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Abstract—Angiotensin II (Ang II) is implicated in the proinflammatory process in various disease situations. Thus, we sought to determine the role of Ang II in early inflammation-induced fibrosis of pressure-overloaded (PO) hearts. PO was induced by suprarenal aortic constriction (AC) at day 0 in male Wistar rats, and they were orally administered 0.1 mg/kg per day candesartan every day from day -7. This was the maximum dose of candesartan that did not change arterial pressure in hypertensive rats with AC (AC rats). In AC rats, cardiac angiotensin-converting enzyme (ACE) activity was transiently enhanced after day 1 and peaked at day 3, declining to lower levels by day 14, whereas serum ACE activity was not changed. In AC rats, PO induced early fibroinflammatory changes (monocyte chemoattractant factor [MCP]-1 and transforming growth factor [TGF]-β expression, perivascular macrophage accumulation, and fibroblast proliferation), and thereafter, left ventricular hypertrophy developed, featuring myocyte hypertrophy, intramyocardial arterial wall thickening, and perivascular and interstitial fibroses. Candesartan suppressed the induction of MCP-1 and TGF-β and reduced macrophage accumulation and fibroblast proliferation in PO hearts. Candesartan significantly prevented perivascular and interstitial fibrosis. However, candesartan did not affect myocyte hypertrophy and arterial wall thickening. In conclusion, a subdepressor dose of candesartan prevented the MCP-1–mediated inflammatory process and reactive myocardial fibrosis in PO hearts. Ang II might play a key role in reactive fibrosis in hypertensive hearts, independent of arterial pressure changes. (*Hypertension*. 2004;43[part 2]:499-503.)

Key Words: angiotensin II ■ fibrosis ■ hypertension, experimental ■ macrophages ■ inflammation

More clinical attention has been paid to diastolic dysfunction as the major cause of congestive heart failure, especially in patients with hypertension, because 30% to 50% of patients who are diagnosed with congestive heart failure show normal systolic function.[1,2] The histologic features of hypertensive cardiac remodeling are myocyte hypertrophy and reactive myocardial fibrosis that extends from the perivascular space into the intermuscular interstitium, and these are basically adaptive changes to pressure overload (PO).[3] However, excessive myocyte hypertrophy and disproportionate myocardial fibrosis are major determinants of increased myocardial stiffness and impaired pumping capacity in patients with chronic hypertension.[4]

We have shown in rats that a suprarenal abdominal aortic constriction (AC) produces cardiac hypertrophy associated with diastolic dysfunction that is characterized by a rapid progression of reactive fibrosis by way of transforming growth factor (TGF)-β–dependent mechanisms.[5] Perivascular fibroinflammatory changes (macrophage accumulation and fibroblast proliferation associated with TGF-β induction) were induced by AC within the first week.[5,6] In this model, inhibition of macrophage infiltration by blocking intercellular adhesion molecule-1 function not only abolished fibroblast activation and TGF-β induction but also prevented reactive

fibrosis, though not affecting arterial pressure and myocyte hypertrophy.[6] Thus, it was suggested that the early fibroinflammatory process would be a new target for prevention and treatment of excessive myocardial fibrosis and diastolic dysfunction in hypertensive hearts. However, the trigger of the fibroinflammatory process had remained undetermined in our previous studies.

Recently, a role for angiotensin II (Ang II) has been suggested in triggering the inflammatory responses and cardiovascular remodeling in various disease situations, including atherosclerosis, arteriosclerosis, and myocardial infarction.[7–9] Earlier studies reported that a depressor dose of angiotensin type I (AT$_1$) receptor blockers (ARBs) ameliorated myocyte hypertrophy and myocardial fibrosis in hypertensive, hypertrophied hearts.[10] However, because in those studies arterial pressure was decreased to normal levels by ARB treatment, it was impossible to dissect out the AT$_1$ function–blocking effect from the arterial pressure–lowering effect. Moreover, little was known regarding the involvement of the Ang II–mediated inflammatory process in hypertensive cardiac remodeling. In this regard, we have shown that a subdepressor dose of candesartan, an ARB, reduces perivascular fibrosis in the PO heart.[11] However, it remained

unknown in this model whether cardiac renin-angiotensin system (RAS) activation occurs and whether the subdepressor dose of candesartan deactivates early inflammatory changes, such as monocyte chemoattractant factor (MCP)-1 induction and macrophage accumulation. Furthermore, we determined whether a subdepressor dose of candesartan prevents not only perivascular but also interstitial myocardial fibrosis induced by AC.

Methods

The study protocol was approved by the institutional committee for the ethics of animal care and treatment. At day 0, male Wistar rats (300 to 400 g; Japan SLC, Shizuoka, Japan) underwent AC or sham operation after anesthesia with sodium pentobarbital (50 mg/kg IP).[12] From day −7, rats received orally the denoted dose of candesartan or vehicle every day. Rats were randomized to the following 3 groups. Sham rats received vehicle and sham operation; AC rats received vehicle and AC; and AC+Cand rats received candesartan and AC. Blood pressure was measured in the unrestricted, conscious state through a heparinized indwelling catheter.[12] Unless otherwise indicated, 5 rats were studied in each group for each time point.

To investigate the pressure change–independent effects of AT_1 receptor blocking, the maximal dose of candesartan that did not change arterial pressure was determined: rats received candesartan 0.03, 0.1, or 0.5 mg/kg per day orally or vehicle every day from 7 days before to 14 days after AC. AC elevated arterial pressure, from 134 ± 10 mm Hg at day 0 to 212 ± 19 mm Hg at day 14, in the vehicle-treated rats. The 3 doses of candesartan did not affect arterial pressure at day 0. At day 14, 0.5 mg/kg per day candesartan significantly decreased hypertension induced by AC (189 ± 18 mm Hg, $P < 0.05$ versus vehicle-treated rats), whereas 0.03 and 0.1 mg/kg/ per day candesartan did not attenuate the AC-induced hypertension (215 ± 14 and 209 ± 17 mm Hg, respectively). Accordingly, 0.1 mg/kg per day candesartan was used throughout the following experiments.

ACE Activity

Rats were killed with an overdose of intraperitoneal pentobarbital. Blood was drawn from the right ventricular cavity for measurements. Thereafter, the left ventricle (LV) was immediately excised, snap-frozen in LN_2, and stored at $-80°C$ until use. Frozen LV tissue was homogenized with FastPrep (ThermoSavant). Angiotensin-converting enzyme (ACE) activity was measured in the sera and homogenates.[13]

Morphometry

Rats were killed with an overdose injection of intraperitoneal pentobarbital and were perfusion-fixed with 4% glutaraldehyde in Hank's solution at 100 mm Hg. The LV was excised, weighed, and then processed for histologic and immunohistologic studies.[5] To evaluate myocyte hypertrophy and myocardial fibrosis, 3 independent hematoxylin-and-eosin–stained and 3 Mallory-Azan–stained sections of each rat were scanned and analyzed with a digital image analyzer. The shortest transverse myocyte diameter was measured in 50 nucleated transverse sections of myocytes in each tissue section.[14] The percent area of myocardial fibrosis was calculated as previously described.[13] In each rat, >40 small (internal diameter, <200 μm) and >10 large (>200 μm) arteries were examined for perivascular fibrosis and vascular wall thickening (wall-to-lumen ratio), as described elsewhere.[13]

Immunohistostaining

Paraffinized sections were subjected to immunohistostaining with an antibody for ED-1 (Chemicon International) and a commercially available detection system (DAKO). In situ bromodeoxyuridine (BrdU) labeling was performed to identify the proliferating cells.[15] BrdU+ spindle-shaped cells were defined as proliferating fibro-

Arterial Pressure and Cardiac Hypertrophy

	Day After AC			
	0	1	3	14
Systolic arterial pressure, mm Hg				
Sham	122 ± 12	—	—	129 ± 13
AC	134 ± 10	$149 \pm 10*$	$181 \pm 13*$	$212 \pm 19*$
AC+Cand	129 ± 13	$149 \pm 10*$	$178 \pm 15*$	$210 \pm 14*$
LVW/BW, mg/g				
Sham	2.37 ± 0.25	—	—	2.42 ± 0.19
AC	2.41 ± 0.13	—	—	$3.34 \pm 0.28*$
AC+Cand	2.41 ± 0.32	—	—	$3.32 \pm 0.30*$
Myocyte diameter, μm				
Sham	11.2 ± 0.5	-	-	11.5 ± 0.6
AC	—	—	—	$15.7 \pm 0.8*$
AC+Cand	—	—	—	$15.6 \pm 0.6*$

Abbreviations and groups are as defined in text. Data are mean$\pm$SD, n=6. *$P < 0.05$ vs sham.

blasts.[5] The labeled cells were counted at 200× magnification in 4 independent entire cross sections from each animal.

Quantitative mRNA Expression Analysis

Total RNA was extracted from unfixed hearts (n=3 per group).[5] Real-time reverse transcription-polymerase chain reaction (RT-PCR; TaqMan) was performed with the relative-standard-curve method.[5] Aliquots (25 ng) of total RNA were RT and amplified in triplicate with a commercially available kit (TaqMan EZ RT-PCR, PE Biosystems). PCR conditions and the nucleotide sequences of primers and TaqMan probes for TGF-β were as previously described.[5] The sequences of the primers and TaqMan probe for MCP-1 were as follows: forward primer, 5′-CTCAGCCAGATGCAGTTAATGC-3′ and reverse primer, 5′-AG-CCGACTCATTGGGATCAT-3′; and for the TaqMan probe, 5′-TCACCTGCTGCTACTCATTCACTGGCA-3′. The expression level of the target gene was normalized to that of glyceraldehyde 3-phosphate dehydrogenase in each sample, and relative changes in the target gene were expressed as the n-fold increase relative to control rats.

Statistical Analysis

Each quantitative analysis was performed by a single observer in a blinded fashion. One-way ANOVA followed by Scheffe F test was performed for statistical comparisons. A value of $P < 0.05$ was considered significant.

Results

In AC rats, systolic arterial pressure was rapidly and sustainedly elevated, and significant LV hypertrophy, as assessed by the ratio of LV weight to body weight (LVW/BW), developed at day 14 (Table). Arterial pressure and LVW/BW did not change in sham rats. As shown in the Table, 0.1 mg/kg per day candesartan had no effect on the increase in LVW/BW after AC, suggesting that LV hypertrophy was not affected by this dose of candesartan.

Activation of Cardiac ACE Activity

In AC rats, cardiac ACE activity was transiently enhanced after day 1, peaked at day 3, and then declined to a lower level, which was significantly higher than the basal level, by day 14 (Figure 1A). The AC-induced ACE activation was not affected by the subdepressor dose of candesartan. Serum
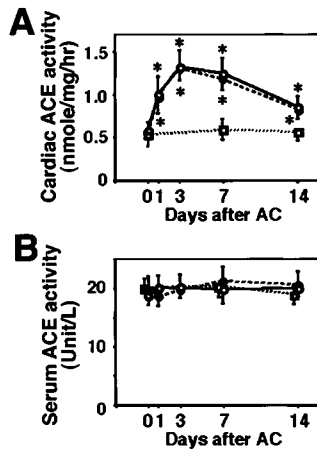
Figure 1. Temporal changes in ACE activities of rat hearts (A) and sera (B). □, Sham-operated, vehicle-treated rats; ○; vehicle-treated AC rats; and ●, candesartan-treated, AC rats. Bar=SD, n=5. *$P<0.05$ vs day 0.

ACE activity was unchanged throughout the observation period, irrespective of candesartan treatment (Figure 1B).

Effects of Subdepressor Dose of Candesartan on Early Fibroinflammatory Changes

Early inflammatory changes were investigated in AC rats at day 3, because in our previous study, the peak number of ED1^{+} macrophage accumulation was observed in PO hearts at day 3.[6] Immunohistostaining for ED1 revealed marked perivascular accumulation of macrophages at day 3, accompanied by myocardial expressions of MCP-1, the major regulator of macrophage recruitment into inflammatory tissues (Figures 2A and 3). Apparently, macrophage infiltration was observed in the perivascular space of both small and large intramyocardial arteries to a similar extent. However, only limited numbers of macrophages were found in the intermuscular interstitium of PO hearts during the observation period. Candesartan not only significantly suppressed MCP-1 induction but also reduced macrophage accumulation in the PO heart.
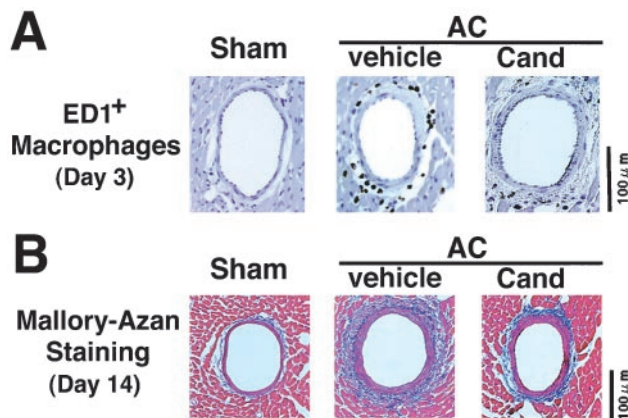

Figure 2. Histologic studies. A, Representative immunohistostaining for ED1^{+} macrophages at day 3. AC-induced perivascular accumulation of macrophages (stained brown) was reduced by the subdepressor dose of candesartan (Cand). B, Representative microphotographs of Mallory-Azan–stained cross sections of intramyocardial artery at day 14. Candesartan markedly ameliorated AC-induced perivascular fibrosis (stained blue). Sham indicates sham-operated, vehicle-treated rats.
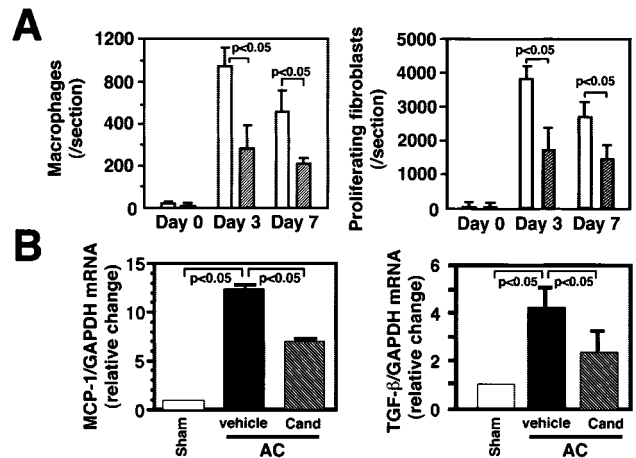

Figure 3. Effects of subdepressor dose of candesartan (Cand) on early inflammatory and fibrotic changes. A, Time course of the number of ED1^{+} macrophages or BrdU[+] proliferating fibroblasts is shown for vehicle-treated (open column) and candesartan-treated (hatched column) AC rats. Bar=SD, n=5. B, Expression levels of MCP-1 and TGF-β at day 3 were expressed as the n-fold change compared with sham rats. Bar=SD, n=3. GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase.

In addition, the effects of candesartan on the early fibrotic process were studied at day 3 (Figure 3). PO activated fibroblast proliferation and upregulated myocardial TGF-β at the mRNA level, which are the determinant factors of myocardial fibrosis in this model.[5] The AC-induced fibroblast proliferation and TGF-β upregulation were significantly inhibited by candesartan. Irrespective of candesartan treatment, neutrophils and T lymphocytes were scarcely found in the perivascular and intermuscular spaces in PO hearts during the observation period.

Effects of Subdepressor Dose of Candesartan on Late Cardiac Remodeling

AC induced myocyte hypertrophy, and transverse myocyte diameter was significantly greater in AC rats by 1.4-fold versus sham rats at day 14 (Table). Also, reactive myocardial fibrosis developed, which extended from the perivascular space to the intermuscular interstitium, and the percentage of myocardial fibrosis reached 9% at day 14 (Figures 2B, 4A, and 4B). In addition to perivascular fibrosis, medial wall thickening developed in both small and large intramyocardial arteries in AC rats (Figures 2B and 4C). Candesartan significantly attenuated the AC-induced perivascular and interstitial fibrosis (Figure 4A and 4B). The inhibitory effects of candesartan on perivascular fibrosis were observed in small and large arteries to a similar extent. In contrast, AC-induced myocyte hypertrophy (Table) and arterial wall thickening (Figure 4C) were not affected by the subdepressor dose of candesartan.

Discussion

Recently, we have shown that AC rats show transient perivascular macrophage accumulation, which in turn triggers TGF-β–mediated perivascular fibrosis, in the early phase, leading to reactive myocardial fibrosis and diastolic dysfunction in
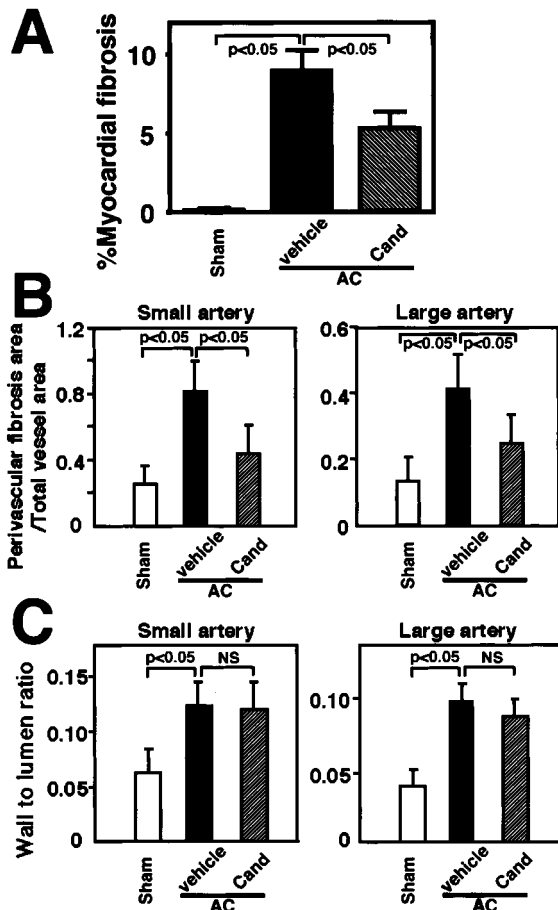
Figure 4. Effects of subdepressor dose of candesartan (Cand) on late myocardial remodeling at day 14. Candesartan ameliorated AC-induced interstitial fibrosis (A) and perivascular fibrosis of both small and large intramyocardial arteries (B). Medial wall thickness (wall to lumen ratio) was not affected by candesartan in either small or large arteries (C). Bar=SD, n=5. NS indicates no statistical significance.

the later phase.[5,6] However, the changes that occur earlier than macrophage accumulation and TGF-β induction have remained undetermined. There is accumulating evidence that Ang II plays a proinflammatory role in various disease situations.[7–9] Thus, the role of Ang II as a local inflammation mediator was investigated in AC-induced myocardial remodeling in the present study.

Myocardial ACE Activation

In humans, local Ang II is produced from angiotensin I by the tissue proteases ACE and chymase. In contrast, because rat chymase cleaves angiotensin I to inactive peptides, ACE is the major enzyme of the tissue Ang II production system in rats. In this model, we have shown that plasma renin activity is not significantly changed until day 28.[5] Accordingly, cardiac ACE activity was evaluated as an indicator of tissue RAS activation in the present study. To the best of our knowledge, there are few data regarding the changes in cardiac ACE activity in the early phase of PO.

As shown in Figure 1A, PO increased cardiac ACE activity without changing serum ACE activity, consistent with previous studies focusing on ACE activity in the chronic phase.[16]

However, the present study demonstrated for the first time that PO rapidly activated cardiac ACE immediately after induction of hypertension and that the activation was no longer sustained, despite the persistent elevation of arterial pressure. These observations might indicate that rapid ACE activation is associated with an abrupt change in arterial pressure and suggest some important roles for tissue Ang II in the early phase of AC-induced myocardial remodeling.

Effects of Subdepressor Dose of Candesartan on Cardiac Remodeling

Earlier studies demonstrated that depressor doses of ARBs ameliorated both myocyte hypertrophy and myocardial fibrosis in hypertensive rat hearts.[10] However, it has remained undetermined whether the inhibitory effects on cardiac remodeling are attributable directly to the functional blocking of the AT$_1$ receptors or simply to the depressor effects. Thus, to investigate the pressure-independent effects of candesartan, the maximal dose of candesartan that did not change arterial pressure was chronically administered to PO rats in the present study.

The subdepressor dose of candesartan significantly reduced perivascular and interstitial fibrosis (Figure 4A and 4B) while having no effects on LV and myocyte hypertrophy as well as vascular hypertrophy (the Table), indicating that Ang II induces reactive fibrosis in part through mechanisms that are independent of the pressor effect. These observations are consistent with the notion that myocardial fibrosis and myocyte hypertrophy are independently regulated each other: myocardial fibrosis is mediated by many interacting factors, including mechanical (mechanical stretch and high coronary perfusion pressure), humoral, and paracrine/endocrine factors, whereas mechanical stretch acts in a crucial role in myocyte hypertrophy.[4,17] Because Ang II can upregulate TGF-β and the extracellular matrix in cultured cardiomyocytes and fibroblasts,[18] Ang II might be one of the major paracrine/endocrine proinflammatory factors. As shown in Figure 4, the subdepressor dose of candesartan was unable to completely inhibit perivascular and interstitial fibrosis. Although we did not evaluate this issue in the current study, it is possible that greater prevention from fibrosis occurs when both Ang II- and mechanical stress–dependent mechanisms are inhibited by higher administered doses of candesartan that are used to decrease arterial pressure. Also, it is likely that higher doses of candesartan would ameliorate myocyte and vascular hypertrophy.

Mechanism of Ang II-Induced Fibroinflammation

PO-induced MCP-1 induction and perivascular macrophage infiltration were reported by Capers et al.[19] We have demonstrated similar findings as well. Capers et al suggested that both Ang II- and mechanical strain–mediated mechanisms are responsible for MCP-1 induction in the hypertensive arterial wall. The most important findings of this study were that a subdepressor dose of candesartan reduced early fibroinflammatory changes in response to PO and attenuated myocardial fibrosis (Figures 2 through 4). It is noteworthy that TGF-β induction and fibroblast proliferation were eliminated by candesartan concomitantly with the inhibition of perivascular

macrophage infiltration. Recently, we have demonstrated that macrophage infiltration is an early key event for reactive myocardial fibrosis, especially perivascular fibrosis, in this model.[6] Also, it has been shown that Ang II supports leukocyte transmigration via AT_1 receptor–dependent, but arterial pressure-independent, mechanisms.[20–22] Infiltrated macrophages are known to produce a variety of cytokines and growth factors, which in turn amplify the inflammatory process and activate tissue fibrosis.[18,23] Taken together, the present study provides in vivo evidence that Ang II might play an important role in early fibrotic changes and the resultant reactive myocardial fibrosis in PO hearts by activating the macrophage-mediated inflammatory process. It is noteworthy that the PO-dependent activation of cardiac ACE was not affected by the low dose of candesartan, which significantly inhibited MCP-1 induction and macrophage infiltration (Figure 1A), suggesting that tissue RAS activation is not a downstream event of these early inflammatory changes in this model. Of course, Ang II is not the only proinflammatory mediator. Recent studies have suggested that PO itself is a strong proinflammatory factor, because mechanical strain can induce inflammatory cytokines, growth factors, and oxidative stress, as well as tissue RAS in the vessel wall.[24] The interplay of these factors might regulate tissue Ang II production and the fibrotic process in PO hearts. Further studies are needed to address this issue.

Perspectives

The present study suggests that ARBs might have effects beyond blood pressure lowering on myocardial fibrosis by inhibiting the Ang II-mediated fibroinflammatory process in hypertensive hearts. Moreover, our findings raise the possibility that ARBs improve not only systolic but also diastolic dysfunction in hypertensive, hypertrophied hearts.

References

1. Vasan RS, Benjamin EJ, Levy D. Prevalence, clinical features and prognosis of diastolic heart failure: an epidemiologic perspective. *J Am Coll Cardiol.* 1995;26:1565–1574.
2. Vasan RS, Larson MG, Benjamin EJ, Evans JC, Reiss CK, Levy D. Congestive heart failure in subjects with normal versus reduced left ventricular ejection fraction: prevalence and mortality in a population-based cohort. *J Am Coll Cardiol.* 1999;33:1948–1955.
3. Brilla CG, Weber KT. Reactive and reparative myocardial fibrosis in arterial hypertension in the rat. *Cardiovasc Res.* 1992;26:671–677.
4. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium: fibrosis and renin-angiotensin-aldosterone system. *Circulation.* 1991;83:1849–1865.
5. Kuwahara F, Kai H, Tokuda K, Kai M, Takeshita A, Egashira K, Imaizumi T. TGF-β function blocking prevents myocardial fibrosis and

diastolic dysfunction in pressure-overloaded rats. *Circulation.* 2002;106:130–135.
6. Kuwahara F, Kai H, Tokuda K, Niiyama H, Tahara N, Kusaba K, Takemiya K, Jalalidin A, Koga M, Nagata T, Shibata R, Imaizumi T. Roles of intercellular adhesion molecule-1 in hypertensive cardiac remodeling. *Hypertension.* 2003;41:819–823.
7. Brasier AR, Recinos A 3rd, Eledrisi MS. Vascular inflammation and the renin-angiotensin system. *Arterioscler Thromb Vasc Biol.* 2002;22:1257–1266.
8. Phillips MI, Kagiyama S. Angiotensin II as a pro-inflammatory mediator. *Curr Opin Invest Drugs.* 2002;3:569–577.
9. Schiffrin EL, Touyz RM. Multiple actions of angiotensin II in hypertension: benefits of AT_1 receptor blockade. *J Am Coll Cardiol.* 2003;42:911–913.
10. Nicoletti A, Heudes D, Hinglais N. Left ventricular fibrosis in renovascular hypertensive rats: effect of losartan and spironolactone. *Hypertension.* 1995;26:101–111.
11. Tokuda K, Kai H, Kuwahara F, Imaizumi T. Sub-depressor dose of angiotensin type-1 receptor blocker inhibits TGF-β-mediated perivascular fibrosis in hypertensive rat hearts. *J Cardiovasc Pharmacol.* 2003;42(suppl):S61–S65.
12. Kuwahara F, Kai H, Tokuda K, Shibata R, Kusaba K, Tahara N, Niiyama H, Nagata T, Imaizumi T. Hypoxia-inducible factor-1α/vascular endothelial growth factor pathway for adventitial vasa vasorum formation in hypertensive rat aorta. *Hypertension.* 2002;39:46–50.
13. Takemoto M, Egashira K, Tomita H, Usui M, Okamoto H, Kitabatake A, Shimokawa H, Sueishi K, Takeshita A. Chronic angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor blockade: effects on cardiovascular remodeling in rats induced by the long-term blockade of nitric oxide synthesis. *Hypertension.* 1997;30:1621–1627.
14. Kai H, Muraishi A, Sugiu Y, Nishi H, Seki Y, Kuwahara F, Kimura A, Kato H, Imaizumi T. Expression of proto-oncogenes and gene mutation of sarcomeric proteins in patients with hypertrophic cardiomyopathy. *Circ Res.* 1998;83:594–601.
15. Shibata R, Kai H, Seki Y, Kato S, Morimatsu M, Kaibuchi K, Imaizumi T. A role of Rho-associated kinase in neointima formation after vascular injury. *Circulation.* 2001;103:284–289.
16. Schunkert H, Jackson B, Tang SS, Schoen FJ, Smits JF, Apstein CS, Lorrel BH. Distribution and functional significance of cardiac angiotensin converting enzyme in hypertrophied rat hearts. *Circulation.* 1993;87:1328–1339.
17. Brecher P. Angiotensin II and cardiac fibrosis. *Trends Cardiovasc Med.* 1996;6:193–198.
18. Border WA, Ruoslahti E. Transforming growth factor-β in disease: the dark side of tissue repair. *J Clin Invest.* 1992;90:1–7.
19. Capers Q IV, Alexander RW, Lou P, De Leon H, Wilcox JN, Ishizaka N, Howard AB, Taylor R. Monocyte chemoattractant protein-1 expression in aortic tissues of hypertensive rats. *Hypertension.* 1997;30:1397–1402.
20. Mervaala EMA, Müller DN, Park J-K, Schmidt F, Löhn M, Breu V, Dragun D, Ganten D, Haller H, Luft FC. Monocyte infiltration and adhesion molecules in a rat model of high human renin hypertension. *Hypertension.* 1999;33(part II):389–395.
21. Strawn WB, Gallagher PE, Tallant EA, Ganten D, Ferrario CM. Angiotensin II AT_1-receptor blockade inhibits monocyte activation and adhesion in transgenic (mRen2)27 rats. *J Cardiovasc Pharmacol.* 1999;33:341–351.
22. Pastore L, Tessitore A, Martinotti S, Toniato E, Alesse E, Bravi MC, Ferri C, Desideri G, Gulino A, Santucci A. Angiotensin II stimulates intracellular adhesion molecule-1 (ICAM-1) expression by human vascular endothelial cells and increases soluble ICAM-1 release in vivo. *Circulation.* 1999;100:1646–1652.
23. Jiang Y, Beller DI, Frendle G, Graves DT. Monocyte chemoattractant protein-1 regulates adhesion molecule expression and cytokine production in human monocytes. *J Immunol.* 1992;148:2423–2428.
24. Nicoletti A, Michel JB. Cardiac fibrosis and inflammation: interaction with hemodynamic and hormonal factors. *Cardiovasc Res.* 1999;41:532–543.