Sirolimus-Eluting Stents to Abolish Intimal Hyperplasia and Improve Flow in Porcine Arteriovenous Grafts
A 4-Week Follow-Up Study

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Background—The patency of arteriovenous (AV) expanded polytetrafluoroethylene (ePTFE) hemodialysis grafts is severely compromised by intimal hyperplasia (IH) at the venous anastomosis and in the venous outflow tract. We addressed the potential of primary placement of a sirolimus-eluting stent (SES) in a validated porcine model.

Methods and Results—In 25 pigs, ePTFE AV grafts were created bilaterally between the carotid artery and the jugular vein, whereupon a self-expandable nitinol stent (14 SESs and 11 bare-metal stents) was implanted over the venous anastomosis in 1 of the 2 grafts. After exclusion of technical failures and 1 unilateral occlusion, 16 pigs (9 SESs and 7 bare-metal stents) were included for further analysis. After 28 days, we measured graft flow and performed quantitative angiography. The pigs were then euthanized, and grafts with adjacent vessels were excised for histological analysis. Minimal luminal diameter was substantially larger in the SES group compared with unstented controls (5.9 ± 0.2 versus 3.8 ± 0.4 mm, respectively, P < 0.01), which was accompanied by more prominent graft flow (SES, 1360 ± 89 mL/min versus unstented, 861 ± 83 mL/min, P = 0.05). IH at the venous anastomosis was 77% less in the SES group compared with unstented controls (0.44 ± 0.05 versus 1.92 ± 0.5 mm², respectively, P < 0.01), whereas IH increased markedly when bare-metal stents were used (5.7 ± 1.4 mm², P = 0.05).

Conclusions—SESs in the venous outflow of AV grafts significantly reduce IH and increase vessel diameter and graft flow compared with unstented grafts. These findings suggest that SESs have the potential to improve primary patency of AV grafts in hemodialysis patients. (Circulation. 2005;111:1537-1542.)

Key Words: transplants n sirolimus n stents n hyperplasia n renal dialysis

Hemodialysis access complications constitute a major cause of morbidity in hemodialysis patients. In the United States, approximately 60% of the 265,000 hemodialysis patients depend on an expanded polytetrafluoroethylene (ePTFE) graft for permanent vascular access.1 The failure of hemodialysis access grafts is predominantly because of progressive intimal hyperplasia at the venous anastomosis, resulting in a decline of graft flow, which ultimately gives rise to graft thrombosis.2 The current 1- and 2-year primary patency rates of ePTFE grafts are as low as 50% and 25%, respectively.3 The urgent need for new strategies to improve patency rates of arteriovenous (AV) grafts for hemodialysis is further emphasized by the ongoing global epidemic of diabetes,4 as a result of which a sharp increase in the incidence of end-stage renal diseases can be expected within the next few decades.

The introduction of sirolimus-eluting stents (SESs) has underscored the ability of local therapeutic strategies to prevent intimal hyperplasia (IH). Recently, the efficacy of these devices has been illustrated in coronary arteries, in which the in-stent restenosis rate remained low for up to 2 years after stenting.5,6 The intimal hyperplastic response in the venous outflow of AV grafts is characterized primarily by a local increase in vascular smooth muscle cell (VSMC) proliferation, comparable to the proliferative responses of in-stent restenosis in coronary arteries.7,8 Until now, there have been no reports with regard to the use of drug-eluting stents in the venous outflow of AV grafts. In the present study, we assessed the effects of SESs on luminal vessel diameter, graft flow, and intimal hyperplasia in the venous outflow tract of ePTFE AV grafts.

Methods

Study Design
Twenty-five female Landrace pigs weighing 52.2 ± 4.6 kg were studied. ePTFE AV grafts were created bilaterally between the
carotid artery and the internal jugular vein. In each pig, we placed a self-expandable endovascular nitinol stent (SMART, Cordis Corp) over the venous anastomosis in 1 of the 2 grafts at a randomly determined side. The contralateral AV graft served as a nonstented control. We used 14 SESs and 11 bare-metal stents. Directly after AV grafting, after stent placement, and before euthanasia, we quantified graft flow and determined the angiographic appearance of the grafts. All animals were euthanized on day 28 after graft placement, whereupon the grafts were excised for histological analysis. The study protocol was approved by the Ethical Committee on Animal Experimentation of the University Medical Center Utrecht, and animal care followed established guidelines. The cosponsors of the study (Cordis Corp) had no role in study design, performance of the experiments, data collection, data analysis, data interpretation, or writing of the report.

Anesthesia
Before operation and termination, the animals were fasted overnight and premedicated with intramuscular ketamine hydrochloride 10 mg/kg, midazolam 0.4 mg/kg, and atropine 0.5 mg and intravenous thiopental sodium 4 mg/kg. Subsequently, they were intubated and ventilated with a mixture of O2 and air (1:2). An ear vein was used for continuous administration of 0.3 mg · kg⁻¹ · h⁻¹ midazolam, 2.5 μg · kg⁻¹ · h⁻¹ sufentanil, and 50 μg · kg⁻¹ · h⁻¹ pancuronium.

AV Graft Implantation
The ePTFE AV grafts were created bilaterally between the carotid artery and the internal jugular vein as described previously. In short, intravenous heparin 5000 IU was administered before manipulation of the vessels. Papaverine 5 mg/mL was applied locally to prevent vascular spasm. Next, the carotid artery was clamped, and a standardized 8-mm arteriotomy was performed. An end-to-side anastomosis using the ePTFE graft was created at an angle of 45°. All ringed ePTFE grafts were 5 mm in diameter and 7 cm in length (W.L. Gore & Associates). The venous anastomosis was created in a similar manner.

Stent Placement
After completion of the AV grafts, 1 graft was selected randomly for stent placement. A 7F sheath was placed in both grafts, and angiograms of the venous outflow tract were obtained by contrast injection through the sheath. Subsequently, a self-expandable nitinol stent (SMART-stent, Cordis) was placed via the sheath over the venous anastomosis of the predetermined graft, extending both into the proximal jugular vein and into the ePTFE graft (Figure 1). After stenting, a second angiogram was obtained. All stents were 20 mm in length and had an unconstrained diameter of 6 mm, which was 1 mm oversized relative to the ePTFE graft and 2 mm oversized relative to the diameter of the jugular vein. The bare-metal stents were commercially available stents, and the SESs were custom-made, non–FDA-approved devices provided by the manufacturer, using the same stent platform as that of the bare-metal stents. Sirolimus (rapamycin, Wyeth Ayerst) is a natural macrolide immunosuppressant that inhibits cytokine- and growth factor–mediated proliferation of VSMCs. The stent coating had a thickness of 5 μm and was composed of an elastic copolymer combined with sirolimus in a 30:70 drug:copolymer weight ratio. The amount of drug per vessel area was 90 μg/cm².

Flow measurements were performed before and after stent placement, using a 4-mm perivascular flow probe (Transonic Systems). Graft flow was calculated as flow through the artery proximal to the arterial anastomosis minus the flow through the distal artery. All graft implantations and terminations were performed during the morning to minimize the potential effects of circadian variability in graft flow.

Antiplatelet Therapy
Starting 6 days before the operation, the pigs received acetylsalicylic acid 80 mg/d. Clopidogrel 225 mg was given 1 day before operation and continued at a dose of 75 mg/d until termination. After an unexpectedly high incidence of early bilateral graft thrombosis (50%) in the first 8 pigs, an intravenous bolus of 10 mg abciximab was added to the anticoagulant regimen.

Quantitative Angiography
Angiograms of the venous runoff were obtained directly after AV grafting, after stent placement, and before euthanasia (Philips, BV Pulsera). Vessel diameters were measured on unsubtracted digital images and calibrated to a steel marker 55 mm in length that was carefully positioned adjacent to the region of interest during imaging, whereby special care was taken to place the marker parallel to the image intensifier. Luminal diameters were taken at the toe of the venous anastomosis and 0.5 cm proximal to the toe of the venous anastomosis. The minimal luminal diameter along the stented venous segment, and the corresponding contralateral unstented segment was measured. Quantitative angiographic analysis was performed using Image J V.1.31 software (National Institutes of Health).

Tissue Preparation and Histological Analysis
On the 28th day of follow-up, pigs were anesthetized as described previously. Heparin 5000 IU was administered before manipulation of the vessels. After we determined graft flow using the perivascular flow probe, the ePTFE graft was cannulated and angiograms were obtained. The grafts and adjacent vessels were then perfused with saline for 3 minutes. Subsequently, the grafts were perfused with formalin at physiological pressure (100 mm Hg). After 2 minutes, both sides of the arteries and veins were ligated, allowing pressure fixation of the vessels. Subsequently, the pigs were euthanized, and the grafts and adjacent vessels were excised and immersed in formalin for 24 hours. Subsequently, the jugular veins with the adherent ePTFE grafts were embedded in methyl methacrylate (MMA) for further histological analysis. Sections were cut with a diamond-coated saw at the center of the venous anastomosis and 0.5 cm proximal to the toe of the venous anastomosis. For morphometric analysis, sections were stained with hematoxylin-eosin. With the highest magnification that allowed visualization of the entire vein section in one field, the intimal and medial areas and the area inside the external elastic lamina (total vessel volume) were traced manually.

Statistical Evaluation
Data are presented as mean±SEM. SPSS 11.0 was used for all statistical calculations. Comparisons between the paired groups (SES-stented veins versus unstented veins and bare-metal–stented veins versus unstented veins) were performed using the Wilcoxon test. A probability value of P<0.05 was considered significant.
Results
Fifty ePTFE grafts were implanted successfully in 25 pigs. The first 8 pigs were operated on without abciximab (4 SES versus unstented and 4 bare-metal stent versus unstented). In view of the high rate of early graft thrombosis (50%) of both the stented (2 SES and 2 bare-metal stent) and unstented sides, we added abciximab to minimize platelet adhesion after bilateral graft cannulation. Because thrombosis occurred not only on the stented side (either drug-eluting or bare-metal) but also on the unstented side, we concluded that the enhanced thrombotic occlusion rate of the grafts reflected early thrombosis after graft cannulation (for angiography and/or stent placement) rather than being stent- or intimal hyperplasia–related. Indeed, after having added 1 single dose of abciximab during the cannulation procedure, early graft thrombosis occurred in only 1 of 17 ensuing pigs. Notably, subanalysis of grafts obtained from pigs with or without abciximab showed no systematic difference between intimal areas, luminal diameters, and graft flows.

For final paired analysis of the AV graft parameters, 9 pigs (5 SES and 4 bare-metal stent) had to be excluded. Five pigs were excluded because of bilateral early thrombotic graft occlusion (ie, old thrombi restricted to the ePTFE grafts) and 3 pigs with bilateral graft infection (characterized by local abscess formation). These “technical failures” had to be excluded for further analysis, because reliable angiographic and morphometric analysis is impossible in these grafts. We also excluded 1 pig with a recent thrombotic occlusion at the side of a bare-metal–stented graft, because the latter precluded paired analysis of angiographic and morphometric measurements. Data from 16 pigs with bilateral patent grafts (9 SES-stented vein versus unstented vein and 7 bare-metal–stented vein versus unstented vein) were included for final analysis.

Quantitative Angiography
Directly after SES placement, the mean luminal diameter increased by 44% at the proximal jugular vein (SES-stented, 5.2±0.4 mm versus unstented, 3.6±0.5 mm, P=0.04). Bare-metal stenting resulted in a comparable increase in luminal diameter at the proximal vein (bare-metal–stented, 5.1±0.2 mm versus unstented, 3.2±0.2 mm, P=0.02). At the venous anastomosis, no significant differences in luminal diameter were observed (data not shown).

At termination, the luminal diameter of the SES segments was consistently larger in the SES group compared with unstented grafts, whereas diameters in bare-metal–stented segments no longer differed from those of the unstented group (Figure 2).

Flow Measurements
Stent placement over the venous anastomosis did not affect postprocedural graft flow (SES, 1124±117 mL/min; bare-metal stent, 1165±138 mL/min; and unstented, 1032±73 mL/min).

At termination, a significantly higher graft flow was observed in the SES-stented vein (1350±87 mL/min) compared with the unstented vein (861±83 mL/min, P=0.05). Graft flow in bare-metal–stented veins (925±124 mL/min)

did not differ significantly from unstented veins (750±97 mL/min, P=0.24).

Morphometric Analysis
At the venous anastomosis, a 77% reduction in intimal area was observed in SES-stented compared with unstented veins, whereas intimal area more than doubled in bare-metal–stented veins (Table). The medial area was not significantly different between groups. At the proximal vein (5 mm proximal to the toe of the anastomosis), a marked reduction in intimal area in the SES group was observed as well. Total vessel volume at the proximal vein was significantly larger in the SES group and the bare-metal–stented group compared with the unstented veins (Table). Representative sections from the venous anastomosis and the proximal vein are shown in Figure 3.

Discussion
In the present study, we show for the first time that SESs reduce intimal hyperplasia at the venous outflow tract of ePTFE grafts. Concomitantly, both luminal diameter at the outflow tract and graft flow increase significantly. Because luminal diameter and graft flow have been shown to be strong predictors for future AV graft survival, the present findings imply that primary stenting using SESs may result in prolongation of primary patency of hemodialysis AV grafts.

SESSs and Vascular Adaptation
The intimal hyperplastic response in the venous outflow tract of AV grafts is characterized predominantly by an increase in VSMC proliferation, comparable to the proliferative responses observed in the process of in-stent restenosis in coronary arteries. In coronary arteries, SESs have been shown to dramatically reduce in-stent restenosis in both animal15 and clinical16 studies. The present study is the first to demonstrate that SESs also suppress IH in veins at the outflow tract of AV grafts. The observed reduction in IH is most likely a result of the antiproliferative effect of sirolimus, which corresponds to the decreased expression of proliferat-
ing cellular nuclear antigen in porcine coronary arteries on SES placement.\textsuperscript{13} In addition, the antiinflammatory and antimigratory properties of sirolimus\textsuperscript{14,15} may have contributed to the inhibitory effect, because a foreign-body response to synthetic ePTFE graft also contributes to cytokine release and subsequent proliferation and migration of VSMCs.\textsuperscript{16,17}

In contrast to SESs, bare-metal stents were associated with enhanced IH formation in the venous outflow tract. These results are in line with previous animal studies showing a 2-fold increase in IH in bare-metal–stented compared with unstented vein grafts.\textsuperscript{18} The significant increase in IH in our bare-metal–stented group may be related in part to overstretch injury after the placement of an oversized stent, because vascular overstretching has been shown to promote IH.\textsuperscript{19}

Whereas only SESs were associated with an increased intraluminal diameter at termination, the dilatory effect of the self-expandable stent resulted in a larger total vessel volume in both SESs and bare-metal stents compared with unstented veins. The combination of reduced IH with an increased vessel volume after SES placement automatically translates into a larger intraluminal diameter, which is an important determinant for graft flow. Graft flow has a direct predictive value for graft patency, as illustrated by a pronounced increase in graft thrombosis if graft flow falls below 600 mL/min in hemodialysis patients.\textsuperscript{20} Hence, the 57% increase in graft flow in the SES group compared with unstented veins at termination may bear direct consequences for graft patency.

The coating of the SESs, consisting of a mixture of synthetic polymers and sirolimus, allows gradual drug release in a controlled concentration and at controlled times over a period of 30 days.\textsuperscript{21} In the coronary circulation, the release of sirolimus during the first month after stenting was effective in abolishing restenosis without signs of a “catch-up phenomenon” during a 2-year follow-up period.\textsuperscript{6} Evidently, effective protection against IH at an early phase is able to convey protection for a period exceeding the release of the stent-related drug release. In the case of AV grafting, the first few weeks constitute a particularly vulnerable period, in which the vascular response to altered hemodynamics, graft-vein compliance mismatch, and surgery-induced injury coincide with the arterialization process of the recipient vein.

The favorable characteristics of the AV-grafted, SES-containing veins after this most critical time frame might be expected to exert beneficial effects exceeding the period studied here, potentially translating into prolonged patency of SES-treated AV grafts. A long-term follow-up study needs to be performed to determine whether the beneficial effects of SESs exceed the period of the stent-related drug release.

**SESSs and Arterialization**

Similar to balloon angioplasty in (coronary) arteries,\textsuperscript{22} severe loss of medial VSMCs in the recipient vein is observed during the first few days after vein grafting.\textsuperscript{23} A subsequent increase in tunica media area is mandatory to withstand arterial pressure. Theoretically, this arterialization response may be hampered by sirolimus, because it requires proliferation of VSMCs. Furthermore, the effect of SESs on the recovery of the tunica media

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### Results of Stenting

<table>
<thead>
<tr>
<th>Summary of Morphometric Analysis</th>
<th>SES (n=9)</th>
<th>Unstented</th>
<th>Difference</th>
<th>P</th>
<th>Bare Stent (n=7)</th>
<th>Unstented</th>
<th>Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Center of venous anastomosis</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intimal area, mm$^2$</td>
<td>0.44±0.05</td>
<td>1.92±0.5</td>
<td>−77%</td>
<td>0.01</td>
<td>5.70±1.4</td>
<td>2.55±0.4</td>
<td>+224%</td>
<td>0.05</td>
</tr>
<tr>
<td>Medial area, mm$^2$</td>
<td>1.40±0.2</td>
<td>1.17±0.2</td>
<td>+20%</td>
<td>0.48</td>
<td>1.72±0.2</td>
<td>1.15±0.2</td>
<td>+50%</td>
<td>0.13</td>
</tr>
<tr>
<td>Intima/media ratio</td>
<td>0.37±0.2</td>
<td>1.55±0.3</td>
<td>−76%</td>
<td>0.01</td>
<td>3.41±0.7</td>
<td>2.62±0.7</td>
<td>+30%</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>5 mm proximal to venous anastomosis</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Intimal area, mm$^2$</td>
<td>0.28±0.1</td>
<td>0.93±0.2</td>
<td>−70%</td>
<td>0.01</td>
<td>4.30±1.3</td>
<td>0.68±0.2</td>
<td>+632%</td>
<td>0.02</td>
</tr>
<tr>
<td>Medial area, mm$^2$</td>
<td>2.26±0.4</td>
<td>1.72±0.3</td>
<td>+31%</td>
<td>0.17</td>
<td>2.30±0.2</td>
<td>2.07±0.3</td>
<td>+11%</td>
<td>0.61</td>
</tr>
<tr>
<td>Intima/media ratio</td>
<td>0.12±0.04</td>
<td>0.56±0.1</td>
<td>−79%</td>
<td>0.01</td>
<td>1.93±0.5</td>
<td>0.30±0.12</td>
<td>+643%</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Total vessel volume, mm$^2$</strong></td>
<td>33.19±2.7</td>
<td>24.10±5.5</td>
<td>+38%</td>
<td>0.05</td>
<td>33.33±3.0</td>
<td>20.13±3.9</td>
<td>+66%</td>
<td>0.04</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM. The difference between groups is expressed as a percentage. A positive difference signifies an increase in one of the experimental groups (SES grafts and bare-stented grafts, respectively) compared with unstented (control) grafts.

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![Figure 3. Representative sections of SES-stented (A and D), unstented (B and E), and bare-metal–stented (C and F) veins at anastomosis and at proximal vein. At both locations, SES-stented vein shows less intimal hyperplasia compared with unstented vein. By contrast, bare-metal–stented vein shows increased intimal hyperplasia. Hematoxylin-eosin staining. Magnification ×12.5.](http://circ.ahajournals.org/).
might be more pronounced in veins, because the venous vessel wall is much thinner than the arterial wall, resulting in higher local concentrations of sirolimus in the venous vessel wall. Notably, no significant differences in medial areas were observed between the 3 groups, indicating that, at least in the present AV graft vein model, sirolimus does not exert negative side effects on medial thickening.

**Early Graft Thrombosis**

In pigs, potent antiplatelet therapy is necessary to prevent early thrombogenic graft failure. In previous studies, administration of clopidogrel and acetylsalicylic acid was sufficient to prevent early graft thrombosis, resulting in 100% patency at 4 weeks after surgery. In the present study, graft puncturing strongly increased the risk of early graft thrombosis to 50%. This increase in early graft thrombosis is procedure-related rather than stent-related, because it occurred bilaterally in all cases. Interestingly, the addition of a single dose of 10 mg abciximab dramatically reduced early graft thrombosis to 7%. These results are in line with the reported efficacy of abciximab in preventing early thrombotic events on percutaneous coronary interventions after myocardial infarction. Administration of abciximab had no effect on vascular parameters other than early graft thrombosis. These results are in concordance with previous studies showing no effect of abciximab on IH per se.

**Study Limitations**

The validity of using animal vascular intervention studies as a model for the human situation has been a matter of debate, because the time course of the vascular healing response after stenting is much longer in humans compared with animals. Notwithstanding these apparent differences, pigs have been used frequently as a model to study cardiovascular diseases because of their analogous vascular anatomy, size, and physiology. In this respect, it is interesting to note that the reduction in late lumen loss and in-stent restenosis in human trials using SESs have thus far even exceeded the reported effects of SESs in short-term follow-up studies in pigs, supporting the relevance of our present findings for the human situation.

Several risk factors, including the uremic milieu and the hemodialysis procedure itself, may have a clear impact on vascular changes and IH formation. These additional risk factors have not been included in the present model. Therefore, one should be cautious in extrapolating these results to the human situation.

**Conclusions**

SESs but not bare-metal stents reduce intimal hyperplasia and improve graft flow and luminal diameter at the outflow tract of porcine AV grafts. These favorable changes at a 28-day follow-up period raise expectations with regard to the effects of SESs on long-term patency of AV grafts. A long-term follow-up study needs to be performed to determine whether the beneficial effects of SESs in AV grafts also result in an increased patency rate. Because AV graft stenosis occurs primarily within the first 2 to 3 cm of the venous outflow tract, the present findings indicate that primary stenting using SESs may be a valuable strategy to improve patency rates of AV grafts in hemodialysis patients.

**Acknowledgments**

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**References**


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