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Effect of Angiotensin II on STAT3 mediated atrial structural remodeling

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Abstract. – OBJECTIVE: Atrial fibrillation (AF) has been identified to contribute significantly to the morbidity and mortality of cardiovascular disease patients. The atrial structural remodeling is a hallmark of AF and the molecular mechanisms underlying this remain unclear. Hence the objective of the present study is to determine the role of angiotensin II (Ang-II)/Ang-II type 1 (AT1) receptor – STAT3 signaling pathway on – atrial structural remodeling.

MATERIALS AND METHODS: The method of this study involves incubation of atrial myocytes, with Ang-II, to increase the level of apoptosis expressions by Tunel assay and the expression of apoptosis related factors like caspase 3 and 8 release of cytochrome C from mitochondria to cytosol by western blot test after OGD pre-treatment.

RESULTS: Atrial myocytes were shown to simulate the ischemia, hypoxia and atrial fibrillation. When incubated with Ang-II, (inhibited by losartan) the improvement was observed in the expression of caspase-3 and caspase-8. Ang-II also significantly promoted the transfer of cytochrome C levels from the mitochondria to the cytoplasm and this transfer was observed to be inhibited by losartan and WP1066. Ang-II incubation showed improved transcriptions of collagens and MMP expressions in atrial fibroblasts. In cultured atrial myocytes and fibroblasts, Ang-II induced tyrosine and serine phosphorylation of STAT3 showing interaction with MMP1 and MMP2 and DNA promoter sequences in atrial fibroblasts. The complete sequence was observed to have an affinity to be inhibited by losartan and WP1066.

CONCLUSIONS: Ang-II/AT1 receptor/STAT3 is an important signaling pathway in the atrial structural remodeling, Ang-II enhances the apoptosis of atrial parenchyma and deposition of atrial ECM, which might contributes to atrial fibrillation.

Keywords:
Atrial fibrillation, Atrial structural remodeling, Angiotensin II, Signal Transducers and Activators of Transcription (STAT).

Introduction

Atrial fibrillation (AF) is one of the most common arrhythmias encountered in cardiology related clinical practice[1-3] and remains the major cause for morbidity and mortality in humans[4]. During the process of development of AF structural changes in atria have been reported to occur, which include perinuclear accumulation of glycogen, alterations in connexin expression, and changes in shape of mitochondria[5-7]. myocytic hypertrophy, interstitial fibrosis[8] and apoptosis of myocytes[9], resulting in an increased conduction of heterogeneity and contribute in facilitating recurrence of AF.

The treatment of AF is most often based on its pathogenesis[10]. The structural remodeling of AF refers to architectural deterioration of the arrhythmogenic substrate.

Even though multiple factors have been implicated during the signaling processes of structural remodeling of AF which includes angiotensin II (Ang-II), transforming growth factor-β [TGF-β1], and platelet-derived growth Factor, etc, the precise signaling process of AF structural remodeling is still unclear.

Recently, the activation of the renin-angiotensin II pathway has been identified to be involved in structural remodeling of AF[11-13], both in animal models and in biopsies from patients with AF and its inhibition has been shown to attenuate the formation of fibrosis and diminish the incidence of AF[13-15].

Angiotensin II is said to induce myocardial tissue and vasculature remodeling via the activation of the mitogen-activated protein kinases (MAPK)[16,17] and NAD(P)H oxidases[18]. Recently, signal transducers and activators of transcription (STAT) pathway, has been identified to be involved in vascular atherosclerosis, ventricular hypertrophy[19-22] and atrial fibrosis[23]. The activation of this pathway by Ang-II has been observed to mediate cardiac myocytes and fibroblasts[23], and showed its effects on myocardial infarction[24].

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Signal transducers and activators of transcriptions (STAT) were originally discovered as latent cytoplasmic transcription factors that mediate cellular responses to diverse cytokines and growth factors[25-30]. STAT family is required for diverse biological processes including embryonic development and adult homeostasis, as well as differentiation, proliferation, survival and apoptosis[31].

Hence, in the present study, an attempt was made for the first time, to study atrial myocytes and fibroblasts *in vitro* to characterize the status of Ang-II-STAT signaling pathway in atrial structural remodeling.

Materials and Methods

Western Blot

The extract of cytosolic and cytoskeleton proteins were performed according to the manufacturer's instructions (Chemicon Compartment Protein Extraction Kit, Millipore, Billerica, MA, USA). Western blotting was performed according to standard protocols. Low-molecular-weight marker (Cell Signaling Technology) and 50 μg of protein from samples were separated on 10% or 12% SDS gels by SDS-PAGE. Separated protein was transferred to a poly (vinylidenedifluoride) membrane that was blocked at room temperature for 1 hour in Tris-buffered saline with 0.2% Tween 20 (TBS-T) containing 5% skim milk and probed with primary antibodies overnight at 4°C. The diluted concentrations of the primary antibodies (Abcam Cambridge, Cell Signaling Technology, Billerica, MA, USA) were as follows: STAT3/phospho Y705, 1:200; STAT3/phospho S727, 1:250; STAT3, 1:200; caspase 3, 1:250; caspase 8, 1:250; cytochrome C, 1:200; β-actin, 1:500. Secondary antibodies (Cell Signaling Technology) included horse radish peroxidase-labeled and were diluted 1:1000 with 0.2% TBS-T and 1% skim milk and incubated for 1 hour at room temperature. Protein bands on Western blots were visualized using ECL Plus (Amersham, Arlington Hts, IL, USA). Relative band densities of proteins in Western blots were normalized against β-actin.

Construct ion of oxygen and glucose deprivation model

The myocytes in culture plates were washed with 2 ml D-Hanks balanced solution two times. The culture plates was put into the box, filled with mixed gas of 95% N_2 and 5% CO_2, and sealed the box at 37°C incubated for 4 hours. Then, changed the D-Hanks solution with complete medium, warmed at 37°C and equilibrated with 5% CO_2 incubator for reoxygenation.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays were carried out using the EpiQuik™ Chromatin Immunoprecipitation Kit (Epigentek Group, Farmingdale, NY, USA) following the manufacturer's protocol. STAT3-associated chromatin fragments were immunoprecipitated using anti-STAT3 antibodies. Four pairs of primers were designed based on the 8000 bp upstream sequence of immune precipitated promoter fragments for amplification Primer sequence for MMP-1, MMP-2, Collagen I & III designated includes:

- Sense: 5' TCAGTACCAAGGACGTTTG 3'
- Antisense5'TTTTAAGCTAGCCCTTGCT3' for 235 bp MMP-1 promoter;
- Sense: 5'ATGGCCACCTCTTTAAAGC 3' ,
- Antisense 5' CCAGGGCATCGTTATTAGG 3' for 229bp MMP-2 promoter;
- Sense: 5' CTTCTTCCAGATGAGCCTG3',
- Antisense: 5'GTGGTCAGTTCCAAAGGAT3' for 230bp collagenIpromoter;
- Sense: 5' GTCTCTGCAAACAGGGTGG 3',
- Antisense: 5'AAAACCTTCACGTTTCCTG 3' for 232bp collagen III promoter;

TUNEL reaction and DAPI staining

Cells were fixed in 4% poly formaldehyde, and then rinsed two times with phosphate buffered saline (PBS), treated in 0.1% Triton X-100 for 5 minutes. Join the mixture of enzyme buffer and labeling buffer at the ratio of 1:9, remove the mixture, drop the TUNEL reaction solution, reaction at 37°C, damp, dark environment 60 min. Cleaning 3 times, then adding 50 μl DAPI (4',6-diamidino-2-phenylindole, dilactate) buffer solution (DAPI 0.01 mg/ml), reaction in the dark environment 5 min. Photographed under a fluorescence microscope in the same view: green fluorescent (fracture of DNA by TUNEL staining) blue fluorescence (hyperchromatic nuclei by DAPI staining).

RNA Extraction and Quantitative Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was prepared by using Isogen, and treated with DNase. First-strand cDNA synthesis was performed according to the manufacturer's protocol. PCR was carried out with 1.5 mM Mg-

Cl_2, 250 mM dNTPs, 0.2 mM each primer and 0.04 U/ml EX Taq polymeras. For RT-PCR, primer sequences and the expected size of amplification products are as follows:

- Sense: 5' TCTGCCAGGTAAACTTGATGC 3'
- Antisense: 5'ATTCCAGGGAAATCTTCT-GCT 3' for 297 bp MMP-1;
- Sense: 5' TGGAAGCATCAAATCGGACTG 3' ,
- Antisense: 5' GAAAGTAGCACCTGGG-AGGGA 3' for 243 bp MMP-2;
- Sense: 5' GGTCCCAAAGGTGCTGATGG 3',
- Antisense 5' GACCAGGCTCACCACGGTCT 3' for 175 bp collagen I;
- Sense: 5' CGAGGTGACAGAGGTGAAAGA 3',
- Antisense: 5' AACCCAGTATTCTCCGCTCTT 3' for 336 bp collagen III;
- Sense: 5' GCATCCATGAAACTACATTCA 3'
- Antisense: 5' ACAGTCCGCCTAGAAG-CATTT 3' for 320 bp β-actin.

A mathematical model was used to determine the relative quantification of target genes compared with the β-actin gene.

Tunel assay

Apoptosis in the atrial tissue was determined using an *In situ* cell death detection kit (Roche Diagnostics, Indianapolis, IN, USA). Briefly, sections were cut from paraffin blocks at 44-μm thickness and mounted onto slides. Sections were dewaxed in xylene and rehydrated through a graded series of alcohols. Sections were immersed in 0.1% TX-100 in 0.1% sodium citrate buffer for 8 min at room temperature. Following rinsing, sections were incubated with TUNEL buffer (containing Tris, 0.7 M NaCaco, $CoCl_2$, 10% BSA in water) for 10 min at room temperature. Sections were then incubated with the reaction mixture as specified by the manufacturer's instructions. Subsequently, tissue sections were incubated with anti-fluorescein antibody labeled with alkaline phosphatase in a humidified chamber for 1 h. Apoptotic cells were visualized with precipitating substrate fast red in 0.1 M Tris-HCl for 15 min at room temperature. Sections were counter stained with Lillie-Mayer's haematoxylin and blued in lithium carbonate before being mounted in glycerol. Apoptotic cells were viewed under light microscopy.

Statistical Analysis

Data are expressed as means±SE. ANOVA, Student *t* test were used to determine statistical significance and p value < 0.05 was considered statistically significant.

Results

Angiotensin II induced apoptosis of atrial myocytes

Apoptosis has been observed in cardiomyopathic conditions including human dialated cardiacmyopathy (DCM) and ischaemic cardiomyopathy (ICM)[32,33] in pacing-induced canine heart failure[34,35]. Studies have shown a definite relationship between apoptosis of cardiomyocytes and atrial structural remodeling[9]. This study showed the effect of Ang-II on apoptosis of atrial myocytes and Ang-II receptor blocker, losartan inhibited Ang-II-induced apoptosis of myocytes through oxygen and glucose deprivation model where atrial myocytes were shown to simulate the ischemia, hypoxia and atrial fibrillation (Figure 1). This report also examined the level of apoptosis by Tunel assay and the expressions of apoptosis-related factors by western blot in atrial myocytes.

Cleavage and activation of caspases play a central role in the initiation and execution of apoptosis[36,37] where caspase-8 is an initiator and caspase-3 is the key executive. After preconditioning with oxygen and glucose deprivation model, Atrial myocytes were incubated with Ang-II, (inhibited by losartan) and observed improvement in the expression of caspase-3 and caspase-8 (Figure 2). Ang-II also significantly promoted the transfer of cytochrome C levels from the mitochondria to the cytoplasm and this transfer was observed to be inhibited by losartan (Figure 3).

Angiotensin II increases expressions of collagens and MMPS in atrial fibroblasts

Accumulation of extracellular matrix (ECM) and fibrosis are important structural changes in AF[38,39]. The predominant matrix proteins in myocardial ECM are the collagens, which are deposited in the myocardial interstitium in a fibrillar architecture to ensure myocardial stability and organization[40]. Increased collagen deposition has been well documented in AF patients compared with control subjects[41]. Of the 5 different collagen isoforms found in the heart, fibrillar collagen type I and III comprise approximately 85% of the cardiac interstitium[42,43]. Matrix metallo-proteinases (MMPs), a key enzyme family, involved in fibrosis. It is known that MMPs-degraded physiological collagens are replaced by fibrous interstitial deposits of various unorganized ECM proteins. A number of experimental and clinical studies have illustrated that MMP levels are associated with atrial fibrosis in AF patients[44,45]. We used collagen

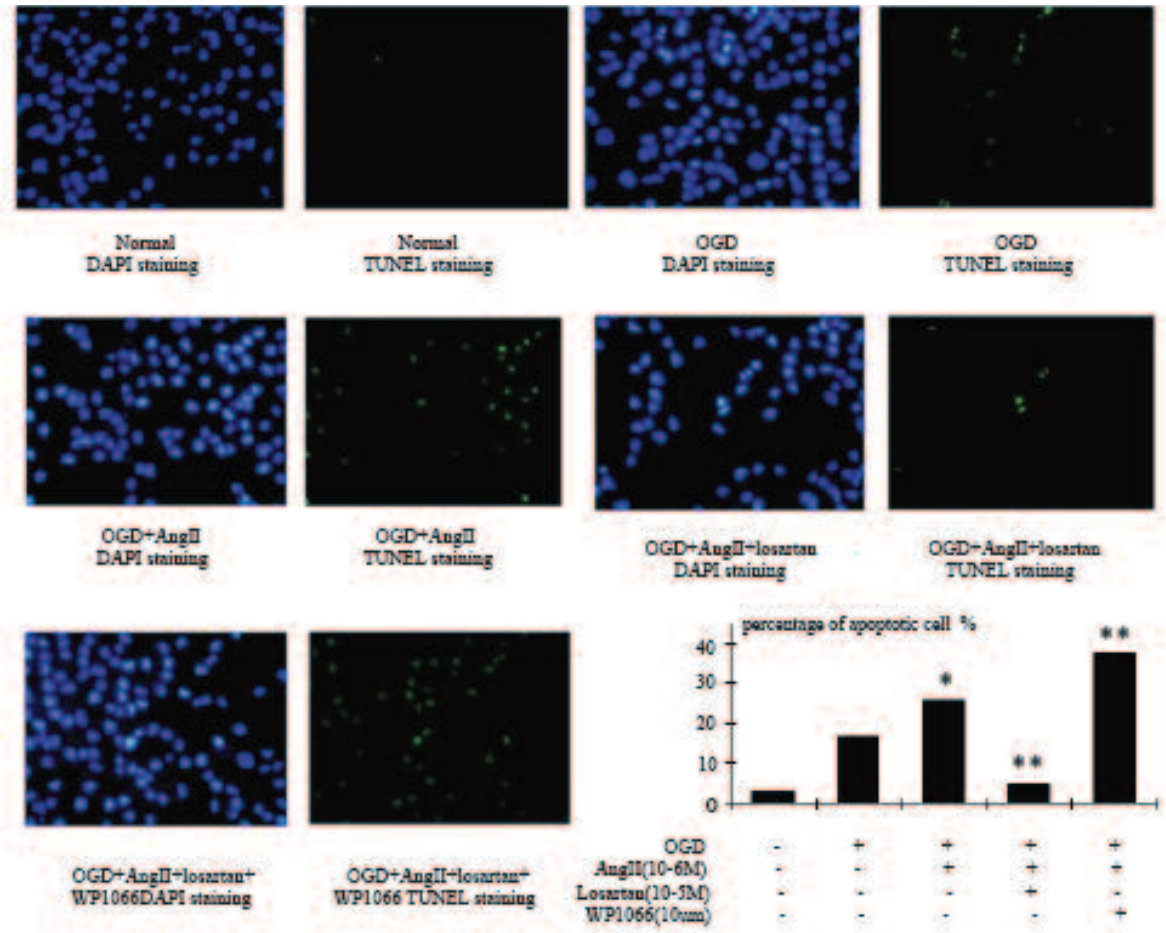

Figure 1. Representation of Ang-II induced apoptosis of atrial myocytes. Blue fluorescent: hyperchromatic nuclei by DAPI staining. Green fluorescent: fracture of DNA of apoptotic atrial myocytes by TUNEL staining in the same view. Bottom: Graph representing quantification by percentage of apoptotic cells N=3 per experiment; data are mean±SD. *$p < 0.05$ vs atrial myocytes + OGD **$p < 0.05$ vs myocytes+OGD+Ang-II. WP1066: STAT3 inhibitor; Losartan: Angiotensin II receptor bloker; OGD: oxygen and glucose deprivation.

I and III, MMP1 and MMP2 expression to represent fibrosis in atrial fibroblasts[44-47]. To investigate the effect of Ang-II on atrial fibrosis, we examined the expression of collagen type I, collagen type III and MMP1, MMP2 in atrial fibroblasts with RT-PCR. In this study Ang-II significantly increased the levels of collagen I and III and after stimulation (Figure 4). Similarly improved expression was observed with MMPs 1 and 2 after stimulation with Ang-II (Figure 4). Losartan inhibited both collagens and MMPs activities induced by Ang-II in fibroblasts.

Dual role of Ang-II-induced STAT3 pathway on atrial structural remodeling

In both atrial myocytes and fibroblasts, stimulation with Ang-II (10^{-6} mol/L) significantly induced tyrosine 705 phosphorylation and serine 727 phosphorylation of STAT3 (Figure 5). The phosphorylation of STAT3 in both atrial myocytes and fibroblasts can be inhibited with ARB: losartan and STAT3 inhibitor: WP1066. These results suggest STAT3 pathway is activated by Ang-II in atrial myocytes and fibroblasts.

In many cancer cells and tissues, STAT3 has been described as mediator for survival anti-apoptosis. To examine the role of STAT3 in Ang-II-induced apoptosis of atrial myocytes, A STAT3-specific inhibitor: WP1066 was used, after preconditioning with Oxygen and glucose deprivation model, incubation with WP1066 increased significantly Ang-II-induced apoptosis in atrial myocytes (Figure 1). WP1066 also was observed to promote Ang-II-induced expressions of caspase-3 and caspase-8 (Figure 2) and transfer of cytochrome C from the mitochondria to the

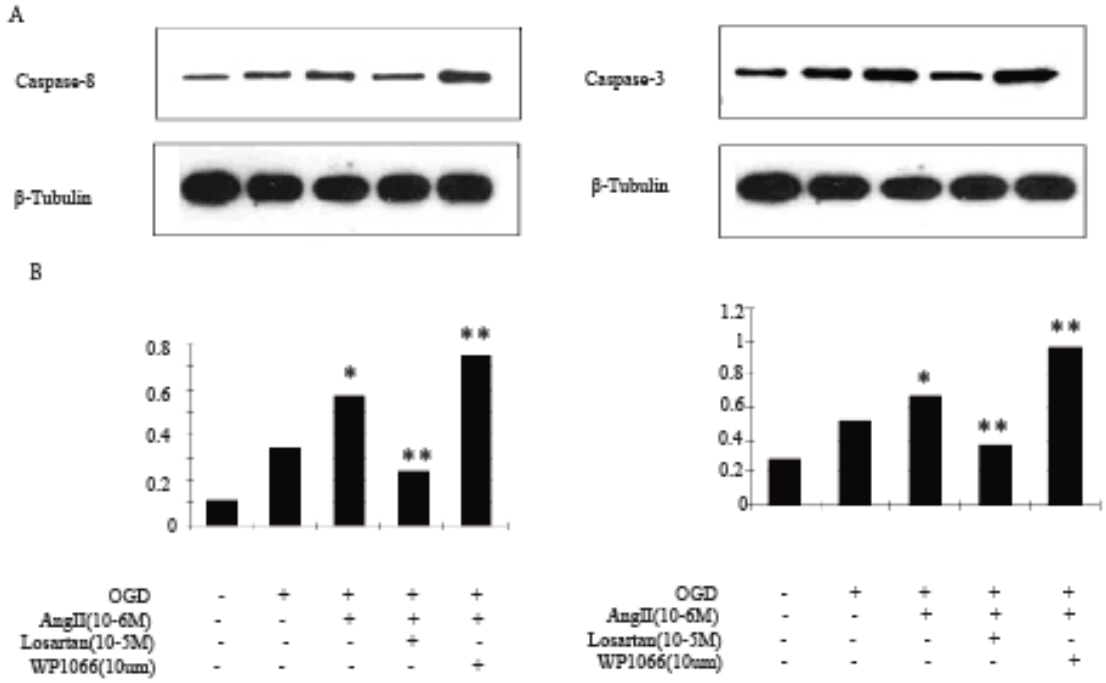

Figure 2. Oxygen and glucose deprivation model representing incubation of Atrial myocytes with Ang-II and its inhibition by losartan showing improved expression of caspase-3 and caspase-8. **A**, Representating expression of caspase-3 measured by western blot. **B**, Representating expression of caspase-8 measured by western blot. **C**, Quantified expression of caspase-3 measured by densitometry. **D**, Quantified expression of caspase-8 measured by densitometry. N=3 per experiment; data are mean±SD. *$p < 0.05$ vs atrial myocytes+OGD **$p < 0.05$ vs myocytes+OGD+Ang-II. WP1066: STAT3 inhibitor; Losartan: Angiotensin II receptor bloker; OGD: oxygen and glucose deprivation.

cytoplasm (Figure 3). Ang-II-induced apoptosis was not STAT3-dependent (not blocked by STAT3 inhibitor WP1066, but promoted by WP1066). These results indicate STAT3 protects atrial myocytes from apoptosis.

Binding of Ang-II-induced STAT-3 to MMPS DNA promoter sequences

Chromatin immuno precipitation (ChIP) binding assays were employed to determine the DNA-STAT3 binding activity. The nuclear proteins from Ang-II-induced atrial fibroblasts were observed to directly associate with DNA sequence of MMP1 and MMP2 (Figure 6). Because the affinity of antibody which, captured protein is anti-STAT3 antibody, two pairs of primers were used in the ChIP analysis and were designed according to the promoter sequences of MMP1 and MMP2. The above association was induced by Ang-II, which was inhibited by losartan and WP1066. We also investigated the interaction of STAT3 with DNA promoter sequence of collagen I and collagen III, but no binding between them was observed (data not shown).

Discussion

In the present study, demonstrated the role of STAT3 by regulation of Ang-II induced atrial structural changes which were attenuated by losartan. This was the first study to report the requirement of STAT3-DNA binding activity for Ang-II-induced MMP1 and MMP2 transcription in atrial fibroblasts.

Ang-II and atrial structural remodeling

Among the plethora of identified fibrogenic factors, the renin angiotensin system, especially Ang-II has been implicated to play an important role in the development of atrial remodeling during AF[23,47]. In the present study; we also found a much higher level of Ang-II in atrial samples of AF patients than in those of without AF. Ang-II may mediate multiple responses including cell growth, inflammation, cardiac apoptosis-fibroblast proliferation, transformation and extracellular matrix (ECM) deposition[48-49]. The present study demonstrated that Ang-II incubation induced profound increases of collagen synthesis atrial myocytes

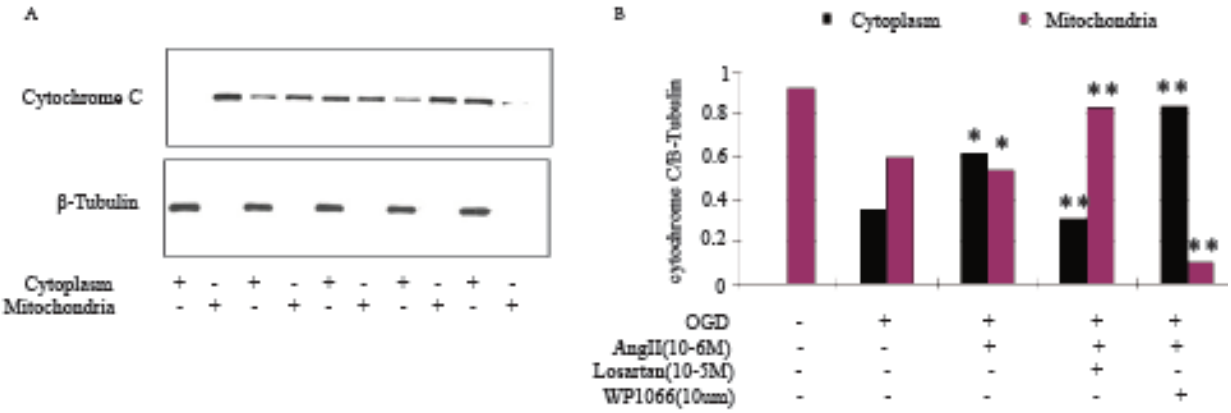

Figure 3. Atrial myocyte showing the transfer of cytochrome C levels from the mitochondria to the cytoplasm in atrial myocytes after OGD pretreatment and its inhibition by losartan. **A**, Release of cytochrome C levels from the mitochondria to the cytoplasm in atrial myocytes after OGD pretreatment measured by western blot. **B**, Quantity of Cytochrome C levels from the mitochondria to the cytoplasm in atrial myocytes after OGD pretreatment measured by densitometry. N=3 per experiment; data are mean±SD. *$p < 0.05$ vs atrial myocytes+OGD **$p < 0.05$ vs myocytes+OGD+Ang-II. WP1066: STAT3 inhibitor; Losartan: Angiotensin II receptor bloker; OGD: oxygen and glucose deprivation.

and apoptosis in atrial fibroblasts, strongly suggesting that Ang-II advance the progression of atrial fibrosis and apoptosis. Ang-II participates in the development of AF-induced myocardial fibrosis could occur through activation of AT1 and AT2 receptors[49-54]. AT1 receptor antagonism significantly attenuates fibrosis process of atrial fibrillation in dogs[51]. Consistent with our *in vitro* observations, we found that AT1 receptor antagonist: losartan inhibited the Ang-II-induced increase in collagens and MMPs expressions in atrial fibroblasts and apoptosis in atrial myocytes, implicating an AT1 receptor-specific mechanism for the Ang-II activation of atrial remodeling signaling pathway.

Multiple signaling pathways underlying Ang-II-induced atrial structural remodeling

Ang-II activates multiple intracellular second messenger Molecules which induce cardiac remodeling. These molecules include: GATA, AP-1, SMAD[47,48] and STAT3[23]. In our study not only incubation with Ang-II *in vitro* but also Ang-II infusion *in vivo* significantly improved the phosphorylation of STAT3.

Ang-II also improved the level of atrial apoptosis both *in vitro* and *in vivo* trials, which is inhibited by AT1 receptor antagonist: losartan. So our study supports the hypothesis that Ang-II might promote atrial apoptosis though STAT3 signal pathway. Surprisingly, after blockade of STAT3 with WP1066, apoptosis of atrial myocytes increased markedly. This could be explained by reporting involvement of multiple pathways in Ang-II-induced atrial remodeling downstream signaling[23,47,54]. These pathways interact with one another resulting in either induction or inhibition of apoptosis. Most of these pathways are involved as one of the Ang-II downstream signaling pathways. In atrium–apoptosis induction pathways are dominant and play a leading role. Thus, Ang-II showed as apoptosis induction, while STAT3 pathway acts as an anti-apoptotic regulator, which plays a minor role.

STAT3 play dual role in Ang-II-induced atrial structural remodeling

With regard to Ang-II-induced atrial structural remodeling by STAT3 activation, 2 distinct mechanisms were identified to operate in atrial myocytes and fibroblasts. In atrial fibroblasts, activation of STAT3 by Ang-II probably required Rac1-induced autocrine or paracrine factors and the activation of JAKs[53,54]. In the present study blockade of STAT3 with WP1066, decreased Ang-II-induced genes transcription of fibrogenic factors and attenuated composition of collagen. These results, illustrated that STAT3 pathway promoted the progression of atrial fibrosis and acted as a positive regulator of Ang-II-induced atrial structural remodeling. In atrial myocytes, caspases family proteins are involved in the apoptosis which occurs in various cardiopathies[55-58]. Inhibition of STAT3 signaling was reported to induce apoptosis in lymphoma[59] and malignant cells[60]. In present study, inhibition of

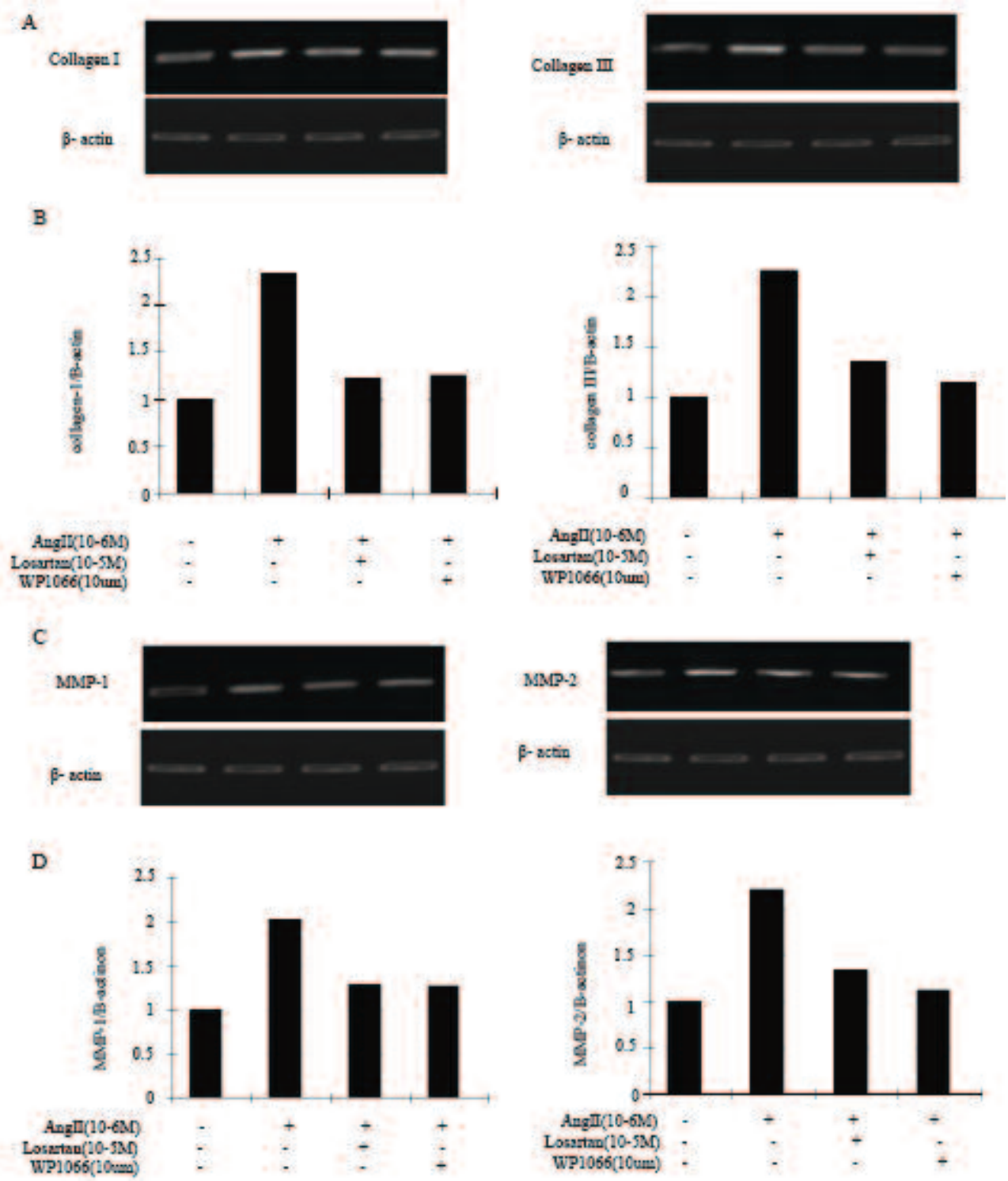

Figure 4. Angiotensin II showing increased expressions of collagens and MMPS in atrial fibroblasts and its inhibition by losartan and WP1066. **A**, Representing transcriptions of collagen I, III measured by RT-PCR. **B**, Quantification of collagens measured by densitometry. **C**, Representing transcriptions of MMPs 1, 2 measured by RT-PCR. **D**, Quantification of MMPs measured by densitometry. N=3 per experiment; data are mean±SD. $*p < 0.05$ vs controled atrial fibroblasts $**p < 0.05$ vs Ang-II-treated atrial fibroblasts. WP1066: STAT3 inhibitor; Losartan: Angiotensin II receptor bloker;

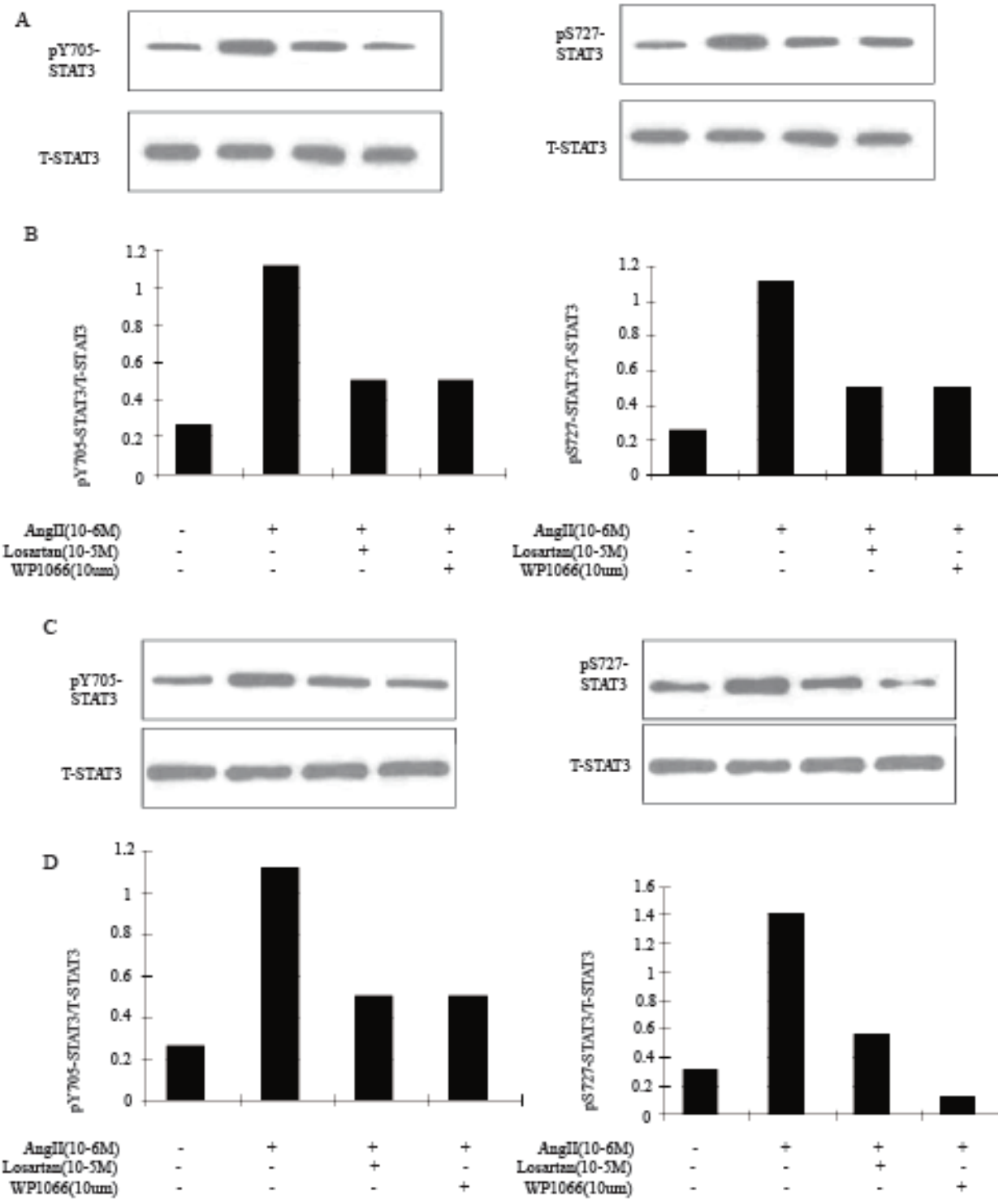

Figure 5. Represention of Ang-II induced phosphorylation of STAT3 in both atrial myocytes and fibroblasts and its attenuation by losartan and WP1066. ***A***, Represention of Ang-II induced phosphorylation of STAT3 in atrial myocytes and its attenuation by losartan and WP1066 western blot. ***B***, Quantification of Ang-II induced phosphorylation of STAT3 in atrial myocytes and its attenuation by losartan and WP1066 by densitometry. ***C***, Represention of Ang-II induced phosphorylation of STAT3 in atrial fibroblasts and its attenuation by losartan and WP1066 western blot. ***D***, Quantification of Ang-II induced phosphorylation of STAT3 in atrial fibroblasts and its attenuation by losartan and WP1066 by densitometry. N=3 per experiment; data are mean±SD. *$p < 0.05$ vs controled atrial fibroblasts **$p < 0.05$ vs Ang-II treated atrial fibroblasts. WP1066: STAT3 inhibitor; Losartan: Angiotensin II receptor bloker; p-STAT3: phosphorylated STAT3.

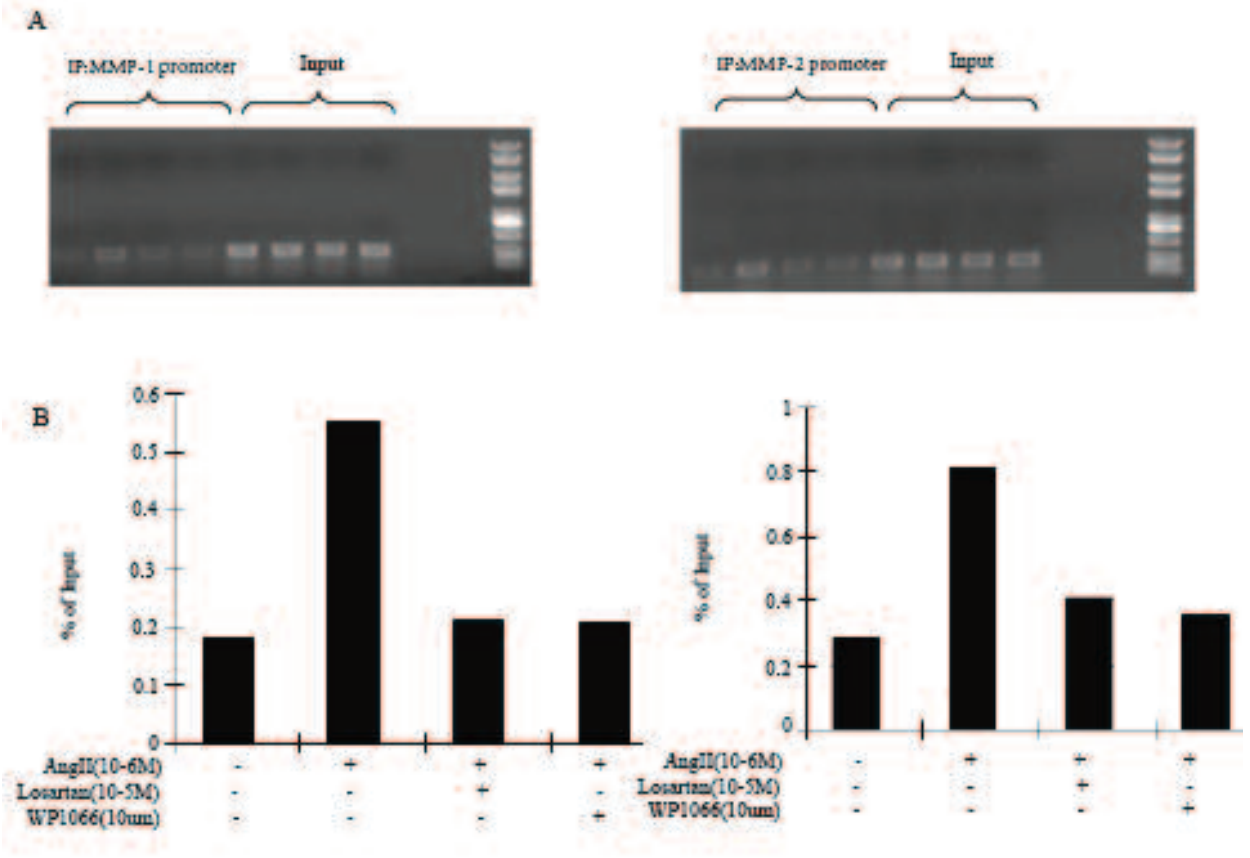

Figure 6. Interaction of STAT-3 with MMP1 and MMP2 DNA promoter sequences in atrial fibroblasts by chromatin immunoprecipitation (ChIP)-binding assay. **A**, Representation of lysates of atrial fibroblasts with DNA sequences of MMP1, immune precipitated with anti-STAT3 antibody and subjected for PCR. **B**, Lysates of atrial fibroblasts with DNA sequences of MMP1 were immunoprecipitated with anti-STAT3 antibody and were quantified by densitometry. **C**, Representation of lysates of atrial fibroblasts with DNA sequences of MMP2, immune precipitated with anti-STAT3 antibody and subjected for PCR. **D**, Lysates of atrial fibroblasts with DNA sequences of MMP2 were immunoprecipitated with anti-STAT3 antibody and were quantified by densitometry. N=3 per experiment; data are mean±SD. *$p < 0.05$ vs controled atrial fibroblasts ** $p < 0.05$ vs Ang-II treated atrial fibroblasts. WP1066: STAT3 inhibitor; Losartan: Angiotensin II receptor bloker;

STAT3 with WP1066 markedly improved Ang-II-induced gene expression of caspase3 and caspase8, promoted apoptosis of atrial myocytes after OGD preconditioning. This suggests that contrary to the pro fibrotic function, STAT3 pathway also protect atria from apoptosis and acted as a negative regulator of Ang-II-induced atrial structural remodeling (Figure 7).

STAT3-DNA binding activity was required for Ang-II-induced MMP expression

Our experimental results provide direct evidence that in atrial fibroblasts, the increased MMP-1, MMP-2 expressions could be attributed to elevated STAT3 activity, and that STAT3-DNA binding activity is required for Ang-II-induced MMP-1, 2 expressions in atrial fibroblast. The activity of MMPs is subject to four levels of regulation, including transcriptional regulation, mRNA stability, activation of proenzyme, and inhibition or activation of enzyme through the tissue inhibitors of metalloproteinases[61-68]. Our chromatin immuno precipitation (ChIP) identified a binding of STAT3 with MMP-1 and 2 promoter sequence after Ang-II stimulation in atrial fibroblasts. Phosphorylated STATs (P-STAT) bind to DNA-response elements named interferon-(gamma)-activated sequence-3 (GAS-3) in the promotion of target genes and activate specific gene expression programs[25]. Several groups have studied the transcriptional regulation of MMPs by other transcription factors[62,69-71].

Previous researches[72] have proved STAT3 becomes phosphorylated and achieves efficient induction of MMP-1 promoter by interacting with

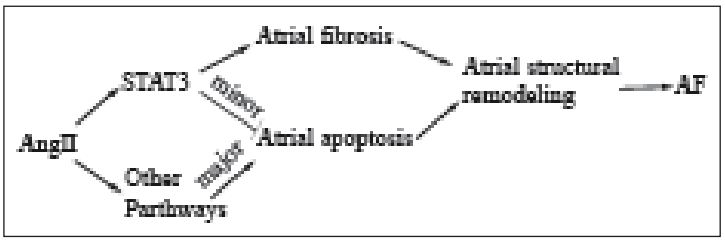

Figure 7. Signal transduction pathways of Ang-II-induced atrial structural remodeling.

c-JUN and AP-1 in T24 bladder cancer cells. In our study, the result that the affinity of STAT3 with MMP1and 2 promoter sequence was attenuated by WP1066 and losartan further proved STAT3-DNA binding activity was required for Ang-II-induced MMP expression. Xie et al[73] identified a high-affinity STAT3-binding element mapped between bp _617 and _610 of the proximal MMP-2 promoter. Mutation of this STAT3-binding element significantly eliminated MMP-2 promoter trans-activation by constitutively activated STAT3, indicating that STAT3-binding element within the proximal MMP-2 promoter was required for activation.

Conclusions

The present study provides compelling experimental evidence that Ang-II/AT1 receptor/STAT3 is an important signaling pathway in the atrial myocardium. Ang-II affects intracellular signaling cascades in various atrial cells, and advances, apoptosis of atrial parenchyma and deposition of atrial ECM resulting in atrial arrhythmias. The results of this study provide newer insights into the understanding of the mechanisms of Ang-II-induced myocardial remodeling and novel therapeutic targets of AF

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Blaauw Y, Crijns HJ. Atrial fibrillation: insights from clinical trials and novel treatment options. J Intern Med 2007; 262: 593-614.
2) Burstein B, Nattel S. Atrial fibrosis: mechanisms and clinical relevance in atrial fibrillation. J Am Coll Cardiol 2008; 51: 802-809.
3) Wyse DG, Gersh BJ. Atrial fibrillation: a perspective: thinking inside and outside the box. Circulation 2004; 109: 3089-3095.
4) Beyerbach DM, Zipes DP. Mortality as an endpoint in atrial fibrillation. Heart Rhythm 2004; 1: B8-18.
5) Frustaci A, Chimenti C, Bellocci F, Morgante E, Russo MA, Maseri A. A Histological substrate of atrial biopsies in patients with lone atrial fibrillation. Circulation 1997; 96: 1180-1184.
6) van der Velden HM, van Kempen MJ, Wijffels MC, van Zijverden M, Groenewegen WA, Allessie MA, Jongsma HJ. Altered pattern of connexin40 distribution in persistent atrial fibrillation in the goat. J Cardiovasc Electrophysiol 1998; 9: 596-607.
7) Ausma J, Dispersyn GD, Duimel H, Thoné F, Ver Donck L, Allessie MA, Borgers M. Changes in ultra structural calcium distribution in goat atria during atrial fibrillation. J Mol Cell Cardiol 2000; 32: 355-364.
8) Allessie M, Ausma J, Schotten U. Electrical, contractile and structural remodeling during atrial fibrillation. Cardiovasc Res 2002; 54: 230-246.
9) Aimé-Sempé C, Folliguet T, Rücker-Martin C, Krajewska M, Krajewska S, Heimburger M, Aubier M, Mercadier JJ, Reed JC, Hatem SN. Myocardial cell death in fibrillation and dilated human right atria. J Am Coll Cardiol 1999; 34: 77-82.
10) Nattel S, Li D, Yue L. Basic mechanisms of atrial fibrillation: very new insights into very old ideas. Annu Rev Physiol 2000; 62: 51-77.
11) Goette A, Staack T, Rocken C, Arndt M, Geller JC, Huth C, Ansorge S, Klein HU, Lendeckel U. Increased expression of extracellular signal-regulated kinase and Angiotensin-converting enzyme in human atria during atrial fibrillation. J Am Coll Cardiol 2000; 35: 1669-1677.
12) Willems R, Sipido KR, Holemans P, Ector H, Van de Werf F, Heidbuchel H. Different patterns of angiotensin II and atrial natriuretic peptide secretion in a sheep model of atrial fibrillation. J Cardiovasc Electrophysiol 2001; 12: 1387-1392.
13) Li D, Shinagawa K, Pang L, Leung TK, Cardin S, Wang Z, Nattel S. Effects of angiotensin-converting enzyme inhibition on the development of the atrial fibrillation substrate in dogs with ventricular tachypacing-induced congestive heart failure. Circulation 2001; 104: 2608-2614.

14) Anne W, Willems R, Van der Merwe N, Van de Werf F, Ector H, Heidbuchel H. Atrial fibrillation after radiofrequency ablation of atrial flutter: preventive effect of angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, and diuretics. Heart 2004; 90: 1025-1030.

15) Savelieva I, John Camm A. Atrial fibrillation and heart failure: natural history and pharmacological treatment. Europace 2004; 5: S5-19.

16) Yano M, Kim S, Izumi Y, Yamanaka S, Iwao H. Differential activation of cardiac c-Jun amino-terminal kinase and extracellular signal-regulated kinase in angiotensin II–mediated hypertension. Circ Res 1998; 83: 752-760.

17) Sugden PH, Clerk A. Cellular mechanisms of cardiac hypertrophy. J Mol Med 1998; 76: 725-746.

18) Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. Circ Res 2000; 86: 494-501.

19) Marrero MB, Schieffer B, Paxton WG, Duff JL, Berk BC, Bernstein KE. The role of tyrosine phosphorylation in angiotensin II-mediated intracellular signalling. Cardiovasc Res 1995; 30: 530-536.

20) Marrero MB, Schieffer B, Paxton WG, Heerdt L, Berk BC, Delafontaine P, Bernstein KE. Direct stimulation of Jak/STAT pathway by the angiotensinII AT1 receptor. Nature 1995; 375: 247-250.

21) Bhat GJ, Thekkumkara TJ, Thomas WG, Conrad KM, Baker KM. Angiotensin II stimulates sis-inducing factor-like DNA binding activity: evidence that the AT1A receptor activates transcription factor-STAT91 and/or a related protein. J Biol Chem 1994; 269: 31443-31449.

22) Mascareno E, El-Shafei M, Maulik N, Sato M, Guo Y, Das DK, Siddiqui MA. JAK/STAT signaling is associated with cardiac dysfunction duringischemia and reperfusion. Circulation 2001; 104: 325-329.

23) Tsai CT, Lai LP, Kuo KT, Hwang JJ, Hsieh CS, Hsu KL, Tseng CD, Tseng YZ, Chiang FT, Lin JL. Angiotensin II activates signal transducer and activators of transcription 3 via Rac1 in atrial myocytes and fibroblasts: implication for the therapeutic effect of statin in atrial structural remodeling. Circulation 2008; 117: 344-355.

24) El-Adawi H, Deng L, Tramontano A, Smith S, Mascareno E, Ganguly K , Castillo R, El-Sherif N. The functional role of the JAK-STAT pathway in post-infarction remodeling. Cardiovasc Res 2003; 57: 129-138.

25) Darnell JE Jr. STATs and gene regulation. Science 1997; 277: 1630-1635.

26) Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 1994; 264: 1415-1421.

27) Schindler C, Darnell JE Jr. Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. Annu Rev Biochem 1995; 64: 621-651.

28) Ihle JN, Kerr M. Jaks and STATs in signaling by the cytokine receptor super family. Trends Genet 1995; 11: 69-74.

29) Horvath CM, Darnell JE. The state of the STATs: recent developments in the study of signal transduction to the nucleus. Curr Opin Cell Biol 1997; 9: 233-239.

30) Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. Annu Rev Biochem 1998; 67: 227-264.

31) Levy DE, Darnell JE Jr. STATs: transcriptional control and biological impact. Nat Rev Mol Cell Biol 2002; 3: 651-662.

32) Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW, Khaw BA. Apoptosis in myocytes inendstage heart failure. N Engl J Med 1996; 335: 1182-1189.

33) Olivetti G, Quaini F, Sala R, Lagrasta C, Corradi D, Bonacina E, Gambert SR, Cigola E, Anversa P. Acute myocardial infarction in humans is associated with activation of programmed myocyte cell death in the surviving portion of the heart. J Mol Cell Cardiol 1996; 28: 2005-2016.

34) Liu Y, Cigola E, Cheng W, Kajstura J, Olivetti G, Hintze TH, Anversa P. Myocyte nuclear mitotic division and programmed myocyte cell death characterize the cardiac myopathy induced by rapid ventricular pacing in dogs. Lab Invest 1995; 73: 771-787.

35) Leri A, Liu Y, Malhotra A, Li Q, Stiegler P, Claudio PP, Giordano A, Kajstura J, Hintze TH, Anversa P. Pacing-induced heart failure in dogs enhances the expression of p53 and p53-dependent genes in ventricular myocytes. Circulation 1998; 97: 194-203.

36) Reed JC. The survivin saga goes in vivo. J Clin Invest 2001; 108: 965-969.

37) Budihardjo I, Oliver H, Lutter M, Luo X, Wang X. Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol 1999; 15: 269-290.

38) Kumagai K, Nakashima H, Urata H, Gondo N, Arakawa K, Saku K. Effects of angiotensin II type 1 receptor antagonist on electrical and structural remodeling in atrial fibrillation. J Am Coll Cardiol 2003; 41: 2197-2204.

39) Chiu YT, Wu TJ, Wei HJ, Cheng CC, Lin NN, Chen YT, Ting CT. Increased extracellular collagen matrix in myocardial sleeves of pulmonary veins: an additional mechanism facilitating repetitive rapid activities in chronic pacing-induced sustained atrial fibrillation. J Cardiovasc Electrophysiol 2005; 16: 753-759.

40) Spinale FG. Myocardial matrix remodeling and the matrix metalloproteinases: influence on cardiac form and function. Physiol Rev 2007; 87: 1285-1342.

41) Anter E, Callans DJ, Wyse DG. Pharmacological and electrical conversion of atrial fibrillation to sinus rhythm is worth the effort. Circulation 2009; 120: 1436-1443.

42) Polyakova V, Miyagawa S, Szalay Z, Risteli J, Kostin S. Atrial extracellular matrix remodelling in pa-

tients with atrial fibrillation. J Cell Mol Med 2008; 12: 189-208.

43) Thomas L, McKay T, Byth K, Marwick TH. Abnormalities of left function after cardioversion: an atrial strain rate study. Heart 2007; 93: 89-95.

44) Xu J, Cui G, Esmailian F, Plun-kett M, Marelli D, Ardehali A, Odim J, Laks H, Sen L. Atrial extracellular matrix remodeling and the maintenance of atrial fibrillation. Circulation 2004; 109: 363-368.

45) Anné W, Willems R, Roskams T, Sergeant P, Herugers P, Holemans P, Ector H, Heidbüchel H. Matrix metalloproteinases and atrial remodeling in patients with mitral valve disease and atrial fibrillation. Cardiovasc Res 2005; 67: 655-666.

46) Qian Y, Meng J, Tang H, Yang G, Deng Y, Wei D, Xiang B, Xiao X. Different structural remodelling in atrial fibrillation with different types of mitral valvular diseases. Europace 2010; 12: 371-377.

47) He X, Gao X, Peng L, Wang S, Zhu Y, Ma H, Lin J, Duan DD. Atrial fibrillation induces myocardial fibrosis through angiotensin II type 1 receptor-specific Arkadia-mediated downregulation of Smad7. Circ Res 2011; 108: 164-175.

48) Schroder D, Heger J, Piper HM, Euler G. Angiotensin II stimulates apoptosis via TGF-beta1 signaling in ventricular cardiomyocytes of rat. J Mol Med 2006. 84: 975-983.

49) Boldt A, Scholl A, Garbade J, Resetar ME, Mohr FW, Gummert JF, Dhein S. ACE-inhibitor treatment attenuates atrial structural remodeling in patients with lone chronic atrial fibrillation. Basic Res Cardiol 2006; 101: 261-267.

50) Nakashima H, Kumagai K. Reverse-remodeling effects of angiotensin II type 1 receptor blocker in a canine atrial fibrillation model. Circ J 2007; 71: 1977-1982.

51) Hirayama Y, Atarashi H, Kobayashi Y, Takano T. Angiotensin-converting enzyme inhibitors are not effective at inhibiting further fibrous changes in the atria in patients with chronic atrial fibrillation: speculation from analysis of the time course of fibrillary wave amplitudes. Jpn Heart J 2004; 45: 93-101.

52) Chrysostomakis SI, Karalis IK, Simantirakis EN, Koutsopoulos AV, Mavrakis HE, Chlouverakis GI, Vardas PE. Angiotensin II type 1 receptor inhibition is associated with reduced tachyarrhythmia-induced ventricular interstitial fibrosis in a goat atrial fibrillation model. Cardiovasc Drugs Ther 2007; 21: 357-365.

53) Pelletier S, Duhamel F, Coulombe P, Popoff MR, Meloche S. Rho family GTPases are required for activation of Jak/STAT signaling by G protein coupled receptors. Mol Cell Biol 2003; 23: 1316-1333.

54) Faruqi TR, Gomez D, Bustelo XR, Bar-Sagi D, Reich NC. Rac1 mediates STAT3 activation by autocrine IL-6. Proc Natl Acad Sci USA 2001; 98: 9014-9019.

55) Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW, Khaw BA. Apoptosis in myocytes in end-stage heart failure. N Engl J Med 1996; 335: 1182-1189.

56) Mallat Z, Tedgui A, Fontaliran F, Frank R, Durigon M, Fontaine G. Evidence of apoptosis in arrhythmogenic right ventricular dysplasia. N Engl J Med 1996; 335: 1190-1196.

57) Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, Quaini E, Di Loreto C, Beltrami CA, Krajewski S, Reed JC, Anversa P. Apoptosis in the failing human heart. N Engl J Med 1997; 336: 1131-1141.

58) Misao J, Hayakawa Y, Ohno M, Kato S, Fujiwara T, Fujiwara H. Expression of Bcl-2 protein, an inhibitor of apoptosis, and Bax, an accelerator of apoptosis in ventricular myocytes of human hearts with myocardial infarction. Circulation 1996; 4: 1506 –1512.

59) Aoki Y, Feldman GM, Tosato G. Inhibition of STAT3 signaling induces apoptosis and decreases surviving expression in primary effusion lymphoma. Blood 2003; 101: 1535-1542.

60) Turkson J, Zhang S, Mora LB, Burns A, Sebti S, Jove R. A novel Platinum compound inhibits constitutive STAT3 signaling and induces cell cycle arrest and apoptosis of malignant Cells. J Biol Chem 2005; 280: 32979-32988.

61) Overall CM, Wrana JL, Sodek J. Transcriptional and post-transcriptional regulation of 72-kDa gelatinase/type IV collagenase by transforming growth factor-beta 1 in human fibroblasts. Comparisons with collagenase and tissue inhibitor of matrix metalloproteinase gene expression. J Biol Chem 1991; 266: 14064-14071.

62) Harendza S, Pollock AS, Mertens PR, Lovett DH. Tissue-specific enhancer-promoter interactions regulate high level constitutive expression of matrix metalloproteinase 2 by glomerular mesangial cells. J Biol Chem 1995; 270: 18786-18796.

63) Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI. Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. J Biol Chem 1995; 270: 5331-5338.

64) Mauviel A. Cytokine regulation of metalloproteinase gene expression. J Cell Biochem 1996; 53: 288-295.

65) Yu AE, Hewitt RE, Kleiner RE, Stetler-Stevenson RE. Molecular regulation of cellular invasion--role of gelatinase A and TIMP-2. Biochem Cell Biol 1996, 74: 823-831.

66) Bian J, Sun Y. Transcriptional activation by p53 of the human type IV collagenase (gelatinase A or matrix metalloproteinase 2) promoter. Mol Cell Biol 1997; 17: 6330-6338.

67) Mertens PR, Harendza S, Pollock AS, Lovett DH. Glomerular mesangial cell-specific transactivation of matrix metalloproteinase 2 transcription is mediated by YB-1. J Biol Chem 1997; 272: 22905-22912.

68) Lee AY, Akers KT, Collier M, Li L, Eisen AZ, Seltzer JL. Intracellular activation of gelatinase A (72-kDa type IV collagenase) by normal fibroblasts. Proc Natl Acad Sci USA 1997; 94: 4424-4429.

69) Harendza S, Pollock AS, Mertens PR, Lovett DH. Tissue-specific enhancer-promoter interactions regulate high level constitutive expression of matrix metalloproteinase 2 by glomerular mesangial cells. J Biol Chem 1995, 270: 18786-18796.

70) Mertens PR, Steinmann K, Alfonso-Jaume MA, Ennia A, Sun Y, Lovett DH. Combinatorial interactions of p53, activating protein-2, and YB-1 with a single enhancer element regulate gelatinase A expression in neoplastic cells. J Biol Chem 2002; 277: 24875-24882.

71) Yan C, Wang H, Boyd DD. ATF3 represses 72-kDa type IV collagenase (MMP-2) expression by antagonizing p53-dependent trans-activation of the collagenase promoter. J Biol Chem 2002, 277: 10804-10812.

72) Itoh M, Murata T, Suzuki T, Shindoh M, Nakajima K, Imai K, Yoshida K. Requirement of STAT3 activation for maximal collagenase-1 (MMP-1) induction by epidermal growth factor and malignant characteristics in T24 bladder cancer cells. Oncogene 2006; 25: 1195-1204.

73) Xie TX, Wei D, Liu M, Gao AC, Ali-Osman F, Sawaya R, Huang S. STAT3 activation regulates the expression of matrix metalloproteinase-2 and tumor invasion and metastasis. Oncogene 2004; 23: 3550-3360.