Angiotensin II Regulation of Collagen Type I Expression in Cardiac Fibroblasts
Modulation by PPAR-γ Ligand Pioglitazone

Kui Chen, Jiawei Chen, Dayuan Li, Xingjian Zhang, Jawahar L. Mehta

Abstract—Angiotensin II (Ang II)–mediated stimulation of fibroblast growth and collagen type I synthesis is believed to be an important component of the cardiac remodeling process in hypertension and chronic ischemia. Ang II–mediated oxidative stress could be important in enhanced fibroblast growth and collagen formation. Accordingly, we postulated that the PPAR-γ ligand, pioglitazone, which is known to modulate oxidative stress, would alter Ang II–induced formation of collagen type I in cardiac fibroblasts. Cardiac fibroblasts were treated with different concentrations (10⁻⁸ to 10⁻⁶ M) of Ang II for different times (6 hours, 12 hours, and 24 hours). Ang II increased the expression of collagen type I in a concentration- and time-dependent fashion (P<0.01 versus control). Ang II also decreased the expression and activity of matrix metalloproteinase (MMP)-1 (MMP-1, P<0.05 versus control). These effects of Ang II were attenuated by pretreatment of cells with pioglitazone (10 μmol/L). Ang II stimulated the intracellular generation of reactive oxygen species (ROS), and this effect was also attenuated by pioglitazone. Ang II treatment activated the redox-sensitive transcription factor NF-κB, and pioglitazone pretreatment blocked this effect of Ang II. Ang II also activated another transcription factor, AP-1, but this effect of Ang II was not modulated by pioglitazone. In other experiments, we observed that trolox, the water soluble analog of vitamin E, attenuated the effects of Ang II on the expression of collagen type I and MMP-1, in a manner similar to pioglitazone. Thus, pioglitazone attenuates Ang II-mediated collagen type I synthesis in cardiac fibroblasts. The effects of pioglitazone are mediated by the modulation of ROS release and redox-sensitive transcription factor NF-κB. (Hypertension. 2004;44:655-661.)

Key Words: angiotensin II ▪ cardiac function ▪ collagen

Heart failure is the end result of cardiovascular disease states, such as hypertension and myocardial ischemia.1 Heart failure is characterized by abundant accumulation of matrix proteins in the extracellular space. Among the extracellular matrix (ECM) proteins, collagens constitute up to 85%–90%.2,3 Collagens are proteins that consist of a triple helix of polypeptide chains and globular domains, and comprise a family of proteins of at least 19 genetically distinct types, among which type I and type III constitute two-thirds.2,3 Collagen type I is usually present in the form of thick fiber with a high tensile strength. Therefore, collagen type I content is considered a major determinant of myocardial stiffness.3,4 Fibroblasts are the major source of collagen in the myocardium.3,5 Proliferation of cardiac fibroblasts and deposition of collagen are directly associated with both systolic and diastolic (especially the latter) heart failure.6 Collagen type I accumulation in the heart depends not only on its production, but also on its degradation, which is performed by proteinases, such as matrix metalloproteinase-1 (MMP-1).3

Activation of the renin-angiotensin system with synthesis and release of angiotensin II (Ang II) is a key feature of hypertension and myocardial ischemia, and both conditions lead to cardiac remodeling comprising myocyte hypertrophy and fibroblast proliferation.7,8 Most of the known effects of Ang II in the heart are believed to be mediated by the activation of its type 1 (AT₁) receptors.8 AT₁ receptors are widely present on cardiac fibroblasts, and Ang II via AT₁ activation mediates fibroblast proliferation and ECM production.9,10 Indeed, long-term intravenous infusion of Ang II is associated with the development of hypertension and cardiac fibrosis.10,11 In recent studies, we showed that Ang II via AT₁ activation stimulates oxidative stress in human coronary artery endothelial cells.12 Whether inhibition of oxidative stress can modulate the profibrotic effects of Ang II is not known.

 Peroxisome proliferator-activated receptors (PPAR) are a family of at least 3 nuclear receptors (α, β/δ, and γ).13,14 After activation by the ligand, PPARs heterodimerize with retinoic X receptor and recognize PPAR response element which

Received June 10, 2004; first decision June 28, 2004; revision accepted August 17, 2004.
From the Departments of Internal Medicine and Physiology and Biophysics, University of Arkansas for Medical Sciences, and the Central Arkansas Veterans Healthcare System, Little Rock.
Correspondence to J.L. Mehta, MD, PhD, Division of Cardiovascular Medicine, University of Arkansas for Medical Sciences, 4301 W. Markham St, Slot 532, Little Rock, AR 72205, E-mail MehtaJL@uams.edu
© 2004 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org DOI: 10.1161/01.HYP.0000144400.49062.6b
exists in the promoter of target genes. The ligand-activated PPAR functions as a transcription factor and regulates target gene expression. It has been shown that PPAR plays a critical role in lipid catabolism, peroxisome proliferation, and adipogenesis. Several investigators have shown that PPAR-γ ligands reduce myocardial infarct size after coronary artery ligation. Shiomi et al provided evidence that pioglitazone attenuates cardiac remodeling after chronic ischemia. In recent studies, we showed that the PPAR-γ ligand pioglitazone blocks the effect of several mediators of tissue injury, such as oxidized low density lipoprotein (ox-LDL), Ang II, and tumor necrosis factor-α (TNF-α), on endothelial cells.

In the present study, we examined the hypothesis that pioglitazone blocks the effect of Ang II on collagen type I and MMP-1 expression in cardiac fibroblasts as a basis of its effect on cardiac remodeling.

Materials and Methods

Cell Culture

Rat cardiac fibroblasts were prepared and cultured as described earlier. In brief, rat hearts were removed from anesthetized Sprague-Dawley male rats (200 to 250 g, Jackson Laboratories, Bar Harbor, ME). The ventricles were isolated, minced, and digested in a 0.25% collagenase solution (37°C, 1 hour). After digestion, the cells were pelleted and suspended in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 1% penicillin and streptomycin and 10% fetal bovine serum (FBS). The suspension was transferred to 56.7 cm² dishes (Nuncbrand). After a 60-minute incubation period, cells that were weakly attached or unattached were removed, and the attached cells were grown to confluence. After 2 to 3 days, the confluent cells were detached by trypsin and seeded on new dishes. The purity of these cultured cardiac fibroblasts was greater than 95% on the basis of positive staining for vimentin and negative staining for smooth muscle actin and von Willebrand factor (vWF, Sigma). Third to fifth passage cardiac fibroblasts were used for all experiments. Cardiac fibroblasts were treated with different concentrations of Ang II (10⁻⁸, 10⁻⁷, and 10⁻⁶ M) for 6 to 24 hours to determine the expression of collagen type I and MMP-1.

In parallel experiments, cells were pretreated with pioglitazone (10 μmol/L) for 30 minutes followed by treatment with Ang II (or left untreated) in the presence of pioglitazone. This concentration of pioglitazone in previous studies was shown to result in maximal inhibition of reactive oxygen species (ROS) release. In other experiments, fibroblasts were pretreated with trolox (10 μmol/L), a water-soluble analog of vitamin E, before exposure to Ang II.

All experimental procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee.

Measurement of Intracellular Generation of Reactive Oxygen Species

Intracellular ROS generation was measured by the method described by Usui-Fukai et al. In brief, cardiac fibroblasts were cultured with DMEM containing 10% calf serum until they grew to 60% confluence; then they were cultured in DMEM containing 0.1% calf serum for 48 hours. Cells were then treated with Ang II (10⁻⁷ M) for 12 hours, and the ROS-sensitive fluorophore DCF-DA (10 μmol/L) added. Intracellular ROS generation was measured using Fluoromax-3 spectrophuorometer with an excitation set at 485 nm and emission at 530 nm. This method involves conversion of the nonfluorescent dichlorofluorescein diacetate to the highly fluorescent compound 2,7-dichlorofluorescein (DCF) in the cells. In parallel studies, cells were pretreated with pioglitazone for 12 hours followed by treatment with Ang II or left untreated.

Collagen Synthesis Assay

Collagen synthesis was determined by measuring ³H-proline incorporation as described earlier. In brief, cardiac fibroblasts were made quiescent by culture in serum-free DMEM for 48 hours. These cells were then cultured in DMEM supplemented with 2.5% FBS, 50 μg/mL ascorbate and L-[2,3,4,5-³H] proline (1 μCi/mL, Amersham Biosciences) and treated as mentioned above. After treatment, cells were made quiescent by culture in serum-free DMEM for 48 hours. These cells were then cultured in DMEM supplemented with 2.5% FBS, 50 μg/mL ascorbate and L-[2,3,4,5-³H] proline (1 μCi/mL, Amersham Biosciences) and treated as mentioned above. After treatment, cells

Figure 1. Top left panel, Ang II and expression of collagen type I. Incubation with Ang II (10⁻⁸ to 10⁻⁶ M for 12 hours) increased the expression of collagen in a concentration-dependent manner in cardiac fibroblasts. Top right panel, The upregulation of collagen by Ang II (10⁻⁷ M) occurred in a time-dependent manner. Collagen protein band density was normalized by its control. Top panels are representative experiments. Bottom panels are summary of data (mean±SD) from 5 independent experiments.

Figure 2. Ang II and expression of MMP-1. MMP-1 expression was markedly decreased by treatment of fibroblasts with low concentration of Ang II (10⁻⁷ M). The reduced expression of MMP-1 with low concentration of Ang II (10⁻⁷ M) was evident when the incubation time was only 6 hours. With the increase in incubation time, the reduction in MMP-1 expression was still evident. The right panel shows that Ang II decreases both 42 kDa (MMP-1) and 62 kDa (MMP-2) bands. Top panels are representative experiments. Bottom panels are summary of data (mean±SD) from 5 separate experiments.
were washed with PBS and ice-cold 10% trichloroacetic acid (TCA). The cells were solubilized and the cell extracts analyzed in a liquid scintillation counter. The count represented the amount of newly synthesized collagen. The data were normalized to cell number.

Protein Preparation and Analysis by Western Blot

Collagen fibrils from each group were pellet and extracted in iced cell lysis buffer (Cell Signaling Technologies). Cell lysates were centrifuged at 15,000g for 15 minutes at 4°C and the supernatants from each group (45 μg protein per lane) were separated by 10% SDS-PAGE (for MMP-1) and 8% nondenatured-PAGE (for collagen type I) and then transferred to nitrocellulose membranes. After incubation in blocking solution (5% nonfat milk, Sigma), membranes were incubated with primary antibodies overnight at 4°C. Primary antibodies used were anticollagen type I (Rockland) and anti-MMP-1 (Oncogene). Membranes were washed with 1×TBST solution and then incubated with secondary antibody (1:5000 dilution, Amersham Life Sciences) for 2 hours. The membranes were detected with the ECL system (Amersham Life Sciences) and relative intensities of bands were analyzed by Scan-gel-it software.23

Collagenase Activity Assay

Collagenase zymography was performed as recently described.24 Essentially, the conditioned culture medium was collected from the dishes and 10 μL of the medium was subjected to electrophoresis in SDS polyacrylamide gel containing 0.1% gelatin under nonreducing conditions. The gels were soaked in 2.5% Triton-X100 for 60 minutes and then washed with water for 60 minutes to remove SDS. The gels were then incubated in a developing buffer containing 50-mm/L Tris, pH 7.4, 5-mm/L CaCl₂, and 0.02% sodium azide for 18 hours at 37°C. After incubation, the gels were stained with Coomassie blue and photographed.

Electrophoresis Mobility Shift Assay

Nuclear extracts were prepared according to the method published previously.25 In brief, cultured cells were washed with ice-cold Tris-buffered saline twice and pelleted. Then the pellets were suspended in 400 μL of solution A (10 mmol/L HEPES, 10 mmol/L KCl, 0.1 mmol/L EDTA, 0.1 mmol/L EGTA, 1 mmol/L dTT, 1 mmol/L PMSF, 1 mmol/L leupeptin, pH 7.9) for 5 minutes. After a brief vortex and centrifugation, the pellets were suspended and incubated in 150 μL of solution B (20 mmol/L HEPES, 0.4 mol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L dTT, 1 mmol/L PMSF, 1 mmol/L leupeptin) for 30 minutes. After centrifugation, the supernatants were transferred to a clear tube and stored in −80°C. EMSA was performed as described previously. In brief, 10 μg of nuclear extract was incubated with radio-labeled probes for 30 minutes. The probes used were oligonucleotides containing nuclear factor-κB (NF-κB) binding sites (5′-AGT TGA GGG GAC TTT CCC AGG C-3′, 3′-TCA ACT CCC CTG AAA GGG TCC G-5′) or activator protein-1 (AP-1) binding sites (5′-CGC TTG ATG AGT CAG CCG GAA-3′, 3′-GC GAC AAC TAC TCA GTC GGC CTT-5′).

The protein/DNA complexes were separated by 4% acrylamide gel. After autoradiography, the relative intensities of bands were analyzed by Scan-gel-it software.23

Data Analysis

All data represent the mean of duplicate samples from at least 3 separately performed experiments. Data are presented as mean±SD. Statistical significance was determined in multiple comparisons among independent groups of data using ANOVA followed by F-test. A probability value of ≤0.05 was considered significant.

Results

Ang II and the Expression of Collagen Type I and MMP-1 in Cardiac Fibroblasts

Ang II treatment increased the expression of collagen type I protein in a time- and concentration-dependant fashion in cardiac fibroblasts (Figure 1). Concurrent with the increase in collagen expression, MMP-1 expression decreased (P<0.01 versus control). This effect, ie, reduction in MMP-1, was evident when cells were treated with Ang II for as short a period of time as 6 hours (Figure 2 left). In all subsequent experiments the expression of collagen or MMP-1. Pioglitazone pretreatment also attenuated the effect of Ang II on the activity of both MMP-1 and -2. Top panels are representative experiments. Bottom panels are the summary of data (mean±SD) from 5 separate experiments.

Figure 3. Pioglitazone and Ang II–induced collagen type I expression and MMP-1 expression/activity in cardiac fibroblasts. Incubation with Ang II (10⁻⁷ M) for 12 hours markedly reduced the expression of collagen and reduced the expression of MMP-1. Pretreatment of cardiac fibroblasts with pioglitazone (10 μmol/L) for 30 minutes almost completely blocked the effect of Ang II. Note that pioglitazone alone had no effect on the basal expression of collagen or MMP-1. Pioglitazone pretreatment also attenuated the effect of Ang II on the activity of both MMP-1 and -2. Top panels are representative experiments. Bottom panels are the summary of data (mean±SD) from 5 separate experiments.

Figure 4. Pioglitazone and Ang II–induced collagen synthesis (3H-proline incorporation) in cardiac fibroblasts. Ang II treatment (10⁻⁷ M, 12 hours) markedly stimulated collagen synthesis in cardiac fibroblasts. Pretreatment of cells with pioglitazone attenuated this effect of Ang II. Again, pioglitazone alone had no effect on collagen synthesis. Protein synthesis was normalized for fibroblast cell count. The data (mean±SD) are summary of 5 separate experiments.
experiments, the concentration of Ang II was kept at $10^{-7}$ M and incubation time at 12 hours.

Next we examined if the effect of Ang II on MMP expression was associated with change in MMP activity. We noted a significant reduction in MMP activity in cardiac fibroblasts when the cells were treated with Ang II (both $P<0.05$) (Figure 2, right). There were 2 distinct bands on zymography, a 62 kDa band, reflecting MMP-2, and another 42 kDa band reflecting MMP-1. The latter band was quite faint and the signal was only one-third to one-half of the MMP-2 band. Nonetheless, signals for both MMP-1 and MMP-2 fell further on treatment of cardiac fibroblasts with Ang II.

Modulation of Ang II-Induced Expression of Collagen Type I and MMP-1 by Pioglitazone

As mentioned above, Ang II treatment markedly increased the expression of collagen and decreased the expression of MMP. These effects of Ang II were almost completely blocked by the pretreatment of cardiac fibroblasts with pioglitazone (both $P<0.05$ versus Ang II treatment). Pioglitazone pretreatment also reversed the effect of Ang II on MMP activity in fibroblasts. Notably, pioglitazone alone had no effect on the basal level of ROS generation. Results of a representative experiment as well as summary of fluorescence intensity are shown in this figure.

Modulation of Ang II-Induced Collagen Synthesis by Pioglitazone

Compared with control, Ang II treatment ($10^{-7}$ M, 12 hours) enhanced collagen synthesis in cardiac fibroblasts ($P<0.01$). Pretreatment of cells with pioglitazone attenuated this effect of Ang II ($P<0.05$ versus Ang II treatment). Notably, pioglitazone alone had no effect on basal collagen synthesis (Figure 4).
Ang II-Induced ROS Generation in Cardiac Fibroblasts and Modulation by Pioglitazone

There was minimal ROS generation in cardiac fibroblasts in the control state. However, on treatment with Ang II, there was a marked increase in intracellular production of ROS, which was inhibited by pretreatment of cardiac fibroblasts with pioglitazone. Pioglitazone alone had no effect on basal ROS generation. Results of a representative experiment, as well as summary of data from multiple experiments are shown in Figure 5.

To determine whether a known antioxidant would alter the effects of Ang II, fibroblasts were treated with trolox before exposure to Ang II. As shown in Figure 6, trolox pretreatment significantly blocked the effect of Ang II on collagen type I and MMP-1 expression. These effects of trolox and pioglitazone were qualitatively similar.

Pioglitazone and Ang II-Induced Activation of NF-κB and AP-1 in Cardiac Fibroblasts

Ang II treatment significantly enhanced the activation of transcription factor NF-κB (P<0.05 versus control). This effect of Ang II was blocked by the pretreatment of cardiac fibroblasts with pioglitazone (P<0.05 versus no pretreatment).

Ang II treatment also significantly induced the activation of another transcription factor AP-1 (P<0.05 versus control), but this effect was not modulated by the pretreatment of cells with pioglitazone. Pioglitazone alone had no effect on the basal activation of either NF-κB or AP-1 (Figure 7).

Discussion

In this study, we show that Ang II increases the expression and synthesis of collagen type I in cardiac fibroblasts. Concurrently, Ang II decreases MMP-1 expression and activity. Importantly, we show that the PPAR-γ ligand pioglitazone almost completely blocks these effects of Ang II. We also show a marked increase in ROS release when fibroblasts are exposed to Ang II, and pioglitazone reduces Ang II-induced ROS generation. Lastly, we show that Ang II activates the transcription factors NF-κB and AP-1, but pioglitazone pretreatment only inhibits the activation of NF-κB.

Effect of Ang II on Collagen Synthesis and Degradation

Studies in rat and human cardiac fibroblasts have shown that Ang II stimulates cell proliferation and collagen synthesis.26–29 The increase in synthesis and expression of collagen in response to Ang II in cardiac fibroblasts was confirmed in the present study. Because collagen content of ECM depends not only on its production, but also on its degradation, which relies on enzymes such as MMP-1,3 we conducted studies to determine the expression of MMP-1. We found that Ang II decreased the expression of MMP-1, even when cardiac fibroblasts were incubated with Ang II for a short period of time (6 hours). The 10⁻⁷ M concentration of Ang II, which is likely to be present in the ischemic heart, significantly inhibited MMP-1 expression. Importantly, collagenase activity was reduced by the treatment of fibroblasts with Ang II in concert with a decrease in its protein expression. It is logical to assume that the reduction in collagenase activity would result in less collagen degradation. This may well be the explanation for the profibrotic effect of Ang II in the myocardium in hypertension, as well as during chronic ischemia, when the renin-angiotensin-system is activated.
A comment needs to be made regarding the identification of MMP-1 and MMP-2 on zymography here. The signal for MMP-1 was considerably weaker than that for MMP-2, but there was a consistent reduction in both signals when fibroblasts were treated with Ang II, and pioglitazone pretreatment reversed the effects of Ang II. We were guided in the detection and recognition of MMP-1 and MMP-2 bands by the work of Yu et al.\textsuperscript{38} and Watanabe et al.\textsuperscript{39}

Brilla et al.\textsuperscript{40} in 1994 first showed that Ang II decreases collagenase activity in cardiac fibroblasts. This effect of Ang II could be blocked by the AT\(_1\) receptor antagonist losartan. More recently, Peng et al.\textsuperscript{31} showed that Ang II decreases TNF-α-stimulated MMP-2 activity and increases the expression of MMP inhibitor (TIMP-1) in neonatal rat cardiac fibroblasts. These effects of Ang II on cardiac fibroblasts are different from the effects of Ang II in endothelial cells\textsuperscript{32} and cardiac myocytes,\textsuperscript{33,34} wherein Ang II enhances MMP expression/activity. Thus, Ang II may affect MMP expression and activity differently in different cell types. Nonetheless, its effects on fibroblasts tend to favor collagen synthesis.

**Ang II–Induced Oxidative Stress and the Modulatory Effect of Pioglitazone**

NADPH oxidase is a major source of ROS in vascular and cardiac tissues, and Ang II stimulates NADPH oxidase.\textsuperscript{35} It has been postulated that the increase in ROS is an important mechanism by which Ang II contributes to the pathogenesis of vascular disease,\textsuperscript{36,37} and perhaps cardiac remodeling. Sano et al.\textsuperscript{38} showed that Ang II stimulates ROS generation in cardiac fibroblasts, and we confirmed this phenomenon in the present study. In another recent study,\textsuperscript{12} we observed that pioglitazone blocks ROS generation in response to ox-LDL, TNF-α, and Ang II in human coronary artery endothelial cells. Several investigators have shown that pioglitazone attenuates the expression and activity of NAD(P)H oxidase subunits (p22phox, p47phox, gp91phox).\textsuperscript{39,40} We postulate that Ang II induces ROS generation in cardiac fibroblasts by activating NADPH oxidase and possibly other pathways, but this remains to be defined. Nonetheless, we observed that pioglitazone attenuated Ang II–enhanced ROS generation. In parallel experiments, pioglitazone modulated the effect of Ang II on collagen as well as MMP-1 expression in cardiac fibroblasts. We suggest that the inhibition of ROS generation is, in large part, the basis of the observed effects of pioglitazone. The evidence for this suggestion comes from experiments wherein fibroblasts were pretreated with trolox. This water soluble analog of vitamin E exerted qualitatively similar effects as pioglitazone on collagen type I and MMP-1 expression in response to Ang II.

Many cytokines can transduce intracellular signals that stimulate the proteolytic breakdown of IκBα, a cytoplasmic inhibitor of transcription factor NF-κB.\textsuperscript{41,42} In turn, NF-κB is rapidly translocated to the nucleus, where it regulates the expression of many related genes. NF-κB is a redox-sensitive transcription factor, which is usually activated by oxidative stress.\textsuperscript{43} We have previously shown that NF-κB is involved in the activation of endothelial cells in response to Ang II through increased intracellular oxidative stress.\textsuperscript{12} Now, we show that Ang II activates NF-κB in cardiac fibroblasts. It was the activation of NF-κB, which was blocked by the pretreatment of cells with pioglitazone. This observation, together with the data on the measurement of ROS generation, suggests that oxidative stress is one of the important mediators of the effects of Ang II on cardiac fibroblasts, and pioglitazone attenuates the effects of Ang II by blocking ROS release and related transcription factor activation.

In addition to NF-κB, we showed the activation of AP-1 in response to Ang II. In at least one previous study,\textsuperscript{34} activation of AP-1 by Ang II in cardiac tissues was described, although it is not clear if the preparation contained fibroblasts. Hattori et al.\textsuperscript{44} showed that Ang II activates AP-1 in vascular smooth muscle cells, and this effect is not affected by pioglitazone. In our study in pure cardiac fibroblasts, we observed that Ang II stimulated the activation of AP-1, and pioglitazone pretreatment did not modulate this effect of Ang II. Schenk et al.\textsuperscript{45} found that transient expression or exogenous application of thioredoxin (an important cellular protein oxidoreductase with antioxidant activity) resulted in a dose-dependent inhibition of NF-κB activity. AP-1-dependent transactivation, in contrast, was strongly enhanced by thioredoxin. As such, a possible explanation for the lack of effect on AP-1 is that pioglitazone inhibits the ROS production induced by Ang II not only by enhancing antioxidant capacity of cells, but also by its own antioxidant effect.

**Perspectives**

This study shows that Ang II stimulates collagen synthesis and activity, and attenuates the expression of collagen-degrading enzyme MMP-1 in rat cardiac fibroblasts. We propose that these effects of Ang II are mediated, in large part, by Ang II-stimulated ROS generation. Because the hemodynamic stress of hypertension can be excluded in the present in vitro studies, it can be safely assumed that it is the presence of Ang II (and associated ROS generation) that is the basis of enhanced collagen synthesis by cardiac fibroblasts. Importantly, modulation of the effects of Ang II in cardiac fibroblasts by pioglitazone may have important therapeutic implications in disease states characterized by enhanced collagen synthesis in the heart.

**Acknowledgments**

These studies were supported in part by funds from Takeda Pharmaceuticals.

**References**


Angiotensin II Regulation of Collagen Type I Expression in Cardiac Fibroblasts: Modulation by PPAR-γ Ligand Pioglitazone
Kui Chen, Jiawei Chen, Dayuan Li, Xingjian Zhang and Jawahar L. Mehta

Hypertension. 2004;44:655-661; originally published online October 4, 2004;
doi: 10.1161/01.HYP.0000144400.49062.6b
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/44/5/655

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/