Novel Mechanisms of Valsartan on the Treatment of Acute Myocardial Infarction Through Inhibition of the Antiadhesion Molecule Periostin

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Abstract—Our previous study demonstrated that periostin, an extracellular matrix protein, plays an important role in left ventricular remodeling through the inhibition of cell–cell interactions. Because the gene regulation of periostin has not yet been examined, we focused on the effects of angiotensin (Ang) II and mechanical stretch, because Ang II and mechanical stretch are related to cardiac remodeling after myocardial infarction. First, we examined the effects of Ang II on periostin in myocytes and fibroblasts in vitro. Ang II significantly increased periostin through phosphatidylinositol 3-kinase, c-Jun N-terminal kinase, p38, and extracellular signal-regulated kinase 1/2 pathways in myocytes and fibroblasts (P<0.05). On the other hand, mechanical stretch also significantly increased periostin expression (P<0.05). This increase was inhibited partially, but significantly, by an Ang II receptor blocker, valsartan, and inhibited almost completely by valsartan with the neutralization antibodies for transforming growth factor-β and platelet-derived growth factor–BB (P<0.05). Therefore, we further examined periostin expression in vivo. Periostin expression was significantly increased in infarcted myocardium (P<0.05), and treatment with valsartan significantly attenuated it at 4 weeks after myocardial infarction (P<0.05), accompanied by a significant improvement in cardiac dysfunction (P<0.05). Overall, the present study demonstrated that Ang II, as well as mechanical stretch, stimulated periostin expression in both cardiac myocytes and fibroblasts, whereas valsartan significantly attenuated the increase in periostin expression. The inhibition of periostin by valsartan might especially contribute to its beneficial effects on cardiac remodeling after myocardial infarction. (Hypertension. 2007;49:1409-1414.)

Key Words: angiotensin II type 1 receptor blockers ■ myocardial infarction ■ adhesions ■ fibrosis ■ ventricular remodeling

Cardiac remodeling after myocardial infarction (MI) results in ventricular dysfunction, which contributes to a poor outcome and high mortality.1 The use of angiotensin (Ang)-converting enzyme inhibitors in patients with MI has improved survival and reduced the rates of major cardiovascular events,2 and Ang II receptor blockers (ARBs) were expected to prevent cardiac remodeling, like Ang-converting enzyme inhibitors. The Valsartan in Acute Myocardial Infarction Trial demonstrated that an ARB, valsartan, was as effective as a proven regimen of an Ang-converting enzyme inhibitor captopril in improving survival and reducing cardiovascular mortality in patients who suffered an MI.3,4 Treatment with captopril or valsartan resulted in similar changes in cardiac volume and ejection fraction after MI,3 whereas treatment with captopril after MI significantly reduced left ventricular (LV) enlargement.2

On the other hand, periostin is a novel secreted and putative soluble extracellular matrix protein5 and is known to be expressed in bone and to a lesser extent in lung, kidney, and heart valves but is not found in normal blood vessels.6–8 Periostin was also identified in another screen comparing altered patterns of gene expression in response to MI, and it was found to be elevated and coclustered with several isoforms of collagen, laminin, and fibronectin within the adult rat heart.9 Our previous study demonstrated that overexpression of periostin in the rat heart led to cardiac enlargement with cardiac dysfunction, accompanied by a significant increase in fibrosis. Notably, one of the molecular mechanisms is inhibition of fibroblast cells attachment. Indeed, inhibition of periostin using antisense technology improved cardiac enlargement, dysfunction, and survival in the salt-sensitive Dhal rat.10 These data suggest that periostin may play a pivotal role in extracellular matrix deposition, fibrosis, and tissue remodeling after MI. It is valuable to elucidate which factors affect cardiac periostin, because no study has elucidated the relation among Ang II, mechanical stretch, and...
peristin. In the present study, we examined whether Ang II or mechanical stretch regulates peristin expression.

**Methods**

**Stimulation of Cardiac Myocytes and Cardiac Fibroblasts**

Primary cultures of neonatal myocytes and fibroblasts were prepared from 1-day–old Wistar-strain rats as described previously. At 48 hours after plating, the medium was replaced by DMEM with 0.1% FBS. Then, 24 hours later, cultures were subjected to treatment with vehicle, Ang II (10^{-6} mol/L), transforming growth factor (TGF)-β1 (5 ng/mL), platelet-derived growth factor (PDGF)-BB (1 ng/mL; Sigma), or mechanical stretch. For experiments, we used valsartan (Novartis Pharma AG), anti-TGF-β antibody (Chemicon), anti-PDGFB antibody (R&D Systems), phosphatidylinositol 3-kinase inhibitor LY294002 (10^{-6} mol/L; n=6); LY, LY294002 (phosphatidylinositol 3-kinase inhibitor; n=6); PD, PD98059 (mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 inhibitor; n=7); SB, SB203580 (p38 mitogen-activated protein kinase inhibitor; n=6); SP, SP600125 (c-Jun N-terminal kinase inhibitor; n=4).

**Rat MI Model**

Male Lewis rats were used for induction of MI. The next day, the rats were randomized to the valsartan group or control, as detailed in the online data supplement (available at http://hyper.ahajournals.org). This animal experiment was approved by the Osaka University Animal Care Committee.

**Physiological Studies**

Four weeks after MI, transthoracic echocardiography study (Core Vision Pro SSA-350A) and pressure study (model SPR-470, Millar Instruments Inc) were performed as described previously. Once the hemodynamic studies were completed, the rats were euthanized and the hearts randomly allocated to either morphological or molecular studies.

**Immunohistochemical Analysis**

At 7 days post-MI, hearts were fixed with freshly prepared 4% paraformaldehyde, paraffin embedded, and sectioned for immunohistochemical examination. We performed hematoxylin/eosin staining, Masson’s trichrome staining, and peristin immunohistochemical staining using polyclonal peristin antibody.

**Quantitative Real-Time PCR**

Total RNA was isolated as described previously. In each experiment, rat β-actin RNA was amplified as a reference standard. PCR primers were used as detailed in the online data supplement.

**Western Blotting**

Preparation of protein extract and Western blotting were performed as described previously. Anti-mouse OSF-2/periostin antibody (R&D Systems) and anti-α tubulin antibody (Santa Cruz Biotechnology) were used.

**Statistical Analysis**

Values are expressed as mean±SE. ANOVA and test followed by Bonferroni adjustment for multiple comparisons were used for comparisons of >2 groups. A P value was considered to indicate significance of mean differences.

**Results**

**Effects of Ang II on Periostin Expression in Cardiac Myocytes and Fibroblasts**

Ang II significantly induced periostin mRNA expression in cardiac myocytes and fibroblasts evaluated by real-time PCR, and, expectedly, valsartan almost completely blocked it (P<0.05; Figures 1A and 2A). Because the signal transduction pathway to induce peristin has not yet been clarified, we elucidated the molecular mechanisms of the induction of peristin by Ang II. In myocytes, PD98059 and SB203580 partially, but significantly, inhibited the upregulation of peristin protein induced by Ang II (P<0.01), whereas LY294002 and SP600125 normalized it (P<0.01; Figure 2B).

**Effects of Mechanical Stretch on Periostin Expression in Cardiac Myocytes and Fibroblasts**

Next, we also tested the effects of mechanical stretch on periostin mRNA evaluated by real-time PCR. Mechanical
by mechanical stretch. Consistent with previous reports,18,19 we demonstrated that mechanical stretch significantly increased periostin expression in both cardiac myocytes and fibroblasts (Figure S1A and S1B). Interestingly, blockade of Ang II by valsartan partially, but significantly, blocked the mechanical stretch-induced increase in periostin mRNA expression in both cardiac myocytes and fibroblasts (P<0.05; Figure S1A and S1B).

To address why valsartan partially inhibited mechanical stretch-induced periostin expression, we examined the induction of various growth factors, such as TGF-β and PDGF-BB, by mechanical stretch. Consistent with previous reports,18,19 we demonstrated that mechanical stretch significantly increased TGF-β and PDGF-BB expression in both cardiac myocytes and fibroblasts, but treatment with valsartan did not attenuate the increase in TGF-β and PDGF-BB induced by mechanical stretch (Figure S2). TGF-β or PDGF-BB significantly increased periostin expression in both cells evaluated by real-time PCR (P<0.05; Figure S3). Furthermore, to prove the direct participation in periostin expression by TGF-β and PDGF-BB, we performed mechanical stretch with each neutralization antibody in both cells. They partially, but significantly, attenuated the increase in periostin expression in both cells (P<0.05). Moreover, when valsartan was added to the neutralization antibodies, they completely blocked the upregulation of periostin by mechanical stretch in both cells (P<0.05; Figure 3A and 3B). Direct stimulation by TGF-β and PDGF-BB induced by mechanical stretch might have contributed to the increase in periostin gene expression, independent of the action of Ang II.

In Vivo Regulation of Periostin After MI
To further study the role of periostin, we examined the expression of periostin in a rat MI model. In normal heart, in situ hybridization of periostin in the rat heart demonstrated that periostin expression was only detected in the cardiac valves in the normal heart (data not shown), consistent with a previous article. In the infarcted area, as shown in Figure 4A through 4C, periostin protein (brown staining) was readily stained with anti-periostin antibody. The area stained positive for periostin was consistent with interstitial fibrous tissue in the infarcted area. Together with in vitro data, periostin protein was mainly localized in fibroblasts and less in cardiac myocytes. Interestingly, periostin mRNA expression in the myocardium was elevated from day 1 after MI, reached a peak on day 7, and gradually normalized (periostin/mRNA ratio; control: 1.00; day 7: 2.90; 0.54; P<0.05 versus control). In contrast, in the noninfarcted myocardium, periostin expression was not significantly increased as compared with that in sham-operated rats (data not shown).

Finally, we examined the effects of Ang II blockade on cardiac periostin expression, because our in vitro data dem-
onstrated that Ang II and mechanical stretch significantly decreased cardiac periostin expression \((P<0.05)\). Valsartan treatment significantly improved cardiac enlargement and function of hearts after MI \((P<0.05)\) without the change of both heart rate and blood pressure (Table). More importantly, administration of valsartan significantly attenuated the increase in periostin expression in the infarcted area (periostin/β-actin mRNA ratio; sham: 1.00±0.21; control [MI]: 3.63±0.55; valsartan [MI]: 1.99±0.51; \(P<0.05\) versus control). Ang II might have directly contributed to the increase in cardiac periostin through the Ang II type 1 receptor.

**Discussion**

LV enlargement is a predictor of survival in humans with heart failure, such as that because of ischemic heart failure, hypertension, valvular disease, or dilated cardiomyopathy. Therefore, the progression of LV enlargement leads to severe heart failure. Although numerous factors have been reported to be involved in LV hypertrophy, only a few have been identified to contribute to ventricular dilation. Our previous data clearly demonstrated that overexpression of periostin in the heart promoted cardiac enlargement, resulting in heart failure through the inhibition of cell attachment. On contrary, periostin secreted by carcinoma was reported to be a ligand for αVβ3 and αVβ5 integrins. We cannot explain the difference, but it seems to have occurred corresponding with the difference between cardiac cells and carcinoma. Originally, periostin was identified to be involved in the process of heart failure, using subtractive hybridization. As a result, the periostin gene was shown to be highly expressed in a heart failure model. It encodes a protein composed of 838 amino acids and has a typical signal sequence, followed by a cysteine-rich domain, a 4-fold repeated domain, and a C-terminal domain. The 4-repeat domain of periostin shows homology to insect fasciclin I, a protein implicated in neural cell–cell adhesion, and to human βig-h3, a protein induced by TGF-β binding to various collagens. Importantly, periostin inhibited the cell–cell interaction between myocytes and fibroblasts. Therefore, periostin might cause cell–cell slippage of cardiac myocytes and/or fibroblasts. Interestingly, 

![Figure 4. Periostin expression in rat MI model. A. Hematoxilin and eosin (HE) staining (purple and red) for myocytes and Masson's trichrome (MTC) staining (blue) for fibrosis in the border zone of MI of the left ventricle at 7 days after MI. B, Immunohistochemical staining for periostin (brown) and hematoxilin staining (purple). Periostin on the fibroblasts was observed in border zone of MI of the left ventricle. C, Negative control (IgG) with hematoxilin staining (purple).](image-url)
other groups demonstrated overexpression of periostin in pressure-overloaded heart, 25 MI heart, 8 and human heart with ischemic cardiomyopathy, hypertrophic cardiomyopathy, or dilated cardiomyopathy. 24 Thus, it is noteworthy to study the regulation of periostin in cardiac tissues.

The present study documented that 2 important factors related to MI, Ang II and mechanical stretch, upregulated cardiac periostin expression. Numerous previous studies revealed that these 2 factors are involved in the pathogenesis of MI and heart failure. In both cardiac myocytes and fibroblasts, Ang II and mechanical stretch increased periostin mRNA expression. In addition, in myocytes, PD98059 and SB203580 partially, but significantly, inhibited, whereas LY294002 and SP600125 normalized the upregulation of periostin induced by Ang II. On the other hand, LY294002, SB203580, SP600125, and PD98059 normalized the upregulation of periostin induced by Ang II in cardiac fibroblasts (Figure 2B). In contrast, the increase in periostin expression by mechanical stretch was partially, but not completely, inhibited by an ARB, valsartan. Because mechanical stretch activated the Ang II type 1 receptor without the involvement of Ang II 26 but also increased various growth factors, such as TGF-β 18 and PDGF-BB, 19 valsartan might show partial inhibition of periostin. Although at least mechanical stress, Ang II, TGF-β, and PDGF-BB increased periostin expression, their relative contribution is still unclear in the present study. Further studies are necessary to investigate the other cytokines or stimulation that increase periostin.

Positive regulation by Ang II and mechanical stretch was also confirmed by in vivo experiments. In a rat MI model that exhibited an increase in cardiac Ang II and mechanical force, periostin was significantly increased in the infarcted area. More importantly, the inhibitory effect of Ang II blockade on periostin expression was confirmed in the rat MI model. The present study demonstrated that valsartan significantly inhibited the increase in periostin expression, accompanied by improvement of cardiac dysfunction, whereas the inhibitory effect of valsartan on periostin expression was not related to change in blood pressure and heart rate. Direct inhibition of the action of Ang II by valsartan might have contributed to the inhibition of periostin. These beneficial effects of valsartan were also confirmed by clinical evidence from the Valsartan in Acute Myocardial Infarction Trial that valsartan was as effective as a proven regimen of an Ang-converting enzyme inhibitor captopril in improving survival and reducing cardiovascular mortality in patients who suffered from MI. 14–27, 28 It is unclear whether the beneficial effect of valsartan through the decrease in periostin is the class effect of ARBs or not, and additive experiments are needed.

Overall, the present study demonstrated that Ang II and mechanical stretch significantly stimulated periostin expression (P<0.05), whereas blockade of Ang II by valsartan significantly inhibited the increase in periostin expression both in vitro and in vivo (P<0.05). The results of treatment with valsartan in an MI model especially suggest that inhibition of periostin by valsartan might contribute to the beneficial effects of valsartan on cardiac remodeling. Because an increase in periostin expression is known to occur in hearts with ischemic cardiomyopathy, hypertrophic cardiomyopathy, or dilated cardiomyopathy, the inhibition of periostin by an ARB might provide a new therapeutic strategy for heart failure.

Perspectives
Mechanical stress, Ang II, TGF-β, and PDGF-BB increased periostin expression. Valsartan can partially, but not completely, inhibit periostin expression in an MI heart, so inhibition of periostin by neutralized antibody may inhibit it more perfectly. The neutralized antibody for periostin with or without ARBs may be beneficial for MI treatment.

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Disclosures
None.

References


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Methods on line supplement

Animal Models of Myocardial infarction

Male Lewis rats weighing 200 to 220 g were used for induction of MI. MI was produced by ligation of the left coronary artery. Briefly, in the anesthetized condition, rat chest was opened through a left lateral thoracotomy, and the left coronary artery was ligated approximately 2 to 3 mm from its origin with a 6-0 silk suture. The next day, the rats were randomized to the valsartan group or control group and implanted with an subcutaneous osmotic mini-pumps (Alzet, ALZA Pharmaceuticals, Palo Alto, California) filled with valsartan administered at 5 mg · kg\(^{-1}\) · day\(^{-1}\) (valsartan group), or saline (control group). We selected this dose of valsartan since it did not decrease blood pressure significantly under normal conditions or after MI. Sham-operated rats underwent a similar procedure without coronary ligation (sham group). This animal experiment was approved by the Animal Care Committee of Osaka University.

PCR primers list

(1) rat periostin
Sense, 5'-AAGTCATTCAAGGCAGTCTTC-3';
antisense, 5'-GTCTCCCTGAAGCAGTCTTT-3';

(2) rat b-actin
Sense, 5'-GCCCTGGCTCCTAGCACC-3';
antisense, 5'-CCACCAATCCACACAGAGTACTTG-3'

(3) rat TGF-b
Sense, 5'-GACCCATCGCAGTAC-3'
antisense, 5'-CCAGTGACGTCAAAAGACAG-3'

(4) rat PDGF-BB
sense, 5’-GATCCGCTCCTTTGATGATC-3’
antisense, 5’-GTCTCACACTTGCATGCCAG -3’

Legends for on line supplement figures

**Figure.S1** Effect of Ang II blockade by valsartan on periostin expression in cardiac myocytes (A) and fibroblasts (B) evaluated by real time PCR
C; control, Val (-); stretch without valsartan (10^{-6}M), Val (+); stretch with valsartan, *P<0.05 vs. Val (-) (stretch without valsartan)
Each experiment was performed at least three times.

**Figure.S2** Effect of Valsartan on mechanical stretch induce TGF-b (A and C) &PDGF-B(B and D) expression on cardiac myocytes (A and B) and cardiac fibroblasts (C and D).
C; control, (-); mechanical stretch without valsartan, V; stretch with valsartan *P<0.05 vs. (-); mechanical stretch without valsaratin, Each experiment was performed at least three times.

**Figure.S3** Effect of TGF-b or PDGF-BB on periostin mRNA expression in cardiac myocytes (A and B) and fibroblasts (C and D)
C; control (vehicle) 12h; 12 hours after TGF-b or PDGF-BB stimulation, 24h; 24 hours after TGF-b or PDGF-BB stimulation,*P<0.05 vs. C (control), Each experiment was performed at least three times.
Figure S1

A

Periostin mRNA/β-actin in myocytes

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stretch (60min)

B

Periostin mRNA/β-actin in cardiac fibroblasts

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stretch (120min)