Original Article

Augmentation of Pulse Wave Velocity Precedes Vascular Structural Changes of the Aorta in Rats Treated with N^{ω}-Nitro-L-Arginine Methyl Ester

Hisako KAMEYAMA, Kazuo TAKEDA*, Tetsurou KUSABA, Hiromichi NARUMIYA, Syuji TANDA, Noriko KUWAHARA, Keiko YAMADA**, Keiichi TAMAGAKI, Mitsuhiko OKIGAKI, Tsuguru HATTA, and Susumu SASAKI

We examined the relationship between structural changes of the aorta and pulse wave velocity (PWV), and the effects of antihypertensive treatments on PWV in N^{ω}-nitro-L-arginine methyl ester (L-NAME)-treated rats. Twelve-week-old Wistar-Kyoto (WKY) rats were divided into the following groups, all of which received drug treatment in their drinking water: an untreated control group (n=36), an L-NAME-treated group (0.7 mg/ml) (n=32), an L-NAME and angiotensin converting enzyme (ACE) inhibitor (ACEI)-treated group (imidapril: 0.4 mg/ml) (n=8), and an L-NAME and hydralazine-treated group (0.2 mg/ml) (n=10). PWV was measured at the same blood pressure (BP) level as in the control group and the wall-to-lumen ratio of the thoracic aorta was evaluated in all groups. In the L-NAME group, PWV increased compared with the value in the control group, at the same time that BP was increasing. After the third day of treatment, PWV was higher in the L-NAME group than in the control group after adjusting BP to the control level, while the wall-to-lumen ratios were equal between the two groups. After the first week of treatment, not only the adjusted PWV, but also the wall-to-lumen ratios were greater in the L-NAME group than in the control group. With administration of antihypertensive agents, both PWV and the thickening of the aortic wall were reduced, but there was no significant difference between the ACEI and hydralazine-treated groups. In conclusion, in a rat model of nitric oxide (NO) synthesis inhibition, the increase in PWV preceded the vascular structural changes, while antihypertensive treatment reduced both changes. There was no significant difference between treatments with ACEI and hydralazine in this model. (*Hypertens Res* 2005; 28: 439–445)

***Key Words*: pulse wave velocity, N^{ω}-nitro-L-arginine methyl ester, vascular changes**

Introduction

Pulse wave velocity (PWV) reflects arterial stiffness, and correlates positively with several markers for atherosclerosis (*1*, *2*). It has been considered a useful method for assessing early-stage atherosclerosis. Increased arterial stiffness, reflected by an increased PWV, leads to impaired perfusion of the peripheral organs and increased cardiac afterload. Recent epidemiological studies have shown that independently of confounding factors such as age and blood pressure (BP), aortic PWV is a predictor of cardiovascular mortality in patients with hypertension (*3*) and in those with end-stage renal failure (*4*). PWV is easily and non-invasively measured using an automatic device and the validity and reproducibility have been proved to be considerably high (*5*). For these reasons, PWV is widely

From the Division of Hypertension and Nephrology, Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan; *Kyoto Industrial Health Association, Kyoto, Japan; and **Department of Internal Medicine, Kameoka Shimizu Hospital, Kameoka, Japan.
Address for Reprints: Hisako Kameyama, M.D., Division of Hypertension and Nephrology, Department of Internal Medicine, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kawaramachi Hirokoji, Kamigyo-ku, Kyoto 602–8566, Japan. E-mail: hisako-k@koto.kpu-m.ac.jp
Received November 15, 2004; Accepted in revised form March 15, 2005.

Table 1. Body Weight, Peripheral SBP and HR in the Control, L-NAME Treated, L-NAME+Hydralazine, and L-NAME+ACEI Treated Groups

	Control				L-NAME			Hyd	ACEI
	0 week	3 days	1 week	3 weeks	3 days	1 week	3 weeks	3 weeks	3 weeks
n	8	8	12	8	7	12	13	10	8
Body weight(g)	350±0	354±4	370±3	398±5	350±3	351±1†	390±5	388±6	375±4†
SBP(mmHg)	107±4	107±4	110±3	115±5	116±4*	134±7†	147±7†	112±5	110±5
HR(bpm)	436±12	434±14	444±9	451±8	358±10†	384±9†	404±11†	449±11	408±12*

Values are mean±SEM. *$p<0.05$ *vs.* the age-matched control group; †$p<0.01$ *vs.* the age-matched control group. L-NAME, N^{ω}-nitro-L-arginine methyl ester; Hyd, hydralazine; ACEI, angiotensin converting enzyme inhibitor; SBP, systolic blood pressure; HR, heart rate; bpm, beats/min.

used as a marker of cardiovascular risk (*6*) and a surrogate end point of cardiovascular diseases.

However, PWV is influenced by age-associated atherosclerosis and BP. The same value of PWV among patients with different backgrounds, including age and cardiovascular risk factors, does not reflect the same degree of vascular damage. For this reason, an absolute PWV value is not always acceptable to use for the assessment of atherosclerosis in subjects with different conditions. Guerin *et al*. have reported that the loss of aortic PWV responsiveness to BP-lowering reflects more advanced vascular lesions (*4*). The response of PWV to therapy, including BP-lowering, may be more predictive of cardiovascular events than the absolute value of PWV.

The relationship between PWV and vascular structural changes is not fully understood. Katsuda *et al*. demonstrated the value of PWV in predicting changes along the aorta in hypercholesterolemic rabbits. PWV was shown to reflect the extent and severity of the atherosclerotic lesion, and to increase as a result of the progression of sclerotic lesions with aging in these rabbits (*7*). In other studies, the association between PWV and aortic structure was investigated in estrogen-treated rats (*8*) and apolipoprotein E-deficient mice (*9*). In those reports, increased PWV in apolipoprotein E-knockout mice was shown to be due to endothelial dysfunction and elastic destruction in the vascular wall. In estrogen-treated rats, increase in PWV was associated with left ventricular hypertrophy, and estrogen increased the medial thickness-to-internal diameter ratio of the thoracic aorta.

The relationship between changes of PWV independent of BP and vascular structure has not yet been clarified. Fitch *et al*. reported that increased vascular smooth muscle tone and vascular remodeling contribute to an increase in PWV independently of BP in chronic N^{ω}-nitro-L-arginine methyl ester (L-NAME)-treated rats; however, they did not perform morphological analysis of the vasculature (*10*).

Our goals were 1) to examine the relationship between aortic PWV and vascular structural changes, 2) to determine whether different antihypertensive treatments have different effects on aortic stiffness and on arterial geometry in rats under conditions of the inhibition of nitric oxide (NO) synthesis in the early stage of atherosclerosis after the third week of L-NAME administration.

Methods

Animals, Experimental Protocol

All experiments were conducted on 12-week-old male Wistar-Kyoto rats obtained from Shimizu Laboratory Supplies (Kyoto, Japan). The study was conducted with approval from, and in accordance with, the Kyoto Prefectural University of Medicine Guide for the Care and Use of Laboratory Animals. Animals were kept in a room with controlled temperature (24°C) and lighting (13:11-h light-dark cycle) with free access to food and tap water.

Four groups of rats were studied. The control group ($n=36$) received chow and tap water. The second group (L group) ($n=32$) received L-NAME (0.7 mg/ml: Nacalai Tesque Co., Kyoto, Japan) in its drinking water. The third group (L+ACEI group) ($n=8$) received L-NAME and an angiotensin converting enzyme (ACE) inhibitor (ACEI) (L-NAME: 0.7 mg/ml; imidapril: 0.4 mg/ml; Tanabe Pharmaceutical Co., Tokyo, Japan) in its drinking water. The fourth group (L+Hyd group) ($n=10$) received L-NAME and hydralazine (L-NAME: 0.7 mg/ml; hydralazine: 0.2 mg/ml; Nacalai Tesque Co.) in its drinking water. These doses of imidapril and hydralazine were chosen based on their previously reported ability to normalize the L-NAME-induced increase in systolic blood pressure (SBP) (*11*).

In all groups, peripheral BP and heart rate (HR) were measured by the tail-cuff method (MK model 2003, Muromachi Kikai Co., Ltd., Tokyo, Japan). Measurements of central arterial pressure, HR, and aortic PWV were made with a Millar Mikro-tip pressure transducer (2.5Fr, SPC-721; Millar Instruments, Houston, USA) on days 0, 3, 7, and 21 in the control group, on days 3, 7, and 21 of administration in the L group and on day 21 of administration in the L+ACEI group and the L+Hyd group. The rats were euthanized for morphometric analyses after measurements of aortic PWV.

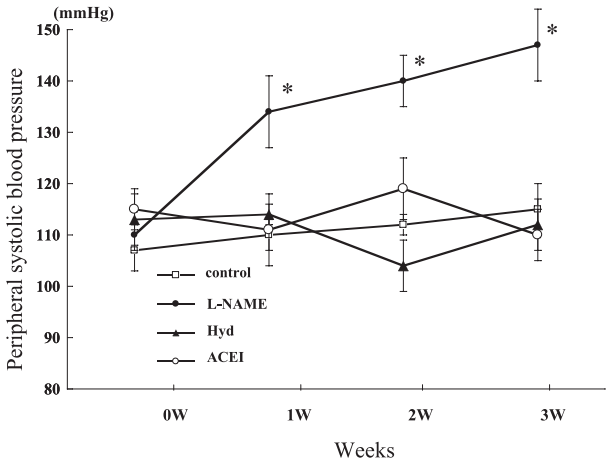

Fig. 1. *Time-related changes in peripheral systolic blood pressure in the control, L-NAME treated, L-NAME+hydralazine treated, and L-NAME+ACEI treated groups.* *p < *0.05* vs. *0 week.*

Surgical Preparation and Hemodynamic Measurements

Rats were anesthetized with an intraperitoneal injection of urethane (1.25 g/kg). The left common carotid artery was exposed. For measurements of central arterial pressure, HR, and PWV, a Millar Mikro-tip pressure transducer with dual high-fidelity pressure sensors located at the distal end and 50 mm from it was implanted *via* the left common carotid artery. Pulse pressure waves from the two transducers were simultaneously imported to an amplifier (Polygraph 366 System; NEC Medical Systems, Ltd., Tokyo, Japan) and the analog signal was fed into a portable microcomputer with an analog-to-digital converter at a sampling rate of 4,800 Hz. When arterial pressure and HR had been stable for 30 min, the pressure waves were stored for at least 30 min. Data were recorded for 10 s and analyzed with software made by Colin Medical Technology Corporation (Aichi, Japan). Systolic arterial pressure, diastolic arterial pressure, mean arterial pressure and HR were calculated from the proximal pressure wave. The transit time for the pulse wave moving from the aortic arch to the abdominal aorta was obtained from the foot-to-foot delay between the simultaneously recorded pressure waves. PWV was calculated from the transit time and fixed distance between the two recording sites (50 mm). The right femoral vein was catheterized with polyethylene tubing for acute administration of hydralazine.

After baseline measurements had been made, rats were infused with hydralazine. When the central systolic arterial pressure had been decreased by hydralazine to the value in the controls (about 100 mmHg), hemodynamic measurements were performed again, because PWV is known to be significantly affected by the BP level. After the experiment was completed, each animal was euthanized with an overdose of urethane and perfused for 30 min with 10% formaldehyde-containing phosphate-buffered saline. A 1-cm sample of the thoracic descending aorta was excised, immersed in 10% formaldehyde solution, dehydrated in graded ethanol solutions, and embedded in paraffin. The paraffin slices were stained with hematoxylin-eosin (HE).

Histological Analysis

On HE staining, short-axis images of the thoracic aorta were studied to evaluate the thickening of the aortic wall. The inner border of the lumen and the outer border of the tunica media were traced in each image with HE staining. Areas encircled by the tracings (Scion Image, Scion Corporation, Frederick, USA) were measured and the wall-to-lumen ratio was then calculated.

Statistical Analysis

Data are expressed as the mean±SEM. Comparisons of the mean values were performed by Student's *t*-tests. Differences were considered statistically significant when the *p*-value was <0.05. The statistical analysis was performed with StatView software.

Results

Body weights at 1 week in the L group and at 3 weeks in the L+ACEI group were significantly lower than those of the age-matched control group (Table 1).

The changes in the SBP measured by the tail-cuff method are shown in Fig. 1. In the L group, SBP increased progressively. No significant changes in SBP were observed in the control group, the L+Hyd, or the L+ACEI groups. Compared with the level in the control group, HR was reduced in the L and L+ACEI groups (Table 1). Tachycardiac responses to hypotension may be impaired in L-NAME-treated rats. Scrogin *et al*. reported that L-NAME treatment alters baroreflex function (*12*).

PWV increased in the L group compared with that in the control group at the same time that BP was increasing (Fig. 2A). After BP was lowered to the control level (central arterial pressure of about 100 mmHg) by acute infusion of hydralazine in order to avoid the effect of BP, PWV was still increased in the L group after the third day of treatment compared with that in the control group (Fig. 2B).

The wall-to-lumen ratio of the thoracic aorta in the L group was not increased after the third day of treatment (0.352±0.005) compared with that in the control group (0.330±0.005) but was increased after the first week of treatment (after 1 week: 0.431±0.018; after 3 weeks: 0.41±0.011) compared with that in the control group (after 1 week: 0.318±0.006; after 3 weeks: 0.295±0.01) (Fig. 2C). Thus, although PWV had already increased after the third day of treatment in the L group, the thickening of the aortic wall had

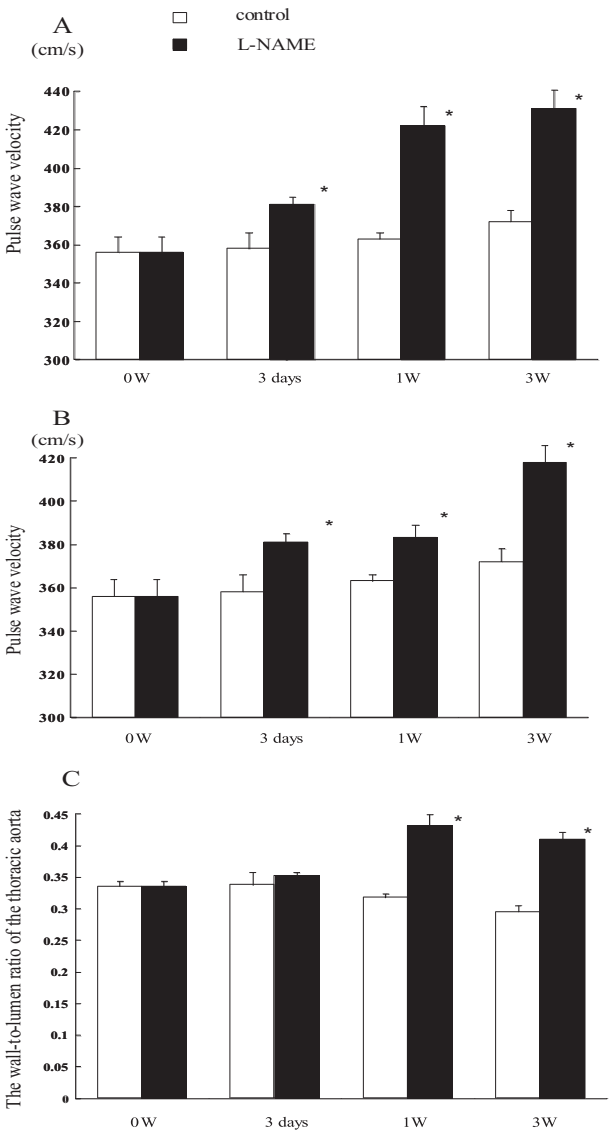

Fig. 2. *Pulse wave velocity before BP-lowering (A), pulse wave velocity after BP- lowering (B) and the wall-to-lumen ratio of the thoracic aorta (C) after 3 days, 1 week and 3 weeks in the control and L-NAME groups.* *$p < 0.05$ vs. *the age-matched control group by Student's* t-*test.*

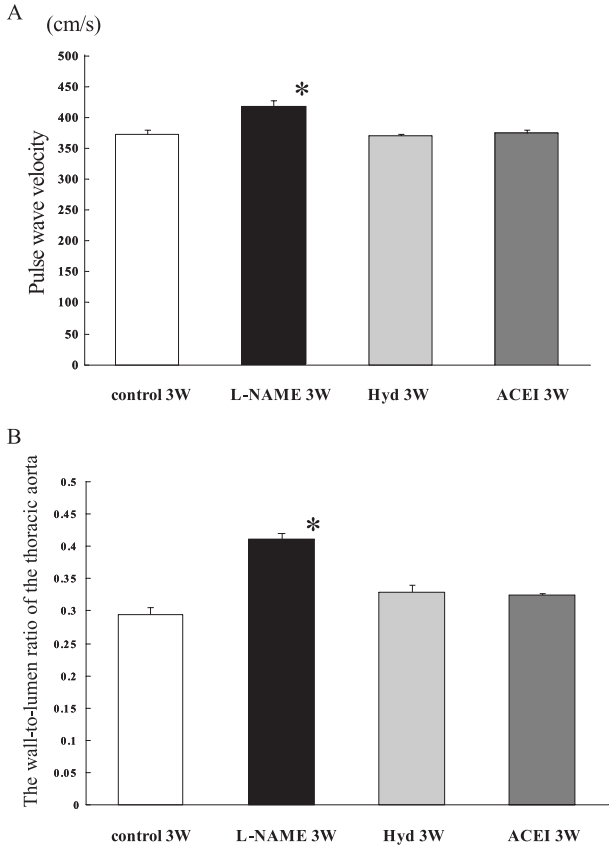

Fig. 3. *Pulse wave velocity (A), and the wall-to-lumen ratio of the thoracic aorta (B) in the control, L-NAME treated, L-NAME+hydralazine treated, and L-NAME+ACEI treated groups. The data were obtained when the systolic arterial pressure level was equal to that in the control group.* *$p < 0.05$ vs. *the age-matched control group by Student's* t-*test.*

not yet occurred.

In the L+ACEI and L+Hyd groups, PWV remained at the same level as in the control group after the third week of treatment (Fig. 3A). Also in both of these treatment groups, PWV and vascular structural changes were reduced by the 3-week treatment (Fig. 3). Light micrographs taken of the thoracic aorta with HE staining for the control, L, L+ACEI and L+Hyd groups are shown in Fig. 4.

Discussion

In a clinical study, Taniwaki *et al*. reported a positive correlation between aortic PWV and carotid intima-media thickness in patients with type 2 diabetes mellitus (*13*). Nakamura *et al*. reported that baPWV correlated with abdominal aortic calcification as a predictor of cardiovascular mortality (*14*). In experimental studies, Farrar *et al*. demonstrated in monkeys that an atherogenic diet increased PWV and aortic intimal area, while an atherosclerosis regression diet decreased both parameters (*15*). Wang *et al*. reported that the increase in PWV in old apolipoprotein E-knockout mice is related to both the lack of endothelial NO and the elastic destruction caused by atherosclerotic lesions (*16*). However, there have been few experimental studies investigating the correlation between PWV and vascular structural changes in early-stage atherosclerosis. Therefore, we used a rat model of chronic inhibition of NO synthesis by administration of L-NAME, since it has been shown that decreased NO activity due to endothelium dysfunction contributes to the progression of early-stage atherosclerosis (*17*, *18*). Moreover, the process of L-NAME-induced atherosclerosis is divided into an early inflammation

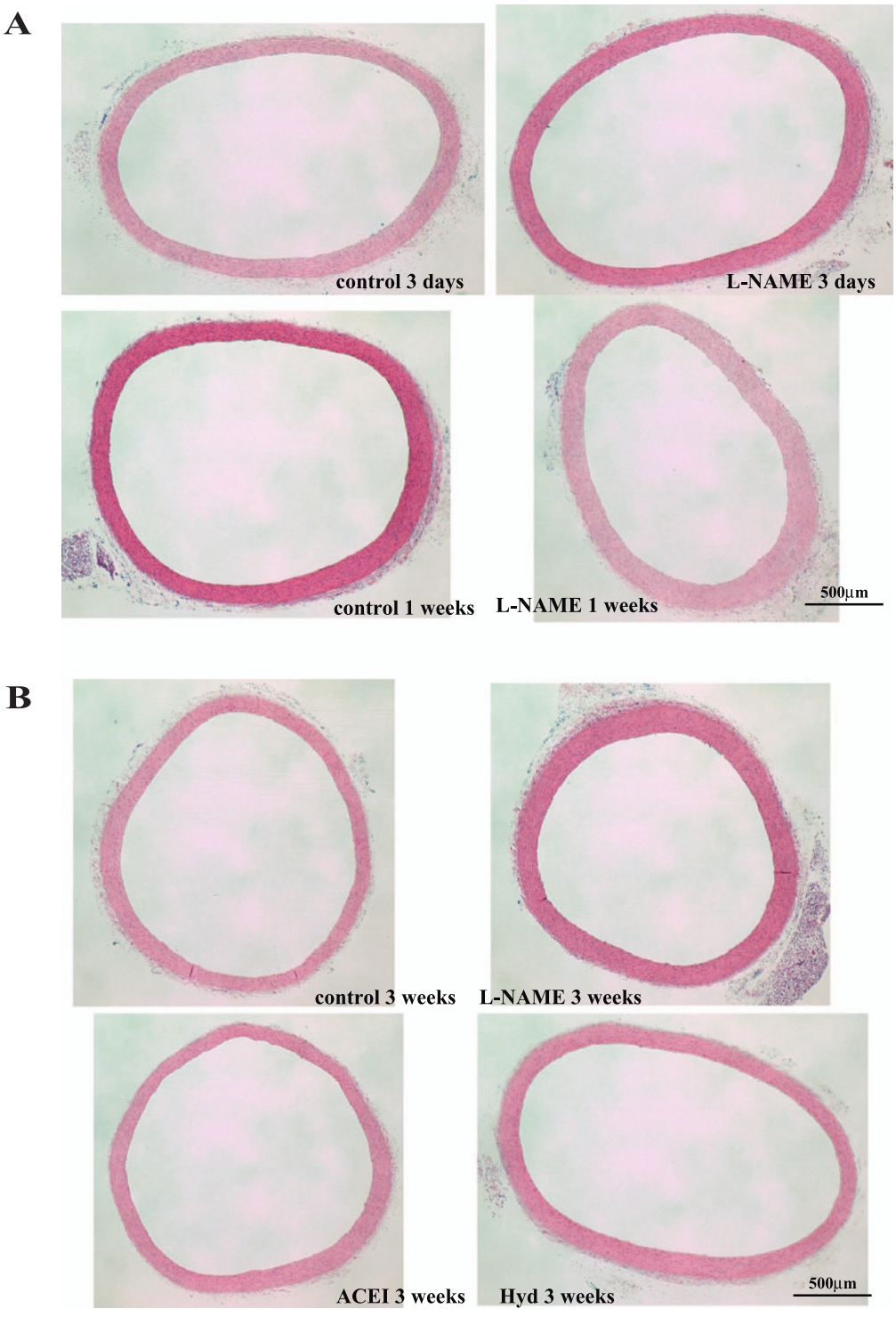

Fig. 4. *Light micrographs taken of the aorta with HE staining for A: the control and L-NAME groups after the third day and 1 week of treatment and B: the control, L, L+ACEI and L+Hyd groups after 3 weeks of treatment. Bar: 500 μm.*

stage within the first week of L-NAME administration and a late remodeling stage after 4 weeks of treatment (*19–22*). We investigated early changes (within 3 weeks) of atherosclerosis in this model.

We compared aortic PWV at the same BP level in all groups because changes in BP are known to affect PWV. After the third day of L-NAME administration, the PWV after adjusting BP to the control level increased compared with that

in the control group; however, the wall-to-lumen ratio of the thoracic aorta did not increase. After the first week of L-NAME administration, PWV after adjusting BP to the control level was higher and the wall-to-lumen ratio was greater compared with those in the control group. This suggests that an increase in PWV precedes structural changes (the thickening of the aorta) in the progression of atherosclerosis.

According to the Moens-Korteweg equation, PWV is defined as follows:

$$PWV^2 = E \cdot h/2r \cdot \rho,$$

where E is Young's modulus, h is arterial wall thickness, r is internal radius, and ρ is the density of blood. PWV is influenced by 2 factors, the vascular geometry and the viscoelastic properties of the wall. After the first week of L-NAME administration, the rise in the wall-to-lumen ratio contributed to an increase in PWV. The increase in PWV without medial thickening after the third day of L-NAME administration may have been caused by other factors. Although it has been reported that changes in smooth muscle tone do not have a major impact on aortic stiffness (*23*), we cannot exclude this possibility. Furthermore, changes in the ratio of collagen to elastin rather than lipid deposition are known to affect the elastic behavior and function of arterial walls (*24*, *25*), while Marque *et al*. reported that improvement in arterial stiffness by captopril and hydrochlorothiazide treatment in old spontaneously hypertensive rat (SHR) is not related to scleroprotein (*26*). Whether the proportion of elastin to collagen influences arterial stiffness may depend on age and other conditions. Because we did not analyze the arterial wall composition or vascular tone, we did not elucidate the contribution of these factors to the changes of PWV in this study.

Numerous pharmacological studies have analyzed the effects of different classes of antihypertensive agents on arterial stiffness. Most studies have reported that ACE inhibitors were more effective than other antihypertensive agents for improving aortic PWV (*27*). Angiotensin II has been shown to be a potent vasoconstrictor, and it also promotes vascular proliferation *via* induction of several growth factors and cytokines. ACE inhibitors prevent aortic stiffness by inhibiting aortic collagen accumulation (*28*). However, in the present study, no significant differences in either PWV or structural changes were found between the ACEI-treated group and the hydralazine-treated group. Takemoto *et al*. showed that the structural changes in the aorta caused by long-term administration of L-NAME were reduced by administration of ACEI, but not hydralazine (*21*). The difference may be related to the fact that younger rats (12-week-old) were used in our study while older rats (20-week-old) were used in their study, and that the duration of administration of L-NAME was shorter in our study (3 weeks) than in their study (8 weeks). Albaladejo *et al*. showed that aortic hypertrophy was prevented to a similar degree by treatment with hydralazine or ACEI (quinapril) in the SHR model (*29*). The aorta, a rather elastic artery, is mainly sensitive to BP, whereas in peripheral arteries, which are rather muscular, hypertrophy may be modulated mainly by nonhemodynamic factors, such as autonomic nervous system activity. PWV was also improved in both ACEI- and hydralazine-treated rats in this study, probably because it may be strongly affected by distending pressure, *i.e.*, BP, in early-stage atherosclerosis. Arterial stiffness may not be affected by the difference in the BP-reduction mechanism between antihypertensive agents in early-stage atherosclerosis.

The importance of sufficient, prompt BP reduction has been emphasized in several clinical studies (*30*). Meta-analysis shows that lowering BP is the most important intervention to prevent cardiovascular events (*31*). The outcome of this study supports this idea. In our study, PWV was almost the same during the interval between 3 days and 7 days in the experiments. However, there were differences in the vascular structural changes at these times. This means that PWV has two components, namely, functional and structural changes, and the contribution of these two components to PWV may differ according to the stage of atherosclerosis.

In conclusion, we demonstrated that 1) increases in PWV preceded the structural changes in the aorta and 2) there was no significant difference in the effects on either PWV or vascular structure between treatments with ACEI and hydralazine in rats under condition of the inhibition of NO synthesis. However, the applicability of these data to humans is uncertain because of biological variability and differences between animal models and human subjects.

References

1. Nitta K, Akiba T, Suzuki K, *et al*: Assessment of coronary artery calcification in hemodialysis patients using multidetector spiral CT scan. *Hypertens Res* 2004; **27**: 527–533.
2. Okamura T, Moriyama Y, Kadowaki T, Kanda H, Ueshima H: Non-invasive measurement of branchial-ankle pulse wave velocity is associated with serum C-reactive protein but not with alpha-tocopherol in Japanese middle-aged male workers. *Hypertens Res* 2004; **27**: 173–180.
3. Laurent S, Boutouyrie P, Asmar R, *et al*: Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; **37**: 1236–1241.
4. Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM: Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation* 2001; **103**: 987–992.
5. Yamashina A, Tomiyama H, Takeda K, *et al*: Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurements. *Hypertens Res* 2002; **25**: 359–364.
6. Yamashina A, Tomiyama H, Arai T, *et al*: Brachial-ankle pulse wave velocity as a marker of atherosclerotic vascular damage and cardiovascular risk. *Hypertens Res* 2003; **26**: 615–622.
7. Katsuda S, Hasegawa M, Kusanagi M, Shimizu T: Comparison of pulse-wave velocity in different aortic regions in

relation to the extent and severity of atherosclerosis between young and older Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits. *Clin Sci* (*Lond*) 2000; **99**: 393–404.
8. Tatchum-Talom R, Martel C, Marette A: Influence of estrogen on aortic stiffness and endothelial function in female rats. *Am J Physiol Heart Circ Physiol* 2002; **282**: H491–H498.
9. Wang YX, Miller MH, Vergona R, *et al*: Increased aortic stiffness assessed by pulse wave velocity in apolipoprotein E-deficient mice. *Am J Physiol Heart Circ Physiol* 2000; **278**: H428–H434.
10. Fitch RM, Vergona R, Sullivan ME, Wang YX: Nitric oxide synthase inhibition increases aortic stiffness measured by pulse wave velocity. *Cardiovasc Res* 2001; **51**: 351–358.
11. Kubo-Inoue M, Egashira K, Usui M, *et al*: Long-term inhibition of nitric oxide synthesis increases arterial thrombogenecity in rat carotid artery. *Am J Physiol Heart Circ Physiol* 2002; **282**: 1478–1484.
12. Scrogin KE, Hatton DC, Chi Y, Luft FC: Chronic nitric oxide inhibition with L-NAME: effects on autonomic control of the cardiovascular system. *Am J Physiol* 1998; **274**: R367–R374.
13. Taniwaki H, Kawagishi T, Emoto M, *et al*: Correlation between the intima-media thickness of carotid artery and aortic pulse-wave velocity in patients with type 2 diabetes. Vessel wall properties in type 2 diabetes. *Diabetes Care* 1999; **22**: 1851–1857.
14. Nakamura U, Iwase M, Nohara S, Kanai H, Ichikawa K, Iida M: Usefulness of brachial-ankle pulse wave velocity measurement: correlation with abdominal aortic calcification. *Hypertens Res* 2003; **26**: 163–167.
15. Farrar DJ, Bond MG, Riley WA, Sawyer JK: Anatomic correlates of aortic pulse wave velocity and carotid artery elasticity during atherosclerosis progression and regression in monkeys. *Circulation* 1991; **83**: 1754–1763.
16. Wang Y, Halks-Miller M, Vergona R, *et al*: Increased aortic stiffness assessed by pulse wave velocity in apolipoprotein E-deficient mice. *Am J Physiol Heart Circ Physiol* 2000; **278**: H428–H434.
17. Drexler H: Endothelial dysfunction: clinical implications. *Prog Cardiovasc Dis* 1997; **39**: 287–324.
18. Loscalzo J, Welch G: Nitric oxide and its role in the cardiovascular system. *Prog Cardiovasc Dis* 1995; **38**: 87–104.
19. Numaguchi K, Egashira K, Takemoto M, *et al*: Chronic inhibition of nitric oxide synthesis causes coronary microvascular remodeling in rats. *Hypertension* 1995; **26**: 957–962.
20. Takemoto M, Egashira K, Tomita H: 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.
21. 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.
22. Tomita H, Egashira K, Kubo-Inoue M, *et al*.: Inhibition of NO synthesis induces inflammatory changes and monocyte chemoattractant protein-1 expression in rat hearts and vessels. *Arterioscler Thromb Vasc Biol* 1998; **18**: 1456–1464.
23. Niederhoffer N, Marque V, Lartaud-Idjouadiene I, Duvivier C, Peslin R, Atkinson J: Vasodilators, aortic elasticity, and ventricular end-systolic stress in nonanesthetized unrestrained rats. *Hypertension* 1997; **30**: 1169–1174.
24. Safar ME, London GM, Asmar R, Frohlich ED: Recent advances on large arteries in hypertension. *Hypertension* 1998; **32**: 156–161.
25. Intengan HD, Schiffrin EL: Vascular remodeling in hypertension: role of apotosis, inflammation, and fibrosis. *Hypertension* 2001; **38**: 581–587.
26. Marque V, Grima M, Kieffer P, Capdeville-Atkinson C, Atkinson J, Lartaud-Idjouadiene J: Withdrawal reveals lack of effect of prolonged antihypertensive treatment on intrinsic aortic wall stiffness in senescent spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol* 2002; **29**: 898–904.
27. Mahmud A, Feely J: Effect of angiotensin receptor blockade on arterial stiffness: beyond blood pressure reduction. *Am J Hypertens* 2002; **15**: 1092–1095.
28. Benetos A, Levy BI, Lacolley P, Taillard F, Duriez M, Safar ME: Role of angiotensin and bradykinin on aortic collagen following converting enzyme inhibition in spontaneously hypertensive rats. *Arterioscler Thromb Vasc Biol* 1997; **17**: 3196–3201.
29. Albaladejo P, Bouaziz H, Duriez M, *et al*: Angiotensin converting enzyme inhibition prevents the increase in aortic collagen in rats. *Hypertension* 1994; **23**: 74–82.
30. Julius S, Kjeldsen SE, Weber M, *et al*: Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: the VALUE randomized trial. *Lancet* 2004; **363**: 2022–2031.
31. Staessen JA, Wang J-G, Thijs L: Cardiovascular protection and blood pressure reduction. *Lancet* 2001; **358**: 1305–1315.