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The Relationship Between Re-Endothelialization and Endothelial Function After DES Implantation: Comparison Between Paclitaxcel Eluting Stent and Zotarolims Eluting Stent

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Background: Several studies have reported re-endothelialization and endothelial function after drug-eluting stent (DES) implantation; however, the relationship between re-endothelialization and endothelial function after DES implantation has not been investigated yet. Methods: A total of 14 patients underwent evaluation of re-endothelialization by optical coherence tomography (OCT) and endothelial function by incremental Ach infusion at 9 months after DES implantation (ZES: $N = 7$, PES: $N = 7$). The neointimal thickness (NIT) inside each strut, strut coverage, and malapposition at every 1 mm cross-section were evaluated by OCT and the endothelial function was estimated by measuring the coronary vaso-reactivity in response to acetylcholine (Ach) infusion into coronary arteries. Results: Zotarolims eluting stent (ZES), compared with paclitaxcel eluting stent (PES), showed more homogeneous neointimal coverage of stent struts and low rate of malapposition. Vasoconstriction in response to Ach in the peri-stent region was also less pronounced in ZES than PES. In particular, vasoconstriction was more often observed in cases with inhomogeneous neointimal coverage of stent struts in the PES group. Conclusions: Our findings suggest that endothelial function seems to be better preserved with ZES than PES, and homogeneous neointimal coverage of stent struts seem to be associated with the preserved endothelial function.

Key words: re-endothelialization; endothelial function; TAXUS; endeavor

INTRODUCTION

Recent reports have raised concerns that drug-eluting stent (DES) implantation may cause delayed or absent endothelialization of traumatized coronary vessel walls that could result in coronary endothelial dysfunction [1,2]. A recent human autopsy study showed that the most important histological and morphometric predictors of late stent thrombosis (LST) were endothelial coverage and the ratio of incomplete neointimal coverage of stent struts after DES implantation [3].

In a series of 8,000 DESs, Daemen et al. reported a consistent 0.6% LST rate over the first 3 years post-stenting [4]. LST is a various factorial phenomenon [5]; however, one significant factor is failure of stent strut re-endothelialization [6].

A zotarolimus-eluting stent (ZES) (Endeavor, Medtronic CardioVascular, Santa Rosa, CA), a cobalt chromium-based thin-strut stent with a phosphorylcholine biocompatible polymer and a shorter drug-elution time of within 2 weeks, have been developed as a second-generation DES and is reported to promote rapid and uniform healing of the endothelium [7]. It has been shown to have a significantly lower prevalence of uncovered struts and malapposed struts than a sirolimus-eluting stent (SES) or a paclitaxel-eluting

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stent (PES), called "first-generation DES" at more than 12 months follow-up on optical coherence tomography (OCT) [8].

However, it is not clear whether neointimal strut coverage detected by OCT can be used as a surrogate for vessel healing after DES implantation.

Changes in coronary endothelial function may be important prognostic factors in the long-term clinical outcomes of patients after DES implantation. In this study, we evaluated the relationship between neointimal strut coverage or malapposition detected by OCT and coronary vasomotion in response to acetylcholine at 9 months after ZES implantation.

TABLE I. Baseline and Procedural Characteristics

	ZES ($N=7$)	PES ($N=7$)	P-value
FU month	10.6 ± 1.9	8.6 ± 1.5	NS
Male gender (%)	100.0%	71.4%	NS
Age	61.6 ± 10.9	69.7 ± 6.2	NS
DM (%)	14.3%	28.6%	NS
HL (%)	57.1%	85.7%	NS
HT (%)	71.4%	71.4%	NS
Target			
LAD/LCx/RCA	5/0/2	6/1/0	NS
prox REF (mm)	2.96 ± 0.29	2.96 ± 0.33	NS
dis REF (mm)	2.49 ± 0.36	2.38 ± 0.34	NS
Stent size (mm)	3.11 ± 0.28	3.18 ± 0.31	NS
Stent length (mm)	19.7 ± 4.2	19.4 ± 6.3	NS

Values are expressed as mean ± SD, or n (%).
ZES, zotarolimus eluting stent; PES, paclitaxcel stent.

METHODS

Study Design and Samples

The baseline clinical characteristics are summarized in Table I. A total of 14 patients who were diagnosed with stable angina and were treated with a single stent (ZES: $N=7$, PES: $N=7$) for a de novo single lesion in the coronary arteries. Eligible subjects were older than 20 years of age, with stable or unstable coronary syndromes. Exclusion criteria were left main disease, ongoing/recent myocardial infarction, previous target vessel stenting, creatinine >2.0 mg/dl, ejection fraction <30%, no suitable anatomy for OCT (ostial lesions and extreme tortuosity). Dual antiplatelet therapy with aspirin and clopidogrel was maintained for 9 months to follow. For all procedures, IVUS-guided stenting was performed. Stenting was performed up to full stent-vessel wall apposition.

Evaluation of Endothelial Function

At 9-month follow-up, endothelial function was estimated by measuring the coronary vasoreactivity in response to Ach infusion into the coronary arteries (Ach1: 20 μg; Ach2: 50 μg; Ach3: 100 μg for 1 min, performed at an infusion rate of 5 ml/min); a 5-min interval was allowed between doses (Fig. 1). When the maximum dose was reached, an intracoronary bolus injection of nitroglycerin (200 μg) was administered. Fujii et al. reported changes in vasomotor reaction 3 months after ZES implantation [9]. It changed over 25% in mean vessel diameter at distal segment after intracoronary infusion of acetylcholine with incremental dose of 100 μg, therefore we defined spasm as % change of vasoreactivity at stent distal position > 25% by angiography, and we measured neointimal hyperplasia thickness on each strut between two groups having spasm or not.

Evaluation of Re-Endothelialization by OCT

After evaluation of endothelial function, OCT was performed to assess the re-endothelialization after DES implantation. The OCT images were obtained with the M2 OCT imaging system (LightLab Imaging, Westford, MA). Motorized pullback OCT imaging was performed at a pullback rate of 1.0 mm/sec. Images were acquired at 15 frames/sec, displayed with a color look-up table and digitally archived. The OCT measurements were performed with LightLab OCT imaging proprietary software with a mouse-based Interface. The neointimal thickness (NIT) inside each strut, strut coverage, and malapposition at every 1 mm cross-section were evaluated. We defined as follows: uncovered slice: three continuous uncovered stent struts were observed in the same slice (Fig. 2) [10].

Statistical Analysis

All statistical analyses were performed using statistical package for the social sciences (SPSS) ver.20 (SPSS, Chicago, IL). Quantitative data were expressed as mean ± standard deviation and were analyzed by student's t-test. A two-tailed P of <0.05 was considered statistically significant.

RESULTS

The baseline clinical characteristics are summarized in Table I. Although DES were not randomly selected, baseline characteristics were similar between two stent

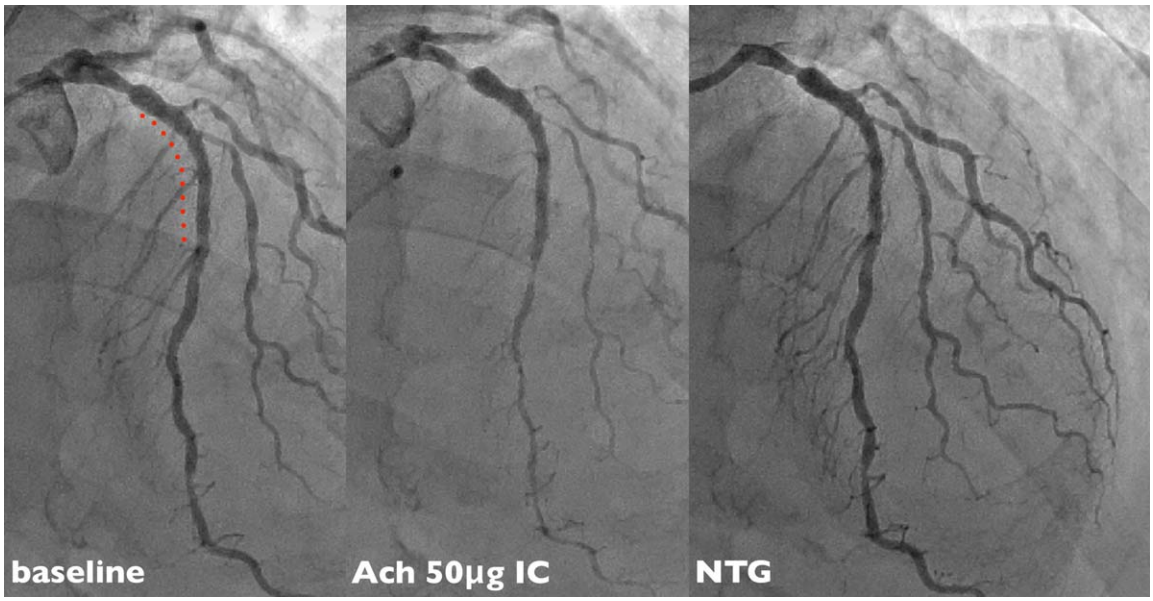

Fig. 1. Coronary vasoreactivity in response to Ach infusion into coronary arteries. Left, the first imaging without nitroglycerine. Center, imaging after acetylcholine injected. Right, imaging after nitroglycerine injected. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

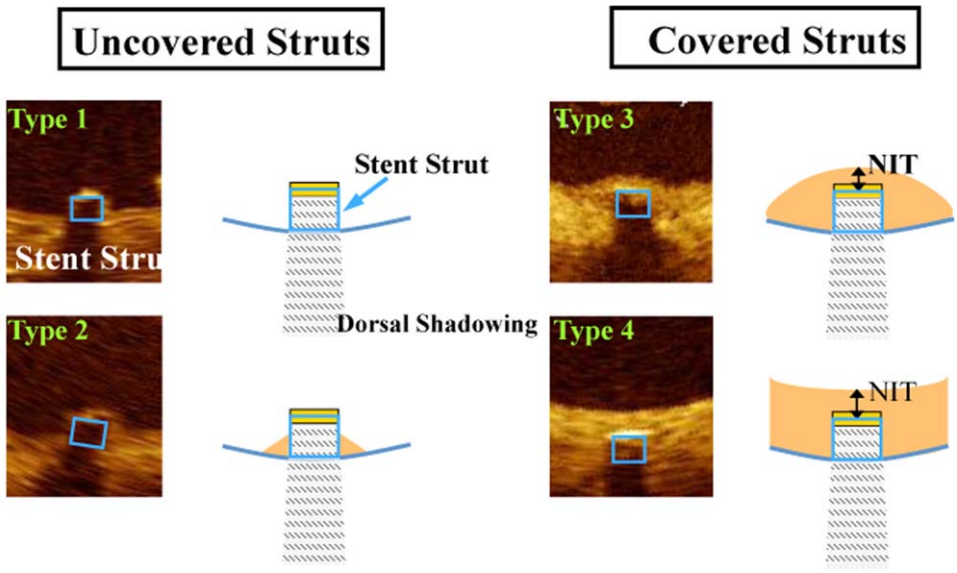

Fig. 2. Stent struts classification by intracoronary optical coherence tomography: type 1-malapposed/uncovered strut, type 2-protuding/uncovered strut, and type 3/4-embedded strut. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

groups. In the PES group, the prevalence of diabetes and dyslipidemia, and the average age was higher than ZES group. In addition, ZES group was all male. However, the mean stent diameter and stent length was similar between the two groups.

Comparison of vasoreactivity between ZES and PES is summarized in Fig. 3. The incidence of acetylcholine-induced spasm was higher in the PES group, also if a proximal portion and a distal portion compared with ZES group.

Strut-based evaluation of re-endothelialization by OCT is summarized in Table II. The ZES group demonstrated a significantly greater magnitude of neointimal coverage than the PES group (0.27 ± 0.14 mm vs. 0.17 ± 0.18 mm, $P < 0.01$), and a low rate of malapposition (0 vs. 2.7%, $P < 0.01$). The ZES group also

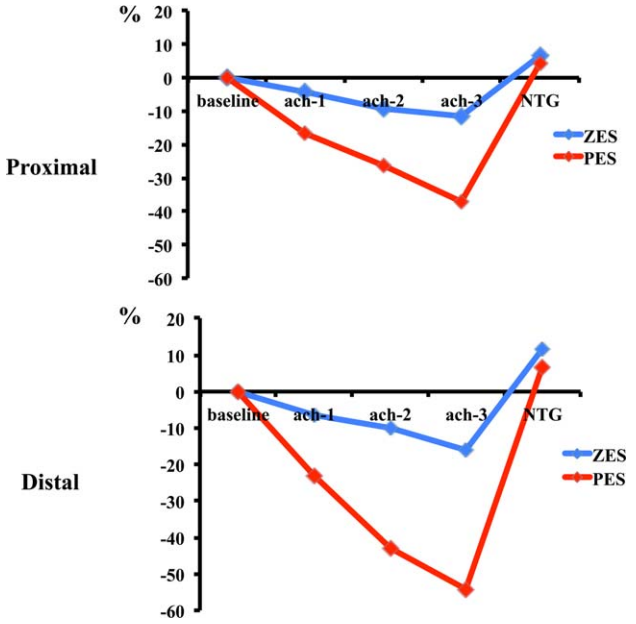

Fig. 3. Comparison of Vasoreactivity between ZES and PES. Mean coronary artery segment luminal diameter change at 9 month's follow-up after intracoronary infusion of acetylcholine (Ach) with incremental doses of 20 (ach-1), 50 (ach-2), 100 (ach-3) μg and nitroglyceline (NTG) for individual patients. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II. Angiographic and OCT Analysis

	ZES ($n=7$)	PES ($n=7$)	P-value
Spasm (+) (%)	28.6	57.1	
Strut-based analysis	$n=1590$	$n=897$	
Coverage (%)			<0.0001
1/2	0	13.4	
3/4	100	86.6	
Malapposition (%)	0	2.7	<0.0001
NIT (mm)	0.27 ± 0.14	0.17 ± 0.18	<0.0001
	Spasm (+)	Spasm (–)	
Coverage (%)			<0.0001
1/2	11.1	1.3	
3/4	88.9	98.7	
Malapposition (%)	2.7	0	<0.0001
NIT (mm)	0.21 ± 0.19	0.25 ± 0.14	<0.0001

Evaluation of re-endothelialization by OCT. Spasm was defined as %change of vasoreactivity at stent distal position >25%. Values are given as mean ± SD, or n (%).

NIT, neointimal thickness; OCT, optical coherence tomography; ZES, zotarolimus eluting stent; PES, paclitaxcel stent.

showed a lower neointima-free frame ratio than did the PES group.

Spasm was defined as percent change of vasoreactivity at a distal position >25% of the stent as described above. Vasoconstriction in response to Ach in the peristent region was also less pronounced in the ZES than PES (28.6 vs. 57.1%, $P<0.01$). In the spasm group, there was a lower and inhomogeneous neointimal coverage than in the nonspasm group (0.21 ± 0.19 mm vs. 0.25 ± 0.14 mm, $P<0.01$). In particular, vasoconstriction was more often observed in cases with inhomogeneous neointimal coverage of stent struts in the PES group.

DISCUSSION

This is the first study for Japanese to evaluate the association between re-endothelialization and endothelial function by acetylcholine infusion at 9 months after ZES implantation. This study demonstrated that the degree of vasoconstriction in response to acetylcholine was associated with the number of uncovered struts and malapposition detected by OCT.

Although intrastent thrombosis is rare, it has an extremely serious prognosis and a reported mortality reaching 45% [11]. Noncoverage strut is one causal factor of intrastent thrombosis, especially in cases of resistance to or premature discontinuation of dual antiplatelet therapy. A recent autopsy study showed that the most important histological and morphometric predictors of LST were endothelial coverage and the ratio of uncovered to total stent struts after DES implantation [3,12]. Ko et al., previous OCT studies in patients with DES-related very late stent thrombosis (VLST), reported that in 15.8% of patients were uncovered stent struts observed [13]. Therefore, identification of neointima over stent struts could provide important information to predict LST. There is, however, a lack of instruments for the in vivo study of re-endothelialization. Under these circumstances, OCT would seem to be most reliable, providing precise, reproducible, strut-by-strut measurements [14]. However, it is not quite clear to whether complete neointimal coverage of stent struts can be established as a surrogate for vessel healing after implantation of DES.

The vascular endothelium plays a critical role in the regulation of arterial function through the synthesis and elaboration of a number of antiatherogenic factors such as nitric oxide (NO). Acetylcholine evokes a NO-mediated vasodilatory response in healthy arteries via muscarinic endothelial membrane receptors, but this effect is blunted, and paradoxical vasoconstriction is observed with endothelial dysfunction [15]. Therefore, coronary angiography after intracoronary acetylcholine infusion is one of the most used methods for evaluating coronary endothelial function after DES implantation. In our study, neointimal coverage was not complete with significant difference in the spasm group.

A histopathological study showed that re-endothelialisation was complete 3 months after bare-metal stent (BMS) implantation [16]. However, there is some report of abnormal coronary vasoconstrictive responses with acetylcholine after implanted first-

generation DES [17]. It has been reported that inhibition of smooth muscle cell of proliferation and endothelial regeneration [18], and local coronary inflammation caused delayed re-endothelialisation of stent and vessel walls [19]. These findings suggest that the coverage of stent struts with neointimal hyperplasia may offer protective advantage for early vessel healing.

Our data show greater neointimal hyperplasia at 9 months in the ZES group (0.27 vs. 0.17 mm in the PES group). This value is not inferior to previous angiographic findings based on late loss. Pocock et al., in 11 randomized trials, reported between 0.60 and 0.61 mm late loss with ZES and 0.30–0.49 mm with PES at 1 year [20].

In the literature, significantly less endothelial strut coverage was more apparent with earlier stent designs of Sirolimus eluting stent (SES) or PES relative to the more recent ZES [21]. It is considered that the drug dose/polymer combination and release properties may also affect endothelial cell proliferation and migration. However, seven randomized trials demonstrated that ZES showed more in-stent restenosis, late lumen loss "in stent" and "in segment" [22]. One should be careful with the implantation of small size ZES; but conversely, it will be convenient when obliged to discontinue dual antiplatelet therapy.

However, noncoverage struts could not be a sufficient condition of the development LST, because there was a significant difference between the rate of deficient coverage in vivo on OCT and the rate of LST. A randomized controlled trial has reported that the rate of incident LST after ZES was 0.9% at the 5-year follow-up [23]. It may be a determining risk factor such as malapposed stent struts, rupture of lipid-laden neointima inside the DESs, or premature discontinuation of antiplatelet therapy. These remarks lead us to continue dual antiplatelet therapy beyond the first 6 months after DES implantation in patients who do not have a high risk of hemorrhage, in line with the latest report [24].

Our present study showed OCT to be an eligible safety tool for investigation re-endothelialization and partial strut coverage. Some randomized comparative OCT trials may provide prognostic factors with the resolution of poststenting healing and may help to determine the term of dual antiplatelet therapy in the future.

STUDY LIMITATIONS

There were three major limitations of this study. First, this study was not a prospective, randomized, controlled study. Secondly, no baseline OCT data were available. Because this is a clinical retrospective study, we cannot show the vasoreactivity in the reference vessel on account of the insurance. Finally, this study was a single-center study with relatively small population and might have a risk of selection bias, therefore baseline patient characteristics becomes the huge difference in one difference. There are huge differences in gender, age, and risk factors of diabetes and dyslipidemia, but it is a limit to exclude significant difference because of small sample size. There is a possibility that higher severity patient in the PES group, but QCA data has no significant difference between two stent groups. However, we evaluated about 1000 stent struts and it seems to be enough to evaluate the relationship between neointimal strut coverage and coronary vasomotion.

CONCLUSION

Our findings suggest that endothelial function can be better preserved in association with ZES than PES and homogeneous neointimal coverage of stent struts can be associated with the preserved endothelial function.

It is conceivable that the second-generation DES, "ZES" could be more beneficial under specific clinical conditions, such as large vessels, patients who cannot continue dual antiplatelet therapy for a certain reason.

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