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The impact of triple anti-platelet therapy for endothelialization and inflammatory response at overlapping bioabsorbable polymer coated drug-eluting stents in a porcine coronary model

Keun-Ho Park [a,b], Myung Ho Jeong [a,b,*], Jong Min Kim [b], Dae Sung Park [b], Jung Ha Kim [b], Kyung Seob Lim [b], Ki Hong Lee [a,b], Doo Sun Sim [a,b], Hyun Ju Yoon [a,b], Nam Sik Yoon [a,b], Kye Hun Kim [a,b], Hyung Wook Park [a,b], Young Joon Hong [a,b], Ju Han Kim [a,b], Youngkeun Ahn [a,b], Jeong Gwan Cho [a,b], Jong Chun Park [a,b], Jung Chaee Kang [a,b]

[a] The Heart Center of Chonnam National University Hospital, South Korea
[b] The Heart Research Center of Designated by Korea Ministry of Health and Welfare, South Korea

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ABSTRACT

Background: This study was conducted to evaluate the endothelialization and the inflammatory responses depending on the administration duration of triple anti-platelet therapy at overlapping bioabsorbable polymer coated biolimus-eluting stents (BESs) in a porcine coronary model.

Methods: We successfully deployed 36 overlapping BESs for the left anterior descending coronary and left circumflex artery or right coronary artery in 18 non-injured pigs. Total pigs were divided into 3 groups (12 overlapping stents of 6 pigs in each group) as follows: group I received aspirin 100 mg and clopidogrel 75 mg daily for 8 weeks, group II received aspirin 100 mg and clopidogrel 75 mg daily for 8 weeks and cilostazol 200 mg daily for initial 4 weeks, and group III received aspirin 100 mg, clopidogrel 75 mg, and cilostazol 200 mg daily for 8 weeks. Follow-up coronary angiograms and histomorphometric and histopahtologic analyses at overlapping and non-overlapping segments were performed respectively.

Results: Inflammation score was similar between overlapping and non-overlapping segments in all pigs (1.2 ± 0.33 vs. 1.1 ± 0.17, $p = 0.117$). The neointima area (NA) and percent area stenosis (%AS) at overlapping segments were not significantly different among the 3 groups. However, at non-overlapping segments, NA and %AS in group III were significantly smaller than those in group I (2.3 ± 0.50 mm^2 vs. 1.8 ± 0.43 mm^2, $p = 0.037$; 48.9 ± 12.85% vs. 37.7 ± 9.08%, $p = 0.031$).

Conclusions: Our study shows that BES appears to be reliable on the inflammatory response at overlapping segments as well as non-overlapping segments. Long-term administration of cilostazol is more effective in reducing neointimal formation at non-overlapping segments of BESs in a porcine coronary model.

1. Introduction

It is well known that bare-metal stents (BMS) have the high restenosis rates and poor clinical outcomes in diffuse long coronary disease requiring overlapping stents [1–3]. However, since drug-eluting stents (DES) were introduced, the incidences of restenosis have been substantially reduced [4–7]. Nonetheless, the fatal complication such as stent thrombosis is still an unresolved problem [8,9]. Particularly, it has been reported that the arterial healing may be delayed due to excessive inflammatory reactions at the overlapping DES segments in diffuse long coronary lesion [10]. However, most studies were conducted in the first generation DES era such as sirolimus-eluting stents (SES; Cypher®, Cordis/Johnson and Johnson, Warren, NJ, USA) or paclitaxel-eluting stents (PES; Taxus®, Boston Scientific Co., Natick, MA, USA). It is not known that the arterial healing and inflammatory response after the overlapping biolimus A9-eluting stents (BES, BioMatrix®, Biosensors Interventional Technologies Pte Ltd., Singapore) which were recently developed as a 3rd generation DES contained the bioabsorbable polymer with eluting the rapamycin series, biolimus A9.

Cilostazol selectively inhibits phosphodiesterase type III which is released from the platelet, and it thereby raises the intracellular concentration of cAMP and calcium. Therefore, it suppresses the platelet aggregation and relaxes the vascular smooth muscle cells, thus having a vasodilatory effect and suppresses the proliferation of injured vascular endothelium. It has been reported that compared to standard dual anti-platelet therapy (DAP; aspirin and clopidogrel), triple anti-platelet

* Corresponding author at: Korea Acute Myocardial Infarction Registry, Heart Research Center Nominated by Korea Ministry of Health and Welfare, Chonnam National University Hospital, 671 Jaebongro, Dong-gu, Gwangju 501-757, South Korea. Tel.: +82 62 220 6243; fax: +82 62 228 7174.
E-mail address: myungho@chollian.net (M.H. Jeong).

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therapy (TAP; aspirin, clopidogrel and cilostazol) significantly reduced late loss at 6 months after DES implantation in patients with long coronary lesions [11]. However, the optimal duration of cilostazol administration is not clear, and particularly, the endothelialization and inflammatory response of DES according to the administration duration of cilostazol have not been reported yet.

Therefore, our study was aimed to evaluate and compare the endothelialization and the inflammatory response between overlapping and non-overlapping segments of BESs depending on the duration of TAP administration in a porcine coronary model.

2. Materials and methods

2.1. Experimental groups

Total 24 overlapping BESs of 12 pigs were divided into 3 groups as follows (8 overlapping BESs of 4 pigs in each group):

Group I Aspirin 100 mg/day and clopidogrel 75 mg/day were administered for 2 months after the overlapping BESs.
Group II Aspirin 100 mg/day and clopidogrel 75 mg/day were administered for 2 months and cilostazol 200 mg/day additionally for the initial one month after the overlapping BESs.
Group III Aspirin 100 mg/day, clopidogrel 75 mg/day, and cilostazol 200 mg/day were administered for 2 months after the overlapping BESs

The flow chart of study was depictured in Fig. 1.

2.2. Experimental animals and procedure

Our animal study was approved by the Ethics Committee of Chonnam National University Medical School and Chonnam National University Hospital (CNU IACUC-H-2010-17), and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Study animals were female swine weighing 25–30 kg. From 5 days prior to experiments, aspirin (100 mg/day) and clopidogrel (75 mg/day) were administered to all pigs. On the procedure day, pigs were anesthetized with ketamine [20 mg/kg intramuscularly (IM)] and xylazine (2 mg/kg IM). They received IM ketamine every 30 min and supplemental oxygen continuously through an oxygen mask and saline was injected through the ear vein. Subcutaneous 2% lidocaine was administered at the cut-down site, left or right carotid artery was surgically exposed and a 7 French sheath was inserted. Continuous hemodynamic and electrocardiographic monitoring were performed throughout the procedure. Heparin (5000 units) was administered intravenously as a bolus prior to the procedure, the target coronary artery was engaged using standard 7 F guiding catheter and both coronary angiograms were performed. Two BESs were implanted in the proximal to mid portion of the left anterior descending artery (LAD) with a 5–8 mm overlap (double strut density) and the overlapping segment was dilated using stent balloon for 10 s. After then, we also deployed overlapping BESs for left circumflex artery (LCX) or right coronary artery (RCA) by same manners.

At the eight weeks after overlapping stent implantation, the pigs underwent follow-up angiography in the same views. After then, they were sacrificed using an intracoronary injection of 20 mL of potassium chloride. The heart was extracted and the coronary arteries were pressure-perfusion fixed at 110 mmHg in 10% neural buffered formalin overnight. The arteries were step-sectioned, processed routinely for light microscopy, and stained for histological analysis.

2.3. Histopathological and histomorphometric analyses

Histopathological and morphometric analyses of each stented segments were performed by an independent experienced observer. The specimens were embedded in methyl methacrylate and sections were cut with a low-speed diamond wafer mounted to a Buehler Isomet saw (Buehler Ltd., Lake Bluff, IL, USA), leaving the stent wires intact in the cross sections to minimize potential artifacts caused by removal of the stent wires. Sections of 50 to 100 μm thickness were obtained at 1 mm intervals and stained with hematoxylin-eosin (H&E) for histological analysis.

Measurements of the histopathologic sections were performed using a calibrated microscope, digital video imaging system, and microcomputer program (Visus 2000 Visual Image Analysis System, IMT Tech, CA, USA). The external elastic lamina area (EEL, mm^2), the internal elastic lamina area (IEL, mm^2), the lumen area (mm^2), and the neointima area (NA, mm^2) were measured in more than 3 areas of the overlapping (the mid-portion of stented segment) and of the non-overlapping (the proximal and the distal portions of stented segment) segments in each coronary artery. Morphometric analysis of NA for a given vessel was calculated as the measured IEL area minus the lumen area. Histopathologic percent area stenosis (%AS) was calculated as $100\times[1-(\text{lumen area}/\text{IEL area})]$.

2.4. Evaluation of arterial injury, inflammation, and fibrin scores

Arterial injury at each strut site was determined by the anatomic structures penetrated by each strut. A numeric value from 0 to 3 was assigned, as previously described by Schwartz et al. [12]: 0 = no injury, 1 = break in the internal elastic membrane, 2 = perforation of the media, and 3 = perforation of the external elastic membrane to the adventitia. The average injury score for each segment was calculated by dividing the sum of injury scores by the total number of struts at the examined section.

The inflammation score for each individual strut was graded as follows: 0 = no inflammatory cells surrounding the strut, 1 = light, non-circumferential lymphohistiocytic infiltrate surrounding strut, 2 = localized, moderate to dense cellular aggregate surrounding the strut non-circumferentially, and 3 = circumferential dense lymphohistiocytic cell infiltration of the strut [13]. The score for each section was obtained from the mean of the individual strut scores. Strut-associated fibrin content also was assessed and data expressed as the number of struts.

2.5. Statistical analysis

The data are presented as mean value ± SD. The mean morphometric and histologic data between overlapping and non-overlapping segments were compared using Student's t-test. $p<0.05$ was considered statistically significant. The mean morphometric and histologic data among groups were compared by one-way ANOVA with post hoc analysis for multiple comparisons. When the error variance of the dependent variable was not equal across groups (the lumen area and injury score at overlapped segments, inflammation score at non-overlapped segments), an overall comparison by group was made using the Kruskal–Wallis test. Intergroup differences were assessed where appropriate using the Mann–Whitney U test and adjusted using the Bonferroni method. Statistical analyses were carried out using the commercially available software (Statistical Package for the Social Sciences Version 18, Chicago, IL, USA).

3. Results

The overlapping BESs were successfully implanted for LAD and LCX or RCA of all pigs. Each stent size (2.75–3.0 mm) and length (11–14 mm) were similar among 3 groups. After follow-up angiography 8 weeks later, the histopathologic analyses of overlapping and non-overlapping segments with specific staining were evaluated and illustrated in Fig. 2

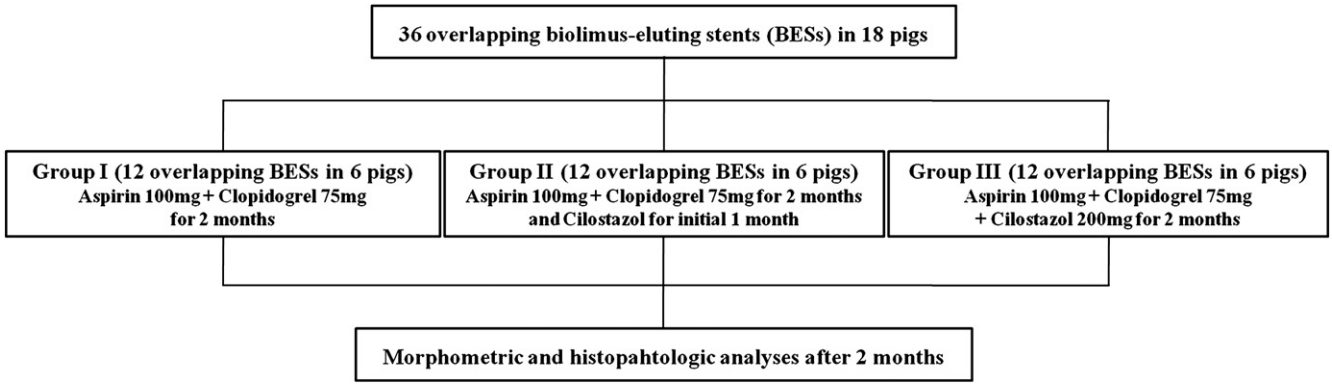

Fig. 1. The flow chart of our study.