Effects of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 on cell proliferation and apoptosis in ECV304 endothelial cells[1]

Yu-gang DONG[2], Dan-dan CHEN, Jian-gui HE, Yong-yuan GUAN[3]

Department of Cardiology, First Affiliated Hospital, Zhongshan University, Guangzhou 510080;
[3]Department of Pathology, Medical College of Zhongshan University, Guangzhou 510089, China

KEY WORDS peroxisome proliferator-activated receptor; atherosclerosis; endothelial cells; apoptosis; transcription factor AP-1; NF-kappa B; prostaglandins

ABSTRACT

AIM: To investigate the effects of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) on cell proliferation and apoptosis in ECV304 endothelial cells and related molecular mechanism. **METHODS:** MTT, Hoechst33258, TUNEL, Flow cytometry, DNA ladder, RT-PCR, Western blot, and electrophoretic mobility shift assay (EMSA) analysis were employed. **RESULTS:** The 15d-PGJ_2 induced apoptosis in ECV304 endothelial cells in a dose-dependent manner (the percentage of apoptosis was enhanced from 10.0 %±1.3 % to 32.8 %±1.6 %), which was accompanied by inhibition of NF-κB and AP-1 DNA binding activity, down-regulation of c-*myc*, upregulation of *Gadd45* and *p53*, and activation of p38 kinase. However, the expression of *p21* was found no significant change. **CONCLUSION:** peroxisome proliferator-activated receptor gamma ligand, 15d-PGJ_2, can inhibit proliferation and induce apoptosis in ECV304 endothelial cells through different mechanisms.

INTRODUCTION

Atherosclerosis is a multifactorial disease in which the occurrence of lesions may result in ischemia of the heart, brain, and extremities. Nevertheless, the mechanism of atherosclerosis remains unclear. Endothelial damage or dysfunction is considered the initiating cause of atherogenesis. Recent studies have shown that increased apoptosis of endothelial cell (EC) has been observed in atheromatous plaques[1]. To observe the change of EC apoptotic pathways in atherosclerosis may enable a greater understanding of disease pathogenesis.

Expression of peroxisome proliferator-activated receptor (PPAR) has been shown in atherosclerotic lesions and macrophage foam cells, which suggests that PPAR may affect atherosclerogenic process[2]. PPAR form a subfamily of the nuclear receptor superfamily, and three isoforms of PPAR have been identified thus far: PPARα, PPARδ, and PPARγ. The PPAR is ligand-dependent transcription factor that regulate target gene expression by binding to specific peroxisome proliferator response elements (PPRE) in enhancer sites of regulated genes. Each receptor binds to its PPRE as a heterodimer with a retinoid X receptor (RXR). Upon binding an agonist, the conformation of a PPAR is altered and stabilized such that a binding cleft is created and recruitment of transcriptional coactivators occurs. The result showed an increase in gene transcription[3]. Activation of peroxisome proliferator-activated receptors induces apoptosis of endothelial cells, human mono-

[1] Project supported by the Provincial Science Foundation of Guangdong, China (2000).
[2] Correspondence to Yu-gang DONG. Phn 86-20-8775-5766, ext 8151. E-mail yugangdong@gzsums.edu.cn
Received 2002-11-22 Accepted 2003-04-30

cyte-derived macrophages and smooth muscle cells[4-6]. Transcription of affected genes may also indirectly modulated by PPAR via interference with other transcription factor pathways. Activation of PPAR negatively interferes with the NF-κB, a signal transducer and activator of transcription (STAT), and Activator protein 1 (AP-1) pathways[5-7]. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) has been demonstrated to be a natural ligand of the PPARγ. It has been reported that 15d-PGJ_2 induced endothelial apoptosis[4], however the mechanisms have been unclear yet. The aim of the present work was to measure the effects of 15d-PGJ_2 on cell proliferation and apoptosis in ECV304 endothelial cells, and further investigate its possible molecular mechanisms.

MATERIALS AND METHODS

Drugs and reagents RPMI-1640 medium, RNase A, agarose gel, 1 kb DNA ladder, proteinase K, propidium iodide (PI), and MTT were purchased from Gibco Co. The 15d-PGJ_2 was purchased from Cayman Chemical Co. Other reagents, antibodies and reagent boxes were purchased from Sigma Chemical Co.

Cell culture The ECV304 human endothelial cell line was purchased from China Center for Type Culture Collection (CCTCC). The cell line was cultured in RPMI-1640 medium containing 10 % fresh bovine serum, benzylpenicillin (100 kU/L), and kanamycin (0.1 g/L) at 37 ºC in 5 % CO_2-95 % air atmosphere.

MTT assay ECV304 human endothelial cells growing on 96-well plates were treated with 15d-PGJ_2 (5-20 μmol/L) for 48 h, and untreated cells served as a control. Ten μL of the stock solution (2.5 g/L) of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) was added to each well. After 1 h of incubation at 37 ºC, the medium was removed, 50 μL of the extraction buffer (10 % Trition-X100; HCl 0.1 mol/L) was added, and plates were gently shaken for 30 min at room temperature. The optical densities were measured at 570 nm.

Hoechst 33258 stainning Cells were fixed with 4 % formaldehyde in phosphate buffered saline (PBS) for 10 min, stained by Hoechst 33258 for 1 h, and were subjected to fluorescence microscopy. Apoptotic cells were identified by nuclear condensation and/or fragmentation.

TUNEL assay TUNEL assay was performed by the apoptosis detection system. ECV304 cells were fixed by 4 % paraformaldehyde in PBS overnight at 4 ºC, and were washed three times with PBS and then were permeabilized by 0.2 % Triton X-100 in PBS for 15 min on ice. After washing twice, cells were equilibrated at room temperature for 15-30 min in equilibration buffer (potassium cacodylate 200 mmol/L, dithiothreitol 0.2 mmol/L, bovine serum albumin 0.25 g/L, and cobalt chloride 2.5 mmol/L in Tris-HCl 25 mmol/L, pH 6.6) and then incubated in the presence of pluorescein-12-dUTP 5 μmol/L, dATP 10 μmol/L, edetic acid 100 μmol/L, and terminal deoxynucleotidyl transferase at 37 ºC for 1.5 h in dark. The tailing reaction was terminated by standard saline citrate (SSC). The samples were washed three times with PBS and were analyzed by fluorescence microscopy. At least 1×10^3 cells were counted, and the percentage of TUNEL-positive cells was determined.

Flow cytometry For DNA content analysis, 15d-PGJ_2 (5 -20 μmol/L) was added to ECV304 endothelial cells in mid-logarithmic phase (1×10^9 cells/L). Cells (1×10^6) were harvested at indicated time, pelleted, washed with phosphate-buffered saline (PBS), and resuspended in PBS containing PI 20 mg/L and RNase A 1 mg/L. Fixed cells (1×10^6) were examined per each experimental condition by flow cytometry, and the percentage of degraded DNA was determined by the number of cells displaying subdiploid (sub-G_1). DNA divided by the total number of cells was examined. Cell cycle analysis was performed in the same experimental conditions and distributions used the CellFit program. All measurements were carried out under the same instrumental settings.

Ladder detection assay After induction of apoptosis, cells (5×10^6 per sample, both attached and detached cells) were lyzed with 150 μL hypotonic lysis buffer (edetic acid 10 mmol/L, 0.5 % Triton X-100, Tris-HCl, pH 7.4) for 15 min on ice and were precipitated with 2.5 % polyethylene glycol and NaCl 1 mol/L for 15 min at 4 %. After centrifugation at 25 000×*g* for 10 min at room temperature, the supernatant was incubated in the presence of proteinase K (0.3 g/L)at 37 ºC for 1 h and precipetated with isopropanol at -20 ºC. After centrifugation, each pellet was dissolved in 10 μL of Tris-edetic acid (pH 7.6) and electrophoresed on a 1.5 % agarose gel containing ethidium bromide. Ladder formation of oligonucleosomal DNA was detected under ultraviolet light.

Western blot analysis The cells were lysed in lysis buffer Hepes 25 mmol/L, 1.5 % Triton X-100, 1 %

sodium deoxycholate, 0.1 % SDS, NaCl 0.5 mol/L, edetic acid 5 mmol/L, NaF 50 mmol/L, sodium vanadate 0.1 mmol/L, phenylmethylsulfonyl fluoride (PMSF) 1 mmol/L, and leupeptin 0.1 g/L (pH 7.8) at 4 ºC with sonication. The lysates were centrifuged at 20 000×*g* for 15 min and the concentration of the protein in each lysate was determined with Coomassie brilliant blue G-250. Loading buffer (Tris-HCl 42 mmol/L, 10 % glycerol, 2.3 % SDS, 5 % 2-mercaptoethanol and 0.002 % bromophenol blue) was then added to each lysate, and then the samples were electrophoresed on a SDS-polyacrylamidel gel. Proteins were transferred to nitrocellulose and incubated sequentially with antibodies against c-fos, c-jun, IκB, c-myc, p53, Gadd45, and p21, and then with peroxidase-conjugated secondary antibodies in the second reaction. Detection was performed with enhanced chemiluminescence reagent.

RT-PCR Total RNA was extracted from cells using TRIzol™. RT-PCR for *c-myc*, *p21*, *p53*, *Gadd45*, and *beta-actin* mRNA was performed as described[7].
beta-actin:sense 5' ATCTGGCACCACACCTTCTAC AATGAG-CTGCG-3',
antisense 5' CGTCATACTCCTGCTTGCTGATCCA-CATCTGC-3'.
c-*myc*: sense 5' ATCTGGCACACACCTTCTACAATGA-GCTGCG 3'
antisense 5' CCCCTCAGTGGTCTTCCCTAC 3'
p21: sense 5' ATGACTGAGTATAAACTTGTGG 3'
antisense 5' TCACATGACTATACACCTTGTC 3'
p53: sense 5' ATGGAGGATTCACAGTCGGA 3'
antisense 5' TCAGTCTGAGTCAGGCCCCA 3'
Gadd45: sense 5' ATGACTTTGGAGGAATTCTCGG 3'
antisense 5' TCACCGTTCGGGGAGATTAATC 3'

Electrophoretic mobility shift assay (EMSA) Nuclear extracts were prepared from ECV304 cells treated with 15d-PGJ_2. Synthetic double-strand oligonucleotides of consensus NF-κB binding sequence, GATCCCAACGGCAGGGGA, and the oligonucleotie of consensus AP-1 binding sequence, CGCTTGATGA-GTCAGCCGAA, were end-labeled with γ[^{32}P]ATP using T4 polynucleotide kinase. Nuclear extract was incubated with the labeled probe in the presence of poly (dI-dC) in a binding buffer containing 20 mmol/L *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid at room temperature for 30 min. For supershift assays, a total of 0.2 µg of antibodies against p65 subunit of NF-κB were included in the reaction. DNA-protein complexes were resolved by electrophoresis in a 5 % non-denaturing polyacrylamide gel, which was dried and visualized by autoradiography.

P38 activity assays[8] P38 MAP kinase activity assays were carried out using an upstate biotechnology MAPKAP kinase 2 immunoprecipitation-kinase assay kit, according to the manufacturer's instructions and using [^{32}P]ATP (Amersham UK).

Statistical analysis The data were mean values of at least three different experiments and expressed as mean±SD. Student's *t*-test was used to compare data. $P<0.05$ was considered statistically significant.

RESULTS

Effect of 15d-PGJ2 on cell proliferation The 15d-PGJ_2 induced a dose-dependent inhibition of ECV304 endothelial cell growth in the range of 5-20 µmol/L. Compared with the control group, greater than 50 % mean inhibition was achieved at 15d-PGJ_2 20 µmol/L (Fig 1).

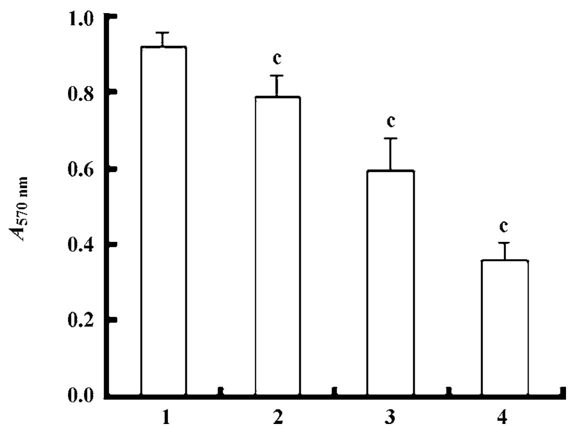

Fig 1. Effect of 15d-PGJ_2 on proliferation in ECV304 endothelial cells. (1): control; (2-4): 15d-PGJ_2 5, 10, 20 µmol/L. *n*=3. Mean±SD. $^cP<0.01$ *vs* control.

Hoechst staining Under the fluorescent microscope, ECV304 endothelial cell at 48 h after treatment with 15d-PGJ_2 showed the morphological changes apoptosis characteristic (Fig 2).

TUNEL assay To confirm that the effect of various concentrations of 15d-PGJ_2 (5-20 µmol/L) on apoptosis in ECV304 endothelial cells, TUNEL analysis was performed. The 15d-PGJ_2 induced apoptosis of ECV304 cells in a dose-depended manner , and the percentage of apoptosis was enhanced from 10.0 %±1.3 % to 32.8 %±1.6 % (Fig 3).

Cell cycle phase distribution ECV304 endothe-

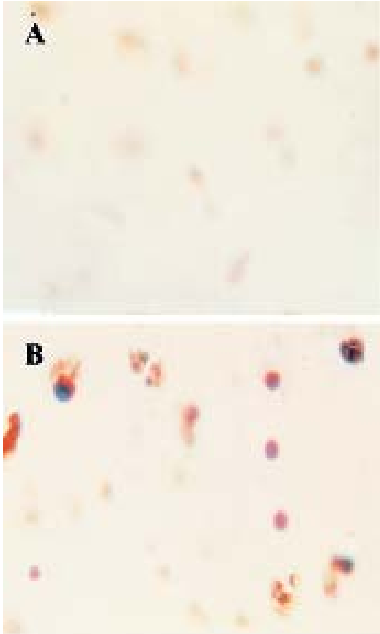

Fig 2. The fluorescant microphotograph: showing condensation and segmentation of nucleus in ECV304 endothelial cell at 48 h after treatment with 15d-PGJ$_2$. A) Control; B) 15d-PGJ$_2$ 10 μmol/L. Hoechst stain, ×200.

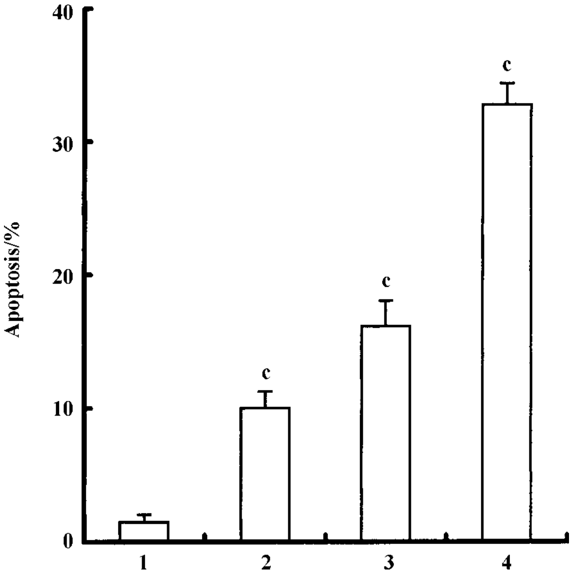

Fig 3. Effect of 15d-PGJ$_2$ on apoptosis in ECV304 endothelial cells. (1): control; (2-4): 15d-PGJ$_2$ 5, 10, and 20 μmol/L. *n*=4. Mean±SD. $^cP<0.01$ *vs* control.

lial cells were accumulated in G_0/G_1 phase, after exposure to different concentrations of 15d-PGJ$_2$ (5-20 μmol/L) for 24 h. There was a significant decrease in the number of cells in S phase. No significant change of cell number was observed in the number of cells in G_2/M phase (Tab 1).

Tab 1. Effect of 15d-PGJ2 on ECV304 cells cycle. *n*=3. Mean±SD. $^bP<0.05$ *vs* control.

15d-PGJ$_2$ /μmol/L	Cell number/%		
	G_0/G_1	S	G_2/M
0	29±2	41±3	29±1
5	38±3^b	32±4^b	29±2
10	51±3^b	21±1^b	28±2
20	74±5^b	2±2^b	25±3

Ladder detection assay Agarose gel electrophoresis exhibited DNA ladder formation in ECV304 endothelial cells after exposure to different concentrations of 15d-PGJ$_2$ (5-20 μmol/L) for 48 h. Compared with the control group, the DNA laddering was clearly observed by the treatment with 15d-PGJ$_2$ (Fig 4).

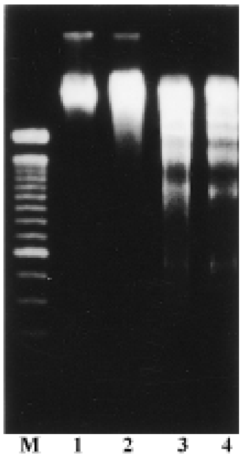

Fig 4. DNA ladder pattern formation of ECV304 endothelial cells. Lane M: DNA marker; Lane 1: control; Lanes (2-4): 15d-PGJ$_2$ 5, 10, and 20 μmol/L.

Effects of 15d-PGJ$_2$ on the expression of c-*myc*, *p21*, *p53*, and *Gadd45* RT-PCR analysis showed that the expression of c-*myc* mRNA was down-regulated, whereas the expressions of *p53* mRNA and *Gadd45* mRNA were up-regulated (Fig 5). Meanwhile, Western blot showed the same changes in protein expressions (Fig 6). Both RT-PCR analysis and Western blot analysis showed no significant changes in the expression of *p21*.

Effects of 15d-PGJ$_2$ on DNA binding activity of NF-κB and AP-1, and on expression of c-jun, c-fos and IκBα EMSA was performed with nuclear extracts prepared from control or treated cells exposed to 15d-

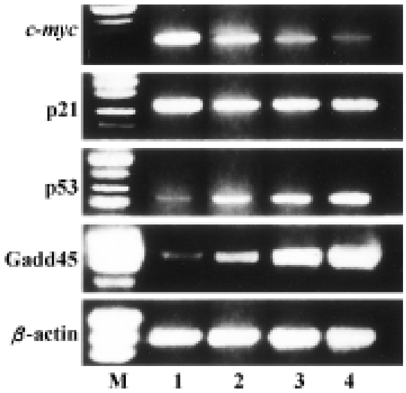

Fig 5. Effect of 15d-PGJ2 on the expression of c-*myc, p21, p53,* and *Gadd45* mRNA in ECV304 endothelial cells. Lane M : DNA marker; Lane 1: control; Lanes 2-4: 15d-PGJ$_2$ 5, 10, and 20 μmol/L.

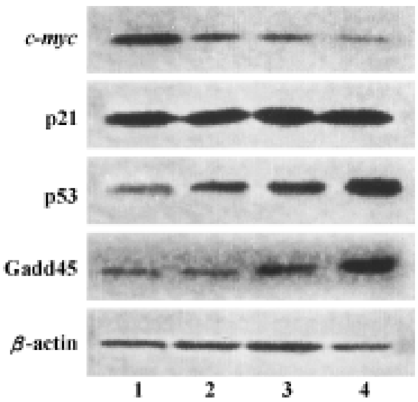

Fig 6. Effect of 15d-PGJ$_2$ on the expression of c-myc, p21, p53, and Gadd45 protein in ECV304 endothelial cells. Lane M : DNA marker; Lane 1: control; Lanes 2-4: 15d-PGJ$_2$ 5, 10, and 20 μmol/L.

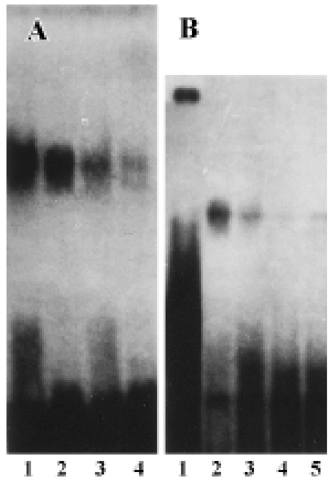

Fig 7. Effect of 15d-PGJ$_2$ on DNA binding activity of AP-I (A), NF-κB (B) . (A): Lane 1: control; Lanes 2-4: 15d-PGJ$_2$ 5, 10, and 20 μmol/L; (B): Lane 1: supershift; lane 2: control; Lanes 3-5: 15d-PGJ$_2$ 5, 10, and 20 μmol/L.

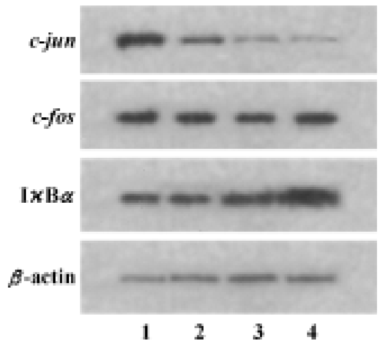

Fig 8. Effect of 15d-PGJ$_2$ on the expression of c-jun, c-fos, and IκBα protein in ECV304 endothelial cells. Lane 1: control; Lanes 2-4: 15d-PGJ$_2$ 5, 10, and 20 μmol/L.

PGJ$_2$ at various concentrations for 6 h. DNA binding activities of NF-κB and AP-1 were almost completely inhibited in 15d-PGJ$_2$-treated cells compared with untreated cells (Fig 7). Western blotting showed that the degradation of IκBα and the expression of c-jun were inhibited by 15d-PGJ$_2$ in a dose-dependent manner (Fig 8).

Effect of 15d-PGJ$_2$ on activity of p38 kinase The activity of p38 kinase in ECV304 endothelial cells was increased with the increase of 15d-PGJ$_2$ 5 -20 μmol/L (Fig 9).

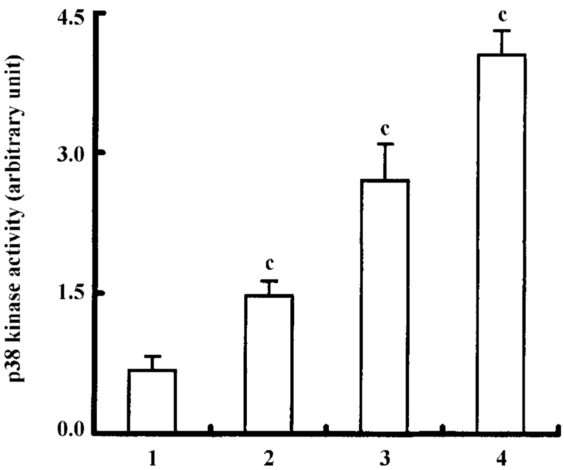

Fig 9. Effect of 15d-PGJ$_2$ on activity of p38 kinase in ECV304 endothelial cells. *n*=4. Mean±SD. ^{c}*P*<0.01 *vs* control. (1): control; (2-4): 15d-PGJ$_2$ 5, 10, and 20 μmol/L.

DISCUSSION

Endothelial cell (EC) apoptosis is an initiating event in atherogenesis since EC apoptosis compromises vasoregulation and increases SMC proliferation, migration, and blood coagulation. In addition, EC overlying vascular lesions increase expression of pro-apoptotic proteins, such as Fas and Bax, while decrease levels of anti-apoptotic factors. Therefore, understanding EC apoptotic pathways altered in atherosclerosis may enable a greater understanding of disease pathogenesis and foster the development of new therapies[1].

PPAR have an impact on the development of atherosclerosis. The expression of the PPAR was found in endothelial cells, macrophage foam cells, smooth muscle cells of human, and atherosclerotic lesions[2]. PPAR play important roles in fatty acid metabolism and storage in liver and adipose tissue, respectively. Furthermore, PPAR have a potentially important effect of inhibiting growth and migration of vascular cells[9]. PPAR not only control plasma levels of cholestrerol and triglyceride concentrations, but also exert an activity at the level of the vascular wall, which might contribute to the atherosclerotic processes.

Our present study demonstrated that 15d-PGJ_2 inhibited the growth of ECV304 endothelial cells in a dose-dependent manner. This finding is in agreement with previous studies demonstrating the ability of 15d-PGJ_2 to inhibit proliferation in HUVEC and ECV304 cells[4]. The possible mechanism might include two aspects: 1)15d-PGJ_2 had an impact on cell cycle phase. In our present study, flow cytometry analysis of ECV304 cells treated with 15d-PGJ_2 for 24 h revealed the accumulation of the cells at the G_0/G_1 peak, and the reduction of cells in S phase. So we could infer that 15d-PGJ_2 inhibited the proliferation of ECV304 endothelial cells by arresting cell cycle; 2) 15d-PGJ_2 induced apoptosis in ECV304 endothelial cells. Hoechst 33258 staining showed that the morphological change characteristic for apoptosis could be observed in ECV304 cells at 48 h after treatment with 15d-PGJ_2. Meanwhile, TUNEL and DNA ladder analysis both confirmed that 15d-PGJ_2 induced apoptosis of ECV304 endothelial cells in a dose-dependent manner.

The mechanism of 15d-PGJ_2 inhibition on cell proliferation and induction on apoptosis is still unclear. Some oxidation-sensitive transcription factors, such as NF-κB, the AP-1 complex, and c-*myc* are activated in atherosclerotic lesions and are involved in expression of early response genes that propagate atherogenic vascular changes[9-11].

Signal-dependent activation of IκB kinase (IKK) results in phosphorylation and rapid degradation of IκB. Free NF-κB migrates into the nucleus and activates expression target gene[12]. AP-1 consists of a variety of dimers composed of member of the Fos, Jun, and ATF families of proteins. All kinds of nuclear receptor can affect the activity of AP-1 by inhibition of the JUN family member (c-jun) and the FOS family member (c-fos)[13]. NF-κB and AP-1 play important roles in cell apoptosis[5,14]. In our study, EMSA analysis showed that 15d-PGJ_2 strongly inhibited the DNA binding activity of NF-κB. Accordingly, Western blot analysis confirmed that 15d-PGJ_2 inhibited the degradation of IκBα. So we could infer that 15d-PGJ_2 inhibited the DNA binding activity of NF-κB by inhibiting the degradation of IκBα. In addition, EMSA analysis also showed that 15d-PGJ_2 distinctly inhibited the DNA binding activity of AP-1. Furthermore, Western blot analysis showed that 15d-PGJ_2 could decrease the expression of c-jun protein. It suggests that 15d-PGJ_2 inhibit the DNA binding activity of AP-1 by inhibiting the expression of c-jun protein. All results suggested that 15d-PGJ_2 induced apoptosis of ECV304 endothelial cells, which may be through inhibiting NF-κB and AP-1 activation pathways.

The proto-oncogene, c-*myc* plays a pivotal role in cell proliferation. *C-myc* has two coupled function: proliferation and apoptosis. These opposing roles of c-*myc* require that other products should inteact with c-*myc* to determine the final outcome of cell[15]. *P53* is one of the most frequently studied tumor suppressing genes. *P53* seems to be responsible for the arrest of cell in the G_1 phase of the cell cycle. *P5*3 induces cell-cycle arrest or apoptosis in many types of cells and activates the transcription of target genes including *p2*1 and *Gadd45*[16]. *Gadd45* is a *p53*-regulated growth arrest gene. Evidence suggests that it plays an important role in regulation of cell growth. *Gadd4*5 may be involved in the G_2/M checkpoint[17]. Both *Gadd45* and *p53* are the target genes of c-*myc.* c-*myc* has been shown to down-regulate both basal and stress-induced *Gadd45* expression[18]. Therefore, in order to understand the mechanism of 15d-PGJ_2 inducing apoptosis in ECV304 cells, we applied RT-PCR and Western blot to investgate the expression of c-*myc, p53, Gadd45,* and *p21*. Both RT-PCR and Western blot analysis showed that 15d-PGJ_2 could decrease the expression of *c-myc*, and induce the expression of *p53* and *Gadd45*. Whereas,15d-PGJ_2 has no effect on the expression of *p21*. So we were able to infer that the down-regulation of c-myc, the up-regulation of *p53* and *Gadd4*5 play important roles in the apoptosis of ECV304 cells induced by 15d-PGJ_2.

Recent studies have shown the possible involvement of MAPK (mitogen-activated protein kinase) pathway. P38 kinase belongs to the MAPK superfamily, which acts as a signaling molecule in cellular apoptosis. Takekawa and Saitohave[19] proposed the following model to the JNK/p38-mediated apoptotic pathways: *p53* is activated in response to genotoxic stress, then causes transcriptional up regulation of *Gadd45*, and *Gadd45* interacts with MTK1 (mitogen-activated protein three kinase 1) to set into motion the JNK/p38-mediated apoptotic pathway. The proposed model implicites *p53* reside at upstream of JNK/p38 pathways and suggests transcriptional and translational dependence for JNK and p38 kinase activation. In our study, p38 kinase analysis showed that the activity of p38 kinase in ECV304 endothelial cells increased after the treatment with 15d-PGJ_2. It was possible that 15d-PGJ_2 induced apoptosis in ECV304 cells by activating p38 kinase, which was activated by the up-regulation of *p53* and *Gadd45*.

In conclusion, 15d-PGJ_2 was able to inhibit ECV304 cell proliferation and induce apoptosis of ECV304. The effect was related to the inhibition of the DNA binding activities of NF-κB and AP-1; on the other hand, the effect was associated with the down-regulation of *c-my*c, up-regulation of *p5*3 and *Gadd45*, and activation of p38 kinase.

REFERENCES

1 Choy JC,Granville DJ, Hunt DW, Mcmanus BM. Endothelial cell apoptosis: biochmical characteristics and potential implications for atherosclerosis. J Mol Cell Cardiol 2001; 33: 1637-90.

2 Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, *et al.* Expression of the peroxisome proliferation-activated receptor Proc Natl Acad Sci Proc Natlγ (PPAR gamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. Proc Natl Acad Sci USA 1998; 95: 7614-9.

3 Toel B , Daid EM. The mechanisms of action of PPARs. Annu Rev Med 2002; 53: 409-35.

4 Bishop-Bailey D, Hla T. Endothelial cell apoptosis induced by the peroxisome proliferator-activated receptor (PPAR) ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. J Biol Chem 1999; 274: 17042-8.

5 Chinetti G, Griglio S, Antonucci M, Torra IP, Delerive P, Majd Z, *et al.* Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages. J Biol Chem 1998; 273: 25573-80.

6 Staels B, Koenig W, Habib A, Merval R, Lebrel M, Torra IP, *et al.* Activation of human aortic smooth-muscle cells is inhibited by PPAR α but not by PPAR α activators. Nature 1998; 393: 790-3.

7 Philippe D, Karolien DB, Sandrine B, Wim VB, Jeffrey MP, Trank JG, *et al* . Peroxisome proliferation-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. J Biol Chem 1999; 274: 32048-54.

8 Cosulich S, James N, Roberts R. Role of MAP kinase signalling pathways in the mode of action of peroxisome proliferators. Carcinogenesis 2000; 21: 579-83.

9 Willa AH, Ronald EL. PPAR γ and Atherosclerosis effects on cell growth and movement. Arterioscler Thromb Vasc Biol 2001; 21: 1891-5.

10 Maulik N, Sasaki H, Addya S, Das DK. Regulation of cardiomyocyte apoptosis by redox-sensitive transcription factors. FEBS Lett 2000; 485: 2-12

11 Dang CV. *C-myc* target genes involved in cell growth, apoptosis, and metabolism. Mol Cell Biol 1999; 19: 1-11.

12 Baeuerle PA, Henkel T. Function and activation of NF-κB in the immune system. Annu Rev Immunol, 1994; 12: 141-79.

13 Caelles C, Gonzalez-Sancho JM., Munoz A. Nuclear hormone receptor antagonism with AP-1 by inhibition of the JNK pathway. Genes Dev 1997; 11: 3351-64.

14 Wang C, Mayo MW, Baldwin AS. TNF and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. Science 1996; 274: 784-9.

15 Hoffman B, Lieberman DA. The proto-oncogene c-*myc* and apoptosis. Oncogene 1998; 17: 3351-7.

16 Wang XW, Zhan Q, Coursen JD, Khan MA, Kontrry HU, Yu L, *et al.* GADD45 induction of a G_2/M cell cycle checkpoint. Proc Natl Acad Sci 1999; 96: 3706-11.

17 Li HL, Ren XD, Zhang HW, Ye CL, Lu JH, Zheng PE, *et al.* Synergism between heparin and adriamycin on cell proliferation and apoptosis in human nasopharyngeal carcinoma CNE2 cells. Acta Pharmacol Sin 2002; 23: 167-72.

18 Aundson SA, Zhan Q, Penn LZ, Fownace AJ. Myc suppresses induction of the growth arrest *gadd34*, *gadd45* and *gadd153* by DNA-damaging agents. Oncogene 1998; 17: 2149-54.

19 Takekawa M, Saito H. A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. Cell 1998; 95: 521-30.