Neointimal Thickening After Stent Delivery of Paclitaxel: Change in Composition and Arrest of Growth Over Six Months

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OBJECTIVES

The purpose of this study was to determine long-term effects of stent-based paclitaxel delivery on amount, rate and composition of neointimal thickening after stent implantation.

BACKGROUND

Paclitaxel prevents vascular smooth muscle cell proliferation and migration in vitro and in vivo. These actions, coupled with low solubility, make it a viable candidate for modulating vascular responses to injury and prolonged effects after local delivery. We asked whether local delivery of paclitaxel for a period of weeks from a stent coated with a bioerodible polymer could produce a sustained reduction in neointimal hyperplasia for up to six months after stenting.

METHODS

Stainless steel stents were implanted in the iliac arteries of rabbits after endothelial denudation. Stents were uncoated or coated with a thin layer of poly(lactide-co-ε-caprolactone) copolymer alone or containing paclitaxel, 200 μg.

RESULTS

Paclitaxel release in vitro followed first-order kinetics for two months. Tissue responses were examined 7, 28, 56 or 180 days after implantation. Paclitaxel reduced intimomedial cell proliferation three-fold seven days after stenting and virtually eliminated later intimomedial thickening. Six months after stenting, long after drug release and polymer degradation were likely complete, neointimal area was two-fold lower in paclitaxel-releasing stents. Tissue responses in paclitaxel-treated vessels included incomplete healing, few smooth muscle cells, late persistence of macrophages and dense fibrin with little collagen.

CONCLUSIONS

Poly(lactide-co-ε-caprolactone) copolymer-coated stents permit sustained paclitaxel delivery in a manner that virtually abolishes neointimal hyperplasia for months after stent implantation, long after likely completion of drug delivery and polymer degradation. (J Am Coll Cardiol 2000;36:2325–32) © 2000 by the American College of Cardiology

Attempts to attenuate restenosis after angioplasty using systemic therapies have met with frustration. Driven by dual goals of high local concentrations but low systemic exposure, local delivery methods have been developed. As endovascular stenting has become widespread, stents have been proposed as ready means for local drug delivery. To date, however, experimental stent-based drug delivery has not been able to inhibit stent-induced intimomedial thickening (1–4) although it has shown efficacy in reducing experimental stent-induced thrombosis (1–3,5).

Limitations facing stent-based antirestenotic therapies include complex multifactorial cellular and extracellular matrix responses to stent-induced injury, adverse and exaggerated tissue responses to materials bound to stents and brevity of contact between many delivered agents and target vascular tissue. We asked whether many of these limitations might be overcome via stent-based delivery of paclitaxel, a diterpenoid compound and the active agent in the antineoplastic drug Taxol. Paclitaxel has diverse mechanisms of action, including microtubule stabilization, arrest of cell mitosis, retardation of cell migration and immunomodulation (6) and has been reported to reduce vascular cell proliferation and migration in vitro and in vivo (7–11). Paclitaxel is also highly lipophilic and poorly soluble in aqueous solution, making it an excellent candidate for sustained delivery from stents and prolonged deposition in atherosclerotic vessels. We used a stent-based polymer delivery system to determine whether sustained delivery of paclitaxel for weeks after stenting could alter intimomedial thickening for months.

METHODS

Stents. Stainless steel stents (9 mm long 7-cell NIR stents, Medinol Inc.) were left bare or coated with poly(lactide-co-ε-caprolactone) copolymer (pLA/pCL) with or without paclitaxel 200 μg/stent, the maximum quantity possible without disrupting the polymer scaffold’s integrity. After sterilization, paclitaxel content for each stent was assessed gravimetrically using the known mass proportions of paclitaxel and pLA/pCL in the coating. Stents with calculated paclitaxel content >220 μg or <180 μg were discarded.
In vitro paclitaxel release kinetics. In vitro assays were performed to assess the kinetics of paclitaxel release from pLA/pCL-coated stents over 56 days. Fifteen stents were coated with pLA/pCL loaded with paclitaxel, sterilized and had initial paclitaxel load calculated gravimetrically (207 ± 2 μg). Each stent was incubated in 15 mL of calf serum (Gibco BRL, Life Technologies) at 37°C with constant agitation at 120 rpm. Serum was changed twice daily for one day and every one to two days thereafter to ensure sink conditions. Three stents each at 1, 7, 14, 28 and 56 days were removed from their release vials and analyzed for residual paclitaxel content. Residual paclitaxel was extracted using THF:DMAC, 50:50 by volume, and the polymer precipitated in H2O:ACN:Acetic acid; 90:10:0.1 (by volume). The resultant supernatant was analyzed for paclitaxel content by HPLC (Metachem Taxsil column), and the percent of initial paclitaxel released from each stent was calculated.

Surgical procedure and tissue processing. All animal care conformed to the “Position of the American Heart Association on Research Animal Use,” was conducted in facilities accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC) and was approved by the Institutional Animal Care and Use Committee (IACUC) of MIT. Twenty-eight New Zealand White rabbits (Millbrook Farm Breeding Labs), 3 to 4 kg, were fed a rabbit chow and water ad libitum. Aspirin (Sigma Chemical Co, 0.07 mg/mL) was added to drinking water one day before surgery and continued throughout the experiment. Under anesthesia with ketamine (Fort Dodge Laboratories, 35 mg/kg IM) and xylazine (Miles Inc., 5 mg/kg IM), the endothelium of both iliac arteries was denuded using a 3F embolectomy catheter (Baxter Health Care Corp.) (2,12–16). A metal stent mounted coaxially on a 3-mm angioplasty balloon (10 mm Bandit, Boston Scientific Corp.) was passed retrogradely into each iliac artery and expanded (15 s, 8 atm), yielding balloon:artery ratios of 1.1 to 1.2:1 (2,12,17). All animals received a single intravenous bolus of standard heparin (100 U/kg, Elkins-Sinn Inc.) at the time of stenting.

All animals survived until planned sacrifice. Iliac arteries were harvested 3, 7, 28, 56 and 180 days after stenting. Bromodeoxyuridine (BrdU) was administered (50 mg/kg intravenously, Sigma Chemical Co.) 1 hour before sacrifice. Under anesthesia with sodium pentobarbital, the inferior vena cava was opened and perfusion performed via left ventricular puncture with Ringer’s lactate solution followed by 4% paraformaldehyde. Stented arterial segments were embedded in methyl methacrylate mixed with n-butyl methacrylate (Sigma Co.) and sectioned at 5 μm thickness (13–15,18). Sections were taken from each end and the middle of each stent. Vessels from animals sacrificed after 180 days were also sectioned just proximal and distal to the stent.

Of 23 arteries implanted with uncoated stents, 2 were harvested at 7, 10 at 28, 7 at 56 and 4 at 180 days. The ten arteries that were implanted with stents coated with pLA/pCL alone included two harvested at 7, four at 28 and four at 56 days. Fifteen arteries receiving pLA/pCL-coated paclitaxel-releasing stents included two harvested at 7, four at 28, five at 56 and four at 180 days.

Histological analysis. All histological analyses were performed by investigators blinded to stent type. Sections stained with Verhoeff’s tissue elastin stain underwent computer-assisted digital planimetry. Injury scores (19) and neointimal, medial and external elastic lamina areas were quantified for three cross sections per stent (proximal end, middle and distal end), and the results were averaged. For nonstented regions intimal:medial area ratios were calculated. Proliferating medial cells were identified immunohistochemically by uptake of BrdU (mouse anti-BrdU, DAKO Co.), intimal macrophages by RAM-11 (DAKO Co.), smooth muscle cell (SMC) by α-actin (mouse anti-α-SMC α-actin, DAKO Co.), endothelial cells by factor VIII related antigen (von Willebrand’s factor [vWF], goat anti-human factor VIII, ICN Biomedicals, Inc.) and hyaluronan by a biotinylated proteoglycan fragment (20) (gift of Dr. G. Underhill, Georgetown School of Medicine). Standard immunohistochemical protocols were followed as previously described (13–15) with fibrin identified by Mallory’s PTAH staining and collagen by picrosirius-red stain observed under cross-polarized light (21).

Medial or intimal cell density was calculated by dividing the number of nuclei by the medial or intimal area, the number of BrdU positive or RAM-11 positive cells counted and their prevalence in the media or intima calculated. Smooth muscle cell, fibrin, hyaluronan and collagen staining were scored from 0 to 4 (0 = no stain present, 1 = very weak, 2 = weak, 3 = moderate and 4 = intense) for staining intensity and distribution.

Endothelial regrowth. The rate and completeness of endothelial regrowth after denuding injury in this model is variable and occurs late (17). Arteries harvested 180 days after stent implantation were stained for vWF and scored from 0 to 4 (0 = no endothelial cells present, 1 = <25%, 2 = 25 to 50%, 3 = 50 to 75%, 4 to >75%) based on the number of quadrants covered with vWF positive cellular staining. To examine specifically the effects of bare or paclitaxel-releasing stents (n = 4 each) on endothelial cells in vivo, stent implantation was performed in four animals in vessels not subjected to balloon denudation. This method is characterized uniformly by complete anatomic endothelial restitution after three days (13,16). En face examination of the completeness of endothelial surface coverage was per-
formed using silver staining (13,16). The area of intrastrut surface covered with endothelium was measured using digital planimetry and expressed as a percent of the total stented segment surface area (16).

Statistics. All data are presented as mean ± standard error. Comparisons between the three treatment groups at each time point used analysis of variance with Bonferroni/Dunn correction for multiple comparisons. Data from the two treatment groups examined at the six-month time point were compared using Student t test. Values of p < 0.05 were considered significant.

RESULTS

Stent-based paclitaxel release. Paclitaxel release from pLA/pCL-coated stents was measured in serum. Release followed first-order kinetics with minimal early burst and little interstent variability: 36 ± 10, 55 ± 6, 63 ± 2, 78 ± 1, 91 ± 1% released after 1, 7, 14, 30 and 56 days, respectively.

Intimal thickening. The intimal response to stent implantation at all time points was markedly altered by stent-released paclitaxel. Qualitative differences were seen in intimal composition and quantitative differences in intimal mass. After seven days, the thin neointima of control or pLA/pCL-coated stents were highly cellular, comprising α-actin-positive SMC, RAM-11-positive macrophages and extracellular matrix. In contrast, cellular organization was absent after seven days in paclitaxel-treated arteries; the internal elastic lamina remained largely exposed, covered not by neointima but only by adherent inflammatory cells. The areas around stent struts were fibrin-dense and relatively free of infiltrating SMC (Fig. 1, A to C).

Neointimal area was markedly reduced 28, 56 and 180 days after implantation of paclitaxel-releasing stents. Twenty-eight days after stent placement, the intima measured 0.98 ± 0.11 and 1.28 ± 0.10 mm² in arteries receiving uncoated and pLA/pCL-coated stents, respectively (p = NS). This was reduced over 50% to 0.40 ± 0.07 mm² in stents releasing paclitaxel (p = 0.004 and 0.0007 compared with uncoated and pLA/pCL-coated stents, respectively, Fig. 2, Table 1). The reduction in neointimal area was sustained 56 days after stent implantation (neointimal areas 1.35 ± 0.20 and 1.36 ± 0.13 mm² in uncoated and pLA/pCL-coated stents, respectively, reduced by 60% to 0.50 ± 0.07 mm² in stents releasing paclitaxel, p = 0.003 and 0.006 compared with uncoated and pLA/pCL-coated stents, Fig. 1, D to I, and 2, Table 1). The effect of paclitaxel persisted 180 days after stent implantation (neointimal area 0.56 ± 0.06 and 0.96 ± 0.11 mm² in arteries receiving paclitaxel-releasing or uncoated stents, respectively, p = 0.036, Fig. 3, Table 1). Injury scores were low and did not differ between groups or over time.

There was no significant change in neointimal size in arteries receiving paclitaxel-releasing stents between 56 and 180 days (0.50 ± 0.07 and 0.56 ± 0.06 mm², respectively, Fig. 2, Table 1) despite this period extending well beyond

Figure 1. Photomicrographs show rabbit iliac arteries stained with Verhoeff’s tissue elastin stain after balloon denudation and stent implantation. Seven days after stenting, a thin cellular neointima separates the lumen from the internal elastic lamina in uncoated stents (A) and stents coated with poly(lactide-co-ɛ-caprolactone) (B). In arteries receiving stents coated with poly(lactide-co-ɛ-caprolactone) containing paclitaxel (C), no intimal thickening is seen although the media retains its cellularity. Fifty-six days after balloon denudation and stent implantation, neointimal thickening had progressed in uncoated stents (D, G) and stents coated with poly(lactide-co-ɛ-caprolactone) (E, H) but remained almost undetectable in stents coated with poly(lactide-co-ɛ-caprolactone) releasing paclitaxel (F, I). Original magnifications: A to F = 150×, G to I = 18×.
the anticipated in vivo completion of paclitaxel release and degradation time for pLA/pCL. In uncoated stents neointimal area fell slightly between 56 and 180 days (1.35 ± 0.20 vs. 0.96 ± 0.11 mm², p = 0.20).

To examine for exacerbation of neointimal hyperplasia at stent margins, segments of artery immediately adjacent to the stent were examined in subjects sacrificed 180 days after stent implantation. Intimal:medial area ratios did not differ between arteries receiving uncoated stents (0.99 ± 0.18) and those receiving paclitaxel-releasing stents (1.03 ± 0.17, p = NS).

Tissue responses. Differences in histologic responses to control and paclitaxel-releasing stents were as pronounced as the quantitative differences in neointimal area. Twenty-eight, 56 and 180 days after stent implantation, the neointima in vessels receiving paclitaxel-releasing stents was thin and sparsely cellular. Present on the luminal surface were adherent inflammatory cells with no overlying intimal thickening. Seven days after stent implantation, the time of peak intimal and medial cell proliferation in this model (14), uncoated stents provoked medial and intimal cell proliferation rates (BrdU positive cells) of 0.11 ± 0.01% and 0.96 ± 0.11%, respectively. These rates were reduced in paclitaxel-releasing stents three-fold to 0.03 ± 0.03% and 0.29 ± 0.15%, respectively.

The inflammatory response was evaluated using immunohistochemical identification of RAM-11 positive tissue monocyte/macrophages (Table 1). There was no significant difference in intimal macrophage number between arteries receiving uncoated and pLA/pCL-coated stents after 7, 28

Table 1. Tissue and Cellular Responses to Stent Implantation 7, 28, 56 or 180 Days After Stent Implantation

<table>
<thead>
<tr>
<th>Time after Stenting</th>
<th>Uncoated</th>
<th>pLA/pCL-coated</th>
<th>pLA/pCL-coated Paclitaxel-releasing</th>
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<tr>
<td></td>
<td>7 day macrophages (# cells RAM-11+)</td>
<td>50 ± 19</td>
<td>63 ± 9</td>
</tr>
<tr>
<td>28 day intima (mm²)</td>
<td>0.98 ± 0.11</td>
<td>1.28 ± 0.10</td>
<td>0.40 ± 0.07*</td>
</tr>
<tr>
<td>media (mm²)</td>
<td>0.25 ± 0.04</td>
<td>0.19 ± 0.03</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>macrophages (# cells RAM-11+)</td>
<td>64 ± 8</td>
<td>41 ± 5</td>
<td>120 ± 30†</td>
</tr>
<tr>
<td>56 day intima (mm²)</td>
<td>1.35 ± 0.20</td>
<td>1.36 ± 0.13</td>
<td>0.50 ± 0.07*</td>
</tr>
<tr>
<td>media (mm²)</td>
<td>0.38 ± 0.10</td>
<td>0.34 ± 0.12</td>
<td>0.13 ± 0.01‡</td>
</tr>
<tr>
<td>macrophages (# cells RAM-11+)</td>
<td>57 ± 9</td>
<td>82 ± 14</td>
<td>168 ± 39†</td>
</tr>
<tr>
<td>180 day intima (mm²)</td>
<td>0.96 ± 0.11</td>
<td>0.56 ± 0.06§</td>
<td>0.15 ± 0.02§</td>
</tr>
<tr>
<td>media (mm²)</td>
<td>0.25 ± 0.03</td>
<td>0.14 ± 0.01 §</td>
<td>0.15 ± 0.02§</td>
</tr>
<tr>
<td>macrophages (# cells RAM-11+)</td>
<td>6 ± 4</td>
<td>157 ± 25§</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.004 compared with uncoated and pLA/pCL-coated; † p < 0.04 compared with uncoated and pLA/pCL-coated; ‡ p = 0.07 compared with uncoated; § p < 0.04 compared with uncoated.

pLA/pCL = poly(lactide-co-Σ-caprolactone).
or 56 days. In uncoated stents there was a 10-fold fall in the number of intimal macrophages between 56 and 180 days. In paclitaxel-releasing stents, intimal macrophage numbers were elevated after 28 and 56 days and did not fall substantially between 56 and 180 days (Table 1). Macrophages were not seen in the arterial media.

We measured medial cross sectional area and cell density in each group to assess possible late effects of inhibition of medial SMC proliferation. Twenty-eight days after stent implantation medial areas did not differ between groups (Table 1). By 56 days medial area had fallen in arteries receiving paclitaxel-releasing stents. After six months medial area was still lower in arteries treated with paclitaxel (0.15 ± 0.02 mm²) compared with uncoated stents (0.25 ± 0.03 mm², p < 0.04). Medial cell densities, however, did not differ significantly between treatment groups at any time. Despite medial thinning and reduced cell density after 180 days in paclitaxel-treated vessels, no histologic evidence of aneurysm formation, medial necrosis or vessel rupture was seen in any artery. Overall arterial size (external elastic lamina area) was no different in arteries receiving paclitaxel-releasing stents compared with uncoated stents at any point in time.

After 28 days the neointima within uncoated stents was rich in SMC, collagen and hyaluronan, with little fibrin (Table 2, Fig. 4, A to C). In contrast, in stents releasing paclitaxel the neointima had markedly fewer SMC, less hyaluronan and collagen and extensive fibrin (Table 2, Fig. 4, G to I). Between 28 and 180 days arteries receiving uncoated stents gained neointimal collagen and lost hyaluronan (Table 2, Fig. 4, D to F), while those receiving paclitaxel-releasing stents showed persistent fibrin with sparse neointimal collagen and few SMC (Table 2, Fig. 4, J to L).

**Endothelial cell regrowth.** Because of the potent inhibitory effects of stent-released paclitaxel on medial SMC

![Figure 4](image-url)

*Figure 4.* Photomicrographs of rabbit iliac arteries after stent implantation. Serial sections from four arteries stained for collagen (picrosirius red), fibrin (Mallory’s PFAH) and hyaluronan (b-PG). In arteries with uncoated stents, collagen deposition within the neointima (yellow-orange viewed under polarized light) intensifies between 28 days (A) and 180 days (D). With paclitaxel-releasing stents, although medial collagen is present, neointimal collagen is sparse after 28 (G) and 180 days (J). Neointimal fibrin (extracellular blue staining) is sparse in arteries receiving uncoated stents after 28 (B) and 180 days (E). In contrast, intense extracellular fibrin persists in vessels receiving paclitaxel-releasing stents at 28 (H) and 180 days (K). Hyaluronan (brown staining) diminishes in arteries with uncoated stents between 28 (C) and 180 days (F). In paclitaxel-releasing stents, hyaluronan persists without change between 28 (I) and 180 (L) days. *Sites of stent struts. Original magnification 120×.*

### Table 2. Neointimal Extracellular Matrix Composition 28 or 180 days After Stent Implantation

<table>
<thead>
<tr>
<th>Stent Coating</th>
<th>Fibrin (0–4) 28 days</th>
<th>Fibrin (0–4) 180 days</th>
<th>Collagen (0–4) 28 days</th>
<th>Collagen (0–4) 180 days</th>
<th>Hyaluronan (0–4) 28 days</th>
<th>Hyaluronan (0–4) 180 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td>3.0 ± 0.6</td>
<td>2.0 ± 0.0</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>pLA/pCL</td>
<td>1.7 ± 0.3</td>
<td>2.0 ± 0.6</td>
<td>2.3 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pLA/pCL-coated paclitaxel-releasing</td>
<td>4.0 ± 0.0</td>
<td>4.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>1.7 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td>2.7 ± 0.3</td>
</tr>
</tbody>
</table>

*pLA/pCL = poly(lactide-co-Σ-caprolactone).*
proliferation and intimal cell accumulation and the reported lower sensitivity of endothelial cells to inhibition by paclitaxel in vitro (7), we investigated effects of stent-based paclitaxel release on endothelial restoration. Endothelial regeneration was variable and incomplete even 180 days after denudation and uncoated stent-implantation. However, the degree of vWF-positive cellular coverage was lower in arteries implanted with paclitaxel-releasing stents than in those receiving uncoated stents (1.0 vs. 2.7 quadrants covered, respectively).

As we have previously reported, complete endothelial regrowth does occur within three days if vessels are not denuded before stenting (13). In this setting stent-based paclitaxel release from pLA/pCL-coated stents did not delay endothelial regrowth, with 100% of the surface covered with endothelium after three days in all control stents and all pLA/pCL-coated paclitaxel-releasing stents.

**DISCUSSION**

Challenges that have faced stent-based drug delivery include identifying a biological target, a material platform that does not itself exacerbate proliferative, thrombotic or inflammatory responses and an agent that can be delivered from the platform so as to affect the chosen target. Our experiments demonstrate that sustained delivery of paclitaxel for a period of a few weeks from an endoluminal stent markedly attenuates stent-induced intimal thickening for at least six months. In addition to quantitative reductions in neointimal thickening, stent-based paclitaxel delivery also yields large qualitative alterations in vascular responses to injury.

**Prior studies.** Previous work has addressed paclitaxel’s effects on vascular repair after injury. Paclitaxel reduced rat arterial SMC migration in vitro by 50% at 0.5 nM and by 100% at 100 nM (8). In vivo experiments demonstrated that systemic administration of paclitaxel for five days (with peak plasma concentrations of 50 to 60 nM) reduced neointimal area by 70% 11 days after rat carotid artery injury. Axel et al. (7) demonstrated that brief exposure to paclitaxel inhibited growth and migration of human arterial SMC at concentrations of 0.01 to 10 μmol/L and examined in vivo the effects of local delivery of paclitaxel via a microporous balloon. Three groups have reported preliminary data that stent-based paclitaxel delivery reduced stent-induced intimal thickening up to 28 days after arterial injury (9–11).

**Postulated mechanism of action.** Paclitaxel’s efficacy may reflect both its breadth of activities as well as its relative insolubility. Paclitaxel interferes with cellular migration and proliferation primarily by stabilizing microtubules (6). Through this mechanism and others (22), it may interfere with cells’ capacities to maintain shape, move, transmit intracellular signals and effect intracellular transport. In addition, paclitaxel may alter inflammatory cell activity, directly linked to the genesis of stent-induced neointima (14,15), by enhancing macrophage production of nitric oxide, prostaglandins or other cytokines (23–29).

**Tissue response.** Histologic characterization of tissue responses in the presence of paclitaxel indicates arrest of healing. Fibrin remains present for months, with little of the collagen deposition or SMC infiltration characteristic of uncoated stents. Of note is the sustained presence of inflammatory cells as part of this delayed healing. The number of monocyte/macrophages in the neointima of arteries receiving uncoated stents fell dramatically after two months, while in stents releasing paclitaxel even after six months, macrophages persisted in the neointima in high numbers. Although previous reports from our group have connected early monocyte/macrophage recruitment with intimal thickening (14,15), it does not appear as if the late presence of these cells, i.e., between two and six months after stent implantation, has the same implications. Of concern, such incomplete healing may prolong the period of vessel wall thrombogenicity, as has been suggested after endovascular radiation therapy (30). While late thrombotic events and delayed tissue growth were not observed in this study, limitations of the model employed—with ligated femoral arteries, consequent low flow velocities and lower shear-forces—will be essential to consider in translating our findings to clinical evaluation. In contrast with responses after stent-based radiation therapy, vessel segments at the margins adjacent to the paclitaxel-releasing stent did not reveal evidence of any marginal exacerbation or “candy-wrapper effect.”

**Comparison with other platforms and agents.** Previous attempts to combat restenosis with stent-based drug delivery have met with mixed results. While several biodegradable and nonbiodegradable polymers have been shown to provide a reservoir of sustained drug release in other clinical settings, their use in coating endoluminal stents has typically engendered a significant inflammatory and proliferative tissue response (3,4,31,32). This response may be attributed to effects of the polymer material or disruption of the dynamic physiology of the luminal surface with bulky material layers. Some stent-based drug delivery systems have reduced the rate of thrombosis after injury (1–3,5) but have failed to reduce either intimal thickening in experimental models (1–3) or clinical restenosis (33). The polymer material (pLA/pCL) used for stent coating in the current experiments provoked no detectable increase in stent-induced inflammation 56 days after implantation when degradation had likely peaked.

Six months after stent implantation, no rebound enhancement of intimal thickening after paclitaxel release and pLA/pCL degradation was seen. This strongly suggests either that a two-month period of paclitaxel release in this experimental model is sufficiently long to counteract the cellular signals and mediators that follow stent-induced arterial injury or that paclitaxel remains present and active within the vessel wall long after delivery from the stent. Because of the gap between completion of drug release and six-month follow-up, our data make unlikely a late catch-up...
phenomenon whereby paclitaxel-treated arteries would develop intimal thickening after cessation of drug activity.

**Study limitations.** This report of abrogation of intimal thickening after stent-implantation warrants confirmation and extension in alternative animal models. In particular, studies of paclitaxel in settings of underlying arterial disease, e.g., lipid-rich lesions or in settings of higher flow or more severe deep arterial injury, e.g., the porcine coronary tree, will be essential. In addition, although posing significant technical challenges, studies of paclitaxel release, serum concentration and tissue deposition in vivo will help elucidate the dynamic nature, location and duration of the presence of released drug, which may enhance mechanistic understanding of how paclitaxel inhibits intimal growth. Such studies will be central to assessing safe and effective doses of stent-based paclitaxel, as well as determining potential risks of systemic toxicity in future clinical applications. Equally challenging will be characterization of pLA/pCL degradation in vivo. Finally, the six-month studies that addressed the hypothesis of long-term efficacy of a combined system of polymer material and drug together, compared with uncoated control stents, did not include polymer-only stents. The absence of vascular responses to stents coated with polymer material alone as late as 56 days, when material degradation is likely complete, makes the possibility of a later toxic polymer effect unlikely.

**Conclusions.** There has been long-standing hope that stents might be able to carry with them their own ammunition for attenuating stent-induced restenosis. To this end, changes in stent design and material, or addition of radiation-emitting isotopes, have been proposed. Perhaps the most inherently appealing, yet technically challenging, approach has been to add local drug treatment to the stent, enabling it to treat directly the injured vessel. Our data, demonstrating that stent-based delivery of paclitaxel can virtually eliminate stent-induced tissue growth for months after cessation of drug delivery, validate this approach as a potential prevention for in-stent restenosis.

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