In Vivo Plaque Characterization Using Intravascular Ultrasound–Virtual Histology in a Porcine Model of Complex Coronary Lesions

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Objective—To determine the accuracy of detection of different tissue types of intravascular ultrasound–virtual histology (IVUS-VH) in a porcine model of complex coronary lesions.

Methods and Results—Coronary lesions were induced by injecting liposomes containing human oxidized low-density lipoprotein into the adventitia of the arteries. IVUS-VH imaging was performed in vivo at 8.2±1.6 weeks after injection. A total of 60 vascular lesions were analyzed and compared with their correspondent IVUS-VH images. Correlation analysis was performed using linear regression models. Compared with histology, IVUS-VH correctly identified the presence of fibrous, fibro-fatty, and necrotic tissue in 58.33%, 38.33%, and 38.33% of lesions, respectively. The sensitivity of IVUS-VH for the detection of fibrous, fibro-fatty, and necrotic core tissue was 76.1%, 46%, and 41.1% respectively. A linear regression analysis performed for each individual plaque component did not show strong correlation that would allow significant prediction of individual values.

Conclusions—In a porcine model of complex coronary lesions, IVUS-VH was not accurate in detecting the relative amount of specific plaque components within each individual corresponding histological specimen. (Arterioscler Thromb Vasc Biol. 2007;27:387-393.)

Key Words: animal model ■ intravascular ultrasound ■ vulnerable plaque

Complex atherosclerotic plaques containing specific morphological features appear to be responsible for the majority of major coronary events. Therefore, the accurate identification and characterization of these lesions has become one of the major challenges of interventional cardiology. Several catheter-based imaging devices designed and built to characterize such features are currently undergoing development. Mainly because of the lack of a large animal model of atherosclerotic vascular disease, the initial development of these technologies is based on the construction of mathematical algorithms based on data acquired from ex vivo evaluation of postmortem human arterial specimens. Therefore, the translation of these algorithms of detection into an in vivo setting may not be universally accurate.

Virtual histology is an intravenous ultrasound (IVUS)-based technology that studies the spectral analysis of the radio frequency signals backscattered from the plaque during IVUS imaging and reconstructs the morphology of the plaque through color-coded maps. Although this technique has been validated in vitro and ex vivo in human coronary arteries and preliminary clinical experience has been published, little data have been published on histological correlates of images acquired in vivo. In the present study, we describe the histological characteristics of complex coronary lesions analyzed in vivo using intravascular ultrasound–virtual histology (IVUS-VH) in a porcine model of disease.

Methods

Experimental Animal Model

The local institutional animal care and ethics committee approved the protocol related to this study. All animals were obtained from the M.D. Anderson Cancer Center Science Park in Bastrup, Texas, and received humane care in compliance with the Animal Welfare Act and the “Principles of Laboratory Animal Care” formulated by the Institute of Laboratory Animal Resources (National Research Council, revised 1996). A total of 5 purpose-bred pigs initially underwent balloon overstretching (Maverick angioplasty balloon with length of 20 mm) at a 1:1.3 ratio and were maintained on a high-cholesterol diet (2% cholesterol, 20% lard, and 1.5% sodium cholate). Two weeks after the initial intervention, intra-mural delivery of lipids was performed in 2 major epicardial coronary arteries in 3 different but adjacent anatomic locations (30 injected segments, 10 arteries) as previously described. The atherogenic mixture consisted of a liposome-based formulation of cholesterol esters and human ox-
dized low-density lipoprotein 1. For the endovascular delivery of the lipids we used a microsyringe infusion catheter (Mercator MedSystems, San Leandro, Calif). After coronary angiography, the injection site was selected after IVUS analysis of the coronary artery. The lipemic solution was loaded into a syringe and connected to the injection lumen of the catheter. The catheter was placed over a coronary wire in the chosen segment and the intramural delivery of the atherogenic mixture was performed over a 20-mm arterial segment. Quantitative coronary angiography (Quartus; Siemens Medical Imaging) was performed at baseline and immediately before termination. After the procedure, all pigs were returned to the recovery rooms and maintained on a high-cholesterol diet for 7 to 10 weeks after injection.

IVUS Imaging Analysis

All IVUS images were acquired using a 20-MHz Volcano Eagle Eye™ IVUS catheter (Volcano Therapeutics Inc, Rancho Cordova, Calif). At follow-up, systemic anticoagulation using heparin and intracoronary nitroglycerine was used before imaging. Once the coronary lesion was identified, the IVUS catheter was inserted distal to the lesion and manually pulled back to assess the severity and length of the lesion. The IVUS catheter was then placed distal to a side branch (distal fiduciary landmark site) and automatic pullback was performed at a rate of 1 mm/sec. The location of the IVUS catheter was determined using continuous fluoroscopy during the entire pullback time and by recording anatomic landmarks seen during IVUS imaging. To achieve adequate images, an average of 2 pullbacks per artery was performed and the best play loop was chosen based on imaging resolution and quality. Continuous EKG monitoring was performed during the procedure to gate IVUS frames for analysis. A complete morphometric analysis was performed in 532 independent IVUS frames using standard definitions. All measurements were automatically derived from the Volcano Invision Gold imaging system software.

IVUS-VH data were recorded to the imaging system hard drive and then extracted and archived for analysis. The analysis was based on border contour calculation from gray scale. The same tissue maps provided by the software (dark green = fibrous, light green = fibro-fatty, red = necrotic core and white = dense calcium) were used to analyze each independent frame. Once the total length of the lesion was determined, a 20-mm vascular segment containing the vascular lesion was selected for analysis. This segment was then divided in equal 2.0-mm subsections, generating a total of 10 series of cross-sections per vascular segment.

Gray level median of echogenicity analysis was performed by visually and mathematically matching histological slides with the same gray scale IVUS frames used for VH analysis. The gray scale intensities of the plaque (external elastic lamina area = lumen area) were recorded and a gray scale histogram produced. The histogram was divided into tertiles corresponding to the lower, mid, and upper echogenic regions within the IVUS image. These regions were then color coded (dark green = fibrous, light green = fibro-fatty, red = necrotic core and white = dense calcium) and compared with all 3 different tissue types. To determine the sensitivity of detection of the technique, each detected component was compared with the corresponding histological slide after the same methodology used for the IVUS-VH analysis.

All IVUS frames were analyzed independently by 2 observers and were used for comparison with corresponding histological sections. These observers performed IVUS quantitative analyses for each cross section independently (interobserver variability). In case of disagreement, the observers reanalyzed the IVUS image and reached a consensus diagnosis. The observers then repeated a blinded analysis after an interval of 2 weeks (intraobserver variability).

Histological Analysis

At last follow-up, each pig was euthanized, heart was retrieved, and the coronary circulation was anterogradely perfused with 1 L of heparinized normal saline at 100 mm Hg to remove residual blood. Individual coronary arteries were excised and the matching fiduciary distal landmark macroscopically identified. Sequential 2-mm sections were obtained starting at the same anatomic position in which IVUS pullback was started (distal to proximal) in a 20-mm arterial segment. A total of 10 corresponding histologic blocks (2-mm-thick) were generated and placed in individual histology cassettes labeling their orientation and landmarks for future reference. The tissue was then preserved frozen in liquid nitrogen (LN) and sent for histological staining. Serial 5-μm sections were cut from each histological block and stained with hematoxylin and eosin, Oil-red-O, Masson trichrome/elastin stain, and Movat pentachrome. Immunohistochemical staining was performed using a myeloperoxidase-labeled streptavidin–biotin method from Neomarkers (Fremont, Calif) to detect the presence of macrophages. Sections were also immunolabeled with an anti-factor VIII antibody followed by a standard avidin-biotin peroxidase complex assay Vector ABC Elite Kit (Vector Laboratories) to stain for neovessels. Slides were developed with DAB (Zymed Laboratories) and counterstained with 10% hematoxylin. After staining, 24-bit, full-color digital images were acquired at a magnification to include the entire vessel cross-section (2×) using an Olympus U-CMAD3 camera (Melville, NY) attached to an Olympus BX41 microscope (Melville, NY) at a resolution of 1024×768 pixels. Morphometric analysis was performed using Image-Pro plus 4.5 (Media Cybernetics, Silver Spring, Md). The characterization of each individual morphological component was performed using standard definitions previously published. Areas of densely packed collagen were labeled as fibrous and those with significant lipid interspersed in collagen were labeled as fibro-lipidic. Necrotic regions were defined as areas of the plaque not occupied by collagen and containing cholesterol clefts, foam cells, and micro-calculifications. Calcium deposits without adjacent necrosis were identified as calcium. The individual total plaque area and individual plaque components were recorded for each individual histological slide and the sum of all of the components matched to the total plaque area.

Statistical Analysis

The data are presented as mean±SD for parametric data. Standard statistical methods were used by using SigmaStat 3.1 software for comparison between the groups. Correlation between histology and virtual histology values were evaluated by using linear regression models. The degree of agreement between the inter-observer and intra-observer variability were quantified using linear regression models and Bland-Altman plots.

Results

Demographic and Angiographic Data

A total of 30 coronary segments (15 in right coronary arteries and 15 in left anterior descending arteries) were injected in 5 pigs that were maintained on a high-cholesterol diet for a total of 8.2±1.6 weeks. The mean total cholesterol level achieved during the follow-up period was consistently between 300 to 400 mg/dL, among all animals. All arterial segments were followed angiographically at last follow-up. There was no evidence of ulceration, calcification, or thrombus formation on any of the lesions. Quantitative coronary angiography showed a mean percentage diameter stenosis of 13.7%±5% and a mean lesion length of 16.6±6.9 mm.

In Vivo IVUS-VH Analysis

IVUS was performed in vivo in all the lesions at baseline, 2 weeks, and at euthanization. Both intra-observer and inter-observer variability were minimal and within acceptable limits for the detection of luminal and media adventitial boundaries as shown in Figure 1. At last follow-up, the mean IVUS pullback length analyzed was 27.5±6.2 mm. IVUS classified these lesions as eccentric, echolucent, and with no evidence of calcification. These lesions were consistently
non-occlusive (41.3\% \pm 8.0\% plaque burden) and located in remodeled vessels (remodeling index, 1.01 \pm 0.08). The mean vessel area was 16.7 \pm 3.0 \text{mm}^2 and the mean plaque area was 6.9 \pm 1.9 \text{mm}^2. IVUS-VH analysis categorized these lesions as having large amount of fibrous tissue (mean: 55.5\% \pm 22.4\%) and lesser amounts of fibro-lipidic (mean, 11.4\% \pm 10.7\%), necrotic (mean, 17.7\% \pm 13\%), and calcific (mean, 14.4\% \pm 16\%) tissue.

**Histological Evaluation of Vascular Lesions**

A total of 60 coronary slides were included in the histological analysis. The mean plaque area (0.99 \pm 0.59 \text{mm}^2 versus 0.05 \pm 0.03 \text{mm}^2; P<0.01) and the mean percentage of plaque area (58.9\% \pm 14\% versus 7.5\% \pm 4.9\%; P<0.01) were significantly different compared with noninjected segments. All lesions were classified as eccentric and fibro-lipidic vascular lesions (mean percentage area for fibrous tissue 40.3\% \pm 12.8\% and 16.8\% \pm 8.19\% for fibro-lipidic). The presence of lipid deposits colocalized with the presence of tissue macrophages (mean macrophage count per histological slide was 32.7 \pm 13.4) and adventitial neovascularization (mean count per plaque 33.1 \pm 12.8). There was no evidence of intra-plaque hemorrhage or calcium deposits in any of the lesions (Figure 2).

**Characterization of Plaque Components by VH**

A histology–IVUS-VH correlation analysis was performed in 60 vascular lesions. Because none of the histological sections showed evidence of significant calcification, the correlation analysis was performed for all 3 remaining tissue types. IVUS-VH correctly identified the presence of fibrous tissue in 35 of 60 lesions (58.3\%), fibro-fatty tissue in 23 of 60 lesions (38.3\%), and necrotic core tissue in 23 of 60 lesions (38.3\%). The sensitivity of IVUS-VH for the detection of fibrous, fibro-fatty, and necrotic core tissue was 76.1\%, 46\%, and 41.1\%, respectively. A linear regression analysis performed for each individual plaque component did not show strong correlation that would allow significant prediction of individual values (Figure 3). Similar analysis performed using gray level median of echogenicity demonstrated an improved sensitivity for detection of different plaque components to 86.9\%, 79.5\%, and 89.7\% for fibrous, fibro-fatty, and necrotic core tissue, respectively (Figure 4).

**Discussion**

The main objective of this study was to determine the accuracy of detection of different tissue types by IVUS-VH in a porcine model of complex coronary lesions. In this model, lipid-rich atherosclerotic lesions are induced by delivering a mixture of cholesterol esters and human oxidized low-density lipoprotein into the adventitia of coronary arteries using an endovascular microsyringe infusion catheter. Longitudinal follow-up using IVUS has shown that the lesions are fully identifiable within 4 weeks after the delivery of lipids.\textsuperscript{18,22} By 7 weeks, histological sections of these plaques show progres-
sion of the lesions with evidence of focal lipid deposition and intra-plaque macrophage infiltration.\textsuperscript{22,23} In this study, at 10 weeks, the lesions formed were eccentric, contained a prominent fibro-lipidic component (>10\% of area), significant amount of intraplaque macrophage infiltration (>25 macrophages per field), and abundant adventitial vasa vasorum formation when compared with the control sites. No evidence of significant calcification was seen in any of the analyzed lesions. A very important feature seen in our model is the ability to consistently reproduce these lesions. In general, all the lesions had a similar size, length, and histological composition, which allow the reliable comparison of data acquired during IVUS imaging.

Our study demonstrated that conventional gray scale IVUS was capable of identifying and quantifying all vascular lesions at 10 weeks. Using the same IVUS frames used for the IVUS-VH analysis, gray level median of echogenicity analysis was able to improve the characterization of all plaque components with an acceptable degree of accuracy. IVUS-VH analysis of the same images acquired in vivo consistently detected fibrous tissue as the most prevalent tissue type found in these lesions (mean, 55.5\% ± 22.4\%). Also, similar proportions of fibro-lipidic (mean, 11.4\% ± 10.7\%), necrotic (mean, 17.7\% ± 13\%), and calcific (mean, 14.4\% ± 16\%) tissue were also detected by IVUS-VH. Although the sensitivity of IVUS-VH for the detection of fibrous tissue was high, the specificity was poor because of high false-positive rates. Specificity of IVUS-VH for detection of fibro-fatty plaque was 80\% and sensitivity was 46\%. Sensitivity and specificity for detection of necrotic core were both modest at 41.1\% and 50\%, respectively. In addition, the presence of calcium was consistently detected despite the fact this component was absent in all histological specimens (Figure 5). A linear regression analysis between IVUS-VH and histology showed that in our model, IVUS-VH was not accurate in detecting the area of each specific component within the corresponding histological specimen. Therefore, in our study, sensitivity and specificity of IVUS-VH in detecting different tissue types were not as robust as had been validated in previous ex vivo studies.

Our findings may have significant implications in regard to the methodology currently used to validate catheter-based plaque detection technologies. Most of the preliminary development of algorithms for the detection and characterization of atherosclerotic plaques is performed using animal models of disease.\textsuperscript{6,24,25} It is possible that because of the biological differences that exist between animals and humans, the different tissue types contained in animal atherosclerotic lesions may not be similar to the lesions seen in human disease. However, distinct histological patterns such as the formation of fibrotic tissue or fibro-lipidic tissue do not differ much between species, and many other catheter-based technologies have been validated on the detection of these specific components in porcine models of disease.\textsuperscript{22,26} The inherent technological limitations of IVUS may have played an important role in our findings. It is possible that the early vascular lesions seen in our model could not be fully characterized because of the limited resolution of IVUS. In our experiments, although vascular lesions were identified in all the cases, it is possible that some of the plaque components may have been difficult to discern because of insufficient complexity of the lesions. However, the degree of...
A major limitation needing to be addressed is the applicability of data that have been validated in ex vivo human tissue in detecting plaque components in an in vivo environment. Although standard definitions were used to analyze the data, the proper histological definitions that need to be included in the process of imaging validation have not been adequately defined. In addition, the potential artifacts in size and morphology created by handling and processing ex vivo samples needs to be taken into consideration at the time of tissue analysis. It is also theoretically possible that the

Figure 3. Linear regression analysis. Percentage plaque composition comparison of histology vs IVUS-VH.
acoustic signals acquired in an already processed tissue are different to the signals acquired in vivo, making the translation of data problematic.

In summary, to our knowledge, the present study represents the first in vivo correlation study of IVUS-VH with histology in an animal model of complex coronary lesions. In our study, IVUS-VH was not able to accurately detect the area of each specific component within the corresponding histological specimen. These findings highlight the importance of unifying criteria for histological definitions and animal model development. The development of an animal model with atherosclerotic lesions that share similar morpho-

Figure 4. Plaque characterization based on gray level median of echogenicity. A, VH analysis of one of the plaques in vivo. B, The same frame displayed analyzed using gray scale median values. C, The distribution of gray scale intensities within the plaque. D, The sensitivity of detection of different tissue components by this technique.

Figure 5. Plaque harvested from the left anterior descending artery in a pig at 10 weeks of follow-up. A, Movat pentachrome section (2×) showing a fibrolipidic plaque with no evidence of calcification. B, Corresponding IVUS-VH image acquired in vivo showing significant amount of necrotic core and calcification.
logical features with human disease remains key for the further development of catheter based imaging modalities.

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Disclosures
None.

References
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