ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 3, 2007

DOI: 10.1089/ars.2006.1456

Forum Original Research Communication

Effects of PGC-1α on TNF-α–Induced MCP-1 and VCAM-1 Expression and NF-κB Activation in Human Aortic Smooth Muscle and Endothelial Cells

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ABSTRACT

Increased oxidative stress in vascular cells is implicated in the pathogenesis of atherosclerosis. Reactive oxygen species (ROS) induce vascular inflammation via the proinflammatory cytokine/NF-κB pathway. Several lines of evidence suggest that peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α) is an important regulator of intracellular ROS levels. However, no studies have examined the effects of PGC-1α on this process. We investigated the effects of PGC-1α on inflammatory molecule expression and activity of the redox-sensitive transcription factor, NF-κB, in vascular cells. PGC-1α expressed in human aortic smooth (HASMCs) and endothelial cells (HAECs) is upregulated by AMP-activated protein kinase activators, including metformin, rosiglitazone and α-lipoic acid. Tumor necrosis factor-α (TNF-α), a major proinflammatory factor in the development of vascular inflammation, stimulates intracellular ROS production through an increase in both mitochondrial ROS and NAD(P)H oxidase activity. Adenovirus-mediated overexpression of the PGC-1α gene in HASMCs and HAECs leads to a significant reduction in intracellular and mitochondrial ROS production as well as NAD(P)H oxidase activity. Consequently, NF-κB activity and MCP-1 and VCAM-1 induced by TNF-α are suppressed. Our data support the possibility that agents stimulating PGC-1α expression in the vasculature aid in preventing the development of atherosclerosis. *Antioxid. Redox Signal.* 9, 301–307.

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease that involves the interplay of various soluble mediators, monocytes, endothelial cells, and vascular smooth muscle cells (9, 24). The initial step in atherosclerosis is the recruitment of mononuclear cells into subendothelial space (24). One of the underlying mechanisms of leukocyte recruitment is a series of complex interactions between circulating leukocytes and vascular cells. Stimulation of vascular cells by cytokines initiates a signaling cascade leading to NF-κB–dependent expression of genes encoding chemokine and adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1) (2, 4). These changes accelerate vascular endothelial cell damage and vascular smooth muscle cell proliferation and migration. Thus, vascular expression of MCP-1 and VCAM-1 induced by the proinflammatory cy-

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tokine/NF-κB pathway is a key mechanism in the development of atherosclerosis.

It is well established that increased oxidative stress in vascular cells plays an important role in the pathogenesis of atherosclerosis (6, 8). Reactive oxygen species (ROS) induce a local inflammatory response through release of various cytokines, including tumor necrosis factor (TNF)-α, from monocytes (12). Generation of intracellular ROS in vascular cells by TNF-α initiates a signaling cascade leading to NF-κB–dependent gene expression, and induces a local inflammatory response through production of various chemokines and adhesion molecules (3, 14). Although the relative contribution of the individual ROS generating systems in the vasculature is currently ambiguous, both cell membrane NAD(P)H oxidase and the mitochondrial electron-transport chain play significant roles in ROS generation by TNF-α (10, 13, 14, 21). Previous studies demonstrate that adenovirus-mediated overexpression of uncoupling protein-2 (UCP-2) in human aortic smooth muscle cells (HASMCs) decreases ROS generation by inhibiting both pathways (22) and suppresses NF-κB activation by fatty acids in human aortic endothelial cells (HAECs) (19). Moreover, peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α) induces UCP-2 expression in vascular cells (26). Collectively, these results support the hypothesis that PGC-1α decreases TNF-α–induced NF-κB activation and MCP-1 and VCAM-1 expression in vascular cells by inhibiting intracellular ROS generation.

PGC-1α is a transcriptional coactivator, identified as an upstream regulator of mitochondrial number and function (26). Several reports show that PGC-1α is involved in the control of ROS production by mitochondria (16, 25, 26). Recently, a direct role for PGC-1α in vascular endothelial cells was proposed from the finding that its expression is associated with an increase in mitochondrial antioxidant defense (16, 26).

Vallea *et al*. (26) showed that overexpression of PGC-1α in vascular endothelial cells trigger an increase in mitochondrial antioxidative enzymes and suppress ROS production. Moreover, Kukidome and colleagues (16) suggested that active AMP-activated protein kinase (AMPK) reduces ROS generation via PGC-1α expression under hyperglycemic conditions.

In this study, we investigated the effects of PGC-1α on TNF-α–stimulated MCP-1 and VCAM-1 expression, as well as NF-κB activity in the vascular cell lines, HASMC and HAEC.

MATERIALS AND METHODS

Materials

Recombinant human TNF-α was purchased from R&D systems (Minneapolis, MN). Radiochemicals ([α-32P]dCTP, [γ-32P]ATP) were from Amersham Biosciences (Little Chalfont, U.K.). Metformin was acquired from Sigma. Rosiglitazone and α-lipoic acid were provided by GlaxoSmithKline (U.K.) and Viatris GmbH & Co. KG (Frankfurt, Germany), respectively.

Cell culture

HASMCs were isolated from the thoracic aorta of kidney transplantation donors by using the explant method, as described previously (1). Tissue collection was approved by the Ethics Committee of the Institution. Cells were cultured in DMEM (Gibco BRL, MD) containing 20% fetal bovine serum (FBS) (Hyclone, Logan, UT). In each preparation, HASMC's purity was determined by positive staining with smooth muscle–specific α-actin monoclonal antibodies (Santa Cruz). All cells were used within passages 5 and 6. At 90% confluence, cells in 100-mm dishes were serum-starved for 24 h with DMEM containing 0.5% serum and 5.5 m*M* D-glucose. HAECs were obtained from BioWhittaker Inc. (Walkersville, MD) and maintained in endothelial basal medium (EBM; BioWhittaker) supplemented with various growth factors and 2% FBS. Cells were passaged more than 3 times before use in experiments, and subsequently processed for nuclear protein or RNA extraction, as described later.

Preparation of recombinant adenovirus

Full-length human PGC-1α cDNA was inserted into the *Hind*III/*Bam*HI site of the pAd-YC2 shuttle vector (7). Shuttle vectors containing human PGC-1α and the rescue vector, pJM17, were co-transfected into human embryonic kidney 293 (HEK-293) cells, which were cultured on 24-well plates the day before transfection. After 12–15 days, recombinants were identified by polymerase chain reaction (PCR) (7), after which they were amplified in HEK-293 cells, and purified and isolated by using CsCl (Sigma). Preparations were collected and desalted, and titers were determined by measuring plaque counts. Control adenovirus devoid of PGC-1α (Ad-Null) was additionally prepared and identified by using this protocol.

Northern blot analysis

Total RNA was isolated from cells by using TRI reagent (Sigma), according to the manufacturer's instructions. Aliquots (20 μg) of total RNA from each sample were loaded onto 1.0% formaldehyde-agarose gels. After electrophoresis, RNA was transferred to Hybond-N^+ nylon membrane (Amersham). After transfer, membranes were cross-linked by using UV crosslinker 1800 (Stratagene, La Jolla, CA), and hybridized to random-primed ^{32}P-labeled probes at 65°C overnight. The membrane was washed with wash solution I (2 × SSC, 0.05 % SDS solution) and wash solution II (0.1 × SSC, 0.1 % SDS solution), air-dried, and exposed to autoradiography film.

Measurement of intracellular H_2O_2 production

Cells were seeded onto a six-well plate. At 90% confluence, cells were incubated in DMEM medium containing 1% FBS for 24 h. Cells treated with 100 MOI (multiplicity of infection) of adenovirus containing the PGC-1α gene (Ad-PGC-1α) or Ad-Null were cultured for 24 h. After exposure to TNF-α for 30 min, 10 μ*M* 2′,7′-dichlorofluorecin diacetate (DCF-DA; Sigma), a H_2O_2-sensitive fluorescent

probe, was added, and cells were cultured for 30 min. H_2O_2 production was quantified by using an AxioCam MRc5 Carl Zeiss fluorescence microscope (Thornwood, NY) at an excitation wavelength of 488 nm and emission wavelength of 515 nm.

Measurement of mitochondrial ROS

To evaluate the direct production of mitochondrial ROS in HASMCs and HAECs, specific staining with the reduced MitoTracker Red probe (CM-H2XRos; Molecular probe) was performed. In brief, cells were cultured at 37°C for 24 h under each condition (with 1% FBS), and loaded with 0.2 μ*M* CM-H2XRos at 37°C for 30 min. A fluorescence microscope (AxioCam MRc5; Carl Zeiss) equipped for equifluorescent illumination was used.

Measurement of NAD(P)H oxidase activity

To measure NAD(P)H oxidase activity, cells were washed twice with PBS, lysed with lysis buffer (50 m*M* Tris–HCl, 150 m*M* NaCl, 1 m*M* EDTA, 1% Triton X-100, 10% glycerol, 1 m*M* PMSF, 1 mg/ml aprotinin, 1 mg/ml leupeptin), and incubated for 1 h on ice. The lysate was centrifuged at 12,000 *g* for 20 min, and the supernatant obtained. Protein content was determined by using the Bradford method (Bio-Rad, Hercules, CA). NAD(P)H oxidase activity was measured by using lucigenin chemiluminescence (17).

Electrophoretic mobility shift assay

Nuclear extracts were prepared from cells and incubated (6 μg) with ~60,000 cpm of the ^{32}P-labeled NF-κB–binding site oligomer, 5′-AGTTGAGGGGACTTTCCCAGGC-3′ (Santa Cruz), for 20 min at RT. In brief, DNA probes, such as those for NF-κB, were labeled by using [γ-^{32}P]ATP and T4 polynucleotide kinase. After end-labeling, ^{32}P-labeled NF-κB was purified with a NAP-5 column (Amercham, Littlecalfort, U.K.). Protein–DNA-binding reactions were performed at room temperature for 20 min in a total volume of 30 μl. Reaction mixtures contained 6 μg nuclear extract, 100 μg/ml poly dI:dC, 10 m*M* Tris/HCl (pH 7.5), 50 m*M* NaCl, 0.5 m*M* EDTA, 0.5 m*M* DTT, 1 m*M* $MgCl_2$, 4% glycerol, and 60,000 cpm ^{32}P-labeled primer DNA. After incubation, samples were loaded onto 4% native polyacrylamide gels in 0.5 *M* Tris-borate-EDTA buffer and run at 150 V for 2 h. Gels were dried and visualized by autoradiography.

Real-time quantitative RT-PCR

After incubation for 24 h in each condition, total cellular RNA was isolated from cells by using TRIzol reagent, according to the manufacturer's protocol (Life Technologies, Gaithersburg, MD). The LightCycler System (Roche Molecular Biochemicals, Indianapolis, IN) was used to quantify PGC-1α transcripts. PCR reactions were performed by using SYBR Green I master mix, and specific primers for human PGC-1α (5-TCAGTCCTCACTGGTGGACA-3 and 5-TGCTTCGTCAAAAACA G-3) and human β-actin (5- CACCCACACTGTGCCCATCTACGA-3 and 5-CAGCGGAACCGCTCA TTGCCAATGG-3). To assess the specificity of the amplified products, a melting curve analysis was performed after the last cycle.

Statistical analysis

Results were expressed as mean values ± SEM. Variance analysis with a subsequent Duncan's test was used to determine significant differences in multiple comparisons. A p value of <0.05 was considered statistically significant. All experiments were performed at least three times.

RESULTS

Adenovirus-mediated PGC-1α gene overexpression inhibits TNF-α–induced MCP-1 and VCAM-1 expression in vascular cells

In view of the lack of reports on PGC-1α expression in HASMCs. We initially examined whether PGC-1α is basally expressed in this cell line. RT-PCR results disclose that primary cultured HASMCs express PGC-1α mRNA (data not shown). To determine whether PGC-1α affects MCP-1 and VCAM-1 expression in vascular cells, we generated an adenoviral vector containing human PGC-1α cDNA (Ad-PGC-1α). HASMCs and HAECs were infected with Ad-PGC-1α at concentrations of 10, 50, and 100 MOI. Notably, Ad-PGC-1α suppressed TNF-α–induced MCP-1 and VCAM-1 mRNA expression in HASMCs and HAECs in a dose-dependent manner, whereas Ad-Null had no effect (Fig. 1).

Ad-PGC-1α inhibits TNF-α–induced intracellular ROS production

We determined the effects of PGC-1α on TNF-α–induced ROS production in HASMCs and HAECs. TNF-α (5 ng/ml) stimulated the production of H_2O_2 in HASMCs and HAECs, compared with that observed under basal conditions. Infection with Ad-PGC-1α (100 MOI) led to complete inhibition of TNF-α–induced H_2O_2 production to the basal level. In contrast, Ad-Null did not influence the H_2O_2 level (Fig. 2).

Ad-PGC-1α inhibits TNF-α–stimulated mitochondrial ROS production and NAD(P)H oxidase activity

To elucidate the mechanism by which PGC-1α inhibits TNF-α–induced ROS production, we examined the effects of Ad-PGC-1α on mitochondrial ROS and NADPH oxidase activity, two major sources of TNF-α–induced intracellular ROS. As shown in Fig. 3, MitoTracker Red fluorescence in HASMCs and HAECs significantly increased with 5 ng/ml TNF-α, compared with the control. TNF-α–induced fluorescence was completely suppressed in the presence of Ad-PGC-1α, indicating abolishment of mitochondrial ROS production. In addition, infection of HASMCs and HAECs with Ad-PGC-1α partially but significantly decreased TNF-α–induced NAD(P)H oxidase activity. However, infection with Ad-Null had no effects on this parameter (Fig. 4).

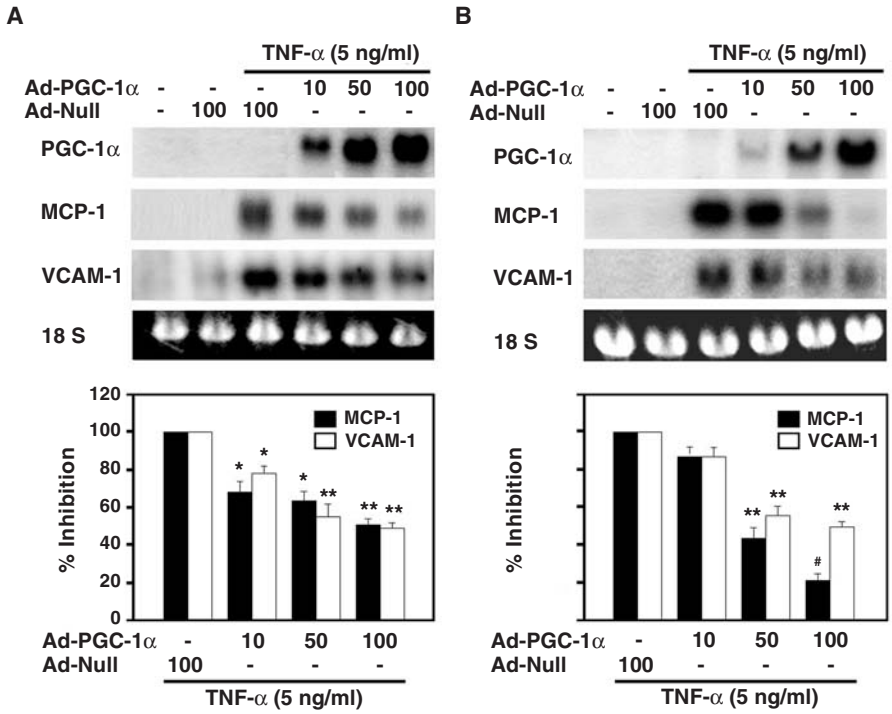

FIG. 1. Effects of Ad-PGC-1α on MCP-1 and VCAM-1 mRNA expression. HASMCs (**A**) and HAECs (**B**) were infected with indicated doses (MOI) of Ad-PGC-1α or Ad-Null for 2 h and treated with TNF-α (5 ng/ml) for 24 h. Total RNA (10 μg) was hybridized with MCP-1, VCAM-1, and PGC-1α probes for Northern blot analysis. The bars represent fold increase, compared with TNF-α–treated control. Data are expressed as mean ± SEM of three separate measurements (lower panel). Statistical significance was determined as $*p < 0.05$, $**p < 0.01$, and $^{\#}p < 0.001$, compared with TNF-α and Ad-Null.

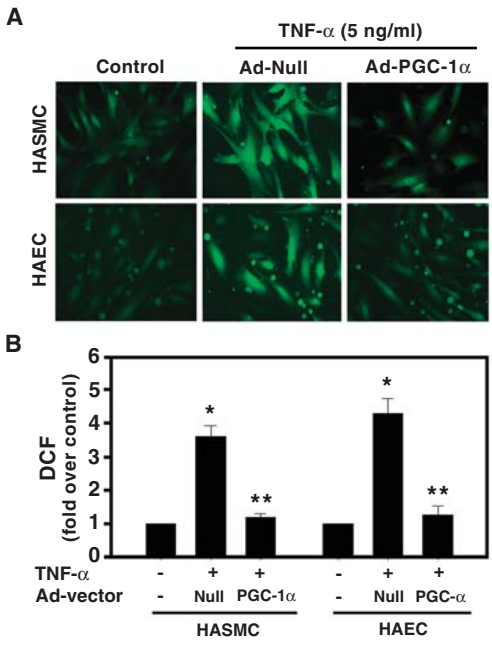

FIG. 2. Effects of Ad-PGC-1α on H_2O_2 production in response to TNF-α. (**A**) HASMCs and HAECs were infected with 100 MOI of Ad-PGC-1α or Ad-Null. Cells were further treated with TNF-α (5 ng/ml) for 30 min and processed for fluorescence microscopy by using the oxidant-sensitive probe 2′,7′-dichlorofluorescein diacetate. Fully confluent fields of HASMCs and HAECs were randomly selected, and fluorescence was quantified with the NIH Image program at a magnification of ×100. (**B**) Data are expessed as mean ± SEM of three separate measurements. Statistical significance was determined as $*p < 0.001$, compared with basal conditions, and $**p < 0.001$, compared with TNF-α and Ad-Null.

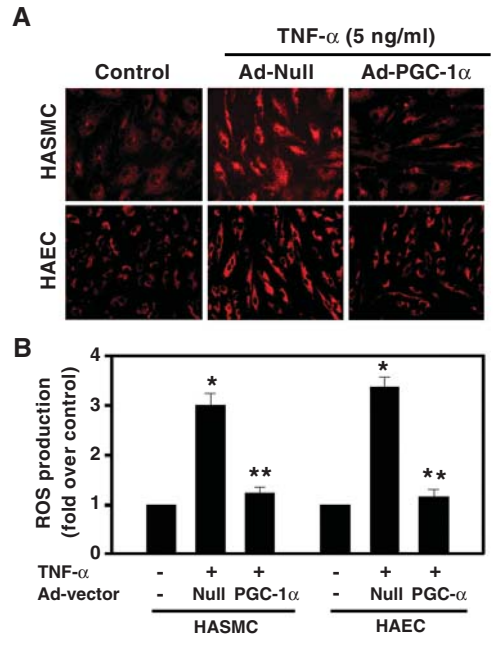

FIG. 3. Effects of Ad-PGC-1α on mitochondrial ROS production. (**A**) HASMCs and HAECs were infected with 100 MOI of Ad-PGC-1α or Ad-Null for 2 h and treated with TNF-α (5 ng/ml) for 24 h. Mitochondrial ROS production was detected by using MitoTracker Red CM-H2XRos. Fully confluent fields of HASMCs or HAECs were randomly selected, and fluorescence was quantified by using the NIH Image program. The magnification was ×100. (**B**) Data are expessed as mean ± SEM of three separate measurements. Statistical significance was determined as $*p < 0.001$, compared with basal, and $**p < 0.001$, compared with TNF-α and Ad-Null.

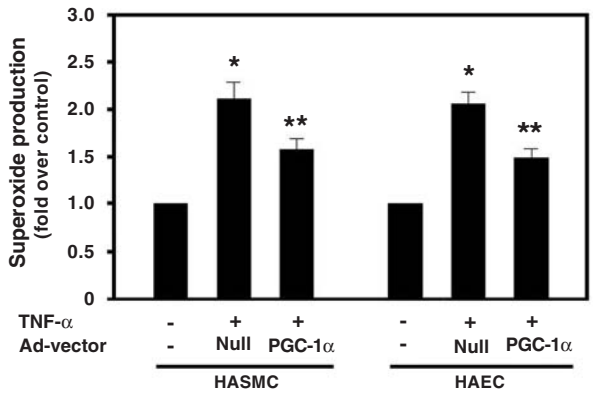

FIG. 4. Effects of Ad-PGC-1α on NAD(P)H oxidase activity. (**A**) HASMCs and HAECs were infected with 100 MOI of Ad-PGC-1α or Ad-Null for 2 h and treated with TNF-α (5 ng/ml) for 24 h. NAD(P)H oxidase activity was determined, as described in Materials and Methods. (**B**) Data are expessed as mean ± SEM of three separate measurements. Statistical significance was determined as $*p < 0.01$, compared with basal conditions, and $**p < 0.05$, compared with TNF-α and Ad-Null.

Ad-PGC-1α inhibits TNF-α–stimulated NF-κB activation

Because NF-κB activation by intracellular oxidative stress is critical for the expression of MCP-1 and VCAM-1 in vascular cells, we examined whether Ad-PGC-1α inhibits TNF-α–stimulated NF-κB activity. Notably, TNF-α (5 ng/ml) markedly increased NF-κB–DNA binding activity in HASMCs and HAECs. This binding activity was attenuated by Ad-PGC-1α in a dose-dependent manner, whereas Ad-Null had no effect (Fig. 5).

AMPK activators increase PGC-1α mRNA expression in HASMCs

Metformin, rosiglitazone, and α-lipoic acid activate AMPK (11, 15, 28). Furthermore, recent studies disclose that activation of AMPK leads to enhanced PGC-1α expression in vascular endothelial cells (16). Previously, we reported that α-lipoic acid stimulates AMPK activity in HAECs (20). The data collectively suggest that metformin, rosiglitazone, and α-lipoic acid increase PGC-1α expression in vascular endothelial cells. Real-time RT-PCR findings from the present study indicate that metformin, rosiglitazone, and α-lipoic acid significantly increase PGC-1α mRNA expression in HASMCs in a dose-dependent manner (Fig. 6). Additionally, we observed that metformin, rosiglitazone, and α-lipoic acid increased PGC-1α expression in HASMCs after exposure to TNF-α (data not shown).

DISCUSSION

We demonstrated that PGC-1α expressed in HASMCs is upregulated by AMPK activator including metformin, rosiglitazone, and α-lipoic acid. Adenoviral transfer of the PGC-1α gene to HASMCs significantly suppressed intracellular ROS production, NF-κB activation, and expression of MCP-1 and VCAM-1 induced by TNF-α. Similar data were obtained with HAECs.

While findings on ROS production in response to TNF-α are inconsistent in general, recent studies show that TNF-α

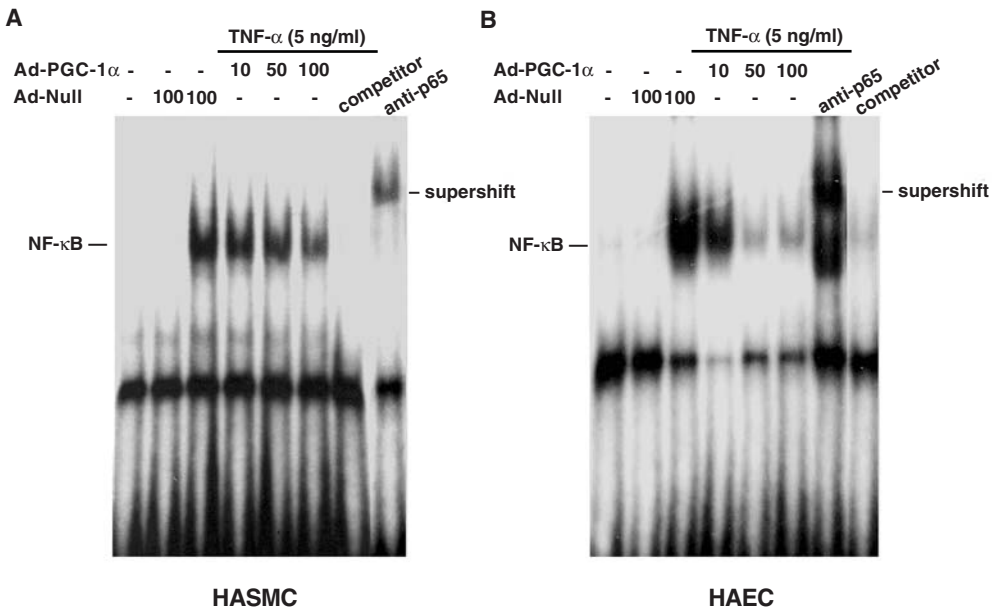

FIG. 5. Effects of Ad-PGC-1α on NF-κB binding activity. HASMCs (**A**) and HAECs (**B**) were infected with indicated doses (MOI) of Ad-PGC-1α or Ad-Null for 2 h and treated with TNF-α (5 ng/ml) for 24 h. Nuclear extracts were prepared, as described in Materials and Methods, and protein (6 μg) was incubated with radiolabeled NF-κB probe.

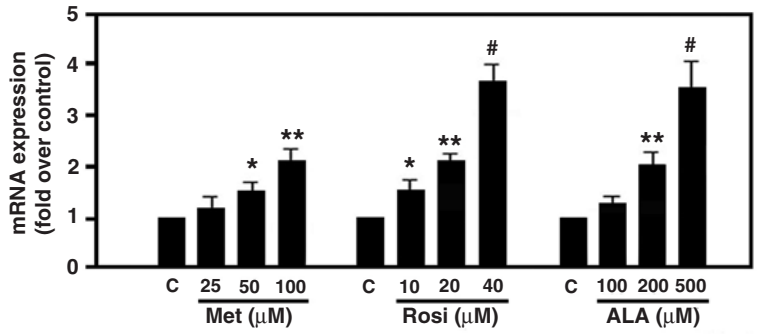

FIG. 6. Effects of metformin, rosiglitazone, and α-lipoic acid on PGC-1α mRNA expression in HASMCs. HASMCs were treated with the indicated doses of metformin, rosiglitazone, or α-lipoic acid for 24 h. PGC-1α mRNA expression was quantified by using real-time RT-PCR (n = 5). C, control; Met, metformin; rosi, rosiglitazone; ALA, α-lipoic acid. Data are expressed as mean ± SEM of three separate measurements. Statistical significance was determined as $*p < 0.05$, $**p < 0.01$, and $\#p < 0.001$, compared with control (basal expression and normalizes hyperglycemia-induced mitochondrial ROS production via AMPK activation. The present study showed that agents that activate AMPK, metformin, rosiglitazone, and α-lipoic acid additionally enhance PGC-1α expression in vascular smooth muscle cells. Although possibly another pathway exists by which metformin, rosiglitazone, or α-lipoic acid inhibits TNF-α–induced activation of NF-κB and expression of MCP-1 and VCAM-1 (18), the present data support one possible mechanism by which these AMPK activators prevent vascular inflammation.

In summary, we show that adenovirus-mediated transfer of PGC-1α inhibits TNF-α–induced NF-κB activation as well as MCP-1 and VCAM-1 expression in both HASMCs and HAECs. Based on the results, we propose that agents stimulating PGC-1α expression in the vasculature aid in preventing the development of atherosclerosis.

stimulates ROS generation from mitochondria (10, 13, 14) and plasma membrane NAD(P)H oxidase (21). In accordance with previous data, we showed that TNF-α induces ROS from both mitochondria and NAD(P)H oxidase. PGC-1α in vascular endothelial cells increases mitochondrial antioxidant gene expression and reduces ROS generation (26). As expected, overexpression of PGC-1α was associated with inhibition of TNF-α–induced mitochondrial ROS. Moreover, PGC-1α partially but significantly decreased TNF-α–increased NAD(P)H oxidase activity. The mechanism by which PGC-1α decreases NAD(P)H oxidase activity in HASMCs and HAECs remains to be established. However, our current findings are consistent with previous data showing that overexpression of UCP-2 in HASMCs decreases NADPH oxidase activity (22). Additionally, a recent publication shows that PGC-1α in vascular cells increases UCP-2 levels (26). Therefore, the effect of PGC-1α on NAD(P)H oxidase activity may be partly explained by the increase in UCP-2 expression in the presence of PGC-1α.

NF-κB, a major transcription factor in the development of atherosclerosis, is activated by intracellular ROS induced by various stimuli. NF-κB activity in vascular cells is associated with the activation of genes responsible for increased transcription of adhesion molecules, cytokines, and chemokines (3, 4, 23, 25). Previously, we demonstrated that inhibition of intracellular ROS production by UCP-2 in HAECs blocked lysophosphatidylcholine and linoleic acid–induced NF-κB activation (19). Because UCP-2 is a target gene of PGC-1α in vascular cells (26), these data raise the possibility that PGC-1α inhibits NF-κB activation, which is stimulated by ROS. To date, no reports on the relation between PGC-1α and NF-κB activation and chemokine expression induced by TNF-α have been documented. Here, we show that PGC-1α expression in vascular cells leads to the inhibition of TNF-α–induced NF-κB activation and MCP-1 and VCAM-1 expression. These findings collectively suggest that upregulation of PGC-1α prevents TNF-α–induced vascular inflammation by reducing intracellular oxidative stress and NF-κB activation.

Several lines of evidence disclose that metformin, rosiglitazone, and α-lipoic acid inhibit vascular inflammatory molecule expression, including MCP-1 and VCAM-1 (5, 18, 27). In addition, metformin, rosiglitazone, and α-lipoic acid activate AMPK (11, 15, 28). Recently we demonstrated that α-lipoic acid activates AMPK in HAECs and prevents endothelial dysfunction (20). More recently, Kukidome *et al*. (16) demonstrated that metformin increases PGC-1α gene expression and normalizes hyperglycemia-induced mitochondrial ROS production via AMPK activation. The present study showed that agents that activate AMPK, metformin, rosiglitazone, and α-lipoic acid additionally enhance PGC-1α expression in vascular smooth muscle cells. Although possibly another pathway exists by which metformin, rosiglitazone, or α-lipoic acid inhibits TNF-α–induced activation of NF-κB and expression of MCP-1 and VCAM-1 (18), the present data support one possible mechanism by which these AMPK activators prevent vascular inflammation.

In summary, we show that adenovirus-mediated transfer of PGC-1α inhibits TNF-α–induced NF-κB activation as well as MCP-1 and VCAM-1 expression in both HASMCs and HAECs. Based on the results, we propose that agents stimulating PGC-1α expression in the vasculature aid in preventing the development of atherosclerosis.

ACKNOWLEDGMENTS

This work was supported by Korea Science & Engineering Foundation through NRL Program (M10600000271-06J000-27110), Brain Korea 21 Project in 2006, and the grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (00-PJ3-PG6-GN07-0001).

ABBREVIATIONS

AMPK, AMP-activated protein kinase; HAECs, human aortic endothelial cells; HASMCs; human aortic smooth muscle cells; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; PGC-1α, peroxisome proliferator activated receptor γ coactivator-1α; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; UCP-2, uncoupling protein-2.

REFERENCES

1. Ahn JD, Morishita R, Kaneda Y, Kim HS, Chang YC, Lee KU, Park JY, Lee HW, Kim YH, and Lee IK. Novel E2F decoy oligodeoxynucleotides inhibit in vitro vascular smooth muscle cell proliferation and in vivo neointimal hyperplasia. *Gene Ther* 9: 1682–1692, 2002.
2. Parks JL, McQuillan JJ, and Iademarco MF. TNF-alpha and IL-4 synergistically increase vascular cell adhesion molecule-1 expres-

sion in cultured vascular smooth muscle cells. *J Immunol* 159: 4532–4538, 1997.
3. Bourcier T, Sukhova G, and Libby P. The nuclear factor kappa-B signaling pathway participates in dysregulation of vascular smooth muscle cells in vitro and in human atherosclerosis. *J Biol Chem* 272: 15817–15824, 1997.
4. Bustos C, Hernandez-Presa MA, Ortego M, Tunon J, Ortega L, Perez F, Diaz C, Hernandez G, and Egido J. HMG-CoA reductase inhibition by atorvastatin reduces neointimal inflammation in a rabbit model of atherosclerosis. *J Am Coll Cardiol* 32: 2057–2064, 1998.
5. Caballero AE, Delgado A, Aguilar-Salinas CA, Herrera AN, Castillo JL, Cabrera T, Gomez-Perez FJ, and Rull JA. The differential effects of metformin on markers of endothelial activation and inflammation in subjects with impaired glucose tolerance: a placebo-controlled, randomized clinical trial. *J Clin Endocrinol Metab* 89: 3943–3948, 2004.
6. Cai H and Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 87: 840–844, 2000.
7. Choi YK, Kim YJ, and Park HS. Suppression of glomerulosclerosis by adenovirus-mediated IL-10 expression in the kidney. *Gene Ther* 10: 559–568, 2003.
8. Dhalla NS, Temsah RM, and Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertens* 18: 655–673, 2000.
9. Dzau VJ, Braun-Dullaeus RC, and Sedding DG. Vascular proliferation and atherosclerosis: new perspectives and therapeutic strategies. *Nat Med* 8: 1249–1256, 2002.
10. Fiers W, Beyaert R, Declercq W, and Vandenabeele P. More than one way to die: apoptosis, necrosis and reactive oxygen damage. *Oncogene* 18: 7719–7730, 1999.
11. Fryer LG, Parbu-Patel A, and Carling D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. *J Biol Chem* 277: 25226–25232, 2002.
12. Guha M, Bai W, Nadler JL, and Natarajan R. Molecular mechanisms of tumor necrosis factor alpha gene expression in monocytic cells via hyperglycemia-induced oxidant stress-dependent and -independent pathways. *J Biol Chem* 275:17728–17739, 2000.
13. Hennet T, Richter C, and Peterhans E. Tumour necrosis factor-α induces superoxide anion generation in mitochondria of L929 cells. *Biochem J* 289: 587–592. 1993.
14. Hughes G, Murphy MP, and ledgerwood EC. Mitochondrial reactive oxygen species regulate the temporal activation of nuclear factor κB to modulate tumour necrosis factor-induced apoptosis: evidence from mitochondria-targeted antioxidants. *Biochem J* 389: 83–89, 2005.
15. Kim MS, Park JY, Namkoong C, Jang PG, Ryu JW, Song HS, Yun JY, Namgoong IS, Ha J, Park IS, Lee IK, Viollet B, Youn JH, Lee HK, and Lee KU. Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nat Med* 10: 681–682, 2004.
16. Kukidome D, Nishikawa T, Sonoda K, Imoto K, Fujisawa K, Yano M, Motoshima H, Taguchi T, Matsumura T, and Araki E. Activation of AMP-Activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells *Diabetes* 55: 120–127, 2006.
17. Lee HS, Son SM, Kim YK, Hong KW, and Kim CD. NAD(P)H oxidase participates in the signaling events in high glucose-induced proliferation of vascular smooth muscle cells. *Life Sci* 72: 2719–2730, 2003.
18. Lee KM, Park KG, Kim YD, Lee HJ, Kim HT, Cho WH, Kim HS, Han SW, Koh GY, Park JY, Lee KU, and Lee IK. Alpha-lipoic acid inhibits fractalkine expression and prevents neointimal hyperplasia after balloon injury in rat carotid artery. *Atherosclerosis* (in press).
19. Lee KU, Lee IK, Han J, Song DK, Kim YM, Song HS, Kim HS, Lee WJ, Koh EH, Song KH, Han SM, Kim MS, Park IS, and Park JY. Effects of recombinant adenovirus-mediated uncoupling protein 2 overexpression on endothelial function and apoptosis. *Circ Res* 96: 1200–1207, 2005.
20. Lee WJ, Lee IK, Kim HS, Kim YM, Koh EH, Won JC, Han SM, Kim MS, Jo IH, Oh GT, Park IS, Youn JH, Park SW, Lee KU, and Park JY. Alpha-lipoic acid prevents endothelial dysfunction in obese rats via activation of AMP-activated protein kinase. *Arterioscler Thromb Vasc Biol* 25: 2488–2494, 2005.
21. Meier B, Radeke HH, Selle S, Younes M, Sies H, Resch K, and Habermehl GG. Human fibroblasts release reactive oxygen species in response to interleukin-1 or tumour necrosis factor-α. *Biochem J* 263: 539–545, 1989.
22. Park JY, Park KG, Kim HJ, Kang HG, Ahn JD, Kim HS, Kim YM, Son SM, Kim IJ, Kim YK, Kim CD, Lee KU, and Lee IK. The effects of the overexpression of recombinant uncoupling protein 2 on proliferation, migration and plasminogen activator inhibitor 1 expression in human vascular smooth muscle cells. *Diabetologia* 48: 1022–1028, 2005.
23. Richimod A. NF-κB, chemokine gene transcription and tumor growth. *Immunology* 2: 664–674, 2002.
24. Ross R: Rous-Whipple award lecture. Atherosclerosis: a defense mechanism gone awry. *Am J Pathol* 143: 987–1002, 1993.
25. St-Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, and Spiegelman BM. Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells. *J Biol Chem* 278: 26597–26603, 2003.
26. Vallea I, Alvarez-Barrientosb A, Arzab E, Lamas S, and Monsalve M. PGC-1α regulates the mitochondrial antioxidant defense system in vascular endothelial cells. *Cardiovasc Res* 66: 562–573, 2005.
27. Zhang WJ and Frei B. Alpha lipoic acid inhibits TNF-alpha-induced NF-kappaB activation and adhesion molecule expression in human aortic endothelial cells. *FASEB J* 15: 2423–2432, 2001.
28. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, and Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108: 1167–1174, 2001.

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Date of first submission to ARS Central, September 17, 2006; date of acceptance, September 26, 2006.

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5. Antero Salminen, Kai Kaarniranta. 2011. AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. *Ageing Research Reviews* . [CrossRef]
6. Ian S. Patten, Zolt Arany. 2011. PGC-1 coactivators in the cardiovascular system. *Trends in Endocrinology & Metabolism* . [CrossRef]
7. Chan-Jung Liang, Shu-Huei Wang, Yung-Hsiang Chen, Shih-Sheng Chang, Tong-Long Hwang, Yann-Lii Leu, Ying-Chih Tseng, Chi-Yuan Li, Yuh-Lien Chen. 2011. Viscolin reduces VCAM-1 expression in TNF-#-treated endothelial cells via the JNK/NF-#B and ROS pathway. *Free Radical Biology and Medicine* **51**:7, 1337-1346. [CrossRef]
8. Qiong Yuan, Lei Chen, Da-Xiong Xiang, Yuan-Jian Li, Chang-Ping Hu. 2011. Effect of resveratrol derivative BTM-0512 on high glucose-induced dysfunction of endothelial cells: role of SIRT1. *Canadian Journal of Physiology and Pharmacology* **89**:10, 713-722. [CrossRef]
9. Antero Salminen, Juha M. T. Hyttinen, Kai Kaarniranta. 2011. AMP-activated protein kinase inhibits NF-#B signaling and inflammation: impact on healthspan and lifespan. *Journal of Molecular Medicine* **89**:7, 667-676. [CrossRef]
10. Sergey Dikalov. 2011. Cross talk between mitochondria and NADPH oxidases. *Free Radical Biology and Medicine* . [CrossRef]
11. Wei Xu, Ting Guo, Yan Zhang, Xiaohong Jiang, Yongxian Zhang, Ke Zen, Bo Yu, Chen-Yu Zhang. 2011. The inhibitory effect of dexamethasone on platelet-derived growth factor-induced vascular smooth muscle cell migration through up-regulating PGC-1# expression. *Experimental Cell Research* **317**:8, 1083-1092. [CrossRef]
12. S. Stein, C. Lohmann, N. Schafer, J. Hofmann, L. Rohrer, C. Besler, K. M. Rothgiesser, B. Becher, M. O. Hottiger, J. Boren, M. W. McBurney, U. Landmesser, T. F. Luscher, C. M. Matter. 2010. SIRT1 decreases Lox-1-mediated foam cell formation in atherogenesis. *European Heart Journal* **31**:18, 2301-2309. [CrossRef]
13. Joseph A. Baur. 2010. Biochemical effects of SIRT1 activators. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1804**:8, 1626-1634. [CrossRef]
14. Yun Jung Lee, Mi Kyoung Moon, Sun Mi Hwang, Jung Joo Yoon, So Min Lee, Kwan Soo Seo, Jin Sook Kim, Dae Gill Kang, Ho Sub Lee. 2010. Anti-Inflammatory Effect of Buddleja officinalis on Vascular Inflammation in Human Umbilical Vein Endothelial Cells. *The American Journal of Chinese Medicine* **38**:03, 585-598. [CrossRef]
15. Hua Lu, Ji-Xin Shi, Dong-Mei Zhang, Jie Shen, Yi-Xing Lin, Chun-Hua Hang, Hong-Xia Yin. 2009. Hemolysate-induced Expression of Intercellular Adhesion Molecule-1 and Monocyte Chemoattractant Protein-1 Expression in Cultured Brain Microvascular Endothelial Cells via Through ROS-dependent NF-#B Pathways. *Cellular and Molecular Neurobiology* **29**:1, 87-95. [CrossRef]
16. Yan Zhang, Weiwei Xu, Xiaoyu Li, Yibo Tang, Peng Xie, Yong Ji, Leming Fan, Qi Chen. 2008. ASSOCIATION BETWEEN PPARGC1A GENE POLYMORPHISMS AND CORONARY ARTERY DISEASE IN A CHINESE POPULATION. *Clinical and Experimental Pharmacology and Physiology* **35**:10, 1172-1177. [CrossRef]
17. Won Sun Park, Eun A. Ko, In Duk Jung, Youn Kyoung Son, Hyoung Kyu Kim, Nari Kim, So Youn Park, Ki Whan Hong, Yeong-Min Park, Tae-Hoon Choi, Jin Han. 2008. APE1/Ref-1 promotes the effect of Angiotensin II on Ca2+-activated K+ channel in human endothelial cells via suppression of NADPH Oxidase. *Archives of Pharmacal Research* **31**:10, 1291-1301. [CrossRef]
18. Meei Ling Sheu, Chih Kang Chiang, Keh Sung Tsai, Feng Ming Ho, Te I Weng, Hsiao Yi Wu, Shing Hwa Liu. 2008. Inhibition of NADPH oxidase-related oxidative stress-triggered signaling by honokiol suppresses high glucose-induced human endothelial cell apoptosis. *Free Radical Biology and Medicine* **44**:12, 2043-2050. [CrossRef]

19. Domokos Gerö, Csaba Szabó. 2008. Poly(ADP-ribose) polymerase: a new therapeutic target?. *Current Opinion in Anaesthesiology* **21**:2, 111-121. [CrossRef]
20. Dr. Eiichi Araki , Jun-Ichi Miyazaki . 2007. Metabolic Disorders in Diabetes Mellitus: Impact of Mitochondrial Function and Oxidative Stress on Diabetes and Its Complications. *Antioxidants & Redox Signaling* **9**:3, 289-291. [Citation] [Full Text PDF] [Full Text PDF with Links]
21. Won Gu Jang, In-Kyu Lee, Eun Jung Kim, Seong Yeol Ryu, Bo-Wan Kim, Jung-Guk Kim. 2007. Transcriptional Regulation of Insulin and CXCL10 Gene by Peroxisome Proliferator Activated Receptor # Coactivator-1#. *The Journal of Korean Diabetes Association* **31**:4, 326. [CrossRef]