Coronary Endothelial Dysfunction Distal to Stent of First-Generation Drug-Eluting Stents

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Objectives This study sought to evaluate the relationship between coronary endothelial function and neointimal coverage after drug-eluting stent (DES) implantation.

Background The mechanisms of endothelial dysfunction after DES implantation remain to be fully elucidated. We hypothesized that poor neointimal coverage after DES implantation may be associated with endothelial dysfunction distal to the stent site.

Methods Sixty-six stable angina patients treated with a first-generation DES were enrolled. At 9-month follow-up, coronary endothelial function was evaluated with intracoronary infusion of incremental doses of acetylcholine (10⁻⁸, 10⁻⁷, and 10⁻⁶ mol/l) and nitroglycerin (200 μg). Vascular responses at the segments proximal and distal to the stent site were angiographically and quantitatively measured. At the same time, the degree of neointimal coverage was evaluated using coronary angioscopy and classified into 4 grades: 0 (no coverage) to 3 (full coverage).

Results We divided the subjects into poor-coverage (grades 0 to 1, n = 33) and good-coverage (grades 2 to 3, n = 33) groups. Acetylcholine induced dose-dependent coronary vasoconstrictions in both groups. At the segment distal to the stent, the magnitude of vasoconstriction to acetylcholine in the poor-coverage group was significantly greater than in the good-coverage group (p < 0.001), whereas vasomotor responses proximal to the stent and vasodilation by nitroglycerine were similar between the 2 groups.

Conclusions Coronary endothelial dysfunction distal to the stent was associated with poor neointimal coverage after DES implantation. (J Am Coll Cardiol Intv 2012;5:966–73) © 2012 by the American College of Cardiology Foundation

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The penetration of drug-eluting stents (DES), compared with bare-metal stents (BMS), has significantly reduced in-stent restenosis and target lesion revascularization after percutaneous coronary intervention (1,2). Despite these benefits, there is no evidence that DES improve mortality rates when compared with BMS use (1,2). Furthermore, the concerns of late stent thrombosis (LST) and very late stent thrombosis (VLST) after DES implantation have arisen (3,4). Although the incidences of LST and VLST are low, they occur steadily at a constant rate of 0.4% to 0.6% per year up to 3 years after first-generation DES implantation (sirolimus-eluting stents [SES] and paclitaxel-eluting stents [PES]) (5,6) and may be life threatening. The mechanisms of LST and VLST have not been fully elucidated. Several factors may be involved, such as patient-related issues, lesion characteristics, and procedural-related variables, as well as cessation of dual antiplatelet therapy (7). Previous histopathological studies have suggested that poor re-endothelialization may be associated with LST and VLST after DES implantation (8,9). Recently, several clinical studies have revealed coronary endothelial dysfunction at segments adjacent to the first-generation DES implanted site (10–12). Endothelial dysfunction is a well-known factor for thrombosis. Thus, poor re-endothelialization at the stent site and endothelial dysfunction adjacent to the stent site may work together to produce LST and VLST. In this context, we previously reported in the canine model of acute coronary syndrome that endothelium function was impaired distal to the thrombotic site of coronary arteries (13). However, as far as we know, there have been no clinical studies available in which poor re-endothelialization and adjacent endothelial function were investigated simultaneously in humans in vivo.

To evaluate the magnitude of endothelialization in human coronary arteries has been challenging. Imaging modalities such as intravascular ultrasound or coronary angiography are unsatisfactory. In this regard, coronary angiography has been used to visually evaluate and inspect the macroscopic pathology at the stent site. Several coronary angiographic observational data revealed poor neointimal coverage and thrombus formation at the first-generation DES implanted site (14–16). However, endothelial function was not examined in these studies and therefore the association of neointimal coverage with endothelial function has been unknown.

Accordingly, the objective of this study was to clarify the relationship between neointimal coverage and coronary endothelial function in patients treated with first-generation DES.

**Methods**

**Study protocol.** From January 2009 to June 2010, 66 patients diagnosed as having stable angina and treated with a single DES for a de novo lesion were enrolled in this study. SES (Cypher, Cordis Corporation, Miami Lakes, Florida) were implanted in 40 patients, and PES (Taxus, Boston Scientific Corporation, Natick, Massachusetts) were implanted in 26 patients. All stents were implanted using standard percutaneous coronary intervention techniques. Follow-up coronary angiography, coronary endothelial function evaluation, and coronary angioscopy were performed 9 months after percutaneous coronary intervention. The following subjects were excluded from this study: acute and old myocardial infarction; clinical or angiographic history of coronary vasospasm; previous coronary bypass graft surgery; left main coronary artery lesion; bifurcation lesion requiring 2 stents; chronic total occlusions; in-stent restenosis lesion; angiographic in-stent restenosis by follow-up angiography; symptomatic congestive heart failure; severe left ventricular dysfunction (ejection fraction <30%); and severe valvular heart disease. This study was approved by the institutional review board of Kurume University, and all patients provided written informed consent.

**Medication regimen.** All patients received aspirin (100 mg/day) and clopidogrel (75 mg/day) during the follow-up period. Statin and renin-angiotensin system inhibitor, including angiotensin-converting enzyme inhibitor and angiotensin receptor blocker were administered daily to all patients. These drugs may have salutary effects on coronary endothelial function (17–19).

**Evaluation of coronary endothelial function.** Coronary endothelial function was estimated by measuring coronary vasomotion in response to acetylcholine (Ach) at 9-month follow-up. All vasoactive medications, including calcium channel blockers, long-acting nitrates, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and beta-blockers, were discontinued at least 48 h before the test. After baseline angiography, endothelium-dependent vasomotor response was evaluated by intracoronary infusion of incremental doses of Ach at 10⁻⁸, 10⁻⁷, and 10⁻⁶ mol/l for 2 min. At least 3 min was allowed between each infusion. If clinically needed, a temporary pacemaker was inserted through the femoral vein. Subsequently, endothelium-independent vasomotor response was tested after an intracoronary bolus infusion of nitroglycerin (NTG) (200 µg). Angiography was repeated every 30 s for 2 min after each drug infusion. The maximal vasomotor responses to Ach and NTG infusion were determined by quantitative coronary angiography with a
Quantitative coronary angiography measurements were performed by an independent blinded reviewer. Two segments, 5 to 15 mm proximal and distal to the stent, were analyzed. Additionally, as a reference, we evaluated an angiographically normal segment in another vessel. If the stent was in the right coronary artery, an angiographically normal segment as far away as possible from the stent was analyzed as the reference. The same segments were identified by anatomic landmarks and assessed at each measurement. Changes in the vessel diameter in response to Ach and NTG infusion were calculated as the percentage of changes versus the baseline coronary diameter.

Angioscopic procedures and evaluation. Angioscopy was performed after assessment of endothelial function. We used a balloon occlusion type of coronary angioscopy (VecmovaNEO, FiberTech, Tokyo, Japan). Details about the procedure and specifications for these devices have been described elsewhere (20). Briefly, the angioscopic fiber was placed distal to the stent and was manually pulled back from distal to proximal segment of the stent under careful angioscopic and angiographic guidance. When the field of view was flushed clear of blood with lactated Ringer’s solution, inflation of the occlusion balloon was constantly maintained. Each angioscopic image acquisition took from 15 to 25 s, and all sequences were recorded on digital video disks for subsequent offline analysis. Angioscopic images were evaluated with a focus on the following: 1) the dominant degree of neointimal coverage over the stent; 2) existence of thrombus inside the stent; and 3) presence of heterogeneous neointimal coverage in the entire stent (Fig. 1). The degree of neointimal coverage over the stent was classified into 4 grades as previously described (14–16): grade 0, fully visible stent struts similar to immediately after stent implantation; grade 1, stent struts with very thin neointima, but protruding into the lumen and transparently visible; grade 2, stent struts embedded by neointima but seen translucently; grade 3, stent struts fully embedded and not visible. Thrombus: red thrombus formation inside the stent site. Heterogeneous coverage: various neointimal coverage grades within a stent segment. *The position of the guidewire.

Statistical analysis. Statistical analysis was performed using the SPSS (version 11.0, SPSS Inc., Chicago, Illinois). All continuous data are given as mean ± SD or median (interquartile range), according to their normal or not normal distribution. Before statistical analysis, normalcy and homogeneity of the variances were tested using Kolmogorov-Smirnov test and Levene test. Categorical variables are presented as number or percentage. To analyze the association between the neointimal coverage and the vascular response to Ach, we performed Spearman rank
correlation test. Comparisons within groups on percentage of changes in vessel diameter were performed by analysis of variance for repeated measurements. For intergroup comparisons, unpaired t test or Mann-Whitney U test was applied in continuous variables, and chi-square test or Fisher exact test was used in categorical variables. The group and location differences on percentage of changes in vessel diameter were tested by 2-way analysis of variance for repeated measurements. To identify factors that were independently associated with endothelial dysfunction, linear regression analyses were used. Including only variables with a p < 0.05 on simple linear regression test, forward stepwise multivariate regression analysis was performed. A 2-tailed p value <0.05 was considered statistically significant.

Results

The degree of vasoconstriction to $10^{-8}$, $10^{-7}$, and $10^{-6}$ mol/l Ach at the distal segment to the stent had a significant correlation with the grade of neointimal coverage, respectively (Ach $10^{-8}$, Spearman rank correlation coefficient $rs = 0.24$, p = 0.04; Ach $10^{-7}$, rs = 0.37, p < 0.01; and Ach $10^{-6}$, rs = 0.29, p = 0.01), but not at the proximal segment to the stent. For further evaluation, we divided our subjects into the 2 groups according to the grade of neointimal coverage as previously described (14). There were 33 patients in the good-coverage group (grades 2 to 3) and 33 in the poor-coverage group (grades 0 to 1). The baseline patient, lesion, and procedural characteristics were similar in the 2 groups (Table 1). Follow-up data are listed in Table 2.
Because the data of in-stent late loss did not show normality and homogeneity of variance, we have reported medians (interquartile range) of in-stent late loss and performed Mann-Whitney U test for comparison. There was a trend toward greater in-stent late loss in the good-coverage group than in the poor-coverage group, but it was not statistically significant ($p = 0.07$) (Table 3). In addition, no adverse cardiac events occurred during the follow-up period, and no patients showed in-stent restenosis on follow-up angiography.

**Coronary endothelial function.** In both groups, Ach induced dose-dependent vasoconstrictions significantly at the segments both proximal ($p < 0.01$) and distal ($p < 0.01$) to the stent. Vasoconstrictions to Ach were significantly greater at the segment distal to the stent than at the one proximal to the stent in both groups ($p < 0.01$). At the segment proximal to the stent, coronary vasomotor responses to Ach were similar between the 2 groups ($p = 0.50$) (Fig. 2). In contrast, vasoconstrictions to Ach distal to the stent in the poor-coverage group were significantly greater than in the good-coverage group ($p < 0.001$) (Fig. 2). Endothelium-independent vasodilation to NTG distal to the stent was similar between the 2 groups (Fig. 2). Comparing SES and PES, coronary vasomotor responses to Ach and NTG were similar at the segments both proximal and distal to the stent (proximal: $p = 0.97$; distal: $p = 0.47$).

**Angioscopic findings.** The distribution of dominant neointimal coverage grading is shown in Table 3. Thrombus and heterogeneous coverage were more frequently observed in the poor-coverage group than in the good-coverage group ($p < 0.01$) (Table 3). Comparing SES and PES, PES showed a higher incidence of thrombus and heterogeneous coverage than SES did (thrombus: 57.7% [15 of 26] vs. 22.5% [9 of 40], $p < 0.01$; heterogeneous coverage: 53.9% [14 of 26] vs. 12.5% [5 of 40], $p < 0.01$, PES vs. SES, respectively).

### Table 2. Patient Characteristics at Follow-Up

<table>
<thead>
<tr>
<th></th>
<th>Poor-Coverage Group (n = 33)</th>
<th>Good-Coverage Group (n = 33)</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>122.7 ± 11.3</td>
<td>122.2 ± 8.6</td>
<td>0.85</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>67.4 ± 7.3</td>
<td>68.8 ± 8.1</td>
<td>0.47</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>82.3 ± 23.9</td>
<td>89.9 ± 23.2</td>
<td>0.19</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>53.8 ± 11.4</td>
<td>49.5 ± 12.0</td>
<td>0.14</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td>6.0 ± 0.5</td>
<td>6.2 ± 1.0</td>
<td>0.34</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statin</td>
<td>33 (100)</td>
<td>33 (100)</td>
<td>1.00</td>
</tr>
<tr>
<td>Antidiabetic drug</td>
<td>13 (39.4)</td>
<td>16 (48.5)</td>
<td>0.62</td>
</tr>
<tr>
<td>ACEI or ARB</td>
<td>33 (100)</td>
<td>33 (100)</td>
<td>1.00</td>
</tr>
<tr>
<td>Calcium-channel blocker</td>
<td>17 (51.5)</td>
<td>15 (45.5)</td>
<td>0.81</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>18 (54.6)</td>
<td>15 (45.5)</td>
<td>0.62</td>
</tr>
<tr>
<td>Aspirin + clopidogrel</td>
<td>33 (100)</td>
<td>33 (100)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Values are mean ± SD or n (%). Abbreviations as in Table 1.

### Table 3. Angiographic and Angioscopic Findings at Follow-Up

<table>
<thead>
<tr>
<th></th>
<th>Poor-Coverage Group (n = 33)</th>
<th>Good-Coverage Group (n = 33)</th>
<th>p Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-stent late loss, mm</td>
<td>0.10 (0-0.38)</td>
<td>0.40 (0.03-0.60)</td>
<td>0.07</td>
</tr>
<tr>
<td>Grade of neointimal coverage (SES/PES)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>9 (7/2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>24 (14/10)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>17 (12/5)</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>16 (7/9)</td>
<td>—</td>
</tr>
<tr>
<td>In-stent thrombus, %</td>
<td>20 (60.6)</td>
<td>4 (12.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SES/PES</td>
<td>9/11</td>
<td>0/4</td>
<td>—</td>
</tr>
<tr>
<td>Heterogeneous coverage, %</td>
<td>14 (42.4)</td>
<td>5 (15.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>SES/PES</td>
<td>5/9</td>
<td>0/5</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are median (interquartile range), n, or n (%). PES = paclitaxel-eluting stent(s); SES = sirolimus-eluting stent(s).
Independent factors of endothelial dysfunction distal to the stent after first-generation DES implantation. A linear regression analysis was performed to determine the factors of vasomotor response to Ach (10^{-6} mol/l) distal to the stent. Type of stent (SES or PES), existence of heterogeneous neointimal coverage, and patient and lesion variables were not associated with the vasomotion to Ach distal to the stent. The presence of intrastent thrombus, poor coverage (grade 0 or 1) or good coverage (grade 2 or 3), and the grade of neointimal coverage (grades 0 to 3) were significantly associated with the magnitude of vasoconstriction to Ach (10^{-6} mol/l) distal to the stent in a simple linear regression (p < 0.001, p < 0.005, and p < 0.01, respectively). In a stepwise multivariate regression analysis, the presence of intrastent thrombus and the poor coverage or good coverage were determined as the independent factors of endothelial dysfunction distal to the stent after first-generation DES implantation (p < 0.001 and p = 0.04, respectively) (Table 4).

Discussion

The present study demonstrated that coronary vasoconstriction in response to Ach at the segment distal to the stent site was significantly greater in the group with poor neointimal coverage estimated by coronary angioscopy than that in the group with good neointimal coverage at 9-month follow-up after first-generation DES implantation. In this study, in-stent thrombus formation and heterogeneous neointimal coverage were more frequently observed in the poor-coverage group than in the good-coverage group. Our findings suggest that coronary endothelial function distal to the stent could be deteriorated by the impaired arterial healing at the stent after DES implantation.

Coronary endothelium-dependent vasomotor response after DES implantation. In the present study, vasomotor response to Ach distal to the stent was significantly different between the poor-coverage group and the good-coverage group. The questions are raised whether this difference has been affected by the systemic factors or the stent type. In these respects, first, the difference in vasomotor responses was not due to the atherosclerotic risk factors, medications, lesion characteristics, and procedural-related factors, because they were similar in the 2 groups at both baseline and follow-up. Second, this vasomotor response distal to the DES site was not caused by systemic vascular response, because vasoconstrictive responses to Ach were similar between the 2 groups and vasodilation by NTG were similar between the 2 groups at the reference segment. Third, vasomotor responses to Ach or NTG were similar between SES and PES. Therefore, vasoconstriction to Ach distal to the stent could be related to a local-regional response regardless of the stent type.

Table 4. Independent Factors of Endothelial Dysfunction Distal to the Stent* After First-Generation DES Implantation

<table>
<thead>
<tr>
<th></th>
<th>Simple Regression</th>
<th>Stepwise Multivariate Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p Values</td>
</tr>
<tr>
<td>Presence of thrombus</td>
<td>-0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Poor-coverage group/</td>
<td>0.41</td>
<td>0.001</td>
</tr>
<tr>
<td>good-coverage group†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Vascular motion in response to acetylcholine (10^{-6} mol/l) at segment distal to drug-eluting stent (DES). †Poor coverage = grade 0 or 1; good coverage = grade 2 or 3.
Under healthy endothelial conditions, Ach causes vasodilation by inducing the release of nitric oxide. By contrast, if endothelial dysfunction exists, paradoxical vasoconstrictive response to Ach is observed because of a disturbed nitric oxide release and vasoconstriction of the vascular smooth muscle (22). In the present study, the vasoconstriction to Ach distal to the stent was more intense in the poor-coverage group than in the good-coverage group, whereas the vasodilator response to NTG was similar between the 2 groups, which indicated functional impairment of the endothelium and preserved vascular smooth muscle dilator function. In the previous studies, although coronary vasoconstriction to Ach was observed in both SES and PES, endothelium-dependent vasomotion in BMS was preserved at 6 to 9 months after stenting (10–12). A possible explanation for this difference may be that re-endothelialization is nearly completed after BMS implantation (23), but not after DES implantation (8,9). Accordingly, endothelium-dependent vasoconstriction distal to the stent may be associated with the incomplete re-endothelialization and/or endothelial dysfunction at the stented site after SES and PES implantation.

**Arterial healing and re-endothelialization after DES implantation.**

Delayed arterial healing is a well-known phenomenon after SES and PES implantation in the previous pathological or imaging-modality studies (8,9,14–16). In this study, coronary angiography revealed the individual variation of arterial healing within SES or PES. Most notably, the prevalence of thrombus was more frequent in the poor-coverage group than in the good-coverage group, because it has been reported that not only the lack of neointimal coverage in stent struts correlates with thrombus formation (24), but also that the presence of uncovered struts without re-endothelialization is the most powerful predictor of thrombus formation (9). Detection of poor neointimal coverage and/or thrombus formation by coronary angiography in this study could indicate the existence of impaired re-endothelialization, although it may be difficult to confirm the impaired re-endothelialization in clinical settings. Thus, there could be differences in terms of in-stent re-endothelialization between the poor-coverage group and the good-coverage group.

**Re-endothelialization at the stent site and endothelium-dependent vasomotor response distal to the stent.**

The underlying mechanisms of impaired endothelium-dependent vasomotor response distal to the DES site have not been well recognized. Our data demonstrate that the severities of endothelium-dependent vasoconstriction distal to the stent were associated with the impaired arterial healing, such as poor neointimal coverage and thrombus formation at the stent site, whereas endothelium-dependent vasomotion proximal to the stent was comparable in all subjects. Possible mechanisms for impaired endothelial function distal to the stent are considered as follows. First, the antiproliferative drugs may have locally diffused through vaso vasorum to the nonstented distal segments, leading to impaired endothelial function distal to the stent after DES implantation (25). Second, we have previously demonstrated in the canine model of acute coronary syndromes that the adhesive interaction between activated platelets and leukocytes plays an important role in endothelial injuries of the coronary artery distal to the thrombotic site. Namely, the lack of full endothelialization of the DES site would predispose thrombus formation at the stent site. The thrombi formed at the stent site release several vasoactive substances that are shed into the distal site of the stent and would impair the distal endothelial function as we demonstrated in the canine model (13,26). Although the pathophysiology at the DES site was different from that at the artificially created stenosis in our previous canine model of acute coronary syndromes, the common pathophysiological findings between them may be localized inflammatory reactions at the culprit site. Thus, several cytotoxic substances released from the DES site might be responsible for impaired endothelial function distal to the stent.

**Study limitations.** First, this study was a relatively small, nonrandomized single-center study. Second, our study ended at 9 months after DES implantation, thus our results refer to this specific point. Moreover, it remains unknown whether endothelial dysfunction associated with DES would persist beyond 9 months. Finally, the clinical outcome of our results in the long term remains unknown. To overcome the previously mentioned limitations, a prospective, randomized multicenter, long-term follow-up study will be required.

**Clinical implication.** It is important to detect coronary endothelial dysfunction in patients with coronary artery disease, because coronary endothelial dysfunction has been indicated as an independent predictor of atherosclerotic disease progression and cardiovascular event rates (27). Furthermore, the previous histopathological study documented that the occurrence and existence of uncovered struts complicated by a dysfunctional endothelium remain the primary cause of LST or VLST in DES (8,9). The present study is a first report to reveal the relationship between delayed arterial healing and coronary endothelial function within the first-generation DES.

**Conclusions**

This study demonstrates that coronary endothelial dysfunction distal to the stent is associated with poor neointimal coverage at the stent site at 9 months after first-generation DES implantation.
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REFERENCES

Key Words: angioscopy ■ drug-eluting stent ■ endothelial function ■ neointima.