Rapamycin-coated expanded polytetrafluoroethylene bypass grafts exhibit decreased anastomotic neointimal hyperplasia in a porcine model

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Objective: **We tested the hypothesis that rapamycin coated onto, and eluted from, expanded polytetrafluoroethylene (ePTFE) grafts would diminish neointimal hyperplasia in a porcine model.**

Methods: **Rapamycin (also called sirolimus) was coated onto the luminal surface of 6-mm-internal-diameter thin-walled ePTFE grafts by using an adhesive polymer that allows timed release of the drug. An adhesive polymer that allows timed release of rapamycin from ePTFE was developed with commercially available chemicals and applied on 6-mm ePTFE grafts. Graft integrity was characterized by scanning electron microscopy, and rapamycin levels were quantified by using high-performance liquid chromatography. Twenty-two mongrel pigs were randomized into three groups: untreated ePTFE (n = 6), adhesive-only coated ePTFE (n = 6), or adhesive- and rapamycin-coated ePTFE (n = 10). End-to-side unilateral aortoiliac bypasses were performed by using 6-mm-internal-diameter ePTFE grafts and standardized anastomotic lengths. Unilateral end-to-side aortoiliac ePTFE grafts (6-mm internal diameter) were inserted by using polypropylene sutures, 6-0 proximally and 7-0 distally; all anastomoses were 12 mm long. All animals received aspirin (325 mg orally) daily. All animals were given oral aspirin (325 mg) daily beginning on the day before surgery. At 28 days, the animals were killed, and the grafts were explanted in continuity with the adjacent aortic cuff and the outflow iliac artery. Variables compared between groups included graft patency, distal anastomotic length and cross-sectional narrowing, and intimal thickness at the arterial-graft junction indexed to the adjacent graft thickness. Microscopic analysis was performed with hematoxylin and eosin and Masson trichrome stains on paraffin sections. A pathologist blinded to experimental groups graded sections for collagen deposition, neointima formation, inflammatory cellular infiltrates, medial necrosis, and aneurysmal degeneration.**

Results: **All animals survived until they were killed without clinical evidence of limb ischemia or graft infection. Preplanned *t* tests in the context of one-way analysis of variance showed no difference in outcome measures between the untreated ePTFE and adhesive-only coated ePTFE groups; therefore, they were combined in further comparisons with the adhesive- and rapamycin-coated ePTFE group. The Rapamycine eluting expanded polytetrafluoroethylene group had longer anastomoses (85.6% vs 60.6% of the initial anastomotic length maintained; $P < .0001$) and less cross-sectional narrowing in the outflow graft (16.2% vs 28.5%; $P = .0007$) when compared with the other two groups by using two-tailed Student *t* tests. There was no evidence of medial necrosis or aneurysmal degeneration. All patent grafts had complete endothelialization on hematoxylin and eosin sections. Rapamycin was detectable and quantifiable in the arterial wall at 28 days after implantation.**

Conclusions: **Rapamycin can be coated onto and eluted from ePTFE by using a nonionic polymer and a simple coating technique. At 4 weeks after implantation, the rapamycin-eluting ePTFE grafts demonstrate gross, pathologic, and morphometric features of diminished neointimal hyperplasia when compared with non–drug-eluting ePTFE. Four weeks after implantation in a porcine model, rapamycin-eluting ePTFE grafts demonstrated gross, pathologic, and morphometric features of diminished neointimal hyperplasia when compared with untreated and adhesive-only coated ePTFE grafts. (J Vasc Surg 2005;42:980-8.)**

Clinical Relevance: **Rapamycin-eluting ePTFE grafts decrease neointimal hyperplasia in a porcine model. Further studies are needed to evaluate whether patency will be improved. Rapamycin-eluting ePTFE grafts may allow the use of prosthetic grafts in situations in which autologous vein is unavailable and in which neointimal hyperplasia is pronounced, such as in small-diameter (<6-mm) vessels typical of infrapopliteal interventions.**

Although autologous saphenous vein is the best conduit for peripheral arterial reconstruction, 30% of patients do not have this option as a result of prior vein harvest, trauma, or phlebitis.[1] Synthetic grafts made of expanded polytetrafluoroethylene (ePTFE) have been used as substitutes but have low patencies in vessels with diameters less than 6 mm because of early thrombosis or late graft failure from neointimal hyperplasia.[2] Infrapopliteal ePTFE grafts have primary patency rates at 4 years as low as 12%.[3]

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Competition of interest: none.

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Restenosis after percutaneous transluminal angioplasty (PTA) is a multifactorial response to local injury involving elastic recoil, negative arterial remodeling, and neointimal formation. Stent technologies help to overcome elastic recoil and negative arterial remodeling associated with vessel injury, but there continues to be a 20% to 50% rate of restenosis because the continuing pressure exerted by stents against the vessel wall stimulates an increased arterial proliferative response.[4] One approach to combat neointimal hyperplasia uses the elution of drugs with antiproliferative properties at the site of vessel injury. Coronary stents that elute rapamycin at the site of angioplasty have reduced neointimal hyperplasia, as evidenced by a decreased incidence of major adverse coronary events and by a reduction in binary restenosis, defined as a greater than 50% diameter stenosis of the target lesion.[5-8] Stents have not performed as favorably in the infrainguinal circulation.

Expanded PTFE is able to withstand the biomechanical forces that are exerted on it in the peripheral circulation without the structural damage (such as fractures) that has been reported when stents are placed in the superficial femoral artery.[9] Nontextile ePTFE grafts are manufactured by an expansion process that transforms an initial full-density polytetrafluoroethylene (PTFE) matrix into a structure composed of PTFE nodes interconnected by fine fibrils, which allow tissue ingrowth. The resulting expanded tube contains approximately 15% pure PTFE and 85% air by volume. The PTFE polymer is, for the most part, chemically inert; moreover, the grafts exhibit little tendency to dilate, have a strong electronegative luminal charge, and are hydrophobic until wetted by body fluids.[10] Coating ePTFE must not change the handling characteristics of the prosthetic because poor healing, inflammation, and thrombosis may result. We hypothesized that rapamycin eluted from prosthetic grafts would decrease neointimal hyperplasia by reducing tissue ingrowth and preserving anastomotic diameter. Thus, we attempted to coat ePTFE with rapamycin to see whether, by local elution, anastomotic neointimal hyperplasia could be decreased without increasing thrombosis or delaying healing.

MATERIALS AND METHODS

Rapamycin (Supelco, Bellefonte, Pa) was coated onto ePTFE by using methodology developed at the University of Tennessee Health Sciences Center. The details of the coating technique have been submitted for an institutional patent. Precut segments of 6-mm thin-walled ePTFE grafts provided by Impra/Bard Peripheral Vascular (Tempe, Ariz) were coated with rapamycin for 1 cm at both ends on the luminal surface. The rapamycin was suspended in a mixture of methacrylates. The coating was 5 to 10 μm thick. The ultrastructure of the ePTFE was assessed by scanning electron microscopy. The rapamycin was eluted over a 30-day period. The concentration of rapamycin was 250 $\mu g/cm^2$ or 1 mg of rapamycin per bypass graft. Grafts were sterilized with ethylene oxide before implantation. In preliminary experiments, rapamycin-eluting grafts were sent for drug quantification both before and after ethylene oxide sterilization.

Preimplantation and postexplantation grafts, blood, and tissues were sent to the high-performance liquid chromatography (HPLC) Drug Monitoring Laboratory at the University of Texas Medical School at Houston for rapamycin level quantification. Analyses were performed with HPLC/UV assays developed by Dr Kimberly L. Napoli, the director of the laboratory.[11] Whole blood was collected on the first three postoperative days and at death to quantitate systemic exposure to rapamycin. To confirm elution of rapamycin from the ePTFE and deposition of drug in the native arterial wall, explanted grafts and adjacent iliac artery were snap-frozen in liquid nitrogen. The tissues were packaged on dry ice and sent for rapamycin quantification. The kinetics of elution were extrapolated by killing nine animals (n = 3 per time point) on postoperative days 7, 14, and 28. These animals were separate from the 22 animals used to evaluate changes in neointimal hyperplasia.

All animal care and procedures were performed in accordance with the guidelines of the University of Tennessee's Institutional Animal Care and Utilization Committee. The animal procedures and care complied with the *Guidelines for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 80-23, revised 1985). Twenty-two mongrel pigs (Nichols Hog Farm, Olive Branch, Mississippi) were housed in the animal care facility of the Department of Comparative Medicine at the University of Tennessee. All animals were male and weighed from 17 to 27 kg. Food and water were provided ad libitum. Animals were fed 325 mg of aspirin daily from the day before surgery until death. Before surgery, animals were given 1 g of cefazolin intravenously and cephalexin 500 mg by mouth twice a day for the first five postoperative days. Aortoiliac bypass grafts using 6-mm thin-walled ePTFE were performed under general anesthesia through a midline laparotomy. Anesthesia was induced with intramuscular Telazol reconstituted with xylazine and was maintained with 1% isoflurane (Rhone-Poulenc, Bristol, UK). Before arterial clamping, a bolus of heparin (110 U/kg) was administered intravenously, after which supplemental heparin (55 U/kg) was given at 30-minute intervals until the completion of surgery. The anastomoses were 12 mm long and had an end-to-side configuration. The aortic anastomoses were performed with 6-0 polypropylene, and the iliac anastomoses were performed with 7-0 polypropylene. The intervening native iliac artery was doubly ligated. The animals were divided into three groups: animals that received bypasses with uncoated ePTFE (U-eP; n = 6), animals that received bypasses with adhesive-coated ePTFE (A-eP; n = 6), and animals that received bypasses with rapamycin-eluting ePTFE (R-eP; n = 10). All animals were killed on postoperative day 28.

At death, the bypass grafts and adjacent iliac and aortic segments were removed in continuity. The iliac artery was opened longitudinally along the vessel wall opposite the anastomosis, pinned to in vivo dimensions, and placed in 10% formalin. Immediately after explantation, the length of

the distal anastomosis was measured (heel to toe), and the percentage of maintained anastomotic length was calculated (explant anastomotic length/12). Two surgeons not blinded to experimental groups measured the explanted anastomotic length and averaged their values for each animal. Specimens were not perfusion-fixed because samples were thin, usually 3 to 5 mm thick, which allowed for rapid formalin fixation. All specimens were collected and processed in a similar fashion. Absolute measurements were not evaluated. Variables examined relative proportions or ratios between groups. After a minimum of 24 hours of soaking in 10% formalin (Baxter Diagnostics, McGaw Park, Ill), the iliac segments were cross-sectioned at the heel and placed in cassettes. Paraffin processing was performed by pathology technicians from the Department of Pathology at the University of Tennessee Health Sciences Center. After paraffin embedding, two to three (5 μm each) sections were stained with hematoxylin and eosin (H&E) or Masson trichrome and used for morphometric analysis or pathologic grading.

Morphometric comparisons were made by using Image J (version 1.30). This software was downloaded from the National Institute of Health's Web site (http://rsb.info.nih.gov/ij/). Measurements were made from cross sections taken at the heel of the iliac anastomoses. Tissue blocks were generated from here because the hyperplastic response was most pronounced to the naked eye, and the sectioning resulted in a circular cross section of the ePTFE that allowed for consistency during pathologic grading and morphometric analysis. H&E-stained or Masson trichrome–stained paraffin sections were mounted on slides and viewed with the ×2 objective on a Zeiss microscope (Carl Zeiss Inc, Oberkochen, Germany). Fields were photographed with a Camedia D-540 ZOOM digital camera (Olympus, Melville, New York). The digital images were analyzed with Image J to determine differences in morphometric criteria of neointimal hyperplasia. The morphometry analysis was performed by a researcher blinded to experimental groups.

The percentage of cross-sectional narrowing attributed to neointimal hyperplasia and the degree of neointimal thickness at the arterial-graft junction indexed to ePTFE graft thickness were compared between groups of animals. The percentage of cross-sectional narrowing was calculated by dividing the neointimal area by the area bound by the inner table of the cross-sectioned ePTFE. The neointimal area was calculated by subtracting the luminal area from the area bound by the inner table of the cross-sectioned ePTFE:

$$\%\ \text{Cross-sectional narrowing} = \text{internal ePTFE area} - \text{luminal area/internal ePTFE area}$$

The intimal thickness index was calculated by dividing the thickness of neointima at the heel of the iliac anastomosis by the cross-sectional thickness of the ePTFE graft. The measurement was performed where the internal elastic lamina of the native artery was disrupted by the polypropylene suture used to perform the anastomosis. Measurements for percentage of cross-sectional narrowing and intimal thickness index are presented as mean ± SEM.

Table I. Comparison of gross pathologic and morphometric parameters (postoperative day 28) of neointimal hyperplasia between animals treated with uncoated (U-eP) and adhesive-coated expanded polytetrafluoroethylene (A-eP) and with rapamycin-eluting expanded polytetrafluoroethylene (R-eP)

Variable	*U-eP and A-eP (n = 12)*	*R-eP (n = 10)*	P *value*
% Initial anastomotic length	60.6% ± 2.3%	85.6% ± 2.5%	<.0001
% Cross-sectional narrowing	28.5% ± 2.7%	16.2% ± 3.0%	.007
Intimal thickness index	1.75 ± 0.13	1.22 ± 0.14	.01

Semiquantitative histologic grading of H&E and Masson trichrome sections was performed by a pathologist blinded to experimental groups (C.R.H.). Features examined included endothelialization, spindle cell ingrowth, and neointimal formation. Grading was performed as follows: 0, none of the luminal circumference of the graft involved; 1, less than 25% of the luminal circumference of the graft involved; 2, 25% to 75% of the luminal circumference of the graft involved; and 3, greater than 75% of the luminal circumference of the graft involved. Immunostains for α-actin and factor VIII–related antigen to identify smooth muscle cells and endothelial cells were not performed. The pathologist examined sections with the ×40 power objective and identified endothelial cells on the basis of surface location and flattened cellular morphology. Spindle cells were characterized with Masson trichrome as elongated cells with purple cytoplasm that populated regions of extracellular matrix (ECM). Ingrowth of spindle cells was used to correlate with vascular smooth muscle cell (VSMC) migration. The presence of spindle cells and ECM, which stains blue or pink with Masson trichrome, depending on collagen content, was used to designate areas of neointimal formation on the ePTFE inner surface.

Statistical analysis was performed with SAS 9.0 (SAS Institute Inc, Cary, NC) statistical software. First, preplanned contrasts in the context of one-way analysis of variance (that are equivalent to two-tailed *t* tests with the square root of the mean square error used as the pooled standard deviation) were used to demonstrate that there was no statistical difference between the U-eP and the A-eP groups. Next, the data were combined into one control group (n = 12). Finally, data from the R-eP group (n = 10) were then compared with data from the combination of the U-eP and A-eP groups (n = 12; Table I) by using two-tailed unpaired *t* tests with equal variances. Differences were considered significant at $P < .05$. Variables are presented as mean ± SEM.

Reproducibility of measurements was assessed for the percentage of cross-sectional narrowing, ePTFE graft thick-

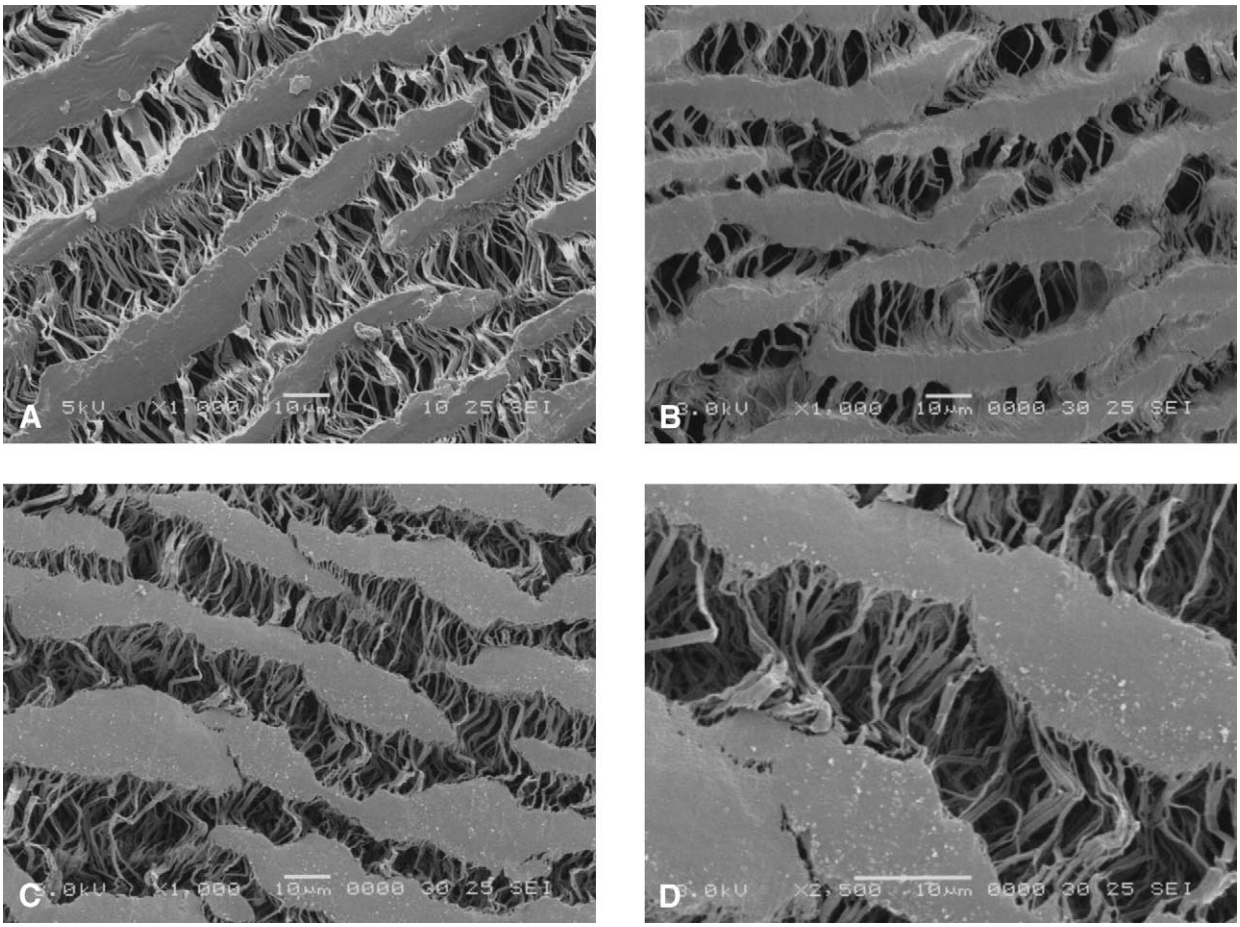

Fig 1. Scanning electron assessment of grafts. **A,** Uncoated expanded polytetrafluoroethylene (ePTFE; *U-eP;* original magnification, ×1000). **B,** Adhesive-coated ePTFE (*A-eP;* original magnification, ×1000). **C,** Rapamycin-eluting ePTFE (*R-eP;* original magnification, ×1000). **D,** R-eP (original magnification, ×2500).

ness, and ePTFE internal graft area. The pooled within-animal standard deviation for percentage of cross-sectional narrowing was 3.5%; the intraclass correlation coefficient was 0.84, indicating excellent reproducibility. For ePTFE graft thickness and area, the pooled within-animal standard deviations were 0.08 mm and 1.40 mm^2, respectively. For ePTFE graft thickness, the reproducibility was poor; however, the reproducibility of the ePTFE internal graft area was moderate, with an intraclass correlation coefficient of 0.71.

RESULTS

Examining sections of coated grafts with scanning electron microscopy, we found that the coating was adherent to the nodal islands of the ePTFE and did not obliterate the internodal fibrils (Fig 1). The scanning electron microscopy studies showed that rapamycin was encapsulated in the matrix of polymeric adhesives. Rapamycin appeared as speckling on nodal islands. The scanning electron microscopy studies did not show any other differences between rapamycin-coated grafts and those coated with adhesive alone. Handling characteristics of the graft material were maintained during suturing and implantation.

All animals survived until they were killed, with palpable pulses in the operated hindlimb and patent grafts. None of the animals developed wound or graft infections. Weight gain was similar. Aneurysmal degeneration was absent, and all grafts were well incorporated.

We compared animals that underwent bypass with uncoated ePTFE grafts with those that underwent bypass with adhesive-coated ePTFE to see whether the vehicle for the rapamycin was responsible for any changes in the degree of neointimal hyperplasia. There were no differences in the length of the iliac anastomosis, the percentage of cross-sectional narrowing, or the intimal thickness index between the two groups at explantation (Table II). The coating seemed inert and was not associated with a propensity for inflammation or thrombosis. The data for the two groups were then combined to form one control group (U-eP and A-eP) for comparison with the R-eP animals.

Table II. Comparison of gross pathologic and morphometric parameters (postoperative day 28) of neointimal hyperplasia between animals treated with uncoated (U-eP) and adhesive-coated expanded polytetrafluoroethylene (A-eP)

Variable	*U-eP (n = 6)*	*A-eP (n = 6)*	P *value*
% Initial anastomotic length	57.3% ± 3.2%	63.8% ± 3.2%	.17
% Cross-sectional narrowing	27.4% ± 4.0%	29.7% ± 4.0%	.68
Intimal thickness index	1.71 ± 0.20	1.79 ± 0.18	.78

Table III. Pathologic grading of anastomotic cross sections for neointimal hyperplasia

Variable	*U-eP and A-eP (n = 12)*	*R-eP (n = 10)*	P *value*
Neointimal formation	2.5 ± 0.2	0.8 ± 0.2	<.0001
Spindle cell ingrowth	2.3 ± 0.3	0.8 ± 0.2	.0007
Endothelialization	3.0 ± 0.0	3.0 ± 0.0	1

U-eP, Uncoated expanded polytetrafluoroethylene; *A-eP,* adhesive-coated expanded polytetrafluoroethylene; *R-eP,* rapamycin-eluting expanded polytetrafluoroethylene; *p = NS,* numerical score.

At death, animals treated with rapamycin-eluting ePTFE grafts had longer explanted iliac anastomoses (R-eP, 10.3 ± 0.26 mm; U-eP and A-eP, 7.3 ± 0.15 mm; $P < .0001$). In addition to having longer anastomoses (85.6% vs 60.6% of initial anastomotic length maintained; $P < .0001$), the R-eP animals had less cross-sectional narrowing in the outflow graft (16.2% vs 28.5%; $P = .0007$) and decreased intimal thickness indexed to ePTFE (Table I).

Complete endothelial coverage of ePTFE was noted in all groups. R-eP animals had less spindle cell ingrowth and neointimal formation. They received lower scores than U-eP and A-eP animals (Table III). Medial necrosis and aneurysmal degeneration were absent, and there were no differences in cellular infiltration between the experimental groups.

Preliminary experiments with waterbaths showed that the adhesive coating was subject to aqueous attack with dissolution over a 30-day period. Scanning electron microscopy samples of explanted anastomoses showed coverage of the internal surface of the ePTFE with cells and biologic debris. The adhesive coating could not be visualized, and comparisons of adhesive integrity and thickness between A-eP and R-eP samples could not be made. It was assumed that the neointima formed on ePTFE as dissolution of the adhesive polymeric matrix was occurring.

The ethylene oxide sterilization process did not cause loss of rapamycin before implantation. Similar drug levels were detected despite sterilization. Similarly, the rapamycin fraction was detected 18 minutes after instillation of the sample onto the HPLC column, irrespective of sterilization. Table IV shows rapamycin levels obtained from grafts, explanted arteries, and blood sampled at various time points. Preliminary HPLC experiments showed that the coating process resulted in loading of 1 mg of rapamycin per ePTFE graft before implantation. Results from 9 animals that were killed temporally to help determine elution kinetics showed that most rapamycin eluted off the grafts by 1 week. Levels decreased from 1000 μg before implantation to 26.7 μg at 1 week after explantation. Drug (2.9 μg/g) was detected in the adjacent arterial wall on postoperative day 7 and persisted until death. Despite being present in the arterial wall on postoperative day 28, rapamycin did not reach levels associated with systemic toxicity. Rapamycin was not detectable in blood after postoperative day 3.

DISCUSSION

Intimal hyperplasia is initiated by endothelial damage. Neointimal hyperplasia represents the response of VSMCs to physical, chemical, and humoral factors in regions of dysfunctional endothelial regulation. VSMCs are induced to migrate from the media to the intima, where they proliferate and deposit ECM.[12] Research on the development of neointimal hyperplasia has focused on the prevention of arterial restenosis after PTA and implantation of vascular grafts. The endothelium is disrupted at vascular anastomoses and at sites of PTA. Use of stents can prevent recoil and remodeling in treated arteries but does not eliminate neointimal hyperplasia. Struts from implanted stents incite an inflammatory response in the adjacent artery. This response perpetuates restenosis by initiating cytokine release from infiltrating cells. Vascular grafting with ePTFE also elicits neointimal hyperplasia through alterations in wall shear, flow, and compliance mismatch between the native artery and the prosthetic.[12-14]

Pharmacologic manipulation of VSMC migration and proliferation and ECM production is one approach in the treatment of restenosis after vascular intervention. Rapamycin is a macrocyclic, lipophilic lactone with immunosuppressive antibiotic activity derived from the actinomycete *Streptomyces hygroscopicus.* Rapamycin is approved by the US Food and Drug Administration for the prophylaxis of renal transplant rejection. Rapamycin has many properties that make it a good agent to counteract neointimal hyperplasia. Rapamycin binds to its cytosolic receptor FK506 binding protein (FKBP-12) and inhibits the mammalian target of rapamycin. The mammalian target of rapamycin is a ubiquitous signal transduction kinase that is responsible for cell-cycle progression. Mammalian target of rapamycin inactivation results in a reduction of cyclin-dependent kinases and increased levels of $p27^{kip1}$, a cyclin-dependent kinase inhibitor. The net effect is to cause G_1-S arrest in proliferating cells such as T cells and VSMCs.[15,16] In addition to inhibiting cellular proliferation, rapamycin inhibits the migration of VSMCs into areas of vascular injury.[17] In pigs, rapamycin needs to be present in the vessel wall for 14 days after injury to be effective.[5,18] The rate of neointimal proliferation in stented porcine coronary arteries is greatest

Table IV. Quantification of rapamycin drug levels in graft, artery, and blood

Variable	*Day 0*	*Day 1*	*Day 2*	*Day 3*	*Day 7*	*Day 14*	*Day 28*
Graft (μg) (n = 3)	1000	N/A	N/A	N/A	26.7 ± 1.4	12.8 ± 5.0	0.005 ± 0.001
Artery (μg/g) (n = 3)	0	N/A	N/A	N/A	2.9 ± 0.8	1.3 ± 0.2	0.025 ± 0.007
Blood (ng/mL) (n = 3)	16.9 ± 1.8	9.3 ± 0.2	2.0 ± 0.1	1.9 ± 0.2	0	0	0

N/A, Not applicable.

at 14 days after injury. The neointima at this point begins to become populated by VSMCs in a proteoglycan-rich matrix. To be effective, rapamycin needs to be present during the time when the stimulus for VSMC migration and proliferation exists. Hydrophobic drugs such as rapamycin may achieve higher mean tissue concentrations in the intima because they are less likely to diffuse back into the circulation, thus facilitating longer exposure in the area of injury.[19,20]

Drug elution has been used with excellent results in coronary interventions. Paclitaxel- and rapamycin-eluting stents significantly reduce the incidence of restenosis and late loss of arterial luminal diameter. Major adverse cardiac events such as myocardial infarction, death, and target lesion/vessel revascularization are also decreased with drug-eluting stents.[21,22] Patients treated with bare metal stents require more frequent coronary interventions. There is no difference in mortality or incidence of acute myocardial infarction, but studies to date have not included data to detect changes in these end points.[22] Review of the literature indicates that drug-eluting stents reduce event rates by 40% to 60% at 12 months.[22]

Drug-eluting stents have not performed as well in the infrainguinal circulation. Stenoses and occlusions are more common in the femoropopliteal region than in the coronary arteries. In addition, lesions here tend to be multiple, long, heavily calcified, and endophytic. Approximately 90% of the time, peripheral arteries can undergo successful angioplasty, but recurrence is common: restenosis occurs in up to 80% of cases after 1 year. Stenting femoropopliteal vessels after balloon angioplasty has not substantially improved patency. Nitinol stents may improve these results because they are less prone to external compression and elicit less neointimal hyperplasia than more rigid balloon-expandable stents.[23-26] Sirolimus-eluting (rapamycin-eluting), nitinol-expandable SMART (Cordis/Johnson & Johnson, Warren, New Jersey) stents in the peripheral circulation have been evaluated in two trials. These trials have the acronym SIROCCO, which stands for sirolimus-coated Cordis SMART nitinol self-expandable stent for the treatment of obstructive superficial femoral artery disease. SIROCCO I had promising early results, with 0% restenosis in the drug-eluting arm at 6 months; however, stent fractures were reported in 6 of 33 patients (3 in each treatment group).[27] The 18-month results were mixed, with the slow-eluting rapamycin stent having 0% restenosis but the fast-eluting stent having 33% restenosis. By 24 months, both slow- and fast-eluting coated stents failed to show a difference from uncoated stents and had a binary restenosis rate of 40%.[28] The SIROCCO II trial 18-month data also failed to show superiority, with a total in-stent binary restenosis rate of 20.7% for the rapamycin-eluting stent and 17.9% for the uncoated stent arm. Stents in the peripheral arteries of the lower extremities treat longer, more calcified lesions in arteries with relatively low flow rates. Stents in the periphery experience increased biomechanical forces including elongation, rotation, and radial compression as a result of the anatomy of the femoropopliteal vasculature. The attendant stent deformation may result in stent fractures and neointimal proliferation.[9] The greater propensity for neointimal hyperplasia may require higher levels of drug than can be eluted locally from coated stents. Also, the use of self-expanding stents presents new challenges for drug loading and delivery that do not pertain to coronary stent technology and that may limit the dose of available drug. Some might question the impetus to pursue improvement in peripheral circulation stenting in view of the relative success that has been achieved by open surgery. The 70% to 80% 5-year patency achieved with bypass surgery (vein and prosthetic) may be hard to surpass.[29]

Although metal alloy technology is optimized to decrease the propensity for stent deformation and fracture, improving the performance of prosthetic grafts is still warranted. Prosthetic grafts are not prone to structural damage and maintain excellent handling characteristics, but small-diameter (<6-mm) grafts are prone to thrombosis. Low shear and flow separation at prosthetic anastomoses cause the release of growth factors that result in VSMC proliferation.[12,14] Coating methods that exploit the hydrophobic nature of the graft and the electronegativity of the graft surface while avoiding denaturation of the pharmacologic agent used to modify neointimal hyperplasia are necessary so that the thromboresistance and biocompatibility of the ePTFE can be maintained.

In these experiments, the reduction in neointimal hyperplasia seen with the rapamycin-eluting ePTFE grafts was encouraging because the maintenance of anastomotic length and the decreased percentage of cross-sectional narrowing may translate to improved patency, especially in small-diameter ePTFE grafts, which are most prone to failure. Equally important is the fact that the coating process does not seem to influence thrombogenicity or alter arterial healing, as evidenced by complete endothelialization in all experimental groups. The results need to be evaluated at longer intervals, because despite apparent safety, the efficacy seen at 28 days may not persist. Carter et al[30] found that rapamycin-eluting stents inhibited intimal hyperplasia for 30 days; however, long-term inhibition was

not sustained, presumably because cellular proliferation occurred despite increased levels of $p27^{kip1}$. In their work, rapamycin remained present in the arterial wall (0.32 ng/mg) at 90 days; however, although increased levels of $p27^{kip1}$ were detected, there was also increased expression of proliferating cell nuclear antigen, and this raised the possibility that factors stimulating neointimal formation were not inhibited by rapamycin's effects on the cell cycle.[31-33] In the present experiment, rapamycin was deposited in the adjacent arterial wall on postoperative day 7 (2.9 ng/mg or 2.9 μg/g). At death, arterial levels of rapamycin decreased to 0.025 ng/mg. The continued suppression of neointimal hyperplasia despite a 10-fold decrease in drug concentration when compared with the study of Carter et al raises questions about the minimum doses and durations of exposure that are required for initiation and maintenance of rapamycin's therapeutic effect. It is not known what minimal tissue level of rapamycin needs to be present to achieve a measurable decrease in neointimal hyperplasia. The current therapeutic rapamycin level (8-17 ng/mL) is derived from the blood of patients receiving prophylaxis against kidney transplant rejection. Low tissue levels, as evidenced by a lack of rapamycin in the blood after postoperative day 3, make systemic toxicity unlikely.

Another cautionary note pertains to the extrapolation of data from a porcine model to humans. The response of peripheral porcine arteries to injury is not as well characterized and seems less vigorous than that in coronary arteries.[34] Humans and pigs exhibit differences in their responses to rapamycin. Preclinical studies showed a 50% reduction in neointimal formation at 30 days, and early human trials showed 80% to 90% inhibition at 6 months.[6] This discrepancy may be explained by differences in species arterial wall substrates. Human trials consisted of atherosclerotic vessels, which have more abundant FKBP-12 receptors, whereas porcine arteries were normal and had lower levels of FKBP-12.[30,35] Pigs may represent a tougher model for demonstrating decreases in neointimal hyperplasia with rapamycin because of their relative paucity of rapamycin receptors. Paclitaxel-eluting stents used in porcine models have also shown modest changes in neointimal hyperplasia. Preclinical porcine data for the TAXUS SR (Boston Scientific Corporation, Natick, Massachusetts) stent failed to show a reduction in neointimal hyperplasia at 28 to 180 days, whereas human clinical data showed reductions in restenosis at 9 months and a maintenance of effect up to 3 years after drug-eluting stent implantation.[36] Regardless of the limitations, porcine coronary arteries respond to injury by producing a neointima within 28 days that is similar to that in humans. The amount of neointima produced is proportional to the degree of injury.[37] Despite limitations in establishing efficacy, the porcine model is good for establishing safety of an intervention.[37]

In conclusion, the results of this study confirm the feasibility and safety of coating ePTFE with rapamycin. At 28 days, rapamycin-eluting ePTFE grafts demonstrate diminished gross, pathologic, and morphometric features of neointimal hyperplasia. These results are with early evaluation of neointimal hyperplasia after implantation and need to be assessed with longer follow-up to confirm maintenance of efficacy. The data from this study support the deposition of locally eluted rapamycin into the arterial wall and its persistence in the artery even after drug has been eluted from the prosthetic (Table IV). Pharmacologic inhibition of VSMC function by rapamycin needs to be maintained at least until endothelial coverage is achieved at anastomoses. Arterial injury causes endothelial dysfunction, VSMC proliferation and migration, phenotype alteration, and ECM deposition during the first 2 to 4 weeks in a porcine model. In humans, the period of arterial healing is longer, and rapamycin needs to be present and active in perianastomotic tissue at least until endothelialization is complete. Alterations in the kinetics of rapamycin elution may be required to allow longer exposure of rapamycin to vessels adjacent to treated anastomoses. Unlike metal stents, which experience fractures and subsequent neointimal formation when exposed to the biomechanical forces in the femoropopliteal circulation, rapamycin-eluting ePTFE grafts may prove superior because handling characteristics and biocompatibility are preserved. It remains to be seen whether decreased neointimal proliferation with rapamycin-eluting ePTFE grafts will translate into improved patency that will allow more frequent use of prosthetic grafts in situations in which autologous material is not available and neointimal hyperplasia is prevalent.

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DISCUSSION

Dr Randolph Geary (Winston-Salem, NC). Thank you. This was an interesting manuscript, and I enjoyed having the opportunity to look through it last night after the cigar reception. You are to be commended on a very nice presentation. I really did enjoy that.

Drug-eluting stents have clearly been one of the greatest advances in interventional cardiology to date, with extraordinarily low rates of restenosis even at late times in real-world patients with multiple risk factors for restenosis and complex lesions. Thus, it is only logical that this technology be applied to other vascular beds, such as the renal and superficial femoral artery and other vascular devices beyond stents. Thus, the group from Memphis has taken a logical next step by coating the ends of PTFE grafts with a polymer that elutes sirolimus, and they have shown reduced anastomotic intimal hyperplasia in a graft iliac anastomosis. The data appear sound, and they justify their conclusions in the context of a nonatherosclerotic animal model with normal arteries. To help put these data into clinical context, however, I have a few questions.

Do the authors feel that the aortoiliac model will accurately predict the biology below the inguinal ligament, where we have the greatest problem with maintaining graft patency? Clearly, the hemodynamics and the biology there are much different. With this in mind, a second question, then. In looking at the coated stent trials in the superior femoral artery, we see at late times that the restenosis rates are no better in sirolimus-eluting and in bare metal stents in the SIROCCO trial. This may be related in part to the duration of the drug elution or to a multitude of other factors, such as stent fractures. The coating that you used and the amount of drug that you used—was it similar to what we see with the drug-eluting stents? Or have you used a much higher dose, something that is not directly comparable to those clinical data from the superficial . . . (end of cassette) . . . graft anastomosis but the incorporation of the PTFE, which is so important in graft healing, as well on the outside of the graft. Did you notice any differences in the amount of inflammation either within the lesion or around the graft?

Again, I enjoyed your presentation and thank you very much for the opportunity to discuss the paper.

Dr Cagiannos. Thank you for your comments. I am actually going to start in reverse order.

With respect to inflammation, grafts were well incorporated, and, in fact, on visual examination at explantation, you couldn't really tell differences between rapamycin-eluting grafts and our uncoated grafts. We also did have our pathologists examine sec-

tions and count the number of cells per square millimeter. Those data are not presented, but there were no differences with respect to either the type of cell that infiltrated the graft and the tissue around the graft or the number of cells that infiltrated. These findings went against the anti-inflammatory nature of rapamycin.

We coated our grafts with 1 mg of rapamycin (sirolimus). The SIROCCO trial utilized SMART stents coated with 2.5 mg of rapamycin. The coronary stents are loaded with about 140 μg/cm^2 of rapamycin, giving a range of about 185 to 200 μg of sirolimus. Our grafts are in the mid range. We don't have as much sirolimus as the SIROCCO trial, and we don't have as little as the coronary stents. Some of the differences with respect to our graft are that we have a fairly rapid elution of rapamycin but still have maintenance of drug in the artery. I think some of the coronary literature shows that the concentration of rapamycin in the artery is more and that there is more sirolimus remaining on the stents.

With respect to the SIROCCO trial, I don't think they have done kinetics to see how much rapamycin is in the tissue or the grafts, obviously, because the grafts are still in the patients.

With respect to the validity of this model in the infrainguinal circulation, the greatest impetus for neointimal hyperplasia occurs in ePTFE grafts, where you are less than 6 mm. We used 6-mm grafts. To really test this model, one needs to use smaller grafts, about 4 mm, which can be done in pigs and can have a longer follow-up. I think the end-to-side anastomotic model is a valid model because it is normally performed in infrainguinal bypasses and elicits some of the shear stresses and compliance mismatches that are seen. This is a nonatherosclerotic model, but it does elicit neointimal hyperplasia in a relatively short period of time. Review of the literature shows that pigs may be more resistant to rapamycin because of a relative decrease in the cytosolic receptor, the FK506 binding protein receptor. If one sees changes in this, it may mean that there is the potential for, and in fact a greater chance of, seeing results with humans.

I hope that these comments answer your questions and again thank you for your comments.

Dr Scott Berceli (Gainesville, Fla). Two questions. First, you focused on the distal anastomosis. Did you look in the midgraft region, and were there differences? If there weren't, what are your thoughts on that? Is it related to an interplay with the hemodynamics that occur that may sort of affect the healing, or is it in some way related to the endothelialization that may occur at the distal anastomosis but not more in the center of the graft?

As for my second question, I think the _____'s lab over the last couple of years has shown that with the amount of intimal hyperplasia that develops in prosthetic grafts, as opposed to vein grafts, the healing occurs with a combination of both proliferation and significant apoptosis, which you do not see in vein grafts. Have you sort of looked to see whether the mechanism for decreased intimal hyperplasia is related to a difference in cell number, and if so, is it related to differences in proliferation or apoptosis?

Dr Cagiannos. We did do other studies that looked at our proximal anastomosis and also had sections through the mid graft. We showed decreased neointimal hyperplasia both at the proximal and distal anastomoses, in the midgraft morphometrically, and on gross visual inspection. Our grafts were 6 cm long.

With respect to differences in apoptosis, we did not perform terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling staining to examine for apoptosis. However, we did do some bromodeoxyuridine immunostaining and showed that there are decreased regions of bromodeoxyuridine uptake in the hyperplastic tissue. Interestingly, the number of cells that are actually proliferating per square millimeter is the same, but there were decreased areas of proliferation. At this point, it appears that we do not block cellular proliferation with rapamycin, but that we decrease regions in the neointimal tissue where it occurs. I hope those answer some of your questions.

Dr Mitchell Goldman (Knoxville, Tenn). You looked at the intimal hyperplasia of the graft, but did you look at the intimal hyperplasia of the vessel?

Dr Cagiannos. We did not do that directly. The equivalent measurement of that was looking at the maintenance of anastomotic length. When we took those anastomoses and sectioned them open, the anastomotic length is the result of the vessel growing inward, and I think that was our indirect measurement of vessel neointimal hyperplasia. We did not look at neointimal formation in the outflow iliac vessel morphometrically, but grossly, we did not appreciate the white overgrowth of tissue typical of this lesion in this location.

Dr Goldman. Because, in fact, when we look at graft failures, the neointimal hyperplasia that vexes us most is really just distal to the anastomosis and not in the graft.

Dr Cagiannos. That is a good point, and we do have sections that can still be examined. I think that will be something that can be done in the future.