

## PRE-CLINICAL RESEARCH

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# Evaluation of Efficacy and Dose Response of Different Paclitaxel-Coated Balloon Formulations in a Novel Swine Model of Iliofemoral In-Stent Restenosis

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**Objectives** The authors aimed to validate a novel iliofemoral in-stent restenosis (ISR) model for the efficacy evaluation of paclitaxel-coated balloons (PCB) using the familial hypercholesterolemic swine (FHS).

**Background** Most of the validation work regarding PCB technologies has been performed in the coronary territory of juvenile domestic swine. Although invaluable for safety evaluation, this model is not suited for the evaluation of the efficacy of peripheral PCB technologies.

**Methods** Twenty-four iliofemoral segments in 12 FHS underwent balloon injury and self-expanding stent placement. After 21 days, the resulting ISR lesions were treated with either 1  $\mu\text{g}/\text{mm}^2$  dose ( $n = 8$ ), or 3  $\mu\text{g}/\text{mm}^2$  dose ( $n = 8$ ) PCB (Cotavance, Bayer Pharma AG/MEDRAD, Indianola, Pennsylvania), or with an identical uncoated control balloon ( $n = 8$ ).

**Results** At termination (28 days after treatment), the percent diameter stenosis by quantitative vascular analysis in the control group was higher ( $31.2 \pm 13.7\%$ ) compared with the 1  $\mu\text{g}/\text{mm}^2$  ( $19.3 \pm 14.0\%$ , 38% reduction) and 3  $\mu\text{g}/\text{mm}^2$  ( $8.6 \pm 10.7\%$ , 72% reduction) PCB groups. Intravascular ultrasound analysis showed 36% (1  $\mu\text{g}/\text{mm}^2$  dose,  $p = 0.04$ ) and 55% (3  $\mu\text{g}/\text{mm}^2$  dose,  $p < 0.01$ ) reductions in neointimal volume stenosis. In the histological analysis, the control group showed the highest degree of percent area stenosis ( $65 \pm 14.3\%$ ). The reductions in percent area stenosis was 13.2% ( $p = 0.5$ ) and 26% ( $p = 0.04$ ) in the 1  $\mu\text{g}/\text{mm}^2$  and 3  $\mu\text{g}/\text{mm}^2$  dose groups, respectively.

**Conclusions** The FHS model of iliofemoral ISR demonstrated a dose-dependent effect on the inhibition of neointimal proliferation of a clinically validated PCB technology. This model represents a positive step toward the efficacy evaluation of PCB in the peripheral vascular territory. (J Am Coll Cardiol Intv 2012;5:1081–8) © 2012 by the American College of Cardiology Foundation

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Paclitaxel-coated balloon (PCB) technologies have been clinically introduced as an alternative therapeutic option to drug-eluting stents in selected clinical settings (1–5). Pre-clinical studies have shown that following balloon inflation, short-term transfer of paclitaxel is achieved and long-term tissue levels are maintained over time (6–9). Regardless of its clinical use in peripheral vessels, most of the pre-clinical validation work involving these technologies have been performed in the coronary territory of juvenile domestic swine (6–8). Although this model is well suited for the evaluation of device safety, it has inherent limitations when it comes to the evaluation of efficacy (10,11). Specifically, there are little data in regard to the optimal drug and coating formulation for the treatment of peripheral vessels. In the present study, we aimed to validate a novel iliofemoral in-stent restenosis (ISR) model for the efficacy evaluation of PCB using the familial hypercholesterolemic swine (FHS).

### Abbreviations and Acronyms

**%DS** = percent diameter stenosis

**EEL** = external elastic lamina

**FHS** = familial hypercholesterolemic swine

**IEL** = internal elastic lamina

**ISR** = in-stent restenosis

**IVUS** = intravascular ultrasound

**MLD** = minimal lumen diameter

**NV** = neointimal volume

**PCB** = paclitaxel-coated balloon(s)

**RVD** = reference vessel diameter

### Methods

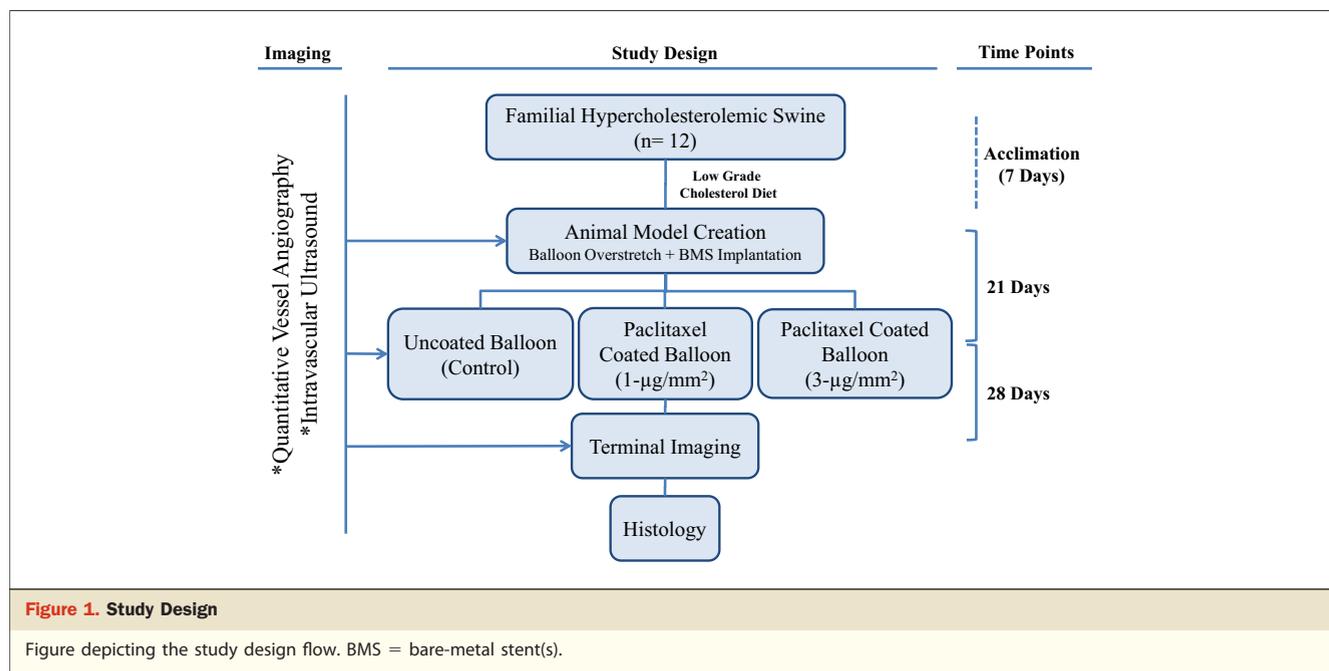
**Device description.** All balloons used in this study had identical material and manufacturing characteristics. In the PCB group, 2 different paclitaxel-iodopromide concentrations were tested (Fig. 1). One group included the original Cota-vance PCB with Paccocath technology at a regular dose (3  $\mu\text{g}/\text{mm}^2$ , Bayer Pharma AG/MEDRAD, Indianola, Pennsylvania) currently used in clinical practice in Europe. The other PCB group differed by the total paclitaxel concentration (1  $\mu\text{g}/\text{mm}^2$ ). The control group consisted of identical uncoated balloons. All balloons used in the study

were 6 or 7 mm  $\times$  20 mm, and the self-expanding stents implanted after initial balloon inflation were 6 or 7 mm  $\times$  20 mm (Luminexx stent, Bard Peripheral Vascular, Tempe, Arizona).

**Hypercholesterolemic swine model.** A total of 12 FHS (7.3  $\pm$  0.7 months of age) obtained from the University of Wisconsin Department of Animal Sciences were used in this study. For this study, the cholesterol levels of the animals at baseline varied from 301 to 652 mg/dl (mean cholesterol level: 409.3  $\pm$  93.4 mg/dl). The FHS carries a liver low-density lipoprotein receptor deficiency bearing a homozygous mutation in 1 allelic mutant gene, *Lpib5*, at the apolipoprotein B locus, and as a consequence, naturally develops hypercholesterolemia (>240 mg/dl) and atherosclerosis, even if maintained under a low-cholesterol, low-fat diet (12,13). Within 2 years, these animals develop complex coronary stenotic lesions containing fibrous caps,

necrotic cores, cholesterol clefts, granular calcium deposits, and neovascularization, among others (12,14). By the third year, plaque neovascularization and hemorrhage are common, with a high potential of plaque rupture (12,14). For this study, animals were maintained on a low-grade cholesterol diet (0.6%) to increase the cholesterol levels and to accelerate the disease process. Despite maintaining a low cholesterol diet, at treatment (21 days), the mean cholesterol level of the animals had increased by 104% (835.3  $\pm$  155.9 mg/dl). At terminal procedure (49 days), the cholesterol level was 931  $\pm$  173.6 mg/dl). In this study, relatively younger animals were used on the basis of previous studies that demonstrated the accelerated progression of neointimal proliferation following vascular injury despite the absence of significant atherosclerotic burden found at this age (15,16).

**In-stent restenosis model development.** The study was approved by the Institutional Animal Care and Use Committee of Skirball Center for Cardiovascular Research. All animals received standard care outlined in the study protocol and in accordance with the Animal Welfare Act and the “Guide for Care and Use of Laboratory Animals” formulated by the Institute of Laboratory Animal Resources (National Research Council, NIH Publication No. 85-23, revised 1996). A total of 12 castrated male juvenile FHS with a mean weight of 43.5  $\pm$  2.0 kg were included in this study. A week before the initial arterial injury of the iliofemoral territory, all animals were started on a low-grade, 0.6% cholesterol diet supplementation. Dual antiplatelet therapy consisting of clopidogrel (150-mg loading dose and 75-mg maintenance dose) and oral aspirin (325-mg loading dose and 150-mg maintenance dose) was initiated 1 day before the arterial injury procedure and maintained for the entire duration of the study. Seven days following diet supplementation (Day 0), general anesthesia was induced with xylazine and Telazol (tiletamine/zolazepam). After reaching an adequate anesthetic level, the animals were intubated and maintained with inhaled 1% to 3% isoflurane. Surgical access was obtained via the carotid artery with general sterile technique. Before catheterization, heparin (5,000 to 10,000 U) was injected to maintain an activated clotting time >250 s. Nitroglycerin was administered intra-arterially to prevent and relieve vasospasm. Intravascular ultrasound (IVUS) (iLab IVUS system; Boston Scientific, Natick, Massachusetts) was used for vessel sizing. Over-sized balloon inflation using 1.3:1.0 balloon-to-artery ratio was applied to the target area followed by self-expanding bare-metal stent implantation. After 21 days, control angiography and IVUS imaging of previously injured arterial segments were performed and then the PCB or control balloons were allocated to the target vessels per the treatment matrix. The balloons were inflated within the stented segment for a total of 60  $\pm$  2 s with a target of 120% overstretch. Four weeks later



(Day 49), terminal angiography and IVUS imaging of all treated sites were done.

**Angiographic analysis.** Quantitative vascular analysis was done at all time points using CAAS software (Pie Medical Imaging, Maastricht, the Netherlands). The following parameters were measured using the guiding catheter as a standard for calibration: minimal lumen diameter (MLD) within the treated segments, and reference vessel diameters (RVD) taken from the proximal and distal portions of the treated sites. The balloon and stent-to-artery ratios were calculated. Percent diameter stenosis (%DS) at pre-procedure (Day 21) and follow-up (Day 49) were calculated as:  $(1 - [MLD/RVD]) \times 100\%$ , and the late loss was calculated as  $MLD \text{ at follow up} - MLD \text{ at post inflation}$ .

**IVUS analysis.** IVUS pullback images were obtained and analyzed using a peripheral ultrasound catheter (Atlantis SR Pro 40 MHz Imaging Catheter, Boston Scientific) and a commercially available measurement analytical system (iLab, Boston Scientific). Using fluoroscopy, the IVUS catheter was placed distal to the site at which the devices were deployed, and the automated pullback was performed at a speed of 1 mm/s, covering 10 mm proximal and distal to the implantation site. The starting position of the IVUS catheter was determined by fluoroscopy and located by anatomical landmarks in live image during the pullback. EchoPlaque software version 3.0.48 (Indec Medical Systems, Santa Clara, California) was used for the analysis of the acquired imaging pullbacks. Cross-section measurements of the lumen area, stent area, and vessel area were applied to every 1 mm of the total 20-mm-long treated site to generate the following volumetric parameters: lumen

volume, stent volume, vessel volume, neointimal volume (NV), and percent volume stenosis.

**Histological analysis.** The histological analysis was conducted by an independent pathology laboratory (Alizee Pathology, Thurmont, Maryland). After obtaining terminal imaging, the animals were given a bolus of 10,000 U of heparin, unless the activated clotting time within the preceding 15 min was  $>1,000$ . The animals were then euthanized under anesthesia by intravenous injection of a commercially available euthanasia solution, Euthasol (Virbach AH, Inc. Fort Worth, Texas) 0.2 ml/kg. Subsequently, the treated vessels were harvested and then immersed in normal buffered formalin 10%. For light microscopy, all treated vessels were embedded in methylmethacrylate and then 40- to 50-  $\mu\text{m}$  sections from the proximal, mid, and distal portions of each stented segment were obtained. These sections were stained with hematoxylin and eosin, and elastic trichrome. The cross-sectional areas (external elastic lamina [EEL], internal elastic lamina [IEL], and lumen area) of each section were measured. These measurements were used to calculate vessel layer areas with the following formulas:  $\text{medial} = \text{EEL} - \text{IEL}$ ;  $\text{neointima} = \text{IEL} - \text{lumen}$ ;  $\text{percent area stenosis} = [1 - (\text{lumen area}/\text{IEL area})] \times 100$ .

The degree of vascular injury was evaluated according to a commonly used injury score (0 score: IEL intact, to 3 score: EEL lacerated with disruption of the media). Peritubular inflammation was scored according to the number of cells around the struts (0 score:  $<25\%$  struts with 10 or fewer inflammatory cells, to 4 score: 2 or more struts associated with granulomatous inflammatory reactions. Adventitial inflammation was scored from 0 (no inflammation

in adventitia) to 3 (moderate peripheral inflammatory infiltration or focally marked in >50% of adventitial area). Fibrin deposits were scored from 0 (none to focal residual fibrin involving any portion of the artery) to 3 (heavy deposition of fibrin involving >25% of the circumference of artery or surrounding >50% of stent struts). Endothelial cell coverage was scored according to the percentage of lumen circumference covered by the endothelium (0: <25%, 1: 25% to 75%, 2: 75% to 95%, and 3: >95%).

**Statistical analysis.** Statistical analysis was conducted using the statistical software SigmaPlot v. 11.0 (Build 11.2.0.5, SYSTAT Software, San Jose, California). Summary statistics; means and standard deviations were calculated using Microsoft Excel spreadsheets (Excel 2007, v12, Microsoft, Redmond, Washington). All continuous data were analyzed by conducting an analysis of variance (ANOVA) to determine significant difference ( $p < 0.05$ ) between groups. If a difference was detected by ANOVA, and the data were considered normally distributed and variances were homogeneous, a Holm-Sidak test was run to compare test subjects and the control. Otherwise, the data were analyzed by Kruskal-Wallis ANOVA and Dunn's test. For the semiquantitative (ordinal) histological data, the Kruskal-Wallis test (with Dunn's method) was performed. A value of  $p \leq 0.05$  was considered statistically significant.

## Results

**Angiographic analysis.** Before injury, the average angiographic vessel diameters were comparable among all groups (control:  $4.42 \pm 0.54$  mm,  $1 \mu\text{g}/\text{mm}^2$ :  $4.77 \pm 0.53$  mm, and  $3 \mu\text{g}/\text{mm}^2$ :  $4.59 \pm 0.92$  mm,  $p = 0.76$ ). Angiographic analysis demonstrated that the degree of injury achieved in all groups was comparable (balloon-to-artery ratio = control:  $1.25 \pm 0.1$ ,  $1 \mu\text{g}/\text{mm}^2$ :  $1.24 \pm 0.09$ , and  $3 \mu\text{g}/\text{mm}^2$ :  $1.34 \pm 0.15$ ,  $p = 0.11$ ). Also, the final stent-to-artery ratio was similar among all the groups (control:  $1.11 \pm 0.14$ ,  $1 \mu\text{g}/\text{mm}^2$ :  $1.11 \pm 0.1$ , and  $3 \mu\text{g}/\text{mm}^2$ :  $1.19 \pm 0.15$ ). A summary of angiographic data is presented in Table 1.

At the time of PCB treatment (21 days), all the baseline angiographic variables were comparable among all groups. The mean baseline %DS was similar among all treated groups (Table 1). In addition, the mean final balloon inflation diameters were also comparable (control:  $4.8 \pm 0.6$  mm,  $1 \mu\text{g}/\text{mm}^2$ :  $4.7 \pm 0.7$  mm, and  $3 \mu\text{g}/\text{mm}^2$ :  $5.0 \pm 0.8$  mm,  $p = 0.77$ ) when measured at their maximal inflation point, leading to a similar angiographic MLD immediately post vessel treatment (control:  $3.5 \pm 1.0$  mm,  $1 \mu\text{g}/\text{mm}^2$ :  $3.5 \pm 1.0$  mm, and  $3 \mu\text{g}/\text{mm}^2$ :  $3.7 \pm 0.8$  mm,  $p = 0.86$ ).

Terminal angiographic evaluation demonstrated a lower %DS in the  $3 \mu\text{g}/\text{mm}^2$  treatment group ( $p = 0.03$ ) and the  $1 \mu\text{g}/\text{mm}^2$  ( $p = 0.2$ ) compared with the control uncoated balloon. The  $3 \mu\text{g}/\text{mm}^2$  dose balloon reduced stenosis by

**Table 1. Angiographic Data at Baseline, Time of Balloon Treatment, and Last Follow-Up**

	Control Group Uncoated	$1 \mu\text{g}/\text{mm}^2$ Dose	$3 \mu\text{g}/\text{mm}^2$ Dose	p Values
Day 0 (baseline)	n = 8	n = 8	n = 8	
RVD, mm	$4.4 \pm 0.5$	$4.8 \pm 0.5$	$4.6 \pm 0.9$	0.76
BAR	$1.3 \pm 0.1$	$1.24 \pm 0.01$	$1.3 \pm 0.2$	0.11
SAR	$1.1 \pm 0.1$	$1.1 \pm 0.1$	$1.2 \pm 0.12$	0.06
Day 21 (procedure)	n = 8	n = 8	n = 8	
MLD, mm	$3.1 \pm 1.1$	$3.1 \pm 1.1$	$3.7 \pm 0.8$	0.53
%DS	$31.1 \pm 23.7$	$34.7 \pm 21.9$	$20.5 \pm 12.7$	0.46
Balloon diameter, mm	$4.8 \pm 0.6$	$4.7 \pm 0.7$	$5.0 \pm 0.8$	0.77
Post-treatment MLD, mm	$3.5 \pm 1.0$	$3.5 \pm 1.0$	$3.7 \pm 0.8$	0.86
Day 49 (follow-up)	n = 8	n = 8	n = 8	
MLD, mm	$2.8 \pm 0.48$	$3.7 \pm 0.7^*$	$4.1 \pm 0.8^*$	0.01
%DS	$31.2 \pm 13.7$	$19.3 \pm 14.1$	$8.6 \pm 11^*$	0.01
LL, mm	$0.6 \pm 0.9$	$-0.2 \pm 0.58$	$-0.5 \pm 0.8$	0.09

Values are mean  $\pm$  SD. \* $p < 0.05$  versus uncoated.  
%DS = percent diameter stenosis; BAR = balloon to artery ratio; LL = late loss; MLD = minimal lumen diameter; RVD = reference vessel diameter; SAR = stent-to-artery ratio.

72% compared with the control group, whereas the  $1 \mu\text{g}/\text{mm}^2$  dose reduced restenosis by 38%. In addition, the final MLD was significantly higher in the  $3 \mu\text{g}/\text{mm}^2$  ( $4.08 \pm 0.8$  mm) and  $1 \mu\text{g}/\text{mm}^2$  ( $3.69 \pm 0.73$  mm) groups compared with the control balloon ( $2.8 \pm 0.5$  mm,  $p = 0.01$ ). As a result, there was a significant reduction in angiographic late loss in both PCB groups compared with the uncoated balloon group (Table 1).

**IVUS analysis.** A summary of IVUS data is presented in Table 2. IVUS analysis revealed that the percentage volume stenosis at the time of PCB treatment (Day 21) was comparable among the groups (control:  $37.6 \pm 25.3\%$ ,  $1 \mu\text{g}/\text{mm}^2$ :  $38.8 \pm 26.1\%$ , and  $3 \mu\text{g}/\text{mm}^2$ :  $28.9 \pm 15.7\%$ ,  $p = 0.52$ ). At termination (49 days), both PCB groups showed significantly lower NV and percent volume stenosis as compared with the control group ( $p = 0.001$  for both parameters). In addition, the lumen volume at termination was significantly higher in both PCB groups compared with the control balloon group (Table 2, Fig 2). In the  $3 \mu\text{g}/\text{mm}^2$  dose group, both NV and % volume stenosis were reduced by 56% ( $p = 0.001$ ) compared with the control group. The  $1 \mu\text{g}/\text{mm}^2$  group showed 34.3% ( $p = 0.1$ ) and 35.7% ( $p = 0.04$ ) reductions in NV (Fig. 2B) and % volume stenosis, respectively. After adjustment for the degree of NV found at termination in comparison with the pre-treatment values (Day 21), compared with the control group ( $163.8 \text{ mm}^3 \pm 90$ ), the least change in neointimal proliferation was found in the  $3 \mu\text{g}/\text{mm}^2$  treatment group ( $2.4 \pm 74 \text{ mm}^3$ ,  $p = 0.008$ ), followed by the  $1 \mu\text{g}/\text{mm}^2$  group ( $31.1 \text{ mm}^3 \pm 97 \text{ mm}^2$ ,  $p = 0.02$ ). IVUS analysis demonstrated that the pattern of neointimal inhibition was homogeneous throughout the length of the stent in both PCB groups (Fig. 3).

**Table 2. Summary of IVUS Data at Time of Treatment and Last Follow-Up**

	Control Group Uncoated	1 $\mu\text{g}/\text{mm}^2$ Dose	3 $\mu\text{g}/\text{mm}^2$ Dose	p Values
Day 21	n = 7	n = 8	n = 8	
LV, $\text{mm}^3$	346.6 $\pm$ 166	334.2 $\pm$ 176	386.8 $\pm$ 127	0.89
SV, $\text{mm}^3$	547.6 $\pm$ 80	534 $\pm$ 103	539.1 $\pm$ 113	1
VV, $\text{mm}^3$	677.4 $\pm$ 118	667.2 $\pm$ 116	680.7 $\pm$ 162	0.98
NV, $\text{mm}^3$	201 $\pm$ 135	199.8 $\pm$ 132	152.2 $\pm$ 85	0.43
%VS	37.6 $\pm$ 25.3	38.8 $\pm$ 26.1	28.9 $\pm$ 15.7	0.52
Day 49	n = 8	n = 8	n = 8	
LV, $\text{mm}^3$	242.5 $\pm$ 71	383.2 $\pm$ 124*	485.8 $\pm$ 161*	0.02
SV, $\text{mm}^3$	594.3 $\pm$ 125	614 $\pm$ 130	640.4 $\pm$ 140	0.97
VV, $\text{mm}^3$	784.7 $\pm$ 180	807.7 $\pm$ 172	831.2 $\pm$ 185	0.98
NV, $\text{mm}^3$	351.7 $\pm$ 131	230.9 $\pm$ 115	154.7 $\pm$ 33*	0.001
$\Delta\text{NV}$	163.8 $\pm$ 90	31.1 $\pm$ 97	2.4 $\pm$ 74	—
%VS	58.3 $\pm$ 11.6	37.4 $\pm$ 17.0*	25.5 $\pm$ 8.6*	0.001

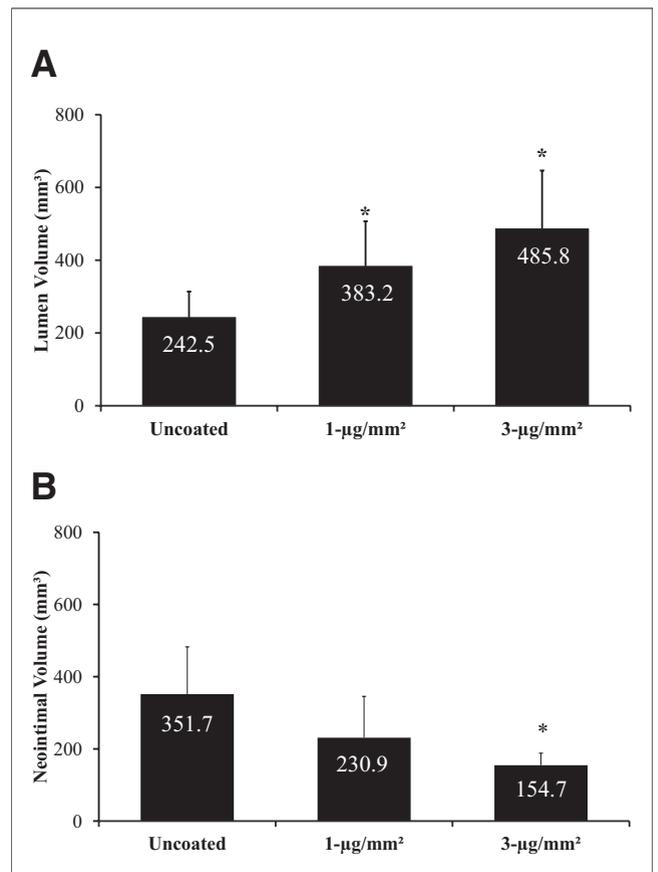
Values are mean  $\pm$  SD. The neointimal volume increase is presented as a difference between NV at Day 21 and NV at Day 49. \*p < 0.05 versus uncoated.  
%VS = percent volume stenosis; IVUS = intravascular ultrasound; LV = lumen volume; NV = neointimal volume; SV = stent volume; VV = vessel volume;  $\Delta\text{NV}$  = neointimal volume increase.

**Histological evaluation.** A summary of histological data is presented in Table 3. The histomorphometric analysis showed a constant IEL area (stent area) between all treatment groups (control;  $26.3 \pm 4.9 \text{ mm}^2$ ,  $1 \mu\text{g}/\text{mm}^2$ :  $27.3 \pm 4.0 \text{ mm}^2$ , and  $3 \mu\text{g}/\text{mm}^2$ :  $27.9 \pm 5.7 \text{ mm}^2$ ). Compared with the uncoated control, the  $3 \mu\text{g}/\text{mm}^2$  dose increased the lumen area by 65% ( $p = 0.04$ ) and decreased the percentage area stenosis by 26% ( $p = 0.04$ ). Meanwhile, in the  $1 \mu\text{g}/\text{mm}^2$  dose group, although there was a trend towards efficacy, the difference did not reach statistical significance (lumen area control:  $8.9 \pm 3.4 \text{ mm}$  vs.  $1 \mu\text{g}/\text{mm}^2$ :  $11.7 \pm 3.4 \text{ mm}$ ,  $p = 0.7$ ) and percent area stenosis (control:  $65.0 \pm 14.3\%$  vs.  $1 \mu\text{g}/\text{mm}^2$ :  $56.4 \pm 14.3\%$ ,  $p = 0.7$ ). The neointimal thickness of the control ( $1.3 \pm 0.5 \text{ mm}$ ) was slightly higher than that of  $1 \mu\text{g}/\text{mm}^2$  dose ( $1.01 \pm 0.4 \text{ mm}$ ,  $p = 0.5$ ) but considerably higher than the  $3 \mu\text{g}/\text{mm}^2$  dose ( $0.8 \pm 0.2 \text{ mm}$ ,  $p = 0.08$ ). No significant effect of treatment on EEL and medial area was observed (Table 3). The vessel wall injury was proportional between the control group ( $0.5 \pm 0.5$ ) and both test groups ( $1 \mu\text{g}/\text{mm}^2$ :  $0.4 \pm 0.5$ , and  $3 \mu\text{g}/\text{mm}^2$ :  $0.3 \pm 0.2$ ). There was a higher degree of fibrin deposits in the PCB ( $1 \mu\text{g}/\text{mm}^2$  dose:  $0.7 \pm 0.5$ , and  $3 \mu\text{g}/\text{mm}^2$  dose:  $0.6 \pm 0.4$ ) as compared with the control group ( $0.2 \pm 0.2$ ). The struts were almost completely covered with endothelium at 28 days follow-up in all studied groups as shown in Table 3. The average peristrut inflammation score was lower in the control group ( $0.5 \pm 0.9$ ,  $p = 0.83$ ) compared with the  $1 \mu\text{g}/\text{mm}^2$  dose ( $0.9 \pm 1.0$ ) and  $3 \mu\text{g}/\text{mm}^2$  dose ( $0.5 \pm 0.6$ ) groups. The mean adventitial inflammation in the control group ( $0.6 \pm 0.6$ ,  $p = 0.95$ ) was also comparable to that of the  $1$  and  $3 \mu\text{g}/\text{mm}^2$  doses ( $0.6 \pm 0.4$  and  $0.7 \pm 0.6$ ), respectively.

## Discussion

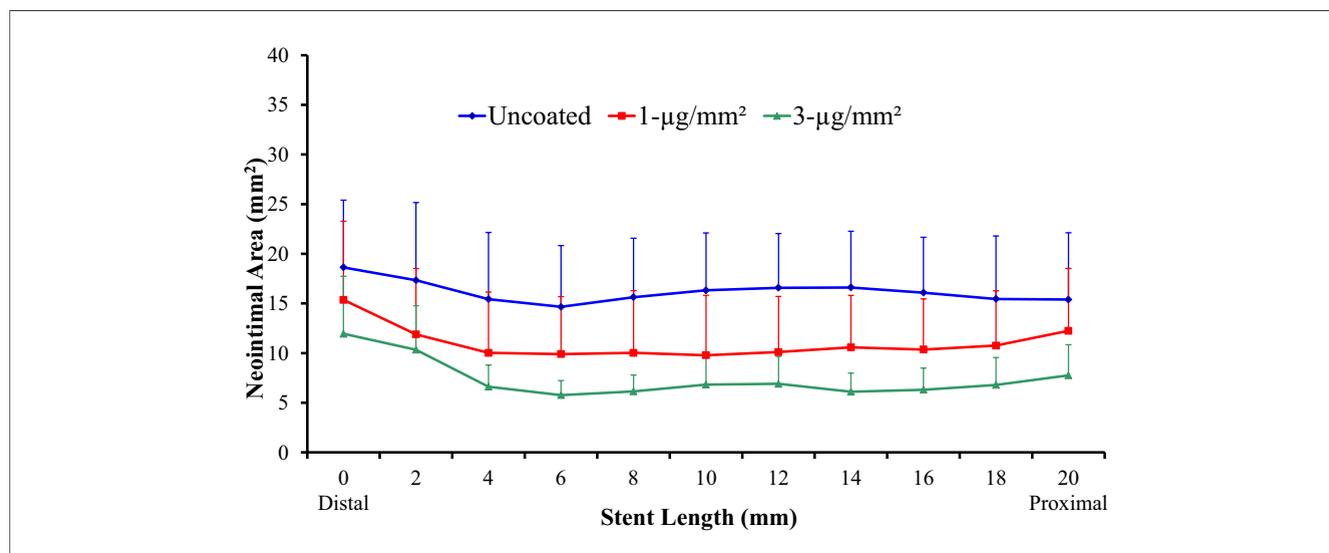
Although PCB technologies have been already tested in different experimental and human clinical trials (1–5,7), there are still little data in regard to the optimal dose and coating formulation for peripheral vascular applications. It has been proposed that the biological efficacy of PCB depends on the optimal development of a drug formulation and specific coating methods (17). Similarly, there are not enough published data to suggest that similar drug coating formulations previously validated in the swine coronary territory would be equally effective in peripheral vascular applications. The peripheral vascular territory is biologically different, and it is known to be subject to different mechanical forces and to react differently from endovascular therapies compared with other vascular beds (18).

Predicting clinical efficacy in nondiseased animal models has proven to be challenging (10,11). The biological nature and progression of neointimal formation in a normal swine artery greatly deviates from what is observed in human atherosclerotic



**Figure 2. Lumen Volume and Neointimal Volume at Last Follow-Up by IVUS**

There is a clear dose-response effect ( $1 \mu\text{g}/\text{mm}^2$  vs.  $3 \mu\text{g}/\text{mm}^2$ ) of both PCB technologies in comparison with the uncoated control group. (A) Lumen volume; (B) neointimal volume. \*p < 0.05. IVUS = intravascular ultrasound; NV = neointimal volume.



**Figure 3. Pattern of In-Stent Neointimal Distribution Along the Treated Segment Analyzed by IVUS**

The homogeneous distribution pattern suggests a uniform biological effect following initial delivery. IVUS = intravascular ultrasound.

lesions. Thus, in this study, we chose to use a diseased and clinically relevant model of ISR in the peripheral territory of the FHS. This model provides a metabolic profile and a vascular territory similar in diameter and anatomic configuration to the human peripheral arteries. However, due to the short duration of the study, low-grade cholesterol diet supplementation and vascular injury were added to accelerate the disease process. The use of balloon and stent overstretch has been previously described by Schwartz et al. (19) and subsequently has been extensively used for the validation of safety of stents in the swine. Previous data published by our laboratory have shown that the FHS model displays a different pattern of neointimal proliferation and healing following bare-metal stent coronary implantation compared with the domestic swine model (15,16). The utility of this model has been demonstrated in the evaluation of efficacy of drug-eluting stents (20) and peripheral drug-coated balloons (16). We then hypothesized that the combination of the intrinsic metabolic defect of the FHS and vessel wall injury would be sufficient to demonstrate efficacy differences following the use of different PCB treatment dosages when compared with an uncoated control balloon (Fig. 4).

The results of this study demonstrated a dose-dependent response on neointimal inhibition of the 2 tested PCB dosages as compared with the uncoated control balloons. This response was consistent and statistically different across all analytical methods used in this study. Both quantitative vascular analysis and IVUS analysis demonstrated a dose-dependent effect on inhibition of neointimal formation when compared with the uncoated control group. A considerably lower angiographic late loss was achieved in both paclitaxel-treated groups (1 µg/mm<sup>2</sup>:  $-0.2 \pm 0.56$  mm,

3 µg/mm<sup>2</sup> dose:  $-0.5 \pm 0.8$  mm) compared with the uncoated balloon group ( $0.6 \pm 0.9$  mm). However, the 1 µg/mm<sup>2</sup> dose PCB did not seem to be as effective as the 3 µg/mm<sup>2</sup> dose in inhibiting neointima. Although the 1 µg/mm<sup>2</sup> dose consistently reduced neointimal formation in comparison with the control uncoated balloon, it remained statistically inferior when compared with the 3 µg/mm<sup>2</sup> dose PCB. The angiographic findings seen using the 3 µg/mm<sup>2</sup> dose appears to be consistent with the findings seen in human trials using the same Cotavance Paccocath technology, whereby after 6 months, this dose reduced angiographic late loss by 50% ( $0.5 \pm 1.1$  mm) and 76% ( $0.4 \pm 1.2$  mm) in the FemPac (Femoral Paclitaxel) and THUNDER (Local Taxane with Short time Exposure for Reduction of Restenosis in Distal Arteries) trials, respectively (1,4).

At 49 days, the injury scores were similarly low in both tested groups, indicating homogeneity of the method used and the similar biocompatibility of the tested technology. The injury scores in the PCB group tended to be the response to both mechanical injury and drug effect. The degree of biocompatibility seemed to be confirmed by the low inflammation scores whose means were almost identical (<1.0 [ $<25\%$  with  $>10$  inflammatory cells] in all tested groups). Although no statistical difference was found among the groups, there was a trend towards greater fibrin deposits in both PCB groups as compared with the uncoated control group. The fibrin presence in the tissue is a typical finding for technologies that use antiproliferative agents, reflecting the biological effects of drug transfer to the vessel wall (21–24). The presence of fibrin at long term has been considered a marker of delayed healing, and it has been associated with adverse clinical events (25–27). In the

**Table 3. Histomorphometry and Qualitative Histological Analysis at Last Follow-Up**

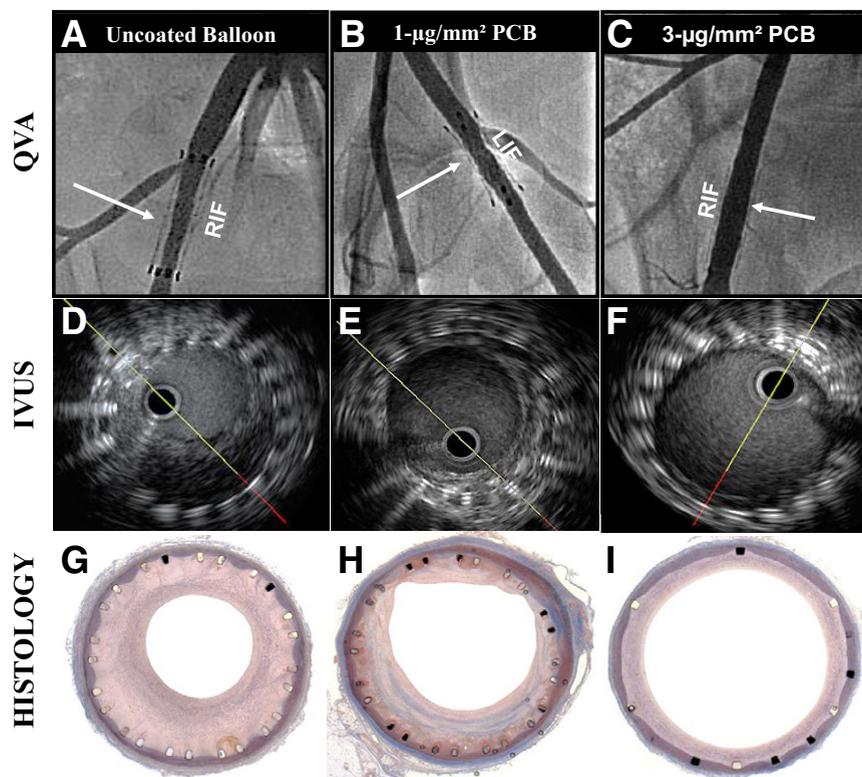
	Control Group Uncoated	1 $\mu\text{g}/\text{mm}^2$ Dose	3 $\mu\text{g}/\text{mm}^2$ Dose	p Value
Lumen area, $\text{mm}^2$	8.9 $\pm$ 3.4	11.7 $\pm$ 3.4	14.6 $\pm$ 4.3*	0.04*
IEL area, $\text{mm}^2$	26.3 $\pm$ 4.9	27.3 $\pm$ 4.0	27.9 $\pm$ 5.7	0.98
EEL area, $\text{mm}^2$	29.3 $\pm$ 5.2	30.4 $\pm$ 4.6	31.4 $\pm$ 6.6	0.96
Medial area, $\text{mm}^2$	3.0 $\pm$ 0.4	3.1 $\pm$ 1.0	3.5 $\pm$ 1.3	0.77
Neointimal area, $\text{mm}^2$	17.4 $\pm$ 7	15.6 $\pm$ 5.2	13.3 $\pm$ 3.2	0.37
% area stenosis	65.0 $\pm$ 14.3	56.4 $\pm$ 14.3	47.9 $\pm$ 8.8*	0.04*
Peristrut inflammation	0.5 $\pm$ 0.9	0.9 $\pm$ 1.0	0.5 $\pm$ 0.6	0.83
Adventitial inflammation	0.6 $\pm$ 0.6	0.6 $\pm$ 0.4	0.7 $\pm$ 0.6	0.95
Endothelial coverage	3.0 $\pm$ 0.1	2.9 $\pm$ 0.2	2.8 $\pm$ 0.4	0.42
Fibrin deposition	0.2 $\pm$ 0.2	0.7 $\pm$ 0.5	0.6 $\pm$ 0.4	0.13
Vessel wall injury	0.5 $\pm$ 0.5	0.4 $\pm$ 0.5	0.3 $\pm$ 0.2	0.96

Values are mean  $\pm$  SD. Percent area stenosis was determined by:  $(1 - \text{lumen area}/\text{IEL area}) \times 100$ .  
 \* $p < 0.05$  versus uncoated.  
 EEL = external elastic lamina; IEL = internal elastic lamina.

present study, fibrin scores were consistently low, with a maximal level of 0.7 (where 1.0 represents mild fibrin deposition involving <10% of the circumference of the

artery, or around <25% of stent struts). In addition, no negative effects of the drug formulation were found with regard to other histological parameters such as inflammation, IEL, EEL, and smooth muscle cell loss.

**Study limitations.** Several limitations were present in the current study. First, there are clear differences in the anatomical and biological composition between human atherosclerotic lesions and relatively normal arteries seen in the FHS. In addition, there are no established parameters to compare the efficacy results found in this study to clinical trial results, hence making it difficult to extrapolate these results to humans. However, it is important to highlight that the 3  $\mu\text{g}/\text{mm}^2$  dose PCB group, efficacious in this study, is the drug formulation successfully used in humans and presently used in clinical practice. Also, although long-term positive clinical data have been already presented using this technology, the long-term vascular effects of the use of this technology, especially in the setting of the chronic presence of self-expanding stents, have not yet been tested in this model. At the present time, the domestic swine remains the standard experimental model for the



**Figure 4. Representative Images at 49 Days Follow-Up in Angiography, IVUS, and Histology**

Representative images at 49 days follow-up in angiography (A, B, and C), intravascular ultrasound (IVUS) (D, E, and F), and histology (G, H, and I). The uncoated balloon group showed the highest degree of neointimal proliferation (A, D, and G) within stents (pointed arrows). An evident decrease in neointimal formation was observed in both the 1  $\mu\text{g}/\text{mm}^2$  (B, E, and H) and 3  $\mu\text{g}/\text{mm}^2$  (C, F, and I) paclitaxel-coated balloon (PCB) groups, the latter showing the highest degree of neointima inhibition. LIF = left iliofemoral artery; QVA = quantitative vascular angiography; RIF = right iliofemoral artery.

evaluation of device safety. This model is cost effective and well characterized for the validation of safety of coronary devices. However, the utility of the domestic swine model for the evaluation of device efficacy is very limited. Therefore, the FHS-ISR peripheral model may provide additional value in the validation of efficacy (i.e., dose response) of peripheral vascular technologies.

## Conclusions

In summary, a novel iliofemoral model of ISR based on the FHS demonstrated the biological efficacy of several PCB formulations in a dose-dependent fashion. The efficacy effect seems to be comparable to what has been presented in human clinical trials of superficial femoral artery intervention using this technology (1,4). The efficacy response observed in this study suggests that this model may represent a step forward towards the evaluation of efficacy of peripheral vascular technologies.

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**Key Words:** familial hypercholesterolemic swine ■ iliofemoral arteries ■ in-stent restenosis ■ paclitaxel-coated balloon.