Comparison of Inflammatory Response After Implantation of Sirolimus- and Paclitaxel-Eluting Stents in Porcine Coronary Arteries

Gregory J. Wilson, MD; Gaku Nakazawa, MD; Robert S. Schwartz, MD; Barbara Huibregtse, DVM; Bradley Poff, DVM; Thomas J. Herbst, PhD; Donald S. Baim, MD; Renu Virmani, MD

Background—Although both sirolimus (CYPHER) and paclitaxel (TAXUS) drug-eluting stents have demonstrated efficacy and safety in clinical trials, human autopsy data have raised concerns about long-term healing and the potential for local inflammatory reactions.

Methods and Results—Overlapping stents (CYPHER drug-eluting stents, Bx SONIC bare metal stents, TAXUS drug-eluting stents, and Liberté bare metal stents) were implanted in noninjured coronary arteries of 58 domestic swine. Histopathological evaluation of proximal, overlapped, and distal stented segments was determined with emphasis on inflammation at 30, 90, and 180 days. Circumferential granulomatous inflammation in all stented segments was defined as inflammation consisting of macrophages, multinucleated giant cells, lymphocytes, and granulocytes, including many eosinophils, adjacent to almost all struts. Circumferential granulomatous inflammation was more prevalent in CYPHER (9 of 23, 39%; P=0.01) and control bare metal stents (0 of 44) in the combined 90- and 180-day cohorts. Only CYPHER specimens showed marked adventitial inflammation (P=0.0025) and fibrosis (P=0.0055) accompanied by extensive remodeling. Fibrin deposition within neointima and medial smooth muscle cell death were greater (both P<0.001) in TAXUS than CYPHER at 30 days, with more fibrin in TAXUS than CYPHER through 90 days (P<0.05).

Conclusions—Although these data cannot be directly extrapolated to humans, the high prevalence in this porcine model of diffuse granulomatous inflammation seen with CYPHER stents, persisting at 180 days and associated with extensive remodeling of the artery, and persistent para-strut fibrin deposition with TAXUS stents emphasize the need for further investigation of biocompatibility with these and other novel combination drug/polymer drug-eluting stents. (Circulation, 2009;120:141-149.)

Key Words: angioplasty ▪ coronary disease ▪ inflammation ▪ pathology ▪ stents

The polymer-based sirolimus (CYPHER) and paclitaxel (TAXUS) drug-eluting stents (DES) have been placed in several million patients since their approval by the US Food and Drug Administration in 2003 and 2004, respectively. Each has been studied in randomized clinical trials compared with bare metal stent (BMS) controls, with a pooled patient-level analysis of 9 such trials (4 CYPHER, 1748 patients; 5 TAXUS, 3513 patients) by Stone et al showing a 0.7% to 0.9% incidence of stent thrombosis within the first year for each DES and its BMS control. After 1 year, depending on the definitions used, both CYPHER and TAXUS show a slight but statistically significant numerical excess in very late stent thrombosis by roughly 1 event per 500 patient-years compared with their respective BMS controls. Despite this finding, there were no significant differences between either DES and its BMS control in cumulative rates of death or myocardial infarction through 4 years, with both CYPHER and TAXUS stents markedly reducing the incidence of angiographic target lesion revascularization compared with BMS for the indications studied in these clinical trials. However, postapproval data suggest a higher risk of both early and late stent thrombosis plus death or myocardial infarction under “off-label use” conditions than in the narrower conditions studied in the randomized trials.2

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The observation of a numerical excess in very late stent thrombosis with DES coincides with pathological analysis of CYPHER and TAXUS stents explanted at autopsy, which...
suggests delayed arterial healing for both CYPHER and TAXUS DES compared with BMS of similar implant duration. Joner et al.\(^1\) compared 32 DES (either CYPHER or TAXUS) from 23 individuals who died >30 days after implantation with 36 matched BMS from 25 individuals. In an expansion of the above autopsy database to 46 individuals, Finn et al.\(^2\) recently reported that a lack of full endothelial strut coverage in any given section is the best single correlate of thrombosis. In another study, Finn et al.\(^3\) reported that hypersensitivity reactions (including lymphocytes, macrophages, multinucleated giant cells, and eosinophils) occurred in 5 of 105 autopsy cases, 4 of which were in CYPHER stents showing pervasive inflammatory cells throughout the stented arterial segment; in contrast, the 1 TAXUS stent case had only focal infiltrates of eosinophils involving a few struts. When the tissue-based inflammatory reactions were diffuse, as seen in hypersensitivity, the artery was often aneurysmally dilated and thrombosed.\(^5,6\)

Although human data are the ultimate test of DES biocompatibility, animal models offer the possibility of obtaining detailed systematic insights into the tissue response to DES under controlled conditions. The present study was undertaken to compare the inflammatory response, especially granulomatous inflammation (GI), for TAXUS and CYPHER stents implanted in porcine coronary arteries.

**Methods**

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication 85–23, revised 1996). Stent implantations were performed at MPI Research Inc (Mattawan, Mich), accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

**Stenting Protocol**

Stainless steel balloon-expandable DES (8-mm length, 3.0- to 3.5-mm diameter), either CYPHER (Cordis Corp, Miami Lakes, Fla) or TAXUS (Boston Scientific Corp, Natick, Mass), or bare metal stents (BMS) of the same platform (Bx SONIC and Liberté, respectively) were implanted in an overlapping fashion (either both DES or both BMS) in the left anterior descending, left circumflex, and right coronary arteries of female crossbred swine (Genetiporc, LLC, Alexandria, Minn) with a 4±1-mm target overlap (double strut density) and an 11- to 13-mm target stent length. Animals were euthanized after implant durations of 30, 90, and 180 days. Three groups of pigs received overlapping CYPHER stent pairs and Bx SONIC BMS control stent pairs at 30 days (10 pigs), 90 days (11 pigs), and 180 days (10 pigs), and another 3 groups received overlapping TAXUS stent pairs and Liberté BMS control stent pairs for the same time points (9 pigs at each time point). When anatomy permitted, each pig received 3 overlapping stent pairs, either 1 DES and 2 BMS or 2 DES and 1 BMS. Each group provided 13 to 16 DES and 12 to 16 BMS controls for evaluation. The stents, including overlapped segments, were, after initial placement, postulated with a high-pressure noncompliant balloon as necessary to achieve a targeted ratio of stent to artery diameter of ~1:1:1, as assessed by quantitative coronary angiography.

**Antithrombotic Regimen**

All animals were pretreated with aspirin (325 mg) and Plavix (75 mg) PO for 3 days before and on the day of implantation. Aspirin and Plavix (75 mg) PO were then administered daily until the day of euthanasia. Intraarterial heparin (100 to 250 IU/kg) was administered at the induction of anesthesia, intraoperatively for implantation, and at the terminal procedures.

**Tissue Harvest and Processing for Histology and Scanning Electron Microscopy**

At 30, 90, and 180 days, animals were anesthetized and angiography was performed. After euthanasia, hearts were harvested and labeled, and arteries were pressure-perfusion fixed with 10% formalin. Stented vessels were dissected from the myocardium, allowing adequate proximal and distal reference vessel length in addition to the stented segment, and radiographed with a high-resolution Faxitron. Six (3 DES and 3 BMS) nonoccluded stents were randomly selected from each group for scanning electron microscopy. For histology, explanted coronary arteries were dehydrated; embedded in plastic; sectioned at proximal, overlapped, and distal locations and proximal and distal to the stented segment; and stained with hematoxylin and eosin and elastic trichrome stains. In selected cases, Luna staining was performed to illustrate eosinophil infiltration.

After dissection of the stented coronary arteries, each heart was sliced transversely at ~1-cm intervals from base to apex. In all hearts, transmural samples of the right coronary, left anterior descending, and left circumflex arteries downstream of the stent implant were obtained and stained with hematoxylin and eosin to evaluate changes of ischemia.

**Morphological Evaluation**

Morphological analysis of the stented sections (proximal, overlapped, distal) was performed blindly by light microscopy using standard ordinal grading assessments (described in Table I of the online-only Data Supplement) and is presented for luminal thrombus, endothelialization, strut tissue coverage, para-strut inflammation, including granuloma formation, medial smooth muscle cell loss, and para-strut fibrin deposition. A vessel injury score was determined with the method of Schwartz et al.\(^7\) The number of struts with surrounding GI was determined. GI was defined as consisting of localized collections of macrophages, giant cells, lymphocytes, and granulocytes with or without numerous (>10 cells per ×40 objective field) eosinophils (Figure 1). Three regional patterns of GI were further defined as follows: unifocal GI, consisting of 1 focus of para-strut GI involving no more than 1 quadrant (90°) of the circumference; multifocal GI, consisting of ≥2 foci of para-strut GI involving no more than 3 quadrants (270° maximum) of the circumference; and circumferential GI (CGI), consisting of several foci of para-strut granulomas involving all 4 quadrants of the circumference (Figure 2). Extension of inflammation into the adventitia and adventitial reactive fibrosis were each graded as 0 (absent) or 1 (present, mild or severe). Strut malapposition, defined as a distance of at least 50 μm between the strut and intact internal elastic lamina, was assessed for each in-stent section.

**Morphometric Evaluation**

Computer-assisted morphometric analysis of the in-stent sections was performed on high-resolution images of the stented arterial cross sections using a combination of automated and manual techniques with calibration. The following measurements were determined for the proximal, overlapped, and distal sections: luminal area (mm²), external elastic lamina (EEL) area (mm²), and stent area (mm²). Strut-to-lumen measurements from the edge of the strut farthest from the lumen to the lumen boundary were made and averaged for each section to determine intimal thickness. Through these measurements, stent-based percent area stenosis was determined.

**Statistical Analysis**

All morphological and morphometric data were statistically analyzed on a segment-by-segment basis (proximal, overlapped, distal) at each time point (30, 90, 180 days), comparing CYPHER with TAXUS and each DES with its corresponding BMS control at Boston Scientific through the use of SAS version 8.02 (SAS Institute Inc, Cary, NC). The Wilcoxon exact test was used for continuous parameters. Ordinal data were ranked and ANOVA was performed.
for comparisons between device groups. Fisher’s exact test and Chi-square tests were used for granuloma scoring. Thirteen of 131 stented vessels examined by histology were excluded from statistical analysis because of a lack of stent overlap or excessive overlap (>/=6 mm). A value of P<0.05 was considered significant. No adjustments were made for multiplicity of comparisons, which is a limitation of the analysis. The issue of potential correlation among stents implanted into different coronary arteries in the same pig is addressed through hierarchical and mixed-model analyses in the online-only Data Supplement, which provides further details on the statistical analysis and comments on the results.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

All stents were successfully deployed without dissection or thrombosis. All animals remained alive for the duration of the study. Repeat angiography at euthanasia showed 2 coronary arteries, each containing overlapped CYPHER stents, with 100% occlusion and healed myocardial infarction downstream of each of these vessels in the heart of one 90-day animal. The rest of the stents were patent at all time points with no stent migration or aneurysms observed.

Morphometry: EEL Area, Area Percent Stenosis, and Intimal Thickness

The only stent group that showed marked variation resulting from increasing EEL area was the CYPHER stent at 90 and 180 days (Figure 3). All other groups had mild variability (Figure 3). Intimal thickness was assessed in the overlapped section (Table 1) and was significantly less in TAXUS compared with CYPHER at 30 days (P<0.0001). This was also true for the proximal (P<0.0001) and distal (P<0.0001) sections (data not shown). In addition, the intimal thickness in TAXUS compared with BMS control at overlapped sections was significantly less (P=0.016) at 30 days. CYPHER intimal thickness at 30 days was not significantly different from its BMS control. However, at 90 and 180 days, there was greater (P=0.0008 at overlap) intimal thickness in TAXUS compared with its BMS control (Table 1). Similar results to TAXUS were observed in CYPHER compared with its BMS control at 90 and 180 days. The intimal thickness mirrored area percent stenosis (Table 1). Thus, after initial neointimal suppression at 30 days, neointimal formation was greater at 90 and 180 days in TAXUS. Area percent stenosis, including all stented sections, ranged from 22% to 63% for TAXUS and from 12% to 47% for its BMS control. The variability with CYPHER was most striking, with area percent stenosis ranging from 20% to 100% compared with 18% to 73% for its BMS control.

Histological Evaluation

Granulomas, Adventitial Inflammation, and Fibrosis

The percent of struts with granulomas was significantly greater in the CYPHER stents at 90 and 180 days compared with TAXUS stents or BMS control stents (Figure 4). Para-strut leukocyte infiltration ranged from mild to severe (Figure 1) in the TAXUS and CYPHER stents at 30, 90, and 180 days. Nine of 23 CYPHER-stented vessel segments (39%) at 90 and 180 days showed CGI in all 3 sections compared with 1 of 21 in TAXUS (5%; P=0.01, Fisher’s exact test). Five CYPHER-stented vessels (22%) had multifocal GI in at least 1 section. Another 39% of CYPHER stents showed only mild leukocyte infiltration around stent struts. BMS controls showed predominantly mild para-strut inflammation. Two notable exceptions were a Bx SONIC at 90 days, which had 2 stented sections with CGI, and a Liberté at 90 days, which had multifocal GI and unifocal GI in 1 section each.

Severe extension of inflammation into the adventitia in >1 sections also was significantly more prevalent for CYPHER (9 of 36) compared with TAXUS (0 of 32; P=0.0025) and was associated with severe adventitial fibrosis in 8 CYPHER
(22%) compared with 0 TAXUS (0%; \(P=0.0055\)) stents. The 1 TAXUS stent with CGI at 90 days showed inflammatory cell infiltration with mild extension into the adventitia and mild adventitial fibrosis (Figure 5).

**Eosinophil Infiltration of Granulomas**

Eosinophil infiltration exceeding 10 cells per high-powered field was observed in 97% of granulomas in the CYPHER arm. The percentage of granulomas with this level of eosinophils in the TAXUS stents was significantly less than CYPHER at 90 and 180 days (\(P=0.0003\) and \(P=0.0004\), respectively; Figure 4). Granulomas exceeding 10 cells per high-powered field of eosinophil infiltration were seen in 2 BMS. One additional Bx SONIC at 90 days showed 2 para-strut granulomas.

**Para-Strut Fibrin and Strut Malapposition**

Fibrin scores were significantly greater for both TAXUS and CYPHER compared with their respective BMS controls (\(P<0.0001\) for both) at 30 days, with TAXUS showing more

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**Table 1. Comparison of Morphometric Measurements at 30, 90, and 180 Days**

<table>
<thead>
<tr>
<th>Time Point, d</th>
<th>Bx SONIC BMS</th>
<th>CYPHER DES</th>
<th>(P), SONIC vs CYPHER</th>
<th>TAXUS DES</th>
<th>Libé BMS</th>
<th>(P), TAXUS vs Libé</th>
<th>(P), CYPHER vs TAXUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal thickness (overlapping segments), mm (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.37±0.11 (7)</td>
<td>0.37±0.16 (13)</td>
<td>0.49</td>
<td>0.21±0.03 (11)</td>
<td>0.32±0.12 (9)</td>
<td>0.016</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>90</td>
<td>0.33±0.13 (12)</td>
<td>0.66±0.51 (10)</td>
<td>0.02</td>
<td>0.48±0.20 (10)</td>
<td>0.24±0.05 (7)</td>
<td>0.0007</td>
<td>0.74</td>
</tr>
<tr>
<td>180</td>
<td>0.25±0.05 (9)</td>
<td>0.41±0.20 (11)</td>
<td>0.02</td>
<td>0.45±0.13 (8)</td>
<td>0.25±0.08 (11)</td>
<td>0.0008</td>
<td>0.31</td>
</tr>
<tr>
<td>Area stenosis (overlapping segments), % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>40±10 (7)</td>
<td>40±14 (13)</td>
<td>1.00</td>
<td>25±4.9 (11)</td>
<td>36±11 (9)</td>
<td>0.0074</td>
<td>0.0003</td>
</tr>
<tr>
<td>90</td>
<td>38±14 (12)</td>
<td>58±24 (10)</td>
<td>0.0009</td>
<td>50±16 (10)</td>
<td>27±3.9 (7)</td>
<td>0.0007</td>
<td>0.74</td>
</tr>
<tr>
<td>180</td>
<td>28±5.5 (9)</td>
<td>43±18 (11)</td>
<td>0.02</td>
<td>47±7.8 (8)</td>
<td>30±7.7 (11)</td>
<td>0.0008</td>
<td>0.31</td>
</tr>
<tr>
<td>Percentage of struts with malapposition (all segments), % (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1±4 (10)</td>
<td>0±0 (13)</td>
<td>0.44</td>
<td>10±10 (11)</td>
<td>0±0 (9)</td>
<td>0.0006</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>90</td>
<td>7±16 (13)</td>
<td>4±11* (9)</td>
<td>0.84</td>
<td>1±2 (11)</td>
<td>3±6* (9)</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>180</td>
<td>9±10 (10)</td>
<td>7±10* (10)</td>
<td>0.59</td>
<td>1±3 (10)</td>
<td>1±2 (11)</td>
<td>0.74</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Wilcoxon exact test is used throughout the table. Values are given as mean±SD. For intimal thickness and area stenosis, n equals overlapped segments evaluated via histology. For percentage of struts with malapposition, n equals number of stented vessels. \(P<0.05\) indicates a significant difference.

*Malapposition was measured only where internal elastic lumina was readily visible.
para-strut fibrin at 30 days than CYPHER \( (P=0.0005) \). TAXUS also showed significantly higher fibrin scores than CYPHER at 90 days \( (P=0.01) \) and 180 days \( (P=0.04; \text{Table 2}) \). Although higher para-strut fibrin scores persisted for TAXUS compared with its BMS control at 90 days \( (P=0.0002) \), they declined to borderline significance \( (P=0.06) \) at 180 days. Fibrin scores were higher in overlapping than nonoverlapping segments at 30 days in both DES (Figure 6) but were not significantly different between overlapping and nonoverlapping segments at 90 or 180 days in either TAXUS or CYPHER stents. At 30 days, strut malapposition of 50 to 100 μm was observed in TAXUS stents involving 10±10% of struts, predominantly at overlap, but was not observed in CYPHER \( (P=0.0013) \) or bare Liberté stents (Table 1). Strut malapposition was not observed in TAXUS stents at 90 and 180 days. The frequency of strut malapposition assessed for CYPHER stents was not significantly different from TAXUS stents at 90 or 180 days (Table 1), but the measurement of malapposition required an intact internal elastic lamina, which was not present when severe GI occurred, affecting CYPHER stents disproportionately.

### Injury Score and Changes in the Media

Overall, the injury scores were low except in stents with granulomas and were observed predominantly in the DES arm, particularly the CYPHER stents. The only statistically significant differences were observed in CYPHER versus Bx SONIC at 90 and 180 days, with scores in the CYPHER arm reaching \( >1 \) (Table 2). When stents with granulomas were excluded, there were no significant differences between any of the arms at 30, 90, and 180 days. The degree of medial

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**Figure 4.** Percentage of struts showing granuloma formation and percentage of granulomas showing \( \geq 10 \) eosinophils per high-powered \((\times 40 \text{ objective}) \) field. Bar charts show data for CYPHER and TAXUS DES and their respective BMS controls Bx SONIC and Liberté. Those comparisons showing statistical significance \((\chi^2 \text{ test})\) are presented.

**Figure 5.** CYPHER vs TAXUS comparison of CGI in all 3 stented vessel segments: proximal, overlap, and distal. A, H, Radiographs of CYPHER- (A) and TAXUS- (H) stented segments. Histological sections through a CYPHER stent are shown in B (proximal), C (overlap), and D (distal). In all 3, the CGI extends into the adventitia, where there is severe adventitial fibrosis and a greatly expanded vessel cross-sectional area (positive remodeling). Histological sections through the 1 TAXUS-stented vessel segment that showed CGI in the proximal (E), overlap (F), and distal (G) sections show less extensive inflammatory activity and less positive remodeling than seen with CYPHER. All hematoxylin and eosin–stained sections are shown at the same magnification.
smooth muscle cell loss, considered to be due predominantly to previous cell death, was greater for TAXUS than its BMS control \((P<0.001)\) at 30, 90, and 180 days. TAXUS also showed greater medial smooth muscle cell loss than CYPHER at 30 days \((P<0.001)\) for proximal, overlapped, and distal sections. Small foci of fibrin deposition in the media, interpreted as a possible marker of previous smooth muscle cell death, were observed in TAXUS stents at 30 days at overlap but not with the other stents or at any other time points. The histology of 2 stented coronary arteries with extensive granuloma formation and changes in the media are compared with their appearance on angiography in Figure 7.

### Strut Tissue Coverage, Luminal Thrombus, and Endothelialization

All struts showed tissue coverage. Luminal microthrombi were seen on scanning electron microscopy in 1 pair of overlapped CYPHER stents explanted at 180 days, but they

#### Table 2. Morphological Scoring

<table>
<thead>
<tr>
<th>Time Point, d</th>
<th>Bx SONIC BMS</th>
<th>CYPHER DES</th>
<th>P, SONIC vs CYPHER TAXUS DES</th>
<th>Libérté BMS</th>
<th>P, TAXUS vs Libérté</th>
<th>P, CYPHER vs TAXUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0 (10)</td>
<td>15 (13)</td>
<td>0.49</td>
<td>0 (11)</td>
<td>0.10</td>
<td>0.48</td>
</tr>
<tr>
<td>90</td>
<td>15 (13)</td>
<td>64 (11)</td>
<td>0.03</td>
<td>18 (11)</td>
<td>0.10</td>
<td>0.081</td>
</tr>
<tr>
<td>180</td>
<td>0 (10)</td>
<td>58 (12)</td>
<td>0.0053</td>
<td>50 (10)</td>
<td>0.012</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Percentage of vessels with granulomas, % (n)**

<table>
<thead>
<tr>
<th>Time Point, d</th>
<th>Percentage of struts with fibrin (n)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>33±17 (10) 86±12 (13) &lt;0.0001 94±6 (11) 15±11 (9) &lt;0.0001 0.11</td>
</tr>
<tr>
<td>90</td>
<td>20±19 (13) 24±18 (11) 0.44 49±23 (11) 5±6 (10) 0.0002 0.013</td>
</tr>
<tr>
<td>180</td>
<td>4±5 (10) 4±5 (12) 0.93 12±14 (10) 1±2 (11) 0.048 0.28</td>
</tr>
</tbody>
</table>

**Fibrin score (n)†**

<table>
<thead>
<tr>
<th>Time Point, d</th>
<th>Injury score including granuloma (n)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.1±0.1 (10) 0.4±0.7 (13) 0.59 0.1±0.1 (11) 0.1±0.1 (9) 0.45 0.50</td>
</tr>
<tr>
<td>90</td>
<td>0.4±0.6 (13) 1.4±1.1 (11) 0.028 0.5±0.8 (11) 0.2±0.3 (10) 0.45 0.11</td>
</tr>
<tr>
<td>180</td>
<td>0.3±0.3 (10) 1.3±1.0 (12) 0.0059 0.9±0.8 (10) 0.4±0.4 (11) 0.12 0.47</td>
</tr>
</tbody>
</table>

**Injury score without granuloma (n)†**

<table>
<thead>
<tr>
<th>Time Point, d</th>
<th>Percentage of vessels with granulomas, % (n)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0 (10) 15 (13) 0.49 0 (11) 0.10</td>
</tr>
<tr>
<td>90</td>
<td>15 (13) 64 (11) 0.03 18 (11) 0.10</td>
</tr>
<tr>
<td>180</td>
<td>0 (10) 58 (12) 0.0053 50 (10) 0.012</td>
</tr>
</tbody>
</table>

**Values are mean±SD. n Equals number of stented vessels except for injury score without granulomas, for which n equals the number of stented vessels minus those showing granulomas. \(P<0.05\) indicates a significant difference.**

*Fisher exact test.
†Wilcoxon exact test.

Figure 6. Fibrin scoring comparing overlapped with nonoverlapped stented vessel segments. Bar charts compare fibrin scores of TAXUS with CYPHER DES overlapped and nonoverlapped segments at 30, 90, and 180 days. Fibrin scores were significantly (Wilcoxon text) greater at overlap than nonoverlap for both TAXUS and CYPHER at 30 days.
covered <5% of the flow surface. No thrombi were observed in the non-CYPHER arms of the study. Endothelial cell coverage exceeded 90% of the luminal surface.

Strut Fractures
Seven pairs of overlapping stents (1 CYPHER, 4 Bx SONIC, 2 TAXUS Liberté) showed strut fractures on high-resolution radiography. On detailed histological analysis, none of the fractured stents showed severe inflammation, stenosis, or changes suggestive of increased local drug effect.

Discussion
This study demonstrates that CYPHER stents implanted in noninjured common swine coronary arteries frequently provoked an intense, extensive granulomatous, eosinophil-rich inflammatory response with circumferential vessel involvement in all stented sections, in association with destruction of both internal elastic lamina and EEL, that extended into the adventitia, leading to marked vessel expansion and adventitial fibrosis (Figure 5). Inflammation also was observed in TAXUS stents and BMS controls for the same platform but at a much lower prevalence and with substantially less intensity. Although the intense inflammatory response described above, with circumferential vessel involvement, was not always associated with neointimal hyperplasia, stented segments usually showed positive vessel remodeling with expansion of the EEL area (Figure 7). Conversely, fibrin deposition was significantly greater in TAXUS than CYPHER at 30, 90, and 180 days; it was associated with mild (50 to 100 μm) strut malapposition in the overlapped segments, but only at 30 days.

CYPHER Stents
The CYPHER stent has been studied previously in swine. At 28 days, Suzuki et al⁸ showed minimal inflammation with no mention of GI. Carter et al⁵ reported arterial inflammation characterized by giant cells, which gradually increased from 90 to 180 days, but again no mention was made of a granulomatous reaction. Finn et al⁵ compared the CYPHER stent with BMS control and reported a higher grade of inflammation in CYPHER stents at 180 days; they also mentioned the presence of granulomas in 14% of vessels at 30 days, 43% at 90 days, and 60% at 180 days. These findings in CYPHER stents are very similar to those in the present study, in which the prevalence of granulomata was 15%, 64%, and 58% at these 3 time points, respectively. Eosinophilic inflammation has been reported in rabbit and pig models by Finn et al.⁵ Sirolimus itself has been associated with a hypersensitivity reaction but with a very low incidence, and it is an unlikely cause given the low levels of inflammation seen during the 30- to 60-day period of drug release from the stent. In contrast, the granulomatous inflammatory reaction appears to develop and progress through 90 and 180 days, with at least 60% of porcine arteries showing a granulomatous reaction at those combined time points. Because only 2 of the BxSONIC (the BMS control for CYPHER)–stented vessels showed para-strut granulomas and only 1 showed circumferential involvement, it is unlikely that the stainless steel backbone is the primary driver of the granulomatous response. The coating polymer(s) may be important in causing the granulomatous response, but other substances introduced in stent manufacture cannot be excluded.

TAXUS Stents
The extent of inflammation in the TAXUS stent was mild, and granulomatous reaction was observed in 0% of struts at 30 days, in only 10% of struts (18% of vessels) at 90 days, and in 4% of struts (50% of vessels) at 180 days. At 90 and 180 days, only 1 TAXUS-stented segment (1 of 21, 5%) had circumferential granulomatous involvement of all 3 sections, whereas 25% (9 of 23) of CYPHER stents had similarly extensive para-strut inflammation. Moreover, none of the TAXUS stents showed the severe adventitial inflammation or fibrosis that was prevalent in CYPHER. The stainless steel backbone for TAXUS was relatively free of granulomatous response, with only one of the TAXUS BMS controls so involved. It is unclear what contribution the TAXUS Translute polymer, styrene-b-isobutylene-b- styrene, may make to the observed GI, given the limited prevalence of the inflammatory response observed in the present study and in the small number of human cases examined to date.⁵

Para-strut fibrin was significantly more frequent (percentage of struts with fibrin) in TAXUS than in CYPHER at 30, 90, and 180 days; it was associated with mild (50 to 100 μm) strut malapposition in the overlapped segments, but only at 30 days.

The effectiveness of both drugs in inhibiting neointimal formation is supported by the strong clinical evidence of low restenosis rates for each type of DES in up to 4 years of follow-up.¹ However, in the porcine coronary artery model, both TAXUS and CYPHER stents cause more neointimal formation at 3 months and beyond than their respective control BMS, as demonstrated in the present study, previously reported by Carter et al⁵ for CYPHER, and recently
shown in TAXUS. In the rabbit iliac artery model, Finn et al detected more luminal eosinophils (heterophils) but fewer para-strut giant cells with TAXUS stents at overlap than CYPHER stents at 28 and 90 days in association with incomplete endothelialization. Porcine coronary arteries endothelialize more rapidly than rabbit arteries and are more likely than rabbit arteries to exhibit prominent para-strut granuloma formation.

Vascular Remodeling
The CYPHER stents with severe and diffuse inflammatory response also showed extensive vascular remodeling as observed by wider EEL variability at 90 and 180 days. The area enclosed by the EEL in such severely inflamed CYPHER segments was more than double that of other coronary arteries, which received stents of the same diameter but showed only mild inflammatory activity. Although no significant change in lumen diameter by angiography was detected and no late aneurysm formation was observed, the long-term clinical sequelae of these remodeling changes cannot be determined from this study.

In the TAXUS stents at 30 days, but not at 90 or 180 days, strut malapposition at overlap was seen (up to 100 μm), often in association with small foci of fibrin insudation into the media inferred to be related to smooth muscle cell death. Medial smooth muscle cell death has been reported in the rabbit model to be associated with medial thinning.10 Medial smooth muscle cell death was more frequent with TAXUS than CYPHER stents at 28 and 90 days in association with small foci of fibrin insudation into the media inferred to be related to smooth muscle cell death. Medial smooth muscle cell death was more frequent with TAXUS than CYPHER stents at 28 and 90 days in association with small foci of fibrin insudation into the media inferred to be related to smooth muscle cell death. Medial smooth muscle cell death has been reported in the rabbit model to be associated with medial thinning.10

Arterial Intramural Inflammation in the Porcine Model: Is It Predictive of Events in Humans?
The porcine coronary artery model used in this study endothelializes much more rapidly than the human artery and produces greater neointima at 3 months and beyond in response to both TAXUS and CYPHER DES. These characteristics may limit its usefulness in predicting very late stent thrombosis in humans. However, the porcine coronary model produces the same pattern of granulomatous, eosinophil-rich inflammation seen in some humans after stent implantation. When the inflammation is sufficiently diffuse throughout the stented region, aneurysmal dilation, malapposition, and occlusive thrombosis may occur. Cook et al reported 13 cases (8 CYPHER and 5 TAXUS) of very late stent thrombosis (>1 year) after DES implantation in patients who underwent intravascular ultrasound and compared them with control patients who did not experience stent thrombosis. Patients with very late stent thrombosis had longer lesions, longer stents, more stents per lesion, and more overlapped stents. Additionally, the thrombosed vessels had significantly larger EEL area compared with nonthrombosed DES stents and more frequent incomplete stent apposition (77% versus 12%; P=0.001). Joner et al have reported several pathological mechanisms that may contribute to stent thrombosis, including some factors observed in this preclinical study such as strut malapposition and hypersensitivity reactions.

The increased fibrin scores with TAXUS compared with CYPHER in both the pig coronary artery and rabbit iliac artery models are also seen in humans. This is not associated with stent thrombosis in the pig, which is contrary to what is reported in humans. This may be related to the rapid neointimal coverage in the pig sequestering the fibrin, whereas in humans it may be associated with uncovered struts, which are observed more frequently in stents with late thrombosis.

Study Limitations
This study was limited to CYPHER and TAXUS DES and respective BMS of the same platforms and did not include any stents coated with polymer but no drug. Therefore, the polymer cannot be separated from the presence of drug in this study. CYPHER and TAXUS stents were not implanted in the same pigs to prevent potential conflicting drug interaction, but this did not allow equal distribution between DES types in the same animals. Because the stents were deployed in normal arteries in this study, the results may not be representative of human atherosclerotic disease.

Conclusions
We have observed in a noninjured common swine coronary artery model that TAXUS stents were associated with greater para-strut fibrin deposition and mild strut malapposition at 30 days. Although in the present study fibrin was sequestered in developing neointima and not exposed to flow, fibrin has been observed to be associated with uncovered struts in human autopsy cases at >30 days after implantation. CYPHER stents induced an evolving extensive granulomatous inflammatory reaction that was rich in macrophages, multinucleated giant cells, lymphocytes, and granulocytes, especially eosinophils, significantly more frequently than similarly implanted TAXUS or BMS control stents. This response was seen as early as 30 days after implantation but progressed in frequency and severity through 90 and 180 days. Although the detailed immunobiology of this reaction has not been well established in this study, a localized hypersensitivity/allergic reaction to ≥1 of the DES components may have contributed. Although a much higher prevalence of this inflammatory response was observed in the porcine model than has previously been reported in humans, the similarity in the type of inflammatory response is noteworthy. The present study serves to strengthen the correlation of histopathological changes in the pig with those observed in humans and the potential relationship of these changes to subsequent clinical events.

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References

CLINICAL PERSPECTIVE
The present study compares sirolimus (CYPHER) and paclitaxel (TAXUS) drug-eluting stents implanted in noninjured coronary arteries in domestic swine for 30, 90, and 180 days. There was a much higher prevalence of a diffusely severe inflammatory response with granuloma formation and numerous eosinophils in the CYPHER-stented vessels compared with TAXUS. This distinctive inflammatory pattern, present diffusely through the stented arterial segment and seen repeatedly in the porcine model, has been identified in a small proportion of human autopsy cases involving CYPHER stents in which the diffuse granulomatous inflammation was associated with aneurysmal dilation and thrombosis of the stented coronary artery but to date has been reported in just 1 TAXUS stent with only focal involvement of a few struts. In the porcine coronary artery model, both CYPHER and TAXUS stents were associated with greater para-strut fibrin deposition than their bare metal controls at 30 days, but TAXUS stents showed greater fibrin deposition than CYPHER stents at 30 days persisting through 180 days. As a result of strut deployment in normal arteries combined with rapid smooth muscle cell proliferation and endothelialization in the pig, fibrin deposits were sequestered with the developing neointima and not exposed to flow in both drug-eluting stents. This characteristic of the porcine model limits its translation to the clinical issue of delayed thrombosis with drug-eluting stents. The porcine model does produce the severe, granulomatous inflammatory response seen in humans, but the true prevalence of this response in humans is unknown at this time.
Comparison of Inflammatory Response After Implantation of Sirolimus- and Paclitaxel-Eluting Stents in Porcine Coronary Arteries

Gregory J. Wilson, Gaku Nakazawa, Robert S. Schwartz, Barbara Huibregtse, Bradley Poff, Thomas J. Herbst, Donald S. Baim and Renu Virmani

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SUPPLEMENTAL MATERIAL

Online Supplement

Additional Description of Statistical Methodology with Comments on the Results

All morphological and morphometric data were statistically analyzed on a segment-by-segment basis (proximal, overlap, distal) for each stent group (CYPHER, BxSONIC, TAXUS Liberte, and bare Liberte) at each time point (30, 90, 180 days) at Boston Scientific using SAS Version 8.02.

For all continuous parameters, consisting of intimal thickness, area stenosis and percentage of struts with malapposition presented in Table 1 of the paper, and percentage of struts with fibrin, fibrin score, injury score including granuloma and injury score without granuloma presented in Table 2 of the paper, statistical analysis was performed using two different models each of which addressed the potential correlation of observations for each parameter related to the implantation of stents in more than one artery per pig by grouping observations.

For the comparison of CYPHER with TAXUS drug eluting stents, different devices were implanted in different animals. No pig received both CYPHER and TAXUS stents. Therefore, a hierarchical model was used in which each animal is randomly chosen and nested within each treatment group (CYPHER and TAXUS stenting being the treatments) with each outcome measurement (continuously variable parameter) within each animal (ie for different coronary arteries receiving only CYPHER or TAXUS stents) treated as a replicate.

For the comparison of each drug eluting stent with its counterpart bare metal stent (ie CYPHER vs BxSONIC and TAXUS vs Liberte), different devices were implanted in the same animal. Thus, a mixed model was used in which each animal is set as a block with correlation of devices considered within the animal.

The p value results of these analyses were compared with the simpler analysis of continuous parameters by Wilcoxon exact test presented in Tables 1 and 2. For all 63 comparisons (27 in Table 1 and 36 in Table 2 of the paper, data not shown in this supplement) the conclusions regarding statistical significance (p<0.05) were identical (with the sole exception of injury score including granuloma between TAXUS and Liberte at 180 days of no practical importance) between the Wilcoxon exact test, which ignored correlation due to implantation of multiple stents in the same animal, and the hierarchical and mixed models which took correlation into account by grouping observations. Thus, what correlation did exist did not affect the inferential testing using a simpler test procedure, the Wilcoxon exact test, which did not take correlation into account. A limitation of the hierarchical and mixed models is that their use assumes normally distributed data. The use of the Wilcoxon exact test does not assume normally distributed data and, in fact, the data examined by Wilcoxon failed testing for normality for several of the 63 comparisons.
For the ordinal morphological parameters luminal thrombus, endothelialization, strut-tissue coverage, para-strut inflammation and medial smooth muscle cell loss, described in Online Table 1, the grades for these parameters were ranked and analyzed using for comparisons between CYPHER and TAXUS, CYPHER and BxSONIC and TAXUS and Liberte at each segment (proximal, overlap and distal) and each time point (30, 90, 180 days).

For granulomatous inflammation, the percentage of vessels with granulomas in each group at each time point was analyzed by Fisher’s exact test to compare CYPHER with TAXUS, CYPHER with BxSONIC and TAXUS with Liberte, as shown in Table 2.

CYPHER and TAXUS were compared for prevalence of circumferential granulomatous inflammation in all three (proximal, overlap and distal) sections and severe extension of inflammation into the adventitia in one or more of the three sections using Fisher’s exact test.

There were three sets of comparisons made: 1) between the two drug-eluting stents CYPHER versus TAXUS; 2) between CYPHER and its bare metal control BxSONIC; and 3) between TAXUS and its bare metal control Liberte. Each of these three comparisons has been treated as if independent of the other comparisons. The comparisons central to the study are between CYPHER and TAXUS and a good argument can be made for their independence. No animal received both CYPHER and TAXUS stents. No adjustments were made for multiplicity of comparisons which is a limitation of the analysis.

**Online Table 1. Morphologic Grading Scales**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal thrombus</td>
<td>0 = not present, 1 = &lt;5%, 2 = 5-50%, 3 = &gt;50% lumen area occupied by thrombus; size of any microthrombi estimated</td>
</tr>
<tr>
<td>Endothelialization</td>
<td>0 = &gt;90%, 1 = 75 – 90%, 2 = &lt;75% coverage of lumen circumference by flattened endothelial-like cells (judged in part by spacing of cell nuclei)</td>
</tr>
<tr>
<td>Strut tissue coverage</td>
<td>0 = no struts uncovered, 1 = one or more struts uncovered</td>
</tr>
<tr>
<td>Para-strut inflammation</td>
<td>0 = none, 1 = mild, 2 = moderate, 3 = severe; highest grade (≥1 strut) reported per section; inflammation located separately from struts (e.g., in the adventitia) noted separately Granulomatous inflammation was defined as consisting of localized collections of macrophages, giant cells, lymphocytes, and granulocytes, with or without numerous (&gt;10 cells per 40× objective field) eosinophils</td>
</tr>
<tr>
<td>Medial smooth muscle cell loss</td>
<td>0 = none, 1 = mild, (&lt;25% of medial area), 2 = moderate (25–50%), 3 = extensive (50–75%) and 4 = severe (75–100%)</td>
</tr>
</tbody>
</table>