Histopathological Analysis of Drug Eluting Stents in Porcine Coronary Restenosis Model

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The aim of this study was to compare the two types of drug-eluting stents with histological analysis in porcine coronary restenosis model. Pigs were randomized into two groups in which the coronary arteries (12 pigs, 12 coronaries in each group) had either biolimus-eluting stent with biodegradable polymer (BPS) or sirolimus-eluting stent with durable polymer (DPS). Radiological analysis was performed pre-stent and post-stent implantation and histopathological analysis was assessed at 28 days after stenting. Angiographic finding showed that the coronary artery was more narrowed by neointima proliferation in BPS compared to DPS. In histopathological analysis, the internal elastic lamina (IEL), lumen area, neointima area, percent area stenosis, and fibrin score were significantly different between the two groups (5.1 ± 0.79 mm², 2.8 ± 0.85 mm², 2.3 ± 0.75 mm², 43.9 ± 13.43%, 0.4 ± 0.79 in BPS group vs. 4.2 ± 0.93 mm², 3.0 ± 0.90 mm², 1.2 ± 0.67 mm², 28.1 ± 14.81%, 2.0 ± 0.93 in DPS group, respectively). However, there was no significant difference in injury and inflammation score (1.3 ± 0.40, 1.0 ± 0.31 in BPS group vs. 1.3 ± 0.32, 1.1 ± 0.15 in DPS group, respectively). Our study demonstrated that BPS was not effective to reduce neointima hyperplasia at 1 month after implantation of stents in porcine coronary artery. However, fibrin score of BPS which means delayed arterial healing is very lower than that of DPS.

Key words: drug-eluting stent, porcine restenosis model, neointima, histopathological analysis, percutaneous coronary intervention

Introduction

Drug-eluting stents (DES) have become the standard of treatment for patients undergoing coronary angioplasty in 2002.1-6 Durable polymer was used to coat drugs on stent surface. Polymer-based DES has significantly reduced the rate of target lesion revascularization (TLR) in comparison with bare metal stents (BMS).7-8 However, it has some drawback such as inflammation or late thrombosis.9 Biodegradable polymer stents (BPS) have been developed to overcome the drawback of durable polymer stents (DPS) such as first generation stents. BPS has been showed to be non-inferior compared with DPS in real life practice.10 Therefore, biodegradable polymer based drug elution are increasingly used in patients with acute myocardial infarction. In recent several studies, -limus families are outstanding taxol derivatives.6,11-12 Thus coronary stents with taxol derivatives are slowly dwindling. Therefore we compared the biodegradable biolimus-eluting stent with durable sirolimus-eluting stent in a porcine coronary restenosis model.

Materials and methods

Animal preparation

The animal study was approved by the Ethics Committee of Chonnam National University Medical School and Chonnam National University Hospital (CNU IACUC-H-2010-18), 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 castrated male pigs weighing 20-25 kg. To prevent acute thrombosis after stenting, premedication with aspirin 100 mg and clopidogrel 75 mg per day was given for 5 days before the procedure. On the procedure day, pigs were anesthetized with zolazepam and tiletamine (2.5 mg/kg, Zoletil50®, Virvac, Caros, France), xylazine (3 mg/kg, Rompun®, Bayer AG, Leverkusen, Germany) and azaperone (6 mg/kg, Stresnil®, Janssen-Cilag, Neuss, Germany). They received supplemental oxygen continuously through oxygen mask. Subcutaneous 2% lidocaine at the cut-down site was administered, left carotid artery was surgically exposed, and then a 7 French (F) sheath was inserted. Continuous hemodynamic and surface electrocardiographic monitoring were maintained throughout the procedure. Then
5,000 units of heparin was administered intravenously as a bolus prior to the procedure. The target coronary artery was engaged using standard 7 F guide catheters and control angiograms of both coronary arteries were performed using nonionic contrast agent in two orthogonal views. The stent was deployed by inflating the balloon and the resulting stent-to-artery ratio was 1.3:1. Coronary angiograms were obtained immediately after stent implantation. Then, all equipment was removed and the carotid artery was ligated.

Protocol

The 10 BPS (3.0 × 18 mm, Biomatrix®, Biosensors International Technologies Pte LTD., Singapore) and 10 DPS (3.0 × 18 mm, Cypher® Cordis/Johnson and Johnson, Warren, NJ, USA) were implanted in the proximal left anterior descending (LAD) artery and proximal left circumflex artery (LCX) by randomized manner for 10 pigs (Figure 1). All received 100 mg of aspirin and 75 mg of clopidogrel daily until sacrifice stage. After four weeks of stenting, the animals underwent follow-up angiography in the same orthogonal views. The hearts were removed, and the coronary arteries were pressure-perfusion fixed at 110 mmHg in 10% neutral buffered formalin overnight. Arteries were step-sectioned, processed routinely for light microscopy, and stained for histological analysis.

Coronary angiographic analysis

Coronary angiography was performed pre- and post-stenting at 28 days by fluoroscopy (BV Pulsera, Philips Medical Systems, Andover, MA, USA). In-stent restenosis (ISR) pattern in angiography was classified by the introduced angiographic classification.13

Histopathological analysis

Histopathologic analysis was performed by an experienced cardiovascular pathologist. The specimens were cut with 50 to 100 µm in a thickness, and they were stained with Hematoxylin-Eosin (H&E) for histological analysis. Quantitative measurement of the samples was performed using a calibrated microscope, digital video imaging system, and microcomputer program (Visus 2000 Visual Image Analysis System, IMT Tech). Borders were manually traced for lumen area, area circumscribed by the internal elastic lamina, and the innermost border of the external elastic lamina (external elastic lamina area). Morphometric analysis of the neointimal area for a given vessel was calculated as the measured internal elastic lamina area minus lumen area. The measurements were made on five cross-sections from the proximal and distal ends and the three midpoints of each stented segment. Histopathologic stenosis was calculated as 100 × [1 - (lesion lumen area/lesion internal elastic lamina area)].14

Evaluation of arterial injury

Arterial injury at each strut site was determined by the anatomic structures penetrated by each strut. A numeric value was assigned, as previously described by Schwartz et al.14: 0 = no injury; 1 = break in the internal elastic membrane; 2 = perforation of the media; 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.

Evaluation of inflammation scores, neointimal reaction and fibrin score

With regard to the inflammation score for each individual strut, the grading was 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. The inflammation score for each cross section was calculated by dividing the sum of the individual inflammation scores by the total number of struts at the examined section. Ordinal data for fibrin were collected on each stent section using a scale of 0 to 3 as previously reported (Figure 3).15

Statistical analysis

Statistical analysis was performed with the aid of the commercially available software (SPSS Version 15, Chicago, IL, U.S.A.). The data were presented as mean value ± SD. Unpaired Student’s t test was used for the comparison of the two stent groups. To examine the correlations between the measured histologic variables, regression analysis was applied for each set of measured variables. A value of p < 0.05 was considered statistically significant.

Results

Two kinds of stents were placed randomly for two coronary

Figure 1. Representative images of angiographic view. Horizontal (A) and vertical view (B) showed no significant difference in stent-to-artery ratio between two stent groups. Black and white arrows indicate proximal LAD and LCX, respectively.
arteries per pig. A total of twelve stents including ten BPS and ten DPS, were placed in the proximal LAD and proximal LCX for 12 pigs. Mortality for this study was zero. There was no significant difference in stent-to-artery ratio between two stent groups. In angiographic analysis, ISR patterns after stenting are ISR pattern II (intra-stent) in all view (Figure 1).

In histopathological analysis, various amounts of inflammatory cells and fibrin infiltrate surrounding on each strut section (Figure 2). H&E for measuring quantitative analysis and Carstair's fibrin staining for determining delayed arterial healing by lymphohistiocytes discriminating analysis in both groups was investigated (Figure 3).

Except the injury (1.3 ± 0.40 in BPS group vs. 1.3 ± 0.32 in DPS group, \( p = 0.82 \)) and inflammation score (1.0 ± 0.31 in BPS group vs. 1.1 ± 0.15 in DPS group, \( p = 0.26 \)), quantitative analysis such as internal elastic lamina (IEL), lumen area (LA), neointima area (NA), percent area stenosis, and fibrin score were significant (Table 1).

**Table 1. Results of quantitative analysis in vessel surrounding stents**

<table>
<thead>
<tr>
<th>Variables</th>
<th>BPS (n = 12)</th>
<th>DPS (n = 12)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury score</td>
<td>1.3 ± 0.40</td>
<td>1.3 ± 0.32</td>
<td>NS</td>
</tr>
<tr>
<td>Inflammation score</td>
<td>1.0 ± 0.31</td>
<td>1.1 ± 0.15</td>
<td>NS</td>
</tr>
<tr>
<td>IEL (mm(^2))</td>
<td>5.1 ± 0.79</td>
<td>4.2 ± 0.93</td>
<td>( p &lt; 0.01 )</td>
</tr>
<tr>
<td>LA (mm(^2))</td>
<td>2.8 ± 0.85</td>
<td>3.0 ± 0.90</td>
<td>( p &lt; 0.05 )</td>
</tr>
<tr>
<td>NA (mm(^2))</td>
<td>2.3 ± 0.75</td>
<td>1.2 ± 0.67</td>
<td>( p &lt; 0.01 )</td>
</tr>
<tr>
<td>percent area stenosis (%)</td>
<td>45.9 ± 13.43</td>
<td>28.1 ± 14.81</td>
<td>( p &lt; 0.01 )</td>
</tr>
<tr>
<td>Fibrin score</td>
<td>0.4 ± 0.79</td>
<td>2.0 ± 0.93</td>
<td>( p &lt; 0.01 )</td>
</tr>
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NS, not significant

**Figure 2.** Representative images of H&E staining after 4 weeks of stenting. Specimen BPS implanted (A, \( \times 20 \)) and DPS implanted (B, \( \times 20 \)).

**Figure 3.** Representative Carstair's fibrin staining after 4 weeks of stenting. Specimen BPS implanted (A, \( \times 20 \)) and DPS implanted (B, \( \times 20 \)).

**Figure 4.** Histomorphometric analysis of the stented arteries. Twelve samples were analyzed and each datum point represents the mean ± SD (n = 12). Injury score (A), inflammation score (B), IEL (C), LA (D), NA (E), percent area stenosis (F).
were showed significantly different between the two groups (IEL; 5.1 ± 0.79 mm² in BPS group vs. 4.2 ± 0.93 mm² in DPS group, p < 0.01, LA; 2.8 ± 0.85 mm² in BPS group vs. 3.0 ± 0.90 mm² in DPS group, p < 0.05, NA; 2.3 ± 0.75 mm² in BPS group vs. 1.2 ± 0.67 mm² in DPS group, p < 0.01, percent area stenosis; 45.9 ± 14.81% in BPS group, p < 0.01, fibrin score; 0.4 ± 0.79 in BPS group vs. 2.0 ± 0.93 in DPS group, p < 0.01) (Table 1 and Figure 4).

Discussion

Our study was conducted to compare the biolimus-eluting stent with a biodegradable polymer (BPS), sirolimus-eluting stent and durable polymer (DPS) in porcine coronary restenosis model. Our study shows that biolimus-eluting stent are not effective to reduce neointima hyperplasia at 1 month after implantation of stents in porcine coronary artery. However, fibrin score which means delayed arterial healing is very lower than DPS. After developing of BMS, it was encountered two major complications such as subacute stent thrombosis and late ISR. Suitable antithrombosis medication reduced subacute stent thrombosis rates to approximately 1%. However, the incidence of ISR remained a drawback to the long-term success of BMS implantation. To overcome the limitation of BMS, a stent-based drug delivery system such as DES was developed. DES delivers an appropriate concentration of effective drugs to inhibit ISR without systemic toxicity.

First generation DES (based on durable polymer) have shown significantly decrease ISR rate. However, inhibitory effect of DES on ISR leads to chronic local inflammatory reaction and poor re-endothelialization which is associated to the presence of permanent polymers. The biodegradable polymer biolimus-eluting stent (Biomatrix®) that developed as a third generation DES elutes new sirolimus derivative biolimus A9 from a biodegradable poly lactic acid (PLA) polymer. The durable polymers may be well known to be related with restenosis, inflammation and late stent thrombosis. But BPS releases biolimus A9 into the vessel wall while the PLA polymer is absorbed by contacted vessel tissues. As opposed to our study, some clinical studies have demonstrated that the safety and efficacy of the biolimus-eluting stent with a biodegradable polymer compared to a sirolimus-eluting stent with a durable polymer at 12 month clinical follow-up. The LEADERS study is a randomized trial which compared the safety and efficacy of BPS (Biomatrix®) with DPS (Cypher®) in an “all-comers” patient population. Non-inferior of BPS has been confirmed in the 1–4 year results. During this period, BPS significantly reduces the risk of very late stent thrombosis compared to DPS. Recent clinical study, BPS improve safety and efficacy compared with DPS during long-term follow-up to 4 years in a pooled analysis of individual patient data from the ISAR-TEST 3, ISAR-TEST 4 and LEADERS randomized trial. In real clinical world, BPS has showed more safety and efficacy than DPS. However our study demonstrated opposite result at 1 month follow-up in porcine coronary restenosis model.

Conclusion

Our study demonstrates that biolimus-eluting stent with biodegradable polymer is not effective to reduce neointima hyperplasia at 1 month after implantation of stents in porcine coronary artery. However, fibrin score which means delayed arterial healing is very lower than sirolimus-eluting stent with durable polymer.

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References


