Apoptosis of Endothelial Cells may be Mediated by Genes of Peroxisome Proliferator-activated Receptor γ 1 (PPAR γ 1) and PPAR α Genes

Ikuo Inoue¹, Kenji Hayashi¹, Fumiharu Yagasaki², Koh-ichi Nakamura³, Toshiyuki Matsunaga³, Haiyuan Xu¹, Koh-ichi Inukai¹, Takuya Awata¹, Tsugikazu Komoda³, and Shigehiro Katayama¹

¹Fourth Department of Medicine, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama, Japan

²First Department of Medicine, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama, Japan

³Department of Biochemistry, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama, Japan

Apoptosis in human umbilical vein endothelial cells (HUVECs) was prevented by transfection with the gene for the human full-length peroxisome proliferator-activated receptor α (PPAR α), or acyl-coenzyme A synthetase (AcylCS) into HUVECs. In contrast, ligands/activators of PPAR γ 1 induced apoptosis by a cytochrome c-dependent mechanism in HUVECs transfected with human full-length PPAR γ 1, but not in hepatocytes. Co-transfection of PPAR γ 1 and PPAR α protected the HUVEC apoptosis. The results suggest that the apoptosis of endothelial cells may be mediated by genes of PPAR γ 1 and PPAR α . *J Atheroscler and Thromb*, 2003; 10: 99–108.

Key Words: Apoptosis, Endothelial cells, Peroxisome proliferator-activated receptor γ 1 (PPAR γ 1), PPAR α

Introduction

The peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear receptor superfamily (1). Three types of PPARs have been described in rodents, humans and amphibians: PPAR α , Nuc1 (also called PPAR β or PPAR δ), and PPAR γ . PPAR α is reportedly to be expressed most abundantly in the liver (2), and is associated with β -oxidation in the mitochondria and peroxisomes. Although PPAR β/δ /Nuc1 is expressed ubiquitously (3), its functional role remains to be clarified. PPAR α activation decreases the fatty acid content of cells through β -oxidation of fatty acids, while PPAR γ is a transcription factor expressed selectively in adipose tissue (4), and seems to be associated with the differentiation of adipocytes. PPAR γ activation reportedly results in lipid filling macrophages by the induction of CD36 (5), a class B scavenger receptor.

Recently, PPAR γ has been reported to induce apoptosis in endothelial cells (6) and macrophages (7). In contrast, PPAR α has been demonstrated to suppress the apoptosis of hepatocytes (8), but to induce apoptosis in macrophages (7) and smooth muscle cells (9).

We hypothesized that the apoptosis of endothelial cells may be associated with PPARs. These responses were therefore compared in human umbilical vein endothelial cells (HUVECs) and hepatocytes. Since hepatocytes induce β -oxidation of fatty acids, hepatocytes may not be foam cells. This study was designed to investigate whether or not apoptosis is regulated by PPAR α co-transfection into lipid-filled HUVECs transfected with PPAR γ 1, and whether or not apoptosis is also suppressed by acyl-coenzyme A synthetase (AcylCS), which is involved in β -oxidation.

Material and methods

Cell cultures

Primary cultures of HUVECs (lot #31091) and human hepatocytes (lot #RI-3716) were purchased from Cell Systems Corporation, Kirkland, WA, USA. HUVECs were checked for their ability to produce factor VIII-related antigen by indirect immu-

Address for correspondence: Ikuo Inoue, MD., Fourth Department of Medicine, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-0495, Japan.

E-mail: i1901018@saitama-med. ac. jp

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nofluorescence, and all cells were identified as endothelial cells. Cell cultures were maintained in 25-cm² flasks in a commercial medium (CS-4ZO-500, Dainippon Pharmaceutical Co. Ltd., Osaka, Japan) supplemented with 10% fetal bovine serum (FBS), HEPES (15 mM), acidic-fibroblast growth factor and heparin in an atmosphere of 5% CO₂/95% air at 37°C. At confluence, the cell population was divided after detachment from the flasks with 0.24% EDTA. The medium was changed twice weekly. Most cultures were used within 3 weeks, at the second to third passage. The tissue samples were frozen immediately in liquid nitrogen and stored at -80°C.

Cell treatment

Pioglitazone, dissolved in 0.1% dimethylsulfoxide, was used at a final concentration of 10 μ M. Pioglitazone was a gift from Takeda Chemical Industries, Ltd., Osaka, Japan. Bezafibrate and fenofibric acid were used at a final concentration of 1 μ M. Bezafibrate was kindly provided as sodium-bezafibrate by Kissei Pharmaceutical Co. Ltd., Matsumoto, Japan, and fenofibric acid was provided by Kaken Pharmaceutical Co. Ltd., Tokyo, Japan. Clofibrate was purchased from Sigma, Kirkland, WA, USA. Clofibrate was used at a final concentration of 10 μ M. The cells were incubated with the drugs for 24 h.

DNA sequencing

Direct sequencing of the DNA fragments was performed using an automated sequencer (ABI PRISM 310 Genetic Analyzer, Perkin Elmer, Forstercity, CA, USA). All DNA sequences were confirmed by reading both DNA strands.

Western blotting

Western blotting was performed and specific immunoreactivity detected with an Amersham ECL kit, Amersham Pharmacia Biotech, Buckinghamshire, UK. Briefly, processed samples were subjected to 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes by semi-dry blotting. Antibodies against human PPARs, Bcl-2, Bcl-xL, Bcl-2 associated X protein (Bax), intracellular cysteine protease-3 (caspase-3/CPP32), caspase-9, cytochrome c, and protein-activating factor-1 (Apaf-1) were obtained from Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA. An antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was obtained from Biogenesis Ltd., Poole, UK. Cytochrome c were used cytosolic fractions, which were isolated by ultracentrifugation at 160,000 g for 20 min at 4°C. Membranes were treated overnight with TBS-Tween/5% dry milk and incubated with goat anti-human PPAR antibodies or sheep anti-human Bcl-2, Bcl-xL, Bax, caspase-3/CPP32, caspase-9, or mouse anti-human Apaf-1, and GAPDH for 1 h. After rinsing, membranes were incubated with horseradish peroxidase-conjugated rabbit anti-goat immunoglobulin G antibodies. Antigen detection was performed using a chemiluminescence detection system.

Subcloning of full-length human PPAR α , PPAR δ , PPAR γ 1, retinoid X receptor α (RXR α), and AcylCS DNA fragments

The specific DNA fragments of full-length human PPAR α (Genbank L-02932), PPAR δ (Genbank L-07592), PPAR γ 1 (Genbank L-40904), RXR α (Genbank X-52773) cDNAs were subcloned from skeletal muscle (#9514, Takara Shuzo Co., Ltd., Tokyo, Japan) or liver (#9505, Takara Shuzo Co., Ltd.) into the pCI-neo mammalian expression vector (pCI-neo) (Promega Corporation, Madison, WI, USA). Direct sequencing of the PCR products was performed using an automated sequencer. For PPAR α , the 1407 bp *Sal* I/*Not* I fragment was subcloned into the *Sal* I/*Not* I site of pCI-neo and the vector was named pCI-PPAR α . PPAR δ , or PPAR γ 1 was also subcloned into the *Sal* I/*Not* I site of the vector and the vectors were named pCI-PPAR δ , or pCI-PPAR γ 1, respectively. RXR α was also subcloned into the *Xho* I/*Not* I site of the vector and the vectors were named pCI-RXR α .

A product encoding the entire open reading frame of human AcylCS 2 cDNA (Genbank AF099940) (from bp 47–2067 of the nucleotide sequence) was amplified by PCR using gene-specific forward and mutated reverse primers (10). The forward primer sequence was 5'-GGAGAAGATGCAGACACAGG-3' and the reverse primer was 5'-G (A) GTCATTGAAA (T) CAATATTTAC-3'. p3ACS2, which has been described previously, was used as a template. The amplified fragment was subcloned into a pcDNA3.1/CT-GFP-TOPO mammalian expression vector (Invitrogen Corporation, Carlsbad, CA, USA) to express AcylCS 2-GFP fusion protein. The clone sequenced for the correct insert was named pcACS2.

Transient transfection/co-transfection

Transient transfection of pCI-PPAR α (3 μ g), pCI-PPAR δ (3 μ g), pCI-PPAR γ 1 (3 μ g), pCI-RXR α (3 μ g), or pcACS2 (3 μ g) were performed on 1×10^6 cells in 100-mm dishes using TfxTM-50 Reagent (Promega Corporation) according to the instruction manual, with an internal control reporter (pRL-TK vector) (2 μ g) (Promega Corporation, Madison, WI, USA) to correct for transfection efficiency. After 24 h, cells were stimulated with test drugs for a further 24 h, used to analyse apoptotic cell and lipid extraction.

Transient co-transfection with PPARs

After 12 h pre-transfection of pCI-PPAR γ 1 (1.5 μ g) or pCI-PPAR α (1.5 μ g), cells were co-transfected with pCI-PPAR α (1.5 μ g), pCI-PPAR δ (1.5 μ g), pCI-PPAR γ 1 (1.5 μ g) for 12 h, stimulated with or without test drugs for another 24 h, used to analyse apoptotic cell and lipid extraction.

Accumulation of lipids in cells and stain with Oil red O

The cells, transfected by PPARs with/without ligands, were harvested, extracted with chloroform/methanol (2: 1, v: v), and the mass of total-, esterified-, and free cholesterol in the extract was measured enzymatically by fluorescence

scanning according to our previous method (11). The cells, transfected by PPARs with/without ligands, were fixed with 0.66% paraformaldehyde then stained with Oil red O (Sigma, St. Louis, MO, USA) and haematoxylin (Sigma) according to our previous method (11).

Detection of fragmented DNA on agarose gel

Cells at an initial density of 1×10^5 /100-mm dish were cultured for 24 h in F12 FCS plus fibrate or thiazolidinedione and then harvested, lysed and centrifuged to separate DNA fragments from intact chromatin. Supernatants were precipitated overnight at -20°C with 2 vols of ethanol and centrifuged at 13,000 g for 15 min. The pellets were then incubated for 30 min at 37°C in 500 μl Tris-EDTA buffer supplemented with 100 $\mu\text{g/ml}$ RNase A. Samples were extracted with phenol/chloroform/isoamyl alcohol (25: 24: 1) and once again with chloroform/isoamyl alcohol (24: 1). DNA was precipitated and the pellets were recovered by centrifugation (13,000 g, 15 min). DNA was air-dried, resuspended in 10 μl of Tris-EDTA buffer supplemented with 2 μl of sample buffer (0.25% bromophenol blue, 30% glycerol), and separated electrophoretically on a 1.3% agarose gel. After electrophoresis, the gels were stained with ethidium bromide (1 $\mu\text{g/ml}$), and pictures taken by UV transillumination.

Quantitative analysis of apoptotic cells

The cells, transfected by PPARs with/without ligands, were scraped, transferred into 15 ml Falcon tubes, and centrifuged at 400g for 5 min. Cell pellets were then fixed for 2 h with PBS containing 2% glutaraldehyde. After rinsing twice with PBS, the pellets were resuspended in 2 ml of PBS containing 0.2 mM Hoechst 33258 and incubated for 10 min at room temperature. At least 500 cells from randomly selected fields were counted under a fluorescent microscope to determine the percentage of apoptotic cells.

Moreover, the cells, which were transfected by PPARs with/without ligands, were observed by terminal deoxynucleotide transferase (TdT)-mediated nick end labelling (TUNEL) assay using a commercial kit (Apoptosis *in situ* detection Kit, Wako Junyaku, Tokyo, Japan).

Reporter assay for PPAR activation

Transient transfections for the reporter assay were performed using TfxTM-50 Reagent (Promega Corporation) according to the manufacturer's recommended protocol. COS-1 was used because of its high transfection efficiency. HUVECs was also used in the reporter assay for PPAR activation using Targefect F-1 Reagent (Targeting Systems, Santee, CA, USA) or Optimized Protocol HUVEC NucleofectTM Kit (Amaxa's Biosystems, Köln, Germany). To measure PPARs activation, the PPAR-responsive promoter region for cellular retinol-binding protein type II (CRBP II) (– 670 to + 60) (12) cloned into the *Kpn* I/*Nco* I sites of pGL3-Basic upstream of the luciferase gene (pGL3-Basic) (Promega Corporation), and termed pCRBP II-Luc, was used. The

PPAR-responsive promoter region for CRBP II was similar to the well-known PPAR-responsive promoter region for acyl-coenzyme A oxidase. This promoter contains both a CAAT and TATA sequence motif. The highlighted bases between – 639 and – 605 represent the PPAR-response element. pCRBP II-Luc and pRL-TK, in the presence or absence of pCI-PPAR α , pCI-PPAR δ , or pCI-PPAR γ 1 were co-transfected with pCI-RXR α into COS-1 cells grown in 24-well plates. As a control, cells without ligand were utilized. The total amount of DNA transfected (0.5 μg) was normalized with a carrier DNA (pCI-neo; Promega Corporation). After 24 h, cells were stimulated with test drugs for another 24 h. We used charcoal-dextran-treated medium (#SH30068, HyClone, UT, USA) for luciferase activity. Finally, luciferase activity was normalized to *Renilla* (Promega Corporation) luciferase activity. Both activities were measured according to the manufacturer's instructions (Promega Corporation).

Results

The effect of fibrate and thiazolidinedione on PPARs was determined by a reporter gene assay in COS-1 (Fig. 1) and HUVECs (data not shown). In COS-1 and HUVECs with 10 μM clofibrate, PPAR α - and PPAR δ -mediated transcription of the reporter gene were enhanced. In contrast, PPAR γ 1-mediated transcription of the reporter gene was enhanced in cells with 10 μM pioglitazone. Furthermore, PPAR α -mediated transcription of the reporter gene was enhanced in cells with 1 μM bezafibrate or 1 μM fenofibric acid. Although not shown, the data obtained from HUVECs was similar tendency to COS-1. Based on these data, clofibrate can be used as an activator of PPAR α and PPAR δ , bezafibrate and fenofibric acid as a specific activator of PPAR α , and pioglitazone as a specific activator of PPAR γ 1 in COS-1 and HUVECs.

The morphology of HUVECs transfected with human full-length PPAR α , PPAR δ or PPAR γ 1 gene without drugs is shown in Fig. 2A. No lipid-filled HUVECs transfected with the human full-length PPAR α or PPAR δ gene were observed (Fig. 2A-b, 2A-c). Although many lipid-filled HUVECs transfected with the human full-length PPAR γ 1 gene were observed (Fig. 2A-d). Co-transfection of PPAR γ 1 and PPAR α protected the lipid-filled HUVECs (Fig. 2A-e).

These data are consistent with those obtained from the cells, which were stained with Oil red O (Fig. 2B). No Oil red O-stained HUVECs transfected with the human full-length PPAR α or PPAR δ gene were observed (Fig. 2B-b, 2B-c) although many stained-, and lipid-filled HUVECs transfected with the human full-length PPAR γ 1 gene were observed (Fig. 2B-d). Co-transfection of PPAR γ 1 and PPAR α protected the lipid-filled HUVECs (Fig. 2B-e). The number of lipid droplets in HUVECs transfected with human full-length PPAR γ 1 gene plus the ligand was greater than without the ligand (data not shown).

The morphology of hepatocytes transfected with human

full-length PPAR α , PPAR δ or PPAR γ 1 gene without drugs is shown in Fig. 3. A few lipid-filled hepatocytes transfected with the human full-length PPAR α , PPAR δ or PPAR γ 1 gene were observed. The number of lipid droplets in hepatocytes transfected with human full-length PPAR γ 1 gene plus the ligand was not greater than without the ligand (data not shown).

We determined the total-, esterified-, or free-cholesterol content in HUVECs transfected with human full-length PPAR α , PPAR δ , PPAR γ 1, or AcylCS gene with/without drugs. Although not shown, increased total-, esterified-, and free-cholesterol were detected in HUVECs transfected with human full-length PPAR γ 1, with/without PPARs ligand/activator.

The DNA ladder and TUNEL positive cells were present in HUVECs transfected with PPAR γ 1 (Fig. 4A,B), while the DNA ladder was not present in hepatocytes transfected with PPAR γ 1 (data not shown). There therefore seems to be a difference between HUVECs and hepatocytes with regard to the mechanisms of apoptosis. Moreover, the DNA ladder and TUNEL positive cells were not present in HUVECs transfected with PPAR α or PPAR δ (Fig. 4A, B).

Phase-contrast microscopy was first performed to examine the morphological features of apoptosis in HUVECs transfected by PPARs (Fig. 5, right). On fluorometrical observation (Fig. 5, left) of the nuclei of the HUVECs stained using Hoechst 33258, transfection with PPAR γ 1 showed typical features of apoptosis, including the formation of condensed or fragmented nuclei (Fig. 5E). However,

no formation of condensed or fragmented nuclei was observed in HUVECs transfected by PPAR α or PPAR δ (Fig. 5–C,D). The percentage of apoptotic cells of the total cells significantly increased in HUVECs transfected by PPAR γ 1 (Fig. 6).

Fig. 7 shows the expression of apoptosis family proteins in HUVECs transfected with the PPAR γ 1 gene with/without co-transfection with the PPAR α gene, or the AcylCS gene. Pioglitazone, or bezafibrate was used as a ligand/activator of PPARs. The molecular mass of cytochrome *c* and Apaf-1 was increased in HUVECs transfected with human full-length PPAR γ 1 gene without drugs. A PPAR γ 1-induced increase in cytochrome *c*, and Apaf-1 was decreased by co-transfection with the PPAR α gene or AcylCS gene and with/without the PPAR α ligand/activator, bezafibrate. The molecular mass of Bcl-xL and Bcl-2 was decreased in HUVECs transfected with the PPAR γ 1 gene, but increased by co-transfection with the PPAR α gene, and PPAR α ligand/activator, bezafibrate. The amount of Bax was increased in HUVECs transfected with the PPAR γ 1 gene, but decreased by the PPAR α gene, and PPAR α ligand/activator, bezafibrate. Antibodies against two different epitopes of Bax were used. The activation of caspase 3/CPP32 was assessed by Western blotting (Fig. 7). Caspase processing, determined by either the disappearance of the proform of the enzyme and/or detection of one of the cleavage intermediates or active subunits, was apparent for caspase 3/CPP32 in HUVECs transfected, with the human full-length PPAR γ 1 gene with or without

Fig. 1. Activation of human PPARs in a cell-based reporter gene assay. COS-1 cells were transfected with reporter plasmid pCRBPII-Luc and hRXR α (pCI-RXR α), an internal control reporter (pRL-TK vector), and either hPPAR α (pCI-PPAR α), hPPAR δ (pCI-PPAR δ), or hPPAR γ 1 (pCI-PPAR γ 1). After transfection, cells were treated for 24 h with or without pioglitazone (Pio), clofibrate (Clo), bezafibrate (Beza), fenofibric acid (Feno). Firefly luciferase activities were normalized to *Renilla* luciferase control activities. Values show the ratio of Firefly luciferase activities/*Renilla* control activities in COS-1 cells co-transfected in the presence or absence of the ligand. Experiments were performed in triplicate, and four independent experiments were performed. All data are the mean $\pm$ SD, * p < 0.05 vs without the ligand.

Fig. 2. Morphology (A) of HUVECs transfected with empty vector (a) human full-length PPAR α (b), PPAR δ (c), PPAR γ 1 (d), or PPAR α plus PPAR γ 1 (e) gene, without drugs. The extent of lipid filling of the HUVECs transfected with the PPAR γ 1 gene was greater than that with the PPAR α gene. The cells, which were transfected by PPARs without ligands, were fixed with 0.66% paraformaldehyde then stained with Oil red O and haematoxylin (B). Many lipid-filled HUVECs transfected with the human full-length PPAR γ 1 gene were observed. And Co-transfection of PPAR γ 1 and PPAR α protected the lipid-filled HUVECs.

Fig. 3. Morphology of hepatocytes transfected with empty vector (a), human full-length PPAR α (b), PPAR δ (c), or PPAR γ 1 (d) gene without drugs. The extent of lipid filling of hepatocytes transfected with the PPAR γ 1 gene was slightly greater than that with the PPAR α and PPAR δ genes.

Fig. 4. The DNA ladder (A) and TUNEL assay (B) were shown in HUVECs transfected with PPAR α , PPAR δ , or PPAR γ 1 with/without ligands: pioglitazone (Pio), clofibrate (Clo), fenofibric acid (Feno). The DNA ladder and TUNEL positive cells were present in HUVECs transfected with PPAR γ 1 with/without the ligand. However, the DNA ladder and TUNEL positive cells were not present in HUVECs transfected with PPAR α or PPAR δ with/without ligands.

pioglitazone. The increased activity of caspase 3/CPP32 by PPAR γ 1 was decreased in HUVECs co-transfected with the human full-length PPAR α gene or AcylCS gene, with or without bezafibrate. The increased proform and activated subunit of caspase 9 by PPAR γ 1 were decreased in HUVECs by co-transfecting the human full-length PPAR α gene with or without bezafibrate.

No significant change in the number of apoptotic family members was detected in hepatocytes transfected with the PPAR α and PPAR γ 1 gene, with or without PPAR ligands/activators.

Discussion

These results demonstrate that PPAR γ 1 induces apoptosis in HUVECs, but not in hepatocytes. Moreover, apoptosis was prevented by transfection with the PPAR α , or AcylCS gene.

Recently, we reported that lipid-filled HUVECs treated with 300 μ M eicosapentaenoic acid (EPA) or 300 mM docosahexaenoic acid (DHA), which are PPAR α and PPAR γ ligands/activators, were detached from the dish (13), possibly due to apoptosis. There have been several papers indicating that ω -3 fatty acids such as EPA and DHA induced the apoptosis of activated cells, namely pancreatic cancer cells (14), or leukemic cells (15). If cells have a reduced ability to metabolize or excrete polyunsaturated fatty acids after a high influx of lipids such

Fig. 5. Hoechst 33258 staining of HUVECs transfected with PPAR α (C), PPAR δ (D), or PPAR γ 1 (E). HUVECs with phase contrast (right) and left fluorophotometrical photographs of the HUVECs. A: not transfected, B: empty vector transfected, F: PPAR α /PPAR γ 1 co-transfected, Original magnification, $\times 400$.

as esterified-cholesterol and fatty acids into the cells, the increased amount of lipid may induce apoptosis. Kellner-Weibel *et al.* recently reported that unesterified cholesterol (free cholesterol), which is formed by neutral cholesterol ester hydrolase, is related to apoptosis (16). Their morphological analysis and *in situ* DNA fragmentation assay demonstrated the occurrence of apoptosis, which was inhibited by acyl-coenzyme A: cholesterol acyltransferase.

Other investigators have speculated that cells undergoing apoptosis require cholesterol to maintain complete function of their plasma membrane (17). Intracellular caspase-3/CPP32 protease is a key molecule during apoptosis (18). Caspase-3/CPP32 protease was previously shown to cleave sterol regulatory element-binding proteins (SREBPs), which are activated by decreasing the cholesterol content of the cells. As already mentioned, there seems to be a close relationship between the cell cholesterol content and apoptosis.

There are many pathways for the initiation of apoptosis such as the Fas/Fas ligand pathway, Ca^{2+} influx pathway, pathway to release cytochrome c from mitochondria (19), and ceramide pathway (18). Recently, it was reported that the apoptosis of endothelial cells was induced by a ligand for PPAR γ 1, 15d-PGJ₂ (6). It is beneficial to induce the apoptosis of activated cells, which usually produce many kinds of cytokines. Our data indicate that PPAR γ -induced apoptosis may be due to the release of cytochrome c. Augmented cytochrome c activity or capacity might increase mitochondrial membrane permeability which causes cytochrome c release from the intermembrane space. Moreover, increased cytochrome c may reflect dysfunction of the mitochondria. Mitochondria are pivotal in controlling cell life and death. Moreover, mitochondrial dysfunction will decrease the production of adenosine triphosphate, resulting in cell death and apoptosis.

It remains to be clarified why PPAR γ ligands/activators

Fig. 6. The percentage of apoptotic cells induced by transfection of PPAR α , PPAR δ , or PPAR γ 1. Apoptotic cells induced by PPAR α , PPAR δ , or PPAR γ 1 were counted. Data shown are the mean $\pm$ SD of five independent investigations, * $p < 0.05$ vs control. Controls were obtained from HUVECs transfected with empty vector.

induced the apoptosis of HUVECs and suppressed hepatocyte apoptosis. Our morphological data indicate that normal cells had little lipid filling, while apoptotic cells were filled with lipid (data not shown). Hepatocytes have a high level of β -oxidation of fatty acids and/or cholesterol 7 α -hydroxylase, and this might be one reason why apoptosis does not occur after PPAR γ 1 transfection. If the cell lipid content can be decreased, apoptosis may be suppressed. The activation of PPAR α , a nuclear receptor related to the enzyme for β -oxidation, or AcylCS, also related to the enzyme for β -oxidation, causes a decrease in the fatty

Fig. 7. Expression of apoptosis family proteins (cytochrome c, Apaf-1, Bax, Bcl-xL, caspase-3, caspase-9) by Western blot analysis in HUVECs transfected with the PPAR γ 1 gene, with or without co-transfection with the PPAR α gene or acyl-coenzyme A synthetase (AcylCS) gene. Pioglitazone or bezafibrate was used as the ligand/activator of PPARs. The molecular mass of the apoptosis family members changed in HUVECs transfected with the human full-length PPAR γ 1 gene without drugs. This change in molecular mass of apoptosis family proteins, which was induced by transfection with the PPAR γ 1 gene, was altered by co-transfection with the PPAR α ligands/activators, bezafibrate or with the AcylCS gene.

acid/esterified-cholesterol content of cells. Indeed, the lipid content was decreased by transfection with the gene for PPAR α , or AcylCS, followed by apoptosis inhibition (Fig. 2 and Fig. 7).

Our data showed that the effect of PPAR α on the esterified-cholesterol content was stronger than its effect on AcylCS (data not shown), suggesting that the other specific effect of PPAR α may be related to decreased esterified-cholesterol content. Recently, it has been reported that PPAR α or γ ligand/activators regulated the gene of adenosine triphosphate-binding cassette transporter 1 (ABCA-1) (20,21). In fact, our results suggest that fibrates and thiazolidinedione may foster cholesterol elimination from endothelial cells by stimulating the ABCA-1-mediated export of the lipid, resulting in prevention of lipid accumulation in the endothelial cells.

Interestingly, our data indicate that treatment with PPAR γ ligands/activators, as well as PPAR α ligands/activators, suppressed the apoptosis of hepatocytes (Fig. 3), which was associated with the delayed degradation of I κ B α and I κ B β (unpublished data). Although there have been many studies indicating that apoptosis might be related to inflammation, the reasons for this association are unclear. Some investigators have demonstrated that nuclear factor κ B (NF κ B), may be involved in inflammation during the process of apoptosis in various cells (22).

In summary, these results suggest that the PPAR γ 1 gene induces apoptosis in activated HUVECs (23), but not in hepatocytes, which was prevented by transfection with the PPAR α , or AcylCS gene, and apoptosis may be associated with the cellular esterified-cholesterol.

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