# Inhibitory Signal Transduction of Angiotensin II-induced Vascular Smooth Muscle Cell Proliferation by Rosiglitazone

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# Inhibitory Signal Transduction of Angiotensin II-induced Vascular Smooth Muscle Cell Proliferation by Rosiglitazone

**Directed by Professor Yangsoo Jang**

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## Abstract

# Inhibitory Signal Transduction of ANG II-induced Vascular Smooth Muscle Cell Proliferation by Rosiglitazone

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The ANG II (ANG II) has been implicated as a potential vascular smooth muscle cell (VSMC) growth and fibrosis through the multiple growth promoting signaling pathways and matrix metalloproteinases (MMPs). Thiazolidinediones are known to inhibit VSMC proliferation by increasing the activity of $p27^{Kip1}$ and Retinoblastoma protein (RB) and have an effect to reduce the fibrosis. However, the pathway of inhibition of VSMC and extracellular matrix (ECM) proliferation need to be more elucidated.

Rat aortic VSMCs (6- to 8-week-old Sprague-Dawley rats) were treated with rosiglitazone 1 and 10 μM at 30 minutes before treatment of

Ang II for 24 hours. Western blot analysis was performed to determine the inhibitory effect of expression and activation on the extracellular signal–regulated kinase (ERK) 1/2 and Akt- mammalian target of rapamycin (m-TOR)- 70-kDa S6 kinase (p70S6K)- eukaryocytic initiation factor 4E (eIF4E) binding protein 1 signaling pathway. RT-PCR was performed to evaluate the expression of peroxisome proliferator-activated receptor- γ (PPAR –γ) expression and connective tissue growth factor (CTGF), fibronection and collagen III.

Western blot analysis demonstrated significant inhibition of activation of ERK 1/2, Akt, m-TOR, p70S6K and 4EBP1 in cells treated with rosiglitazone. RT-PCR revealed significantly increased PPAR- γ activity and decreased the CTGF, fibronection and collagen III.

Our present study demonstrates that PPAR-γ activator, rosiglitazone, can directly inhibit the pro-atherosclerotic effect of ANG II on rat aortic VSMCs. Rosiglitazone is capable of attenuating ANG II-induced ECM and CTGF production in rat aortic VSMCs. More importantly, we document that these effects are mediated partly by PPAR-γ activation likely through m-TOR-p70S6 kinase and -4EBP1 system.

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**Key words:** Angiotension II, rosiglitazone, mammalian target of rapamycin (m-TOR)

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## I. INTRODUCTON

Abnormal and excessive growth of vascular smooth muscle cells (VSMCs) such as those in the coronary arteries of the human heart is a hallmark of vascular occlusive disorders such as atherosclerosis and restenosis after balloon angioplasty[1]. In these disorders, VSMCs can undergo hyperplasia or hypertrophy (increases in cell number and size, respectively) in response to a variety of humoral and mechanical stimuli[2]. The vasoconstrictive octapeptide Angiotensin II (ANG II) has been implicated as a potential VSMC growth-inducing agent and acts via at least two G protein-coupled receptor

subtypes, angiotensin 1 ($AT_1$) and angiotension 2 ($AT_2$)[2,3]. Multiple growth promoting signaling pathways are activated by Ang II through AT1 receptors, such as phosphatidylinositol 3-kinase ($PI_3K$) and extracellular signal–regulated kinase (ERK) 1/2[3-8]. PI3K mediates its effects through Akt, 4E-binding protein (BP)1, and 70-kDa S6 kinase (p70S6K)[9].

Thiazolidinediones (TZDs), such as rosiglitazone, are high affinity ligands for peroxisome proliferator-activated receptor- γ (PPAR-γ), a transcription factor of the nuclease hormone receptor superfamily[10]. They are mainly used as insulin sensitizing drugs in type 2 diabetes mellitus. Increasing evidence shows that TZDs not only improve insulin resistance in patients with type 2 diabetes but also exert a broad spectrum of pleiotropic vascular effects in vitro and in animal models[10,11]. Previous studies demonstrated that PPAR-γ activators decreased blood pressure (BP) and cell growth and improved endothelial dysfunction in mesenteric resistance arteries from Ang II–infused rats, thus suggesting that PPAR-γ could contribute to the regulation of different vascular genes in hypertension[12]. Furthermore, Benkirane et al. showed that effect of the PPAR-γ activator, rosiglitazone on chronic Ang II–induced PI3K and mitogen-activated protein kinase (MAPK) signaling was mediated by the inhibition of $PI_3K$/Akt/4E-BP1 and ERK1/2 signaling pathways but did not demonstrated the relation of mammalian target of rapamycin (m-TOR) with rosiglitazone effect[13,14].

Therefore, in this study, we hypothesize that rosiglitazone, a PPAR-ɣ agonist,

may inhibit vascular smooth muscle cell proliferation by the ANG II through the inhibition of Akt-m-TOR-p70S6 kinase or -4EBP1.

## II. MATERIALS AND METHODS

### 1. Materials

Rosiglitazone was a generous gift from GlaxoSmithKlein. All other materials were purchased from commercial suppliers.

### 2. Methods

#### Isolation and culture of rat aortic vascular smooth muscle cells

Rat aortic smooth muscle cells (RAoSMCs) were isolated and purified by previously described methods[15]. The thoracic aortas from 6- to 8-week-old Sprague-Dawley rats were removed and transferred in serum-free Dulbecco's modified Eagle' medium (DMEM; Invitrogen Co, Carlsbad, CA, USA) containing 100 units/㎖ of penicillin and 100 ㎍/㎖ of streptomycin. The aorta was freed from connective tissue, transferred into Petri dish containing 5 ㎖ of an enzyme dissociation mixture containing DMEM with 1 ㎎/㎖ of collagenase type I (Sigma, St. Louis, MO, USA) and 0.5 ㎍/㎖ elastase (USB

Bioscience, Cleveland, OH, USA), and incubated for 30 min and at 37°C. The aorta was the transferred into DMEM and the adventitia was stripped off with forceps under a binocular microscope. The aorta was transferred into a plastic tube containing 5 ㎖ of the enzyme dissociation mixture and incubated for 2 h at 37°C. The suspension was centrifuged (1,500 rpm for 10 min) and the pellet was resuspended in DMEM with 10% fetal bovine serum (FBS). Rat aortic VSMCs were cultured in DMEM supplement with 10% FBS, 100 IU/㎖ penicillin, 100 ㎍/㎖ streptomycin in 75-㎠ flasks at 37°C in a humidified atmosphere of 95% air and 5% $CO_2$ (Forma Scientific, Inc., Marjetta, OH, USA.).

**Cell proliferation assay**

Rat aortic VSMCs were plated in triplicate wells of 96 well plates at a density of 1 x $10^4$ per well. Pretreatment with rosiglitazone was done 30 min prior to exposure to ANG II (0.1 μM) for 24 hrs. After cell treatment, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Sigma, St. Louis, MO, USA) was added to each well to a final concentration of 0.5 mg/mL and was incubated at 37℃ for 3 hr to allow MTT reduction. The formazan crystals were dissolved by adding dimethylsulfoxide (DMSO) and absorbance was measured at the 570 nm with a spectrophotometer. Experiments were performed in triplicate.

**RT-PCR analysis**

The expression levels of various proteins were analyzed by reverse transcription polymerase chain reaction (RT-PCR). Total RNA was prepared by the UltraspectTM-II RNA system (Biotecx Laboratories, Inc., USA) and single-stranded cDNA was then synthesized from isolated total RNA by Avian Myeloblastosis virus (AMV) reverse transcriptase. A 20 μl reverse transcription reaction mixture containing 1 μg of total RNA, 1X reverse transcription buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 1 mM deoxynucleoside triphosphates (dNTPs) 0.5 unit of RNase inhibitor, 0.5 μg of oligo(dT)15 and15 units of AMV reverse transcriptase was incubated at 42℃ for 15 min, heated to 99℃ for 5 min, and then incubated at 0-5℃ for 5 min. PCRs were performed for 35 cycles with 3' and 5' primers based on the sequences of various genes. Primers were as follows:

**PPAR-γ: 5'-TGGAGCCTAAGTTTGAGTTTG-3', 5'-ATCTTCTGGAGCACCTTGG-3' (226 bp)**
**Collagen type III: 5'-AGATGCTGGTGCTGAGAAG-3', 5'- TGGAAAGAAGTCTGAGGAAGG-3' (312 bp)**
**Fibronectin 5'-GTGAA-GAACG-AGGAG-GATGT-G-3', 5'-GTGATGGCGGATGATGTAGC-3' (300 bp)**
**Connective tissue growth factor: 5'-AAGAAGACTCAGCCAGACC-3', 5'-AGAGGAGGAGCACCAAGG-3' (235 bp)**

GAPDH (primers 5'-accacagtccatgccatcac-3' and 5'-tccaccaccctgttgctgta-3' (450 bp)) was used as the internal standard. The signal intensity of the amplification product was normalized to its respective GAPDH signal intensity.

**Immunoblot analysis**

Rat aortic VSMCs were treated with rosiglitazone before the addition of ANG II as stimulus. Cells were washed once in PBS and lysed in a lysis buffer (Cell signaling, Beverly, MA, USA) containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM $Na_2EDTA$, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM $Na_3VO_4$, 1 mg/ml leupeptin and 1 mM PMSF. Protein concentrations were determined using the Bradford protein assay kit (BioRad, Hercules, CA, USA). Proteins were separated in a 12% SDS-polyacrylamide gel and transferred to PVDF membrane (Millipore Co, Bedford, MA, USA). After blocking the membrane with Tris-buffered saline-Tween 20 (TBS-T, 0.1% Tween 20) containing 5% non-fat dried milk for 1 hr at room temperature, membranes were washed twice with TBS-T and incubated with primary antibodies for 1 hr at room temperature or for overnight at 4°C. The following primary antibodies were used: rabbit anti-ERK, mouse anti-phospho ERK (Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-m-TOR, rabbit anti-phospho m-TOR, rabbit anti-p70S6K, mouse anti-phopho p70S6K, rabbit anti-4EBP1, rabbit anti-phospho 4EBP1 (Cell signaling, Beverly, MA, USA). The membrane were washed three times with TBS-T for 10 min, and then incubated for 1 hr at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies. After extensive washing, the bands were detected by enhanced chemiluminescence (ECL)

reagent (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The band intensities were quantified using a Photo-Image System (Molecular Dynamics, Uppsala, Sweden).

**Statistical analysis**

Data are presented as mean ± S.E.M. of more than three separate experiments performed in triplicate. Where results of blots and RT-PCR are shown, a representative experiment is depicted. Comparisons between multiple groups were performed with one-way ANOVA (Analysis of Variance) with Bonferroni's test. Statistical significance was defined as $p < 0.05$ and $p < 0.01$.

# III. RESULTS

## 1. Antiproliferative effect of rosiglitazone in ANG II-stimulated rat aortic VSMCs

ANG II induces a wide variety of vascular events including endothelial activation and dysfunction, cell proliferation and monocyte chemoattraction, which play an important role in atherosclerosis development. In order to investigate whether rosiglitazone might regulate the proliferation of rat aortic VSMCs stimulated by ANG II, cells were pretreated with various concentrations (0 - 100 μM) of rosiglitazone for 30min prior to exposure of ANG II (0.1 μM). Rosiglitazone inhibited ANG II-stimulated proliferation (Figure 1). Namely, these finding shows rosiglitazone has an antiproliferative effect on rat aortic VSMCs stimulated by ANG II.

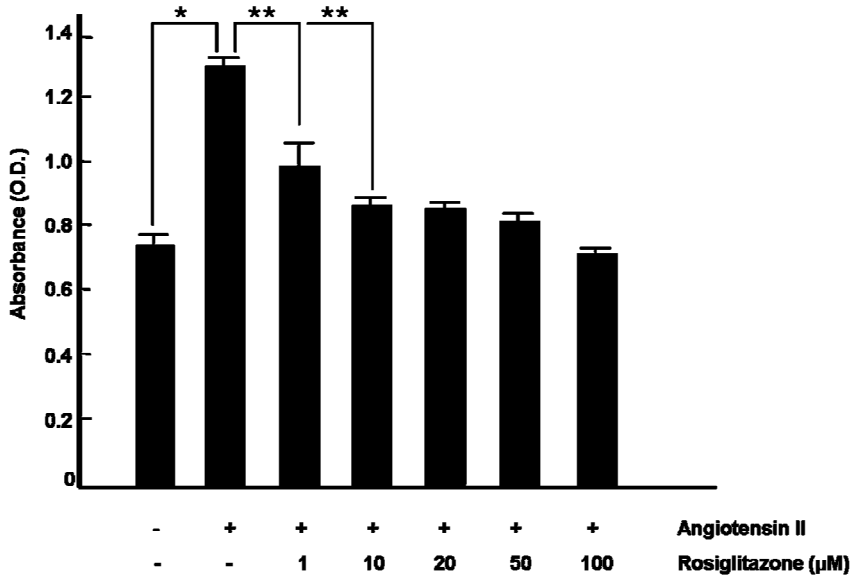


Figure 1. Effect of rosiglitazone on the proliferation of ANG II-induced RAoSMCs. Quiescent RAoSMCs (1.0 x $10^4$ cells per well) were stimulated with 0.1 μM of ANG II. Pretreatment with rosiglitazone was done 30 minutes before exposure to ANG II (0.1 μM) for 24 hours. After cell treatment, cell proliferation was determined by the 3-(4,5-Dimethylthiazol-2-yl) -2,5 -diphenyltetrazolium bromide (MTT) assay according to the concentration of rosiglitazone. 8). $^{*}P< 0.01$; $^{**}< 0.05$.

**2. PPAR-γ expression in ANG II-stimulated rat aortic VSMCs**

To further demonstrate that PPAR-γ activation was involved in the regulation of rosiglitazone in ANG II-induced rat aortic VSMCs proliferation, first, we

studied the expression and activation of PPAR-γ in response to ANG II and rosiglitazone treatment. As shown in Figure 4, ANG II treatment down-regulated PPAR-γ mRNA expression whereas pretreatment of cells with rosiglitazone significantly increased PPAR-γ expression in ANG II-stimulated rat aortic VSMCs.

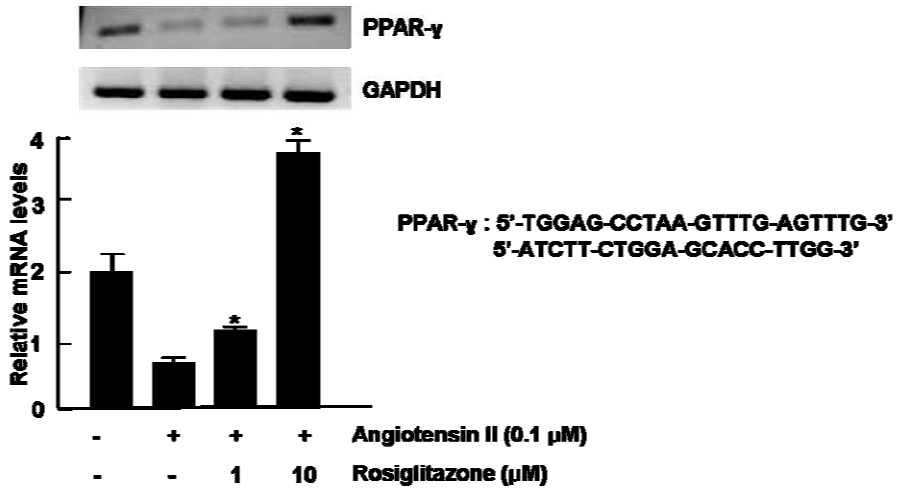


Figure 2. Effect of rosiglitazone on peroxisome proliferator-activated receptor-γ (PPAR-γ) expression of ANG II-induced RAoSMCs. The expression levels of PPAR-γ were analyzed by reverse transcription polymerase chain reaction (RT-PCR). The signal intensity of the amplification product was normalized to its respective GAPDH signal intensity. ).*P< 0.01.

### 3. Effect of rosiglitazone on activation of ERK stimulated by ANG II

In the mechanisms of cellular survival and proliferation, the activation of ERKs plays an important role in gene regulation. ERK is one of dual specificity kinases in MAPKs. Phosphorylation of ERKs (42 and 44 kDa) was detected by immunoblot assay. It has been known that ANG II stimulates the activation of ERK MAPK in rat aortic VSMCs. Thus, we investigated the effect of rosiglitazone on ERK MAPK activation in ANG II-stimulated rat aortic VSMCs. We demonstrated that ERK MAPK activation was seen within 30 min with 0.1 μM of ANG II. These signaling activations were significantly inhibited by pretreatment with 1 and 10 μM of rosiglitazone (Figure 3).

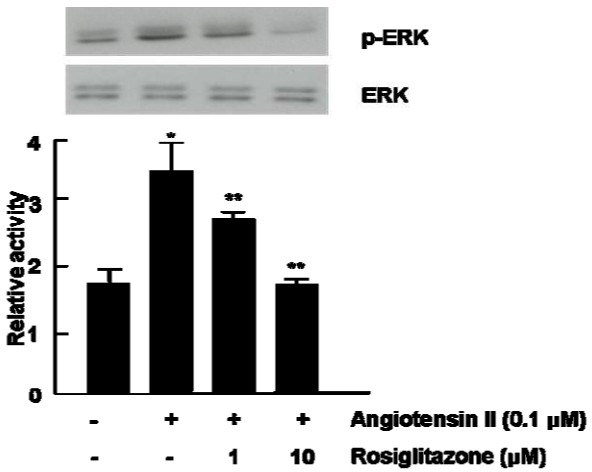


Figure 3. Effect of rosiglitazone on expression and activation of extracellular signal–regulated kinase (ERK) in RAoSMCs. Western blot analysis of ERK

was performed in RAoSMCs exposed to 1-10 μM rosiglitazone before treatment of 0.1 μM ANG II in the DMEM with 0.5% FBS. The band intensities were quantified using a Photo-Image System and the figure revealed the levels of each activity as relative value of the maximal level of ERK. Western blot was performed in triplicate. Results are means ± SE. $^{*}P < 0.01$; $^{**}P < 0.05$.

**4. Inhibitory effects of rosiglitazone on activation of Akt and m-TOR in ANG II-stimulated rat aortic VSMCs**

To confirm the inhibitory effect of various concentrations of rosiglitazone on Akt and m-TOR, the change of Akt and m-TOR was estimated by immunoblot analysis. The 70% confluency of rat aortic VSMCs were made quiescent by serum starvation with 0.1% FBS for 3days. Cells were treated with various concentrations of rosiglitazone before stimulation of 0.1 μM ANG II. The activation of Akt and m-TOR with ANG II stimulus was significantly inhibited by rosiglitazone in a dose-dependent manner. (Figure 4) Compared to cells treated with ANG II alone, cells treated with rosiglitazone before ANG II stimulus demonstrated significant inhibition of Akt and m-TOR phosphorylation.

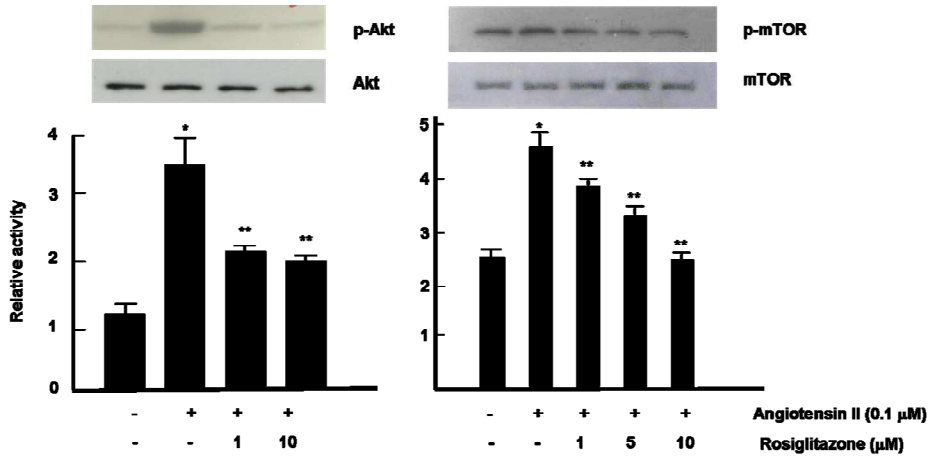


Figure 4. Effect of rosiglitazone on expression and activation of Akt and mammalian target of rapamycin (m-TOR) in RAoSMCs. Western blot analysis of m-TOR was performed in RAoSMCs exposed to 1-10 μM rosiglitazone before treatment of 0.1 μM ANG II in the DMEM with 0.5% FBS. The band intensities were quantified using a Photo-Image System and the figure revealed the levels of each activity as relative value of the maximal level of m-TOR. Western blot was performed in triplicate. Results are means ± SE. $^{*}P<$ 0.01; $^{**}<$ 0.05.

**5. P70S6 kinase and 4EBP1 signaling pathway in rat aortic VSMCs treated with rosiglitazone**

The stimulation of cell proliferation by ANG II in rat aortic VSMCs was blocked by rosiglitazone, which is an inhibitor of m-TOR signaling pathway

that includes the 70-kDa S6 kinase (p70S6k) and plays a key role in cell growth. Furthermore, ANG II triggered dissociation of the translation initiation factor, eukaryotic initiation factor-4E, from its regulatory binding protein 4E-BP1. Stimulation of rat aortic VSMCs with ANG II caused an increase in the activity of p70S6k. The ANG II-enhanced activity was completely suppressed by pretreatment of rat aortic VSMCs with rosiglitazone (1 and 20 μM). ANG II also significantly increased 4E-BP1 phosphorylation, which was significantly inhibited by rosiglitazone (1 and 20 μM, Figure 6). It suggests that rosiglitazone significantly inhibit both the activation of the m-TOR-p70S6 kinase and -4EBP1 system.

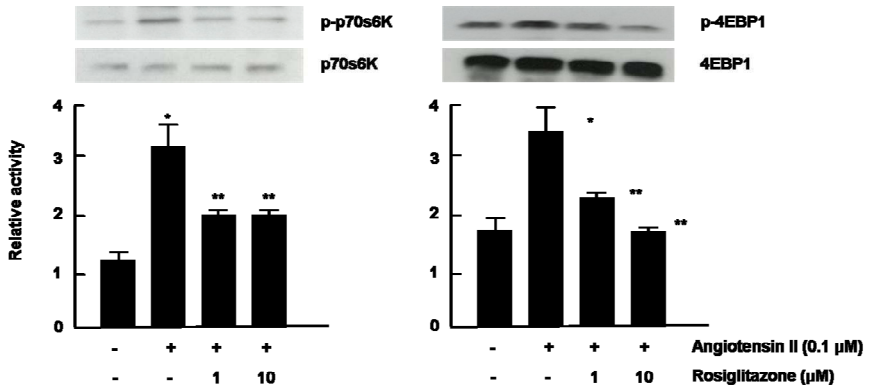


Figure 5. Effect of rosiglitazone on expression and activation of 70-kDa S6 kinase (p70S6) and 4E-binding protein 1 (4EBP 1) in RAoSMCs. Western blot analysis of p70S6 and 4EBP1 was performed in RAoSMCs exposed to 1-10 μM rosiglitazone before treatment of 0.1 μM ANG II in the DMEM with 0.5% FBS. The band intensities were quantified using a Photo-Image System and the figure

revealed the levels of each activity as relative value of the maximal level of p70S6 and 4EBP1. Western blot was performed in triplicate. Results are means ± SE. $^{*}P< 0.01$; $^{**}< 0.05$.

**6. Inhibitory effects of rosiglitazone on expression of CTGF and extracellular matrix in ANG II-stimulated rat aortic VSMCs**

To demonstrate whether a direct cellular mechanism was involved in the regulatory effect of rosiglitazone on ANG II-induced CTGF and ECM expression in rat aortic VSMCs, we performed experiments in cultured RASMCs. As illustrated in Figure 2, ANG II (0.1 μM) treatment for 24 h markedly increased the mRNA expression of CTGF, Col III and FN in rat aortic VSMCs. Rosiglitazone (1and 10 μM) dose-dependently abolished this effect (Figure 6).

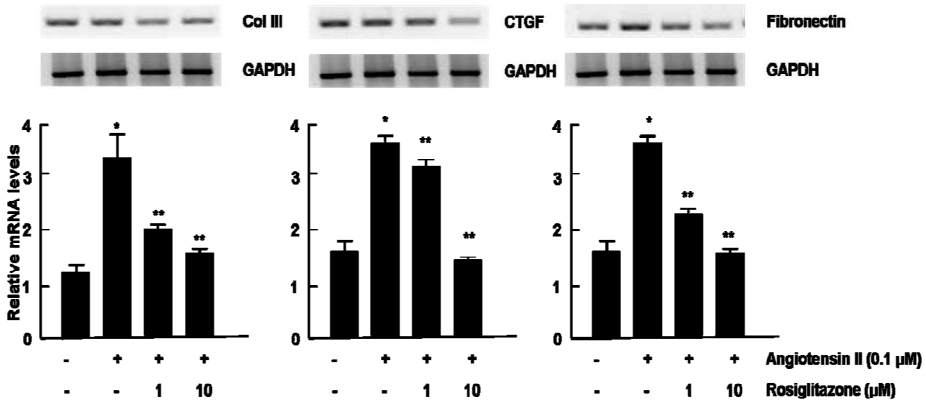


Figure 6. Effect of rosiglitazone on expression of connective tissue growth factor (CTGF) and extracellular matrix of ANG II-induced RAoSMCs. The

expression levels of PPAR-γ were analyzed by reverse transcription polymerase chain reaction (RT-PCR). The signal intensity of the amplification product was normalized to its respective GAPDH signal intensity. Results are means ± SE. $^{*}P< 0.01$; $^{**}< 0.05$.

## IV. DISCUSSION

Our present study demonstrates that PPAR-γ activator, rosiglitazone, can directly inhibit the pro-atherosclerotic effect of ANG II on rat aortic VSMCs. Fibrosis is one of the vascular changes caused by hypertension and diabetic mellitus. In this study, rosiglitazone is capable of attenuating ANG II-induced ECM and CTGF production in rat aortic VSMCs. More importantly, we document that these effects are mediated partly by PPAR-γ activation likely through m-TOR-p70S6 kinase and -4EBP1 system.

In general, diabetes and hyperglycemia alone have not been associated with processes of cell cycle regulation but it has been known that ANG II triggers responses in vascular smooth muscle cells that lead to proliferation, migration, and a phenotypic modulation resulting in production of growth factors and extracellular matrix[3,16,17]. ANG II induces an increase of translation initiation factors, phosphorylation of 4EBP-1 and S6K, critical cell cycle regulators down stream of m-TOR[4,7,18,19]. The mammalian Target of Rapamycin(m-TOR) is a key regulatory kinase that plays a major role in the mammalian cell cycle and is a major pathway in the pathogenesis of neointimal hyperplasia and in stent restenosis[20]. Previous studies have revealed that m-TOR signaling is important in cell and organism growth, cell cycle and proliferation and in aspects of metabolism, as well as gene transcription and transcriptional regulators[4].

Thiazolidinediones such as rosiglitazone are insulin sensitizing agents

treating hyperglycemia by improvement of insulin resistance in type 2 diabetic patients[10]. In addition to increase in insulin sensitivity, studies have shown significant antiproliferative effect of Thiazolidinediones on the vascular tissues[10]. However, the molecular mechanism underlying the antiproliferative effect has not been fully elucidated.

Because m-TOR itself is known to be activated by the Phosphoinositide 3 kinase(PI3Ks)-Akt pathway, we wanted to determine whether Rosiglitazone inhibits vascular smooth muscle cell proliferation through the inhibition of Akt-m-TOR-p70S6 kinase or -4EBP1 pathway and that Akt-m-TOR-p70S6K or -4EBP1 pathway system is a specific, major pathway for VSMC proliferation inhibition[4,19]. Although previous studies showed that effect of the PPAR-γ activator, rosiglitazone on chronic ANG II–induced PI3K and MAPK signaling was mediated by the inhibition of PI3K/Akt/4E-BP1 and ERK1/2 signaling pathways but did not demonstrated the relation of m-TOR with rosiglitazone effect[13,14,21,22]. The results from this study demonstrated that cells treated with rosiglitazone before ANG II stimulus demonstrated significant inhibition of p-m-TOR, p-Akt, p-P70S6K, and p-4EBP1 suggestive of significant inhibition of activation in the Akt-m-TOR-p70S6 kinase and -4EBP1 system.

ANG II has been implicated as a potential vascular smooth muscle cell (VSMC) growth and fibrosis through the multiple growth promoting signaling pathways and MMPs[16,22]. ANG II also involves the vascular fibrosis, which is one of vascular change by hypertension and diabetes mellitus[17]. Gao et al.

demonstrated rosiglitazone inhibit the ANG II-induced VSMCs proliferation and ECM production through the mechanism of reducing the CTGF, which play a role of cell proliferation and apoptosis[23]. This study confirmed that rosiglitazone could significantly reduced the ANG II stimulated CTGF and ECM expression in rat aortic VSMCs.

The present study has several limitations. Firstly, in vivo study was not performed because it was difficult to make an ANG II simulated animal model, therefore we could not reproduced in vivo situations. Secondly, although we found out the inhibition of m-TOR by rosiglitazone, we could not explain this effect whether it caused indirectly by inhibition of upstream pathway or direct effects. But, this study has an implication that the entire inhibitory mechanism was elucidated for angiotension II induced VSMC and ECM proliferation by rosiglitazone.

## V. CONCLUSION

In conclusion, rosiglitazone inhibits vascular smooth muscle cell proliferation because it blocks m-TOR-p70S6 kinase and -4EBP1 system. The inhibitory effect of rosiglitazone on ANG II-induced rat aortic VSMCs may be also mediated by reduction of extracellular matrix production. These findings extend the understanding of the important role of rosiglitazone in vascular fibrosis and provide novel evidence for the beneficial vascular effect of rosiglitazone.

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**ABSTRACT (IN KOREAN)**

## Rosiglitazone 에 의한 ANG II 유도 혈관 평활근 세포 증식의 억제 신호 전달

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Angiotension II는 여러가지 증식에 영향을 주는 신호전달 체계 및 여러 matrix metalloproteinase 등에 의해 혈관 평활근의 증식 및 혈관 벽의 섬유화를 도모하는 것으로 알려진 물질이다. 이에 반해 최근 rosiglitazone으로 알려진 thiazolidinediones은 $p27^{Kip1}$과 retinoblastoma protein (RB)의 활성도를 억제해서 혈관 평활근 세포의 증식을 억제한다고 보고되고 있다. 최근 이러한 기전과 연관된 upstream signaling에 대한 연구가 진행되고 있고 몇몇의 기전은 밝혀진 상태이다. 하지만 세포성장 및 증식을 조절하는데 중추적인 역할을 하는 Akt-m-TOR-P70S6K 에 대한

효과 및 혈관 평활근 및 섬유화에 대한 전반적인 기전에 대한 연구는 미흡한 실정이다. 따라서 본 연구에서 rosiglitazone이 angiotension II로 유도된 혈관 평활근의 증식 및 섬유화의 억제하는 전반적인 기전에 대해서 알아보고자 하였다. angiotension III를 mitogenic stimulus로 사용하였으며 백서 대동맥 평활근 세포에 angiotension II를 처치하기 30 분전 rosiglitazone 1, 10μM를 처치하였다. rosiglitazone이 Akt-m-TOR-P70S6K 신호전달체계 및 섬유화를 억제하는지를 분석하기 위해 western blot 분석과 RT-PCR을 시행하였다. western blot 분석결과 rosiglitzone이 전처치된 대동맥 평활근 세포에서는 p-Akt, p-m-TOR, p-p70S6K의 활성도가 현저하게 감소됨을 관찰하였고 RT-PCR 결과 collagen II, fibronectin, connective tissue growth factor (CTGF)의 mRNA transcript가 rosiglitazone이 전처치된 세포에서 현저히 감소됨을 관찰하였다. 본 연구결과에서 rosiglitazone에 의한 angiotension II에 의한 평활근 세포 증식에 있어 Akt-m-TOR-p70S6K 신호전달체계의 억제가 중요한 역할을 하면 m-TOR 자체로 억제되는 결과를 알 수 있었고 섬유화 역시 억제되는 소견이 확인되었다. 따라서 결론적으로 본 연구에서는

rosiglitazone에 의한 혈관 평활근 세포 증식 억제효과는 in vitro에서 Akt-m-TOR-p70S6K 신호전달체계의 억제를 통하며 CTGF의 억제를 통해 혈관의 섬유화가 억제됨을 알 수 있었다.

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**핵심되는 말: Angiotensin II, rosiglitazone, mammalian target of Rapamycin (m-TOR)**