neointima after balloon injury. Thiazolidinediones, a class of antidiabetic drugs that are specific ligands of PPARγ, inhibit neointima formation after balloon injury. Studies in vitro also demonstrate that PPARγ agonists inhibit VSMC proliferation and migration. It is postulated that the increase in PPARγ expression and activation noted in vascular lesion may function as an endogenous negative feedback element through inhibition of growth factors such as PDGF in vascular disease [20]. 15d-PGJ_2 is a metabolite of prostaglandin, which is abundant in endothelial cells and plays an important role in the regulation of endothelial cell growth and apoptosis. However, the precise mechanism is not clear. Recently, 15d-PGJ_2 was identified as an endogenous ligand of PPARγ. Thus, we hypothesized that 15d-PGJ_2 may inhibit the PDGF expression through activation of PPARγ. In this study, we demonstrated that 15d-PGJ_2 dose-dependently decreased PDGF mRNA and protein expression stimulated by PMA in HUVEC.

Emerging evidence also indicates that 15d-PGJ_2 exerts its function through PPARγ-independent mechanisms. Ward et al. [16] reported that 15d-PGJ_2 induced granulocyte apoptosis through inhibition of IκBα degradation using a PPARγ-independent pathway. In this study, we found that highly specific PPARγ ligands GW7845 and ciglitazone could not mimic the suppression effect of 15d-PGJ_2 on PDGF expression in HUVEC. Moreover, PPARγ-specific antagonist GW9662 cannot reverse the effect of 15d-PGJ_2. Thus, 15d-PGJ_2 exerts its suppression effect on PDGF expression through PPARγ-independent mechanisms.

It was well defined that a GC-rich region in the PDGF-A promoter, which contains positive transcriptional regulator Egr-1 and Sp1 binding element, is responsible for basal and growth factor-stimulated PDGF expression in endothelial cells. To explore the mechanisms that 15d-PGJ_2 inhibits PDGF expression in HUVEC, we observed the effect of 15d-PGJ_2 on Sp1 and Egr1 expression and found 15d-PGJ_2 significantly reduced Sp1 mRNA levels but not Egr-1. This indicated that 15d-PGJ_2 may affect PDGF expression by interfering with Sp1 expression or mRNA stability.

In summary, the present study demonstrated that 15d-PGJ_2 inhibited PDGF-A and PDGF-B expression in HUVEC in a PPARγ-independent manner. This study provides a new insight into the understanding of the role of prostaglandins and their metabolites in vascular diseases.

Acknowledgments

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