The Union of Anti-CD34 Antibody Can Improve the Performance of Drug-Eluting Stents

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Objectives: The authors investigate whether the combination of anti-CD34 antibody with DES is win–win cooperation. Background: DES may reduce the risk of restenosis compared to bare-metal stents (BMS), but they were found to inhibit the healing process of intima. Methods: Fifteen BMS, 17 DES, and 16 combined anti-CD34 antibody and DES were randomly implanted in the coronary arteries of 22 minipigs. Ten minipigs were followed up to 2 weeks. The stenting coronary segments were examined by histological examination and scanning electron microscopy after in vivo coronary angiography and intracoronary optical coherence tomography (OCT) examinations. The other 12 minipigs were followed up to 3 months. Coronary angiography and intracoronary OCT examination were performed in vivo and histological examination was performed on the stenting coronary segments. Results: After 2 weeks, the neointimal covering level of the DES was lower than that in BMS, but the covering level of the combined stents was even better than the BMS. After 3 months, neointimal hyperplasia was significant in the BMS, but not in the other two types of stents. The in-stent late lumen loss of the combined stents even showed a decreasing tendency when compared with the DES. Conclusion: The combination of anti-CD34 antibody and DES can not only well offset the short-term inhibitory effect on re-endothelialization but also slightly enhance the long-term antiproliferative effect.

Key words: anti-CD34 antibody; drug-eluting stents; endothelial progenitor cells; optical coherence tomography

INTRODUCTION

Drug-eluting stents (DES) could dramatically reduce the risk of restenosis after stenting, which was confirmed by randomized trials [1–3] and routine clinical practice [4]. However, the increased risk of late and very late stent thrombosis after DES implantation has attracted people’s attention in recent years [5]. The advantage of DES compared with bare-metal stents (BMS) may be partially offset by its increasing tendency of stent thrombosis [6].

DES was found to be able to delay significantly the formation of a confluent endothelial layer on the stent struts [7,8]. A recent autopsy study showed that the most important histological and morphometric predictors of late-stent thrombosis were the endothelial coverage and the ratio of uncovered to total stent struts after DES implantation [9].

Circulating endothelial progenitor cells (EPCs) mobilized from bone marrow have been shown to be able to contribute to endothelial cell regeneration [10]. Recently, circulating EPCs have also been suggested to guide arterial repairing response after balloon injury [11]. EPCs-capture-mediated re-endothelialization has been confirmed by scanning electron microscopy [12]. CD34 is a relatively special marker on EPCs, and anti-CD34 antibody is commonly used to identify and collect EPCs from

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peripheral blood. Preliminary clinical studies have shown that the use of stents coated with the anti-CD34 antibody was feasible and safe. However, the long-term antiproliferative effect of solely EPCs capturing stents was not satisfactory [13,14]. The authors postulate that the combination of the anti-CD34 antibody and the DES may be a win–win cooperation, which is unknown now.

Intracoronary optical coherence tomography (OCT) is capable of identifying thin layers of neointima with its high resolution (10–20 \( \mu \)m). Several studies have reported the feasibility and the efficacy of OCT evaluation on stent strut coverage [15,16]. In this study, this technique was applied as the in vivo approach to evaluate the short-term and long-term neointimal coverage situation.

METHODS

Animals

Twenty-two Chinese minipigs, weighing 25–45 kg, were used in the study (provided by the Chinese Experimental Minipig Breeding Faculty). The investigation 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). The experimental procedure was approved by the Animal Care and Use Committee of Capital Medical University (No. 20060124).

Stent Design

Three types of stents including BMS, DES, and anti-CD34 antibody and drug-eluting combined stents (ADS) were investigated in the study, which were provided by Lepu (Beijing) Medical Instruments Corporation. The substance of the three types of stents was identically stainless steel and their length was 12 mm. The micropores were made on the strut surface of all stents by patent technology. Sirolimus, an antiproliferative drug, was filled in the micropores of three sides of the strut surface beside the inner one in the DES stents. On the basis of the DES stents, monoclonal anti-CD34 antibodies were immobilized on the inner strut surface in the ADS stents. The schematic diagram of the ADS stents is shown in Fig. 1.

Fig. 1. The schematic diagram of ADS stent. ADS: anti-CD34 antibody and drug-eluting combined stents.

Procedure

The minipigs were fed with ordinary pig feed during the whole experimental period. Operative approach was established in the femoral artery by Seldinger technique after minipigs were anesthetized. During the operation, heparin 100 IU/kg was given through the arterial sheath. Coronary angiography was performed using 6F JL3.5 and JR3.5 guiding catheters. The proximal segments of the three main coronary arteries were chosen for stent implantation if they were not tapering and had no apparent branches. The three types of stents were randomly implanted in the three main coronary arteries. Angiograms were recorded before and after stent implantation. After experimental operation, the artery sheath was removed and the femoral artery was oppressed to stop bleeding. Every pig was given an antiplatelet therapy with 100 mg aspirin and 75 mg clopidogrel daily, from at least 5 days before the stent implantation to the end of the follow-up period.

Quantitative coronary analysis (QCA) was performed offline using special QCA software (CASS; Pie Medical Instruments, Maastricht, The Netherlands) by a single individual who was blinded to the information of stent implantation. Parameters including reference vessel diameter before stenting and in-stent minimal lumen diameter immediately after stenting were measured. Dilation ratio (in-stent minimal lumen diameter immediately after stenting/reference vessel diameter before stenting) was calculated.
Two-Week Follow-Up

Two weeks after stent implantation, intracoronary OCT examination was performed after coronary angiography in 10 of the experimental pigs. An OCT system (Model M2 Cardiology Imaging System, LightLab Imaging, Westford, MA) and a 0.014-inch wire-type optical imaging catheter (ImageWire, LightLab Imaging) were used in this study. After the image wire was positioned through the occlusion balloon distal to the stent, the occlusion balloon (Helios, Avantec Vascular Corp., Sunnyvale, CA) was inflated to 0.4–0.6 atm and heparinized saline (25 IU/ml) was infused through it at 1.0 ml/sec. At the same time, the image wire was pulled distal to proximal at a speed of 1 mm/sec, and cross-sectional images were stored continuously (15 frames/sec) for subsequent analysis.

Cross-sectional OCT images were analyzed at 1-mm intervals. The images were magnified to maximize the accuracy of measurement. If there was no definite neointima on the surface of one stent strut, the authors defined it as an uncovered stent strut. For the covered struts, the distance between the endoluminal surfaces of neointima and stent strut was measured with a measurement line as perpendicular as possible to the strut as neointimal thickness. At every analyzed cross-sectional image, the covered ratio of stent struts (covered struts number/total struts number) and the mean neointimal thickness (sum of neointimal thickness of covered struts/total struts number) were calculated.

After the intracoronary OCT examination, the 10 experimental animals were executed by rapid bloodletting. The stenting coronary segments were taken out carefully and divided into two parts. One part was performed by histological examination after hematoxylin and eosin stain. The injury score and inflammation score were determined according to published methods [17]. The other one was split lengthways and examined by scanning electron microscopy.

Three-Month Follow-Up

Three months after stent implantation, coronary angiography and intracoronary OCT examination were performed in the other 12 experimental pigs.

In QCA, parameters including reference vessel diameter before stenting, in-stent minimal lumen diameter immediately after procedure, in-stent and in-segment minimal lumen diameter at follow-up, diameter stenosis rate (in-stent late lumen loss/in-stent minimal lumen diameter immediately after procedure × 100%) and in-segment stenosis rate (in-segment minimal lumen diameter at follow-up/reference vessel diameter × 100%) were calculated.

Cross-sectional OCT images were analyzed at 3-mm interval. Because all the stent struts were well covered by neointima, the authors only paid close attention to the level of neointimal hyperplasia (NIH). Stent area and lumen area were measured. Percent NIH was calculated as ([stent area – lumen area]/stent area × 100%).

Histological examination was performed on the stented coronary artery segments. The stent area and the lumen area were measured and percent of stenosis was calculated ([stent area – lumen area]/stent area × 100%).

Statistical Analysis

The parameters were expressed as mean value ± standard deviation. All data were analyzed using SPSS software (SPSS 13.0; SPSS, Chicago, IL). The normality of the distribution of various parameters was investigated using the Kolmogorov–Smirnov test. Data transformation was used when the distribution was skewed. A comparison of continuous variables between groups was performed using a one-way ANOVA. For multiple comparisons, the authors performed a post hoc LSD test. A chi-square test was used to compare quantitative data. P-value less than 0.05 was considered statistically significant.

RESULTS

Basic Situations

All experimental pigs gained weight steadily and no pig died unexpectedly throughout the follow-up period. No acute- or late-stent thrombosis was found by coronary angiography, OCT, or histological examination.

Two-Week Follow-Up Results

Twenty stents of the three types were successfully implanted in the coronary arteries of the 10 minipigs. The three types of stents were randomly distributed in the three main coronary arteries (χ² = 0.823, P = 0.286) and their dilation ratio had no difference (P = 0.315) (Table I).

At the 2-week follow-up, the stent struts of the three different types were partially covered by neointima in cross-sectional OCT images. Two-hundred and sixty cross-sectional OCT images of the three types of stents were analyzed. The neointimal covering level of different types of stents is shown in Table I and the representative OCT images of the three types of stents are...
The mean value of the covered ratio of stent struts in the ADS group was highest in the three groups, which was significantly higher than the one in the DES group ($P < 0.05$) and a 32.86% improvement was found. The ADS group showed a higher tendency in covered ratio of stent struts than the BMS group, though it did not reach a significant level ($P = 0.227$). Besides, the mean thickness of neointima in the ADS group was significantly higher than that in the DES group ($P < 0.001$) and the BMS group ($P = 0.001$).

Histological examination showed that thin neointima was found on the stent struts of all the three types of stents. The injury scores and inflammation scores were not different between the three groups ($P = 0.619$ and 0.426, respectively) (Table I). The covering of neointima on the stent struts was more complete in the BMS and the ADS stents than in the DES stents (Fig. 2).

Low-magnification scanning electron microscopy (SEM) images showed that a thin neointimal covering could be found on the stent struts of all types of stents. The neointima on the BMS and the ADS stent struts was smooth, consecutive, and almost complete. The neointima on the DES stent struts was thinner and relatively incomplete. High-magnification SEM images showed that the neointima on the DES stent struts was coarse and discontinuous (Fig. 2).

### Three-Month Follow-Up Results

Twenty-eight stents of the three types were successfully implanted in the coronary arteries of 12 minipigs. The three types of stents were randomly distributed in the three main coronary arteries ($\chi^2 = 0.601$, $P = 0.896$) and the dilation ratio of different types of stents had no difference ($P = 0.275$). QCA showed that the mean value of in-stent late lumen loss in the ADS group was the smallest one among the three groups and was significantly lower than the one in the BMS group.

**TABLE I. The Implantation and the 2-Week Follow-Up Results of the Three Types of Stents**

<table>
<thead>
<tr>
<th></th>
<th>BMS</th>
<th>DES</th>
<th>ADS</th>
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<tbody>
<tr>
<td>The distribution in the three main coronary arteries</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Left anterior descending</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Left circumflex</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Right coronary artery</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Dilation ratio</td>
<td>1.15 ± 0.05</td>
<td>1.10 ± 0.04</td>
<td>1.07 ± 0.04</td>
</tr>
<tr>
<td>The neointimal coverage in OCT images</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The analyzed cross-sections number</td>
<td>78</td>
<td>91</td>
<td>91</td>
</tr>
<tr>
<td>The analyzed struts number</td>
<td>813</td>
<td>936</td>
<td>878</td>
</tr>
<tr>
<td>The covered ratio of stent struts (%)</td>
<td>47.86 ± 4.49</td>
<td>41.82 ± 3.73</td>
<td>55.56 ± 5.65</td>
</tr>
<tr>
<td>Mean thickness of neointima (μm)</td>
<td>44 ± 7.2</td>
<td>32 ± 4.9</td>
<td>89 ± 5.0</td>
</tr>
<tr>
<td>histological results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injury score</td>
<td>0.67 ± 0.21</td>
<td>0.86 ± 0.26</td>
<td>1.00 ± 0.22</td>
</tr>
<tr>
<td>Inflammation score</td>
<td>1.00 ± 0.26</td>
<td>1.29 ± 0.18</td>
<td>0.86 ± 0.26</td>
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</tbody>
</table>

BMS = bare metal stents; DES = drug eluting stents; ADS = anti-CD34 antibody and drug-eluting combined stents; OCT = optical coherence tomography.
group \( (P < 0.05) \). The in-segment stenosis rates had no significant difference among the three groups \( (P = 0.295) \) (Table II).

Obvious neointimal hyperplasia was found in the BMS stents in cross-sectional OCT images, while the neointimal coverage in the DES and the ADS stents was merely moderate (Fig. 3). One hundred and thirty cross-sectional OCT images of the three types of stents were analyzed. The percent NIH of both ADS \( (34.75 \pm 2.64\%) \) and DES group \( (35.63 \pm 2.07\%) \) were significantly lower than that of the BMS group \( (48.28 \pm 3.25\%) \) \( (P < 0.001 \) and \( = 0.001 \), respectively).

Histological examination showed that complete and smooth neointimal coverage was found on all the stent struts. The injury scores and inflammation scores were not different between the three groups \( (P = 0.366 \) and \( 0.378 \), respectively). The neointimal coverage was moderate in the DES and the ADS stents, but obvious neointimal hyperplasia could be seen in the BMS stents (Fig. 3). The percent of stenosis of both DES and ADS groups was significantly lower than that of the BMS group \( (both P < 0.05) \) (Table II).

### DISCUSSION

Sirolimus is thought to play an antirestenosis role through inhibiting the migration and proliferation of smooth muscle cells by blocking cell-cycle progression from G1- to S-phase. Recent studies have shown that DES could also delay the neointimal healing process after endothelial injury. Animal experiments showed that 90 days after stent implantation in coronary arteries, regions of acellular plasma-like collections could be still observed by histological examination in the sirolimous-eluting stents, but not in BMS [18]. A histological study of atherectomy specimens of early in-stent restenosis tissue showed that fibrinoid, a marker of impaired healing, was present only in sirolimus- or paclitaxel-eluting stents as late as 2 years after stent placement, but not in BMS [8]. A large cohort study showed that late stent thrombosis occurred steadily at an annual rate of 0.4–0.6% for up to 4 years after DES implantation [19]. In this study, 2-week follow-up results showed that sirolimus in the DES stent could inhibit the neointimal healing process after stent implantation.

Recent research results suggest that EPCs in the blood play an important role in the repair of tunica intima of artery after its injury [10,11]. In a mouse model of severe vascular injury, sirolimus has been shown to inhibit the differentiation of EPCs into endothelialoid cells [20]. It has been postulated that the inhibitory effect of sirolimus on re-endothelialization after stent implantation is at least partially because of its inhibitory effect on circulating EPCs. The use of the anti-CD34 antibody to capture circulating EPCs onto stent struts has been proved feasible and safe by recent studies [14,21]. Hence, the attachment of the anti-CD34 antibody to the DES stent may be an effective way to redeem its inhibitory effect on intimal healing. At the 2-week follow-up in this study, by intracoronary

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**TABLE II. The Implantation and the 3-Month Follow-Up Results of the Three Types of Stents**

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</tr>
<tr>
<td>Right coronary artery</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Dilution ratio</td>
<td>1.15 ± 0.04</td>
<td>1.06 ± 0.03</td>
<td>1.08 ± 0.02</td>
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<tr>
<td>The QCA results</td>
<td></td>
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<tr>
<td>Reference vessel diameter (mm)</td>
<td>2.31 ± 0.11</td>
<td>2.49 ± 0.14</td>
<td>2.39 ± 0.10</td>
</tr>
<tr>
<td>LMD immediately after procedure (mm)</td>
<td>2.38 ± 0.08</td>
<td>2.44 ± 0.10</td>
<td>2.43 ± 0.16</td>
</tr>
<tr>
<td>LMD during follow-up (mm)</td>
<td>2.03 ± 0.11</td>
<td>2.22 ± 0.10</td>
<td>2.24 ± 0.15</td>
</tr>
<tr>
<td>Late lumen loss (mm)</td>
<td>0.35 ± 0.06</td>
<td>0.23 ± 0.03</td>
<td>0.19 ± 0.06</td>
</tr>
<tr>
<td>Diameter stenosis rate (%)</td>
<td>14.96 ± 2.82</td>
<td>9.32 ± 1.22</td>
<td>7.83 ± 2.50</td>
</tr>
<tr>
<td>In-segment stenosis rate (%)</td>
<td>22.07 ± 4.52</td>
<td>16.38 ± 3.92</td>
<td>13.06 ± 9.94</td>
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<tr>
<td>The histological results</td>
<td></td>
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<tr>
<td>Lumen area (mm²)</td>
<td>2.91 ± 0.22</td>
<td>3.46 ± 0.20</td>
<td>3.39 ± 0.18</td>
</tr>
<tr>
<td>Stent area (mm²)</td>
<td>5.53 ± 0.25</td>
<td>5.15 ± 0.19</td>
<td>5.21 ± 0.16</td>
</tr>
<tr>
<td>Intimal area (mm²)</td>
<td>2.62 ± 0.21</td>
<td>1.69 ± 0.15</td>
<td>1.82 ± 0.15</td>
</tr>
<tr>
<td>Percent of stenosis (%)</td>
<td>46.18 ± 8.25</td>
<td>29.33 ± 6.07</td>
<td>26.65 ± 5.64</td>
</tr>
<tr>
<td>Injury score</td>
<td>0.78 ± 0.22</td>
<td>0.70 ± 0.21</td>
<td>0.11 ± 0.20</td>
</tr>
<tr>
<td>Inflammation score</td>
<td>1.22 ± 0.22</td>
<td>1.70 ± 0.21</td>
<td>1.56 ± 0.29</td>
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</table>

BMS = bare metal stents; DES = drug-eluting stents; ADS = anti-CD34 antibody and drug-eluting combined stents; QCA = quantitative coronary analysis; LMD = minimal luminal diameter.
OCT examination, the authors found that both covered ratio of stent struts and mean thickness of neointima in ADS group were significantly better than those in the DES group, and the mean thickness of neointima in the ADS group was even significantly better than the one in the BMS group. Both histological and SEM examination also showed that the covering level of stent struts by neointima in the ADS stent was much better than that in the DES stent. The rapid re-endothelialization of the ADS stents may reduce the risk of subacute and late-stent thrombosis and shorten the course of dual antiplatelet treatment after stent implantation.

Although the pro-reendothelialization effect of EPCs-capturing stents has been proved, their long-term antirestenosis effect was not superior when compared with the BMS [22]. The authors presumed that EPCs capturing coating covered only the stent struts and theoretically no early functional endothelial lining could be expected in the interstrut space. The antirestenosis effect of DES has been well proved. In this study, the 3-month follow-up results showed that, similar to the DES group, late lumen loss in the ADS group was significantly lower than the ones in the BMS group. Therefore, the antirestenosis effect of DES could be well reserved in the ADS stents. Furthermore, the mean values of both late in-stent lumen loss by QCA and percent NIH by OCT analysis in the ADS group had a slight tendency to decline than the ones in the DES group. The authors postulate that, consistent with previous studies’ results [11,23], the rapid endothelial healing of the ADS stents could also slightly contribute to reduce the long-term risk of restenosis. Considering the fact that the area of endoluminal surface of the stent struts only accounts for a very small proportion of the total area of endoluminal surface of the stented artery segments, the weakness of the anti-restenosis effect of the anti-CD34 antibody is easily understood.

Compared with the relatively low lethality of restenosis [24], the life-threatening late-stent thrombosis is more worrying. That the combination of the anti-CD34 antibody to DES could not only significantly offset its inhibitory effect on re-endothelialization but also slightly enhance its efficacy of antirestenosis is definitely an exciting result. The anti-CD34 antibody and the drug-eluting combined stents may become an excellent type of stent in the future.

Limitations
Because all data in this study were collected from an animal model without spontaneous atherosclerosis, further clinical trials are warranted.

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REFERENCES


