The Ligands/Activators for Peroxisome Proliferator-Activated Receptor α (PPARα) and PPARγ Increase Cu^{2+},Zn^{2+}-Superoxide Dismutase and Decrease p22phox Message Expressions in Primary Endothelial Cells

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We examined the effects of a variety of ligands/activators of the peroxisome proliferator-activated receptor (PPAR) on the expression of the superoxide scavenger enzyme, Cu^{2+},Zn^{2+}-superoxide dismutase (CuZn-SOD), and the superoxide generating enzyme nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) oxidase in primary cultures of human umbilical vein endothelial cells (HUVEC) and human aorta endothelial cells (HAEC). Our data show that 3 types of PPARs, PPARα, PPARβ/δ/Nuc1, and PPARγ are expressed in endothelial cells. Bezafibrate, which is a ligand/activator for PPARα, increased the CuZn-SOD gene expression and protein levels in endothelial cells. Troglitazone and pioglitazone, which are ligands/activators for PPARγ, also induced PPARα gene and protein expression and increased CuZn-SOD gene expression and protein levels in addition to increasing PPARγ gene and protein expression in endothelial cells. Moreover, with treatment of monounsaturated and polyunsaturated fatty acids (PUFA), the CuZn-SOD mRNA levels were positively correlated with PPARα mRNA levels ($r = .872$, $P < .0001$) in primary endothelial cells. In addition, the phorbol myristate acetate (PMA)-stimulated or PMA-nonstimulated 22-kd a-subunit (p22phox) mRNA levels and 47-kd a-subunit (p47phox) protein levels in NADPH oxidase were decreased by treatment with PPARα and PPARγ ligands/activators. These results suggest that PPARα and PPARγ gene and protein expression in endothelial cells may play a physiologic role as radical scavengers, although the details of these mechanisms remain to be established.

THE PEROXISOME proliferator-activated receptor (PPAR) is a member of the steroid hormone receptor superfamily.[1] Three types of PPARs have been described in rodents, humans, and amphibians: PPARα, Nuc1 (also called PPARβ or PPARδ), and PPARγ. PPARα has been reported to be expressed in the liver, retina, digestive mucosa, proximal tubules of kidney, heart, muscle, and brown adipose tissue.[2] However, PPARβ/δ/Nuc1 is ubiquitously expressed.[3] PPARβ has been identified in amphibians, PPARδ was isolated in mice, and Nuc1 was identified in mammals. PPARγ is a transcription factor selectively expressed in adipose tissues[4] and seems to be associated with differentiation of adipocytes.

PPARs are differentially activated by a variety of fatty acids. PPARα is activated by polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA), and a variety of hypolipidemic fibrates such as bezafibrate,[5] clofibrate, fenofibrate,[6] and gemfibrozil.[7] PPARγ is activated by 15-deoxy-delta 12, delta 14 prostaglandin J2 (PGJ2)[8,9] and a variety of hypoglycemic thiazolidinediones, such as troglitazone,[10] and pioglitazone.[11] However, PPARβ/δ/Nuc1-selective ligands have not yet been identified.

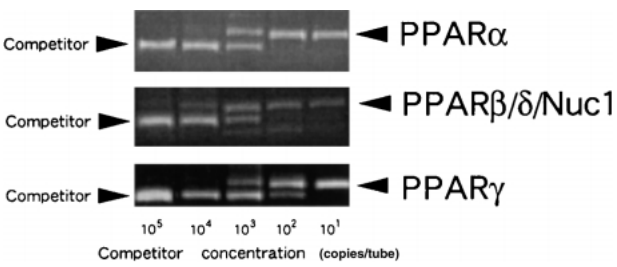

Fig 1. Expression of PPARα (lane 1), PPARβ/δ/Nuc1 (lane 2), and PPARγ (lane 3) mRNA by quantitative RT-PCR in HUVEC with 10% FBS.

Recently, it has been reported that PPARα affects the duration of inflammation induced by leukotriene B4/arachidonic acid.[12] Moreover, it has been reported that PPARγ is expressed at high levels in peritoneal macrophages.[13] These findings suggest that PPARα and PPARγ may play a role in atherosclerotic and inflammatory events, as well as in pathophysiologic alterations of lipid and glucose metabolisms.

Atherosclerosis and inflammation involve a variety of cells including endothelial cells, vascular smooth muscle cells, monocytes, neutrophils, lymphocytes, and platelets. Among these, endothelial cells play a crucial role in the regulation of platelet function, coagulation, and vascular tone, and they initiate the inflammatory response. Interestingly, endothelial dysfunction occurs at an incipient stage of atherosclerosis, particularly when cardiovascular risk factors such as hyperlipidemia, hypertension, and diabetes are present. Recently, we reported that PPARα is expressed in endothelial cells and regulated by hormones, such as dexamethasone and insulin.[14] Moreover, we have found that PPARα is associated with the expression of Cu^{2+},Zn^{2+}-superoxide dismutase (CuZn-SOD),[15] which scavenges reactive oxygen species (ROS), which can be produced by nicotinamide adenine dinucleotide phosphate (reduced form)

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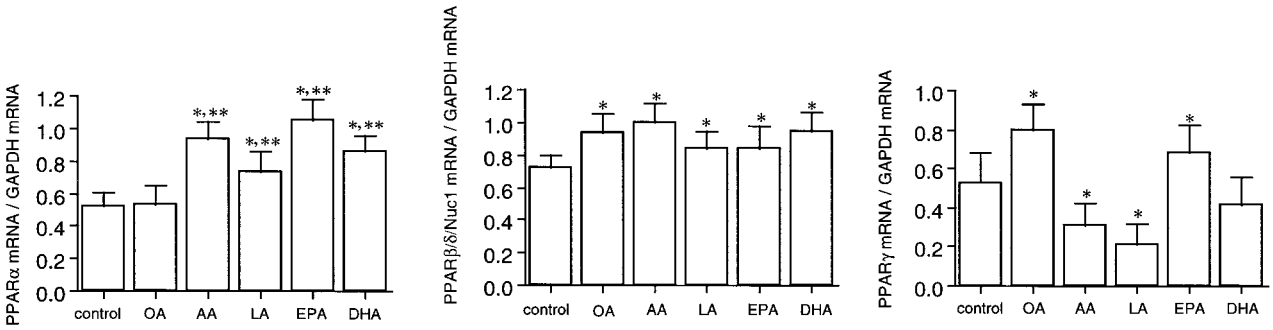


Fig 2. PPARα, PPARβ/δ/Nuc1, and PPARγ mRNA expression after treatment of endothelial cells (HUVEC) with 10% FBS plus 30 μmol/L OA, 30 μmol/L AA, 30 μmol/L LA, 30 μmol/L EPA, or 30 μmol/L DHA. The experiment was performed in triplicate, and 4 independent experiments were performed. All data are normalized by GAPDH levels, and data are the mean ± SD. *$P < .05$ *v* control; **$P < .05$ *v* OA.

(NADPH) oxidase, resulting in the reduction of plasma thiobarbituric acid-reactive substance (TBARS) levels.[5] Oxidative stress in endothelial cells may accelerate atherosclerosis and inflammation.

The purpose of this study was to explore the possibility that the enzyme mRNA and protein levels of CuZn-SOD or NADPH oxidase may change in response to the ligands/activators for PPARα and PPARγ in endothelial cells.

MATERIALS AND METHODS

Primary cultures of human umbilical vein endothelial cells (HUVEC) (lot #31091) and primary cultures of human hepatocytes (HUH) (lot #RI-3716) were purchased from Cell Systems (Kirkland, WA). In addition, human aorta endothelial cells (HAEC) were obtained from 3 commercial sources (lot #RI-375, Cell Systems; lot #14319 and lot #6F0741, Clonetics, San Diego, CA). The endothelial cells were checked for their ability to produce factor VIII-related antigen by indirect immunofluorescence microscopy, and all cells were identified as endothelial cells. Cell cultures were maintained in 25-cm^2 flasks in a commercial medium: CS-4ZO-500, Dainippon Pharmaceutical, supplemented with 10% fetal bovine serum (FBS), HEPES (15 mmol/L), acidic-fibroblast growth factor (FGF), and heparin in an atmosphere of 5% CO_2 and 95% air at 37°C. Some HUVEC were also incubated without FBS. At confluence, the cell population was divided after detachment from the flasks with 0.24% EDTA. The medium was changed twice weekly. Most of cultures were used within 3 weeks, at the third to fifth passage. The tissue samples were immediately frozen in liquid nitrogen and were stored at −80°C until extraction of total RNA.

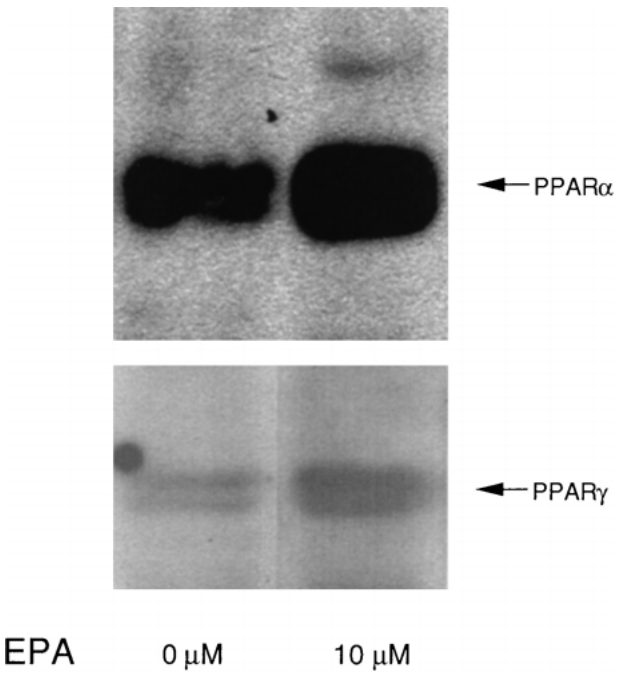


Fig 3. The expression levels of PPARα and PPARγ protein by Western analysis in endothelial cells (HUVEC) treated with or without EPA for 6 hours. The experiment was performed in triplicate, and 4 independent experiments were performed.

Cell Treatments

Oleic acid (OA) (C18:1, ω-9), arachidonic acid (AA) (C20:4, ω-6), linoleic acid (LA) (C18:2, ω-6), EPA (C20:5, ω-3) or docosahexaenoic acid (DHA) (C22:6, ω-3) was added at a final concentration of 10 μmol/L, 20 μmol/L, 30 μmol/L, 100 μmol/L, or 300 μmol/L, respectively, to the cultured endothelial cells. The medium plus the above-mentioned fatty acids was replaced with fresh medium every day. After the cells were incubated with the above-mentioned fatty acids for 6 hours, 12 hours, 24 hours, and 48 hours, the cells were removed and assayed for the enzyme activity, mRNA, and protein expression. Troglitazone or pioglitazone, which was dissolved in 0.1% dimethylsulfoxide (DMSO), was used at a final concentration of 10 μmol/L or 30 μmol/L. Troglitazone was a generous gift from Sankyo, Tokyo, Japan and pioglitazone, a generous gift from Takeda, Osaka, Japan. Bezafibrate, which was used as sodium-bezafibrate, was used at a final concentration of 2 μmol/L, 10 μmol/L, or 30 μmol/L. Sodium-bezafibrate was kindly provided by Kissei Pharmaceutical, Matsumoto, Japan. The cells were incubated with the above-mentioned drugs for 6 hours, 12 hours, and 24 hours.

Reverse Transcriptase-Polymerase Chain Reaction

Preliminary experiments had shown that PPARα, PPARβ/δ/Nuc1, and PPARγ mRNA expression was low, and that accurate measurements would not be possible by Northern analysis. We therefore performed reverse transcriptase-polymerase chain reaction (RT-PCR) to examine PPARα, PPARβ/δ/Nuc1, and PPARγ expres-

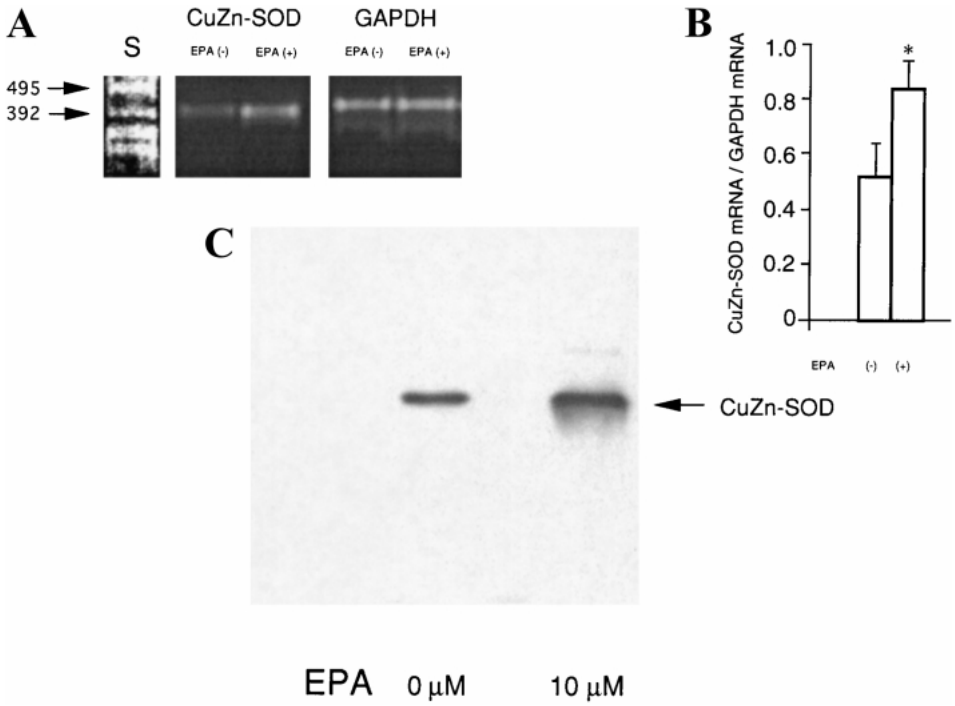


Fig 4. (A) Expression of CuZn-SOD and GAPDH mRNA for 24 hours in endothelial cells (HUVEC). Four independent experiments were performed in triplicate. Values are expressed as relative expression to GAPDH mRNA. (B) Mean ± SD data. *$P < .05$ *v* control. (C) Change in CuZn-SOD protein levels by Western analysis after treatment with EPA for 24 hours in HUVEC.

sion levels, and some experiments were performed by quantitative RT-PCR. Total RNA, which was isolated from HUVEC or HAEC (10^7 cells) using a commercial kit (Isogen; Nippon Gene, Toyama, Japan), was used as a template for DNA synthesis using oligo (dT) primer and a DNA cycle kit (GeneAmp RNA PCR Kit; Perkin Elmer, Foster City, CA) according to the manufacturer's instructions. The RT reaction was performed at 42°C for 15 minutes to maximize cDNA synthesis and was terminated by heating at 99°C for 5 minutes. The resulting cDNA was used as the template for PCR.

Oligonucleotide primers for PPARα RT-PCR were designed to amplify partial cDNA sequences. The synthetic oligonucleotides were obtained from Nippon Flour Mills, Kanagawa, Japan. The primers used for PPARα were 5′-AGA ACT TCA ACA TGA ACA AGG TCA-3′ for the forward primer and 5′-GCC AGG ACG ATC TCC ACA GCA AAT-3′ for the reverse primer. The primers used for PPARβ/δ/Nuc1 were 5′-AGC AGC CTC TTC CTC AAC GAC CAG-3′ for the forward primer and 5′-GGT CTC GGT TTC GGT CTT CTT GAT-3′ for the reverse primer. The primers used for PPARγ were 5′-CCC TCA TGG CAA TTG AAT GTC GTG-3′ for the forward primer and 5′-TCG CAG GCT CTT TAG AAA CTC CCT-3′ for the reverse primer. The primers used for CuZn-SOD were 5′-GGC GTC ATT CAC TTC GAG CAG AAG-3′ for the forward primer and 5′-GGC AAT CCC AAT CAC ACC ACA AGC-3′ for the reverse primer. The primers used for 22-kd a-subunit (p22phox) of NADPH oxidase were 5′-GTT TGT GTG CCT GCT GGA GT-3′ for the forward primer and 5′-TGG GCG GCT GCT TGA TGG T-′ for the reverse primer. Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) mRNA expression was determined as a control.

PCR reactions were performed using the same temperatures for denaturation (94°C, 30 seconds) and extension (72°C, 90 seconds), but an annealing temperature was 50°C for 50 seconds. The number of amplification cycles was 28, 30, 33, 34, and 36 cycles. The PCR products were separated on a 7.5% polyacrylamide gel (NPU, 7.5 type; Atto, Tokyo, Japan). DNA was visualized by ethidium bromide staining at a concentration of 10 mg/mL. The intensities of the bands that corresponded with the mRNA levels were evaluated using an ultraviolet (UV)-light box imaging system (Atto).

DNA Sequencing

Direct sequencing of the RT-PCR products was performed using an automated Sequencer (ABI PRISM 310 Genetic Analyzer; Perkin Elmer, Foster City, CA). All DNA sequences were confirmed by reading both DNA strands.

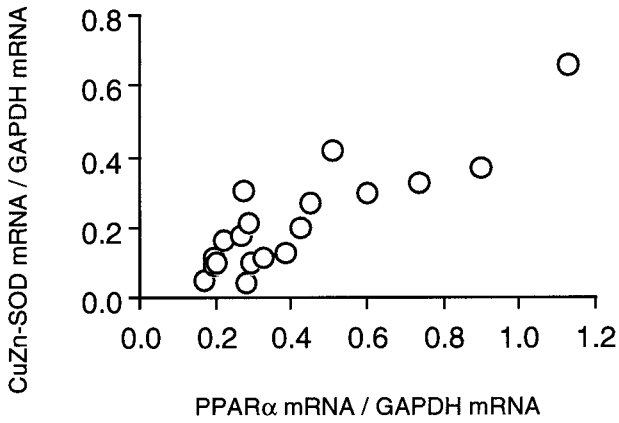


Fig 5. The relationship between expression of CuZn-SOD and PPARα in HUVECs treated with various fatty acids for 12 hours. The experiment was performed in triplicate, and 4 independent experiments were performed. The values obtained were expressed as relative expression to GAPDH mRNA. Data points represent the mean of triplicate determinants, and all points are indicated as (○).

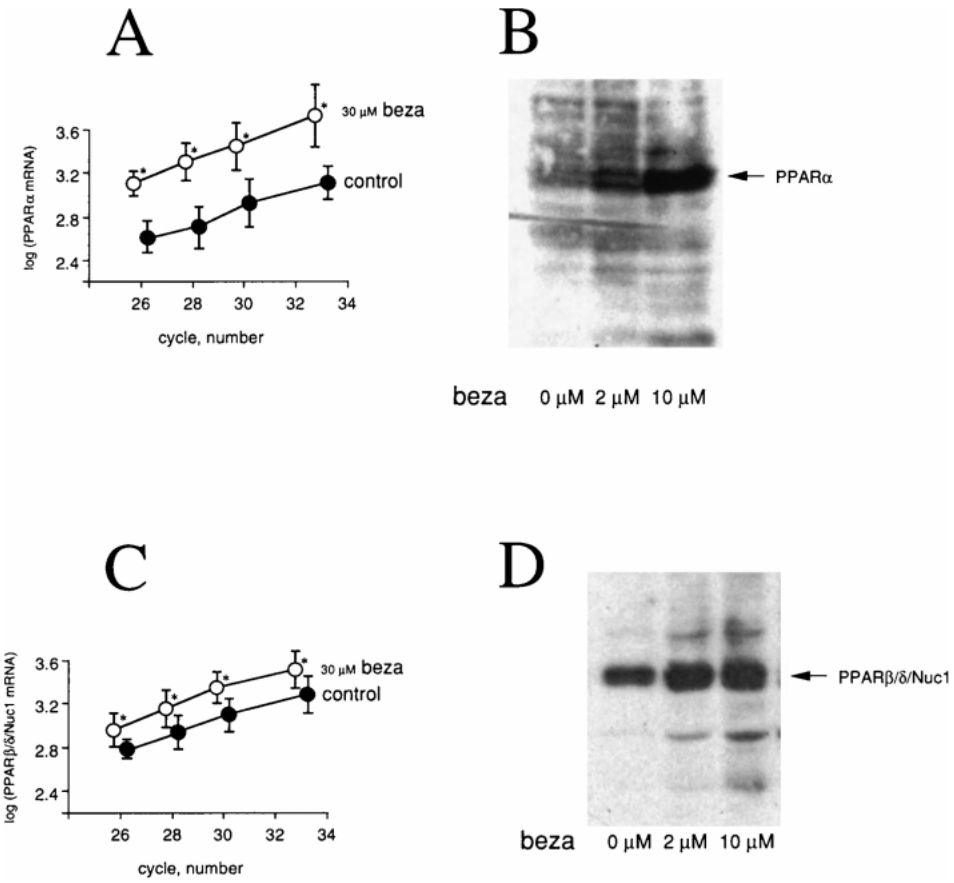


Fig 6. (A) Changes in the logarithm of PPARα mRNA levels by 30 μmol/L bezafibrate (beza) calculated at a variety of PCR cycles. (B) Changes in PPARα protein levels after treatment with 2 and 10 μmol/L beza for 6 hours. (C) Changes in the logarithm of the PPARβ/δ/Nuc1 mRNA levels by 30 μmol/L beza calculated at a variety of PCR cycles. (D) Changes in the PPARβ/δ/Nuc1 protein level after treatment with 2 and 10 μmol/L beza for 6 hours. Control indicates the change in PPARα or PPARβ/δ/Nuc1 mRNA and protein levels by Western analysis in nontreated HAEC. Four independent experiments were performed in triplicate. All data are the mean ± SD. *$P < .05$ *v* control.

Western Blotting for PPARs, CuZn-SOD, and p47phox

Western blotting was performed, and specific immunoreactivity was detected using an Amersham ECL kit (Buckinghamshire, UK). Briefly, processed samples were applied to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Millipore, Tokyo, Japan) by using semidry blotting. Membranes were treated overnight with TBS-Tween/5% dry milk and incubated with goat antihuman PPARs, CuZn-SOD, and p47phox antibodies for 1 hour. Antibody against human PPARs, CuZn-SOD, and p47phox were from Santa Cruz (Santa Cruz, CA), Binding Site (Birmingham, UK), Transduction Laboratories (Lexington, KY), respectively. After washing, membranes were incubated with horseradish peroxidase-conjugated rabbit antigoat monoclonal antibody. Antigen detection was performed with a chemiluminescence detection system.

Statistical Analysis

Parametric data are expressed as the mean ± SD. Differences between groups were evaluated by Scheffé's F test.

RESULTS

Figure 1 shows PPARα, PPARβ/δ/Nuc1, and PPARγ gene mRNA expression by quantitative RT-PCR in HUVEC treated with 10% FBS. PPARα, PPARβ/δ/Nuc1, and PPARγ gene mRNA expressions were found in HUVEC, and the gene expression of PPARγ was weak in comparison with those of PPARα and PPARβ/δ/Nuc1. Figure 2 shows PPARα, PPARβ/δ/Nuc1, and PPARγ mRNA expression after treatment of HUVEC with OA, AA, LA, EPA, or DHA during 6 hours. PPARα gene expression after treatment with AA, LA, EPA, and DHA was significantly higher than after OA treatment in HUVEC (Fig 2). PPARγ mRNA expression was significantly increased by treatment with OA and EPA compared with control (Fig 2). PPARγ mRNA expression was significantly decreased by treatment with AA or LA compared with control (Fig 2). Figure 3 shows the protein expression by Western analysis of PPARα and PPARγ in HUVECs treated with or without the EPA for 6 hours. The protein expression levels of PPARα and PPARγ were significantly increased in HUVECs treated with the EPA during 6 hours. The change of protein expression levels by OA, AA, LA, and DHA were also consistent with the mRNA level (data not shown).

Figure 4 shows the mRNA and protein expression by Western analysis of CuZn-SOD in HUVECs treated with or without the EPA for 24 hours. The mRNA (Fig 4A and B) and protein (Fig 4C) expression of CuZn-SOD were significantly increased in HUVECs treated with the EPA during 24 hours.

Figure 5 shows the relationship between mRNA expression of CuZn-SOD and PPARα in HUVECs treated with 20 μmol/L, 30 μmol/L, 100 μmol/L, or 300 μmol/L OA, AA, LA,

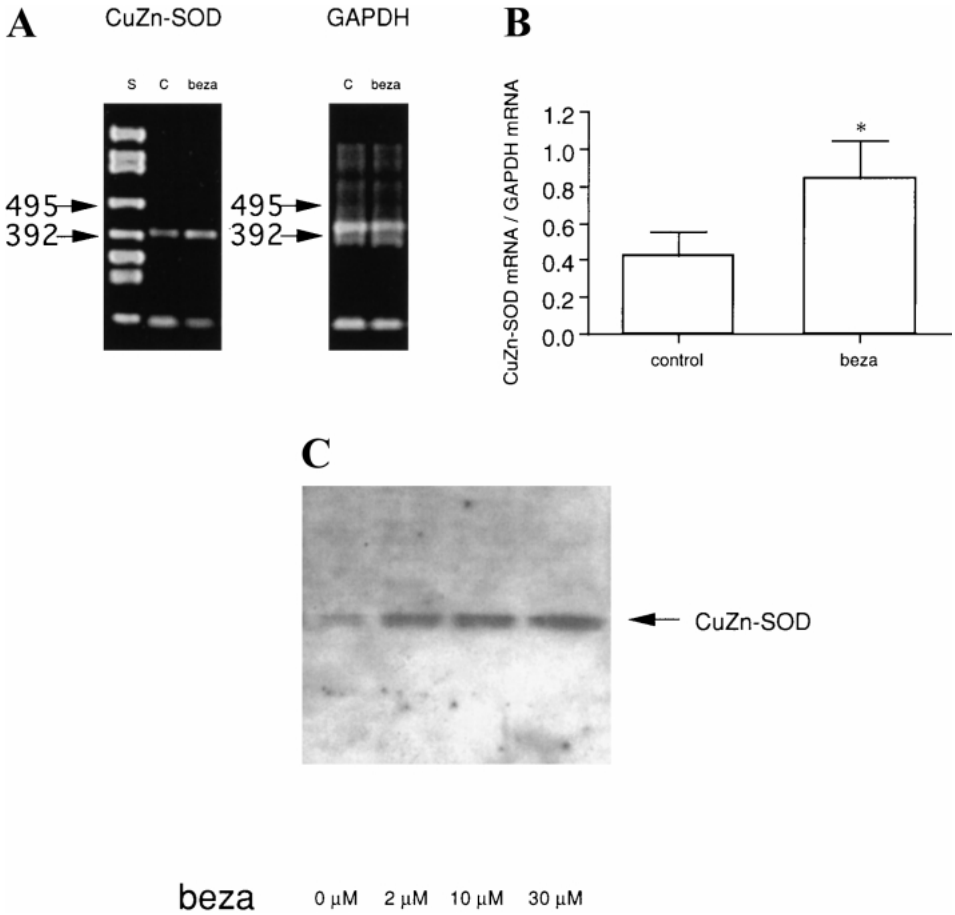


Fig 7. (A) Effect of 10 μmol/L beza on CuZn-SOD mRNA expression. Lane C indicates CuZn-SOD mRNA levels in nontreated HUVEC. (B) Four independent experiments were performed in triplicate. Data (mean ± SD) normalized by GAPDH levels. *$P < .05$ *v* control. (C) Changes in CuZn-SOD protein levels by Western analysis after treatment with 2, 10, and 30 μmol/L beza for 6 hours.

EPA, or DHA for 12 hours. PPARα was strongly associated with CuZn-SOD expression ($r = .872$, $P < .0001$) in HUVECs treated for 12 hours.

Bezafibrate significantly increased PPARα (Fig 6A) and PPARβ/δ/Nuc1 mRNA levels (Fig 6C) in medium without FBS during 6 hours. Western blotting showed an increase in PPARα (Fig 6B) and PPARβ/δ/Nuc1 (Fig 6D) protein levels in patterns consistent with mRNA levels.

Bezafibrate significantly increased CuZn-SOD mRNA expression (Fig 7A and B) and protein levels by Western analysis (Fig 7C). Troglitazone and pioglitazone also increased PPARα mRNA expression (Fig 8) and CuZn-SOD mRNA levels (Fig 9), in addition to PPARγ expression (Fig 10) in medium without FBS during 6 hours.

Figure 11 shows the expression of the 22-kd a-subunit (p22phox). Phorbol myristate acetate (PMA)-stimulated p22phox mRNA levels and treatment with bezafibrate, EPA, troglitazone, or pioglitazone attenuated p22phox mRNA expression in HAEC (Fig 11). Moreover, troglitazone and bezafibrate significantly decreased the PMA-stimulated p22phox mRNA levels in HAEC (Fig 12). To assess the p47phox protein level, which was the other subunit of NADPH oxidase, Western blotting was performed (Fig 13), and the results were consistent with the mRNA level in HAEC (data not shown).

DISCUSSION

Our present study indicates that in addition to PPARα,[14] PPARβ/δ/Nuc1 and PPARγ might be expressed in endothelial cells (Fig 1). Although PPARγ was expressed at lower levels in endothelial cells (Fig 1), expression was induced by the PUFAs (Fig 2) and thiazolidinediones (Fig 10) during 6 hours. These findings indicate that serum PUFAs and tiazolidinediones might have a direct action on endothelial cells, in addition to adipocytes, which may play a role in glucose and lipid metabolism.

Of interest is the observation that both PPARα and PPARγ ligands/activators significantly induced CuZn-SOD gene expression in cultured endothelial cells (Figs 7 and 9). Recently, we have reported that liver CuZn-SOD gene expression shows a significant positive correlation with liver PPARα mRNA levels in vivo after treatment with bezafibrate, a ligand/activator of PPARα.[15] A point to be noted in our present in vitro study is that PPARα gene expression was positively correlated with CuZn-SOD gene expression in the cultured endothelial cells (Fig 5). The expression of CuZn-SOD, which scavenges the free radicals, might be regulated by PPAR. Kim et al[16] have already cloned the 1.5-kb upstream region of the CuZn-SOD gene, demonstrat-

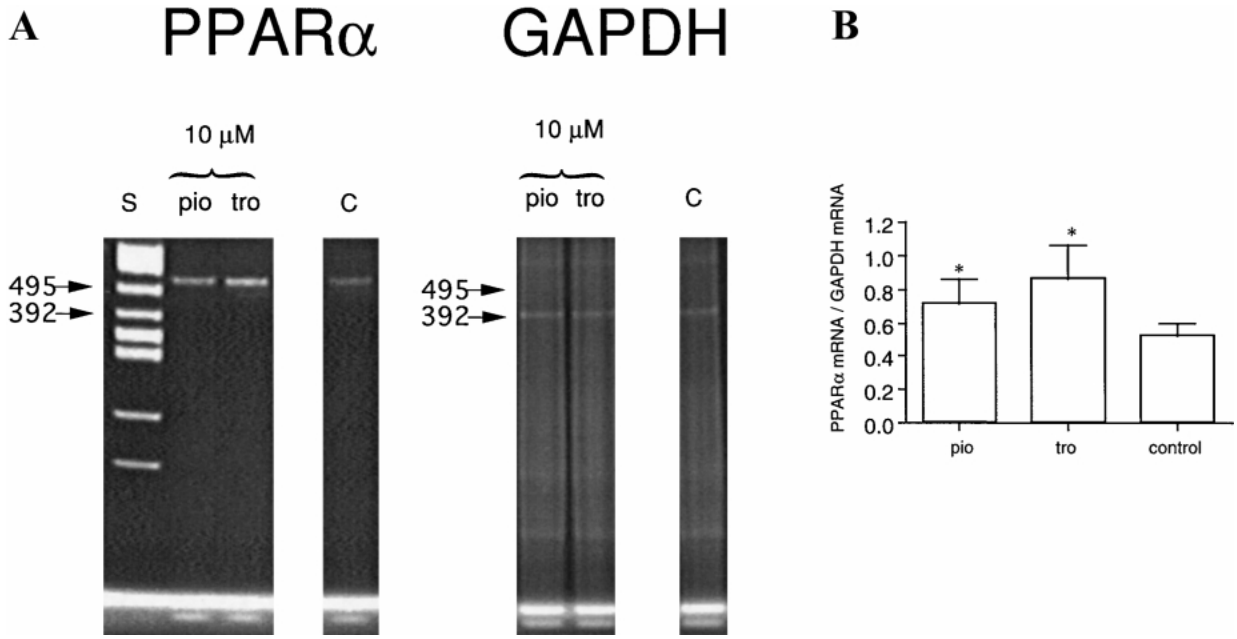


Fig 8. Changes in PPARα mRNA levels after treatment with 10 μmol/L troglitazone (tro) and 10 μmol/L pioglitazone (pio). Lane C indicates PPARα mRNA levels in nontreated HUVEC (A). The experiment was performed in triplicate, and 4 independent experiments were performed. All data are normalized by GAPDH levels, and the data are the mean ± SD (B). *P < .05 *v* control.

ing peroxisome proliferator-activated receptor response elements (PPRE) at −797 region in upstream region of the rat CuZn-SOD gene. PPARα and PPARγ ligands/activators might activate the transcription of the CuZn-SOD gene through binding to PPRE. In fact, our data indicates that CuZn-SOD expression in cultured endothelial cells is increased by the addition of ligands/activators for PPARα (Figs 3, 6, and 8) and PPARγ (Figs 3 and 10) into the culture medium. Reactive oxygen-induced free radicals play an important role as mediators of tissue injury associated with many pathologic conditions, such as inflammatory and ischemic states.

Endothelial cells themselves can release both O_2^- and H_2O_2. These ROS are metabolized to H_2O and O_2 by catalase and CuZn-SOD, which are expressed in endothelial cells, as well as in the liver. It is known that SOD selectively scavenges superoxide anions. Although it has been reported that the administration of exogenous CuZn-SOD was dose-dependently toxic to the tissue,[17] Wang et al[18] recently reported that the overexpression of endogenous CuZn-SOD dramatically prevented injury to ischemic tissue. They referred to the increased expression of CuZn-SOD in the endothelial cells of transgenic mice with overexpression of human CuZn-SOD. Moreover, Fang et al[19]

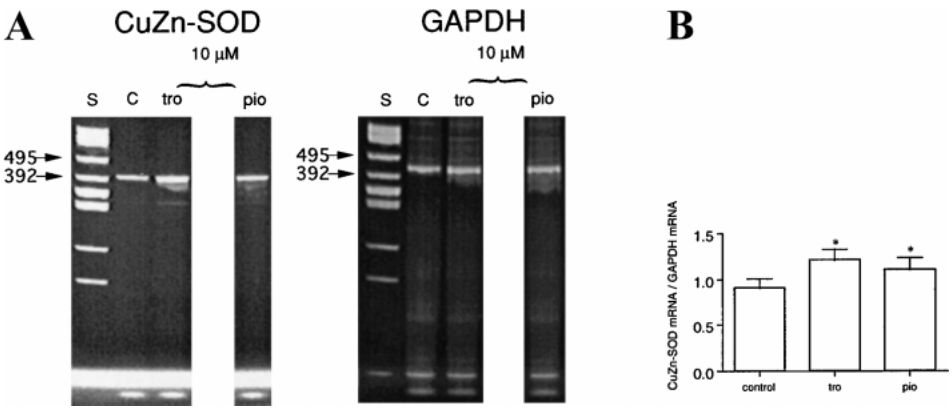
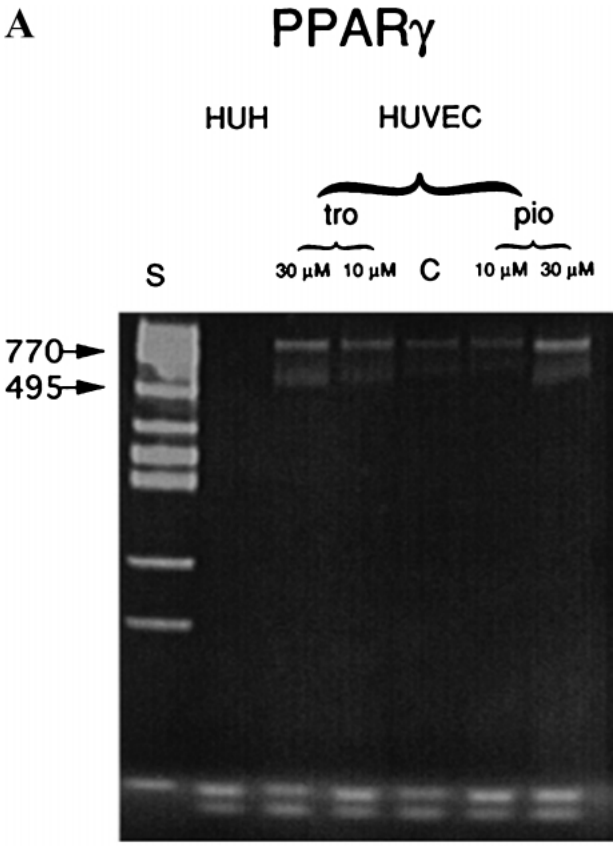


Fig 9. (A) CuZn-SOD mRNA levels after treatment with 10 μmol/L tro and 10 μmol/L pio. Lane C indicates CuZn-SOD mRNA levels in nontreated HUVEC. Four independent experiments were performed in triplicate. (B) Data (mean ± SD) normalized by GAPDH levels. *P < .05 *v* control.



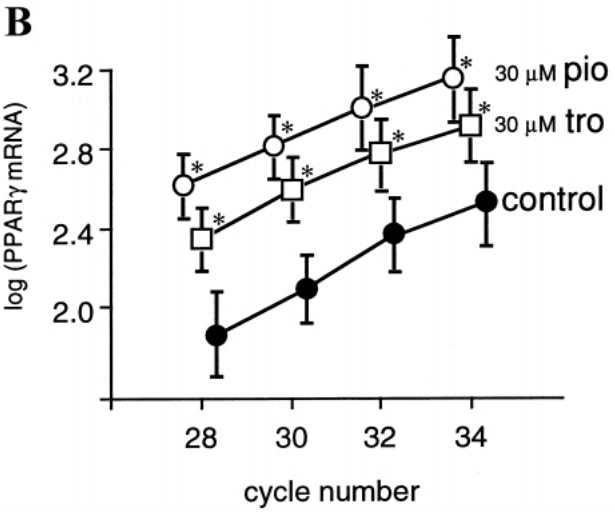


Fig 10. (A) PPARγ mRNA expression after treatment with tro (10 and 30 μmol/L) and pio (10 and 30 μmol/L). Lane C indicates PPARγ mRNA levels in nontreated HUVEC. (B) Primary HUH were used as a negative control for PPARγ expression. The changes in the logarithm of the PPARγ mRNA levels by 30 μmol/L tro or 30 μmol/L pio were calculated at a variety of PCR cycles. Control indicates the change in PPARγ mRNA levels in nontreated HUVEC. The experiment was performed in triplicate, and 4 independent experiments were performed.

also reported that the overexpression of endogenous CuZn-SOD inhibited the elevation of oxidized low-density lipoprotein by endothelial cells. Increasing endogenous CuZn-SOD activity by such a regimen might prevent atherosclerotic changes.

Moreover, it is known that SOD selectively scavenges superoxide anions and can prolong the biological half-life of nitric oxide (NO).[20,21] Davda et al[22] reported that oleic acid, which had little effect on induction of PPARα, reduces the activity of the endothelial nitric oxide synthase (NOS) in vitro. Okuda et al[23] reported that EPA, which could induce PPARα, enhances NO production from HUVEC. Although preliminary, we have found reduced nitrotyrosine content in endothelial cells treated with fibrate and thiazolidinediones. The formation of nitrotyrosine seems to be due to peroxynitrite in injured tissues. A possible mechanism of NO-induced cellular damage is through its interaction with superoxide to produce peroxynitrite, which reacts with tyrosine to form nitrotyrosine in cellular proteins.[24]

Recently, Ricote et al[13] reported that a PPARγ agonist inhibited the induction of inducible NOS (iNOS) mRNA levels in response to interferon-γ in the activated macrophage, resulting in reduction of NO production. Moreover, they have reported that PPARγ also inhibited iNOS expression by blocking the activities of the transcription factors AP-1, signal transducers and activators of transcription (STAT), and nuclear factor (NF)-κB. In contrast, Staels et al[25] reported that PPARα activator inhibited the PMA-stimulated activation of the promoter of the cyclooxygenase-2 (COX-2) gene in smooth muscle cells. COX-2 is rapidly induced upon stimulation by growth factors and cytokines. Recently, Lehmann et al[26] reported that nonsteroidal antiinflammatory drugs, which inhibit both COX-1 and COX-2, also have the effect on PPARα and PPARγ. It is clear that PPARα and PPARγ function in the inflammatory response. This potential action of PPARα and PPARγ might be due, at least in part, to induction of CuZn-SOD. PPAR is activated by a variety of fatty acids and hypolipidemic fibrates and hypoglycemic thiazolidinedione, resulting in an increase in CuZn-SOD gene expression and prevention of atherosclerosis and inflammation in endothelial cells. Moreover, our data indicate that PPARα and PPARγ ligands/activators decrease PMA-nonstimulated (Fig 11) and PMA-stimulated expression of p22phox (Fig 12), which is a critical component of NADPH oxidase.[27] Although we need to determine the activity of NADPH oxidase in addition to CuZn-SOD, the reduction of p22phox mRNA levels might be due to induction of CuZn-SOD expression by the addition of PPARα and PPARγ agonist. Alternatively, the PPARα and PPARγ agonist may have a direct effect on the reduction of p22phox mRNA levels. The above-mentioned pleiotropic function of PPARs might also operate in endothelial cells.

In summary, we have shown that PPARβ/δ/Nuc1 and PPARγ mRNA are expressed in endothelial cells, in addition to PPARα. Further studies should be performed to clarify

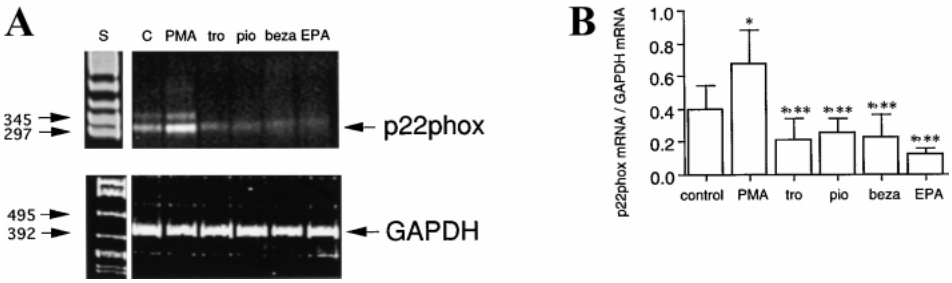


Fig 11. (A) Changes in nonstimulated p22phox mRNA levels after treatment with 30 μmol/L beza, 30 μmol/L EPA, 10 μmol/L tro, or 10 μmol/L pio. Lane C indicates control p22phox mRNA levels in nontreated HAEC. Four independent experiments were performed in triplicate. (B) Data (mean ± SD) normalized by GAPDH levels. *P < .05 v control; **P < .05 v PMA.

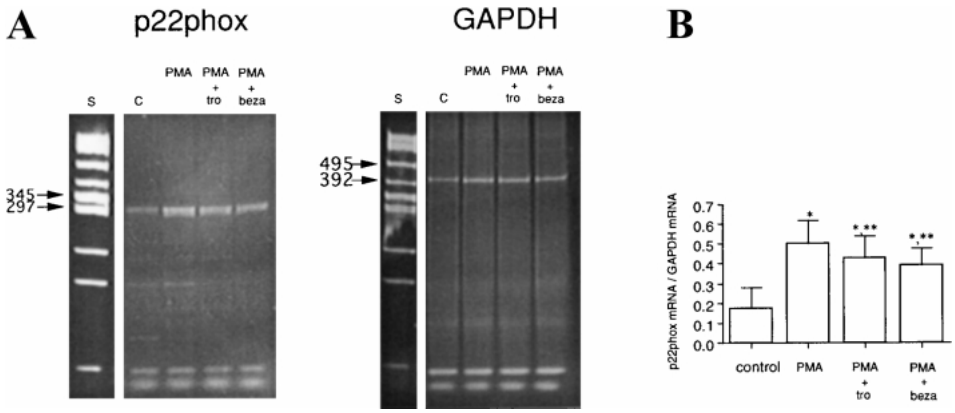


Fig 12. (A) Changes in the PMA-stimulated 22-kd a-subunit (p22phox) mRNA levels after treatment with 30 μmol/L beza or 10 μmol/L tro. Lane C indicates p22phox mRNA levels in nontreated HAEC. Four independent experiments were performed in triplicate. (B) Data (mean ± SD) normalized by GAPDH levels. *P < .05 v control; **P < .05 v PMA.

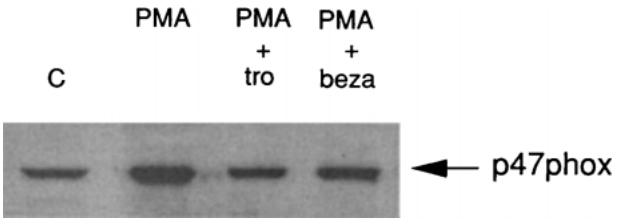


Fig 13. Changes in the PMA-stimulated 47-kd a-subunit (p47phox) protein levels by Western analysis after treatment with 30 μmol/L beza or 10 μmol/L tro. Lane C indicates p47phox mRNA levels in nontreated HAEC.

whether the PPAR genes in endothelial cells confer some benefit in preventing vascular complications. It is quite likely that PPAR activity in endothelial cells may be a regulatory factor in the pathogenesis of hyperlipidemia and atherosclerosis, inflammation,[12] coagulation, and apoptosis[28] through an increase in CuZn-SOD activity.

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