Vascular Responses to Drug-Eluting and Bare Metal Stents in Diabetic/Hypercholesterolemic and Nonatherosclerotic Porcine Coronary Arteries

Raul Llano, MD; Dawn Winsor-Hines, BS; Dhavalkumar B. Patel, MD; Paul S. Seifert, PhD; Damir Hamamdzic, DVM, PhD; Gregory J. Wilson, MD; Hong Wang, MS; Martin G. Keane, MD; Barbara A. Huibregtse, DVM; Robert L. Wilensky, MD

Background—Animal models used to gain insight into the vascular response to drug-eluting stents are generally juvenile and nonatherosclerotic, whereas stents are placed in patients with complex atherosclerosis and comorbidities. Hence, models reflecting these complexities are needed to help elucidate the vascular effects of drug-eluting stents. We compared the vascular responses with bare metal stent (BMS) and paclitaxel-eluting stent (PES) implantation in a diabetic/hypercholesterolemic (DM/HC) porcine model of advanced coronary atherosclerosis with the standard juvenile porcine model.

Methods and Results—Two studies using similar stent procedural protocols were performed in either DM/HC (n = 20) or domestic swine (non-DM/HC, n = 20). Animals pretreated with dual-antiplatelet therapy, underwent BMS or PES implantation (1/artery, 2 stents per animal) and were euthanized 30 or 90 days later. DM/HC resulted in a 24% increase in platelet aggregation (P < 0.05 versus baseline), whereas dual-antiplatelet therapy reduced platelet aggregation in both groups (P < 0.0001). DM/HC pigs developed substantially greater neointimal area versus non-DM/HC pigs, regardless of stent type, (P = 0.004 for BMS at 30 days and P = 0.002 at 90 days, P = 0.005 for PES at 30 days, P = 0.002 at 90 days). Compared with non-DM/HC pigs, reendothelialization was delayed in DM/HC pigs, more so after PES implantation. Increased para-strut leukocytes were observed for PES compared with BMS in the DM/HC pigs at both 30 days (P = 0.023) and 90 days (P = 0.04). As well, increased T-lymphocyte infiltration was seen in the DM/HC pigs.

Conclusions—Stent implantation in a DM/HC swine model provides a metabolic environment closer to human disease, including hyperglycemia, hypercholesterolemia, and increased platelet aggregation. This model augmented differences in the vascular response between PES and BMS that are not as clearly evident in the non-DM/HC swine, including increased neointimal area, delayed reendothelialization, and greater, persistent vascular inflammation. (Circ Cardiovasc Interv. 2011;4:438-446.)

Key Words: animal models of human disease  ■  coronary stent  ■  diabetes mellitus  ■  paclitaxel-eluting stent  ■  restenosis  ■  vascular response

Preclinical evaluations of the vascular effects of stent implantation have been conducted in healthy nonatherosclerotic animals, generally rabbits and pigs. In these models, stent implantation induces a predictable neointimal reaction useful for understanding the response to the materials and design of the stent and delivery system while providing insight into potential safety issues.1,2 With the extended use of drug-eluting stents (DES) in patients with complex atherosclerosis and comorbidities such as diabetes mellitus (DM) and/or hypercholesterolemia (HC),3,4 animal models reflecting these complexities are needed to assess the possible vascular effects. The current study was designed to evaluate the differential vascular responses to stent implantation, either paclitaxel-eluting stents (PES) or bare metal stents (BMS), in a porcine model of complex coronary atherosclerosis5-7 compared with responses in a standard pig model.

Methods

Animal Models
The generated data were produced in 3 independent groups of animals: DM/HC intervention, non-DM/HC intervention, and DM/HC no intervention (Figure 1). All studies enrolled male Yorkshire

Received April 15, 2010; accepted July 14, 2011.
From the Cardiovascular Institute, University of Pennsylvania, Philadelphia, PA (R.L., D.B.P., D.H., M.G.K., R.L.W.); Boston Scientific Corporation, Natick, MA (D.W.-H., P.S.S., H.W., B.A.H.); and the Division of Pathology, Department of Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Ontario, Canada (G.J.W.).
Correspondence to Robert L. Wilensky, MD, Hospital of the University of Pennsylvania, 3400 Spruce St, 9 Gates, Philadelphia, PA 19104. E-mail robert.wilensky@uphs.upenn.edu

© 2011 American Heart Association, Inc.

Circ Cardiovasc Interv is available at http://circinterventions.ahajournals.org DOI: 10.1161/CIRCINTERVENTIONS.110.959957

438
WHAT IS KNOWN

- Virtually all preclinical studies evaluating the safety and potential efficacy of drug-eluting stents have used juvenile, nonatherosclerotic animals.
- Although the efficacy of drug-eluting stents in porcine coronary and rabbit iliac arteries have correlated positively with long-term clinical results, the effects of drug-eluting stents on reendothelialization and vascular inflammation have been underestimated, and the responses to ongoing metabolic conditions have not been fully investigated.

WHAT THE STUDY ADDS

- The use of a diabetic/hypercholesterolemic porcine model of atherosclerosis more clearly differentiates the variable vascular response to bare metal stent and drug-eluting stent implantation.
- Compared with control animals, diabetic/hypercholesterolemic pigs demonstrated greater neointimal area progression after both bare metal stent and drug-eluting stent implantation.
- Diabetic/hypercholesterolemic porcine coronary arteries demonstrated features more similar to the human response to drug-eluting stent implantation: delayed healing, chronic inflammation, and delayed reendothelialization.

Swine. The DM/HC no intervention group was used only for evaluation of the effects of DM/HC and dual-antiplatelet therapy (DAPT) on platelet aggregation over time. Results of the incidence, severity, and morphometric characteristics of coronary lesions of this group have been previously published. The 2 intervention studies used a similar protocol for stent implantation, and in both studies, a single stent was implanted in 1 coronary artery (see below). Twenty pigs were enrolled in the DM/HC intervention, 21 pigs in the non-DM/HC intervention, and insulin was administered when the serum glucose level was the study duration. Heparin was administered periprocedurally to achieve an activated clotting time 300 seconds. After angiography in both intervention studies, the animal was randomized to the time interval between stent implantation and euthanasia (ie, at 30 or 90 days after implantation) as well as to the stented artery. Randomization was performed, by blinded card selection, so that the arteries that were stented were equally distributed between groups. A single stent, either PES (Taxus Liberté) or BMS (Liberté, Boston Scientific Corp, Natick, MA), with a diameter slightly larger than the reference arterial diameter (1.2:1 balloon-to-artery ratio), was implanted in the proximal or midsection of 1 of the 3 epicardial vessels. A second stent (again either PES or BMS) was placed into 1 of the other epicardial vessels so that each animal had a stent placed in 2 separate arteries with each artery randomized to either PES or BMS. As a result, an animal could have a single PES in 2 arteries, a single BMS in both arteries, or a PES in 1 artery and a BMS in the other. The ultimate distribution of stents among the coronary arteries in the 30- and 90-day cohorts in DM/HC and non-DM/HC animals is presented in Table 1. The stents were deployed using a pressure of 12 to 16 atm for 30 seconds. Angiography was performed to determine late luminal loss using quantitative coronary angiography.

Evaluation of Platelet Aggregation

In DM/HC pigs, platelet aggregation was determined before induction of DM/HC, after 20 weeks of DM/HC (before initiation of DAPT), and after 3 days of DAPT. In the DM/HC intervention group, additional assessment was performed before stent implantation and at euthanasia, whereas in non-DM/HC pigs, platelet aggregation was determined before and after 3 days of DAPT (Figure 1). Samples were obtained from the jugular vein, anticoagulated with 3.9% sodium citrate (blood:citrate ratio, 9:1), and diluted 1:1 with preservative-free saline. Aggregation was induced using 2 μg/mL collagen. All tests were performed in duplicate within the 30 minutes of blood sampling, using a Whole Blood Impedance Aggregometer with the extent of platelet activity quantified in Ohms, using AGGRO/LINK software (Chrono-log Corp, Havertown, PA).

Tissue Collection and Evaluation

Histoprocessing was performed at the same facility (Boston Scientific Corporation, Plymouth, MN), and all histological evaluations were performed, in a blinded fashion, using the same scoring system. After cineangiography, the heart was rapidly removed and the coronary arteries were perfused at physiological pressure with saline and thereafter with 10% neutral buffered formalin. The heart underwent high-resolution radiography to identify the location of the stent. Twelve arteries (3 BMS and 3 DES DM/HC, 3 BMS and 3 DES non-DM/HC) underwent scanning electron microscopy (SEM) with the remainder undergoing histological evaluation.

For SEM analysis, arteries were cut lengthwise and the luminal surface was inspected. Specific evaluation for the presence of attached thrombus, endothelial coverage, and strut coverage was performed by an investigator blinded to time and group, using the following scoring system: endothelialization: 0 = >90% of luminal surface covered, 1 = 75% to 90% of the surface covered, and 2 = <75% of luminal surface covered; luminal thrombus: 0 = no thrombus, 1 = thrombus occupies ≤5% of surface, 2 = 5% to 50% of surface, and 3 = >50%; adherent leucocytes: 0 = none, 1 = few, and 2 = numerous. For histological evaluation, each artery was embedded in SPURR plastic and cut into 5 sections (proximal nonstented, proximal stented, midstented, distal stented, and distal nonstented). All sections were stained with hematoxylin and eosin and Comori elastin trichrome. Neointimal area and thickness was measured as previously published. Histological samples were evaluated for the presence of para-strut leukocyte infiltration, internal and external elastic lamina disruption, medial smooth muscle cell loss, and para-strut amorphous material (PAM), using a previously published grading system: para-strut leukocytes: 0 = none present, 1 = mild inflammatory response, 2 = moderate, and 3 = severe; internal elastic lamina and external elastic lamina disruption: 0 = no disruption, 1 = ≤25% disrupted, 2 = 26% to 50% disrupted, 3 = 51% to 75% disrupted, and 4 = >75% disrupted; medial cell loss: 0 = no cell loss, 1 = ≤25% loss of cell density, 2 = 26% to 50% loss, 3 = 51% to 75% loss, and 4 = >75% loss; and PAM: 0 = not present, 1 = mild deposition, 2 = moderate deposition, and 3 = extensive deposition.
Immunohistochemical staining for the presence of CD3+ T-lymphocytes was performed on 3 arteries/stent type/model/time point. Briefly, sections were deplasticized, and a rabbit polyclonal anti-CD3 antibody (Biocare Medical, Concord CA) was incubated with the tissue sections. The antibody binding was detected with UltraMap anti-rabbit alkaline phosphatase (Ventana Medical, Tucson AZ) and ChromoMap Red (Ventana Medical, Tucson AZ).

**Statistical Analysis**

The 2 interventional studies were not designed for time-dependent analysis, and each animal was measured at the 30- or 90-day time point, but not at both. Statistical analysis was performed using SPSS statistical software (SPSS, Chicago, IL). Data are summarized as mean±SD. The paired t test was used to compare the same subject for platelet aggregation over time. The issues of potential correlation among stents implanted into different coronary arteries in the same pig and the measures based on multiple observations in the same stented coronary artery (from 3 histological sections per stented segment) between control and DM/HC were addressed through generalized estimating equations methodology, which takes the correlated histological data and repeated measurements in the same pigs into consideration. Statistical significance for all tests was set at the 0.05 level.

**Results**

The baseline fasting serum glucose level for all pigs averaged 56.5±17.7 mg/dL (DM/HC intervention: 60.2±19.8; DM/HC no intervention: 52.7±14.8) and the mean serum cholesterol level 92.3±16.7 mg/dL (DM/HC intervention: 99.5±14.7; DM/HC no intervention: 85.0±16.0). In non-
DM/HC pigs, glucose and cholesterol levels remained constant, whereas in DM/HC pigs, the mean serum glucose level ranged from 242±102 to 391±108 mg/dL and the mean serum cholesterol level ranged from 400±88 to 563±150 mg/dL (Figure 2). The weight of DM/HC intervention pigs before induction averaged 27.8±4.0 kg. When euthanized at 30 days, the animals weighed 58.8±13.1 kg (6 months after DM/HC induction) and at 90 days, 78.4±18.7 kg (8 months after induction). The non-DM/HC animals weighed, on average, at the time of stent implantation, 41.1±3.5 kg, with 30-day animals weighing 50.6±2.2 kg at death and 90-day animals, at death, weighing 71.2±4.8 kg. Fourteen of the 20 DM/HC intervention pigs required insulin supplementation at least once.

Twenty-two animals successfully achieved the DM/HC state. One animal died during stent implantation as the result of ventricular fibrillation, and a second animal, with persistently elevated platelet aggregation, was found dead in its cage 5 days after stent implantation. Histopathology revealed no occlusive luminal arterial thrombus, although a small myocardial infarction was observed, thought to be secondary to a periprocedural or early postprocedural ischemic event. The remaining 20 animals completed the study without complications (30 days: n=9; 90 days: n=11). All 20 non-DM/HC animals completed the study. Animals that died prematurely were excluded from all analyses.

Table 1. Anatomic Location of Stents in DM/HC and Non-DM/HC Porcine Coronary Arteries

<table>
<thead>
<tr>
<th></th>
<th>RCA</th>
<th>LAD</th>
<th>LCx</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM/HC animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PES</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>BMS</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>90 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PES</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>BMS</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Non-DM/HC animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PES</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>BMS</td>
<td>2</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>90 d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PES</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>BMS</td>
<td>2</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

DM/HC indicates diabetic/hypercholesterolemic; RCA, right coronary artery; LAD, left anterior descending coronary artery; LCx, left circumflex artery; PES, paclitaxel-eluting stent; and BMS, bare metal stent.

Table 2. Whole Blood Platelet Aggregation Over Time

<table>
<thead>
<tr>
<th></th>
<th>DM/HC, No Intervention</th>
<th>DM/HC Intervention</th>
<th>Non-DM/HC Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>11.3±4.8</td>
<td>11.2±4.6</td>
<td>10.2±4.1</td>
</tr>
<tr>
<td>1 mo DM/HC</td>
<td>14.5±4.2</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>3 mo DM/HC</td>
<td>15.4±4.0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>5 mo DM/HC</td>
<td>14.6±4.6</td>
<td>13.6±3.8</td>
<td>...</td>
</tr>
<tr>
<td>After DAPT</td>
<td>...</td>
<td>6.8±4.0 (30 d)</td>
<td>5.4±2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.6±3.0 (90 d)</td>
<td></td>
</tr>
<tr>
<td>After procedure</td>
<td>30 d</td>
<td>7.4±3.8</td>
<td>X</td>
</tr>
<tr>
<td>90 d</td>
<td>9.9±4.4</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

DM/HC indicates diabetic/hypercholesterolemic; DAPT, dual antiplatelet therapy.

In DM/HC animals (n=21) not undergoing an intervention and not treated with DAPT, a significant and persistent increase in platelet aggregation was noted, compared with data obtained before induction of DM/HC, that is, baseline (1 month, P=0.002; 3 months, P=0.0004; 5 months, P=0.0001). For DM/HC animals undergoing intervention, DM/HC increased platelet aggregation (P=0.005) and 3 days of DAPT significantly decreased aggregation (P<0.0001). At the 30- and 90-day time points, an increase in aggregation compared with the post-DAPT (preintervention) time point was noted (30 days, P=0.0031 versus post-DAPT). In non-DM/HC animals undergoing intervention, the DAPT significantly reduced platelet aggregation by 47% (P=0.00001). Values are mean Ohms ±SD.

Platelet Aggregation

At baseline, platelet aggregation was slightly higher in animals that remained non-DM/HC (Table 2). DM/HC resulted in a 24% increase in platelet aggregation at 20 weeks compared with before DM/HC induction (P=0.05, Table 1). The DM/HC pigs that did not undergo stent implantation and were not treated with DAPT exhibited a similar 27% increase in platelet aggregation 1 month after DM/HC induction (P=0.002), with consistently elevated levels over a 5-month follow-up period (P=0.0004, P=0.0001 at 3 and 5 months, respectively, all compared with baseline). Three days of DAPT significantly reduced platelet aggregation in DM/HC intervention pigs by 57% (P<0.0001), although there was a subsequent increase noted 90 days after stent implantation (P=0.0031 versus post-DAPT). In the non-DM/HC pigs, DAPT reduced aggregation by 47% (P=0.00001).

Figure 2. Mean blood glucose (A) and cholesterol concentration (B). No statistically significant differences were observed between animals randomized to the 2 time points.
Differences in Vascular Response to Stent Implantation in DM/HC and Non-DM/HC Pigs

In non-DM/HC pigs, endothelialization assessed by SEM was complete by 30 days in both BMS and PES arteries (Figure 3). In DM/HC pigs, on the other hand, reendothelialization was delayed. Of the 3 BMS DM/HC arteries, 2 exhibited complete endothelial coverage and 1 exhibited 75% endothelial coverage at 30 days, and all BMS arteries had complete coverage at 90 days. In PES DM/HC arteries, 2 exhibited 75% to 90% endothelial coverage at both the 30- and 90-day time points and the other artery demonstrated complete reendothelialization. Complete strut coverage in non-DM/HC arteries was observed at 30 and 90 days. In PES DM/HC arteries, several struts were uncovered in 1 artery at 30 days with full strut coverage at 90 days. In non-DM/HC and DM/HC pigs, no luminal thrombus or microthrombi were present in any arterial section.

Because stent implantation can affect the severity and composition of the underlying atherosclerotic lesion, we evaluated the reference sections for the presence and severity of atherosclerosis in adjacent proximal and distal nonstented sections. Histological evaluation at the time of euthanasia demonstrated that the severity and composition of atherosclerotic plaque was similar in DM/HC vessels randomized to PES and BMS. By histology, 55% of the DM/HC sections, at the time of euthanasia, had atheroma with mean percent stenosis of 12.0±5.5% in animals euthanized at 30 days and 32.8±23.7% at 90 days. The corresponding mean lesion area was 0.85±0.45 mm² at 30 days and 2.30±2.45 mm² at 90 days. All vessels in the non-DM/HC model were without plaque.

Angiographic late lumen loss was statistically similar after PES and BMS at both time points in both models, although it was considerably greater in DM/HC animals (Figure 4). Neointimal area was correspondingly similar in both PES and BMS arteries at each time point in both models; however, neointimal area was significantly greater in the DM/HC arteries, regardless of stent type (Tables 3 and 4 and Figure 5).

Greater numbers of para-strut leukocytes were observed in the DM/HC PES-implanted arteries compared with DM/HC BMS at both 30 and 90 days (P=0.02, P=0.04, respectively); by SEM, none of the BMS placed in DM/HC arteries exhibited adherent leukocytes at either time point, whereas all 3 PES exhibited adherent leukocytes (all grade 1) at both time points. In non-DM/HC swine, there was a moderately increased presence of T-cells in PES arteries at 30 and 90 days, whereas none were observed after BMS implantation (Figure 6). After PES implantation, T-cells were noted in all 3 arterial layers at 30 days, and at 90 days there was a fairly extensive presence of T-cells in the tunica media and adventitia. In DM/HC pigs, T-cells were noted after BMS implantation at 30 days, whereas a greater number of T-cells were noted at 90 days.
days, especially after PES implantation. PES versus BMS was associated with greater internal elastic lamina disruption at 30 days ($P=0.023$, Table 3) and greater external elastic lamina disruption at 90 days ($P=0.0035$, Table 4) in DM/HC pigs. Medial SMC loss was greater in the PES groups in both models at both 30 days ($P=0.007$ for non-DM/HC and $P=0.028$ for DM/HC pigs) and 90 days ($P=0.004$ for non-DM/HC and $P=0.001$ for DM/HC pigs). PAM was also greater with PES than BMS in both models at 30 and 90 days (Tables 3 and 4). The effects on medial smooth muscle cell loss and PAM are expected with PES.8

Discussion
This study was designed to evaluate the vascular effects of stent implantation in a diseased model of complex coronary atherosclerosis and increased platelet aggregation and compare those effects with those observed in the standard porcine model. As such, this is the first preclinical study to directly compare the response to DES and BMS in both diseased and nondiseased coronary artery models. The results in DM/HC arteries demonstrated greater neointimal development after both stent types, delayed reendothelialization, and a more prolonged and pronounced pattern of vascular inflammation. Although the effects of PES implantation compared with BMS were observed in non-DM/HC animals, the diseased model allowed greater discrimination of these effects.

The DM/HC porcine model has several features that make it attractive as a model for assessing the safety and effect of intracoronary therapeutic devices. Rabbits, commonly used for studies evaluating the vascular effects of DES implantation,2,11 are herbivores and do not naturally develop atherosclerosis. The rabbit’s size limits stent placement to the aortic, iliac, or femoral arteries, which have different anatomic and flow properties compared with coronary arteries. A high cholesterol diet is often initiated resulting in cholesterol levels that exceed 1000 mg/dL, and the resulting lesions are largely foam cell rich.12 The standard porcine model uses juvenile pigs without atherosclerosis or other comorbidities. In contradistinction, the DM/HC pig develops coronary lesions with a morphology and physiology similar to human disease. A relatively high percentage of lesions are classified as pathological, with 89% of animals having an advanced lesion 9 months after DM/HC induction.6 Lesion development and progression is dependent in large part on vascular inflammation associated with increased expression of numerous proinflammatory genes, many crucial for macrophage and T-lymphocyte recruitment and functioning and subsequent upregulation of inflammatory mediators.6,7,13,14 Furthermore, DM and HC synergistically result in hypoaclitization of the Akt pathway, resulting in increased cellular proliferation, apoptosis, vasa vasorum neovascularization, inflammation, and advanced atherosclerosis.13 Such lipid-rich atheroma, in the setting of increased numbers of macrophages and T-lymphocytes, reflect increased oxidative stress that mimics human diabetic lesions.15–17

We have demonstrated increased platelet aggregation in DM/HC pigs, a finding also observed in diabetic patients.18 Previous studies have shown that platelets obtained from nonatherosclerotic juvenile pigs demonstrate aggregation to

Table 3. Histological Results at 30 Days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Pigs</th>
<th>DM/HC Pigs</th>
<th>Control Versus DM/HC Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMS</td>
<td>PES</td>
<td>BMS</td>
</tr>
<tr>
<td>Neointimal area, mm²</td>
<td>0.70±0.22</td>
<td>0.78±0.15</td>
<td>2.65±1.24</td>
</tr>
<tr>
<td>Neointimal thickness, mm</td>
<td>0.15±0.02</td>
<td>0.16±0.01</td>
<td>0.33±0.15</td>
</tr>
<tr>
<td>Para-strut leucocytes</td>
<td>1.05±0.12</td>
<td>1.00±0.00</td>
<td>0.0±0.00</td>
</tr>
<tr>
<td>Internal elastic lamina disruption</td>
<td>0.24±0.44</td>
<td>0.24±0.44</td>
<td>0.0±0.00</td>
</tr>
<tr>
<td>External elastic lamina disruption</td>
<td>0.0±0.00</td>
<td>0.10±0.30</td>
<td>0.0±0.00</td>
</tr>
<tr>
<td>Medial smooth muscle cell loss</td>
<td>0.76±0.44</td>
<td>2.10±0.54 ($P=0.007$)</td>
<td>0.0±0.00</td>
</tr>
<tr>
<td>Para-strut amorphous material</td>
<td>0.1±0.30</td>
<td>1.76±0.44 ($P=0.006$)</td>
<td>0.0±0.00</td>
</tr>
</tbody>
</table>

DM/HC indicates diabetic/hypercholesterolemic; PES, paclitaxel-eluting stent; and BMS, bare metal stent.

Values are shown as mean score ± SD, except for neointimal area (mm² ± SD) and neointimal thickness (mm ± SD). For comparisons within each animal model, only $P$ values <0.1 are presented.
collagen similar to that in humans.19,20 The current study builds on this data by demonstrating persistent, increased aggregation to collagen in the setting of DM/HC (Table 1). Also, previous preclinical studies in healthy porcine coronary arteries have reported no significant delay in reendothelialization after DES implantation.8–10 However, reendothelialization occurs rapidly in porcine arteries,21 and the current study demonstrated that unlike arteries in nonatherosclerotic pigs, DM/HC coronary arteries respond with delayed endothelial coverage after PES implantation. Delayed reendothelialization after BMS implantation has also been demonstrated in the LDL receptor–deficient pig model, a model that develops atherosclerotic lesions similar to the DM/HC pig model.22 In the current study, DAPT in the setting of increased platelet aggregation and delayed reendothelialization inhibited development of thrombi in the DM/HC group.

Observations regarding stent performance in a diseased model, regardless of the presence or absence of differences, provide novel information that can inform decisions regarding model choice to address specific questions. For example, one key difference noted was the response to BMS implantation over time. In the non-DM/HC swine, the angiographic late loss and neointimal thickness decreased over time, whereas in DM/HC arteries they increased, suggesting a different healing process. This finding supports data obtained by Tellez et al.,22 in which both parameters decreased between 30 and 90 days after BMS implantation in nondiseased arteries while remaining relatively stable in LDL receptor–deficient porcine arteries. Therefore, the healing response is not equivalent between the diseased and nondiseased models because continued atherosclerotic plaque development was evident in DM/HC pigs at 90 days but not in non-DM/HC swine. Indeed, there are greater similarities between the DM/HC porcine and human responses compared with the nondiseased model, as the DM/HC model demonstrates those features which characterize, and may contribute to the long-term untoward effects of DES implantation in humans, namely delayed healing, chronic inflammation, and delayed reendothelialization.23,24 Such diseased models should be used to evaluate potential safety issues.

Of interest was the lack of difference in morphometric neointimal accumulation after BMS and PES implantation in either model, whereas efficacy of PES has been demonstrated in humans.25 However, in nonatherosclerotic porcine models, neointimal accumulation is similar at the 90-day time point in sirolimus-eluting stents and BMS.26 In this regard, it is important to recall that preclinical animal studies showing a significant reduction in neointimal area at 30 days led to

---

**Table 4. Histological Results at 90 Days**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Pigs</th>
<th>DM/HC Pigs</th>
<th>Control Versus DM/HC Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neointimal area, mm²</td>
<td>BMS</td>
<td>PES</td>
<td>BMS</td>
</tr>
<tr>
<td></td>
<td>0.65±0.17</td>
<td>1.26±0.66</td>
<td>4.04±1.95</td>
</tr>
<tr>
<td>Neointimal thickness, mm</td>
<td>0.14±0.01</td>
<td>0.27±0.15</td>
<td>0.45±0.18</td>
</tr>
<tr>
<td>Para-strut leucocytes</td>
<td>1.0±0.0</td>
<td>1.62±0.74</td>
<td>1.00±0.89</td>
</tr>
<tr>
<td>Internal elastic lamina disruption</td>
<td>0.48±0.51</td>
<td>0.90±1.48</td>
<td>0.86±0.79</td>
</tr>
<tr>
<td>External elastic lamina disruption</td>
<td>0.0±0.0</td>
<td>0.52±1.21</td>
<td>0.19±0.40</td>
</tr>
<tr>
<td>Medial smooth muscle cell loss</td>
<td>0.95±0.22</td>
<td>3.62±0.50</td>
<td>0.62±1.02</td>
</tr>
<tr>
<td>Para-strut amorphous material</td>
<td>0.0±0.0</td>
<td>1.29±1.10</td>
<td>0±0</td>
</tr>
</tbody>
</table>

DM/HC indicates diabetic/hypercholesterolemic; PES, paclitaxel-eluting stent; and BMS, bare metal stent.

---

**Figure 5.** Representative histological cross sections of arteries with bare metal stents (BMS) and paclitaxel-eluting stents (PES) over time. Movat staining, magnification ×2.5. AP indicates atherosclerotic plaque; NI, neointima.
clinical trials that demonstrated efficacy of DES and subsequently to their widespread clinical use. Evaluation of efficacy may be best performed in juvenile, nondiseased porcine coronary arteries at the 30-day time point, as preclinical results have positively correlated with clinical results.

In DM/HC arteries, after implantation of PES, a greater and persistent inflammatory response was observed compared with arteries receiving the BMS. Although inflammatory responses were observed in non-DM/HC arteries as well, the effect was less evident. It has long been recognized that the persistence of both biodegradable and nonbiodegradable polymers within the arterial wall is associated with inflammation. More recently a persistent inflammatory state characterized by the presence of macrophages, lymphocytes, and eosinophils has been observed in patients who died of late stent thrombosis. Implantation of sirolimus-eluting stents has been shown to be associated with increased T-cell infiltrates, findings not observed after BMS implantation. This reaction has been postulated as a local hypersensitivity reaction to the nonerodable polymers.

This study has several potential limitations. The DM/HC porcine model, designed to mimic human disease, produces lesions that are variable in morphology and severity and represents an accelerated model of atherosclerosis that develops more rapidly than human coronary atherosclerosis. DM/HC arteries undergo vascular remodeling so that the atherosclerotic lesion cannot be detected by coronary angiography. Hence, some of the vascular segments were not atherosclerotic at the time of stent implantation. Indeed, given the variability in lesion development, it is possible that a greater number of DM/HC lesions of a particular composition were randomized to one particular stent type than to the other, and therefore precise comparisons between vascular responses to the stent type cannot be made. The observed changes in the DM/HC arteries may have resulted either from the stent or intervened lesion type. Intravascular ultrasonography before stent implantation would assist in determining the presence and composition of underlying lesions, although it is associated with increased risk of ischemia and dissection. Finally, given the importance of diabetic-induced inflammation in the production of advanced lesions, the model may be more relevant to diabetic than nondiabetic atherosclerosis. These limitations, however, do not invalidate the current findings because this study was designed to compare the differential response of diseased versus nondiseased arteries with stent implantation, and therefore the observed differences between the DM/HC and standard models remain valid.

In summary, this study demonstrates the utility of a diseased porcine model of atherosclerosis in differentiating the variable vascular response to BMS and DES implantation. Differences between BMS and PES were augmented in multiple parameters, including the delay in endothelial cell coverage, inflammatory response, and greater neointimal area. Although such changes were noted in non DM/HC arteries, the differences were less stark. Inherent differences in the models, specifically the increased and persistent inflammation in the DM/HC model, support its use to evaluate next-generation DES, as potential differences between newer DES are becoming increasingly difficult to demonstrate. As such, the DM/HC porcine model represents a suitable model to evaluate the effects of DES on vascular architecture.

Potential Clinical Impact

Virtually all preclinical studies evaluating the safety and potential efficacy of DES have used juvenile, nonatherosclerotic animals. As a result, the potential clinical effects of DES on reendothelialization and vascular inflammation have been underestimated, and the responses in the presence of ongoing metabolic conditions have not been fully investigated. This study compared the effects of stent implantation in a diseased DM/HC pig model, which develops human-type lesions, and contrasted the results of such stent implantation to the effects in a standard domestic pig model (non-DM/HC). The results show a more clear distinction between the effects of PES and BMS implantation in DM/HC arteries than in non-DM/HC arteries. The effects of PES included delayed healing, persistent inflammation, and delayed reendothelialization, effects similar to those observed after implantation into humans. Use of such diseased models to evaluate effects of DES may better indicate potential clinical safety issues.

Acknowledgments

We gratefully acknowledge the assistance of Harilla Profka in the care of the animals and performance of the studies.

Sources of Funding

This study was supported by an unrestricted grant from Boston Scientific Corporation (PI: Dr Wilensky).
Disclosures
Drs Winsor-Hines, Seifert, Wang, and Huibregtse are employees of Boston Scientific Corporation.

References
Vascular Responses to Drug-Eluting and Bare Metal Stents in Diabetic/Hypercholesterolemic and Nonatherosclerotic Porcine Coronary Arteries
Raul Llano, Dawn Winsor-Hines, Dhavalkumar B. Patel, Paul S. Seifert, Damir Hamamdzic, Gregory J. Wilson, Hong Wang, Martin G. Keane, Barbara A. Huibregtse and Robert L. Wilensky

Circ Cardiovasc Interv. 2011;4:438-446; originally published online October 4, 2011;
doi: 10.1161/CIRCINTERVENTIONS.110.959957
-Circulation: Cardiovascular Interventions is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-7640. Online ISSN: 1941-7632

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circinterventions.ahajournals.org/content/4/5/438

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Interventions can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Interventions is online at:
http://circinterventions.ahajournals.org//subscriptions/