Inhibition of experimental neointimal hyperplasia and neoatherosclerosis by local, stent-mediated delivery of everolimus

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Introduction: A novel self-expanding, drug-eluting stent was designed to slowly release everolimus in order to prevent restenosis after percutaneous peripheral intervention. The purpose of this experimental animal study was to test the hypothesis that long-term local, stent-mediated delivery of everolimus would reduce neointimal hyperplasia in porcine iliac arteries.

Methods: The iliac arteries of 24 Yucatan mini-swine were percutaneously treated with overlapping 8- × 28-mm self-expanding nitinol stents loaded with everolimus (225 µg/cm² stent surface area) formulated in a poly(ethylene-co-vinyl alcohol) copolymer intended to deliver the drug in a sustained fashion over about 6 months (drug-eluting stent [DES]). Bare nitinol self-expanding stents (bare metal stent [BMS]) were implanted in an identical fashion on the contralateral side to serve as controls. After 3, 6, or 12 months, the animals were sacrificed and the stented arteries perfusion-fixed for histomorphometric analysis.

Results: The chronic presence of everolimus in arterial tissue reduced stent-induced inflammation after 3 months (inflammation score BMS 2.29 ± 0.44 vs DES 0.17 ± 0.17; P = .001) and 6 months (BMS 2.06 ± 0.43 vs DES 0.50 ± 0.5; P = .007), although some late inflammation was observed after drug exhaustion (BMS 1.00 ± 0.25 vs DES 2.56 ± 0.62 after 12 months; P = not significant [NS]). Treatment with locally delivered everolimus significantly reduced neointimal hyperplasia after 3 months (neointimal thickness BMS 0.79 ± 0.20 vs DES 0.37 ± 0.04 mm; P = .03) and 6 months (BMS 0.73 ± 0.14 vs DES 0.41 ± 0.08 mm; P = .05), although the effect had dissipated after 12 months (BMS 0.68 ± 0.11 vs DES 0.67 ± 0.11 mm; P = NS). Remarkably, stent-induced neoatherosclerosis, characterized by the histologic presence of foamy macrophages and cholesterol clefts, was significantly attenuated by treatment with everolimus (atherogenic change scores at 3 months BMS 0.56 ± 0.15 vs DES 0.04 ± 0.04; P = .003; 6 months BMS 0.84 ± 0.23 vs DES 0.00 ± 0.00; P = .004; and 12 months BMS 0.09 ± 0.10 vs DES 0.19 ± 0.19; P = NS).

Conclusions: In this experimental animal model, local arterial stent-mediated delivery of everolimus inhibited the formation of neointimal hyperplasia and neoatherosclerosis during the first 6 months. The effect was transient, however, as arterial morphology and histology appeared similar to control stented arteries after 12 months. (J Vasc Surg 2012;**.[missing]**)

Clinical Relevance: Atherosclerotic vascular disease and its sequelae remain the single greatest killers in the world. Significant advances have been made in the development of endovascular techniques for both coronary and peripheral interventions. Although almost always successful technically, 30% to 50% of stent-based coronary and peripheral interventions will fail during the first year. In the coronary arteries, this problem has largely been circumvented by the development of drug-eluting stents. To date, however, drug-eluting stents have been found to be only marginally helpful in the peripheral circulation. The purpose of this experimental animal study was to test the hypothesis that long-term local, stent-mediated delivery of everolimus would reduce neointimal hyperplasia in porcine peripheral arteries.

Atherosclerosis remains the leading cause of death and disability worldwide. The clinical events generated by atherosclerotic plaques, including myocardial infarction, stroke, renal failure, and amputation are responsible for 17 million deaths per year.1 Given its prevalence and impact, the prevention and treatment of atherosclerosis has been a major focus of the medical community for decades. Proven therapeutic strategies are comprised of three basic categories: lifestyle modification (control of body weight, habitual physical activity, and avoidance of tobacco products), systemic drug therapy (antihypertension, lipid-lowering, platelet inhibition, and/or plaque stabilization), and invasive mechanical recanalization.

The invasive treatment of established atherosclerotic lesions comprises the single largest category of major operations in the world, including over 6 million surgical and/or interventional procedures performed annually. Open surgical procedures designed to remove or exclude plaques have been performed since the 1950s and some operations, such as coronary artery bypass grafting, periph-
eral vascular bypass grafting, and carotid endarterectomy, are ubiquitous. More recently, catheter-based therapies, including percutaneous transluminal angioplasty and stenting have been developed. These minimally invasive techniques can convert plaque-laden, occlusive coronary, peripheral and cerebrovascular segments to widely patent flow channels without the need for open surgery. Although almost always successful technically, 30% to 50% of stent-based coronary and peripheral interventions will fail during the first year due to the pathologic process of neointimal hyperplasia and restenosis. In the coronary arteries, this problem has largely been circumvented by the development of drug-eluting stents (DES), in which a relatively low dose of antiproliferative drug is applied to an intravascular stent for the purpose of inhibiting vascular smooth muscle cell proliferation. DES have also been found to be useful in the tibial arteries as well, showing similar reductions in restenosis and reintervention. In the superficial femoral artery (SFA), however, DES have been largely ineffectual, possibly due to (1) the larger plaque burden and complexity of the SFA, (2) the continual and unpredictable mechanical deformation of the SFA during sitting and walking, and (3) the tendency of the peripheral arteries toward profound neointimal hyperplasia in response to intervention. The purpose of the experimental study described herein was to test the hypothesis that a peripheral DES with a relatively high drug dose and prolonged elution profile would inhibit neointimal hyperplasia in a long-term porcine model of percutaneous peripheral intervention (PPI).

METHODS

Animal operations. Animal operations were conducted at Synecor (Durham, NC), a test facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care and registered with the United States Department of Agriculture to conduct research on laboratory animals. At least 1 day before the stent implant procedure, 325 mg aspirin and 75 mg clopidogrel (Plavix, Sanofi-Aventis, Bridgewater, NJ) were administered to each animal per os as a daily single dose and continued until study termination at the designated time points. After induction of anesthesia, an incision was made in the neck to expose the left carotid artery, an arterial sheath was introduced and advanced into the vessel, and, under fluoroscopy, a 0.035-inch guidewire was used to gain access to target arteries. Heparin (200 IU/kg) was administered intravenously to achieve an activated clotting time of ≥250 seconds. After baseline arterial dimensional measurements were obtained and recorded, DES and bare metal stent (BMS) self-expanding devices were deployed into optimally sized regions of the left and right iliac arteries.

All conditions of testing conformed to the Animal Welfare Act (CFR 9) and its amendments. The protocol was reviewed and approved by the Animal Care and Use Committee at the test facility for compliance with regulations before study initiation.

Intravascular devices. The two test devices used in this experiment were the Dynalink BMS (Abbott Laboratories, Abbott Park, Ill) and the Dynalink-E experimental DES (Abbott). The Dynalink self-expanding stent (BMS) is constructed of nitinol which is super-elastic at body temperature. The 0.008-inch strut thickness design is based on a series of sinusoidal rings that are connected at six locations around the circumference such that the connections are aligned along the length of the stent and positioned 60° from each other.

The Dynalink-E experimental self-expanding DES (Fig 1) is comprised of three components: (1) the Dynalink nitinol stent platform (described above), (2) a copolymer coating matrix of poly(ethylene-co-vinyl alcohol; EVAL Americas, Houston, Tex), and (3) the antiproliferative drug everolimus (Ceritan, Zortress, and Afinitor; Novartis Pharmaceuticals Corporation, Basel, Switzerland). EVAL is a semicrystalline polymer with a glass transition temperature of 55°C and melting point of 180°C. The chemical backbone is a C-C bond and the pendant group is -OH; neither contains hydrolytically or oxidatively labile chemical bonds. The antiproliferative drug, everolimus, is a therapeutic agent originally developed for the prevention of organ transplant rejection, but it is also effective at inhibiting the growth of certain solid tumors. It effectively inhibits experimental vascular smooth muscle cell proliferation, enhances vascular remodeling in animal models, and has been shown to be safe and effective as the drug component of coronary DES. The total drug load of Dynalink-E was 8-mm × 28-mm device contained approximately 1 mg of everolimus.

Pharmacokinetic evaluation. Pharmacokinetic studies were performed to assess everolimus distribution after a total of 64 Dynalink-E device implantations in 14 Yorkshire
Farm swine weighing 39 to 69 kg and 2 Yucatan Miniature swine (180-day time point) weighing 31 to 33 kg. Four devices were implanted in each animal, one each in the bilateral iliac and deep femoral arteries. At each of the time points (1, 3, 7, 14, 28, 56, 90, and 180 days, two animals per time point), whole blood samples were collected (n = 2) and stented arteries were harvested and separated into stent and tissue after death (n = 8). Unstented artery samples, approximately 5 mm in length, were harvested from the distal edge of the device (n = 8).

To assess arterial drug content long-term, 24 overlapped pairs of DES were implanted into the iliac arteries of 12 Yucatan Miniature swine, 10 to 16 months of age and ranging in weight from 44 to 54 kg. Two overlapped DES pairs were implanted in the bilateral iliac arteries of each animal and harvested at 180, 270, and 360 days (n = 8 stents in four animals per time point).

The drug remaining on the harvested stents was dissolved in 4 mL of N,N-dimethyl acetamide, extracted in water-dimethyl aceticamide (1:1) solution, and measured using high pressure liquid chromatography equipped with a 3.0- × 150-mm symmetry shield using a 5-μm particle size column maintained at 50°C with UV detection set at 277 nm. The isocratic mobile phase was composed of 50/50 (v/v) acetonitrile/water at 1.2 mL/minute. Everolimus content in whole blood and arterial tissue homogenate was assayed by liquid chromatography equipped with a SB-C18 (4.6- × 12.5-mm, 5-μm) cleanup column (ambient, 5 mL/minute), an Inertsil ODS-3 (2.1- × 50-mm, 5-μm; GL Sciences, Inc, Torrance, Calif) analytical column (0.4 mL/minute) with mass spectroscopic detection. The lower limit of quantification of the blood and arterial tissue assays was 0.0005 μg/mL and 0.0005 μg/g, respectively.

**Evaluation of long-term vascular response.** To evaluate the long-term vascular response after BMS or DES implantation, 24 overlapping pairs of 8-mm × 28-mm devices were implanted into the iliac arteries of 24 Yucatan Miniature swine 11 to 18 months of age and weighing 35 to 56 kg. Each animal underwent a single interventional procedure during which overlapped stent pairs of BMS or DES were implanted in the bilateral iliac arteries. Each animal received two overlapped BMS in one iliac artery and two overlapped DES in the other. The target stent oversizing ratio (nominal stent diameter-to-artery diameter ratio) was 1.1 to 1.4. Approximately one-third of each device was overlapped within the artery, and the right and left iliac arteries were randomly assigned to receive BMS and DES pairs.

Animals were sacrificed and stented arteries explanted at 3, 6, or 12 months after implantation (eight animals per time point). Stented arteries were excised, dehydrated in a graded series of ethanol, and embedded in methylmethacrylate resin. After polymerization, three 2- to 3-mm sections were sawed from each of the proximal, middle (overlapped), and distal arterial portions, ground to 40 to 50 μm, polished, and stained with toluidine blue and basic fuchsin.

**Histologic assessment** was performed using light microscopy by pathologists blinded to treatment assignment. Peri-strut inflammation was evaluated semiquantitatively using an ordinal scoring scale of 0 to 4 (0 = none to minimal, 1 = mild, 2 = moderate, 3 = marked, and 4 = granulomatous inflammation ≥25% of stent struts). Neointimal proliferation (new formation of intima) was also scored using an ordinal scale of 0 to 4 (0 = no neointimal changes, 1 = focal neointimal collections of foam cells, 2 = focal neointimal collections of foam cells with cholesterol deposition [necrotic core ≥5% of lesion area], 3 = necrotic core with hemorrhage, and 4 = rupture of necrotic core).

Histomorphometric analysis was performed using digital morphometry to measure neointimal thickness, cross-sectional areas, and area stenosis. Cross-sectional areas measured included lumen, internal elastic lamina (IEL), and external elastic lamina. Histomorphometric percent area stenosis was calculated as 100 × (IEL – lumen) + IEL.

**Statistical analysis.** Histologic and morphometric values for the three sections in a given arterial segment were averaged in order to report a single value. Data are presented as mean ± SD unless otherwise noted. The paired t-test (continuous variables) or Wilcoxon/Kruskal-Wallis rank-sum test were used to compare the treatment groups. Tests of significance were two-tailed and significance was established by a value of P ≤ .05.

**RESULTS**

**Implantation and stent sizing.** Devices were implanted in all animals without complication, with oversized ratios of ~1.3 for all groups (3-month group: BMS 1.34 ± 0.05, DES 1.34 ± 0.06; 6-month group: BMS 1.24 ± 0.06, DES 1.26 ± 0.09; and 12-month group: BMS 1.36 ± 0.04, DES 1.35 ± 0.04).

**Pharmacokinetics.** The release profile of everolimus from the Dynalink-E DES is shown in Fig 2. Approximately 50% of the drug was eluted by 14 days then continued at a slower rate over the next 3 months. By the end of 6 months, the drug was almost completely exhausted from the stent (96.9% ± 0.4%).

**Fig 2.** In vivo profile of everolimus release from the drug-polymer coating on the Dynalink-E nitinol self-expanding stent.
Everolimus concentrations in whole blood, stented arterial tissue, and adjacent unstented arterial segments are shown in Fig 3. Whole blood concentrations of everolimus were low and transient and, coupled with available human pharmacokinetic data, suggest that the device can be implanted without adverse systemic drug effects. Everolimus content in stented arteries was initially high (>10 μg/g tissue after implantation) and, although it could still be detected a full year after the procedure, this is atypical of coronary DES. Everolimus could also be detected in unstented adjacent arterial segments, although the amount of drug was one to two orders of magnitude less than in the stented segment.

Long-term vascular responses after BMS and DES implantation. All arteries and stents were patent at all time points; there were no instances of arterial or stent thrombosis in this study. Example histophotomicrographs of overlapped stented arteries harvested at various time points are shown in Fig 4. Histologic and histomorphometric data are given in the Table. There were no significant differences in stent area between the groups; this was expected as the BMS and DES stent platforms were mechanically identical. The Dynalink-E DES significantly inhibited neointimal hyperplasia at both 3 and 6 months, as evidenced by the statistically significant attenuated neointimal thickness (P ≤ .05). The effect was transient, however, as there was no difference in neointimal thickness 1 year after treatment. The decreased neointimal area in DES-treated arteries generated an increased lumen area as well as statistically significant reductions in percent area stenosis. Again, the differences observed at 3 and 6 months were not reproduced after 1 year.

The presence of everolimus attenuated the inflammatory response to stenting at 3 and 6 months. Similar to the morphometric observations, however, there were no statistically significant differences in inflammation score after 1 year; in fact, the DES score was nominally higher due to the occasional presence of granulomata, which were generally not observed after BMS implantation. Last, an unexpected finding of the study was that everolimus inhibited the formation of neoatherosclerosis after stenting (Fig 5). Compared to BMS, there were significant reductions in the atherogenic change scores for DES-treated arteries at both 3 months (BMS 0.56 ± 0.15 vs DES 0.042 ± 0.04; P = .003) and 6 months (BMS 0.84 ± 0.23 vs DES 0.00 ± 0.00; P = .004). Some late atherogenic changes were observed in DES-treated arteries after 12 months such that the differences between BMS and DES were no longer evident (BMS 0.094 ± 0.10 vs DES 0.19 ± 0.19; P = .927).

DISCUSSION

The impact of DES on the effectiveness of percutaneous coronary intervention has been profound. In large-scale clinical trials, coronary DES that elute either sirolimus, paclitaxel, or zotarolimus have been found to reduce restenosis by 70% to 90% as compared to BMS. Second-generation devices that resist thrombosis as well as restenosis quickly followed, and a recent meta-analysis encompassing more than 17,000 patients suggests that second-generation everolimus-eluting coronary stents can be implanted with composite rates of myocardial infarction, target lesion revascularization, and stent thrombosis of only 2.9%, 5.7%, and 0.7%, respectively. These newer-generation DES have been found to be efficacious in other vascular beds as well. For instance, in patients with critical limb ischemia (CLI) from tibial artery occlusive disease, a recent randomized trial comparing everolimus-eluting with bare metal stents revealed that DES significantly enhanced 1-year primary patency (85% vs 54%; P = .0001) and freedom from reintervention (95% vs 65%; P = .005).

Unfortunately, DESs have met with considerably less success in the larger and more complex SFA. For example, the SIROCCO trial (SIROlimus Coated Cordis SMART Nitinol Self-expandable Stent for the Treatment of Obstructive Superficial Femoral Artery Disease), which compared sirolimus-eluting vs bare metal nitinol self-expanding stents in SFA lesions of ~8 cm in length, showed no significant differences in any morphologic or clinical metric after 4 years. To date, the only successful trial of local stent-based drug therapy in the SFA is the Zilver PTX trial in which self-expanding nitinol Zilver stents (Cook Medical, Bloomington, Ind) were sprayed with paclitaxel and compared with nondrug stents in ~6 cm SFA lesions in patients with peripheral arterial disease. The results showed that the 61 patients who failed percutaneous transluminal angioplasty and were treated with “bailout” Zilver PTX stents enjoyed superior 1-year primary patency to the 59 patients treated with “bailout” bare Zilver stents (90% vs 73%; P = .009). However, the larger cohort of 246 patients with short-segment SFA lesions (mean 5.43 cm) exhibited a 1-year primary patency rate of only 83%, and preliminary reports suggest that this rate may fall to 75% by 2 years. Thus, the antirestenotic effect of DES in the SFA...
seems to be considerably less than that observed after percutaneous coronary intervention\(^3\) and represents only a modest improvement over traditional bare metal stenting.\(^{15,18-21}\) This is, perhaps, not surprising given that the Zilver PTX device is not a DES per se. Because Zilver PTX is created by direct application of paclitaxel without a polymer, the 300-\(\mu\)g/cm\(^2\) drug dose is immediately released upon deployment and not sustained over time.\(^{22}\)

In contrast, the device examined in the present preclinical study was designed as a “true” peripheral DES. The Dynalink-E nitinol, self-expanding DES is loaded with 225-\(\mu\)g everolimus per cm\(^2\) stent area. The drug is applied in conjunction with an EVAL polymer designed to slowly release the drug in a diffusion-controlled manner over several months.\(^{23}\) The experimental evidence suggests that this design goal was met; \(\sim 50\%\) of the drug is released by 2 weeks, \(\sim 80\%\) by 3 months, and \(\sim 100\%\) by 6 months. The relatively high drug dose and long elution rate ensure that the drug is durably delivered and sustained within the arterial target tissue. Indeed, everolimus could still be detected in experimental arterial tissue a full year after the index implantation procedure.

Histologic examination of porcine arteries treated with Dynalink-E in this experiment revealed that everolimus exerted the anticipated antiproliferative effect. Arteries treated with overlapped Dynalink-E stents exhibited statistically significant decreased inflammation, neointimal thickness, and percent area stenosis at both the 3- and 6-month time points. Historically, local antiproliferative effects of DES of this magnitude and duration have been notoriously difficult to demonstrate in animal models, which are primarily used to establish device safety rather than device efficacy. Indeed, most published preclinical studies of coronary DES demonstrate decreased neointima formation at 28 days, but the effect is usually transient and cannot be reproduced at longer time points.\(^7,24-26\) The sustained antiproliferative effect of Dynalink-E, lasting up to 6 months in this experiment, can probably be attributed to its exceptionally long elution profile.

Also demonstrated in this study is the novel observation that Dynalink-E DES attenuated the development of experimental arterial neoatherosclerosis. Neoatherosclerosis is a pathologic process characterized by the accelerated formation of new atherosclerotic lesions after intravascular stent implantation. Similar to de novo atherosclerosis, the lesions are typified by foamy macrophage infiltration and cholesterol cleft formation and, in the advanced stages, mature plaques with central necrosis and overlying thrombus.\(^{27}\) An analogous process has been described in human vein grafts in which autologous venous conduits transplanted into the arterial circulation are prone to atherosclerotic degeneration after several years.\(^{28}\)

The etiology of neoatherosclerosis is speculative, although endothelial dysfunction, ongoing inflammation, and chronic mismatch of mechanical compliance almost certainly play a role.\(^{27}\) Given that DES are generally thought to promote neoatherosclerosis,\(^{27}\) the antiatherosclerotic effect of everolimus in this model was an unexpected finding. However, everolimus has been found to exert profound antiproliferative, anti-inflammatory, and antineoplastic effects in a number of preclinical models and clinical disease states, including (1) attenuation of neointimal hyperplasia in preclinical models in which everolimus is delivered in culture;\(^{29}\) orally,\(^6,30\) or via DES;\(^7\) (2) reduction of restenosis after stenting for coronary or peripheral occlusive disease;\(^3,5\) (3) prevention of rejection after solid organ
transplantation, reduction of the severity and incidence of cardiac allograft vasculopathy, (5) suppression of a variety of solid tumors, including breast, colorectal, pancreatic, renal, lung, skin (melanoma), prostate, ovarian, hepatic, and brain (glioblastoma, astrocytoma), and, finally, (6) control of hematologic malignancies such as acute myelogenous and chronic lymphocytic leukemia. In the United States,everolimus has gained approval as adjunctive chemotherapy for advanced renal cell carcinoma, advanced pancreatic neuroendocrine tumors, and subependymal astrocytoma associated with tuberous sclerosis (Afinitor; Novartis). Interestingly, it has recently been suggested that everolimus, when formulated on intravascular biodegradable scaffolds, may actually slow the progression of underlying atherosclerotic plaques. The drug’s well-known inhibitory effect on macrophages has been cited as a possible mechanism, given that transformation of macrophages into lipid-foam cells is an important step in atherogenesis. In a similar fashion, it is not unreasonable to speculate that the related process of accelerated neoatherosclerosis might also be effectively treated with everolimus. In a parallel to most preclinical studies of DES that include longer time points, the favorable cellular effects of Dynalink-E in this experiment were transient. Statistically significant reductions in inflammation, neointimal hyperplasia, and neoatherosclerosis were observed 6 months after stenting; however, no such differences between DES and BMS were detected after 12 months. A similar phenomenon was observed in the clinical study of the Dynalink-E device. The SFA Treatment with Drug-Eluting Stents (STRIDES) trial was a prospective, nonrandomized, single-arm, multicenter clinical study designed to evaluate the safety and performance of Dynalink-E for the treatment of atherosclerotic peripheral arterial disease. One hundred four patients with severe symptomatic vascular disease were enrolled, including significant proportions of patients with CLI (17%), diabetes (39%), and single-vessel outflow (26%). The mean lesion length was 9.0 ± 4.3 cm. The results showed that, after 6 months, primary patency was an impressive 94% ± 2.3%. However, the apparent antiproliferative effect appeared to wane and, by 12 months, primary patency had decreased to a disappointing 68% ± 4.6%.

Table. Histologic results after overlapped BMS or DES implantation in the porcine iliac arteries

<table>
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<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
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<tr>
<td>Stent area, mm²</td>
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<tr>
<td>BMS</td>
<td>42.16 ± 2.25</td>
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<td>DES</td>
<td>39.53 ± 2.12</td>
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<tr>
<td>BMS</td>
<td>0.79 ± 0.20</td>
<td>0.73 ± 0.14</td>
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<tr>
<td>DES</td>
<td>0.27 ± 0.04</td>
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<td>P value</td>
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<td>Lumen area, mm²</td>
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<tr>
<td>BMS</td>
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<td>DES</td>
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<tr>
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<td>Struts with fibrin, %</td>
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<tr>
<td>BMS</td>
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<td>BMS</td>
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<td>BMS</td>
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<td>BMS</td>
<td>18.63 ± 1.25</td>
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<tr>
<td>P value</td>
<td>.188</td>
<td>.704</td>
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BMS, Bare metal stent; DES, drug-eluting stent; NA, not applicable.
Data are presented as means ± SEM; n = 8 for each group and time point.
Whether the loss of patency was due to inadequate drug delivery, too short of an elution profile, persistent chronic outward force of the self-expanding nitinol stent against the vessel wall, or inherent drug resistance of end-stage SFA lesions, it is clear that the development of effective drug-device combinations for the peripheral vasculature is considerably more challenging than for the coronary arteries.

In summary, sustained local arterial delivery of everolimus inhibited the formation of neointimal hyperplasia and neatherosclerosis in a preclinical model of PPI. It is hoped that the persistent problem of peripheral arterial restenosis can be successfully approached through the continued development of devices that facilitate sustained intravascular delivery of antiproliferative agents and that overcome the

Fig 5. Histologic examples of stent-induced neoatherosclerosis. a, Bare-metal stent (BMS) at 3 months. Note the presence of severe neoatherosclerosis, including cholesterol clefts visible as void spaces, surrounding foamy cell accumulation, and fat infiltration. b, BMS at 6 months. c, BMS at 12 months. d, Drug-eluting stents (DES) at 12 months. Sections were stained with toluidine blue basic fuchsin.
inherent problem of placing a permanent self-expanding implant in the dynamic arterial environment of the lower extremity.

**AUTHOR CONTRIBUTIONS**

Conception and design: HZ, AN, RV, LS
Analysis and interpretation: HZ, AN, RV, LS
Data collection: HZ, AN, RV, LS
Writing the article: HZ, AN, LS
Critical revision of the article: HZ, AN, RV, LS
Final approval of the article: HZ, AN, RV, LS
Statistical analysis: HZ, AN, RV, LS
Obtained funding: LS
Overall responsibility: LS

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