Assessment of the Histological Characteristics of Coronary Arterial Plaque With Severe Calcification

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Background: Several studies have shown that rotational atherectomy (RA) is associated with higher rates of the slow-flow phenomenon and that ablated particles may be the possible cause. Intravascular ultrasound (IVUS) has an intrinsic limitation in assessing plaque morphology behind the calcification because of acoustic shadowing. Therefore, the purpose of this study was to investigate plaque characteristics behind severe calcification by histological examination.

Methods and Results: One hundred eight coronary arterial segments from 40 human cadavers (24 males, 16 females, mean age 74±7 years) were examined. Serial images of IVUS were obtained and 18 severe calcified lesions were collected. Experienced observers quantitatively analyzed the lesions by computerized planimetry for fibrous, fibrofatty, calcification, and necrotic tissue area. Histologically, 15 of 18 severely calcified lesions (83%) had an extensive necrotic tissue containing large numbers of cholesterol crystals and microcalcifications; 16 of same 18 severely calcified lesions (89%) had fibrofatty tissue as well as calcification. The necrotic tissue occupied 14±13% and fibrofatty tissue occupied 13±11% of severely calcified lesions.

Conclusion: Necrotic core and fibrofatty tissue components “hidden” behind calcification might cause emboli-induced thrombus formation and distal flow disturbance during RA. (Circ J 2007; 71: 643–647)

Key Words: Coronary arterial plaque; Intravascular ultrasound; Rotational atherectomy; Slow flow

Intravascular ultrasound (IVUS) is a widely used intracoronary imaging modality that enables visualization of the arterial wall during interventional procedures. However, IVUS is limited for assessment of severe calcification components because the bright IVUS signals from calcification make assessment of neighboring tissue difficult as a result of the saturation artifact. Moreover, attenuation of the ultrasound beam by calcification causes acoustic shadowing, which impairs visualization of deeper vessel wall structures.

Rotational atherectomy (RA) has become a widely used treatment of severe calcified lesions;¹⁻⁴ however, several studies have shown that RA is associated with higher rates of the no-reflow/slow-flow phenomenon than other coronary revascularization procedures⁵⁻⁹ Previous studies have shown that the total amount of ablated particles may be a possible cause of slow-flow⁸⁻¹¹ so we hypothesized that the characteristics of the ablated plaque, which may play a critical role in the development of no-reflow/slow flow, could not be visualized by IVUS and may have influenced the results of RA. To test this hypothesis, we investigated by histological examination the characteristics of severely calcified plaque.

Methods

We examined 108 coronary arterial segments from 40 human cadavers (24 males, 16 females, mean age 74±7 years); 6 cases had been diagnosed with ischemic heart disease as the cause of sudden cardiac death related to coronary artery occlusion (15%). The existence of hypertension, hyperlipidemia, and diabetes mellitus were determined, using the following criteria. Hypertension was defined as blood pressure >140/90 mmHg or medication; hyperlipidemia as total cholesterol level >220 mg/dl or triglyceride level >150 mg/dl or medications; diabetes mellitus as plasma glucose level (anytime) >200 mg/dl or medication. The study protocol was approved by the Ethics Committee of Kawasaki Medical School, and written informed consent was given by each family. An approximately 5-cm length of the proximal site of the 3 major coronary arteries (ie, left anterior descending coronary artery, left circumflex artery, and right coronary artery) were obtained from the cadavers at autopsy within 3h of death. The surrounding soft tissues were dissected from each specimen, small arterial perforators and their branches were tied off with sutures, and the distal end of the artery was occluded with a large cork. A 7F sheath was sewn into the proximal end of the artery to complete the closed system. Saline (0.9%), which was kept at 37°C, was infused through the side arm of the sheath. The pressure inside the coronary artery was maintained at a...
physiologic level (60–80 mmHg) with a syringomanometer connected to the infusion.

An IVUS catheter (Atlantis SR Pro® 2.5F, 40-MHz; Boston Scientific, Natick, MA, USA) was inserted through the diaphragm of the sheath and serial images were obtained using an automatic pullback device at a rate of 0.5 mm/s. After IVUS imaging, each coronary artery was pressure-fixed in 10% neutral buffered-formalin. After fixation for 48 h, standard paraffin embedding was performed. In every 400 μm of each coronary artery, 3 series of 4-μm thick sections were cut and stained with hematoxylin–eosin, Masson’s trichrome, and elastica van Gieson stain.

IVUS images were analyzed off-line by commercially available image processing software (Netra 3D IVUS system, ScImage, Los Altos, CA, USA). Using the cross-sectional image the arc of calcification was measured quantitatively and 18 severely calcified lesions, which were defined as having an arc of calcification >180 degrees, were obtained and compared with the corresponding histological images. Experienced observers, unaware of the

Table 1 Clinical Characteristics of the Patients With Calcified Plaque

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Severely calcified (+) (n=10)</th>
<th>Severely calcified (-) (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, years</td>
<td>76±11</td>
<td>70±6</td>
<td>0.143</td>
</tr>
<tr>
<td>M/F</td>
<td>5/5</td>
<td>19/11</td>
<td>0.351</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>3 (30%)</td>
<td>10 (33%)</td>
<td>0.586</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>1 (10%)</td>
<td>3 (10%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>1 (10%)</td>
<td>6 (20%)</td>
<td>0.428</td>
</tr>
<tr>
<td>Hemodialysis, n (%)</td>
<td>2 (20%)</td>
<td>2 (7%)</td>
<td>0.256</td>
</tr>
<tr>
<td>Ischemic heart disease, n (%)</td>
<td>2 (20%)</td>
<td>4 (13%)</td>
<td>0.474</td>
</tr>
</tbody>
</table>

Table 2 Histological Characteristics of Severely Calcified Lesions

<table>
<thead>
<tr>
<th>CSA, cm²</th>
<th>13.2±4.4</th>
<th>8.4±2.8</th>
<th>65±12</th>
<th>4.8±2.6</th>
<th>213±73</th>
<th>3.6±1.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal elastic membrane CSA (mm²)</td>
<td>13.2±4.4</td>
<td>8.4±2.8</td>
<td>65±12</td>
<td>4.8±2.6</td>
<td>213±73</td>
<td>3.6±1.1</td>
</tr>
<tr>
<td>Plaque CSA (mm²)</td>
<td>7.8±2.2</td>
<td>3.6±1.1</td>
<td>4.8±0.6</td>
<td>213±73</td>
<td>3.6±1.1</td>
<td></td>
</tr>
<tr>
<td>Percent plaque area (%)</td>
<td>100</td>
<td>80</td>
<td>60</td>
<td>40</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Lumen area (mm²)</td>
<td>4.8±2.6</td>
<td>3.6±1.1</td>
<td>4.8±0.6</td>
<td>213±73</td>
<td>3.6±1.1</td>
<td></td>
</tr>
<tr>
<td>Calcification degree (°)</td>
<td>213±73</td>
<td>3.6±1.1</td>
<td>4.8±0.6</td>
<td>213±73</td>
<td>3.6±1.1</td>
<td></td>
</tr>
<tr>
<td>Calcification area (mm²)</td>
<td>3.6±1.1</td>
<td>3.6±1.1</td>
<td>4.8±0.6</td>
<td>213±73</td>
<td>3.6±1.1</td>
<td></td>
</tr>
</tbody>
</table>

Fig 1. Histological examples of different tissue types. Fibrous (A, elastica van Gieson), necrotic (B, hematoxylin–eosin (H&E)), calcification (C, H&E), fibrofatty tissue (D, H&E). Scale bars = 100 μm.

IVUS images, using NIH imaging software quantitatively analyzed the lesions by computerized planimetry for fibrous, fibrofatty, calcification, and necrotic tissue areas (Fig 1). Areas of densely packed collagen were termed fibrous and those with lipid interspersed in collagen were defined as fibrofatty. Regions comprising cholesterol clefts, foam cells, and microcalcifications were defined as necrotic. Calcium deposits without adjacent necrosis were identified as calcification. Internal elastic membrane (IEM) cross-sectional area (CSA) and lumen area were measured. Plaque CSA was calculated by subtracting the lumen area from the measured IEM CSA. Percent plaque area was calculated as (plaque CSA/IEM CSA)×100 (%).

Statistical Analysis
To compare cadavers with and without severely calcified lesions, analysis of variance and unpaired t-test were used for continuous variables and Fisher’s exact test for dichotomous variables. Data are expressed as mean value ± SD. A value of p<0.05 was considered significant.

Results
Of 108 coronary arterial segments from 40 human cadav-
ers by IVUS, 18 severely calcified lesions were found in 10 cadavers (25%). The clinical characteristics of cadavers with and without severely calcified lesions are shown in Table 1 and the histological characteristics of the severely calcified lesions are shown in Table 2. Maximum thickness of calcification ranged from 0.08 mm to 1.88 mm. Of the 18 severely calcified lesions 15 (83%) had an extensive necrotic tissue containing large numbers of cholesterol crystals and microcalcifications; 16 of the 18 (89%) had fibrofatty tissue as well as calcification. Fig 2 shows the plaque component analyses from the histological examination. The necrotic tissue comprised 14±13% and fibrofatty tissue 13±11% of the severely calcified lesions. No significant relationships were observed between calcification area and necrotic or fibrofatty tissue area (R=0.054, p=0.831, and R=0.424, p=0.080, respectively).

Representative IVUS and corresponding histological images of coronary plaque associated with severe calcification are shown in Fig 3.

Discussion

The present study demonstrates the histological characteristics of plaque lesions with severe calcification. To the best of our knowledge, this is the first report to clarify the histological characteristics of plaque lesions with the acoustic shadow of severe calcification evaluated by IVUS. This study has now shown the incidence of a necrotic and fibrofatty tissue in severely calcified lesions. A recent in vivo IVUS study with radiofrequency-derived image analysis demonstrated that fibrofatty tissue comprised 19.8–26.3% and necrotic tissue 5.9–9.6% of the lesions in cases...
of severe coronary artery disease. Our results are as high as those from previous reports of coronary segments without severe calcification. Some studies have indicated that coronary arterial calcification is a marker for significant coronary atherosclerosis and that patients with high coronary arterial calcification scores are at an increased risk for coronary events. The results of the present study are compatible with those previous reports. RA has become widely used for the treatment of severe calcified lesion. During ablation of the plaque, microparticles are produced by the advancing burr and experimental studies suggested that these particles pass harmlessly through the distal microcirculation. However, several clinical studies have shown that RA is associated with higher rates (6–15%) of the no-reflow/slow-flow phenomenon than other coronary revascularization procedures and this phenomenon can lead to serious ischemic complications, such as conduction disturbances, myocardial infarction, cardiogenic shock or even death. It has been reported that high-speed rotablation might cause aggregation platelet activation of platelet-rich plasma and distal flow disturbance during RA procedures. Other previous studies have shown that the total amount of the ablated particles may be a possible cause of slow flow during RA procedures. On the other hand, in a scintigraphic study using 99mTc-sestamibi, Koch et al demonstrated that myocardial hypoperfusion during RA was not influenced by the volume of ablated plaque. It is therefore important to clarify the impact of the plaque characteristics during RA. The present study revealed a high incidence of necrotic and fibrofatty tissue in severely calcified lesions. Generally, fatty tissue protruding into the vessel’s lumen may cause luminal thrombus formation in patients with acute coronary syndrome. In addition, the lipid compounds are a predictor of no-reflow during percutaneous coronary revascularization in acute coronary syndrome. Therefore, during RA procedures, these necrotic and fibrofatty tissue components “hidden” behind the calcification on IVUS evaluation might protrude into the vessel lumen and cause emboli-induced thrombus formation, such as in acute coronary syndrome.

Study Limitations
Measurements during IVUS and the histological examination were carried out on strictly corresponding sites at the same distance from the side branches, which were used as anatomical landmarks. However, any discrepancy related to differences in the measurement position for IVUS and histological examination might influence the results. In addition, histological examinations might be influenced by shrinkage artifact during the processing of specimens, despite the use of pressure-fixation.

Because this study is an autopsy study, it was impossible to confirm the direct relationship between plaque components and distal flow disturbance during RA procedures in vivo. However, the incidence of necrotic core and fibrofatty tissue in this study was as high as in previous reports of the lesions associated with severe coronary artery disease.

Conclusions
The present study has demonstrated a high incidence of necrotic and fibrofatty tissue in severely calcified lesions assessed by histological examination. These tissue components “hidden” behind the calcification on IVUS images might cause emboli-induced thrombus formation and distal flow disturbance during RA procedures.

References
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