Characteristic Change in Local Pulse Wave Velocity in Different Segments of the Atherosclerotic Aorta in KHC Rabbits

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Background: Pulse wave velocity, conventionally determined between the carotid and femoral arteries, is a useful measure to estimate stiffness of the aorta. We investigated local pulse wave velocity (LPWV) in different segments in the aorta with relatively early-stage atherosclerosis in relation to the extent and severity of atherosclerotic lesions.

Methods: Pressure waves were recorded in eight aortic positions using two catheters with one or two micrometers to determine LPWV in the ascending aorta, distal end of the aortic arch, proximal, middle, and distal thoracic aortas, and proximal, middle, and distal abdominal aortas in Kurosawa and Kusanagi-hypercholesterolemic (KHC) and normal rabbits aged 10 to 12 months.

Results: The LPWV in the KHC rabbit was greatest in the aortic arch, decreased almost to the normal level in the middle and distal thoracic aorta, increased in the proximal abdominal aorta, and showed almost identical change to that in the normal rabbit in the middle and distal abdominal aortic regions. There was significant difference in LPWV in the aortic arch, proximal thoracic, and proximal abdominal aortas between the two rabbit groups. The sclerotic lesion was prominent in the aortic arch, proximal thoracic aorta, and proximal abdominal aortas. The wall was severely thickened with abundant foam cells. The significant increase in LPWV would be mainly related to the increased wall thickness in these aortic regions.

Conclusions: We can conclude that LPWV reflects well the distribution and severity of atherosclerotic lesion and the increased wall thickness in the local aortic region in which pulse waves were traveled. Am J Hypertens 2004;17:181–187 © 2004 American Journal of Hypertension, Ltd.

Key Words: Aorta, atherosclerosis, local pulse wave velocity, KHC rabbit.

Measurement of pulse wave velocity (PWV) is useful in the clinical assessment of arterial stiffness, which has an intimate connection with such conditions as hypertension, obesity, diabetes mellitus, and end-stage renal disease. Many recent clinical and epidemiologic studies have revealed that the increased PWV was an independent predictor of cardiovascular risk in patients with hypertension and end-stage renal failure. In most studies, pressure or flow waves are measured noninvasively between two arterial sites (eg, the carotid artery and femoral artery). The PWV is, therefore, determined as an averaged velocity between two sites mainly in the entire aorta. If an atherosclerotic lesion is relatively premature or small, PWV may not always reflect an alteration in distensibility of the arterial wall. Atheromatous plaque does not develop diffusely in the aorta, but does focally in a restricted area (eg, mainly around orifices of branch arteries). Sclerotic lesions are dominant mainly in the proximal region of the abdominal aorta in humans and in the ascending aorta and around the orifices of branch arteries in heritable hypercholesterolemic rabbits before progressing to peripheral lesion-free areas with age. However, it has not been demonstrated how PWV in the entire aorta relates to extent and severity of a localized sclerotic lesion along the aorta. We previously measured PWV from the ascending aorta to different sites.
positions along the aorta in the Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbit, but the PWV did not completely represent local velocity in separate aortic segments, such as the proximal, middle, or distal thoracic aortic segment.

In the present study, we measured local pulse wave velocity (LPWV) in seven different aortic segments along the atherosclerotic aorta in KHC rabbits using two catheters with one or two micromanometers and attempted to elucidate to what extent LPWV reflected extent and severity of atherosclerotic lesion in which pulse waves traveled. We also compared LPWV with PWV between the ascending and distal abdominal aortas to test diagnostic sensitivity of PWV clinically used.

**Methods**

**Animals**

Ten KHC and 10 normal Japanese White rabbits of both sexes aged 10 to 12 months (Japan Laboratory Animals, Inc., Tokyo, Japan) were used in the present study. They were given cholesterol-free commercial rabbit food (RC-4, Oriental Yeast, Co., Ltd., Tokyo, Japan) and allowed free access of tap water in an air-conditioned room at a temperature of 22° to 25°C, relative humidity of about 50% before the experiment. The present study was performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

**Pressure Wave Recording and Determination of LPWV**

The rabbits were anesthetized by the intravenous administration of pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, IL) at a dose of 30 mg/kg body weight, fixed in a supine position, and intubated through tracheotomy. Fig. 1 illustrates the schematic arrangement of experimental set-up for pressure wave recording. A catheter with one micromanometer at the tip (SPS-330, Millar Instrument, Inc., Dallas, TX) was introduced from the left common carotid artery and fixed at the tip at the ascending aorta (AA). Another catheter with two micromanometers at the tip at intervals of 40 mm (SSD-796, Millar Instrument) was advanced to the distal end of the aortic arch from the left femoral artery. Pressure waves at the ascending aorta and two adjacent aortic positions were simultaneously measured with a polygraph system by moving a catheter from the distal end of the aortic arch (position 0; P.0) to the distal abdominal aorta (P.6) at intervals of 40 mm. Pressure waves were recorded with a personal computer (Macintosh PowerBook 5300ce, Apple Computer Inc., Cupertino, CA) through an analog-to-digital converter (MacLab System 8s, AD Instruments Inc., Sydney, Australia) at intervals of 0.1 msec. P.1, P.2, and P.3 were located at the proximal, middle, and distal thoracic aortas and P.4, P.5, and P.6 were at the proximal, middle, and distal abdominal aortas, respectively. The distance from the pressure sensor of the catheter fixed at AA to the entry site at the left femoral artery (ΔD AA-FA) was measured in situ as precisely as possible at least twice by lying a thread along the aorta at the center of the adventitial surface before cutting open the aorta longitudinally, and then averaged.

**Determination of LPWV**

Fifty successive computer-recorded pressure waves at two adjacent aortic positions were used for determination of
LPWV. The time at the first peak of the second derivative of each pressure wave was taken as the foot of pressure waves, as it coincided with the rising point of the pressure waves. The LPWV in each aortic segment in the descending aorta was determined as \( \frac{d}{dt} \), where \( d \) (40 mm) was the distance between two adjacent aortic positions and \( t \) was difference in time between the feet of two pressure waves. The distance from P.0 to the entry site of the catheter in the left femoral artery (\( D_{AA-P.0} \)) was 320 mm because we took the distance from P.6 to the entry site as 80 mm in all animals. The distance between AA and P.0 was estimated as \( \frac{(D_{AA-P.6})}{(D_{AA-P.0})} \). Pressure level (mean aortic pressure) was calculated as a cyclic mean of 50 successive computer-recorded pressure waves used for the determination of LPWV.

**Determination of Percent Fractional Lesioned Area**

The aorta was excised from the origin of the ascending aorta to the bifurcation of the common iliac arteries, cut longitudinally, and the intimal surface was photocopied. The outline of atheromatous plaques and entire aortic surface were carefully traced. The aortic surface was divided into seven regions surrounding the two adjacent aortic positions in which pressure waves were measured. Monochromatic images of the intimal surface in the seven aortic regions surrounding the adjacent aortic positions were fed into an image analysis system (Luzex FS, Nireco Corporation, Tokyo, Japan) through a CCD-camera and digitized by the main processor of the image analysis system as reported previously.\(^{16}\) The lesioned and lesion-free areas within each aortic region were displayed as black and white, respectively, by manipulating intensity, brightness, and purity of outline of each plaque and the entire aortic area. Percent fractional lesioned area (PFLA) in each aortic region was determined as a ratio of lesioned area to total surface area in each region. The wall strips excised from the ascending, proximal thoracic, and proximal abdominal aortas were fixed in 10% neutral buffered formalin solution and embedded into paraffin. The strips were sliced at 5 \( \mu \)m and stained with Elastica-van Gieson stain.

**Statistical Analysis**

Systolic (SAP), diastolic (DAP), mean (MAP), and pulse (PP) pressures in the normal and KHC rabbit groups were compared by repeated measures two-way analysis of variance (ANOVA). We tested differences in each parameter between the two rabbit groups at each position using Schefè’s method when significant difference (\( P < .05 \)) was observed in the test using ANOVA. The LPWV was tested by ANCOVA with MAP as a covariate, and then compared between the two rabbit groups at each position using Student \( t \) test with Bonferroni’s correction when significant difference (\( P < .05 \)) was found in the test using ANCOVA. The PWV from AA to P.6 was compared by Student \( t \) test between the two rabbit groups.

**Results**

**SAP, DAP, and PP at Different Aortic Positions**

Fig. 2 shows SAP, DAP, and PP in the normal and KHC rabbit groups at different aortic positions. The MAP level at each aortic position ranged from 108.5 to 112.5 mm Hg in the normal rabbit group and from 120.7 to 123.1 mm Hg in the KHC rabbit group. There was no significant difference in the MAP level among aortic positions in both
rabbit groups ($P > .05$). The SAP ($P < .001$), DAP ($P < .001$), MAP ($P < .001$), and PP ($P < .05$) in the KHC rabbit group at any of aortic positions showed significant increase compared to those in the normal rabbit group.

**Change in LPWV in Different Aortic Segments**

Fig. 3 illustrates changes in LPWV at different aortic segments in the normal and KHC rabbit groups. In the normal rabbit group, LPWV was lowest in the aortic arch (AA–P.0), increased slightly with almost identical values in the proximal (P.0–P.1), middle (P.1–P.2), and distal (P.2–P.3) thoracic aortic segments, and thereafter increased gradually in the proximal (P.3–P.4), middle (P.4–P.5), and distal (P.5–P.6) abdominal aortic segments. In the KHC rabbit group, LPWV was greatest in AA–P.0, decreased gradually almost to the normal level in P.0–P.1 and P.2–P.3, increased only in P.3–P.4, and showed almost identical changes to those in the normal rabbit group in P.4–P.5 and P.5–P.6. Values of LPWV were significantly greater in the KHC rabbit group than in the normal rabbit group in AA–P.0 ($P < .001$), P.0–P.1 ($P < .05$), and P.3–P.4 ($P < .001$) in which sclerotic lesion was relatively dominant. The PWV from AA to P.6, an averaged velocity in the entire aorta was $5.5 \pm 0.1$ m/sec (mean $\pm$ SD) and $6.1 \pm 0.4$ m/sec in the normal and KHC rabbit groups. There was a significant difference in PWV between the two rabbit groups ($P < .001$).

**Features of Atherosclerotic Lesions**

Atheromatous plaques were observed predominantly in the aortic arch and around the orifices of the branch arteries (ie, intercostal, celiac, mesenteric, renal arteries), as reported previously (Fig. 4A). The PFLA determined in different aortic segments was about 84% in AA–P.0 and 47% in P.0–P.1. It decreased to about 22% in P.1–P.2 and P.2–P.3 and increased to about 43% in P.3–P.4 due to the presence of the orifices of the celiac, mesenteric, and renal arteries in this segment. In the middle and distal abdominal aortas, PFLA diminished suddenly to below 5%. Marked intimal thickening with abundant cholesterol-rich foam cells were histologically observed in the ascending, proximal thoracic, and proximal abdominal aortas (Fig. 4B). The inner elastic laminae were partly injured.

**Discussion**

**Change in LPWV in Different Aortic Segments**

We observed characteristic change in LPWV along the aorta in the KHC rabbit group, which LPWV reflected the distribution pattern of the PFLA in different aortic segments proximal to the P.3–P.4. Assuming that the blood is a nonviscous fluid, PWV can be theoretically explained by the Moens-Korteweg equation:

$$\text{PWV} = \sqrt{\frac{K}{Eh/2R}},$$

where $K$, $E$, $h$, and $R$ were a constant, elastic modulus, wall thickness, a density of the blood, and internal radius, respectively. Hasegawa et al. reported no significant difference in the value of $h$ between the normal and hyperlipidemic rabbit groups. We measured the diameter of the aorta in situ in another experiment using an intravascular ultrasonic (IVUS) method in eight normal and eight KHC rabbits aged 10 to 12 months, although they were different from those used for determination of LPWV in the present study (Katsuda et al, unpublished data). The pressure-strain elastic modulus ($P_E$) was determined as $P_E = PP/(D_s - D_d)/D_m$, where $D_s$, $D_d$, and $D_m$ were systolic, diastolic, and mean ($D_s + D_d)/2$ diameters, respectively. Ring sample of the wall 3.0 mm in width was excised from the proximal thoracic and the proximal abdominal aortas where the diameter was measured in situ, and then
weighed with a precision balance. Wall thickness in situ was estimated as \( h = W/(1.06 \times \pi \times D_m \times W_d) \), where 1.06, \( W \), and \( W_d \) were specific weight, sample weight, and sample width. In the proximal thoracic aorta, the value of \( P_E \) in the KHC rabbit group showed a significant decrease by about 20% in average to that of the age-matched control group. In the proximal abdominal aorta, \( P_E \) tended to decrease by about 10% in average to that of the normal rabbit group, which was not significant (\( P > .05 \)). The value of \( h \) in the KHC rabbit group increased significantly by about twofold to that of the control aorta in these aortic portions. There was no significant change in the value of \( D_m \) between the two rabbit groups in these aortic portions, although it increased by about 8% and 5% in the proximal thoracic and the proximal abdominal aortas. The changes in \( P_E \) and \( h \) in vivo due to atherosclerosis showed similar tendency to those observed in the previous ex vivo tensile study in the 10- to 12-month-old KHC rabbits.\(^{16}\) The increased \( L_PWV \) in the proximal thoracic and proximal abdominal aortas would be mainly related to the significant increase in wall thickness due to sclerotic lesion in the young KHC rabbits. We\(^{16}\) previously reported the static rheologic characteristics in the 10- to 12-month-old KHC rabbits. The elastic modulus of the wall at 50% stretching to the initial length did not significantly change and wall thickness was about twofold compared with those of the control rabbit in the ascending aorta. Although the values of \( P_E, h, \) and \( D_m \) were not measured in the ascending aorta, the severely increased \( h \) would mainly contribute to the increased \( LPWV \) in the ascending aorta. In the aortic arch, traveling time of pulse waves must be different between the inner and outer surface of the arch. We determined the distance along the center of the aorta because the sensor at AA was located in most cases almost at the center of the aorta. The \( LPWV \) in the aortic arch was estimated to be a velocity mainly along the center of the vessel. Turbulent flow must also be generated in this region. The hemorheologic effects on \( LPWV \) should be elucidated in future.

In the 10- to 12-month-old KHC rabbits, atherosclerosis was relatively premature as characterized chiefly by abundant foam cells and less proliferation of collagen or elastin fibers. The value of \( P_E \) in the atherosclerotic aorta was small compared to that in the normal aorta. In the early stage of atherosclerosis, increased thickness of the wall may be a principal contributing factor to increased \( LPWV \). Farrar et al\(^{20}\) also demonstrated in the atherosclerotic aorta of cholesterol-fed monkeys that increased wall thickness contributed to an increase in PWV without a significant increase in elastic modulus.

**Effects of SAP, DAP, and PP on LPWV**

Pressure waves consist of the early systolic and late systolic wave components. The former is an orthodromic wave toward the peripheral arterial site and the latter, an antidromic wave toward the heart, generated by reflection mainly at branching sites where arterial impedance mismatch occur.\(^{21}\) Increased SAP and PP have been reported to be due mainly to increased amplitude of the late systolic waves that has been assessed by augmentation index (AIX).\(^{22,23}\) We observed previously in the KHC rabbit that AIX of ascending aortic pressure waves were significantly elevated with the significant increase in total peripheral vascular resistance (TPR) and without change in cardiac output (CO) compared to age-matched control rabbits.\(^{24}\) The increase in SAP and DAP in the 10- to 12-month-old
KHC rabbits would be partly associated with the increase in reflected waves and TPR, respectively.

Pulse pressure is closely related to decreased distensibility of the arterial wall. In the KHC rabbit aged 10 to 12 months, being relatively young, the value of Pp unexpectedly showed a slight or significant decrease compared to that in the normal aorta. This means that the atherosclerotic aorta is soft rather than rigid at a relatively early stage. Abundant cholesterol-rich foam cells seem to be responsible for the viscoelastic characteristic of the wall. Similar findings were reported by Newman et al in cholesterol-fed cockerels. Decreased traveling time of the reflected waves due to the increased PWV possibly shortened the timing that the reflected waves were superimposed on the orthodromic waves. reported that delay time of reflected waves determined by cross-correlation analysis shortened in the 10- to 12-month-old KHC rabbit group (P < .01), although the increase in reflection magnitude in the KHC rabbit group was slight and not significant compared to that in the age-matched control group. This would partly contribute to the increase in PP.

Stiffness of the wall could be affected by the distending pressure. Takazawa et al demonstrated that PWV had a positive correlation with DAP and SAP and that PWV increased by 1.0 m/sec and 1.6 m/sec due to the increases in SAP and DAP by 20 mm Hg. We investigated the relationship between LPWV and SAP and between LPWV and DAP at different aortic positions in the normal and KHC rabbit groups. There was no significant correlation between LPWV and either DAP or SAP at any aortic position in either rabbit group (P > .05 for DAP and SAP in each aortic position). The increase in SAP and DAP levels and their variations were too small to show a positive correlation with LPWV in both rabbit groups, although the precise physiologic mechanism remains unclear. In the present study, the modest hypertension was considered to contribute little to the increase in LPWV, which was indirectly supported by the lack of a significant difference in LPWV in the lesion-free aortic regions.

Comparison of LPWV with PWV in the Atherosclerotic Aorta

There was a significant difference in PWV from AA to P6 between the two rabbit groups. Although PWV as an averaged velocity along the entire aorta, it was useful for estimating the presence of aortic atherosclerotic lesion; we failed to estimate distribution and severity of sclerotic lesions in the present study with PWV. The LPWV reflected precisely the existence of lesions and alteration in the rheologic characteristics of the aortic wall in which pressure waves traveled. The LPWV could be a powerful aid for diagnosing atherosclerosis. When this method is applied clinically to patients with atherosclerotic disease during cardiac catheterization you can estimate distribution and severity of aortic atherosclerosis before autopsy or additional invasive procedures. We should establish a profile of age-related changes in LPWV in the normal and sclerotic aortas in the future.

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References


