Echographical Assessment of the Early Stage of Experimental Atherosclerosis of the Descending Aorta in Rabbits

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To assess the early stage of atherosclerosis of the thoracic descending aorta, we evaluated morphological atheromatous lesions (atherosclerosis) and the stiffness parameter of the artery (β; sclerosis) in 24 male rabbits using echography. Male Japanese white rabbits weighing 2.5–3.0 kg were fed a diet containing 1% cholesterol for 7 (n=8) or 14 weeks (n=8). Rabbits fed a normal diet were used as controls (n=8). Atheromatous lesions were evaluated with intravascular ultrasound (IVUS: Aloka, 20 MHz, 6F). We also calculated β using M-mode echography (7.5 or 10 MHz) and direct aortic pressure measurement. Thickening of the intima-media complex was clearly observed with IVUS in the 14-week group but was not detected in the others. Histologically, only a thin layer of foamy cells on the intima (thickness <20 μm) was observed in the 7-week group. The value of β was significantly increased in both the 7-week (4.7±2.2) and 14-week groups (4.5±0.8) compared with controls (1.7±0.9, both p<0.01). These results suggest that the development of atherosclerosis might be preceded by vascular sclerosis during the early stage of atherosclerosis when the serum cholesterol level is high: at a time when the thin layer of foamy cells could not be detected by conventional IVUS.

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Atherosclerosis is characterized by two major features: fatty degeneration (atherosclerosis) and vessel stiffening (sclerosis). In 1904 Marchand identified a consistent association between fatty degeneration and increased vessel stiffness and coined the term atherosclerosis to describe this combination of features. Atherosclerosis can be evaluated by various methods. For example, contrast angiography has long been used to assess atherosclerotic lesions. However, although this modality provides information on luminal dimensions and intimal irregularities, it is not helpful in assessing detailed morphological changes in the intima or in determining the tissue characteristics of the lesions.

Echographical imaging has recently emerged as a promising diagnostic modality for delineating the extent of atherosclerosis in superficial vessels, especially the carotid arteries and coronary arteries. This technique allows vessel luminal diameter, wall dimension and wall structure to be measured reliably. Transesophageal echocardiography permits visualization of the thoracic descending aorta and evaluation of atherosclerosis and enables sclerosis to be assessed by means of M-mode echography.

The use of the echography to delineate atherosclerosis and sclerosis could have major clinical implications for observation of the regression or progression of atherosclerotic lesions in response to treatment with...
cholesterol-lowering agents or dietary regimens.

In the study described here, we attempted to evaluate atherosclerosis and sclerosis separately in the early stage of atherosclerosis of the aorta using echography in rabbits fed a high-cholesterol diet.

MATERIALS AND METHODS

The experimental protocol was approved by the Animal Studies Committee of Yamaguchi University School of Medicine.

Animal Model and Experimental Preparation

Three groups of Japanese white rabbits (4–8 months old, weighing 2.5–3.0 kg) were studied. The control group of animals (n=8) was fed with standard rabbit chow. The plasma cholesterol level of control animals was 23±14 mg/dl. Age-matched rabbits were fed a high-cholesterol (1%) diet (130 g/24 h; Kyushu Animal Co, Japan) for 7 weeks (7-week group; n=8). During this time, the plasma cholesterol increased to 890±310 mg/dl (p<0.01 compared with control animals). Other rabbits were fed the 1% cholesterol diet for 14 weeks (14-week group; n=8). The plasma cholesterol at the end of this period was 910±190 mg/dl (p<0.01 compared with control animals).

On the day of examination, rabbits were anesthetized with sodium pentobarbital (30 mg/kg by iv injection). A vertical incision was made in the anterior aspect of the neck, and the trachea, right common carotid artery, and external jugular vein were isolated. A tracheotomy was performed, and a 3.5 mm non-cuffed intubation tube was inserted. The rabbits' lungs were mechanically ventilated at 45 breaths per minute and 40 mL tidal volume with an animal ventilator (Harvard Apparatus). The right common carotid artery was cannulated with a polyvinyl chloride catheter (15 cm long; internal diameter 0.5 mm) connected to a Statham P23ID transducer through a 100 cm-long rigid polyvinyl tube, and the catheter was advanced into the ascending aorta for continuous aortic pressure monitoring. The external jugular vein was cannulated with the same catheter for saline infusion.

Evaluation of Atherosclerosis

The intravascular ultrasound (IVUS) imaging system (Aloka; SSD-550, Japan) consisted in a 20 Mhz single mechanical transducer placed at the tip of a 6F catheter. After incising the abdominal wall and isolating the abdominal aorta, the catheter was inserted via the abdominal aorta at the level of renal artery into the descending aorta; in this way cross-sectional ultrasound images of the thoracic descending aorta could be obtained. All IVUS studies were photographed and recorded on a videotape. Gain settings were adjusted and fixed for optimal visualization of the vessel lumen interface.

The highly echogenic area between the leading edge of the intima and the sonolucent zone is called the intima-media complex. We defined the detectable sonolucent zone on cross-sectional echographic images as the thickening of the intima-media complex.

Evaluation of Sclerosis

After opening the left chest wall, the chest cavity was filled with warm echo jell. The thoracic descending aorta, about 2 cm caudal to the aortic arch, was visualized by echocardiography (Aloka; SSD-630, Japan) using a single-element transducer with a frequency of 7.5 or 10 MHz and without aortic compression. From a 2-dimensional echogram of the aorta, we chose a section without atherosis. An M-mode echogram of this part of the thoracic descending aorta was recorded during several cardiac cycles on a strip-chart recorder at a sweep speed of 100 mm/sec with simultaneous aortic pressure. This echo system allows pulsatile changes in aortic diameter to be measured reliably (axial resolution 0.08 mm). We measured minimum aortic dimension (Dmin, mm), maximum aortic dimension during the ejection period (Dmax, mm), and the systolic amplitude of the internal dimension (ΔD=Dmax−Dmin). A stiffness parameter β was calculated using the following equation: β=ln (BP/BPd) (ΔD/Do), where BP is maximum systolic aortic pressure and BPd is minimum diastolic aortic pressure. This parameter β represents a physiologic stiffness index of the vessel independent of the operating level of aortic pressure.
Echographic Findings in Rabbits With Atherosclerosis

![Images of control and 14-weeks group](image)

Fig 1. Representative images of the thoracic descending aorta obtained by intravascular ultrasound. Left (control group): The aortic wall is smooth and uniform and no sonolucent zone can be observed. Right (14-week group): A sonolucent zone is apparent (from 6 to 10 o'clock), which we identified as thickening of the intima-media complex. IMC, intima-media complex; SL, sonolucent zone.

![Images of control and 7-weeks group](image)

Fig 2. Representative M-mode echograms of the thoracic descending aorta. Top: control; bottom: 7-week group. Images obtained in animals from the 7-week group reveal that the instantaneous dimensional change was less than in control animals. In control animals, the aortic wall furthest from the transducer showed almost no motion throughout a cardiac cycle. The near and far aortic walls correspond, respectively, to the left and right lateral walls of the descending aorta. The left lateral aortic wall is covered only with parietal pleura. In contrast, the right lateral aortic wall is backed by thoracic vertebrae, so that the absolute excursion of the right lateral aortic wall appears to be reduced. $D_{\text{max}}$, maximum aortic dimension; $D_{\text{min}}$, minimum aortic dimension; W, aortic wall; L, aortic lumen; AP, aortic pressure.

**Histological Study**

The site in the thoracic descending aorta that was imaged by IVUS was marked by inserting a pin into the adventitia to assess the histological findings.

The rabbits were killed with a lethal injection of sodium pentobarbital. The descending thoracic aorta was excised, washed with saline, and fixed in formalin. The section of the aorta (approximately 0.5 cm) was embedded in paraffin and 4 μm cross-sections of vessels were cut and stained with hematoxylin-eosin or Azan-Mallory stains. Slides were viewed with a Nikon Optiphot microscope. The maximum intimal thickness of each tissue was measured.

**Statistical Analysis**

Data are presented as means±SD. Differences were compared using ANOVA, and differences between groups were evaluated by Student’s t-test. A p-value of <0.05 was considered significant.
RESULTS

Representative intravascular and M-mode echograms of the thoracic descending aorta are shown in Figs 1 and 2.

IVUS Findings

In the control and 7-week groups, IVUS images revealed a smooth intimal surface and a uniformly thick aortic wall with no sonolucent zone. However, in the 14-week group the thickness of aortic wall was not uniform and IVUS clearly revealed eccentric atheromatous plaques, associated with an obvious sonolucent zone in all animals.

M-mode Echogram Findings

![Graph showing the stiffness parameter (β) among the control, 7- and 14-week groups. The value of β in the 7- and 14-week groups was significantly higher than in the control group but did not differ between the groups receiving a high-cholesterol diet.]

The values of β in the 7-week and 14-week groups (4.7±2.2 and 4.6±0.9, respectively) were significantly higher (p<0.01) than in the control group (1.7±0.9). In contrast, the difference in β between the 7- and 14-week groups was not statistically significant (Fig 3). Differences in aortic dimension and pressure between the groups were not statistically significant (Table I).

Histological Findings

In the 14-week group, apparent intimal thickening was observed and the maximum thickness of the endothelium was 150±120μm. In contrast, in the 7-week group, no intimal thickening was observed or only a thin layer of foamy cells was present on the intima and the maximum thickness of the endothelium was 6.0±8.7μm (p<0.01 vs 14-week group) (Fig 4a and b).

DISCUSSION

The major finding in this study is that, during the early stages of atherosclerosis, aortic wall stiffness (sclerosis) increases before intimal atheromatous lesions (atherosis) develop.

The development of ultrasound techniques, including intravascular ultrasound, has enabled detailed investigation of aortic atherosclerosis. In addition, some investigators have reported that the severity of atherosclerosis in the coronary vascular bed is positively correlated with the degree of atherosclerosis in the aorta and other major arterial branches. Thus, it is possible that estimation of the severity of aortic atherosclerosis will allow us to forecast its severity in clinically important vessels, such

TABLE I BLOOD PRESSURE, AORTIC DIAMETER AND ITS STIFFNESS PARAMETER IN EACH GROUP

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>D_max (mm)</th>
<th>D_min (mm)</th>
<th>ΔD (mm)</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>118±26</td>
<td>104±27</td>
<td>4.8±0.9</td>
<td>4.4±0.9</td>
<td>0.38±0.09</td>
<td>1.7±0.9</td>
</tr>
<tr>
<td>7 weeks group</td>
<td>8</td>
<td>110±22</td>
<td>92±18</td>
<td>5.0±0.4</td>
<td>4.8±0.4</td>
<td>0.20±0.06*</td>
<td>4.7±2.2*</td>
</tr>
<tr>
<td>14 weeks group</td>
<td>8</td>
<td>117±20</td>
<td>98±20</td>
<td>4.4±0.8</td>
<td>4.2±0.8</td>
<td>0.17±0.07*</td>
<td>4.6±0.9*</td>
</tr>
</tbody>
</table>

*p<0.01 vs Control

Data are mean±SD. SBP: systolic blood pressure, DBP: diastolic blood pressure, D_max: maximum aortic dimension during ejection period, D_min: minimum aortic dimension, ΔD: D_max-D_min.
as the coronary artery.

IVUS imaging is a new, evolving technique in which a high-frequency transducer is inserted in the tip of a catheter. Using this device it is possible to obtain high-resolution images of vessel structure in real time and to gather valuable information about the extent of atherosclerotic plaques, in relation to the underlying tunica media and adventitia.  

We assessed atherosclerosis in rabbits using this method. In animals fed a high-cholesterol diet for 14 weeks, thickening of the intima-media complex could be observed with IVUS; however, this thickening was not observed in animals fed a high-cholesterol diet for 7 weeks. By comparing IVUS images with histological findings, we were able to measure the thickened intima in the 14-week group, and obtained a mean thickness of 150 μm. In the 7-week group the thin layer of foamy cells (the so-called fatty streak) that was apparent histologically could not be detected with IVUS, and the intimal thickness was approximately 20 μm.
The static mechanical properties of an artery can be expressed as its elasticity. Hayashi et al. suggested that there exists a simple exponential relation between arterial pressure and arterial radius within a physiologic blood pressure range. Based on this relation, they also proposed \( \beta \) as a parameter of arterial stiffness independent of the working range of blood pressure within the physiologic blood pressure range. Aortic elasticity is one of the basic indices for the evaluation of atherosclerosis. Ultrasound imaging has been used to evaluate local vessel sclerosis by measuring local vessel diameter change. In a previous study, we evaluated aortic wall stiffness using the value of \( \beta \) measured by transesophageal M-mode echography. In the experiment described here, we measured the instantaneous dimensional change in rabbit aorta by conventional M-mode echography and direct aortic pressure in order to calculate \( \beta \). This method revealed that impairment of vascular function occurred in the thoracic descending aorta not only in the 14-week group but also in the 7-week group, in which significant atheroma was not observed with IVUS.

It is well known that a cholesterol-rich diet causes atherosclerotic lesions in rabbit’s aorta. Hayashi et al. in an in vitro study, found that arterial stiffness and the elastic modulus of wall material were generally higher in high cholesterol-fed rabbits than in control rabbits. The majority of sclerotic vessels were not calcified but had reduced systolic expansion and abnormally rapid pulse wave propagation, which could be measured noninvasively. Farrar et al. determined pulse wave velocity in cynomolgus monkeys and showed that early atherosclerosis resulted in aortic stiffening that was revealed by increased pulse wave velocity before the development of significant stenotic lesions. Their results support our observations.

No data regarding the value of \( \beta \) in rabbit’s thoracic descending aorta have been published. Hirai et al. used echography to measure \( \beta \) in the abdominal aorta and common carotid artery in humans. Mean values of \( \beta \) obtained in normal control subjects aged 42–80 years were 8.58 for abdominal aorta and 9.17 for the common carotid artery. In our study, the mean value of \( \beta \) in control rabbits aged 4–8 months was 1.7 for the thoracic descending aorta, which is considerably lower than the value reported by Hirai et al. This difference may reflect species and age differences.

Motoi et al. measured instantaneous arterial circumference during a cardiac cycle in human using IVUS, and from this evaluated elasticity. In contrast, we measured the instantaneous dimensional change in rabbit aorta by conventional M-mode echography, because the frame rate of the IVUS system used in our study was not high enough to detect the maximum and minimum aortic dimensions during one cardiac cycle. The rabbit’s heart rate was so rapid (about 250–270 beats/min) that we could not measure the instantaneous change in aortic circumference during a cardiac cycle using an IVUS system with a frame rate of 15 frames/sec (900 frames/min).

In the present study, we found that the IVUS system could not detect the fatty streak that was apparent histologically in the 7-week group. However, at that time the stiffness parameter \( \beta \) of the descending aorta was already impaired, suggesting that evaluation of the vascular stiffness parameter may be more sensitive than morphological assessment of atherosclerosis in diagnosing very early atherosclerosis.

The present data revealed that fibrotic change or intimal thickening could not be detected histologically in the 7-week group even although wall stiffness was increased. It is not known why atherosclerosis is preceded by vascular sclerosis, although the two features are generally thought to progress in parallel. However, it has been reported that, in the presence of a high serum cholesterol level, medial smooth muscle cells change into smooth muscle cells containing non-muscle myosin before the smooth muscle cells migrate into the intima. This phenotypic change in the smooth muscle cells may play an important part in the development of vascular sclerosis before the formation of atherosclerosis. Egashira et al. reported that endothelium-dependent coronary vasomotion was impaired in patients with hypercholesterolemia, and that vasomotion was significantly improved by cholesterol-lowering therapy.

The impairment of endothelium-depen-
dent vasomotion in the presence of high serum cholesterol level may contribute to the development of aortic sclerosis before the formation of atheroma. Further studies are necessary to clarify the detailed mechanisms involved in the development of sclerosis.

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REFERENCES


