Impact of NAD(P)H Oxidase-Derived Reactive Oxygen Species on Coronary Arterial Remodeling:
A Comparative Intravascular Ultrasound and Histochemical Analysis of Atherosclerotic Lesions

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Background—Coronary arterial remodeling, which is a response to the growth of atherosclerotic plaques, is associated with plaque vulnerability. Oxidative stress induced by reactive oxygen species (ROS) via NAD(P)H oxidase in the vasculature also plays a crucial role in the pathogenesis of atherosclerosis-based cardiovascular disease. In this study, the relationship between coronary arterial remodeling and ROS generation was examined by comparing preinterventional intravascular ultrasound findings of atherosclerotic lesions to the histochemical findings of corresponding specimens obtained by directional coronary atherectomy.

Methods and Results—Predirectional coronary atherectomy intravascular ultrasound images of 49 patients were analyzed. The remodeling index was calculated by dividing the target-lesion external elastic membrane cross-sectional area by the reference-segment external elastic membrane cross-sectional area. Expansive remodeling was defined as a remodeling index of >1.0. ROS generation and NAD(P)H oxidase p22phox expression in directional coronary atherectomy specimens were evaluated using the dihydroethidium staining method and immunohistochemistry as the ratio of the positive area to the total surface area in each specimen, respectively. ROS generation and p22phox expression were significantly greater in lesions with expansive remodeling than in lesions without remodeling (0.18±0.12 versus 0.03±0.02, P=0.0001; 0.10±0.08 versus 0.04±0.05, P=0.0039, respectively). Both ROS generation and p22phox expression significantly correlated with the intravascular ultrasound-derived remodeling index (r=0.77, P<0.0001, r=0.53, P<0.0001, respectively).

Conclusions—Simultaneous examination with intravascular ultrasound and immunohistochemistry analyses suggests that NAD(P)H oxidase-derived ROS is related to the coronary arterial remodeling process associated with plaque vulnerability. (Circ Cardiovasc Intervent. 2009;2:196-204.)

Key Words: atherosclerosis ■ remodeling ■ intravascular ultrasound ■ reactive oxygen species ■ oxidative stress

Clinical Perspective on p 204
Oxidative stress induced by reactive oxygen species (ROS) in the vessel wall has an important role in the instability of atherosclerotic plaques and in atherogenesis.7–11 Several...
enzymatic origins of ROS in the vasculature have been proposed, including xanthine oxidase, myeloperoxidase, lipooxygenase, and NAD(P)H oxidase. Among these, NAD(P)H oxidase is a major source of ROS in human coronary arteries. This oxidase system was originally identified as a defense against exogenous microorganisms in phagocytes. Phagocytic NAD(P)H oxidase comprises at least 6 components: the plasma membrane-spanning cytochrome b558 (composed of gp91phox and p22phox), 3 cytosolic components (p67phox, p47phox, and p40phox), and a small G protein, rac. Vascular smooth muscle cells also produce ROS in an reduced nicotinamide-adenine dinucleotide or reduced nicotinamide-adenine dinucleotide phosphate–dependent manner. The significance of NAD(P)H oxidase in the pathogenesis of various cardiovascular diseases is now under intense investigation. Various homologues of phagocytic gp91phox, designated the Nox family, were recently cloned. Among these Nox family members, p22phox is critical for the regulation of NAD(P)H oxidase activity. Knockdown of p22phox via transfection with its antisense oligonucleotide into cultured smooth muscle cells results in decreased NAD(P)H oxidase activity and decreased ROS generation. We previously reported that p22phox is closely associated with plaque vulnerability via enhanced oxidative stress.

Arterial remodeling is a complex process and its precise mechanisms remain to be elucidated. Various cellular responses, including proliferation, phenotypic changes of vascular smooth muscle cells, or deposition of extracellular matrix, may be involved. ROS, especially NAD(P)H oxidase-derived ROS, which have profound influences on these cellular processes, might therefore have a crucial role in the pathophysiology of arterial remodeling. Khatri et al demonstrated a significant role of p22phox in arterial remodeling using transgenic mice overexpressing p22phox in the arterial wall. Therefore, to clarify the association between coronary arterial remodeling and NAD(P)H oxidase-derived ROS in patients with coronary artery disease, we evaluated the relation between preinterventional IVUS findings of the atherosclerotic plaque and ROS generation or p22phox expression in corresponding specimens obtained by directional coronary atherectomy (DCA).

Methods

Patient Population

The study population comprised 49 patients with angina pectoris who were treated with percutaneous coronary intervention using DCA (7 Fr Flexi-Cut L, Abbott Vascular, Abbott Park, Ill) for a de novo lesion in a native coronary artery between May 1, 2000 and July 31, 2008, at Miki City Hospital. Clinical characteristics, including age, sex, risk factors for coronary disease (hypertension, hypercholesterolemia, diabetes mellitus, and smoking), medications, and angio graphic data were available from the medical records and interventional database at our institution. A diagnosis of hypertension, hyperlipidemia, or diabetes was based on the criteria put forth in the guidelines of the Japanese Society of Hypertension, Japan Atherosclerosis Society, or Japan Diabetes Society, respectively. Unstable angina pectoris was defined according to the criteria of Hamm Braunwald, and the angiographic appearance before DCA was evaluated by the classification reported by Ambrose et al. The present study was approved by the hospital ethics committee. Written informed consent was obtained from all patients.

IVUS System and Procedure

Baseline coronary angiography was performed after intracoronary administration of 100 to 200 μg of nitroglycerine. IVUS imaging was then performed before DCA using a commercially available 30- or 40-MHz ultrasound catheter (Boston Scientific, Natick, Mass). The IVUS catheter was advanced to the site with the smallest luminal diameter in the segment where DCA confirmed that the tissue was retrieved from this segment. Proximal and distal references were single slices with the largest lumen and smallest plaque burden within 10 mm proximally and distally, but before any large side branch. At each selected site, the external elastic membrane; CSA, cross-sectional area.

IVUS Analysis

IVUS images were digitized with commercially available software for IVUS image analysis, which runs on an Intel Pentium-based PC system running the Windows NT operating system (NetraIVUS, SClmage Inc, Los Altos, Calif). Two independent operators, who were blinded to the clinical presentation and the histological findings, analyzed the IVUS images. The target lesion was selected as the site with the smallest luminal diameter in the segment where DCA was performed. Images from IVUS pullback performed after DCA confirmed that the tissue was retrieved from this segment. Proximal and distal references were single slices with the largest lumen and smallest plaque burden within 10 mm proximally and distally, but before any large side branch. At each selected site, the external elastic membrane (EEM), lumen, and plaque plus media (P&M=EEM—lumen) cross-sectional area (CSA) were measured. Plaque burden (in percentage) was calculated as P&M CSA divided by EEM CSA. The intra- and interobserver correlation coefficients resulted in r values of 0.99 and 0.97 for the lumen CSA, and r values of 0.99 and 0.96 for the EEM CSA, respectively.

Definitions of Coronary Arterial Remodeling

For the purposes of the present analysis, the remodeling index was calculated as the target-lesion EEM CSA divided by the average of the proximal and distal reference-segment EEM CSA. Expansive remodeling was defined as a remodeling index >1.0. EEM indicates external elastic membrane; CSA, cross-sectional area.

Histological Analysis

Tissue samples obtained during the DCA procedure were immediately embedded in optimal cutting temperature compound (SAKURA Finetechical Co), placed in liquid nitrogen, and stored at...
Table 1. Baseline Patient and Lesion Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Expansive Remodeling (+) (n=23)</th>
<th>Expansive Remodeling (-) (n=26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>64.0±9.4</td>
<td>62.4±10.9</td>
<td>0.592</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>21 (91)</td>
<td>19 (73)</td>
<td>0.100</td>
</tr>
<tr>
<td>Hypertension, n (%)*</td>
<td>12 (52)</td>
<td>7 (27)</td>
<td>0.070</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)†</td>
<td>14 (61)</td>
<td>11 (42)</td>
<td>0.195</td>
</tr>
<tr>
<td>Diabetes, n (%)‡</td>
<td>8 (35)</td>
<td>7 (27)</td>
<td>0.551</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>12 (52)</td>
<td>14 (54)</td>
<td>0.907</td>
</tr>
<tr>
<td>Medication, n (%)</td>
<td></td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>8 (35)</td>
<td>9 (35)</td>
<td>0.990</td>
</tr>
<tr>
<td>ACE inhibitors/ARBs</td>
<td>13 (57)</td>
<td>15 (58)</td>
<td>0.934</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>8 (35)</td>
<td>7 (27)</td>
<td>0.551</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>11 (48)</td>
<td>13 (50)</td>
<td>0.879</td>
</tr>
<tr>
<td>Clinical presentation, n (%)</td>
<td></td>
<td>0.366</td>
<td></td>
</tr>
<tr>
<td>Unstable angina</td>
<td>15 (65)</td>
<td>9 (35)</td>
<td></td>
</tr>
<tr>
<td>Stable angina</td>
<td>8 (35)</td>
<td>17 (65)</td>
<td></td>
</tr>
<tr>
<td>High sensitivity CRP (mg/dL)</td>
<td>0.120 (0.050 to 0.328)</td>
<td>0.195 (0.035 to 0.760)</td>
<td>0.976</td>
</tr>
<tr>
<td>Target coronary artery, n (%)</td>
<td>0.120 (0.050 to 0.328)</td>
<td>0.195 (0.035 to 0.760)</td>
<td>0.976</td>
</tr>
<tr>
<td>Left anterior descending</td>
<td>17 (74)</td>
<td>23 (88)</td>
<td></td>
</tr>
<tr>
<td>Left circumflex</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>5 (22)</td>
<td>2 (8)</td>
<td></td>
</tr>
<tr>
<td>Angiographic stenosis of DCA sites</td>
<td>89.2±8.6</td>
<td>85.2±7.7</td>
<td>0.092</td>
</tr>
<tr>
<td>Angiographic appearance, n (%)</td>
<td></td>
<td>0.182</td>
<td></td>
</tr>
<tr>
<td>Concentric narrowing</td>
<td>6 (26)</td>
<td>7 (27)</td>
<td></td>
</tr>
<tr>
<td>Type 1 eccentric (asymmetric with smooth border)</td>
<td>4 (17)</td>
<td>11 (42)</td>
<td></td>
</tr>
<tr>
<td>Type 2 eccentric (asymmetric with irregular border)</td>
<td>7 (30)</td>
<td>3 (12)</td>
<td></td>
</tr>
<tr>
<td>Multiple irregular narrowing</td>
<td>6 (26)</td>
<td>5 (19)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean±SD, median (interquartile range), or n (%). ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; CRP, C-reactive protein; DCA, directional coronary atherectomy. *Hypertension was defined as a systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of an antihypertensive drug. †Hypercholesterolemia was defined as a total cholesterol level ≥240 mg/dL or medication use. ‡Diabetes was defined as diet controlled and oral agent treated or insulin treated.

Immunofluorescence experiments were performed as described previously.20 Unfixed frozen samples were cut from a given sample and air dried onto slides. Additional serial cryostat sections were stained with hematoxylin-eosin for analysis of morphological details by light microscopy. The tissue slices were fixed with 100% acetone at −20°C for 10 minutes. The sections were incubated with bovine serum albumin (Dako LSAB kit, Dako A/S) for 60 minutes at room temperature and then incubated with primary antibody overnight at 4°C. Rabbit polyclonal antihuman p22phox antibody against a synthetic peptide that corresponded to the p22phox C-terminal region (residues 175 to 194) was used in the present investigation. Mouse monoclonal antihuman CD68 antibody (clone KP-1, Dako) for macrophages and antismooth muscle α-actin antibody (clone 1A4, Dako) were used to analyze cellular composition. The antibody specificity was reported previously.27,28 Texas red-conjugated immunoglobulin was applied as the secondary antibody. The samples were then examined by the laser scanning confocal imaging system. The presence of p22phox was demonstrated by red immunofluorescence. Three independent pathologists who were blinded to the identities of the patients examined the DCA samples. To compare fluorescence signals between different specimens, semiquantitative analysis was performed. All DCA specimens were digitized by a digital camera, and the total area of each section and the surface area occupied by ROS, p22phox, or the cell marker-positive area were outlined using the image analysis software Image J. The fluorescent areas were measured automatically with a fixed threshold. Relative expression was expressed as the ratio of the positive area to the total area. Differences between the 2 groups were analyzed using unpaired Student t test if the distributions were normal. If normality tests failed, the Mann–Whitney U test was used. Categorical variables were reported as frequencies and compared...
using the $\chi^2$ test. Linear regression analyses were performed between the IVUS remodeling index and histological parameters, including the ROS-positive and p22phox-positive areas. Multiple linear regression analysis was calculated to determine independent influences on the IVUS-derived remodeling index. A $P<0.05$ was considered statistically significant.

Results

Baseline Patient and Lesion Characteristics

Expansive remodeling was observed in 23 of the 49 lesions. Baseline patient characteristics are listed in Table 1. Other than clinical presentation, patient characteristics, including medications, did not differ significantly between those having lesions with expansive remodeling and those having lesions without expansive remodeling. Unstable angina pectoris was significantly more frequent in subjects with lesions with expansive remodeling. Unstable angina pectoris was significantly more frequent in subjects with lesions with expansive remodeling. Unstable angina pectoris was significantly more frequent in subjects with lesions with expansive remodeling.

IVUS Measurements of Lesion and Reference Site

Table 2 shows the ultrasound measurements of the lesion and reference site. The parameters of the reference sites were similar between the 2 groups. At the minimum lumen site, lesions with expansive remodeling had a greater EEM CSA, P&M CSA, and plaque burden (in percentage), compared with those without remodeling.

ROS Generation and p22phox Expression in DCA Specimens

Representative micrographs of fluorescence images with dihydroethidium for detection of in situ ROS generation and immunostaining of p22phox in DCA specimens obtained from lesions with and without expansive remodeling are shown in Figures 2 and 3. ROS-positive and p22phox-positive area ratios in DCA specimens from lesions with expansive remodeling were significantly greater than those from lesions without remodeling (0.18±0.12 versus 0.03±0.02, $P<0.0001$, 0.10±0.08 versus 0.04±0.05, $P=0.0039$, respectively; Figure 4). Correlations of the remodeling index with ROS generation and p22phox expression in DCA specimens are shown in Figure 5. Significant positive correlations were observed between the remodeling index and the ROS-positive area ratio ($r=0.77$, $P<0.0001$), and also between the remodeling index and the p22phox-positive area ratio in DCA specimens ($r=0.53$, $P<0.0001$). Furthermore, both ROS generation and p22phox expression correlated significantly with P&M CSA ($r=0.72$, $P<0.0001$, $r=0.32$, $P=0.0250$, respectively; Figure 6).

Multiple linear regression analysis revealed that ROS generation, or plaque burden (in percentage) was independently associated with the remodeling index (Table 3). In all cases, plaque burden (in percentage) was closely associated with the remodeling index ($r=0.63$, $P<0.0001$). The slope of the regression line of the relation between plaque burden (in percentage) and the remodeling index in the high-ROS group (ROS positive area ratio ≥0.05; median value of ROS positive area ratio) was steeper than that of the low-ROS group (ROS positive area ratio <0.05; Figure 7).

Remodeling Index and Cellular Composition in DCA Specimens

The relation between vascular remodeling and cellularity or cellular composition was examined. The cell number in each DCA specimen did not significantly differ between coronary

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Table 2. Intravascular Ultrasound Measurements of Lesion and Reference Site

<table>
<thead>
<tr>
<th></th>
<th>Expansive Remodeling (±) (n=23)</th>
<th>Expansive Remodeling (−) (n=26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average EEM CSA, mm²</td>
<td>16.1±4.8</td>
<td>16.4±3.9</td>
<td>0.795</td>
</tr>
<tr>
<td>Average lumen CSA, mm²</td>
<td>8.2±3.1</td>
<td>8.5±2.2</td>
<td>0.737</td>
</tr>
<tr>
<td>Average P&amp;M CSA, mm²</td>
<td>7.9±2.4</td>
<td>7.9±2.4</td>
<td>0.970</td>
</tr>
<tr>
<td>Average plaque burden, %</td>
<td>49.6±9.3</td>
<td>47.6±8.3</td>
<td>0.434</td>
</tr>
<tr>
<td>Minimal lumen site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EEM CSA, mm²</td>
<td>19.6±6.6</td>
<td>13.9±3.7</td>
<td>0.0005</td>
</tr>
<tr>
<td>Lumen CSA, mm²</td>
<td>2.5±1.1</td>
<td>2.7±1.1</td>
<td>0.522</td>
</tr>
<tr>
<td>P&amp;M CSA, mm²</td>
<td>17.1±6.0</td>
<td>11.2±3.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plaque burden, %</td>
<td>87.0±5.2</td>
<td>79.9±7.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Remodeling index</td>
<td>1.2±0.1</td>
<td>0.8±0.1</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. EEM indicates external elastic membrane; CSA, cross-sectional area; P&M, plaque plus media.
lesions with and without expansive remodeling (supplemental Figure 1). On the other hand, the CD68-positive area in lesions with expansive remodeling was significantly greater than that in lesions without positive remodeling, whereas there was no difference in the antismooth muscle α-actin-positive areas between coronary lesions with and without expansive remodeling. These findings indicate that a macrophage-based inflammatory process may contribute to expansive remodeling of the coronary arteries.

Discussion

In the present study, atherosclerotic plaque specimens obtained by DCA after preinterventional IVUS examination were immunohistochemically analyzed to investigate the association between arterial remodeling and NAD(P)H oxidase-derived ROS. DCA is a catheter-based plaque-debulking device designed to resect and retrieve a part of the atheromatous tissue from the coronary arteries of patients with ischemic heart disease. The use of IVUS during the DCA procedure enables confirmation of the site from which the specimens are obtained. Therefore, IVUS combined with the histological analysis of DCA specimens is a unique method to establish the relation between the ultrasound-derived in vivo findings and the tissue characteristics in the culprit coronary lesion.29,30 In the present study, ROS generation in lesions with expansive remodeling was significantly greater than that in lesions without remodeling, and the degree of arterial remodeling correlated with ROS generation and the expression of NAD(P)H oxidase in the DCA specimens. These findings strongly suggest that ROS derived from NAD(P)H oxidase are crucially involved in the pathogenesis of arterial remodeling in human coronary arteries.

Differences in Lesion Characteristics and IVUS Findings Between Lesions With and Without Expansive Remodeling

In the present study, unstable angina pectoris was statistically more common in patients that had lesions with expansive remodeling than in those having lesions without remodeling. Furthermore, lesions with expansive remodeling had significantly greater EEM CSA, P&M CSA, and plaque burden (in percentage), compared with lesions without remodeling. Previous pathological and IVUS studies have demonstrated that expansive remodeling is frequently observed in culprit lesions of patients with unstable clinical presentation,3,5,31,32 and lesions with expansive remodeling have large atherosclerotic plaques.5,33–35 Our data are consistent with those reported previously. Correlations between coronary arterial remodeling and plaque composition have been investigated. Varnava et al6 analyzed 108 lesions of 88 patients who died...
suddenly of coronary artery disease; lesions with expansive remodeling had a higher lipid content and macrophage count, both of which are markers of plaque vulnerability. Burke et al\textsuperscript{2} also demonstrated that macrophage burden, lipid core size, calcium, and medial atrophy were associated with expansive remodeling in 36 patients who died of severe coronary artery disease. The positive area of CD68, a marker of macrophages, in lesions with positive remodeling was significantly greater than that in lesions without positive remodeling. These findings together suggest that the inflammatory process is involved in vascular remodeling. We previously reported enhanced NAD(P)H oxidase expression and ROS generation in coronary plaques of unstable angina patients compared with those of patients with stable angina.\textsuperscript{10,20} Inflammatory cytokines induced by ROS in coronary plaques could mediate plaque vulnerability by various mechanisms, including the expression of metalloproteinases.\textsuperscript{36} Thus, these findings lead to the hypothesis that arterial remodeling and plaque vulnerability are initiated by the same mechanisms, such as cellular proliferation or an imbalance of metalloproteases and tissue inhibitor of metalloprotease via redox-sensitive pathways.

ROS Generation and p22\textsubscript{phox} Expression in Coronary Lesions With Expansive Remodeling

Expression of p22\textsubscript{phox}, indicating NAD(P)H oxidase activity and ROS generation, was more pronounced in coronary lesions with expansive remodeling than in those without. Furthermore, ROS generation in DCA specimens correlated with the remodeling index and the P&M CSA. The expression of p22\textsubscript{phox} in DCA specimens also positively correlated with the remodeling index and P&M CSA. These findings indicate that ROS derived from p22\textsubscript{phox}-based NAD(P)H oxidase significantly contribute to not only coronary atherogenesis but also to the arterial remodeling process. Atherosclerosis is a complex process and atherosclerotic lesions are composed of various cell types, including smooth muscle cells, fibroblasts, inflammatory cells, and extracellular matrix. The growth and proliferation of these cell types\textsuperscript{17,37} is promoted by the expression of atherogenic gene products such as adhesion molecules and other vascular proinflammatory gene products\textsuperscript{36} induced by enhanced ROS activation of the redox-sensitive signal transduction pathways. The significance of p22\textsubscript{phox} in arterial remodeling was also recently demonstrated in experimental models using p22\textsubscript{phox} transgenic mice, in which p22\textsubscript{phox} overexpression was targeted to vascular smooth muscle cells. Enhanced generation of ROS, smooth muscle cell growth, and neovascularization were observed in the arterial walls of p22\textsubscript{phox} transgenic mice compared with wild-type mice. The carotid flow cessation experimental model revealed significantly more expansive remodeling in p22\textsubscript{phox} transgenic mice compared with that in wild-type mice.\textsuperscript{21} Their findings are very consistent with our clinical observations.

In the present study, plaque burden (in percentage) correlated significantly with the remodeling index, consistent with previous studies.\textsuperscript{5,33–35} Thus, size and volume of atherosclerotic plaques seem to be one of determinants of arterial remodeling. Multiple linear regression analysis, however, revealed that ROS generation or plaque burden (in percentage) was independently associated with the remodeling index. Furthermore, the slope of the regression line between the plaque burden (in percentage) and remodeling index in the high-ROS group was steeper than that in the low-ROS group (Figure 7). These findings suggest that the impact of...
NAD(P)H oxidase-derived ROS on arterial remodeling is independent of that on increasing plaque volume or atherogenesis. The following findings lead us to speculate that ROS have a critical role in vascular remodeling. First, the inflammatory responses associated with ROS generation lead to the release of matrix metalloproteases, which may have a pivotal role in arterial remodeling resulting from the degradation of matrix components within the arterial wall.39–43 Second, generated ROS activate metalloproteases in cultured vascular cells.9 Given the significant role of ROS in metalloprotease regulation, the dysregulation of matrix components by metalloproteases might contribute to arterial remodeling.

Coronary risk factors, including hyperlipidemia, are associated with enhanced vascular ROS.44,45 As described earlier, oxidative stress induced by excess ROS generation is involved in atherogenesis, plaque vulnerability, and arterial remodeling. Thus, oxidative stress is likely to be a common pathway that links risk factors with cardiovascular disease. Previous prospective population studies, however, demonstrated that antioxidant drugs likely have no beneficial effect on cardiovascular disease. The reason for the apparent inability of antioxidants to prevent cardiovascular disease requires further investigation. Simultaneous examination with IVUS and immunohistochemistry analyses, such as in the present investigation, might provide new insights into understanding the pathogenesis of coronary artery disease and might lead to the development of a therapeutic strategy using antioxidants.

### Limitations

The limitations of this study are as follows. First, samples of this study were obtained from lesions with clinically significant stenosis, and may not necessarily reflect focal processes in other lesions, such as a rupture-prone plaque without clinically significant stenosis. Second, ROS generation was assessed by microtopography with dihydroethidium. Several other techniques for the detection of ROS, eg, lucigenin-enhanced chemiluminescence, electron spin resonance, and the cytochrome c reduction method, have been reported and each has advantages and disadvantages regarding sensitivity, specificity, and convenience. Although the generation of ROS should ideally be evaluated by several different methods, we confirmed a good correlation between values measured by microtopography with dihydroethidium and values measured by lucigenin-enhanced chemiluminescence.10 Third, the findings of the present study demonstrated significant correlations between arterial remodeling and ROS generation or p22phox expression; however, these correlations cannot be interpreted as a cause and effect relationship.

Studies using p22phoxnull transgenic mice may provide an answer regarding this issue. As mentioned earlier, the observation of greater expansive remodeling in these transgenic mice in the carotid flow cessation models compared with wild-type mice strongly suggests that ROS derived from NAD(P)H oxidase is causally related to the process of vascular remodeling.

In conclusion, this is the first report of a relationship between local ROS generation and coronary arterial remodeling, and of coronary arterial remodeling related to the expression of p22phox-based NAD(P)H oxidase in these lesions. Taken together, these findings suggest that NAD(P)H oxidase-derived ROS have a significant role in the coronary arterial remodeling process associated with plaque vulnerability in patients with coronary artery disease.

### Acknowledgments

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### Disclosures

None.

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CLINICAL PERSPECTIVE
Arterial remodeling has been considered a biologic phenomenon to maintain constant flow despite increases in atherosclerotic mass. Recent accumulating evidence, however, indicates that coronary arterial remodeling does not necessarily have favorable effects on the cardiovascular system, because it is associated with plaque vulnerability. Arterial remodeling is a complex process in which reactive oxygen species (ROS) are induced, but the precise mechanisms remain to be elucidated. Oxidative stress induced by ROS exerts profound effects on the function of vascular cells, including cellular proliferation, phenotypic changes in vascular smooth muscle cells, and deposition of the extracellular matrix. To clarify the roles of ROS derived from NAD(P)H oxidase in the process of arterial remodeling in patients with coronary artery disease, we evaluated the relation of preinterventional intravascular ultrasound findings of atherosclerotic plaque with ROS generation and NAD(P)H oxidase p22phox expression in corresponding specimens obtained by directional coronary atherectomy. ROS generation and p22phox expression in lesions with expansive remodeling were significantly higher than in lesions without remodeling. Both ROS generation and p22phox expression were significantly correlated with the intravascular ultrasound-derived remodeling index. Coronary expansive remodeling was associated with inflammatory responses. Our observation indicates that NAD(P)H oxidase-derived ROS have a significant role in the coronary arterial remodeling process associated with plaque vulnerability in patients with coronary artery disease. Recent prospective population studies demonstrate that nonspecific antioxidant vitamins likely have no beneficial effect on cardiovascular disease; however, our investigation suggests that NAD(P)H oxidase might be an effective therapeutic target molecule for coronary artery disease.
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Supplemental Figure I: Comparison of (A) cell number, (B) CD-68 positive area ratio, or (C) alpha-actin positive area ratio in specimens obtained by DCA between lesions with and without expansive remodeling.

DCA indicates directional coronary atherectomy.
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