In Vivo Quantitative Tissue Characterization of Human Coronary Arterial Plaques by Use of Integrated Backscatter Intravascular Ultrasound and Comparison With Angioscopic Findings

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Background—The purpose of the present study was to define whether integrated backscatter (IB) combined with conventional intravascular ultrasound (IVUS) makes tissue characterization of coronary arterial plaques possible.

Methods and Results—IB-IVUS was performed in coronary arteries (total 18 segments) of 9 patients at autopsy, and the findings were compared with the histology. RF signals, which were digitized at 2 GHz in 8-bit resolution, were obtained with an IVUS system with a 40-MHz catheter. IB values of the RF signal from the region of interest (ROI) (100-μm depth, 1.4° per line) were calculated by use of a personal computer. IB values on the ROIs were divided into 5 categories, compared with each of the plaque histologies: category 1 (thrombus), −88 < IB ≤ −80; category 2 (intimal hyperplasia or lipid core), −73 < IB ≤ −63; category 3 (fibrous tissue), −63 < IB ≤ −55; category 4 (mixed lesions), −55 < IB ≤ −30; and category 5 (calcification), −30 < IB ≤ −23. On the basis of these categories, we analyzed 5120 ROIs per segment in each ring-like arterial specimen. Color-coded maps of plaques were constructed by use of these IB data and conventional IVUS data, which reflected the plaque histology of autopsied coronary arteries well. Then, the same method was undertaken in 24 segments with plaque from 12 patients in vivo with angina pectoris. Comparisons between coronary angioscopy and IB-IVUS revealed that the surface color of plaques in angioscopy reflected the thickness of the fibrous cap rather than the size of the lipid core.

Conclusions—IB-IVUS represents a new and useful tool for evaluating the tissue structure of human coronary arterial plaques. (Circulation. 2002;105:2487-2492.)

Key Words: ultrasonics ▪ plaque ▪ tissue

In general, coronary arterial plaque in humans consists of 5 tissue characteristics: lipid core with fibrous cap, intimal hyperplasia, fibrous tissue, calcification, and thrombus. Coronary arterial plaque is also classified into stable plaque without progression of stenosis and unstable plaque with progression followed by acute coronary syndrome.1,2 According to a pathological study, it is considered that the features of the former are fibrous tissue or small lipid core with thick fibrous cap. The features of the latter are the presence of a large lipid core with thin fibrous cap, which cause plaque rupture and/or thrombi followed by rapid progression, or intimal hyperplasia, which causes a gradual progressive condition, such as restenosis after percutaneous coronary intervention.2,3 In addition, substantial reduction of acute cardiac events has been shown in most of the lipid-lowering trials, despite only a minimal geometric regression of plaque.3,4 This is because plaque stability is achieved by the removal of lipids from the lipid-rich plaques. There is no clinical evidence of stabilization, however, because of the lack of adequate clinical methods for tissue characterization of coronary arterial plaque. Therefore, it is very important to develop new methods for evaluation of the tissue characteristics of coronary plaques.

At present, there are several approaches to clinically detect tissue characterization of plaques. Conventional echo technique, especially intravascular ultrasound imaging (IVUS), is widely used to determine calcification and the 3 layers of the arterial wall. Differentiation of lipid core from fibrous tissue by use of echo intensity, however, is difficult. Coronary angioscopy is a useful method for detection of thrombi and the color of the surface of the plaque.

It has been reported that integrated backscatter (IB), which is useful for tissue characterization of the myocardium, can
differentiate fibrous tissue and fatty tissue of arteries ex vivo. Recently, we reported that the differentiation of the 7 tissue components of arteries, consisting of thrombus, lipid pool, intimal hyperplasia, fibrosis, mixed lesion, calcification, and the presence of media, was possible in human carotid and femoral arteries in vivo by use of IB combined with conventional 2D echo and that the constructed color-coded maps of the arteries with plaques in the patients during life reflected each of the above histologies well at autopsy of the same patients. There is no known study, however, of IB analysis on coronary arteries in vivo.

Thus, the purpose of the present study was (1) to define whether IB-IVUS can differentiate clinicopathologically the tissue characteristics in human coronary arterial plaque in vivo and (2) to compare IB-IVUS data with angioscopic findings.

Methods

Subjects
For the ex vivo study, 18 coronary arteries with mild or moderate stenosis from 9 autopsied patients (68 to 84 years old; 7 men and 2 women) were used. The causes of death were pulmonary emphysema; pneumonia; old myocardial infarction; and cancers of the lung, tongue, larynx, and pancreas. In the in vivo study, 25 lesions with mild or moderate stenosis from 12 patients with angina pectoris (64 to 76 years old; 10 men and 2 women) in which IB-IVUS was performed were used. Angina-related arteries with severe stenosis in which an IVUS catheter could not be inserted were excluded from the present study. Informed consent was obtained from patients for the in vivo studies and from their relatives for the ex vivo studies in all cases.

Study Protocol
At autopsy, within 8 hours after death, the coronary arteries were dissected and coronary arteries with plaques were subjected to the ex vivo IB imaging of IVUS in 0.9% saline at a temperature of 37°C. To clarify the “rotational” position of the included segment, stainless steel needles were carefully inserted into the coronary arteries to be used as a reference point in the ex vivo and histological studies. Subsequently, the same imaging procedures were repeated 2 days after fixation with 10% buffered formalin. Ring-like arterial specimens obtained at a level similar to that of the ultrasound study after decalcification for 5 hours were embedded in paraffin and cut 4 μm thick transversely perpendicular to the longitudinal axis of the artery. They were stained with hematoxylin-eosin, elastic van Gieson stain, and Masson’s trichrome. In addition, immunohistochemical analysis using anti-actin antibody was performed for the detection of smooth muscle cells. According to the definition of atherosclerotic lesions by the American Heart Association Council on Atherosclerosis, 7 pathological subsets were identified in each region of interest (ROI): thrombus (collections of erythrocytes embedded in a net of platelets), lipid core (extracellular lipid, macrophages, and/or foam cells), intimal hyperplasia consisting of smooth muscle cells that occupied >50% of the sample area, fibrous tissue, mixed lesions (mixed mineral deposits, extracellular lipid, and fibrous tissue), calcification, and the presence of media. These histological determinations were based on the agreement of 2 specialists who were blinded to the ultrasound echo study.

IB System Presets and Data Acquisition
Conventional IVUS images and IB signals were acquired with a commercially available IVUS imaging system to characterize the coronary arterial tissue with a 40-MHz intravascular catheter (Boston Scientific). An IVUS catheter was placed at the site perpendicular to the longitudinal axis of the coronary arteries in the center of the lumen. We used an analog-to-digital converter, which allowed acquisition, storing, and retrieving of signals that were digitized at 2 GHz in 8-bit resolution. Offline analyses of the acquired RF signals were performed by retrieving the previously stored data from the built-in hard-disk drives in the system by use of software we developed for this study. IB was calculated as the average power of the ultrasound backscattered signal from a small volume of tissue by use of fast Fourier transform (FFT) measured in decibels (dB). The FFT analyses were performed by a program that was constructed by use of Visual Basic. The code in the FFT program is shown in the Appendix. Color-coded construction was performed by Noesys. In the present study, we used 256 vector lines per image (1.4 grade per line) and set 20 ROIs of each 100-μm depth on each vector line (total 5120 ROIs per image). The tissue IB values were calibrated by subtracting the IB values from the IB value of stainless steel placed at a distance of 1.5 mm from the catheter. In the ex vivo studies, each site of each tissue characteristic was placed at a distance of ~1.5 mm from the catheter. Offline analysis after the IB values had been retrieved allowed us to set the ROIs one by one, referring to the pathological characteristics by pathological photographs.

Angioscopic Analysis
In an in vivo angioscopic analysis in the patients with angina pectoris, images obtained with a Vecmova (4.5F) catheter (Clinical Supply) were classified into white plaque, light yellow plaque, and yellow plaque. The same segments in which IB-IVUS was performed were analyzed by angioscopy. Angioscopic findings were compared with color-coded maps obtained by the IB-IVUS analysis.

Statistical Analyses
Values are reported as the mean ± SD. The significance of the differences of IB values among tissue characteristics in the arterial wall were tested by ANOVA followed by Fisher’s exact test, which was used for the post hoc test. Correlation among the IB values during life and before and after fixation was tested for significance by Pearson’s correlation coefficient. A value of P < 0.05 was considered to be statistically significant.

Results

Comparison Between Ex Vivo IB Values and Histology
In the conventional IVUS images, the medial layer was easily differentiated as an echo-free zone surrounded by high-intensity signals. IB values obtained at autopsy and after fixation were compared with each other. The correlation was satisfactory (r = 0.87, P < 0.01).

To compare the IB values and their pathologies, a total of 88 sampling sites with typical histology were examined in the 18 arteries of autopsied specimens (Table 1). The typical histologies of these sampling sites were divided into calcification (n = 9), mixed lesion (n = 11), fibrous tissue (n = 17), lipid core (n = 16), intimal hyperplasia (n = 5) and thrombus (n = 6) in the intima, and the media (n = 24). The IB values of

| TABLE 1. Definition of IB Values in Each of 5 Histological Categories in Plaque |
|---|---|
| Histology | Definition, dB |
| Calcification | −30 < IB ≤ −23 |
| Mixed lesion | −55 < IB ≤ −30 |
| Fibrous tissue | −63 < IB ≤ −55 |
| Lipid core or intimal hyperplasia | −73 < IB ≤ −63 |
| Thrombus | −88 < IB ≤ −80 |

Definition of IB values in each histological category was determined on the basis of the mean IB value ±1 SD shown in Figure 1.
these tissues after fixation at autopsy were $-26.7\pm 3.6$, $-40.7\pm 4.6$, $-58.9\pm 3.6$, $-67.5\pm 3.6$, $-70.7\pm 2.7$, and $-84.2\pm 3.8$ in the intima, respectively, and $-74.5\pm 6.2$ in the media (Figure 1). In each of the ex vivo studies, the IB values were highest in calcified plaque and lowest in thrombus (Figure 1). The differences among thrombus, fibrous tissue, mixed lesion, calcification and lipid core, intimal hyperplasia, and media were significant. Lipid core, intimal hyperplasia, and media, however, had similar IB values. The correlation between IB values and each category by use of Spearman’s correlation coefficient by rank was sufficient ($R=0.954$).

**Construction of Color-Coded Maps by Use of IB Values and Conventional 2D IVUS**

To construct IB color-coded maps in the arterial wall, IB values on the ROIs were divided into 5 categories based on the mean calibrated IB values $\pm 1$ SD; category 1 (thrombus), category 2 (intimal hyperplasia or lipid core in the intima and media), category 3 (fibrous tissue), category 4 (mixed lesion), and category 5 (calcification) (Table 1).

On the basis of the above categories, 2D color-coded maps of tissue characterization were constructed in 18 segments of coronary arteries in 9 autopsied patients (Figure 2). We analyzed 5120 ROIs (20 ROIs per vector line $\times 256$ vector lines) in each segment. It was reported that the average attenuation with a 40-MHz frequency catheter was 5.9 dB/mm.$^{9,10}$ Therefore, we corrected each IB value, adding 0.59 dB/0.1 mm when the ROI was located 1.5 mm farther from the catheter and subtracting 0.59 dB/0.1 mm when the ROI was located 1.5 mm closer to the catheter. In category 2, the media and intima were differentiated by use of conventional 2D echo.$^5$ In general, lipid cores (category 2) are pathologically located under fibrous caps consisting of fibrous tissue (category 3) and/or mixed lesion (category 4). The fibrous cap, however, is not generally observed in intimal hyperplasia. Therefore, the presence of ROIs with category 2 under a layer of ROIs with category 3 (fibrous cap) and/or category 4 (mixed lesion) was defined as a lipid core but not as intimal hyperplasia. The thicknesses of the fibrous caps and the areas of the lipid core were measured from the number of ROIs 100 $\mu$m in depth. Large and small lipid core, thin and thick fibrous caps, intimal hyperplasia, fibrous tissue, and mixed lesions reflected each of the pathological findings in the plaque well (Table 2).

**Comparison Between the Findings of IB-IVUS and Angioscopy**

The findings of IB-IVUS and angioscopy were compared in 25 segments of coronary arteries of 12 patients with angina pectoris. As shown in Figure 3, 7 of 9 white plaques in angioscopy showed fibrous plaques without lipid core or plaques with thick fibrous caps ($\geq 500\mu$m in narrowest width). The narrowest width of fibrous cap was 200 to 400 $\mu$m in each of 9 light yellow plaques and $<100\mu$m in 6 of 7 yellow plaques. That is, plaque color depended on the thickness of the fibrous cap. Conversely, the size of the lipid core varied considerably among yellow, light yellow, and white plaques (Figure 3).

**Discussion**

The present system with the commercially available IVUS imaging system (Boston Scientific) and digital-to-analog converter (Wavepro 960, LeCroy) allowed quantitative analysis of the image data after the RF signal had been retrieved and stored to the hard-disk drive without any reconstruction for video recorder images. This offline analysis and the presence of stainless steel needles to clarify the precise position allowed determination of the IB values of ROIs at almost identical areas of interest on the pathological photographs taken after the autopsy. Thus, the 2D structures of the coronary arterial wall based on the IB values were visualized.

**Technical Consideration of IB-IVUS**

According to previous studies on IB values by use of aortic, carotid, and femoral arteries, the IB from the arterial wall is angle dependent.$^{11}$ The IB values of fibrous tissues were higher than those of fatty tissues rich in lipid, which showed low angular scattering.$^{11}$ The present findings in coronary arteries confirmed the above observations. The angle dependence makes tissue characterization unstable when ROIs are not perpendicular to the axis.$^{12}$ Therefore, a catheter should be put at the site perpendicular to the longitudinal axis of the coronary arteries in the center of the lumen, which was done in the present study. Axial resolution depends on the pulse length. In addition, lateral resolution depends on the beam width and the aperture size of the transducer. Increasing the transducer frequency (40 MHz) and high sampling rate (2 GHz) allowed detailed analysis by use of IB measurement. When the frequency of a transducer was 40 MHz and the speed of sound in a tissue was $\approx 1540$ m/sec, the resolution was calculated as 38.5 $\mu$m. In the present study, the size of the ROI was 100 $\mu$m in depth. The presence of the small ROI made the precise measurements of thickness of fibrous caps and the area of the lipid core possible.
It was reported that fixation and processing for histopathological examinations resulted in a decrease in total vessel cross-sectional area and luminal cross-sectional area, but absolute wall area (total vessel cross-sectional area minus luminal cross-sectional area) did not change in vessels with minimal atherosclerotic narrowing. Several studies have documented that formalin fixation does not significantly affect the morphology and quantitative echo character of plaque tissue of human aortic walls. This fact was also confirmed in the present study. Because the IB values of saline are similar to those of uniformly flowing blood, the IB values of each category obtained from the autopsy study by use of saline were used in the in vivo study.

Because intimal hyperplasia and lipid core have similar IB values, it is necessary to use a complex method to differentiate them. This may limit the value of the method for broad usage. The difficulty of differentiation between extracellular lipid and macrophages and/or foam cells limits the value of the IB-IVUS method. It would be more accurate to calculate

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**TABLE 2. Comparison Between Color-Coded Maps Obtained by IB-IVUS in Ex Vivo Study and Pathology**

<table>
<thead>
<tr>
<th>Tissue Structures</th>
<th>IB-IVUS Study/Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large lipid core</td>
<td>6/6</td>
</tr>
<tr>
<td>Small lipid core</td>
<td>4/3*</td>
</tr>
<tr>
<td>Thick fibrous cap</td>
<td>7/7</td>
</tr>
<tr>
<td>Thin fibrous cap</td>
<td>3/3</td>
</tr>
<tr>
<td>Mixed lesion</td>
<td>6/7*</td>
</tr>
<tr>
<td>Fibrous tissue</td>
<td>4/4</td>
</tr>
<tr>
<td>Intimal hyperplasia</td>
<td>4/4</td>
</tr>
<tr>
<td>Calcification</td>
<td>4/4</td>
</tr>
</tbody>
</table>

Large lipid core: >3 mm² in area; small lipid core: ≤3 mm² in area; thick fibrous cap: ≥100 μm at the narrowest width; thick fibrous cap: ≥200 μm at the narrowest width.

*One of 4 small lipid cores in IB-IVUS showed mixed lesions in pathology because of the attenuation phenomenon of calcification.

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**Figure 2.** Color-coded maps of coronary arterial plaques constructed by IB-IVUS and histology, coronary angiography, or angioscopy. A, Autopsy study of coronary arterial plaque. A1, Histological finding with fibrosis, mixed lesion, calcification, and large (right) and small (left) lipid cores (∗) stained with Masson’s trichrome. Bar=1 mm. A2, Conventional IVUS image of same segment as A1. A3, Color-coded map of intima of A1 constructed by IB-IVUS. Note large and small lipid cores (∗, blue) with thick fibrous caps consisting of fibrous tissue (green) and mixed lesion (yellow) indicated by arrowheads. Color-coded map reflects histology of A1 well. B, In vivo study of coronary arterial plaque. B1, Angiography of left coronary artery. Arrow indicates a segment with 60% diameter stenosis. B2, Conventional IVUS image of same segment as shown by arrow in B1. B3, Color-coded map of intima of B1 constructed by IB-IVUS. Note intimal hyperplasia of left side (#) without fibrous cap and diffuse fibrosis of right side (F). B4, Angioscopic finding of plaque at right in B2. Note that white plaque is related to fibrous tissue. C, In vivo study of coronary arterial plaque. C1, Angiography of right coronary artery. Arrow indicates a segment with 40% diameter stenosis. C2, Conventional IVUS image of same segment as shown by arrow in C1. C3, Color-coded map of intima in C1 constructed by IB-IVUS. Note large lipid core (∗) with fibrous cap (arrowhead). Note that fibrous cap (green) at upper side is thin. C4, Angioscopic finding of plaque at top in C1. Note that yellow plaque is related to thin fibrous cap. Red circle, Borderline between vessel lumen and intima obtained by conventional IVUS. Yellow circle, Borderline between intima and media obtained by conventional IVUS. See Construction of Color-Coded Maps by Use of IB Values and Conventional 2D IVUS in Results section for definition of fibrous cap, lipid core, and intimal hyperplasia.
R values for each type of plaque instead of pooling them all together. The categories in the present study, however, were not defined quantitatively. This makes the correlation and validation study subject to erroneous interpretations.

Clinical Implications
The IB-IVUS presented here can enable us to visualize lipid cores, fibrous caps, intimal hyperplasia, fibrous tissue, mixed lesions, and calcification in the plaque of human coronary arteries in vivo. Conversely, coronary angioscopy is a good tool for evaluation of thrombi and surface color of plaques. The present study, however, revealed that the plaque color detected by angioscopy reflected the thickness of the fibrous cap but not the size of the lipid core. Thus, IB-IVUS is considered to be a more precise method for tissue characterization of coronary arterial plaques than coronary angioscopy.

In general, it is easy to insert the IB-IVUS catheter in segments with mild or moderate stenosis but difficult to insert it in segments with severe stenosis. Therefore, IB-IVUS was performed in coronary arteries with mild or moderate stenosis in the present study. When definite calcification is present in the fibrous cap or in the mixed lesion, it is difficult to obtain a precise IB-IVUS image in the outside tissue of the fibrous cap because of the attenuation phenomenon. This was confirmed in the present study. Most cases of acute coronary syndrome, an important cause of mortality in ischemic heart disease, however, occur from unstable plaque consisting of large lipid cores with thin fibrous caps or intimal hyperplasia in which the degree of stenosis is mild or moderate.1,2,16 In these plaques without severe stenosis, calcification of fibrous caps is generally rare or slight. Therefore, IB-IVUS is adequate for the detection of unstable plaques showing mild or moderate stenosis.

Conclusions
A new color-coded mapping method using IB-IVUS is useful to identify atherosclerosis of human coronary arteries in vivo. This technique may play an important role in assessing stable and unstable coronary plaques.

Appendix

Code of FFT Constructed by Use of Visual Basic

Public Sub fftCalc(x() As Double, y() As Double, N As Integer, id As Double)
    Dim i As Integer, i0 As Integer, i1 As Integer, j As Integer, 
    ns As Integer, k As Integer, arg As Integer
    Dim s As Double, c As Double, sc As Double, x1 As Double, y1 As Double
    ns = N/2: sc = 2 * 4 * Atn(1#)/N
    Do While ns > N/128
        arg = 0
        For j = 1 To N Step 2 * ns
            k = N/4
            c = Cos(sc * arg): s = Sin(id * sc * arg)
            For i0 = j To j + ns - 1
                i1 = i0 + ns
                x1 = x(i1) * c - y(i1) * s: y1 = y(i1) * c + x(i1) * s
                x(i1) = x(i0) - x1: y(i1) = y(i0) - y1
                x(i0) = x(i0) + x1: y(i0) = y(i0) + y1
            Next i0
            Do While k >= 1
                arg = arg - k: k = k/2
                If k = 0 Then Exit Do
                Loop
                arg = arg + k
                Next j
                ns = ns/2
                Loop
        If id < 0 Then
            For i = 1 To N
                x(i) = x(i)/N: y(i) = y(i)/N
            Next i
        End If
        j = 1
        For i = 1 To N - 1
            x1 = x(i): x(i) = x(j): x(j) = x1
            y1 = y(i): y(i) = y(j): y(j) = y1
            End If
            k = N/2
            Do While k < j
                k = k/2
                Loop
                x(j) = k
            Next i
        End Sub

Figure 3. Relation between thickness of fibrous cap and thickness of lipid core in in vivo coronary arteries in which IB-IVUS and angioscopy were performed. Note that plaque color in angioscopy depended on thickness of fibrous cap with lipid core.
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References
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