Inflammation and Delayed Endothelization With Overlapping Drug-Eluting Stents in a Porcine Model of In-Stent Restenosis

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Background This study evaluated the inflammatory reaction at the site of overlapping drug-eluting stents (DES) in a porcine model of in-stent restenosis.

Methods and Results Twenty bare metal stents (BMS) (group I; n=10), 20 sirolimus-eluting stents (SES) (group II: n=10), 20 paclitaxel-eluting stent (PES) (group III: n=10), and 10 PES and 10 SES (group IV: n=10) were overlapped in the left anterior descending coronary arteries of 40 pigs. Follow-up coronary angiography and histopathology were performed at 4 weeks after stenting. For the overlapped segments, the minimal luminal diameter at 4 weeks was smaller in group I than in the other groups (1.78±0.13 mm, 2.79±0.09 mm, 2.90±0.04 mm, 2.80±0.07 mm, respectively; p<0.001), and the neointimal area (5.51±0.58 mm², 2.38±0.53 mm², 2.07±0.37 mm², 2.39±0.58 mm², respectively; p<0.001) and area stenosis (68.74±4.02%, 27.79±4.73%, 23.66±3.24%, 27.63±4.07%, respectively; p<0.001) were higher in group I than in the other groups; however, the inflammatory score was higher in group III than in the other groups (1.80±0.42, 2.10±0.32, 2.90±0.31, 2.50±0.52, respectively; p<0.001) and the endothelization score was lower in group III than in the other groups (2.80±0.42, 2.30±0.67, 1.30±0.48, 2.10±0.74, respectively; p<0.001).

Conclusion Compared with BMS, DES inhibit neointimal hyperplasia, but inflammation and poor endothelization occur at the site of overlapping stents. (Circ J 2008; 72: 463–468)

Key Words: Coronary disease; Drug-eluting stent; Restenosis; Stent thrombosis

Diffuse, long coronary lesions are associated with an increased rate of restenosis and poor outcome, despite the development of percutaneous coronary intervention (PCI) techniques. In particular, the implantation of multiple, overlapping, bare metal stents (BMS) has a high restenosis rate. Use of drug-eluting stents (DES) results in a significant and sustained suppression of neointimal proliferation, and has greatly attenuated the relationship between stent length and restenosis rate. As a result, a long DES is usually selected for a diffuse coronary lesion, but if a residual segment of the lesion is left uncovered, additional stenting with some overlap is considered to eliminate the risk of a residual stent gap.

Thus in the case of a diffuse, long coronary lesion, overlapping DES are used for complete lesion coverage, but there has been concern whether the overlapping DES have the effect of increasing the local drug dose or whether they could result in dose-related side-effects, such as delayed arterial healing and promotion of inflammation, as compared with overlapping BMS.

Few histopathological studies have been done on the response to overlapping DES or BMS, and even less so for the overlapping of different DES, such as the sirolimus-eluting stent (SES) or paclitaxel-eluting stent (PES). Therefore, the present study assessed the histopathological response and the effect on neointimal hyperplasia of overlapping DES or BMS in a porcine model of coronary in-stent restenosis (ISR).

Methods

Animal Study Protocol

The animal study was approved by the Ethical Committee of the Chonnam National University Hospital. Study animals were female swine weighing 25–35 kg. To decrease the incidence of acute thrombosis after stenting, premedication with aspirin 100 mg and clopidogrel 75 mg/day was given for 7 days before the procedure. On the day of stent implantation, pigs were anesthetized with ketamine (20 mg/kg intramuscularly) and xylazine (2 mg/kg intramuscularly) and maintained on 3 L/min of supplemental oxygen continuously through a mask. After subcutaneous 2% lidocaine was administered at the cut-down site the left carotid artery was surgically exposed, and a 7F sheath was inserted. Continuous hemodynamic and surface electrocardiographic monitoring was maintained throughout the procedure. After 10,000 units of heparin were administered intravenously as a bolus...
prior to the procedure, the target coronary artery was engaged using standard 7F guiding catheters and control angiograms of both coronary arteries were taken using nonionic contrast agent in 2 orthogonal views.

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=10)</th>
<th>Group II (n=10)</th>
<th>Group III (n=10)</th>
<th>Group IV (n=10)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference diameter</td>
<td>2.83±0.12</td>
<td>2.81±0.10</td>
<td>2.78±0.07</td>
<td>2.80±0.12</td>
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<tr>
<td>Post-stenting diameter</td>
<td>3.11±0.09</td>
<td>3.12±0.07</td>
<td>3.12±0.80</td>
<td>3.14±0.07</td>
<td>0.816</td>
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<tr>
<td>Overlapped segment</td>
<td>9.38±0.84</td>
<td>9.23±0.83</td>
<td>8.74±1.37</td>
<td>8.95±0.37</td>
<td>0.508</td>
</tr>
<tr>
<td>4 weeks after stenting (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target reference diameter</td>
<td>3.01±0.12</td>
<td>2.99±0.75</td>
<td>3.04±0.09</td>
<td>3.01±0.11</td>
<td>0.801</td>
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<tr>
<td>Minimal luminal diameter</td>
<td>1.78±0.13</td>
<td>2.79±0.09</td>
<td>2.90±0.04</td>
<td>2.80±0.07</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Group I vs II, III, IV.
Group I, overlapped bare stent group; Group II, overlapped sirolimus-eluting stent (SES) group; Group III, overlapped paclitaxel-eluting stent (PES) group; Group IV, overlapped SES and PES group.

Table 2  Histopathologic Assessment of Porcine Coronary Arteries at 4 Weeks After Stenting

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=10)</th>
<th>Group II (n=10)</th>
<th>Group III (n=10)</th>
<th>Group IV (n=10)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury score</td>
<td>1.40±0.70</td>
<td>1.60±0.84</td>
<td>1.41±0.69</td>
<td>1.30±0.82</td>
<td>0.758</td>
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<tr>
<td>Lumen area (mm²)</td>
<td>2.50±0.13</td>
<td>6.14±0.41</td>
<td>6.63±0.21</td>
<td>6.17±0.31</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IEL area (mm²)</td>
<td>8.01±0.66</td>
<td>8.53±0.65</td>
<td>8.69±0.46</td>
<td>8.57±0.85</td>
<td>0.126</td>
</tr>
<tr>
<td>Neointima area (mm²)</td>
<td>5.51±0.58</td>
<td>2.38±0.53</td>
<td>2.07±0.37</td>
<td>2.39±0.58</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Area stenosis (%)</td>
<td>68.74±2.02</td>
<td>27.79±4.73</td>
<td>23.66±3.24</td>
<td>27.63±4.07</td>
<td>&lt;0.001*</td>
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<tr>
<td>Inflammatory score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal segment</td>
<td>1.35±0.47</td>
<td>1.40±0.52</td>
<td>1.60±0.52</td>
<td>1.65±0.47</td>
<td>0.460</td>
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<tr>
<td>Overlapped segment</td>
<td>1.80±0.42</td>
<td>2.10±0.32</td>
<td>2.90±0.31</td>
<td>2.50±0.32</td>
<td>&lt;0.001*</td>
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<tr>
<td>Distal segment</td>
<td>1.30±0.48</td>
<td>1.50±0.53</td>
<td>1.45±0.50</td>
<td>1.50±0.47</td>
<td>0.778</td>
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<tr>
<td>Endothelization score</td>
<td>2.80±0.42</td>
<td>2.30±0.67</td>
<td>1.30±0.48</td>
<td>2.10±0.74</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*Group I vs II, III, IV; †Group III vs I, II, IV.
Group I, overlapped bare stent group; Group II, overlapped SES group; Group III, overlapped PES group; Group IV, overlapped SES and PES group.
IEL, internal elastic lamina. Other abbreviations see in Table 1.

A total of 80 stents [20 BMS (Group I; n=10); 20 SES (Group II; n=10); 20 PES (Group III; n=10); 10 PES and 10 SES (Group IV; n=10)] were placed and overlapped in the proximal left anterior descending coronary artery of 40 pigs.

Stent Deployment and Overlap Procedure
Each animal received 1 pair of overlapping DES or BMS. Arterial diameters before and after stenting were measured on the angiograms and the ratio of stent to artery was calculated as 1.3:1. Repeat angiograms were taken immediately after stent implantation, then all equipment was removed and the carotid artery was ligated. All animals received 100mg of aspirin and 75 mg/day clopidogrel orally until death. At 28 days after stenting, the animals underwent repeat angiography in the same 2 orthogonal views and 20 ml potassium chloride was injected into the heart to cause death. The hearts were removed, and the coronary arteries were pressure-perfusion fixed overnight at 70mmHg in 10% neutral buffered formalin. Arteries were step-sectioned, processed routinely for light microscopy, and stained with hematoxylin-eosin for histological analysis.

**Histopathological Analysis**

The specimens were embedded in methylmethacrylate and 50–100μm sections were cut approximately 1 mm apart with a low-speed diamond wafer mounted on a Buehler Isomet saw (Buehler Ltd, Lake Bluff, IL, USA), leaving the stent wires intact in the cross-sections to minimize
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The stent endothelization score was defined as the extent of the circumferential of the arterial lumen covered by endothelium and was scored from 1 to 3 (1: <25%, 2: 25–75%, 3: >75%).

Statistical Analysis

Statistical analysis was performed using commercially available software (SPSS Version 11, Chicago, IL, USA). The data are presented as rates or mean value ± SD. Probability values are 2-sided from Student’s t-test for continuous variables and the Fisher’s exact test for categorical variables. To examine the correlations between the measured histological variables, regression analysis was used for each set of measured variables. A value of p<0.05 was considered statistically significant.

Results

All stents were successfully deployed and all 40 animals survived the 28 days. Repeat angiography at euthanasia showed preservation of stent overlap with no evidence of stent migration.

On the angiograms the mean length of stent overlap was not significantly different between groups. No significant differences were found in the luminal diameter of the overlapping segments after stent implantation. The minimal luminal diameter of group I was significantly less at 28 days after stenting (1.78±0.13 mm vs 2.79±0.09 mm vs 2.90±0.04 mm, vs 2.80±0.07 mm, groups I–IV respectively; p<0.001, Table 1, Fig 1).

Although no significant differences were noted in the injury score and internal elastic lamina area at the overlapping site, the neointimal area (5.51±0.58 mm² vs 2.38±0.53 mm² vs 2.07±0.37 mm² vs 2.39±0.58 mm², groups I–IV respectively; p<0.001) and area stenosis (68.74±4.02% vs 27.79±4.73% vs 23.66±3.24% vs 27.63±4.07%, groups I–IV respectively; p<0.001) were significantly higher in group I than in the other groups (Table 1, Fig 1).

The endothelization score for the overlapping site was significantly lower in group III (2.80±0.42 vs 2.30±0.67 vs 1.30±0.48 vs 2.10±0.74, groups I–IV respectively; p<0.001, Table 2, Figs 1,2).

The inflammatory score of the neointimal area was not significantly different in the non-overlapping sites, but was significantly higher in group III in the region of overlapping (1.80±0.42 vs 2.10±0.32 vs 2.90±0.31 vs 2.50±0.52, groups I–IV respectively; p<0.001, Table 2, Figs 3,4).

Discussion

In this study, though the overlapping of the DES overcame neointimal hyperplasia more effectively, it resulted in a higher degree of inflammatory reaction and delayed endothelization.

Treatment of a diffuse, long, coronary artery lesion with overlapping BMS is associated with high rates of restenosis1–3 whereas the use of DES greatly attenuates neointimal proliferation, so that in patients with a diffuse, long, coronary lesion, implantation of multiple overlapping DES has been associated with a good clinical outcome. Two DES in particular proved effective in large randomized trials: the SES (Cypher stent, Cordis/Johnson & Johnson, NJ, USA) and polymer-based PES (Taxus stent, Boston Scientific

Fig 4. Histopathologic assessment of the overlapped segment. (A) Lumen area (mm²), (B) minimal luminal diameter (mm), (C) neointimal area (mm²), (D) area stenosis (%), (E) endothelization score, (F) inflammation score. Group I, overlapped bare stent group; Group II, overlapped sirolimus-eluting stent (SES) group; Group III, overlapped paclitaxel-eluting stent (PES) group; Group IV, overlapped SES and PES group.

Evaluation of Inflammatory Scores, Neointimal Reaction and Endothelization

With regard to the inflammatory score for each individual strut, the grading was as follows: 0 = no inflammatory cells surrounding the strut; 1 = light, noncircumferential lymphohistiocytic infiltrate surrounding strut; 2 = localized, moderate to dense cellular aggregate surrounding the strut noncircumferentially; and 3 = circumferential dense lymphohistiocytic cell infiltration of the strut. The inflammatory score for each cross-section was calculated by dividing the sum of the individual inflammatory scores by the total number of struts in the examined section.

Potential artifacts caused by removal of stent wires.

Measurements of the sections were performed using a calibrated microscope, digital video imaging system, and microcomputer program (Visus 2000 Visual Image Analysis System). Borders were manually traced for lumen area, the 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 of a given vessel was calculated as the measured internal lamina area minus lumen area. Histopathologic evaluation of a given vessel was calculated as the measured internal elastic lamina area. The measurements were made on 4 cross-sections of each stent. 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: 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 the injury scores by the total number of struts in the examined section.

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have been associated with late thrombosis and death. Recent publication of "real-world" patients suggests that the window of thrombotic risk associated with the new generation of stents extends far beyond that seen with BMS. FDA reports and autopsy findings suggest that DES may be a cause of systemic and intra-stent hypersensitivity reactions that, in some cases, have been associated with late thrombosis and death.10,11 This hypersensitivity or cytotoxicity is thought to associated with the coating drug or polymer12 and lesions showed greater delayed healing and poor endothelialization.

According to current reports of diffuse, long, coronary lesions using overlapping DES, the overlapped segment shows delayed endothelization compared with the non-overlapped segment. Both SES and PES overlaps are known to induce different histopathologic effects, with PES overlap inducing more heterophils, eosinophils and fibrin deposition than SES overlap, whereas SES overlapping is more often associated with giant cell peri-strut infiltration.

In the present study, more severe histological changes occurred in groups II and III, the mono-DES overlap groups, than in the SES plus PES overlap group (group IV). Perhaps the overlapping of the one type of DES increases local drug concentration and thus worsens the inflammation and delayed endothelization of the overlapped site.

The mechanism of delayed endothelization after DES overlap is that the overlapping increases direct contact of the stent struts and arterial tissue, increasing the local drug concentration by the overlap itself, as well as by changing the cell structure of the stent strut. A systemic concentration of 1.1 μg/ml rapamycin was obtained after deployment of 2 SES and was associated with markedly delayed endothelization, enhanced platelet aggregation, and stent thrombosis.

The incidence of stent thrombosis in the modern era of stent deployment varies from a low of 0.5% to 1.9% with BMS implantation, the same as with DES implantation. To prevent stent thrombosis, clopidogrel 75 mg/day should be given for at least 6 months after DES implantation and, ideally, up to 12 months in patients who are not at high risk of bleeding, but there has been debate regarding how long such therapy should continue.

The clinical consequences of stent thrombosis include death in 20–48% or major myocardial infarction in 60–70% of persons who experience this event. Premature discontinuation of antiplatelet therapy is strongly associated with stent thrombosis and some individuals are resistant to antiplatelet therapy. In the real world, more clinical trials and long-term follow-up is necessary to conclude the safety of DES, because some patients and more complex lesions are excluded from large clinical trials.

Furthermore, we should develop a new generation of DES to overcome the problem of polymer-mediated thrombosis or inflammation and to enhance healing after stenting. Future generations of stents will likely be engineered for optimal drug delivery to specific lesions while continuing to refine the BMS platform to enhance acute procedural success. Furthermore, it is likely that novel polymer materials and pharmacologic agents will be chosen for biological activity in specific disease states or vascular beds.

Study Limitations

The main limitation was the relative small number of experimental animals because we used a large-animal model. Also, we did not perform fibrin-specific staining for evaluation of incomplete healing in the overlapped segments. Finally, it is unclear whether delayed endothelial healing was observed more often in PES-only overlap compared with different DES overlapped, whether that was related to the relatively small number of animals or whether the same thing happens in the real world. A larger clinical trial with long-term follow-up is necessary to confirm these findings.

Conclusion

Overlapping of DES inhibited neointimal hyperplasia compared with overlapping BMS in a porcine model of ISR, but poor endothelization because of severe inflammation at the site of overlapped segments occurred, especially with PES.

Acknowledgment

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References