Feasibility of In Vivo Intravascular Ultrasound Tissue Characterization in the Detection of Early Vascular Transplant Rejection

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Background—Unprocessed ultrasound radiofrequency (RF) signal analysis has been shown to distinguish different tissue structures more reliably than gray-scale interpretation of conventional ultrasound images.

Methods and Results—The objective of this study was to test the feasibility of in vivo intravascular ultrasound (IVUS) RF signal analysis in an animal model of allograft rejection. Six cynomolgus monkeys underwent transplantation of 3-cm aortic allograft segments distal to the renal arteries from immunologically mismatched donors. IVUS imaging with a 30-MHz system was performed 84 to 105 days after the operation. RF signals were acquired from cross sections of the recipient and the allograft aortas in real time with a digitizer at 500 MHz with 8-bit resolution. Sixty-five cross sections and 68 regions of interest (31 in host aorta and 37 in allograft) were analyzed in the adventitial layer with a total number of 8568 vectors processed. For each region of interest, a weighted-average attenuation was calculated on the basis of the attenuation and length for each individual vector. Histological examination was performed at every cross section imaged by IVUS. When the gray-scale images of conventional IVUS scored by an independent observer were compared, no distinction between adventitia of the native aorta and allograft was possible. Analysis of the average RF backscatter power also showed no significant difference (70.32 ± 3.55 versus 70.72 ± 3.38 dB). However, the average attenuation of allografts was significantly lower than that of the host aortas (2.64 ± 1.38 versus 4.02 ± 1.16 dB/mm, P < 0.001). Histology demonstrated a marked adventitial inflammatory response in all allografts, with no inflammation observed in the host aortas.

Conclusions—In vivo IVUS tissue characterization can be performed during routine imaging. In this model of transplant vasculopathy, RF attenuation measurements were more sensitive than visual or quantitative gray-scale analysis.

Methods

Animal Preparation
Six adult Macaca fascicularis monkeys weighing between 6.6 and 8.3 kg were anesthetized with ketamine hydrochloride 10 mg/kg SC and maintained with 1.5% to 2% isoflurane. Abdominal aortic graft segments 3 cm in length were exchanged between 3 pairs of mixed lymphocyte reaction–mismatched (average 30.5 ± 17 mismatches) animals. The donor-recipient pairs were selected for maximum alloreactivity on the basis of mixed lymphocyte cultures with donor irradiated lymphocytes and recipient responder lymphocytes. The grafts were implanted distal to the renal arteries (Figure 1). After the operation, the animals were allowed to recover and maintained without immunomodulatory therapy. The study protocol was approved by the Institutional Laboratory Animal Committee.

RF Signal Acquisition
IVUS imaging was performed 84 to 105 days after the operation via a 5F femoral sheath with an automated pullback system at a speed of...
0.5 mm/s. A 2.9F imaging catheter with a 30-MHz transducer (Ultra, BSC/CVIS) was used to obtain cross-sectional images of the vessel wall. Multiple cross sections for RF analysis were acquired in each animal during the IVUS pullback from the host aorta proximal (at distances of 3, 2, and 1 cm of the proximal anastomosis site) and distal (1 and 2 cm of the distal anastomosis) to the allograft as well as from the graft itself (at 5-mm intervals including both anastomosis sites). The RF ultrasound signals from each cross section were sampled in real time at a rate of 500 MHz with 8-bit resolution with a personal computer–based digitizer (Gauge Inc). The unprocessed signal was obtained after preamplification but before time-gain compensation or any other signal manipulation. For each cross section, complete 360° scans, consisting of 256 vectors (received signal from a single acoustic pulse), were acquired digitally and stored to hard disk. IVUS images were recorded on videotape for further analysis.

RF Signal Analysis
By use of the digitized ultrasound RF signals, regions of interest (ROIs) were selected in the adventitial layer of the host aorta and the allograft. Each ROI was made as large as possible (6.7×3.2 in host aorta and 10.9×6.4 mm² in allografts) so that maximum accuracy for attenuation measurements was realized. For each vector within an ROI, the RF signal envelope was determined by a Hilbert transform method. Attenuation was calculated by curve-fitting the envelope data to the following equation by use of linear regression analysis: envelope sample = C×10⁻10αz, where C is a scaling constant, α is the attenuation of the medium in dB/mm, and z is the distance from the catheter. Attenuation is defined as the spatial rate of change of reflected ultrasonic energy. It represents a measurement of relative energy change for an ROI and is not greatly influenced by interposed tissue along the propagation path of the signal. On the basis of the individual vectors’ attenuation and length within the ROI, a weighted-average attenuation was calculated for each ROI. The weighting accounts for the fact that longer vectors provide more accurate attenuation estimates. To rule out any bias in the analysis, the distance of the ROIs from the catheter and the average length of vectors within the ROIs were calculated and compared for the host aorta and the allograft. In addition, average backscatter power of the ultrasonic signal was computed by a time-domain approach for each ROI in both the recipient and graft aorta.

Pathological Studies
After data collection, the allografts and the proximal and distal host aortas were pressure-fixed in situ with formalin and excised. The aorta was cross-sectioned at the sites corresponding to RF data acquisition. The positions of these sites were determined by computation of the length with respect to a reference point (branch or edge of graft segment) based on a known motorized pullback rate. The tissue was immediately processed, embedded in paraffin wax, and stained with hematoxylin-eosin. The perivascular tissue was evaluated for the presence, intensity, and distribution of inflammatory infiltrates, and the intimal layer was assessed for intimal fibroproliferation by a pathologist blinded to the RF data.

Statistical Analysis
Data are given as mean±SD. Student’s t test was used to compare parameters among the groups, and a value of P<0.05 was considered statistically significant.

Results
A total of 65 cross sections were digitized, and 68 ROIs (31 in host aorta and 37 in allograft) were analyzed in the adventitial layer, with 8568 vectors processed. Calculation of the average distance of the ROI from the catheter and the average length of the vectors in the ROI showed no significant difference between recipient aortas and allografts (distance from catheter: 2.51±0.54 versus 2.76±0.65 mm; length of vectors in ROI: 1.14±0.32 versus 1.10±0.53 mm in host aorta and allograft, respectively).

In comparisons of the gray-scale images of conventional IVUS by an independent observer, no distinction of the ROIs in
the adventitial layer between the 2 vessel types was possible (Figure 2). Average backscatter power in the host aortas and the allografts also showed no significant difference (70.32±3.55 versus 70.72±3.38 dB). However, attenuation of the allografts was significantly lower than in the host aortas (2.64±1.38 versus 4.02±1.16 dB/mm, \( P<0.001 \)) (Figure 2, Table). Histological examination of the allografts showed concentric intimal proliferation indicative of transplant vasculopathy. The adventitial and periadventitial layers were thickened and infiltrated by a moderate to severe lymphoplasmacytic cell infiltrate. Sections of the host aortas did not show inflammation or fibrointimal proliferation (Figure 1). The sensitivity of the method in determining allograft rejection was 76%, with a specificity of 71%, based on single cross sections. This could be further improved to a sensitivity of 83% and a specificity of 100% when multiple cross sections (temporal averaging) in the individual animals were included in the analysis.

**Discussion**

This is the first study to use in vivo IVUS RF tissue characterization techniques to discriminate different properties of the vessel wall. Average attenuation distinguished allograft rejection from normal aorta, correlating well with histology. These differences were not appreciable by either a qualitative visual evaluation by IVUS gray-scale images of the ROIs in the adventitia or quantitative conventional backscatter power computations.

IVUS displays the structure of arterial walls qualitatively and has become a standard imaging modality for the assessment of vessel size and general plaque composition.\(^2\) Quantitative evaluation of IVUS images has been performed in vitro using integrated backscatter as an objective index of tissue scattering.\(^3,4\) Lockwood et al.\(^6\) investigated the acoustic properties of human vascular tissues with an ultrasound backscatter microscope measuring both backscatter and attenuation. The average attenuation in the artery wall at 30 MHz was 4 dB/mm. This correlates very well with our measurements of attenuation in normal aorta (4.02±1.16 dB/mm) using a 30-MHz IVUS device, indicating that in vivo IVUS RF analysis of average attenuation may be comparable to acoustic microscopy for this parameter.

The relationship between collagen and ultrasonic attenuation in myocardial tissue has been studied by Mimbs et al.\(^7\) Regions of infarcted and normal myocardium were investigated in 2 animal models. A close relationship between increased collagen content in zones of infarction and increased attenuation could be demonstrated. This finding confirms that changes in tissue composition can result in altered ultrasonic attenuation. In the present study, a significant association between a decrease in average attenuation and the presence of inflammatory cells could be shown. The decrease in attenuation in the presence of increased cellularity may be best explained by an increase in water content accompanying the aggressive inflammatory response, because attenuation is dependent on the protein and water

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**Figure 2.** IVUS RF-derived attenuation (dB/mm) is significantly different for host aorta and allograft. In corresponding IVUS images, no distinction is possible within ROIs placed in adventitial layer.

**Attenuation Measurements for Host Aorta and Allograft for Each Individual Animal**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Host Aorta</th>
<th>Allograft</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.95±0.49</td>
<td>2.49±0.57</td>
</tr>
<tr>
<td>2</td>
<td>4.41±0.61</td>
<td>2.03±0.71</td>
</tr>
<tr>
<td>3</td>
<td>3.88±0.86</td>
<td>2.78±1.80</td>
</tr>
<tr>
<td>4</td>
<td>3.55±0.70</td>
<td>2.12±1.16</td>
</tr>
<tr>
<td>5</td>
<td>3.85±2.39</td>
<td>2.82±1.42</td>
</tr>
<tr>
<td>6</td>
<td>4.16±1.15</td>
<td>3.68±1.63</td>
</tr>
<tr>
<td>Total</td>
<td>4.02±1.16</td>
<td>2.64±1.38</td>
</tr>
</tbody>
</table>

Values represent mean±SD in dB/mm.
content. In a model of myocardial ischemia, O’Brien et al demonstrated that an increase in water content results in a significant reduction of attenuation coefficient.

A potential limitation of our study is the averaging of vectors in each individual ROI. Although it accurately reflects attenuation of the entire ROI, small heterogeneities of the tissue may remain undetected. This factor limits this application to fairly homogeneous tissue types. Native atherosclerosis, which is more heterogeneous in nature, may be difficult to distinguish by this technique. Furthermore, there are certain practical limitations of applying this method in the clinical setting, because it requires a special acquisition unit (personal computer with digitizer) and additional trained personnel to run the system.

In conclusion, this study demonstrates that in vivo IVUS tissue characterization is feasible and in this model is able to differentiate vasculopathic adventitial tissue changes.

Acknowledgments
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References
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