Original Article

Angiotensin II Type 1 Receptor Blocker Prevents Atrial Structural Remodeling in Rats with Hypertension Induced by Chronic Nitric Oxide Inhibition

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The prevalence of atrial fibrillation (AF) increases in patients with hypertension. Angiotensin II is involved in structural atrial remodeling, which contributes to the onset and maintenance of AF in paced animal models. We investigated the role of angiotensin II in atrial structural remodeling in rats with hypertension. Ten-week-old male Wistar-Kyoto rats were randomly divided into 4 groups: a control group (no treatment), an N^{ω}-nitro-L-arginine methyl ester (L-NAME) group (administered L-NAME, an inhibitor of nitric oxide synthase, 1 g/l in drinking water), an L-NAME+candesartan group (L-NAME plus candesartan—an angiotensin II receptor blocker (ARB)—at 0.1 mg/kg/day), and an L-NAME+hydralazine group (L-NAME plus hydralazine at 120 mg/l in drinking water). Eight weeks after treatment, the L-NAME group showed significantly higher systolic blood pressure than the control group (197±12 *vs.*138±5 mmHg, $p<0.05$). Candesartan or hydralazine with L-NAME reduced systolic blood pressure to baseline. Chronic inhibition of NO synthesis increased the extent of fibrosis and transforming growth factor-β expression in atrial tissue, and both of these effects were prevented by candesartan, but not by hydralazine. Cardiac hypertrophy and dysfunction were induced in the L-NAME group, and these effects were also prevented by candesartan, but not by hydralazine. In contrast, the decrease in thrombomodulin expression in the atrial endocardium in hypertensive rats was restored by candesartan and hydralazine. The ARB prevented atrial structural remodeling, a possible contributing factor for the development of AF, in the hearts of rats with hypertension induced by long-term inhibition of NO synthesis. (*Hypertens Res* 2006; 29: 277–284)

***Key Words*: angiotensin II type 1 receptor blocker, atrial fibrillation, nitric oxide, hypertension, atrial remodeling**

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This study was supported by grants for research on Human Genome, Tissue Engineering, and Food Biotechnology (H13-Genome-11) and grants for Comprehensive Research on Aging and Health (H13-21seiki [seikatsu]-23) in Health and Labour Science Research from the Ministry of Health, Labour, and Welfare, Japan.

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Received September 7, 2005; Accepted in revised form January 13, 2006.

Introduction

Atrial fibrillation (AF) is associated with a high risk of cardiovascular morbidity and mortality, and its prevalence is expected to double in the next 50 years (*1*–*5*). Patients with hypertension and chronic heart failure (CHF) are at high risk of developing AF (*2*, *6*). The development and maintenance of AF are associated with changes in electrical and structural properties known as atrial remodeling (*7*, *8*). Kumagai *et al*. demonstrated that rapid atrial pacing caused electrical and structural atrial remodeling (*8*). Li *et al*. demonstrated that CHF induced by rapid ventricular pacing strongly promotes the induction of sustained AF by causing fibrosis, which is a mechanism different from that of AF related to atrial tachycardia (*7*, *9*). Importantly, both rapid atrial and ventricular pacing demonstrated that slowing of atrial conduction velocity is more important than shortening of the atrial effective refractory period in the development of AF, and that slowing of atrial conduction velocity is associated with extensive fibrosis (*8*, *9*). Thus, the prevention of atrial fibrosis is a promising strategy for the management of AF. Although atrial structural remodeling, a possible contributing factor for the development and maintenance of AF, is observed in atrial and ventricular rapid pacing models (*8*, *9*), it remains unknown whether it is involved in hypertensive hearts.

The renin-angiotensin system (RAS) is excessively activated in patients with CHF (*10*–*13*). Recent clinical data have clearly shown that the RAS suppression by treatment with an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin type 1 receptor blocker (ARB) reduces the new onset of AF in patients with CHF (*14*–*16*). Consistent with the clinical data, RAS suppression by ACE inhibitors or ARBs prevents atrial structural remodeling in both rapid atrial and ventricular pacing models (*8*, *17*). Recently, an ARB has been shown to confer better protection against the new onset of AF in hypertensive patients with left ventricular (LV) hypertrophy compared with a β-adrenergic receptor antagonist (*18*), suggesting that angiotensin II may also play an important role in the onset and maintenance of AF in hypertension. In the present study, we examined the occurrence of atrial structural remodeling and the role of angiotensin II in its development using candesartan, an ARB, in the hearts of rats with hypertension induced by long-term inhibition of nitric oxide (NO) synthesis (*19*). Furthermore, since atrial endocardial dysfunction such as a reduced thrombomodulin (TM) expression was previously induced in a rapid atrial pacing model (*20*, *21*), we also investigated the effects of candesartan or hydralazine on TM expression in the atrium in this hypertensive model.

Methods

Materials

Candesartan was provided by Takeda Co., Ltd. (Osaka, Japan), and the other drugs were obtained from Sigma Chemical Co. (St. Louis, USA). All procedures were performed in conformity with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 93–23, revised 1985) and approved by the Osaka University Ethical Committee for Laboratory Animal Use.

Animal Model of Chronic Inhibition of NO Synthesis

Since endothelial dysfunction has been documented in patients with CHF and hypertension (*22*, *23*), which is largely the result of a decrease in the bioavailability of NO (*24*, *25*), we adopted a hypertensive rat model induced by a long-term inhibition of NO synthesis. Ten-week-old male Wistar-Kyoto rats (Charles River, Yokohama, Japan) were randomly divided into 4 groups. The control group ($n=11$) received no treatment. The N^{ω}-nitro-L-arginine methyl ester (L-NAME) group ($n=11$) received L-NAME at 1 g/l in drinking water. The L-NAME+candesartan group ($n=11$) received L-NAME plus candesartan (0.1 mg/kg per day) orally. The L-NAME+hydralazine group received L-NAME plus hydralazine (120 mg/l in drinking water). Body weight (BW) was measured 1 week before and 8 weeks after the initiation of treatment. Blood pressure (BP) and heart rate (HR) were measured by the tail-cuff method 1 week before and 1, 2, 4, and 8 weeks after starting the experiments. All rats were housed, treated, and subjected to euthanasia as described previously (*26*).

Echocardiographic Studies

Transthoracic echocardiography was performed using a machine equipped with a 7.5 MHz transducer (ALOKA Co., Ltd., Tokyo, Japan) after 8 weeks of treatment. Rats were anesthetized intraperitoneally with ketamine (15 mg/kg) and xylazine (5 mg/kg) and subjected to echocardiographic study. We measured echocardiographic parameters including LV end-diastolic dimension (LVDd), LV end-systolic dimension (LVDs), LV fractional shortening (LVFS), and the ratio of early to late filling wave of transmitral pulse-wave Doppler velocity (E/A).

Histopathological and Immunohistochemical Analysis

Eight weeks after treatment, rats in each group were sacrificed for morphometric, immunohistochemical and biochemical analyses. Excised hearts were weighed, separated into atrial and ventricular sections, cut, and stained with hematoxylin-eosin (HE) solution and Masson trichrome solution, and left atrial sections were carefully scanned as described previously (*26*). Each section was scanned at ×200 magnification. For immunohistochemistry, paraffin sections (5 μm thick) were incubated overnight at 4°C with a rabbit polyclonal anti-

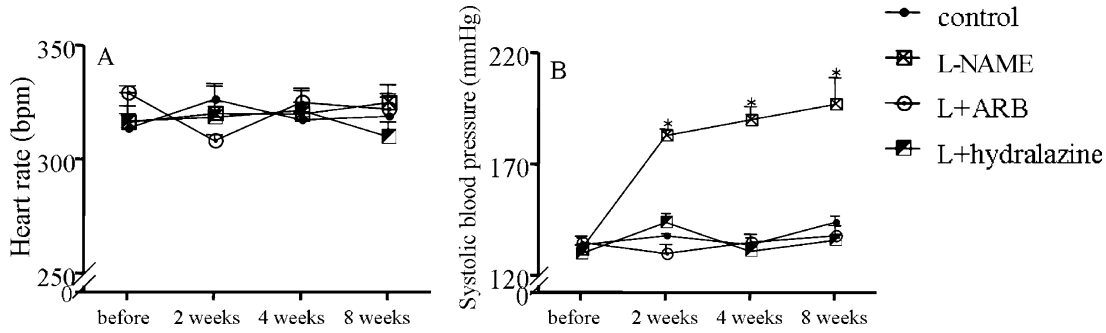

Fig. 1. *Hemodynamic parameters in groups tested. A: Heart rate. B: Systolic blood pressure. L-NAME,* N$^{\omega}$*-nitro-L-arginine methyl ester; L+ARB,* N$^{\omega}$*-nitro-L-arginine methyl ester plus angiotensin II receptor blocker; L+hydralazine,* N$^{\omega}$*-nitro-L-arginine methyl ester plus hydralazine. Data: mean±SEM.* *p < *0.05* vs. *control group.*

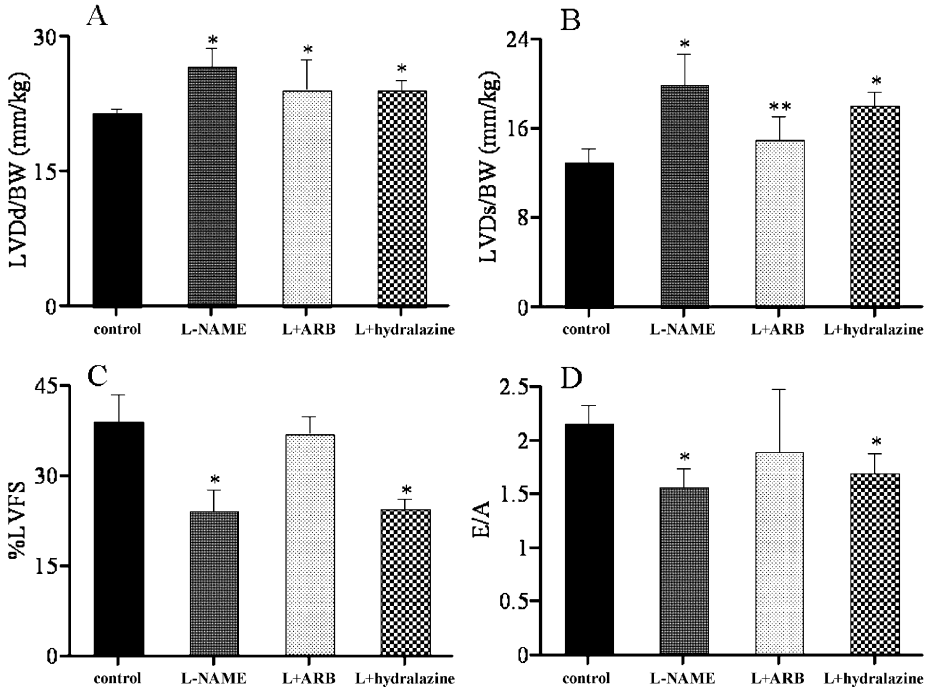

Fig. 2. *Echocardiographic parameters in groups tested. A: Left ventricular end-diastolic dimension (LVDd) corrected by body weight (BW). B: Left ventricular end-systolic dimension (LVDs)/BW. C: Left ventricular fractional shortening (LVFS). D:* E/A *ratio (ratio of early to late filling wave velocity). L-NAME,* N$^{\omega}$*-nitro-L-arginine methyl ester; L+ARB,* N$^{\omega}$*-nitro-L-arginine methyl ester plus angiotensin II receptor blocker; L+hydralazine,* N$^{\omega}$*-nitro-L-arginine methyl ester plus hydralazine. Data: mean±SEM.* *p < *0.05* vs. *control group,* **p < *0.05* vs. *L-NAME group.*

TM antibody (American Diagnostica Inc., Stamford, USA). A goat polyclonal anti-rabbit IgG antibody (DAKO) was used as a secondary reagent.

Real-Time Quantitative Reverse Transcriptase–Polymerase Chain Reaction

Reverse transcriptase–polymerase chain reaction (RT-PCR) of left atrial samples of rat was performed according to the Omniscript Reverse Transcription Handbook (QIAGEN Inc., Hilden, Germany). The rat primers and probes used for quantification of collagen type 1 and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (internal control) were designed according to the manufacturer's protocol (Applied Biosystems, Foster City, USA) and those for transforming growth factor (TGF)-β were designed as described previously (*27*). Real-time quantitative RT-PCR was performed with an ABI PRISM7700 Sequence Detection System (Applied Biosys-

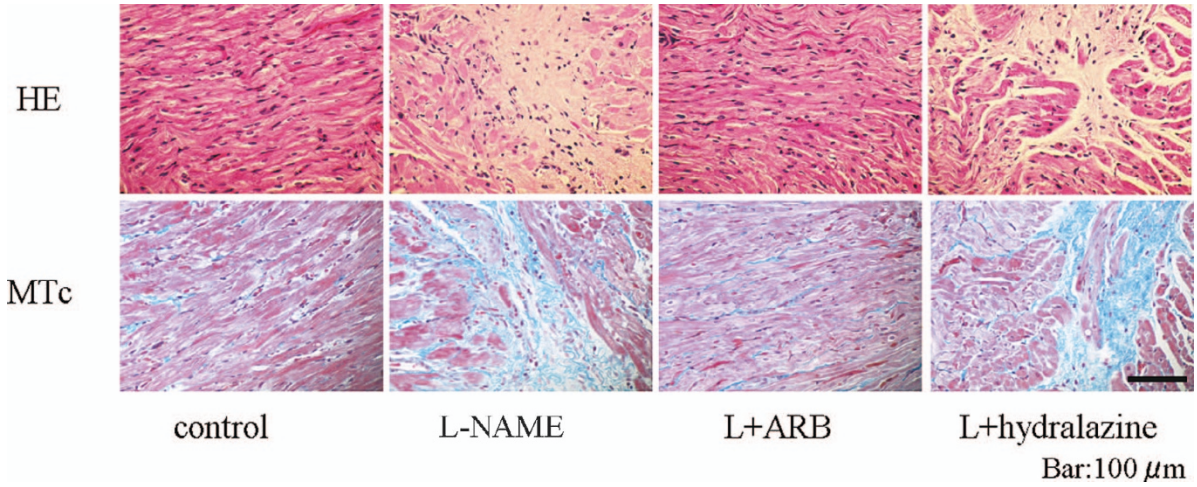

Fig. 3. *Effects of angiotensin II receptor blocker (ARB) on atrial structural remodeling in the hypertensive rat model. Atrial sections were stained with hematoxylin-eosin (HE) and Masson's-Trichrome (MTc). The bar indicates 100 μm. L-NAME,* N$^{\omega}$*-nitro-L-arginine methyl ester; L+ARB,* N$^{\omega}$*-nitro-L-arginine methyl ester plus angiotensin II receptor blocker; L+hydralazine,* N$^{\omega}$*-nitro-L-arginine methyl ester plus hydralazine.*

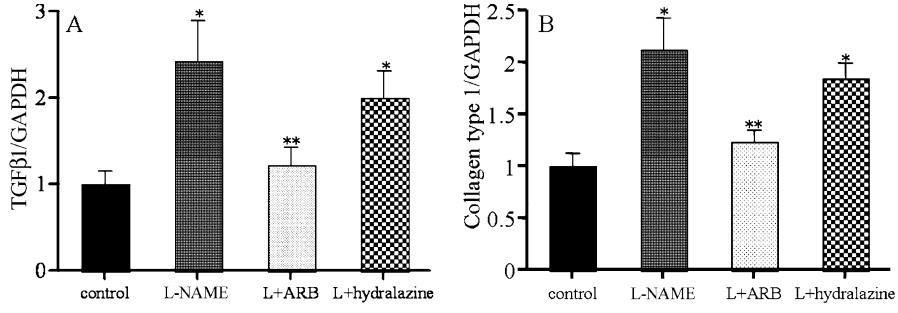

Fig. 4. *Expression of collagen type 1 and transforming growth factor (TGF)-β in atrial tissue in the hypertensive rat model. Each sample of TGF-β 1(A) and collagen type 1(B) was normalized by glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). L-NAME,* N$^{\omega}$*-nitro-L-arginine methyl ester; L+ARB,* N$^{\omega}$*-nitro-L-arginine methyl ester plus angiotensin II receptor blocker; L+hydralazine,* N$^{\omega}$*-nitro-L-arginine methyl ester plus hydralazine. Data: mean±SEM.* *p$<$*0.05* vs. *control group,* **p$<$*0.05* vs. *L-NAME group.*

tems) by the relative standard curve method. The target amount was determined from the relative standard curves constructed with serial dilutions of the control total RNA.

Immunoblot Analysis

Protein extraction and immunoblot analysis of left atrial samples of rats were performed as described previously (*20*), and immunoreactive bands were quantified by densitometry (Molecular Dynamics, Sunnyvale, USA).

Statistical Analysis

Data are expressed as the mean±SEM. Heart weight (HW), BW, hemodynamic variables, collagen type 1 or TGF-β expression normalized by GAPDH and TM protein levels were compared using one-way ANOVA followed by Bonferroni's test for multiple comparisons. Comparisons of the changes of BP among the groups over time were performed by two-way repeated-measures ANOVA followed by Bonferroni's correction, and values of $p<0.05$ were considered to be statistically significant.

Results

Hemodynamic Parameters

HR and systolic BP (SBP) are presented in Fig. 1. The HR was comparable among the 4 groups tested and did not change throughout the study. SBP was comparable among the

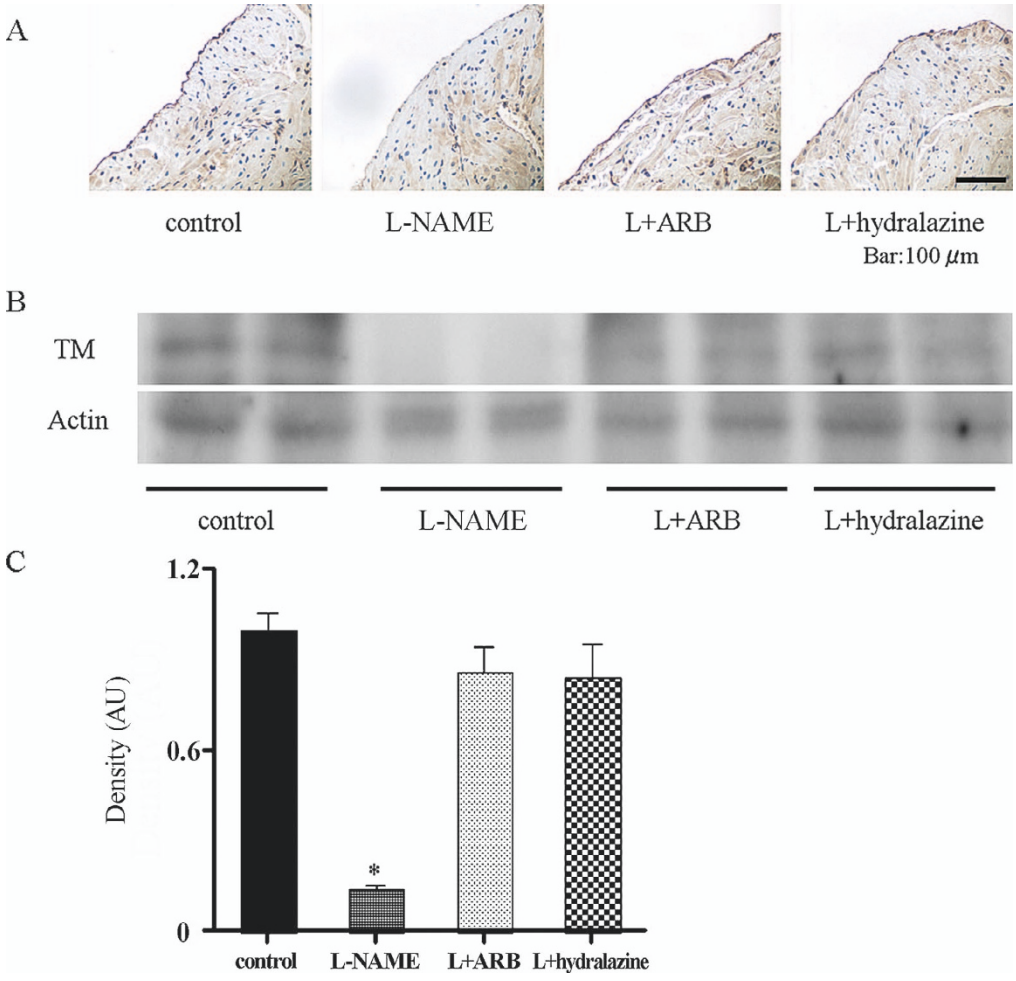

Fig. 5. *Thrombomodulin (TM) expression in atrial tissues in the hypertensive rat model. A: Representative changes of TM expression in the atrial endocardium. The bar indicates 100 μm. B: Immunoblot analysis for TM protein expression in each group tested. C: Quantitative analysis of TM protein expression. L-NAME,* N$^{\omega}$*-nitro-L-arginine methyl ester; L+ARB,* N$^{\omega}$*-nitro-L-arginine methyl ester plus angiotensin II receptor blocker; L+hydralazine,* N$^{\omega}$*-nitro-L-arginine methyl ester plus hydralazine; AU, arbitrary unit. Data: mean±SEM.* *p < *0.05* vs. *control group.* N = *3*.

4 groups before the study. In the L-NAME group, SBP increased progressively and became higher than that in the control group from 2 weeks of treatment onwards. The increase in SBP produced by L-NAME was decreased to the baseline by candesartan or hydralazine.

Effects of Candesartan and Hydralazine on Cardiac Hypertrophy and Dysfunction in Hypertensive Rats

The chronic treatment of rats with L-NAME significantly ($p<0.05$) increased the HW/BW ratio compared with that in the controls (3.50±0.05 *vs*. 2.89±0.02). This increase was prevented by candesartan (2.98±0.14), but not by hydralazine (3.38±0.07).

Quantitative echocardiographic data are presented in Fig. 2. Both LVDd/BW and LVDs/BW in the L-NAME group were significantly larger than in the control group. In the L-NAME group, LVFS was significantly decreased compared with that in the control group, indicating that systolic dysfunction was induced when NO synthesis was chronically inhibited. Candesartan, but not hydralazine, completely restored the reduction of LVFS by treatment with L-NAME. In addition, the *E*/*A* ratio in the L-NAME group was significantly decreased compared with that in the control group, indicating that diastolic dysfunction was also induced when NO synthesis was chronically inhibited. Candesartan, but not hydralazine, completely restored the reduction of *E*/*A* induced by L-NAME.

Effects of Candesartan and Hydralazine on Atrial Structural Remodeling in Hypertensive Rats

The extent of atrial fibrosis in the L-NAME group 8 weeks after treatment was significantly greater than that in the control group. This fibrotic change was prevented by candesartan, but not by hydralazine (Fig. 3). Quantitative analysis by real-time RT-PCR demonstrated that the mRNA levels of collagen type 1 and TGF-β in whole atrial tissue were significantly increased in the L-NAME group, while the increase in either molecule was reversed to the control levels by candesartan, but not by hydralazine (Fig. 4).

Hemodynamic Effects on TM Expression in the Atrium of Hypertensive Rats

Eight weeks after treatment by L-NAME, immunohistological analysis revealed that TM expression on the atrial endocardial surface was markedly decreased (Fig. 5A). Treatment with candesartan or hydralazine comparably reversed the decrease in atrial TM levels induced by chronic inhibition of NO synthesis (Fig. 5B, C).

Discussion

The present study demonstrated that candesartan, an ARB, but not hydralazine, prevented the progression of atrial fibrosis as well as LV hypertrophy and dysfunction in a hypertensive rat model induced by chronic inhibition of NO synthesis. Furthermore, candesartan and hydralazine comparably attenuated the reduction in TM expression in the atrial endocardium in this model. These findings suggest that angiotensin II plays an important role in the pathophysiology of atrial and ventricular structural remodeling in the hypertensive heart model. To our knowledge, the present study is the first to demonstrate a reduction of TM expression in left atrial tissue in hypertensive hearts.

Recent animal models of AF have proposed two principal forms of atrial remodeling: electrical remodeling, which affects cellular electrical properties, and structural remodeling, which alters the architecture of atrial tissue (*7–9*, *28*). Atrial tachycardias cause ionic remodeling while decreasing the atrial refractory period and promoting atrial reentry (*29*, *30*). By contrast, CHF produces extensive atrial interstitial fibrosis compared with atrial tachycardia, which promotes arrhythmogenesis by interfering with atrial conduction (*7*). Although Takemoto *et al*. clearly demonstrated that long-term inhibition of NO synthesis induces fibrotic changes in the left ventricle (*31*), the present study is the first to demonstrate structural atrial remodeling, a possible contributing factor for the development of AF, in the hypertensive heart model. Since we found extensive atrial fibrosis in this hypertension model, it is likely that the morphological changes in the atrium of hypertensive hearts are similar to those found in CHF rather than to those in atrial-tachycardia pacing. These morphological differences in atrial tissue may reflect a difference in the pathophysiology of atrial fibrosis.

Recent clinical trials have demonstrated a reduction in the development and recurrence of AF by ACE inhibitors and ARBs in patients with CHF and hypertension (*14*, *32*). Thus, the inhibition of the RAS is a novel concept for the prevention of AF that may target the underlying abnormalities of cardiac structure and electrical physiology that lead to AF. In the present study, candesartan, an ARB, but not hydralazine, prevented the progression of atrial fibrosis as well as LV hypertrophy and dysfunction in the hearts of hypertensive rats. There are two possible mechanisms by which candesartan prevented atrial structural remodeling in experimental hypertension with LV hypertrophy. One possible mechanism is the prevention of LV hypertrophy that may hemodynamically overload the atrium. LV hypertrophy is associated with a high incidence of AF in hypertensive patients (*6*). Thus, an antihypertensive drug, such as an ACE inhibitor or an ARB that can reverse LV hypertrophy, may effectively prevent the occurrence of AF (*33*). Indeed, in the LIFE study, an ARB, losartan, significantly reduced the incidence of AF compared with a β-adrenergic antagonist (*18*). The other possible mechanism is a direct anti-fibrotic effect of RAS inhibition. In both rapid atrial and ventricular pacing models, there are reports that the RAS inhibition by ACE inhibitors or ARB attenuates the development of atrial fibrosis that leads to the slowing of atrial conduction velocity (*8*, *9*). Candesartan, an ARB, may reduce the extent of atrial fibrosis due to reduction of LV hypertrophy as well as its direct antifibrotic effect in hypertensive hearts. Furthermore, it is likely that an inverse agonist effect by candesartan will be beneficial for preventing activation of angiotensin II type 1 receptor due to atrial stretch (*34*).

Consistent with these atrial morphological changes, quantitative analysis by real-time RT-PCR demonstrated that mRNA levels of collagen and TGF-β increased in this model. TGF-β is a cytokine known to play an important role in stimulating fibrosis (*35*). In a transgenic mouse model, constitutional activation of TGF-β produces atrial-restricted fibrosis and promotes inducibility of AF. Since angiotensin II induces the up-regulation of TGF-β that leads to cardiac fibrosis (*36*), the attenuation of TGF-β by the blockade of angiotensin II type 1 receptor may result in the reduction of fibrosis in atrial tissue.

Thrombin bound to endothelial TM cannot convert fibrinogen to fibrin or activate the anticoagulant protein C. Therefore, TM is considered to be a potent intrinsic anticoagulant factor (*37*), and the loss of TM in atria may lead to an increased risk of thromboembolism. In the hypertensive heart model induced by chronic inhibition of NO synthesis, we found that the expression of TM on the atrial endocardium was reduced. Interestingly, in contrast to the different effects of candesartan and hydralazine on atrial structural remodeling, candesartan and hydralazine comparably reduced systemic BP and restored the expression of TM. Thus, it is likely that atrial expression of TM is affected by hemodynamic fac-

tors such as systemic BP. However, we cannot deny the possibility that the local left atrial pressure may have been different, because the *E*/*A* ratios of the two groups were different. Since little is known about the modulators of TM expression, further investigation will be required into the mechanism by which the atrial expression of TM is regulated.

Patients with hypertension show endothelial dysfunction that may be largely attributable to reduced NO bioavailability (*23–25*). Thus, the hypertension model induced by long-term inhibition of NO synthesis will share the pathophysiology of patients with hypertension. We and other groups demonstrated that angiotensin II plays an important role in the development of LV fibrosis and cardiac inflammation in this model (*25*, *26*, *38*). However, the present study is the first to demonstrate the important role of angiotensin II in atrial remodeling in this hypertensive model. We must examine the role of angiotensin II in atrial structural remodeling in other hypertensive hearts.

A limitation of this study was that we did not demonstrate that atrial fibrosis in this hypertension model was associated with an increased vulnerability to AF or an increased incidence of AF. Future investigation will be required to check the relationship between atrial structural remodeling and the occurrence of AF in this hypertensive heart model.

In conclusion, atrial structural remodeling was observed in a hypertensive rat model. Blockade of angiotensin II type 1 receptor attenuated atrial fibrosis and the reduction in TM in the atrial endocardium. We need to consider pathophysiological atrial remodeling when we treat patients with hypertension.

Acknowledgements

We thank Mr. Hirofumi Yukawa (ALOKA Co., Ltd.) for echocardiographic assistance, Ms. Yukari Arino for secretarial work, and Ms. Hiroko Okuda and Ms. Yoko Nagamachi for technical assistance.

References

1. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D: Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. *Circulation* 1998; **98**: 946–952.
2. Wang TJ, Larson MG, Levy D, *et al*: Temporal relations of atrial fibrillation and congestive heart failure and their joint influence on mortality: the Framingham Heart Study. *Circulation* 2003; **107**: 2920–2925.
3. Minamino T, Kitakaze M, Sanada S, *et al*: Increased expression of P-selectin on platelets is a risk factor for silent cerebral infarction in patients with atrial fibrillation: role of nitric oxide. *Circulation* 1998; **98**: 1721–1727.
4. Minamino T, Kitakaze M, Asanuma H, *et al*: Plasma adenosine levels and platelet activation in patients with atrial fibrillation. *Am J Cardiol* 1999; **83**: 194–198.
5. Minamino T, Kitakaze M, Sato H, *et al*: Plasma levels of nitrite/nitrate and platelet cGMP levels are decreased in patients with atrial fibrillation. *Arterioscler Thromb Vasc Biol* 1997; **17**: 3191–3195.
6. Verdecchia P, Reboldi G, Gattobigio R, *et al*: Atrial fibrillation in hypertension: predictors and outcome. *Hypertension* 2003; **41**: 218–223.
7. Li D, Fareh S, Leung TK, Nattel S: Promotion of atrial fibrillation by heart failure in dogs: atrial remodeling of a different sort. *Circulation* 1999; **100**: 87–95.
8. 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.
9. Shinagawa K, Li D, Leung TK, Nattel S: Consequences of atrial tachycardia-induced remodeling depend on the preexisting atrial substrate. *Circulation* 2002; **105**: 251–257.
10. Cohn JN, Ferrari R, Sharpe N, on behalf of an International Forum on Cardiac Remodeling: Cardiac remodeling—concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. *J Am Coll Cardiol* 2000; **35**: 569–582.
11. Schnee JM, Hsueh WA: Angiotensin II, adhesion, and cardiac fibrosis. *Cardiovasc Res* 2000; **46**: 264–268.
12. Weber KT, Brilla CG: Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. *Circulation* 1991; **83**: 1849–1865.
13. Tanemoto M, Abe T, Obara N, Abe M, Satoh F, Ito S: Successful treatment of severe hypertension with the combination of angiotensin converting enzyme inhibitor and angiotensin II receptor blocker. *Hypertens Res* 2003; **26**: 863–868.
14. Vermes E, Tardif JC, Bourassa MG, *et al*: Enalapril decreases the incidence of atrial fibrillation in patients with left ventricular dysfunction: insight from the Studies of Left Ventricular Dysfunction (SOLVD) trials. *Circulation* 2003; **107**: 2926–2931.
15. Maggioni AP, Latini R, Carson PE, *et al*: Valsartan reduces the incidence of atrial fibrillation in patients with heart failure: results from the Valsartan Heart Failure Trial (Val-HeFT). *Am Heart J* 2005; **149**: 548–557.
16. Miura S, Saku K, Karnik SS: Molecular analysis of the structure and function of the angiotensin II type 1 receptor. *Hypertens Res* 2003; **26**: 937–943.
17. Shi Y, Li D, Tardif JC, Nattel S: Enalapril effects on atrial remodeling and atrial fibrillation in experimental congestive heart failure. *Cardiovasc Res* 2002; **54**: 456–461.
18. Wachtell K, Lehto M, Gerdts E, *et al*: Angiotensin II receptor blockade reduces new-onset atrial fibrillation and subsequent stroke compared to atenolol: the Losartan Intervention for End Point Reduction in Hypertension (LIFE) study. *J Am Coll Cardiol* 2005; **45**: 712–719.
19. Egashira K, Ni W, Inoue S, *et al*: Pravastatin attenuates cardiovascular inflammatory and proliferative changes in a rat model of chronic inhibition of nitric oxide synthesis by its cholesterol-lowering independent actions. *Hypertens Res* 2000; **23**: 353–358.
20. Yamashita T, Sekiguchi A, Iwasaki YK, *et al*: Thrombomodulin and tissue factor pathway inhibitor in endocardium of rapidly paced rat atria. *Circulation* 2003; **108**: 2450–2452.
21. Li-Saw-Hee FL, Blann AD, Lip GY: A cross-sectional and

diurnal study of thrombogenesis among patients with chronic atrial fibrillation. *J Am Coll Cardiol* 2000; **35**: 1926–1931.
22. Kubo SH, Rector TS, Bank AJ, Williams RE, Heifetz SM: Endothelium-dependent vasodilation is attenuated in patients with heart failure. *Circulation* 1991; **84**: 1589–1596.
23. Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990; **323**: 22–27.
24. Node K, Kitakaze M, Yoshikawa H, Kosaka H, Hori M: Reduced plasma concentrations of nitrogen oxide in individuals with essential hypertension. *Hypertension* 1997; **30**: 405–408.
25. Ribeiro MO, Antunes E, de Nucci G, Lovisolo SM, Zatz R: Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. *Hypertension* 1992; **20**: 298–303.
26. Minamino T, Kitakaze M, Papst PJ, *et al*: Inhibition of nitric oxide synthesis induces coronary vascular remodeling and cardiac hypertrophy associated with the activation of p70 S6 kinase in rats. *Cardiovasc Drugs Ther* 2000; **14**: 533–542.
27. Kuwahara F, Kai H, Tokuda K, *et al*: Transforming growth factor-beta function blocking prevents myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. *Circulation* 2002; **106**: 130–135.
28. Hoit BD: Matrix metalloproteinases and atrial structural remodeling. *J Am Coll Cardiol* 2003; **42**: 345–347.
29. Yue L, Feng J, Gaspo R, Li GR, Wang Z, Nattel S: Ionic remodeling underlying action potential changes in a canine model of atrial fibrillation. *Circ Res* 1997; **81**: 512–525.
30. Cha TJ, Ehrlich JR, Zhang L, Nattel S: Atrial ionic remodeling induced by atrial tachycardia in the presence of congestive heart failure. *Circulation* 2004; **110**: 1520–1526.
31. Takemoto M, Egashira K, Usui M, *et al*: Important role of tissue angiotensin-converting enzyme activity in the pathogenesis of coronary vascular and myocardial structural changes induced by long-term blockade of nitric oxide synthesis in rats. *J Clin Invest* 1997; **99**: 278–287.
32. Cohn JN, Tognoni G: A randomized trial of the angiotensin-receptor blocker valsartan in chronic heart failure. *N Engl J Med* 2001; **345**: 1667–1675.
33. Dahlof B, Herlitz H, Aurell M, Hansson L: Reversal of cardiovascular structural changes when treating essential hypertension. The importance of the renin-angiotensin-aldosterone system. *Am J Hypertens* 1992; **5**: 900–911.
34. Zou Y, Akazawa H, Qin Y, *et al*: Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat Cell Biol* 2004; **6**: 499–506.
35. Border WA, Noble NA: Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 1994; **331**: 1286–1292.
36. Kawano H, Do YS, Kawano Y, *et al*: Angiotensin II has multiple profibrotic effects in human cardiac fibroblasts. *Circulation* 2000; **101**: 1130–1137.
37. Esmon CT: The roles of protein C and thrombomodulin in the regulation of blood coagulation. *J Biol Chem* 1989; **264**: 4743–4746.
38. Takemoto M, Egashira K, Tomita H, *et al*: 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.