Differential Response of Delayed Healing and Persistent Inflammation at Sites of Overlapping Sirolimus- or Paclitaxel-Eluting Stents

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Background—Although effective coverage of challenging coronary lesions has warranted the use of overlapping drug-eluting stents, the histopathological response to stent overlap is unknown.

Methods and Results—The arterial reaction to overlapping Cypher or Taxus drug-eluting stents was examined in rabbits with bare metal stents, BxVelocity or Express, serving as controls. Single iliac artery balloon injury was followed by placement of 2 overlapping 3.0-mm-diameter drug-eluting stents or bare metal stents in 60 animals (mean length of overlap, 9.8±3.6 mm). Stented arteries were harvested at 28 and 90 days for histology. Overlapped segments exhibited delayed healing compared with proximal and distal nonoverlapping sites at 28 days. Overlapped segments in Taxus stents induced significantly more luminal heterophils/eosinophils and fibrin deposition than Cypher; peristrut giant cell infiltration, however, was more frequent in the latter. Overlapping bare metal stents also showed mild delayed healing compared with nonoverlapped segments, but not to the same extent as drug-eluting stents. Although neointimal thickness within the overlap was similar in 28- and 90-day Cypher stents, there was a significant increase with Taxus (P=0.03).

Conclusions—Compared with bare metal stents, drug-eluting stents further delay arterial healing and promote inflammation at sites of overlap. Taxus stents induced greater fibrin deposition, medial cell loss, heterophils/eosinophils, and late neointimal hyperplasia. Patients receiving overlapping drug-eluting stents need more frequent follow-up than patients with nonoverlapping stents. (Circulation. 2005;112:270-278.)

Key Words: drugs ■ pathology ■ restenosis ■ stents

A governing principle in the deployment of drug-eluting stents (DES) is to effectively treat the entire lesion.1 This strategy requires the stent to span from healthy to healthy tissue, avoiding the termination of stent edges in grossly diseased segments. For lesions extending beyond the capacity of a single stent, multiple overlapping stents are deployed. Approximately 33% of patients enrolled in the Sirolimus-Eluting Balloon Expandable Stents in the Treatment of Patients with De Novo Coronary Artery Lesions (SIRIUS) and European multicenter, randomized, double-blind study of long atherosclerotic lesions in small coronary arteries (E-SIRIUS) were treated with overlapping stents compared with 29% in TAXUS-VI.2-4

DES raise considerable concerns over local toxicity given that drug and/or polymer concentrations are likely to be significantly higher at sites of stent overlap.5 This notion is based partially on animal studies of sirolimus- and paclitaxel-eluting stents showing a dose-dependent drug response of incomplete arterial healing characterized by persistent fibrin deposition. Undesirable effects, including medial necrosis, increased intimal, and in some cases adventitial inflammation, are reported with paclitaxel-eluting stents.6-8 To date, however, no studies have detailed the histological changes in the arterial wall in response to overlapping drug-eluting or bare metal stents (BMS). Therefore, the present study assessed the short- and long-term responses to stent overlap in a rabbit model of iliac artery stenting.

Methods

This protocol, approved by the Institutional Animal Care and Use Committee of the Armed Forces Institute of Pathology, conformed to the position of the American Heart Association on use of animals in research.
Angioplasty and Stenting Protocol

Balloon injury to a single iliac artery was performed under fluoroscopic guidance in anesthetized New Zealand White rabbits (average weight, 3.8±0.3 kg; range, 3.2 to 4.2 kg). Subsequently, 2 matching overlapping sirolimus- (Cypher, Cordis Corp, Johnson & Johnson Co) or paclitaxel- (Taxus Express, Boston Scientific) eluting stents were deployed at the area of balloon injury. Overlapping BxVelocity or Express stents served as respective controls. Each animal received 1 pair of overlapping DES or BMS. For drug loading, both DES use nonerodible polymers, which consist of a mixture of polyethylene-co-vinyl acetate (PEVA) and poly n-butyl methacrylate (PBMA) in Cypher and poly(styrene-b-isobutylene-b-styrene) (SIBBS) in Taxus stents. Cypher and BxVelocity stents were 18 and 23 mm long; Taxus and Express stents were 16 and 20 mm; and all stents were 3.0 mm in diameter. Individual stents were deployed at their respective nominal pressures (9 to 11 atm, 30-second balloon inflation), and the overlapped segment (overall length, 3.9 to 14.5 mm) was postdilated at 12 atm to ensure complete expansion. Before the stents were deployed, all arteries were of similar diameter with similar amounts of vessel tapering toward the distal segments. Vessel diameters before and after stenting were measured on angiograms at 3 locations, and ratios of stent to artery were calculated.

Antithrombotic Regimen

All animals were pretreated with aspirin 40 mg PO (10 mg/kg) 24 hours before stenting with continued therapy until death. In addition, heparin (150 IU/kg) was administered intra-arterially before catheterization procedures.

Tissue Harvest and Histological Processing

Either 28 or 90 days after receiving stents, animals were anesthetized, and follow-up angiography of the iliac arteries was performed to document patency and position of the overlapping stents. Subsequently, rabbits were euthanized by an overdose of sodium pentobarbital, and the stented arteries were perfusion fixed in situ. The stented arterial segments were radiographed (Faxitron); specimens were embedded in methyl methacrylate; and 3-mm segments from the proximal, middle overlap (×2), and distal ends of the stent were cut with a tungsten carbide knife (Delaware Diamond Knives). Sections (4 μm) were cut on an automated microtome (Leica) and stained with hematoxylin and eosin and Movat Pentachrome. To assess cellular proliferation, animal received bromodeoxyuridine (BrdU) before euthanasia as previously described. To identify eosinophils. Heterophils, the equivalent to human neutrophils, were negative by Luna staining. To evaluate stent endothelialization, SEM was performed on stents implanted in additional rabbits, as described above, for 28 or 90 days. All histological sections were examined blindly. Computerized planimetry was performed as previously described. The percentage of stenosis was calculated with the following formula: neointimal area divided by internal elastic lamina area times 100. The neointimal cell proliferation index (percent proliferating cells) was defined as the ratio of Brdu-positive cells to total cell number and summarized for both neointimal and medial wall. For medial cell density, only spindle-shaped nuclei at and near stent strut sites were counted, and values are expressed as number of medial cells per 1 mm². Ordinal data were collected on each stent section to include fibrin deposition, giant cell reactions, and heterophil/eosinophil infiltration around the stent struts; these data were expressed as the number of struts or by a score of 0 to 3+ in the case of fibrin and total luminal heterophils/eosinophils or peristrut giant cells per section.

SEM Studies

Stents from 10 additional animals receiving overlapping BMS and DES were processed for SEM for evaluation of endothelialization as previously described.

Pharmacokinetics

For pharmacokinetic studies, 12 rabbits received a single sirolimus-(18-mm length) or paclitaxel-(20-mm length) eluting stent. Serial venous blood samples were collected at 0.5, 1, 2, 4, 6, 12, 24, 72, and 192 hours after stent deployment in siliconized tubes containing EDTA; whole blood (sirolimus) or plasma (paclitaxel) was kept frozen at −70°C until analysis. The stented arteries were harvested at 24 hours or 8 days after perfusion with ice-cold Ringer’s lactate at physiological pressure (100 mm Hg). A 3-mm arterial segment just proximal and distal to the stent was removed, weighed, and snap-frozen in liquid nitrogen, along with the tissue surrounding the stent. Blood and tissue samples were analyzed by liquid chromatography and mass spectroscopy and expressed per weight of tissue (Charles River).

Statistical Analysis

Values are expressed as mean±SD. When mean histomorphometric/histological variables were compared between groups, 1-way ANOVA with planned comparisons and Bonferroni’s correction of critical values for multiple assessments were used.

To correct for considerable inhomogeneity of variances (heteroscedasticity) between groups for several of the variables, appropriate monotonic transformations of the heteroscedastic variables were applied, and a modified Levene equal variance test was used to confirm that the transformations had equalized the variances. Variables were transformed as follows: Internal elastic lamina, percent area stenosis, medial thickness, and giant cell counts were all transformed by the logarithmic transformation. Analysis of heterophil/eosinophil counts was transformed by square-root transformation. All other variables were homoscedastic by the Levene test and were analyzed untransformed. All tables and figures display means and SDs of untransformed variables to retain familiar units; however, all statistical tests and the resulting tail probabilities (probability values) were obtained from appropriately transformed variables. These analyses were performed with the NCSS Statistical Software package (J. Hintze, NCSS and PASS, Number Cruncher Statistical Systems, Kaysville, Utah; www.ncss.com; March 2004 release).

To assess differences in tissue levels at 1 and 8 days for sirolimus and paclitaxel, normality of the data was first confirmed by the Shapiro Wilk W test, after which mean values at 2 time points were compared by t test for means with unequal variances. A value of P<0.05 was considered statistically significant.

Results

All stents were successfully deployed without incidence of dissection or thrombosis. All 60 animals (at 28 days, n=30 animals; at 90 days, n=8 animals; pharmacokinetics, n=12; SEM, n=10) remained healthy for the duration of the experiment. Repeated angiography at euthanasia showed preservation of stent overlap with no evidence of stent migration or aneurysm formation. Average ratios of stent to artery were similar among groups (Cypher, 1.40±0.09; BxVelocity, 1.37±0.17; Taxus, 1.30±0.17; Express, 1.46±0.99; P=NS), as was the total length of the stented arterial segments (Cypher, 29.6±2.7 mm; BxVelocity, 27.2±2.9 mm; Taxus, 30.4±3.0 mm; and Express, 32.0±1.8 mm). The mean length of stent overlap was 9.8±3.6 mm without significant differences in length of overlap between experimental groups (mean overlap: Cypher, 9.0±3.9 mm; BxVelocity, 8.5±3.1 mm; Taxus, 10.3±3.2 mm; Express, 11.7±0.2 mm; P=NS). In overlapping segments, average numbers of stent struts per section were greater in Taxus and Express stents (24.3 and 27.8 struts per section) compared with Cypher and BxVelocity stents (18.4 and 16.5 struts per section; P<0.04).
Stent Release of Paclitaxel and Sirolimus

With rare exceptions, circulating levels of paclitaxel at 0.5 hours to 9 days after stent deployment were generally below the detection limit of 0.02 ng/mL. Drug concentration of paclitaxel at the stent treatment site at 1 (n=1005) and 8 (n=1005) days averaged 1.7 and 1.3 ng/mg tissue, respectively (P=NS). In contrast, tissue concentrations of sirolimus at the stented site averaged 4.52 (n=6) and 1.56 (n=6) ng/mg tissue at 1 and 8 days, respectively (P=0.03), whereas whole-blood concentrations peaked at 0.5 hours (13.31 ng/mL; n=6) and declined to 4.58 ng/mL at 24 hours (n=6) and 0.90 ng/mL at 8 days (n=3).

Morphometric Analysis of Overlapping 28- and 90-Day DES

No significant differences were found in the percentage of stenosis, neointimal thickness, and neointimal area in overlapping segments of either DES at 28 days (Table 1 and Figure 1). The internal elastic lamina area in overlapping segments, however, was greater in Taxus stents. In addition, the medial wall thickness was similar in overlapping segments of Taxus compared with Cypher stents. Medial injury was similar in all groups.

Although no significant differences were noted in the percentage of stenosis, neointimal thickness, and neointimal area at overlapping sites between DES harvested at 28 days, a comparison of neointimal thickness in 28- and 90-day Taxus stents showed a lack of sustained neointimal suppression (28 days, 0.08±0.03 mm; 90 days, 0.13±0.02 mm; P=0.03; Figure 1). In contrast, neointimal thickness measurements within overlapping segments of 28- and 90-day Cypher stents were similar.

In BxVelocity stents, the percentage of stenosis was significantly greater in overlapped (25.7±3.5%) compared with nonoverlapped (17.8±5.9%; P=0.001) segments. This was also true for the Express stent (25.2±7.1% versus 17.9±5.0%; P=0.009). In the region of overlap, Cypher stents showed a decrease in neointimal thickness of 26.3% compared with BxVelocity; the reduction between Taxus and Express was 20.0%. These differences were not statistically significant and are probably related to fewer animals receiving control stents and the relative delay in healing at overlapping segments of BMS (Table 1).

Histological Observations From Cypher and Taxus Stents

There was greater evidence of delayed healing in overlapping versus nonoverlapping segments, independently of the DES, as indicated by incomplete endothelialization (assessed by light microscopy and SEM), greater accumulated fibrin, and inflamma-

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**TABLE 1. Comparison of Morphometric Measurements of Overlapping Segments from 28-Day Cypher, BxVelocity, Taxus, and Express Stents**

<table>
<thead>
<tr>
<th>Group, Overlapping Segments</th>
<th>IEL Area, mm²</th>
<th>Neointimal Area, mm²</th>
<th>Area Stenosis, %</th>
<th>Neointimal Thickness, mm</th>
<th>Medial Thickness, mm</th>
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<tbody>
<tr>
<td>Cypher (n=9)</td>
<td>6.95±0.90</td>
<td>1.62±0.50</td>
<td>23.30±6.58</td>
<td>0.14±0.07</td>
<td>0.07±0.02</td>
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<td>P vs BxVelocity</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>BxVelocity (n=6)</td>
<td>7.04±0.60</td>
<td>1.80±0.25</td>
<td>25.65±3.47</td>
<td>0.19±0.03</td>
<td>0.077±0.02</td>
</tr>
<tr>
<td>Taxus (n=9)</td>
<td>8.37±1.21</td>
<td>1.85±0.71</td>
<td>21.55±5.79</td>
<td>0.16±0.06</td>
<td>0.083±0.03</td>
</tr>
<tr>
<td>P vs Express</td>
<td>0.03</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Express (n=6)</td>
<td>7.51±0.61</td>
<td>1.90±0.53</td>
<td>25.18±7.13</td>
<td>0.20±0.10</td>
<td>0.068±0.01</td>
</tr>
<tr>
<td>P, Cypher vs Taxus</td>
<td>0.00003</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

IEL indicates internal elastic lamina.

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**Figure 1.** Iliac artery morphology 28 (A–F) and 90 (G–L) days after placement of overlapping DES in rabbit. Note that neointimal overage of stent struts is greater in nonoverlapping proximal and distal segments vs sites of overlap (middle sections). Movat Pentachrome stain (×20 magnification). Bar graph (M) shows significant increase in neointimal thickness between 28- and 90-day Taxus stents; neointimal thickness (above stent) measurements within overlapping segments of 28- and 90-day Cypher stents were similar.
mation (Figure 2). There was significantly more fibrin in 28-day Taxus compared with Cypher stents (Figure 3). Moreover, heterophils/eosinophils in overlapping segments were more frequent in Taxus stents (89.50±64.1 versus 33.8±29.6 cells; \( P=0.006 \)), whereas peristrut giant cells were more common in Cypher stents (7.9±6.5 versus 1.2±1.2; \( P=0.0007 \); Table 2 and Figure 4). Stents harvested at 90 days showed markedly less fibrin than those harvested at 28 days, and differences among DES were no longer significant (Figure 3). Greater numbers of luminal heterophils/eosinophils and giants cells, however, persisted for both stent types (Figure 4). Overlapping segments from control BMS showed less accumulated fibrin than DES, although significant amounts of fibrin were still apparent in Express stents (Table 2). Although similar numbers of giant cells were found between overlapping BxVelocity and Cypher stents, Express stents evoked significantly more giant cell response than Taxus stents (Table 2). The amount of surface heterophils/eosinophils was markedly greater in both DES (Table 2).

**Histological Observations in BxVelocity and Express Stents**

Overlapped segments from Express stents showed greater fibrin accumulation around stent struts than BxVelocity stents, although the reaction was considerably less than in

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Bar graphs comparing 28-day arterial histological effects at proximal and distal sites of nonoverlapping Cypher and Taxus stents to region of overlap. A, Differing effects on number of luminal heterophils/eosinophils. B, Comparison of percent endothelialization at nonoverlap vs overlap sites. C, Number of struts surrounded by fibrin at these sites.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Photomicrographs showing fibrin deposition (reddish-orange stain) around overlapping stent struts (A–D). Taxus stents display more accumulated fibrin at sites of stent overlap than Cypher at both 28 (A, B) and 90 (C, D) days. Carstair’s stain (×200 magnification). Bar graph (E) showing significantly increased neointimal fibrin deposition in overlapping segments of 28-day Taxus stents; significant differences between DES at 90 days were not apparent.
Cypher or Taxus stents. Luminal heterophils/eosinophils were greater in overlapping Express than BxVelocity sections but were considerably less than that found in overlapping DES segments. Endothelialization, as assessed by light microscopy, was also less in overlapped DES than BMS (Table 2).

Cell Density and Proliferation in Cypher and Taxus Stents
Analysis of BrdU-positive nuclei revealed no significant differences in intima or medial cell proliferation or intimal cell density among DES at any time point (Table 3). In contrast, in overlapped segments, medial cell density near stent struts was significantly less with Taxus compared with Cypher stents ($P=0.04$).

SEM Findings
SEM of 28-day Cypher stents demonstrated endothelial cell coverage involving 63±8% of the stent surface compared with 37±12% in Taxus stents ($P=0.03$; Figure 5). In both DES, endothelial cell junctions were loose and easily separated during dehydration; in particular, the arterial wall close to the stent struts showed adherence of white cells and platelet aggregation. At the overlapping stent sites, the stent struts were rarely covered by endothelial cells; however, endothelialization was greater at the nonoverlapping proximal and distal segments. In contrast, endothelialization in control BxVelocity and Express stents was >80% (Figure 5), with nonendothelialized regions of the surface localized primarily to overlapped stent struts.

Discussion
Despite the relatively avid use of overlapping DES in clinical trials, to the best of our knowledge, this is the first published comparison of overlapping Cypher or Taxus stents on arterial wall pathology. The unique advantage of DES is that they allow higher local drug concentrations at lesion sites while

<table>
<thead>
<tr>
<th>Group, Overlapping Segments</th>
<th>Lumen Endothelialized, %</th>
<th>Fibrin Score (0 to 3+)</th>
<th>Struts With Fibrin, n</th>
<th>Giant Cells per Strut, n</th>
<th>Luminal Heterophil/Eosinophil, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cypher (n=9)</td>
<td>74.8±19.4</td>
<td>1.6±0.7</td>
<td>10.2±4.4</td>
<td>7.9±6.5</td>
<td>33.8±29.6</td>
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<tr>
<td>P vs BxVelocity</td>
<td>0.04</td>
<td>0.0004</td>
<td>0.0005</td>
<td>NS</td>
<td>0.005</td>
</tr>
<tr>
<td>BxVelocity (n=6)</td>
<td>95.7±5.5</td>
<td>0.2±0.3</td>
<td>1.4±1.7</td>
<td>3.3±1.8</td>
<td>0.9±1.5</td>
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<tr>
<td>Taxus (n=9)</td>
<td>66.8±24.7</td>
<td>2.2±0.8</td>
<td>15.6±4.9</td>
<td>1.2±1.2</td>
<td>89.5±64.1</td>
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<tr>
<td>P vs Express</td>
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<td>0.002</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.0007</td>
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<tr>
<td>Express (n=6)</td>
<td>80.4±12.1</td>
<td>1.0±0.5</td>
<td>7.4±4.5</td>
<td>10.5±5.0</td>
<td>14.0±8.0</td>
</tr>
<tr>
<td>P, Cypher vs Taxus</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>0.0007</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Figure 4. Photomicrographs showing heterophils/eosinophils (A, B) and peri-strut giant cells (C, D) in overlapping 28-day Cypher or Taxus stents. Greater numbers of heterophils/eosinophils are seen on luminal surface of Taxus stents (inset in B, ×1000 magnification), whereas Cypher stents show more peri-strut giant cells (arrows). Hematoxylin and eosin stain (×200 magnification). Bar graphs (E, F) representing number of heterophils/eosinophils and giant cells, respectively, in 28- and 90-day Cypher and Taxus stents.
avoiding systemic toxicity. Our findings of persistent inflammation, fibrin deposition, and delayed endothelialization, particularly at overlapping segments (compared with BMS), highly suggest that local arterial toxicity may potentially develop when the surrounding tissue is exposed to inappropriate levels of drug and/or polymer.

Both DES showed signs of delayed arterial healing at nonoverlapping sites, which is in agreement with other published reports of sirolimus- and paclitaxel-eluting stents. In direct contrast, however, delayed healing was more pronounced at overlapping segments. The inflammatory response to either DES was selective so that overlapping Taxus stents induced more fibrin and heterophils/eosinophils compared with Cypher stents. Moreover, although both DES evoked a giant cell reaction near stent struts, the response was greater with Cypher stents. Luminal endothelialization was significantly more complete in overlapping 28-day Cypher than Taxus stents when visualized by SEM. Arterial healing was generally more complete in DES harvested at 90 days; however, Taxus stents failed to provide sustained neointimal suppression. Incomplete healing in both DES was mostly characterized by persistent fibrin and inflammation cell infiltrate characterized by heterophils/eosinophils for Taxus stents and giant cells for Cypher stents. Although no differences were found in cell proliferation or intimal cell density, medial cell density at or near stent struts was significantly lower in both 28- and 90-day Taxus stents.

Notably, the relative delay in healing found in overlapping segments of stainless steel BMS was not nearly to the degree found with DES and was possibly related to the finishing processes in the final preparation of the stent.11 This reaction, however, might have affected neointimal growth in overlapping segments because neointimal thickness measurements between Cypher and BxVelocity and Taxus and Express stents, although lower, were not significant.

### Sirolimus-Eluting Stents

Preclinical studies of sirolimus-eluting stents show a range of biological effects on arterial wall healing, inflammation, and neointimal growth. In a study by Klugherz et al,12 little evidence of increased inflammation or delayed endothelialization was noted with 28-day sirolimus-eluting stents (64 or 196 \( \mu g \) per stent) compared with polymer-coated stents or BMS in rabbit iliac arteries. In another 28-day study, Suzuki et al7 reported higher amounts of accumulated fibrin with sirolimus-eluting stents (180 \( \mu g \) per stent) compared with BMS in porcine coronary arteries, although the degree of endothelialization was similar among groups. Despite disparities in arterial wall pathology, both studies report significant suppression of neointimal growth with sirolimus-eluting stents. More recently, Carter et al6 described the long-term effects of Cypher stents, again in porcine coronary arteries. Arterial inflammation characterized by giant cells gradually progressed from 90 to 180 days with a corresponding increase

<table>
<thead>
<tr>
<th>Group</th>
<th>Intimal Cell Density per 1 mm²</th>
<th>Medial Cell Density per 1 mm²</th>
<th>BrdU, % Intimal</th>
<th>BrdU, % Medial</th>
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<tr>
<td></td>
<td>28 d</td>
<td>90 d</td>
<td>28 d</td>
<td>90 d</td>
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<tr>
<td>Cypher</td>
<td>2754±590</td>
<td>3743±1221</td>
<td>2656±896</td>
<td>2615±716</td>
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<tr>
<td>Taxus</td>
<td>2679±784</td>
<td>2163±406</td>
<td>1105±804</td>
<td>1342±494</td>
</tr>
<tr>
<td>( P )</td>
<td>NS</td>
<td>NS</td>
<td>0.04</td>
<td>0.01</td>
</tr>
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</table>

Medial cell density per 1 mm² refers to near stent struts. Values are mean±SD.

### Figure 5.

SEM of overlapping 28-day BxVelocity (A), Cypher (B–F), Express (G), and Taxus (H–L) stents. Regions of overlap are within horizontal arrows. Overall, there is less surface coverage by endothelial cells in Taxus than Cypher stents, specifically in segment of overlap. Overlapping segments within BxVelocity and Express stents showed far greater endothelialization than DES. Higher-power views of Cypher stents (C–F) from segment of overlap show adherent platelets and inflammatory cells on stent struts and adjoicing neointima. Higher-power images from overlapping segments of Taxus stents (I–L) show greater inflammatory infiltrate (I), polymer sticking and stretching across stent struts (J, arrow), unexpanded struts (K arrow), and irregular distribution of the polymer over stent strut surface (L, arrowheads).
in neointimal formation. Unlike the present study, however, none of the above reports mention neutrophilic/eosinophilic reactions to sirolimus-eluting stents and collectively emphasize the wide therapeutic index for sirolimus in normal vessels.

Our findings are consistent with previous studies in that sites of nonoverlap initially demonstrated minimal delayed healing at 28 days, whereas the late studies (ie, 90 days) showed neointimal healing to be nearly complete with mild inflammation. Our data conflict with previous findings in that we found a dose-dependent increase in local arterial toxicity characterized by delayed endothelialization, fibrin deposition, and inflammation at sites of overlap. The lack of exaggerated heterophilic/eosinophilic response in the overlapping control BxVelocity BMS suggests that overlapping layers of polymer and/or drug may evoke excessive inflammation, which alone can delay healing. This is consistent with the findings of Suzuki et al in that the PEVA/PMBBA nonerodible polymers used in the Cypher stent demonstrated an increase in arterial inflammation as polymer load was increased. However, the total dosage of drug at overlap sites (ie, 300 μg) in the present study is also a consideration.

Paclitaxel-Eluting Stents
Paclitaxel is a cytotoxic drug known to suppress neointimal formation accompanied by persistent fibrin deposition, macrophage infiltration, and an overall decrease in smooth muscle cells at both 28 and 180 days after implantation in rabbit iliac and porcine coronary arteries. Results of overlapping moderate-release Taxus stents (1 μg/mm²) demonstrated a moderate inflammatory response without evidence of eosinophils and increased amounts of fibrin deposition with partially complete endothelialization; unfortunately, no results of neointimal area were reported in this study. The significant increases in internal elastic lamina in overlapping Taxus compared with Cypher stents in the present study is consistent with the earlier findings of Heldman et al, who reported significant increases in luminal area with high-dose paclitaxel-eluting stents. This was attributed in part to medial wall necrosis, smooth muscle cell loss, and arterial dilation, as was noted particularly in overlapping segments of Taxus stents in our study. Alternatively, the radial strength of the Taxus (Express) stent is greater than the Cypher (BxVelocity) stent and may account for the internal elastic lamina differences between these groups (J.A. Ormiston, et al, Green Lane and Mercy Hospitals, Auckland, New Zealand).

We also demonstrated a dose-dependent effect of the slow-release Taxus stent on healing after injury using a stent loaded with 137 μg paclitaxel. Fibrin deposition, inflammatory cell infiltration and inhibition of endothelialization were greater at sites of overlap where the dose is presumed to be twice that of nonoverlap sites. The findings at sites of overlap are consistent with arterial toxicity and indicate a narrow therapeutic index for this drug even when delivered locally. This toxic effect may be amplified by the biphasic drug release profile characterized by an initial burst of drug followed by a constant slow release, which continues to elute the drug even after 180 days. The marked luminal heterophilia/eosinophilia seen may be a reaction to the SIBBS polymer. Although previous stent studies have not shown inflammation to polymers or paclitaxel, the current observation is strengthened by the lack of a significant heterophilic/eosinophilic reaction at sites of overlapping Express BMS in this study. Because all stents used in this study were the commercially used slow-release formulation, a possible explanation for this discrepancy between our findings and those of Boston Scientific may relate to the polymer load at the site of overlap because the commercially available slow-release formulation contains greater polymer weight than the moderate-release formulation.

Clinical Relevance
The relevance of our findings pertains to the widespread use of overlapping DES in major clinical trials. The most critical issue raised by this study relates to the long-term effects of chronic inflammation, including heterophilic/eosinophilic infiltrate, persistent fibrin, and delayed endothelialization in overlapping DES segments. The poor endothelialization may be the result of drug overdose or may be secondary to hypersensitivity reaction (eosinophilic infiltrate) to the polymer; either or both mechanisms are likely responsible.

The importance of a significant delay in endothelialization at sites of overlapping DES is underscored by the recent report of McFadden et al involving 4 cases of human coronary thrombosis occurring >11 months after deployment of a single polymer-based paclitaxel-eluting or sirolimus-eluting stent. Each patient presented shortly after antiplatelet therapy was discontinued. From the present data, an increased thrombotic risk would be predicted with overlapping DES. Because the clinical course of complete endothelialization after placement of overlapping DES is unknown, the duration of and strategies to deal with unanticipated interruption of antiplatelet therapy need to be further investigated. Nonetheless, patients receiving overlapping DES likely require antiplatelet therapy for much longer periods of time than used in major clinical trials.

In addition, clinical experience with the QuaDS-QP2 stent underscores the concern that excessive inflammation may result in an aggressive delayed increase in neointimal growth. Although complete healing after single DES placement in animals is seen only after 90 days, in humans, the time course of healing is beyond the duration of all clinical trials. Morphological examination of atherectomy tissue from a subset of patients receiving QuaDS-QP2 stents showed persistent fibrin deposition, consistent with incomplete healing. These histological findings looked strikingly similar to those seen in animal models with the paclitaxel-eluting stent, although the time course of healing is prolonged in humans. Moreover, the heterophilic/eosinophilic nature of the inflammatory response resembles that reported in humans as a consequence of hypersensitivity response to the polymer after Cypher stent placement, albeit in a milder form.

Although the practice of overlapping DES is common, there is a paucity of information about the long-term (ie, >1
year) clinical consequences of this practice. Early 1-year follow-up for overlapping stents in the SIRIUS trial demonstrated a 5.7% target lesion revascularization rate for 344 patients compared with 4.5% for nonoverlapped Cypher stents in 714 patients.²³ Degertekin et al²⁴ reported a 1-year target vessel revascularization rate of 6.2% for 96 patients with overlapped Cypher stents in their own research registry, although the location of restenosis was not reported. In a series of 368 patients receiving Cypher stents, Colombo et al²⁵ reported 5 cases of restenosis involving patients treated with overlapping stents, although in only 2 cases was the site of renarrowing at the overlap segment. In TAXUS-VI, the target lesion revascularization rate at 6 months was 1.6% and in segment stenosis was 8.1% for overlapping paclitaxel-eluting stents.⁴

The wide therapeutic index of sirolimus may explain why the clinical experience with overlapping Cypher stents thus far remains favorable. Our study revealed almost total reendothelialization at 90 days at sites of nonoverlap despite evidence of incomplete healing at overlapped sites. The recent findings of Carter et al⁶ pertaining to a lack of long-term neointimal suppression and increased inflammation after Cypher stent placement in porcine coronary arteries emphasize the need for careful long-term observation of these patients.

Unfortunately, there is considerably less clinical experience with overlapping Taxus stents. Results of our histological analysis reveal significantly less endothelialization, more medial cell loss, and heterophilic/eosinophilic infiltration in overlapping segments of 28- and 90-day Taxus compared with Cypher stents. The persistent inhibition of cellular repair mechanisms and cytotoxicity at sites of overlap may reduce the long-term (beyond 18 months) therapeutic benefit of paclitaxel. Indeed, although at 28 days neointimal thicknesses for both DES were equivalent at sites of overlap in our rabbit model, there was a significant increase in neointimal thickness in overlapping Taxus segments at 90 days. This disparity between early and late outcomes is similar to that seen with the long-term results with intracoronary de novo brachytherapy in humans, although probably not to the same extent.²⁶,²⁷ Only long-term clinical studies involving angiographic and intravascular ultrasound may reveal the consequences of high-grade persistent inflammation.

### Study Limitations
The assessment of overlapping DES in an animal model using normal nonatherosclerotic arteries may have underestimated the effects of high doses of drug and polymer load on the arterial vasculature because atherosclerotic tissue may intensify the inflammatory response. Current animal models used in the assessment of stents are limited by their ability to replicate human conditions, although results with the rabbit iliac model have been generally representative of human responses, albeit with a different time course of healing. It is still inconclusive whether our results would be similar to those observed in human atherosclerotic coronary arteries.

### Conclusions
This is the first published study to compare the effects of 2 currently Food and Drug Administration–approved overlap-


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