Sustained Reduction of In-Stent Neointimal Growth With the Use of a Novel Systemic Nanoparticle Paclitaxel

Frank D. Kolodgie, PhD; Michael John, BA; Charanjit Khurana, MD; Andrew Farb, MD; Patricia S. Wilson, BS; Eduardo Acampado, DVM; Neil Desai, PhD; Patrick Soon-Shiong, MD; Renu Virmani, MD

Background—Paclitaxel (PXL)—eluting stents in animals cause incomplete healing and, in some instances, a lack of sustained suppression of neointimal growth. The present study tested the efficacy of a novel systemic delivery nanoparticle PXL for reducing in-stent restenosis.

Methods and Results—A saline-reconstituted formulation of PXL stabilized by albumin nanoparticles (nPXL) was tested in 38 New Zealand White rabbits receiving bilateral iliac artery stents. Doses of nPXL (1.0 to 5.0 mg/kg) were administered as a 10-minute intra-arterial infusion; control animals received vehicle (0.9% normal saline). In a follow-up chronic experiment, nPXL 5.0 mg/kg was given at stenting with or without an intravenous 3.5-mg/kg repeat nPXL dose at 28 days; these studies were terminated at 3 months. At 28 days, mean neointimal thickness was reduced (P≤0.02) by doses of nPXL ≧2.5 mg/kg with evidence of delayed healing. The efficacy of a single dose of nPXL 5.0 mg/kg, however, was lost by 90 days. In contrast, a second repeat dose of nPXL 3.5 mg/kg given 28 days after stenting resulted in sustained suppression of neointimal thickness at 90 days (P=0.009 versus single dose nPXL 5.0 mg/kg and controls) with nearly complete neointimal healing.

Conclusions—Although systemic nPXL reduces neointimal growth at 28 days, a single repeat dose was required for sustained neointimal suppression. Thus, this novel systemic formulation of PXL may allow adjustment of dose at the stent treatment site and prove to be a useful adjunct for the clinical prevention of in-stent restenosis. (Circulation. 2002;106:1195-1198.)

Key Words: arteries • drugs • muscle, smooth • restenosis • stents

Paclitaxel (PXL), a potent antineoplastic drug, promotes the assembly of abnormally stable microtubules that interfere with cell migration and replication.1 For the prevention of in-stent restenosis, PXL is thought best delivered locally on a drug-eluting stent to achieve therapeutic concentrations without the risk of systemic toxicity. This rationale has been successfully applied in 28-day animal studies of in-stent neointimal growth; however, there is incomplete healing2–4 and, in some instances, a lack of sustained neointimal suppression.3 Alternative commercial systemic formulations of PXL containing the nonionic surfactant Cremophor EL (polyoxyethylated castor oil) have been previously avoided as systemic therapy for restenosis because of the risk of hypersensitivity reactions (despite prophylaxis) and long infusion rates over a period of 3 to 24 hours.5

Recently, a novel cremophor-free albumin-stabilized nanoparticle formulation of PXL (nPXL) was developed for rapid parenteral delivery.6 Systemic delivery of PXL should allow more uniform drug exposure to the stented arterial segment, treatment of multiple lesions, and adjustment of target dose. The present study assessed the efficacy of systemic nPXL to reduce neointimal growth in a short-term and long-term rabbit model of iliac artery stenting.

Methods

Surgical Procedure

Under anesthesia with intramuscular ketamine (35.0 mg/kg) and xylazine (5.0 mg/kg), male New Zealand White rabbits (2.5 to 3.5 kg; Covance, Denver, Pa) were intubated and maintained on inhaled isoflurane with 100% oxygen. Both common iliac arteries were denuded of endothelium with the use of a balloon catheter before implanting stents. ACS Multilink Duet stents (3.0 mm × 13 mm long) were then deployed in each iliac artery. Stents were expanded with 30 seconds of balloon inflation pressure at 6 atm for secure placement within the vessel. All animals received 40 mg of aspirin orally 24 hours before surgery and each day thereafter.

For 28-day studies, animals received nPXL in dosages of 1.0, 2.5, 3.5, or 5.0 mg/kg immediately after stent implantation. The drug was given as a 10-minute intra-arterial infusion through a balloon catheter positioned at the iliac bifurcation. Infusion of 0.9% normal saline (nPXL vehicle) served as a control. For 90-day chronic studies, rabbits received a single dose of nPXL (5.0 mg/kg) as
Histomorphometry of Iliac Artery Stent Implants in Rabbits Treated With Systemic nPXL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Internal Elastic Lamina Area, mm²</th>
<th>Intimal Area, mm²</th>
<th>Intimal Thickness, mm</th>
<th>Percent Stent Stenosis</th>
<th>Inflammation Score</th>
<th>Intimal Fibrin, %</th>
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<tr>
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<td>nPXL, mg/kg</td>
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<td>1.0</td>
<td>8</td>
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<td>0.10±0.02</td>
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<tr>
<td>2.5</td>
<td>8</td>
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<td>1.30±0.09†</td>
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<td>3.5</td>
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<td>0.09±0.01†</td>
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<tr>
<td>Control</td>
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<td>31.9±4.4</td>
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<td>nPXL, mg/kg</td>
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<tr>
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<td>5.91±0.11</td>
<td>1.23±0.40¶</td>
<td>0.09±0.01#</td>
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<td>0.33±0.14</td>
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</table>

Data are expressed as mean±SEM. n indicates number of stents.

*P<0.007, †P<0.02, ‡P<0.01, §P<0.03, and ¶P<0.05 vs controls in 28-day study.

Histological Processing

Before euthanasia, rabbits received bromodeoxyuridine for monitoring cell proliferation.3 The stented iliac segments were harvested after perfusion fixation with 10% neutral-buffered formalin.

Specimens were embedded in methyl methacrylate. Sections from the proximal, middle, and distal ends of the stent were cut at 4.0 μm and stained with hematoxylin and eosin and Movat pentachrome; specific antibodies were used to identify macrophages and fibrin. Selected stents were processed for scanning electron microscopy for evaluation of endothelialization.

Single-Dose In Vivo Pharmacokinetics

After implanting bilateral iliac artery stents, 5.0 mg/kg of radiolabeled 3H nPXL was infused in 3 rabbits as described above. Blood samples (1.0 mL) were drawn from the jugular vein immediately before stopping the drug infusion, at 15 and 30 minutes, and at 1, 3, 5, 8, 12, 24, and 48 hours. After euthanasia, stented iliac segments were removed and the radioactivity of blood and tissue samples quantified.

Data Analysis

Data are expressed as the mean±SEM. Factorial ANOVA followed by Bonferroni/Dunn was used to determine significant differences in multiple comparisons. A P<0.05 was considered statistically significant.

Results

In Vivo Pharmacokinetics

Pharmacokinetics showed a biphasic profile with an initial rapid decline in concentration followed by a slower elimination phase. Whole blood concentrations of nPXL were maximal at 15 minutes after infusion and ranged from 3.0 to 5.0 μmol/L. At 24 and 48 hours, blood levels were equivalent to 0.12 μmol/L and 0.1 μmol/L, respectively. The total arterial tissue level of nPXL in the stented segments at 48 hours was 1.7±0.1 μg/mg of tissue.

Hematologic Profiles

Animals receiving nPXL at doses ≥3.5 mg/kg experienced a slight decrease in peripheral white blood cell counts, which reach nadir at 3 days (baseline=8.2±1.1×10³/mm versus 3 days=5.3±0.5×10³/mm); all values were within normal limits. Neutrophil counts at nPXL doses ≥2.5 mg/kg decreased approximately 3-fold (baseline=3.7±0.4×10³/mm versus 3 days=1.1±0.6×10³/mm). All hematologic profiles were similar to baseline values by 10 days.

Stent Injury Model

Twenty-Eight Days

At 28 days, there was a significant dose-dependent reduction in mean neointimal thickness with nPXL 2.5 to 5.0 mg/kg compared with control stents, respectively (Table, Figures 1 and 2). The neointima of stented arteries treated with nPXL 3.5 to 5.0 mg/kg showed incomplete healing at 28 days, with frequent areas of mild-to-severe focal fibrin and inflammation. Surface endothelialization was nearly complete (>90%) in control and nPXL 2.5-mg/kg stents. In contrast, focal nonendothelialized areas representing approximately 17±3% and 23±4% of the luminal surface with adherent inflammatory cell and platelets were found in animals receiving nPXL 3.5 and 5.0 mg/kg, respectively (P<0.01 versus control).

Ninety Days

At 90 days, however, a single 5.0-mg/kg dose of nPXL at the time of stenting was ineffective in reducing neointimal growth. In contrast, a single repeat dose of 3.5-mg/kg nPXL given 28 days after stenting resulted in sustained suppression of neointimal thickness at 90 days versus single-dose nPXL or controls. The neointima of control and single-dose 5.0-mg/kg nPXL stents was well healed. Similarly, the neointima at 90 days of stents treated with a single repeat dose of 3.5 mg/kg at 28 days showed near complete healing; only rare sections showed focal fibrin accumulation. SEM analysis...
demonstrated ≥94% endothelialization of luminal surfaces in all arteries independent of treatment.

**Discussion**

Systemic nPXL produced a dose-dependent reduction of neointimal growth at 28 days, accompanied by incomplete healing. Blood levels of approximately 0.1 μmol/L were achieved at 5.0-mg/kg doses and concentrations ≤5.0 mg/kg were well tolerated. A single nPXL dose (5.0 mg/kg) administered at the time of stenting failed to sustain neointimal growth suppression at 90 days. However, a repeat intravenous dose of 3.5 mg/kg at 28 days led to persistent reduction of
neointimal formation at 90 days with almost complete healing.

Both sirolimus- and PXL-eluting stents have been recently presented as a major advance in the prevention of restenosis. A small one-year study and subsequent large clinical trial with sirolimus reported a dramatic reduction of restenosis. Although preclinical animal studies with either of these agents have demonstrated efficacy at 28 days, long-term studies show inconsistent results (and Andrew J. Carter, MD, Providence Heart Institute, Providence, Ore, unpublished data, 2002; Alan W. Heldman, MD, Johns Hopkins University, Baltimore, Md, unpublished data, 2002). In humans, healing of a bare stainless steel stent usually takes between 3 and 6 months, whereas in animals this response is usually complete by 28 days. Although clinical trials with PXL-eluting stents (European evaluation of paclitaxel-eluting stent [ELUTES] and Perspective, Randomized, Double-Blind Comparison of NIR Stents Coated With Paclitaxel in a Polymer Carrier in De Novo Coronary Lesions Compared With Uncoated Controls [TAXUS I]) have shown promise at 6 months (unpublished data, 2001), long-term results are still needed.

Inhibition of neointimal growth by PXL is dependent on the dose and release kinetics of the stent polymer coating. Furthermore, the narrow therapeutic margin of PXL poses difficulties in providing adequate dosages to prevent neointimal proliferation while allowing healing and endothelialization. The strong hydrophobic character of the drug can lead to high arterial wall concentrations that exceed the bulk concentration. The resultant toxicity evidenced by inflammation and/or incomplete healing is most likely responsible for the lack of sustained suppression of neointimal growth with local delivery of PXL in animals.

In contrast, systemic nPXL has a relatively wide therapeutic index. Recent clinical trials in >300 patients with cancer suggest excellent tolerance at relatively high dosages when given intra-arterially or intravenously. In a Phase II clinical trial involving 43 patients who were administered nPXL at doses of 175 mg/m^2, the incidence of grade 4 neutropenia, a common toxicity in patients receiving chemotherapy, was only 7% (Nuhad K. Ibrahim, MD, Anderson Cancer Center, Houston, Tex, unpublished data, 2002). For the clinical prevention of in-stent restenosis, dosages of nPXL approximating 100 mg/m^2 are expected to achieve blood levels of $\geq 0.01$ μmol/L (effective concentrations for reduction of in vitro SMC proliferation and migration) up to 48 hours.

Systemic nPXL allows treatment of multiple stents with a single systemic injection. In addition, repeat doses could be implemented for optimization of neointimal suppression. Finally, the clinician would have the option to select the most appropriate stent for varying lesion lengths, artery sizes, and anatomic locations. In conclusion, these data emphasize the importance of repeat dosing for long-term efficacy of antirestenotic drugs. Systemic antirestenosis therapy that uses formulations with reduced toxicity may overcome some of the limitations of drug-coated stents.

References

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