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Assessment of in vivo Aortic Wall Characteristics at the Early Stage of Atherosclerosis in Rabbits

Katsumi Hironaka

The Second Department of Internal Medicine, Yamaguchi University, School of Medicine, Ube, Yamaguchi 755, Japan
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Abstract To assess whether vascular responsiveness to α-receptor agonist is altered or not at the early stage of atherosclerosis, in vivo aortic pressure-diameter relationship of aorta over a wide range of pressure was analyzed before and after the acute administration of α-receptor agonist (phenylephrine) in 9 hypercholesterolemic fat-fed (7 weeks) rabbits and 8 normal diet-fed (7 weeks) rabbits. There was no significant difference in the aortic wall thickness between both groups, suggesting the early stage of atherosclerosis. Using a modified three element Maxwell model, diastolic stress-strain relationship was computed after applying several assumptions to the actual aortic pressure-diameter relationship. After the intravenous administration of phenylephrine at a rate of 5 μg/ kg/ min, the stress (ordinate)-strain (abscissa) relation curves were shifted to the left, indicating the activation of aortic smooth muscle by phenylephrine. The difference in the stress before and after phenylephrine infusion showed a single peak at a certain strain. The peak difference in the stress was smaller in hypercholesterolemic fat-fed rabbits than in normal diet-fed rabbits, indicating the decreased vascular responsiveness at the early stage of atherosclerosis.

Key words: Hypercholesterolemia, Aortic smooth muscle, Atherosclerosis, Stress-strain relationship, Phenylephrine

Introduction

Aortic wall mainly consists of three components, elastin, smooth muscle and collagen. The only active component, smooth muscle plays a major role in determining the mechanical property of the aorta which is known to influence the cardiac dynamics as an afterload (4).

Recent studies have demonstrated that atherosclerosis is associated with functional abnormalities in contractile function of vascular smooth muscle as well as an impairment of its relaxation response (8, 9, 10, 12). Even at the early stage of atherosclerosis in which no major structural change was observed in the aortic wall, aortic stiffness was significantly increased in hypercholesterolemic fat-fed rabbits (17), suggesting the possible involvement of smooth muscle activity in the altered aortic wall characteristics in hypercholesterolemia.

In the present study, we investigated whether or not the responsiveness of aortic wall to α-receptor agonist (phenylephrine) is altered in hypercholesterolemia, particularly at the

early stage of atherosclerosis.

Materials and Methods

Nine male Japanese white rabbits weighing 3.3 ± 0.1 kg were fed with a diet containing 1 % cholesterol for 7 weeks (Group CH). Eight male Japanese white rabbits weighing 3.2 ± 0.2 kg were fed with a normal diet (Group N).

These rabbits were anesthetized with intravenous 5 % α-chloralose solution (100 mg/kg). A thoracotomy was made at the fifth intercostal space, and the animals were artificially ventilated with a respirator (Harvard Apparatus). A pair of ultrasonic dimension gauges (10 MHz Crystal Biotechs Instrument) was positioned face to face about 20 mm below level of the aortic arch to measure external aortic diameter. A pressure micromanometer (2 F Millars) and a fluid-filled polyvinyl chloride tube (for later calibration of micromanometer) were inserted via the left femoral artery. After the calibration, the pressure micromanometer was advanced to a level just below the aortic dimension gauges. The occluder was placed around the thoracic aorta at least 30 mm distal to the ultrasonic dimension gauges to eliminate the possible dislocation of crystals during the aortic occlusion. A balloon occluder of inferior vena cava was inserted via the right jugular vein. This experimental instrumentations are shown in Fig. 1. Recordings were made by a multi-channel recorder (VR12, Electronics for Medicine) and analog data were digitized at 2 msec interval. The recording took less than 20 s, and therefore a requiring stoppage of the respirator was not more than 20 s. This study was approved by Animal Care Committee of the School of Medicine, Yamaguchi University.

Experimental protocol

After the steady-state recording of hemodynamics, transient inferior vena caval occlusion was carried out, sequentially followed by a transient thoracic aortic occlusion to obtain pressure-diameter relationships over a wide range of aortic pressures (3, 5, 6) as shown in Fig. 2 (control). After confirming the full recovery of the hemodynamics from

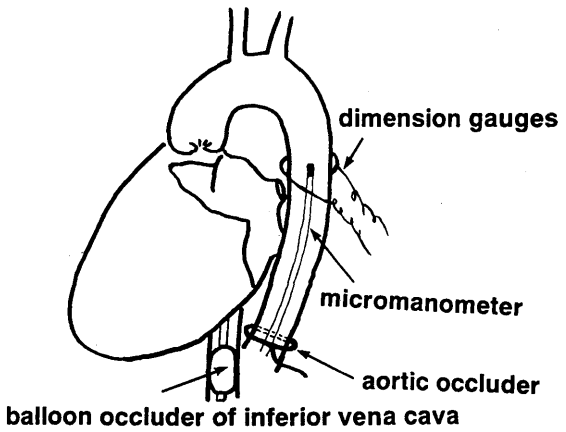

Fig. 1. A pair of ultrasonic dimension gauges was positioned face to face about 20 mm below level of aortic arch, and a pressure micromanometer were inserted via the left femoral artery, positioned just below the dimension gauges. A balloon oclluder of inferior vena cava was inserted via right jugular vein, and aortic occluder was placed at least 30 mm distal to a pair of dimension gauges.

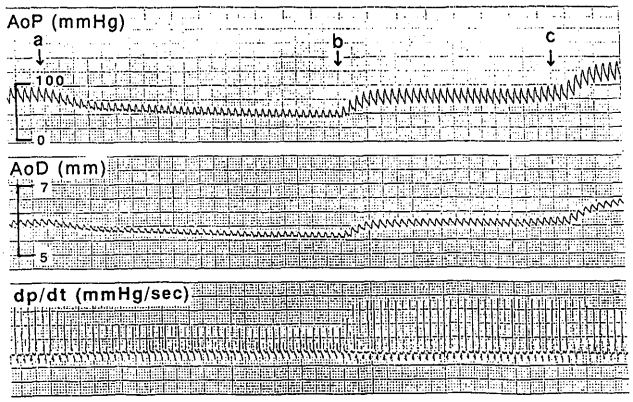

Fig. 2. Low speed recordings of aortic pressure (AoP), aortic diameter (AoD), and dP/dt of AoP. Inferior vena caval occlusion was started at point (a) and released at point (b), sequentially followed by aortic occlusion at point (c). AoP: aortic pressure, AoD: aortic diameter, dp/dt: the first derivative of aortic pressure.

the previous procedure, phenylephrine was infused at a rate of 5 μ g. kg^{-1}. min^{-1} to activate aortic smooth muscle (3). It took 10 to 15 minutes to reach a new steady state after phenylephrine infusion. Both before and after phenylephrine infusion, simultaneous measurements of aortic pressure and external diameter were made under the steady state and during the above occlusion procedure. After the hemodynamic study, the rabbits were sacrificed with a intravenous overdose of α-chloralose solution followed by potassium chloride. The segment of thoracic descending aorta (30 mm) including the site of crystal dimension gauges was carefully dissected free from surrounding tissue and weighed. The aortic segment was tied off at both ends after inserting the polyvinyl chloride tube which was connected with the fluid-filled pressure transducer. The nonstressed midwall radius (R_0) was measured approximately at 25 mmHg of aortic pressure (3).

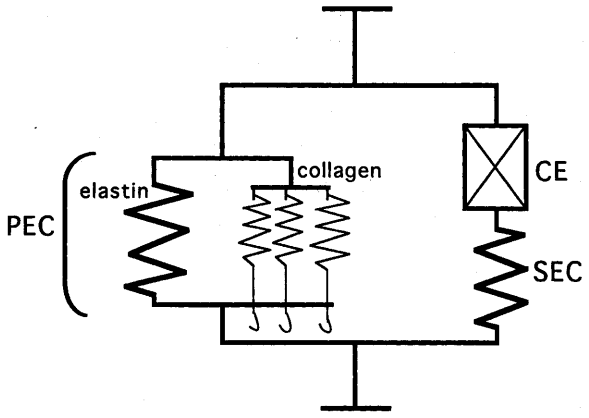

Fig. 3. Schematic representation of modified Maxwell model. SEC, series elastic component; PEC, parallel elastic component; CE, contractile element.
SEC was coupled in series with a viscous force-generating contractile element (CE).

Data analysis

Pressure-independent stiffness index (β) was calculated as;
β = ln (PSP/DP)/(ΔD/DD), where PSP = peak systolic aortic pressure, DP = diastolic aortic pressure, ΔD = difference between maximum systolic and minimum diastolic aortic diameter, and DD = minimum diastolic aortic diameter (7).

Aortic wall thickness was calculated as the difference between the external aortic radius (r_e) and the internal aortic radius (r_i). To estimate ri, the following equation was used:
$r_i = \{r_e^2 - (V/\pi \times L)\}^{1/2}$ (3)

Where V is the volume and L is the length of given aortic segment. V was calculated using the weight of aortic segment and assuming a tissue density of 1.066g/mL. Since V dose not change in vivo, r_i and aortlc wall thickness (r_e-r_i) can be calculated continuously.

Strain (ε) was obtained from the ratio of mid wall radius (R) to nonstressed mid wall radius (R_0): $\varepsilon = R/R_0$ (3) ; where $R=(r_e+r_i)/2$

Stress (σ) was calculated using the following equation:
$\sigma = 2P(r_e \times r_i)^2/(r_e^2-r_i^2) \times 1/R^2$ (3) ; where P is aortic pressure.

To evaluate the mechanics of vascular smooth muscle (VSM), a modified three element Maxwell model (Fig. 3) was used (2). According to this model, the total stress can be written as;
$\sigma = E_E(\varepsilon - \varepsilon_{0E}) + E_C \times f_C \times \varepsilon + \sigma_{SM}$ (3); (E_E; the elastic modulus of elastin fibers, E_C; the elastic modulus of collagen fibers, f_C; the fraction of collagen fibers recruited to support wall stress, ε_{0E}; the strain-axis intercept, ie, the extrapolated value of strain for stress equal to zero), where σ_{SM} is the active stress of smooth muscle supported by the tandem CE- SEC (CE; contractile element, SEC; series elastic component) (3).

Using Barra et al's theory, the computation of active stress by the tandem CE-SCE was made by subtracting the aortic stress-strain relation in the control condition from the aortic stress-strain relation obtained under phenylephrine administration at the same level of strain (3): $\sigma_{SM} = \sigma - \sigma_{PE}$; where σ_{PE} is the stress of parallel elastic component. The diastolic stress-strain relationship in one cardiac cycle was obtained by plotting the stress (ordinate) and strain (abscissa) measured at 2 ms interval from 40 ms after the dicrotic notch of the aortic pressure to 20 ms before the rise of the first derivative of aortic pressure (3, 6).

The diastolic stress-strain relation in the

whole pressure range was then obtained by plotting the diastolic stress-strain values in all cardiac cycles during the aortic-occlusion procedure immediately after the release of inferior vena cava occlusion. The diastolic stress-strain relation in whole range was fitted to a cubic equation before and after phenylephrine infusion ($r > 0.99$).

Statistical analysis

All measurements and calculated values are expressed as mean ± SD. Linear regression analysis was made using the least squares method. Statistical analysis of the difference between Group CH and Group N was conducted using unpaired T test, and paired T test was employed for the difference between before (control) and after (PH) phenylephrine infusion.
Values of $P < 0.05$ were considered statistically significant.

Results

The cholesterol diet markedly increased the plasma levels of total cholesterol (671 ± 271 mg/dl). However, there was no significant difference in the calculated aortic wall thickness between both groups, suggesting the early stage of atherosclerosis (Group N vs Group CH : $2.88\times10^{-1}\pm0.40$mm vs $2.71\times10^{-1}\pm0.41$mm ns).

Hemodynamics and pressure-diameter relationship

A typical example of pressure-diameter relation curve is shown in Fig 4. In Group CH, the pressure-diameter relation curve is shifted toward smaller diameter compared with in Group N and hence the strain at systolic pressure 90 mmHg of Group CH was significantly smaller than that of Group N (Group N vs Group CH : 1.18 ± 0.09 vs 1.08 ± 0.07 $p<0.03$: unpaired T test). After phenylephrine infusion, the pressure-diameter relation curves shifted toward left in both group. However, the extent of the leftward shift was smaller in Group CH.

Table 1 summarizes the hemodynamic parameters. Compared with the hemodynamics before phenylephrine, phenylephrine significantly increased aortic pressure, and decreased the heart rate in both groups ($P<0.01$: paired T test). In Group N, aortic diameter was not increased after phenylephrine infusion, as reflected by the marked leftward shift of pressure-diameter relation curve, whereas increased in Group CH ($P<0.05$: paired T test).

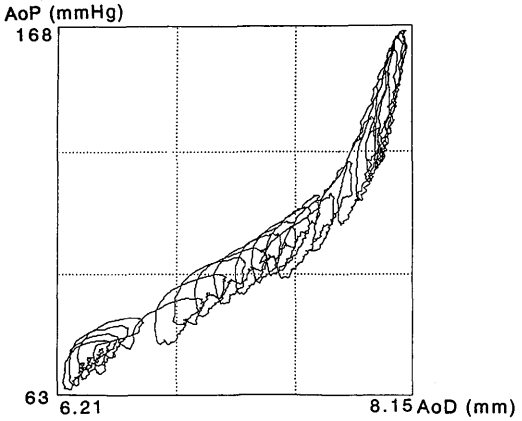

Fig. 4. Pressure-diameter hysteresis loops in all cardiac beats during the release of inferior vena caval occlusion followed by aortic occlusion. So obtained pressure-diameter relationship showed a sigmoid configuration.

Pressure-independent aortic stiffness index (β)

Table 2 shows % aortic excursion and pressure-independent aortic stiffness index (β). In either before or after phenylephrine infusion, excursion of aortic diameter in group CH was significantly smaller than that of group N. The aortic stiffness index (β) was also larger in group CH than in group N.

Stress-strain relationship

A typical example of diastolic stress-strain relation curve in Group N is depicted in Fig 5. After phenylephrine infusion, the diastolic stress-strain relation curve shifted toward left. After fitting the diastolic stress-strain curve to a cubic function, the difference in stress before and after phenylephrine infusion was calculated at the corresponding strain. Then, the difference in the stress, which might be induced by the contraction of vascular smooth muscle in aortic wall, was

Table 1. Hemodynamics before and after phenylephrine infusion.

	Group N	Group CH
HR control (bpm)	256±49	266±37
HR PH (bpm)	233±48*	249±41*
PSP/DP control (mmHg)	86±15/68±14	87±13/68±14
PSP/DP PH (mmHg)	115±21*/90±15*	111±16*/89±13*
PSD/DD control (mm)	6.40±.69/6.14±.67	6.15±.51/5.97±.49
PSD/DD PH (mm)	6.55±.67/6.29±.65	6.48±.73#/6.30±.71#

HR: heart rate, PSP: peak systolic aortic pressure, DP: diastolic aortic pressure, PSD: peak systolic aortic diameter, DD: minimum diastolic aortic diameter. bpm indicates beats per minute.
Values are mean ± SD. * $p<0.01$, # $p<0.05$, vs control value

Table 2. % excursion of aortic diameter and aortic pressure-independent stiffness index (β) before and after phenylephrine infusion.

	Group N	Group CH
%EX. control(%)	4.32±.98	3.16±1.11#
%EX. PH(%)	4.16±.91	2.84±.88*
β control	5.47±1.13	9.11±4.69#
β PH	6.05±1.66	8.92±2.96#

%EX: % excursion of aortic diameter = $\Delta D/DD \times 100$
aortic pressure-independent stiffness index (β) = ln (PSP/DP)/($\Delta D/DD$)
PSP: peak sysiolic aortic pressure, DP: diastolic aortic pressure, DD: minimum diastolic aortic diameter, ΔD: difference between maximum and minimum aortic diameter.
Values are mean ± SD. * $p<0.01$, # $p<0.05$ vs Group N value

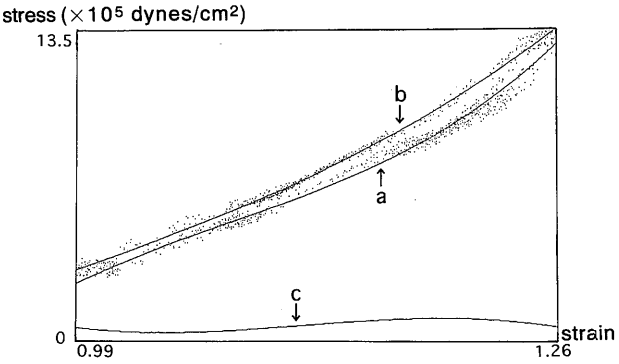

Fig. 5. A typical example of aortic stress-strain relationship during control (a) and activation of vascular smooth muscle by phenylephrine (b). The difference in the stress between (a) and (b) as a function of strain is provided as a new curve (c) which represents the amount of the stress increased by phenylephrine. We defined this curve as "Δ stress-strain curve" which revealed a single peak at a certain strain.

plotted as a function of strain (Δ stress- strain curve). So obtained, Δ stress-strain curve revealed a single peak at certain strain in all rabbits. Although the strain at which the maximum increase in the stress (max Δ stress) was induced by phenylephrine was not changed, max Δ stress was significantly smaller in Group CH compared with in Group N, indicating the decreased vascular responsiveness to phenylephrine in Group CH (Table 3).

Discussion

The major finding of this study is that the aortic smooth muscle responsiveness evaluated by the capability of increasing aortic wall stress upon stimulation by phenylephrine is decreased in hypercholesterolemic rabbit model (the early stage of atherosclerosis)

Table 3. The change in the vascular stress after phenylephrine infusion

	Group N	Group CH
Max Δ stress ($\times 10^4$dyn/cm^2)	14.5±2.2	7.8±4.5※
Strain at Max Δ stress	1.23±.13	1.15±.07

Max Δ stress: The maximum increase in the stress after phenylephrine infusion.
Values are mean ± SD. ※ $p<0.002$, vs Group N value

compared with that in normal rabbit model.

Evaluation of aortic stiffness and vascular responsiveness to phenylephrine

Aortic wall mainly consists of three components elastin, smooth muscle and collagen. Bader et al. reported that the triphasic character of this curve had been attributed to a parallel arrangement of relatively extensible elastin, smooth muscle and highly inextensible collagen (13). In these three components, smooth muscle was the only active component. There are several ways of evaluating aortic stiffness, eg. pressure-independent stiffness index (β), pressure-diameter or volume relationship, and stress-strain relationship.

Previous studies have documented that activation of smooth muscle affects the aortic elasticity (14, 15, 16)

In this study, pressure-diameter relation curve showed a sigmoid shaped curve consistent with the previous report from our institute (6). Stress-strain relation curve derived from original pressure-diameter relation curve also showed a sigmoid shaped curve. After phenylephrine infusion, either the pressure-diameter relationship or the diastolic stress-strain relation curve was shifted toward left, suggesting the activation of aortic smooth muscle since the activation of vascular muscle is known to shift stress-strain relation toward a higher level of stress (1, 3).

Vascular responsiveness to vaso-active agents in atherosclerosis

Atherosclerosis is associated with functional abnormalities in vascular smooth muscle as well as various structural changes, eg, intimal wall thickening and grossly visible alteration or plaques in the vascular wall.

Even at the early stage of atherosclerosis due to hypercholesterolemia when no major structural change in the vascular wall was observed except fatty streak (11), the elasticity of aorta was decreased(17). Consistent with these reports, we also observed the decreased in the elasticity of aorta without a change in the wall thickness, indicating the increased aortic stiffness in the early stage of atherosclerosis.

There are many reports concerning the vascular responsiveness in hypercholesterolemic model to various vaso-active agents, eg. α-agonist, serotonin, and acetylcholine. Endothelium-dependent relaxation is known to be defective in response to acetylcholine in the isolated arteries taken from rabbits or pigs, and this abnormality can be observed even before light and electron microscopic changes of atherosclerosis developed (12). However, there is a controversy about the vascular response to other drugs. Broderick et al. demonstrated that the responsiveness of arterial smooth muscle to adrenergic stimulation was augmented in hypercholesterolemic rabbits. On the other hand, other in vitro studies using the arteries from hypercholesterolemic rabbit (9) or monkey (10) showed an increase in the contractile response of vascular smooth muscle by serotonin, but not by norepinephrine.

In the present study, we observed that α-agonist (phenylephrine) stimulated the vascular response viz. increase in the wall stress both in the hypercholesterolemic rabbit and in the normal rabbit. However, the extent of the increase in the vascular wall stress after phenylephrine infusion was much smaller in the hypercholesterolemic rabbit.

Since the only active component of aortic wall is smooth muscle, the increased aortic stiffness in our model (early stage of atherosclerosis) could be attributable to the hyperresponsiveness of the smooth muscle to several vaso-active agents already existing in the vascular wall at control stage. It is likely that the remaining capability of the aortic

smooth muscle to be activated may be smaller in the hypercholesterolemic rabbit than in normal rabbit. This may be a reasonable explanation why the vascular response to phenylephrine was smaller in the hypercholesterolemic rabbit.

Limitation

The reproductibility of the measurement of aortic pressure-diameter relationship should be addressed. We obtained this relationship during inferior vena caval occlusion followed by aortic occlusion in triplicate. There was no significant difference in the hemodynamic indices (β, wall stress) derived from these three pressure-diameter curves (not shown).

Also, the vascular responsiveness may be affected by anesthesia (3, 18). The influence of anesthesia on central nervous system and vascular system may be different depending on the level of cholesterol or degree of atherosclerosis, resulting in different vascular responsiveness to various vaso-active drugs. With regard to these observations, clearly more works are needed.

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