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Measurement of Atherosclerotic Plaque Volume in Hyperlipidemic Rabbit Aorta by Intravascular Ultrasound

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Abstract

Objectives. Atherosclerotic changes in the rabbit have been evaluated by various methods. Although most previous studies have analyzed atherosclerotic plaque in the femoral, carotid and iliac arteries of rabbits by intravascular ultrasound (IVUS) because of easier access, we established a method for the precise measurement of plaque volume as well as plaque area in the thoracic descending aorta in the Watanabe heritable hyperlipidemic (WHHL) rabbit, which has severe atherosclerosis.

Methods. WHHL and Japanese White (JW) rabbits were used. An IVUS catheter was inserted into the right femoral artery and advanced to the left subclavian artery, which was used as an anatomical landmark. After IVUS image acquisition, the catheter was removed. Vessel volume, lumen volume and plaque volume were analyzed.

Results. Atheroma of the aorta was easily detected in WHHL rabbits by IVUS examination, whereas atherosclerosis was not observed in JW rabbits. The atheroma showed a low-echoic lesion compared to the adventitia, with morphological characteristics similar to human lipid-rich, soft atheromatous plaques. In 15-month-old WHHL rabbits, the vessel volume, lumen volume and plaque volume in the thoracic descending aorta were 815 ± 109, 559 ± 107 and 256 ± 10 mm^3/3 cm, respectively.

Conclusions. We established a method for the precise quantitation of plaque volume by IVUS technology in WHHL rabbits aorta for the first time. This method is useful for evaluating several locally or generally delivered therapeutic agents in a hyperlipidemic animal model.

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Key Words

■ **Atherosclerosis (plaque volume)** ■ **Intravascular ultrasound**
■ **Experimental medicine (rabbits)**

INTRODUCTION

Intravascular ultrasound (IVUS) imaging provides cross-sectional views of the vessel lumen. Techniques for the direct evaluation of atheromas have advanced considerably in the clinic, particularly through the use of IVUS technology.[1] IVUS studies have clearly suggested that lipid-rich plaque is the major target of lipid-lowering therapy.[2,3] In addition, IVUS can be used to examine the structure of arterial walls and has thus become a standard imaging modality for the assessment of plaque size and composition before and after therapy.[4,5] Atherosclerotic lesions in the rabbit artery have been evaluated by IVUS in carotid or iliac arteries after injury or cholesterol-feeding, but not in rabbit aorta.[6–9] Although atherosclerotic lesions in cholesterol-fed rabbit abdominal aorta have been ana-

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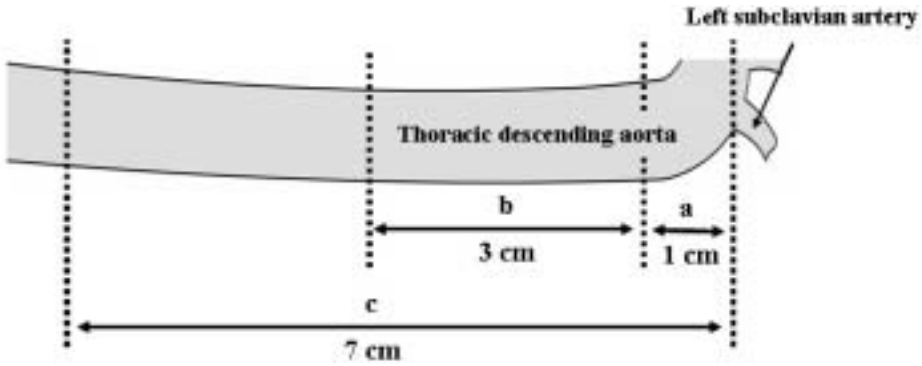

Fig. 1 Region of the rabbit thoracic descending aorta as assessed by intravascular ultrasound

lyzed by IVUS, only plaque area, but not plaque volume, was measured.[10,11]

The Watanabe heritable hyperlipidemic (WHHL) rabbit[12] shows a genetic reduction in low-density lipoprotein receptor function and exhibits hypercholesterolemia.[13] These characteristics are similar to human familial hypercholesterolemia. Since aortic atherosclerosis is observed on about 70% of the aortic surface in the 12-month-old WHHL rabbit and aortic lesions cover most of the aorta above 18 months of age, a method for measuring the plaque volume of the aorta may be useful for evaluating several locally or generally delivered lipid-lowering therapeutic agents.

Therefore, we evaluated atherosclerotic lesions with regard to plaque volume as well as plaque area in the WHHL rabbit, which shows morphological characteristics similar to those of human lipid-rich, soft atheromatous plaque using IVUS.

SUBJECTS AND METHODS

Animal preparation

WHHL and Japanese White (JW) rabbits weighing 2.8−3.2 kg were used for the study. Procedures involving animals and their care were performed in accordance with the guidelines described in the national animal protection law, and were approved by the Fukuoka University Internal Review Committee. Rabbits were anesthetized by intravenous injection of pentobarbital sodium (1 mg/kg) and intramuscular injection of xylazine (4 mg/kg).

IVUS evaluation of the lesion

After anesthesia, incision of the inguinal region was made, and then the right femoral artery was isolated and a ligature was placed around the vessel. The femoral artery was tied at the central side and tip side of the puncture lesion. A mechanical IVUS catheter (2.5F, 40 MHz; Boston Scientific) was inserted. The thread was loosened at the central side and the IVUS catheter was easily advanced to the left subclavian artery. The artery was identified and used as an anatomical landmark for IVUS examination. After being mechanically pulled back 1 cm (**Fig. 1**, position *a*), the probe was positioned about 3 cm over the thoracic aortic lesion (**Fig. 1**, position *b*) and then pulled back for a total distance of 7 cm (**Fig. 1**, position *c*). After IVUS image acquisition, the catheter was removed and the femoral artery was ligated. The wound was closed in a sterile fashion, and the animal was allowed to recover. Accordingly, we could later perform additional investigations by IVUS from the left femoral artery.

Assessment of IVUS

A mechanical IVUS system (ClearView, Boston Scientific) with 40 MHz piezoelectric transducers was used. Images are provided at 30 frames/sec. The ultrasonic transducer was withdrawn inside a steady external sheath (diameter 2.5F = 0.83 mm) using a motorized pullback device at a constant speed of 0.5 mm/sec. All IVUS examinations were recorded on high-resolution super-VHS videotape for later off-line quantitative analysis. Analysis of the IVUS images was performed by an independent observer using computerized planimetry (TapeMeasure, Indec Systems). Cross-sectional areas (CSA) of the lumen and vessel (which is a reproducible measure of the total arterial CSA) were measured at the point of maximal plaque formation. Since media thickness cannot be measured accurately, plaque plus media CSA was used as a measure of the plaque CSA and calculated as exter-

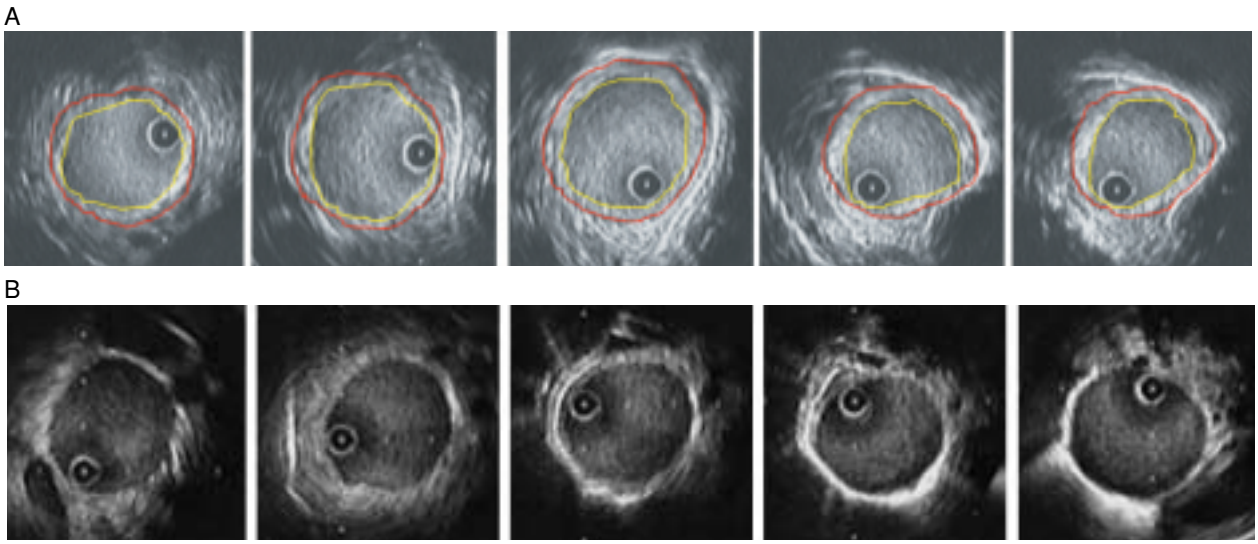

Fig. 2 Intravascular ultrasound images
Intravascular ultrasound images show representative pictures of WHHL (*A*) and JW (*B*) rabbit thoracic descending aorta. An atheroma is observed in the WHHL rabbit, whereas no atherosclerotic change is observed in the JW rabbit. The plaque of the aorta is shown in the space between the *red* and *yellow lines*.

nal vessel CSA minus lumen CSA.

Longitudinal reconstructions of IVUS images were performed using Echo Plaque 3.0 software (Indec Systems).[14] Three-dimensional images were obtained by manual tracing of the lumen area and vessel area; a series of cross-sections of ultrasonographic images was selected exactly 1.0 mm apart along the long axis of the vessel. IVUS analyses included the vessel volume, lumen volume, and plaque volume. Plaque area or plaque volume was calculated as vessel area minus lumen area or vessel volume minus lumen volume, respectively. Measurements were performed in accordance with the standards of the American College of Cardiology and the European Society of Cardiology.[15]

RESULTS

IVUS image of atherosclerosis in WHHL rabbits

Fig. 2−A shows atheroma of the aorta in WHHL rabbit. The lesioned arterial segments show progressive changes, 'soft lipid-rich plaque', with low-echoic lesions compared to the adventitia. Atheroma of the aorta had morphological characteristics similar to those of human lipid-rich, soft atheromatous plaque. On the other hand, atherosclerosis was not observed in JW rabbits (**Fig. 2−B**). In this way, the presence of atherosclerotic lesions was easy to detect, and non-atherosclerotic aorta was also clearly detectable. **Fig. 3** shows the longitudinal reconstruction of IVUS images of the WHHL rabbit aorta. The result was a high-quality image that was suitable for volumetric analyses. After IVUS observation, the area and volume in thoracic descending aorta of WHHL rabbits were determined, as shown in **Table 1**. The vessel volume, lumen volume and plaque volume in 15-month-old WHHL rabbits were 815 ± 109, 559 ± 107 and $256 \pm 10\,mm^3$/3 cm, respectively.

DISCUSSION

IVUS is recognized as the gold standard for imaging of atherosclerosis. IVUS technology is widely used in human peripheral and coronary arteries.[1,4,5] Recent advances in catheter technology have facilitated IVUS examination in vessels of 2 mm in diameter and make this method feasible for imaging vessels in small animals. Although few studies have analyzed plaque of the rabbit aorta by IVUS[10,11] because difficulties have been encountered in advancing the guide wire and imaging catheter through the diseased femoral artery of WHHL rabbits, we established a method for the precise measurement of plaque volume in WHHL rabbit aorta in this study.

Aortic atheroma could be detected in WHHL rabbits by IVUS examination, but atherosclerosis was not observed in JW rabbit aorta. We could easily evaluate the presence of atherosclerotic or non-atherosclerotic lesions in rabbit aorta. The technical

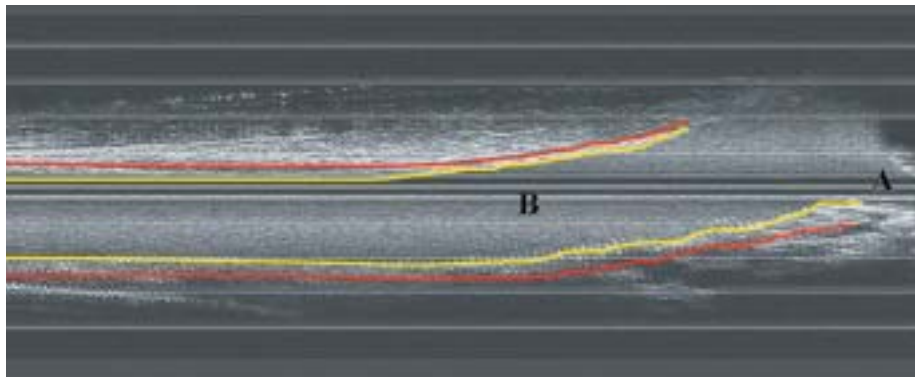

Fig. 3 Longitudinal reconstruction of an intravascular ultrasound image
This high-quality image was suitable for volumetric analyses. Site A and site B indicate the left subclavian artery and the thoracic descending aorta, respectively. The left subclavian artery is used as an anatomical landmark for intravascular ultrasound examination. *Red line* indicates the media of the aorta. The plaque of the aorta is shown in the space between the *red* and *yellow lines*.

Table 1 Intravascular ultrasound variables in the WHHL rabbit aorta

Two-dimensional variables	
Vessel area (mm^2)	33.6±4.9
Lumen area (mm^2)	21.4±4.9
Plaque area (mm^2)	12.2±1.2
Three-dimensional variables	
Vessel volume (mm^3/3 cm)	815±109
Lumen volume (mm^3/3 cm)	559±107
Plaque volume (mm^3/3 cm)	256±10

Values are mean±SD.

and scientific advantages of the present experimental model mainly consist of the possibility of monitoring the same lesion in the aorta over time *in vivo*. Analysis of all parameters of the IVUS measurement should make it possible to evaluate the effects of different drug treatments on plaque volume or composition without sacrificing the animals. Although magnetic resonance imaging (MRI), which is a powerful system for assessing vascular wall structure,[16,17] is a non-invasive alternative to IVUS, MRI is difficult to apply to small animals and is expensive.

Study limitation

A limitation of the present study is the potential sources of error in the IVUS measurements. This may be related to the encroachment of the IVUS catheter in small vessels, which may result in vessel stretching and, consequently, the overestimation of volume. This may occur in very severe stenosis.

CONCLUSIONS

We successfully established a method for the precise quantitation of plaque volume by IVUS technology in WHHL rabbit aorta for the first time. Moreover, this method is a good strategy for evaluating several locally or generally delivered therapeutic agents in a hyperlipidemic animal model.

要　　約

血管内エコー法による高脂血症ウサギの大動脈プラーク体積測定法

岩田　敦　三浦伸一郎　今泉　聡　張　波　朔　啓二郎

目　的: ウサギの動脈硬化性変化はさまざまな方法により測定されている．以前の多くの研究では，血管内エコー法(IVUS)によりアクセスが容易という理由からウサギ大腿・頸・腸骨動脈を評価の対象としていた．今回，我々は，重症の動脈硬化を起こすワタナベ遺伝性高脂血症(WHHL)ウサギの胸部下行大動脈プラーク面積のみでなく，プラーク体積も正確に測定できる方法を確立する

こととした．

方　法: WHHLウサギと日本白色(JW)ウサギを使用した．IVUSカテーテルを右大腿動脈より解剖学的マーカーである左鎖骨下動脈へ進めた．IVUSイメージを取り込み，カテーテル抜去後，血管体積，内腔体積，プラーク体積を解析した．

結　果: IVUSによりWHHLウサギの動脈硬化は容易に描出できたが，JWウサギの場合はできなかった．その動脈硬化巣は，ヒトでみられる脂質リッチな柔らかいプラークのような特徴を持ち，外膜と比較すると低エコー域として認められた．15ヵ月齢のWHHLウサギ胸部下行大動脈の平均血管体積，内腔体積とプラーク体積は，それぞれ815±109，559±107と256±10 mm^3/3 cmであった．

結　論: WHHLウサギ胸部下行大動脈の正確なプラーク体積の測定方法を初めて確立した．この方法は，高脂血症動物モデルにおいて局所や全身投与による薬効評価に有用である．

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