Pathological Analysis of Local Delivery of Paclitaxel Via a Polymer-Coated Stent

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Background—Paclitaxel can inhibit vascular smooth muscle proliferation in vitro, and early studies suggest that paclitaxel may be useful in preventing restenosis. Early and late intimal growth and local vascular pathological changes associated with paclitaxel delivered via stents have not been fully explored.

Methods and Results—Localized drug delivery was accomplished with balloon-expandable stainless steel stents coated with a cross-linked biodegradable polymer, chondroitin sulfate and gelatin (CSG), containing various doses of paclitaxel. CSG-coated stents with paclitaxel (42.0, 20.2, 8.6, or 1.5 \( \mu \)g of paclitaxel per stent), CSG-coated stents without paclitaxel, and uncoated stents (without paclitaxel or CSG) were deployed in the iliac arteries of New Zealand White rabbits, which were killed 28 days after implant. Mean neointimal thickness at stent strut sites was reduced 49% (\( P < 0.0003 \)) and 36% (\( P < 0.007 \)) with stents containing 42.0 and 20.2 \( \mu \)g of paclitaxel per stent, respectively, versus CSG-coated stents without paclitaxel. However, histological findings suggested incomplete healing in the higher-dose (42.0 and 20.2 \( \mu \)g) paclitaxel-containing stents consisting of persistent intimal fibrin deposition, intraintimal hemorrhage, and increased intimal and adventitial inflammation. Stents coated with CSG alone (without paclitaxel) had similar neointimal growth as uncoated stents. In a separate group of rabbits killed at 90 days, neointimal growth was no longer suppressed by CSG-coated stents containing 42.0 or 21.0 \( \mu \)g of paclitaxel.

Conclusions—CSG coating appears to be a promising medium for localized drug delivery. Paclitaxel polymer–coated stents reduce neointima formation but are associated with evidence of incomplete healing at 28 days. However, neointimal suppression was not maintained at 90 days. (Circulation. 2001;104:473-479.)

Key Words: stents ■ restenosis ■ pathology

In-stent restenosis remains a clinical problem and can be expected to increase in incidence as coronary stenting becomes more frequent and is used in less-ideal lesions. Neointimal growth involves dedifferentiation of vascular smooth muscle cells from a contractile state to a secretory state leading to cellular proliferation, migration from the media into the intima, and synthesis of extracellular matrix.1,2 The pathophysiology of restenosis consists of the complex interaction of cytokines and growth factors with cellular and acellular elements.3 Blockade of any one factor is often insufficient to inhibit the restenosis cascade. Therefore, attention has focused on disrupting essential central cellular processes that would subsequently affect “downstream” events that ultimately lead to restenosis.

Microtubules play an essential role in multiple cellular activities that are relevant to the restenosis process, including cell division, motility, transport, and extracellular secretory processes.4 The mechanism of action of paclitaxel consists of polymerization of tubulin, which results in the formation of abnormally stable and nonfunctional microtubules,5,6 thereby inhibiting cellular replication in the G0/G1 and G1/M phases.7 Previous in vitro studies demonstrated inhibition of migration and proliferation of vascular smooth muscle cells by paclitaxel,8,9 and initial promising in vivo studies of systemic and local paclitaxel administration to inhibit intimal growth have been reported.8,10

Paclitaxel has been successfully incorporated into a biodegradable film containing chondroitin sulfate A and gelatin (CSG) for local delivery.11 The primary objective of the present study was to determine whether CSG containing paclitaxel placed on metallic stents could inhibit neointimal growth. Detailed histological analyses of the local arterial effects of paclitaxel-releasing stents were performed. Finally, we sought to establish whether 28-day in-stent neointimal
inhibition would be maintained for 90 days in CSG-coated paclitaxel-releasing stents.

**Methods**

**Preparation of Paclitaxel CSG Films on Stents**

Various concentrations of paclitaxel were deposited on ACS MultiLink stents (0.7 cm length) by evaporation from a volatile solvent. Stents were coated with a gelatin-chondroitin sulfate coacervate film by first dipping the stent into a molten 10% gelatin solution followed by dipping in 1% chondroitin sulfate A plus 0.1% glutaraldehyde. The stents were rinsed 3 times with sterile filtered water and dried overnight before ethylene oxide sterilization. The concentration of paclitaxel retained on the stent was determined after overnight digestion of the CSG film with 286 U/mL collagenase in a PBS solution with 0.5 mmol/L CaCl₂ at 37°C. The mixture was extracted twice with 0.5 mL of chloroform. The chloroform extracts were pooled and evaporated to dryness under a helium stream. The residue was dissolved in 1.0 mL of absolute ethanol. Paclitaxel concentration was measured on a Gilson high-performance liquid chromatograph (HPLC) with a 25x4.6-cm pentafluorophenyl column with ultraviolet detection at 227 nm using a mobile phase consisting of 45% acetonitrile and 55% water with a 1.5 mL/min flow rate. Variation in the paclitaxel dose on stents was ≤10%. The CSG film on the stents degrades in vivo with an approximate first-order rate constant of 0.03 per hour (half-life ~23 hours).

**Iliac Artery Stent Deployment**

Male New Zealand White rabbits were randomly assigned to receive 1 or 2 iliac artery stents. Twenty-four stents coated with CSG film containing various doses of paclitaxel (42.0, 21.0, or 0 µg) were deployed, and 24 control stents (CSG coating alone [no paclitaxel, n=18] or an uncoated bare metal stent [no paclitaxel, no polymeric film, n=6]) were placed. In rabbits that received 2 stents, the second stent was placed in the contralateral iliac artery. Mean body weight for rabbits that received stents with various doses of paclitaxel was 3.0 versus 3.3 kg, respectively; however, angiographic arterial diameters and stent deployment diameters were similar among treatment groups, so that differences in rabbit weight did not translate into differences in arterial size.

**Tissue Processing and Analysis**

The stented portion of the vessel was processed as described previously. Histological sections from the proximal, middle, and distal portions of the stent were stained with hematoxylin-eosin and Movat pentachrome. Sections were digitized with the observer blinded to the treatment group. Neointimal thickness at each strut site was measured by computerized morphometry (IPLab Spectrum software), and mean neointimal thickness for each arterial segment was calculated. The injury score at each stent site was determined, and a mean score for each artery was calculated. The presence or absence of intimal hemorrhage (defined as the presence of confluent collections of extravasated red blood cells) was determined at each stent strut site for all arterial sections. The number of strut sites with hemorrhage per total struts per stent was determined. For example, if there were 36 strut sites examined per artery, and 12 sites had hemorrhage, then 33% of strut sites were associated with hemorrhage. Intimal and adventitial regions between stent struts were examined for the presence or absence of ≥10 inflammatory cells per 400× field. The number of interstrut sites with ≥10 inflammatory cells per total number of interstrut sites was determined. Fibrin deposition around stent struts was assessed as being either present in any of the stented segments or absent in all sections and was confirmed by immunohistochemical staining in selected cases. Medial necrosis was defined by focal areas of smooth muscle cell dropout. To assess intimal cellular proliferation, midsegment sections were stained with anti-BrdU antibody, and BrdU-positive intimal cells were counted as a percent of total cells in eight 400× fields per midsegment section (BrdU-labeling index), as described previously.

**In Vivo Pharmacokinetics**

One CSG-coated stent containing 42.0 µg of paclitaxel was implanted in a rabbit iliac artery with euthanasia, and perfusion fixation was performed at 6 hours, 24 hours, 72 hours, 7 days, and 14 days after stent deployment (n=3 stents per time point). Plasma samples were obtained to determine paclitaxel levels. The stented segment of the artery was excised along with a 5-mm-long arterial segment proximal and distal to the stent. The stent was carefully removed from the arterial tissue. The tissue was then diced, and both the tissue and stent were separately digested in a PBS solution containing 1 mmol/L CaCl₂, 1 mg/mL collagenase, and 0.33 mg/mL BSA. Total stent and arterial tissue paclitaxel content (recovery) was measured in ethanol extracts by direct HPLC injection. All other extracts and digests were cleaned up via solid-phase extraction (CN-Sep, Fisher) before HPLC quantification. Limits for paclitaxel detection with these techniques exceeded 0.02 µg. Paclitaxel plasma levels were measured with solid-phase extraction by HPLC quantification.

**Statistical Analysis**

Numerical data are presented as mean ± SEM. Continuous variables were compared with an ANOVA (t test with Bonferroni correction), and categorical variables were compared with a χ² test. A P value ≤0.05 was considered significant.

**Results**

Stents (n=48) were placed in 41 rabbits (1 stent each in 34 rabbits and 2 stents each in 7 rabbits), and all stents were easily deployed at the relatively low balloon inflation pressures used. Stent oversizing ranged from 1.2 to 1.4 and was similar among treatment groups. There was no evidence of systemic toxicity of locally administered paclitaxel with any dose. One rabbit that received an 8.6-µg-paclitaxel stent died <24 hours after stenting. A complete blood count performed in randomly selected animals showed similar white blood cell counts, hematocrit, and platelet counts among treatment groups (data not shown). No stent thrombosis was observed, and all stents were angiographically patent at 28 days.

**Morphometric Measurements**

There was a significant dose-dependent reduction in neointimal thickness in the 42.0 and 20.2 µg treatment groups compared with the stents containing a lower concentration of
paclitaxel (8.6 and 1.5 μg), the CSG-coated stents without paclitaxel, and the uncoated metallic stents (Figures 1 and 2). The 42.0-μg paclitaxel stents produced a 49% and 48% reduction ($P < 0.0003$) in neointimal thickness versus the CSG-coated stents without paclitaxel and the uncoated metallic stents, respectively. The 20.2-μg paclitaxel stents were associated with a 36% and 35% reduction ($P < 0.007$) in neointimal thickness compared with the CSG-coated stents without paclitaxel and the uncoated metallic stents, respectively.

**Histological Observations**

No cases of stent thrombosis were observed in any of the treatment groups. Injury scores were low (mean <1.0) and similar in all groups. Multiple qualitative and quantitative histological findings suggested incomplete healing or local toxicity at the higher-dose (42.0 and 20.2 μg) paclitaxel-containing stents. Intimal fibrin deposition surrounding stent struts (Figure 3A) was focally present in all 42.0-μg- and 5 of 7 (71%) of the 20.2-μg-paclitaxel stents and only 1 of 6 of the 1.5-μg-paclitaxel stents; intimal fibrin was not observed adjacent to struts in the 8.6-μg-paclitaxel stents, CSG-coated stents without paclitaxel, and uncoated metallic stents ($P < 0.0001$). Focal medial necrosis (Figure 3A) was present in 2 of 6 arteries containing 42.0-μg-paclitaxel stents and 2 of 7 of the 20.2-μg-paclitaxel stents. None of the other stent groups had evidence of medial necrosis ($P = 0.037$).

Intraintimal hemorrhage, defined as the percent of struts per stent with associated hemorrhagic foci, was more frequently observed in 42.0- and 20.2-μg-paclitaxel–coated stents versus stents containing 8.6 and 1.5 μg of paclitaxel.

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**Figure 1.** Neointimal thickness 28 days after deployment of CSG-coated stents containing various doses of paclitaxel, CSG-coated stents without paclitaxel, and uncoated bare stents without paclitaxel.

**Figure 2.** Photomicrographs demonstrating neointimal thickness in arteries 28 days after stent deployment. A, Uncoated (bare) stent without paclitaxel; B, CSG-coated stent with paclitaxel; C, CSG-coated stent containing 1.5 μg of paclitaxel; D, CSG-coated stent containing 8.6 μg of paclitaxel; E, CSG-coated stent containing 20.2 μg of paclitaxel; and F, CSG-coated stent containing 42.0 μg of paclitaxel. Movat pentachrome stain; scale bar 0.12 mm.
and uncoated stents (Figures 3B and 4). There was a significant dose-dependent increase in the percentage of interstrut intimal areas that contained inflammatory cells, expressed as the percent of interstrut sites per stent with inflammatory cells, with all doses of paclitaxel-coated stents compared with CSG-coated stents without paclitaxel (Figures 5 and 6A). The 42.0- and 20.2-μg-paclitaxel–coated stents were associated with greater adventitial inflammation compared with stents containing 8.6 and 1.5 μg of paclitaxel, CSG-coated stents without paclitaxel, and uncoated stents (Figure 6B).

**Intimal Cellular Proliferation**

The 42.0- and 20.2-μg-paclitaxel–coated stents were associated with a significant or borderline significant increase in proliferation versus the 8.6- and 1.5-μg-paclitaxel stents and uncoated bare stents (Figure 7).

**Chronic (90-Day) Study**

In-stent mean intimal thickness was similar among treatment groups (CSG-coated stents containing 42.0 [n=10], 21.0 [n=7], or 0 μg [n=7] of paclitaxel per stent; Figure 8). Morphologically, local toxic effects associated with high-dose paclitaxel stents seen at 28 days were no longer apparent at 90 days. Specifically, the neointima in all groups was well healed, consisting of smooth muscle cells in a proteoglycan-rich matrix; only rare small foci of inflammation were present near struts in all groups. Scanning electron microscopy of the stent surfaces demonstrated complete endothelialization in all treatment groups.

**Pharmacokinetics**

After placement of a 42.0-μg stent in the rabbit iliac artery, total arterial tissue and stent paclitaxel content was 0.75±0.30 and 12.4±4.3 μg at 6 hours, 0.42±0.08 and 4.1±3.1 μg at 24 hours, 0.99±1.21 and 1.3±2.0 μg at 72 hours, and 0.14±0.15 and 0.1±0.2 μg at 7 days, respectively. No paclitaxel was detected on the stent 2 weeks after implant. Plasma paclitaxel levels were 0.03±0.04 μg/mL at 6 hours, 0.002±0.003 μg/mL at 24 hours, and undetectable at 72 hours and at 7 days.
Discussion

In the present study, stents coated with CSG containing 42.0 and 20.2 mg of paclitaxel reduced intimal thickness by 49% and 36%, respectively, 28 days after deployment in rabbit iliac arteries compared with CSG-coated stents without paclitaxel. The present study demonstrated the feasibility and limitations of local arterial delivery of paclitaxel via a stent in doses that were not associated with systemic effects.

Intimal inhibition was lost by 90 days with 42.0- and 20.2-mg-paclitaxel CSG-coated stents, which suggests that paclitaxel delayed but did not prevent in-stent neointimal growth in this model. Several factors may be operative in this loss of effect. Perhaps most importantly, the pharmacokinetic study with a 42.0-µg-paclitaxel CSG-coated stent demonstrated low stent and tissue drug levels by 1 week. In addition, 42.0- and 20.2-µg-paclitaxel CSG-coated stents were associated with increased inflammation and cell proliferation at 28 days. In previous studies of arterial stenting, local inflammation correlated positively with increased neointimal growth.14 Several histological findings suggested that reduced intimal development was associated with incomplete healing and/or a local arterial toxic effect of paclitaxel: intimal hemorrhage, intimal fibrin deposition, intimal and adventitial inflammation, medial necrosis, and increased intimal cell proliferation. Impaired intimal healing is not a specific response to paclitaxel. For example, delayed intimal healing consisting of persistent intimal fibrin deposition, inflammation, and persistently increased cellular proliferation has been seen in arteries treated with radioactive stents.12 These findings suggest that interventions designed to inhibit smooth muscle cell proliferation and extracellular matrix synthesis may share a class effect of delaying rather than preventing restenosis.

The mechanism of vascular toxicity associated with local exposure to paclitaxel is unknown. In vitro studies have shown that paclitaxel inhibits the dynamic reorganization of microtubules that is necessary for mitosis, which in turn prevents formation of the normal mitotic spindle.15 As a result, chromosome movement is impaired, and mitotic arrest and extensive nuclear damage may occur.15 In the gastrointestinal tract, paclitaxel has been associated with mitotic arrest and cellular necrosis.16 In addition, in human leukemia, myeloma, and other solid tumor cell lines, incubation with paclitaxel produces apoptosis with or without inhibition of expression of BCL-2, an oncogene that suppresses apoptosis.15,17

Previous In Vivo Studies of Vascular Applications of Paclitaxel

To date, studies of intimal suppression after arterial injury have been performed in various animal models. Paclitaxel (2 mg/kg), administered intraperitoneally on days 0 to 4 after rat carotid artery balloon endothelial denudation, was associated with a 70% reduction in neointimal area assessed at 11 days.8
Pathological evidence of local arterial toxicity was not reported. After rabbit carotid injury, paclitaxel (10.0 \( \mu \)mol/L) was delivered locally via a microporous balloon catheter.\(^9\) At 28 days, mean intimal wall area, intimal thickening, and percent luminal stenosis was reduced in the paclitaxel group, but differences did not reach statistical significance.\(^8\) Detailed histological analyses of arteries treated locally with paclitaxel were not performed. In another study,\(^9\) paclitaxel was directly applied to Palmaz-Schatz stents (without a biodegradable polymer) and deployed in porcine coronary arteries. Lumen area was increased and neointimal growth reduced in arteries implanted with stents containing 187 \( \mu \)g of paclitaxel. Focal intimal hemorrhage was more commonly seen in arteries implanted with stents containing 15 or 187 \( \mu \)g of paclitaxel.

The use of a polymer-coated stent releasing paclitaxel was recently reported by Drachman et al\(^{10}\); polymer-coated stents containing 200 \( \mu \)g of paclitaxel reduced neointimal growth at 28, 56, and 180 days in rabbit iliac arteries. The finding of sustained neointimal suppression differed from our results in which neointimal growth inhibition was lost by 90 days. Their polymer [poly(lactide-co-\( \varepsilon \)-caprolactone)] differed from the CSG used in the present study, and their paclitaxel dose was more than 4-fold higher than our highest dose, although tissue levels were not established. However, the number of paclitaxel-stents studied by Drachman et al\(^{10}\) at 56 and 180 days was small. Furthermore, intimal and medial cell proliferation rates at 7 days were extremely low (<1.0%) for paclitaxel stents and controls. In contrast, in our experience, cellular proliferation rates in stented rabbit iliac arteries at 7 days are >15%. Similar to our study, Drachman et al\(^{10}\) noted that paclitaxel stents were associated with delayed intimal healing characterized by increased local arterial inflammation and fibrin deposition. Taken together, the studies by Drachman et al\(^{10}\) and Heldman et al\(^{19}\) and the present study support the hypothesis that paclitaxel-releasing stents can reduce in-stent neointimal growth associated with an incompletely healed intimal surface.

It is possible that any potentially toxic effects of paclitaxel are augmented by the presence of a stent acting as a local foreign body, as an explanation for the focal intimal hemorrhage and inflammation seen in the present and other stent studies\(^{10,19}\) in which stents were not used. Furthermore, the present study shows the need for detailed pathological studies of vascular interventions so that the potential benefits (e.g., inhibition of intimal growth) are weighed against potential local toxicity.

**Paclitaxel and Antirestenosis Therapy: Possible Mechanisms**

Paclitaxel, in nanomolar concentration, inhibits platelet-derived growth factor–stimulated rat vascular smooth muscle cell migration and proliferation and causes a dose-dependent increase in microtubule polymerization.\(^8\) From cell culture studies, the mechanism of its inhibition in growth factor-directed migration appears to be via microtubule polymerization-induced interference with smooth muscle cell locomotion and/or shape changes.\(^8\) Cytoskeletal changes in human vascular smooth muscle cells after incubation with paclitaxel include shortened and decentralized microtubules not connected to the perinuclear center, occasional disarrangement of \( \alpha \)-actin fibers, and cells that are rounded up and smaller than normal.\(^9\) Paclitaxel administered locally to balloon-injured rabbit carotid arteries resulted in an increase in artery size (positive remodeling), which was associated with changes in intimal microtubule assembly and impairment in arterial contractile function.\(^{20}\) Taken together, these data support investigations into the use of paclitaxel as a potential therapy for the treatment of restenosis.

**Polymer-Coated Stents With and Without Paclitaxel**

Results of experimental studies of biodegradable polymer stents and nonbiodegradable polymers coated to metallic stents have been disappointing secondary to the development of marked intimal inflammation.\(^{21}\) In the present study, the CSG-coated stents (without paclitaxel) were similar to the uncoated stents with respect to intimal and adventitial thickness, had less frequent intimal hemorrhage, and had a trend toward reduced inflammation. However, whereas the CSG polymeric coating was biocompatible, intimal growth was not suppressed at 90 days with stents containing paclitaxel, and nearly all of the paclitaxel was released by 7 days. A polymer coating that releases paclitaxel at a slower rate may result in prolonged intimal inhibition, but this benefit will need to be weighed against the potential for a more prolonged delay in intimal healing.

**Study Limitations**

This assessment of paclitaxel and CSG-coated stents in a small animal model used normal (nonatherosclerotic) peripheral arteries. All animal models currently used in arterial intervention research are limited in their ability to replicate human conditions. Results with the rabbit iliac model in other types of local arterial therapy (e.g., brachytherapy) have been particularly representative of the responses seen in humans. However, it is uncertain whether results similar to those seen with paclitaxel-eluting polymer-coated stents would be observed in human atherosclerotic coronary arteries.

**Conclusions**

Stents coated with CSG containing paclitaxel suppressed neointimal formation at 28 days in a dose-dependent manner without systemic toxicity. However, there was greater intimal fibrin deposition, intimal hemorrhage, intimal cell proliferation, and intimal and adventitial inflammation associated with paclitaxel-coated stents. These findings indicate delayed healing or local toxicity secondary to paclitaxel exposure. By 90 days, local toxicity associated with paclitaxel resolved, but in-stent neointimal growth suppression was no longer present. These data suggest that additional pharmacokinetic and histological studies of polymeric coatings that delay drug release are needed to better define the therapeutic window of paclitaxel-releasing stents.

**References**

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