Characterization of Lipid-Rich Aortic Plaques by Intravascular Photoacoustic Tomography Ex Vivo and In Vivo Validation in a Rabbit Atherosclerosis Model With Histologic Correlation

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ABSTRACT

BACKGROUND Histologic studies have demonstrated that lipid content and its spatial distribution is related to plaque vulnerability. However, in vivo imaging is still limited. Photoacoustic imaging may provide novel in vivo insights into these lipid-rich plaques.

OBJECTIVES This study sought to examine whether intravascular photoacoustic tomography (IVPAT) allows localization and quantification of lipid content in atherosclerotic plaques.

METHODS Rabbits fed with a high-fat/high-cholesterol diet served as the atherosclerotic model. Catheter-based IVPAT was used to evaluate pixel-based lipid relative concentration (LRC) of the vessel wall. The aorta of 4 groups of rabbits (n = 12) were examined ex vivo with IVPAT after 0, 5, 10, and 15 weeks of a high-fat diet, respectively. Six rabbits underwent 3-dimensional (3D) IVPAT after 20 weeks of the high-fat diet. Three rabbits were examined in vivo using IVPAT without interruption of blood flow. Concentration-based lipid map and quantitative index were calculated. For subsequent histologic correlation, all specimens were evaluated with Oil Red O staining.

RESULTS Cross-sectional LRC maps allowed visualization of concentration and depth information of lipid content in the atherosclerotic plaques. Lipid accumulation within plaque, assessed by the maximum LRC, mean LRC, and high lipid content area correlated to duration of a high-fat diet. Three-dimensional LRC maps enabled overall evaluation of focal plaques in an intact explanted aorta including spatial and structural features. In vivo-obtained LRC maps accurately showed the structure of lipid core with high contrast. Ex vivo and in vivo IVPAT results were highly consistent with histological results.

CONCLUSIONS In an animal model, IVPAT allowed characterization of spatial and quantitative features of lipid-rich plaques. (J Am Coll Cardiol 2014;64:385–90) © 2014 by the American College of Cardiology Foundation

Histological studies have demonstrated that the primary cause of acute cardiovascular events is the rupture of atherosclerotic plaques. Lipid content and its distribution within the plaque influence the propensity of plaques to disrupt flow (1,2). Based on this hypothesis, a series of pivotal clinical trials have proven that lipid-lowering therapy can reduce cardiovascular morbidity and mortality (3,4). However, current intravascular imaging techniques have inherent limitations for characterizing lipid content within atherosclerotic plaques. Thus, there is a need for a method that enables an in situ
quantitative and spatial characterization of lipid content in the vessel wall (5).

As a hybrid imaging technique, photoacoustic tomography provides the volumetric images of tissues with optical contrast and ultrasonic resolution by reconstructing the detected photoacoustic signal (6–8). Intravascular photoacoustic tomography (IVPAT) has great potential for meticulous in vivo examinations of atherosclerotic changes in the vessel wall. Previous studies have demonstrated feasibility of plaque visualization (9–11) with this modality. More recent work has focused on detecting lipid-rich components in atherosclerotic plaques (12,13). However, quantitative and spatial characterization of the lipid content has been incomplete.

In an animal model, we therefore examined the feasibility of a catheter-based IVPAT imaging platform to construct 3-dimensional (3D), concentration-based maps of lipid components in atherosclerotic plaques (Central Illustration). A series of ex vivo and in vivo experiments were performed with subsequent histologic correlation.

METHODS

ANIMALS. The Animal Study Committee at South China Normal University College of Biophotonics in Guangdong, China, approved all animal procedures. New Zealand white rabbits (male, age 3 months, weight 2.3 to 2.8 kg) served as the experimental model of atherosclerosis. Atherosclerotic changes were induced with a high-fat/high-cholesterol (HFC) diet (97% normal chow, 2% lard, and 1% cholesterol).

IVPAT. Figure 1 describes the experimental setup of the IVPAT system and the lipid imaging process. The current system had an approximate 100-μm axial resolution, an approximate 380-μm transverse resolution and more than 2 mm of imaging depth (Online Fig. 1). The system was operated via a custom-made LabVIEW program (National Instruments, Austin, Texas). MATLAB software (Mathworks, Natick, Massachusetts) was used for image construction and index measurement.

EXPERIMENTAL PROTOCOL. In the first set of experiments, IVPAT was used to monitor the development/progression of induced atherosclerosis in 4 groups of rabbits (n = 12) after 0, 5, 10, or 15 weeks of the HFC diet. In subsequent experiments, intact aortas of 6 rabbits were imaged over the entire length after 20 weeks of HFC feeding to obtain 3D lipid relative concentration (LRC) maps. In these experiments, imaging was performed ex vivo, after the animal was sacrificed. Lastly, the aortas of 3 rabbits were examined in vivo at 20 weeks (n = 1) or 25 weeks (n = 2) with IVPAT and magnetic resonance imaging, before sacrificing the animal, as shown in Figure 2. (See the Online Appendix for additional information.)

Following IVPAT imaging, all specimens were evaluated with en-face Oil Red O staining or cross-sectional Oil Red O staining. Maximum LRC, mean LRC, and high lipid content area in the LRC maps were measured by analyzing their entire units with MATLAB. A detailed description of the lipid-imaging process and the experimental protocol is provided in the Online Appendix.
STATISTICAL ANALYSIS. Data are presented as mean ± SD. Correlation between IVPAT and histology was tested with linear regression analysis using Origin (OriginLab Corporation, Northampton, Massachusetts). Bland-Altman tests (14) were performed to determine the agreement between them with GraphPad Prism (GraphPad Software, La Jolla, California).

RESULTS

Our first goal was to assess diet-induced atherosclerosis development or progression as monitored by ex vivo IVPAT versus histology. Figure 3 shows representative LRC maps at the 4 time points (after 0, 5, 10, and 15 weeks on the HFC diet), respectively. Figure 3A demonstrates a low lipid aortic...
FIGURE 3 Diet-Induced Atherosclerosis Development/Progression Monitored by Ex Vivo IVPAT Versus Histology

Representative results of lipid accumulating and arterial wall remodeling in relation to the duration of high-fat diet monitored by IVPAT and histology: 0 weeks (A, B); 5 weeks (C, D); 10 weeks (E, F); or 15 weeks (G, H). Normalized image intensity of LRC maps enabled a quantitative comparison. The scale bar is 1 mm. (I) High lipid area in the aortic wall, measured by IVPAT and histology, was plotted against duration of high-fat diet. (J) Bland-Altman tests for all results (n = 12). HFC = high fat/high cholesterol; other abbreviations as in Figure 1.

Our data demonstrate the feasibility of in vivo IVPAT for characterization of spatial and quantitative features of lipid-rich plaques. Compared with other intravascular plaque imaging modalities, the advantage of IVPAT is the combined utilization of light and sound wave features for image generation.

The theoretical advantage of IVPAT can be derived from the limitations of other imaging modalities, specifically intravascular ultrasound (IVUS), sound wave based) and near-infrared spectroscopy (light wave based) (15). Grayscale IVUS has a high imaging depth that enables a full-field evaluation of

Histology (Figs. 3B, 3D, 3F, and 3H) demonstrated the same characteristics. Both the linear regression analysis ($r = 0.978$, $p < 0.0001$) and the Bland-Altman tests (Fig. 3J) verified excellent correlation between IVPAT and histology.

Next, we sought to assess the characterization of entire length atherosclerotic aortas by ex vivo 3D IVPAT. Longitudinal 3D LRC map (Fig. 4A, Online Video 1) enabled an overall evaluation of an intact aorta affected by large-area plaques after 20 weeks of HFC feeding. Corresponding en-face 3D LRC map (Fig. 4B, Online Video 2) demonstrated a positive relationship between the lipid accumulation and the intima thickening. The en-face histology (Fig. 4C) verified the nonuniform distribution. Furthermore, cross-sectional LRC maps (Figs. 4D and 4E) could visualize lipid components within plaques with content and depth information. Linear regression analysis ($r = 0.972$, $p < 0.0001$) as well as Bland-Altman tests of the other 3 aortic segments (Fig. 4F) demonstrated a high degree of coherence between 3D IVPAT and continuous frozen sections (Online Fig. 4).

LIPID IMAGING BY IN VIVO IVPAT. Magnetic resonance imaging visualizes plaque morphology (Figs. 5A, 5D, and 5G), but it was insufficient to specify lipid content within plaques. IVPAT obtained high-contrast LRC maps at the same imaging planes (Figs. 5B, 5E, and 5H), which simultaneously showed content and distribution of lipid within the plaques. The whole boundary of the lipid-rich intima could be distinguished, which matched well with the histology (Figs. 5C, 5F, and 5I). Further measurements demonstrated that all 3 intima had $>95\%$ maximum LRC and $>40\%$ mean LRC. Although the blood flow was kept during the data acquisition, IVPAT probe could be located by x-ray (Fig. 5J). The relative error between IVPAT and histology was approximately 10% (Fig. 5K).

DISCUSSION

TABLE 1

<table>
<thead>
<tr>
<th>Location</th>
<th>Intima</th>
<th>Adventitia</th>
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<tbody>
<tr>
<td></td>
<td>Max</td>
<td>Mean</td>
</tr>
<tr>
<td>Time</td>
<td>0 Weeks</td>
<td>5 Weeks</td>
</tr>
<tr>
<td>0 Weeks</td>
<td>30.7 ± 5.40</td>
<td>63.3 ± 14.30</td>
</tr>
<tr>
<td>5 Weeks</td>
<td>6.24 ± 2.50</td>
<td>10.43 ± 6.30</td>
</tr>
<tr>
<td>10 Weeks</td>
<td>29.5 ± 1.90</td>
<td>33.2 ± 3.60</td>
</tr>
<tr>
<td>15 Weeks</td>
<td>5.52 ± 1.30</td>
<td>6.12 ± 2.10</td>
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</tbody>
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Values are mean ± SD.
HFC = high fat/high cholesterol; LRC = lipid relative concentration.
plaques with large lipid cores. And virtual histology IVUS can distinguish plaque components. However, detailed assessment of lipid content is limited (16,17). Near-infrared spectroscopy provides several valuable indexes for evaluating lipid content (18,19). However, near-infrared spectroscopy is an en-face imaging mode that only enables the evaluation of lipid from a 2-dimensional image plane (endothelial surface), without depth information.

As a hybrid imaging technique, IVPAT integrates the advantages of optical contrast and ultrasonic resolution (20), which can simultaneously demonstrate the spatial distribution and relative concentration of lipid content in atherosclerotic plaques along entire vessel segments. Furthermore, the unique combination of the optical and ultrasonic components suggests prospective multiple-imaging modalities with IVUS or optical coherence tomography (21) for comprehensively evaluating plaque structures (e.g., calcification, arterial wall remodeling, and fibrous cap).

**STUDY LIMITATIONS.** First, the transverse resolution was not satisfactory, because the laser spot of this optical fiber was relatively large. Second, the repetition frequency of the laser used was 10 Hz, so that

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**FIGURE 4 3D Imaging of Lipid Within Intact Atherosclerotic Aortas by IVPAT**

(A) A longitudinal 3D LRC map of an intact atherosclerotic aorta (20 weeks HFC diet feeding). (B) Corresponding en-face 3D LRC map viewed from inside of the aorta over a 360° field. (C) En-face histology. (D and E) Cross-sectional LRC maps and corresponding histology located at 0 mm and 12.5 mm. (F) Bland-Altman tests of high lipid content area (n = 58) measured from cross-sectional LRC maps and histology. See Online Videos 1 and 2. Abbreviations as in Figures 1 and 3.

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**FIGURE 5 In Vivo IVPAT Imaging of Lipid in the Atherosclerotic Plaques**

Magnetic resonance imaging (MRI) results, LRC map, and histological image of 3 rabbits in vivo examined with in vivo IVPAT after 20 weeks (A to C) or 25 weeks (D to F, G to I) HFC diet feeding. (J) X-ray image showed the location of IVPAT probe within the rabbit aorta. (K) Comparison of the high lipid content area measured by IVPAT and histology. ER = relative error; other abbreviations as in Figures 1 and 3.
the in vivo 3D visualization of lipid content has not been achieved. The in vivo IVPAT results showed slight dislocation, which we thought was caused by the heartbeat-driven vascular deformation during the ultrasonic signal acquisition. Third, current results were based on rabbit atherosclerotic plaques, and further studies should be undertaken on human plaques to assess the capabilities and potential benefits of IVPAT.

CONCLUSIONS

Our data demonstrate feasibility of in vivo IVPAT for characterization of spatial and quantitative features of lipid-rich plaques and encourages further development of IVPAT for basic science research and clinical diagnosis of atherosclerosis.

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REFERENCES


KEY WORDS atherosclerosis, histological imaging, intravascular photoacoustic tomography, lipid

APPENDIX For supplemental information, figures, and accompanying videos and legends, please see the online version of this article.