Vascular Response to Zotarolimus-Coated Balloons in Injured Superficial Femoral Arteries of the Familial Hypercholesterolemic Swine

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Background—Drug-coated balloons are rapidly emerging as a therapeutic alternative for the interventional treatment of peripheral vascular disease. The purpose of this study was to test the hypothesis that an angioplasty balloon coated with the mTOR inhibitor zotarolimus (ZCB) would inhibit neointimal hyperplasia in a novel injury-based superficial femoral artery model in the familial hypercholesterolemic swine.

Methods and Results—A total of 44 familial hypercholesterolemic swine were included (12 designated to study tissue pharmacokinetics and 32 to study safety and efficacy). Fogarty balloon denudation was performed in all superficial femoral artery segments, followed by balloon angioplasty. In the pharmacokinetic study, a total of 24 ZCBs (300 μg/cm²) were used. Zotarolimus was detected in arterial tissue at 5 minutes (162 ng/mg of tissue), 24 hours (5.9 ng/mg of tissue), and 28 days (0.007 ng/mg of tissue) after ZCB inflation. In the safety and efficacy study, superficial femoral artery segments were randomized to either high-dose (600 μg/cm², n = 16), low-dose (300 μg/cm², n = 16), or paired uncoated balloons (high-dose ZCB control, n = 16; low-dose ZCB control, n = 16). At 28 days, the percentage of angiographic stenosis was similar among all tested groups. Histological analysis demonstrated a reduction in neointimal formation in both ZCB groups compared with controls (high-dose ZCB 44% reduction, P = 0.007; low-dose ZCB 22% reduction, P = 0.08). There was no evidence of delayed arterial healing or vascular toxicity in any of the ZCB groups.

Conclusions—The single delivery of zotarolimus via coated balloon is feasible, and therapeutic levels are maintained up to 28 days. The ZCB technology appears to be effective in the reduction of neointimal proliferation in the superficial femoral artery of the familial hypercholesterolemic swine. (Circ Cardiovasc Interv. 2011;4:447-455.)

Key Words: peripheral vascular disease • restenosis • zotarolimus • angioplasty balloon • hypercholesterolemic swine

In recent years, drug-coated balloons (DCBs) have emerged as a therapeutic alternative for the interventional treatment of peripheral vascular disease.1,2 With the use of this technology, the short-term transfer of antiproliferative drugs to the arterial wall appears feasible, thus potentially reducing the untoward effects of the prolonged drug release associated with polymer-based stent technologies.3-6 To date, most of the available data with regard to DCBs relate to the use of paclitaxel-based technologies.1,2,7-11 In small clinical trials, paclitaxel-coated balloons have been shown to be safe and effective in reducing restenosis among patients with coronary in-stent restenosis and de novo peripheral vascular disease.1,2,9,10 Because of its pharmacological and biological profile, zotarolimus has also been proposed as an antiproliferative agent for this particular application.12-14 The aim of the present study was to evaluate the pharmacokinetic profile, safety, and efficacy of a zotarolimus-coated balloon (ZCB) in the prevention of restenosis using a novel model of superficial femoral artery (SFA) stenosis in the familial hypercholesterolemic swine (FHS).

Balloon Description

The ZCB used in the present study consisted of an over-the-wire peripheral balloon catheter (6 × 40 mm; Abbott Vascular) coated with either 600 μg/cm² (labeled as “high dose,” 4.5 mg/balloon; HD-ZCB) or 300 μg/cm² (labeled as “low dose,” 2.3 mg/balloon; LD-ZCB) of zotarolimus. The control balloon was an identical balloon catheter (6 × 40 mm; Abbott Vascular) without the presence of a coating or a drug.
WHAT IS KNOWN

• Drug-coated balloons (DCB) are rapidly emerging as a viable therapy for the interventional treatment of peripheral vascular disease.
• To date, most of the data available with regard to DCB technologies relate to the use of paclitaxel.
• Although small clinical trials have demonstrated the safety and efficacy of this technology among patients with de novo peripheral vascular disease, there is still a need to investigate alternative drugs that display different pharmacological profiles.

WHAT THE STUDY ADDS

• In the present study, we showed for the first time that zotarolimus can be delivered effectively via DCB and that its tissue levels can be maintained over time.
• At 28 days, superficial femoral arteries of a hypercholesterolemic swine treated with a zotarolimus-coated balloon had a significant reduction in neointimal proliferation, with no evidence of delayed healing, compared with the uncoated-balloon group.

Hypercholesterolemic Swine Model

A total of 44 female FHS obtained from the University of Wisconsin, Department of Animal Sciences were used in the present study. The FHS carries a liver low-density lipoprotein receptor deficiency bearing a homozygous mutation in 1 allelic mutant gene, Lpb5 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.15,16 By 2 years, these animals develop eccentric lesions that consist predominantly of macrophage-derived foam cells with admixed smooth muscle cells. By the third year, large areas of necrosis, fibrous cap formation, mononuclear cell infiltration, and intraplaque hemorrhage are commonly seen in these lesions.17 All animals included in the study ranged from 6 to 8 months of age, with an average weight of approximately 45 kg at the time of enrollment.

Study Design

The study was approved by the Institutional Animal Care and Use Committee. All animals received standard care as described by the protocol and according to the Act of Animal Welfare and the “Principles of Care of Laboratory Animals” formulated by the Institute of Laboratory Animal Resources (National Research Council, NIH publication No.85-23, revised 1996). A total of 44 FHS were included (12 were designated to pharmacokinetic study and 32 to safety and efficacy study). A week before initial arterial injury, all animals were started on a low-grade cholesterol supplementation diet that contained 50% of Laboratory Porcine Diet Grower (LabDiet) and 50% of Mod Mini-Pig w/20%Lard/2%Chol/1.5%NaChol (TestDiet) to increase the cholesterol levels and to accelerate the disease process. 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. At day 7, after diet supplementation, animals were anesthetized with isolurane (1% to 3%) via face mask. Surgical access was obtained via carotid artery with a general sterile technique. Before catheterization, heparin (5000–10 000 U) was injected to maintain an activated clotting time >250 seconds. Nitroglycerin was administered intra-arterially to prevent or relieve vasospasm. A broad-spectrum antibiotic (1 g of intravenous cefazolin) was administered immediately before the procedure. SFA injury was induced by denudation of the endothelium with a Fogarty catheter (Arterial Embolectomy Catheter, 4F; Edwards Lifesciences) via carotid artery access (Figure 1). A total of 2 Fogarty balloon passes were applied in each artery, with efforts to maintain constant resistance during balloon withdrawal in an SFA segment 60 mm in length. After the Fogarty denudation, a single inflation of the test (ZCB) and control balloon was applied with the aim of achieving a balloon-to-artery ratio of 1.3:1.0. At the end of the procedure, catheters were removed, carotid arteries were ligated, and the surgical incision was repaired. Animals were allowed to recover and were housed until their designated day of euthanasia. Four of 12 animals designated for the pharmacokinetic study were euthanized 5 minutes after the balloon inflation. In that subgroup of animals, the ZCBs were inflated simultaneously in the left and right SFA for consistency of a very short 5-minute posttreatment follow-up.

Pharmacokinetic Study

The pharmacokinetic study was performed in a separate group of 12 FHS (4 animals with 8 treated sites per time point). Both contralateral SFAs were treated with the ZCB after initial arterial denudation. The arteries were harvested at 5 minutes, 24 hours, and 28 days after balloon inflation and then sectioned into 3 segments as follows: (1) Proximal region containing 10 mm of proximal untreated and 5 mm of treated artery; (2) mid region containing 30 mm of treated artery; and (3) distal region containing 5 mm of treated 10-mm distal artery. The tissue samples were placed in plastic vials, snap-frozen in liquid nitrogen, and stored at −70°C. On drug analysis, the arterial tissue specimens were thawed, weighed, and processed for the measurement of drug concentration by liquid chromatography and mass spectrometry.

Safety and Efficacy Study

A total of 64 arteries of 32 FHS (HD-ZCB=16 arteries, LD-ZCB=16 arteries, HD-ZCB paired uncoated control balloon...
group=16 arteries, and LD-ZCB paired uncoated control balloon group=16 arteries) were included in this study. Blood for plasma cholesterol analysis was collected at 3 time points: (1) At baseline (before the addition of the cholesterol-rich diet); (2) on the day of the SFA denudation and balloon treatment procedure (1 week after the cholesterol-rich diet was administered); and (3) at the study termination (28 days after the procedure). The blood drawn on the day of the procedure was taken before introduction of any of the tested devices into the vasculature. Immediately after Fogarty balloon denudation, all arterial segments were randomized to the inflation of either a ZCB or an identical uncoated control balloon with a predetermined 1:1 distribution. ZCBs and uncoated balloons were used in the contralateral SFA of the same animals. The order of low- and high-dose balloons and the order of treatment sites (right versus left) designated for ZCBs were randomized. Terminal angiography was performed at 28 days of follow-up before the animals were euthanized. Treated arterial segments were harvested for histology and histomorphometry analysis.

Angiographic Quantitative Vessel Analysis

Vessel angiographies were obtained with General Electric Innova digital flat panel angiographic units. Quantitative vessel angiography analysis was performed by an unblinded technician using QAngio XA software version 7.1.14.0 (Medis Medical Imaging Systems). The baseline (pre-ZCB treatment) and 28-day follow-up reference vessel diameters were taken from the proximal and distal portions of the treated segments with the guiding catheter used as a standard for measurement. The balloon-to-artery ratio was calculated. Percent diameter stenosis at follow-up was calculated as \[1 - \left(\frac{\text{minimum lumen diameter}}{\text{reference vessel diameter}}\right)\times 100\%\].

Tissue Harvesting

Target arteries were perfused with formalin before harvesting, and special attention was taken to precisely identify the balloon-treated segments with the support of angiograms and measurements of distances from the arterial side branches. There were 2 sets of sutures that marked the proximal and distal edges of each balloon segment. A line of blue ink was applied to the ventral surface of the right and left SFAs, including a 10-mm proximal segment, a balloon segment, and a 10-mm distal segment. The vessels were then sectioned into 6 separate blocks, with 4 middle blocks in the balloon-treated segment. All blocks were submitted for paraffin processing through a graded series of alcohol and xylene and were paraffin embedded. Each paraffin block was sectioned at 4- to 6-\(\mu\)m intervals, and 2 sections were stained with hematoxylin and eosin and elastin stain (Movat pentachrome).

Histology Evaluation

All sections were evaluated with a semiquantitative scoring system used at CVPath Institute (Gaithersburg, MD). To assess arterial injury and healing, ordinal data were collected for multiple parameters that included platelets/fibrin, proteoglycan/collagen, red blood cells (hemorrhage), calcification, and medial injury. These parameters were quantified with a scoring system of 0 to 4 (0=not identified, 1=trace, 2=mild, 3=moderate, and 4=severe). To evaluate the extent of these changes, the following description was used: For platelets/fibrin—0=none; 1=minimal, focal; 2=mild, multifocal; 3=moderate, regionally diffuse; and 4=severe, marked diffuse or total luminal occlusion; for proteoglycan/collagen deposits and adventitious fibrosis—0=none, 1=<25% of the area, 2=25% to 50% of area, 3=51% to 75% of area, and 4=>75% of area; for the extent of calcifications—0=none; 1=focal, with <10% of the region affected; 2=multifoical, with 10% to 25% of the region affected; 3=regionally with 25% to 30% of the region affected; and 4=regionally diffuse, with >30% of the region affected; for medial injury—0=none, 1=focal disruption of the internal elastic lamina (IEL), 2=widely diffuse disruption of the IEL, 3=complete medial disruption with containment (intact external elastic lamina (EEL)), and 4=complete disruption of the arterial wall involving the media and adventitia; and for hemorrhage—0=none; 1=focal, occasional; 2=minifocal and regional; and 3=regionally diffuse. Pathological changes of thrombosis and necrosis were also recorded. Morphometric measurements were performed on histological sections with computer-assisted software (IP Laboratory for Mac OS X, Scanalytics, Rockville, MD). The cross-sectional areas (outer adventitial border, EEL, IEL, and lumen area) of all sections for each artery were measured. The adventitial area, neointimal area, media area, and percent stenosis were calculated. The following formula was used to calculate stenosis: Stenosis (%) = 100×(1−lumen area/IEL area). The injury index was calculated with the following formula: (Length of IEL fragmentation/total IEL length)×100.

Statistical Analysis

Statistical analysis of angiographic data was performed with SigmaStat 3.1 software (Chicago, IL), and histological data were analyzed with JMP Statistical Software (Cary, NC). Paired t test of means was used as appropriate to calculate the significance of differences between 2 continuous variables. Values are expressed as the mean±SD. \(P \leq 0.05\) was considered statistically significant.

Although 2 tests were performed for each variable compared (low and high dose versus their respective controls), the statistical analysis performed did not control for multiple comparisons. Unpaired t test of means was used to evaluate individual group comparisons as a function of injury level. Score data, including fibrin/platelets, proteoglycans, calcification, adventitial fibrosis, medial injury, and red blood cells (hemorrhage), were expressed as medians with interquartile ranges and compared with a Mann-Whitney test.

Results

Pharmacokinetics Study

The average baseline cholesterol level at the time of balloon injury was 586±125 mg/dL. The mean inflation pressure in all treated sites was 9.5±22 atm. The pharmacokinetic profile of zotarolimus delivered via the balloon to the target arterial tissue is shown in Figure 2. Five minutes after balloon inflation, the mean arterial tissue concentration of zotarolimus was 162 ng/mg of tissue and then decreased to 5.9 ng/mg of tissue within 24 hours. At 28-day follow-up, the drug arterial tissue concentration was still at the detectable level of 0.007 ng/mg of tissue. At short-term follow-up (5 minutes and 24 hours), there was a concentration gradient between the proximal, mid, and distal regions of the treated segment with regard to tissue levels of zotarolimus (Figure 2). At 28 days,
Table 1. Zotarolimus Concentration in Arterial Tissue (ng/mg)

<table>
<thead>
<tr>
<th>Posttreatment Time</th>
<th>Proximal Region (n=8)</th>
<th>Mid Region (n=8)</th>
<th>Distal Region (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>5 min</td>
<td>17.30±10.89</td>
<td>218.57±162.95</td>
<td>251.17±279.35</td>
</tr>
<tr>
<td></td>
<td>(7.05–36.59)</td>
<td>(56.14–463.29)</td>
<td>(5.25–777.20)</td>
</tr>
<tr>
<td>24 h</td>
<td>0.24±0.18</td>
<td>2.22±3.72</td>
<td>15.23±39.54</td>
</tr>
<tr>
<td></td>
<td>(0.11–0.61)</td>
<td>(0.32–11.22)</td>
<td>(0.38–113.05)</td>
</tr>
<tr>
<td>28 d</td>
<td>0.008±0.002</td>
<td>0.007±0.006</td>
<td>0.007±0.008</td>
</tr>
<tr>
<td></td>
<td>(0.005–0.01)</td>
<td>(0.002–0.02)</td>
<td>(0.001–0.025)</td>
</tr>
</tbody>
</table>

Data expressed as mean±SD (including range).

however, the drug appeared to be homogeneously distributed throughout the length of the vessel segment (Table 1).

Safety and Efficacy Study

A total of 64 SFA arteries were treated successfully in 32 animals. All intended SFA vessels were treated with either low or high doses of the ZCB or the control balloon. The mean inflation pressure was 11±3.4 atm and did not differ between groups (P=0.42). Among the 64 treated peripheral sites, 21 (32.8%) displayed residual spasm, 11 (17.2%) intimal dissection, and 3 (4.7%) slow flow after balloon dilation. None of these acute periprocedural findings had a negative influence on the angiographic outcomes at follow-up, except in 1 animal (control group) in which the terminal angiography revealed total occlusion of the previously injured site (right SFA). The average baseline cholesterol level was 425±56 mg/dL in the HD-ZCB control group and 419±68 mg/dL in the LD-ZCB control group, which further increased to 795±279 and 791±310 mg/dL, respectively, at the time of balloon injury. At termination, cholesterol levels decreased to 310 mg/dL, respectively, at 28 days follow-up are shown in Figure 3. The average baseline cholesterol levels over time was related to the cholesterol-rich diet that was implemented through the duration of the study.

Angiographic Analysis

Representative angiograms of HD-ZCB, LD-ZCB, and control groups at day 0, right after balloon treatment, and at 28-day follow-up are shown in Figure 3. The average baseline vessel diameters were similar between the ZCB and control treated segments, with values of 4.13±0.48 mm for HD-ZCB, 4.03±0.46 mm for HD-ZCB uncoated controls (P=0.18), 4.21±0.28 mm for LD-ZCB, and 4.29±0.43 mm for controls (P=0.20). Balloon-to-artery ratios were also similar for the HD-ZCB and paired control at 1.25±0.10 and 1.27±0.10, respectively (P=0.30), and for the LD-ZCB and paired control at 1.27±0.07 and 1.24±0.09, respectively (P=0.16). Minimum lumen diameters at termination were similar among groups (HD-ZCB 2.84±0.61 mm versus controls 2.80±0.94 mm, P=0.44; and LD-ZCB 2.92±0.56 mm versus controls 3.03±0.61 mm, P=0.13). Diameter stenosis was also comparable at termination (HD-ZCB 17.15±10.3% versus controls 18.90±22.58%, P=0.39; and LD-ZCB 15.03±7.0% versus controls 14.74±9.7%, P=0.46). All arteries treated with ZCB were patent except 1 artery in the control group (contralateral to 1 of the pigs that received an HD-ZCB), which was occluded. After exclusion of this occluded control artery, the mean values of the angiographic variables analyzed, including vessel diameter (4.17±0.47 mm), balloon-to-artery ratio (1.25±0.10), minimum lumen diameter (3.02±0.60 mm), and diameter stenosis (14.16±8.16%), did not change significantly.

Histological Evaluation

The results of histological analysis are presented in Table 2, and representative histology slides of ZCB-treated and paired control vessels are presented in Figure 4. Atherosclerotic lesion classification for the histological segments included in the ZCB arms most commonly was described as adaptive...
Intimal thickening in response to injury or intimal xanthoma/fibrofatty plaques with various degrees of macrophage infiltration. There was 1 instance of pathological intimal thickening and a single section of a fibroatheromatous lesion with late necrosis contained by a mature fibrous cap in the HD-ZCB group. All injury scores were comparable among both treated and control groups. The neointimal response to treatment with HD-ZCB (600 \( \mu \text{g/cm}^2 \)) consisted of mild neointimal proliferation composed of smooth muscle cells in a proteoglycan-rich matrix with an atherosclerotic component of various degrees of “foamy” macrophage infiltration. Overall, neointimal growth appeared generally well organized and mild, resulting in an average mean stenosis of 23.5±7.6%, which was significantly less than control vessels at 41.2±20.1% (\( P=0.002 \)). Neointimal area was also significantly less, with a mean value of 0.83±0.37 mm\(^2\) compared with the control vessels at 1.49±0.84 mm\(^2\) (\( P=0.007 \); Figure 5). Neointimal area in the LD-ZCB group was 0.80±0.41 mm\(^2\) compared with the paired control group at 1.03±0.52 mm\(^2\) (\( P=0.08 \)). Mean stenosis was 26.9±12.2% in the LD-ZCB group compared with 27.6±14.5% (\( P=0.82 \)) in the paired control group. The degree of inhibition of neointimal formation was not statistically significant when the 2 ZCB groups were compared. In the HD-ZCB group, the efficacy differences were maintained after the occluded vessel from the control group (right superficial femoral artery) was excluded from the analysis. A separate analysis with adjustment by degree of vascular injury was also performed (Figure 6). In all groups, approximately one third of all analyzed samples had evidence of IEL disruption. In addition, focal medial rupture and complete disruption of the EEL was observed in 9 (56%) of 16 vessels in the HD-ZCB group, 5 (31%) of 16 vessels in the LD-ZCB group, and 12 (38%) of 32 vessels

Table 2. Histomorphometry and Histology Analysis of the ZCB and Contralateral Control Groups (Paired \( t \) Test)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LD-ZCB Uncoated Controls (n=16)</th>
<th>LD-ZCB (n=16) (P vs Controls)</th>
<th>HD-ZCB Uncoated Controls (n=16)</th>
<th>HD-ZCB (n=16) (P vs Controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adventitia area, mm(^2)</td>
<td>4.31±1.28 (0.99)</td>
<td>4.60±1.14 (0.95)</td>
<td>4.62±1.37 (0.95)</td>
<td></td>
</tr>
<tr>
<td>EEL area, mm(^2)</td>
<td>7.30±2.15 (0.002)</td>
<td>7.00±2.57 (0.16)</td>
<td>6.29±1.60 (0.16)</td>
<td></td>
</tr>
<tr>
<td>IEL area, mm(^2)</td>
<td>4.44±1.84 (0.004)</td>
<td>4.05±2.26 (0.36)</td>
<td>3.66±1.38 (0.36)</td>
<td></td>
</tr>
<tr>
<td>Lumen area, mm(^2)</td>
<td>3.41±1.92 (0.01)</td>
<td>2.53±1.32 (0.44)</td>
<td>2.56±1.90 (0.44)</td>
<td></td>
</tr>
<tr>
<td>Medial area, mm(^2)</td>
<td>2.86±0.42 (0.002)</td>
<td>2.56±0.37 (0.01)</td>
<td>2.95±0.48 (0.01)</td>
<td></td>
</tr>
<tr>
<td>Neointimal area, mm(^2)</td>
<td>1.03±0.52 (0.08)</td>
<td>0.80±0.41 (0.07)</td>
<td>1.49±0.84 (0.07)</td>
<td></td>
</tr>
<tr>
<td>Area stenosis, %</td>
<td>27.60±14.46 (0.82)</td>
<td>26.91±12.22 (0.82)</td>
<td>41.22±20.05 (0.002)</td>
<td></td>
</tr>
<tr>
<td>Fibrin/platelets*</td>
<td>0.00 (0.13) (0.98)</td>
<td>0.00 (0.25) (0.98)</td>
<td>0.13 (0.28) (0.4)</td>
<td></td>
</tr>
<tr>
<td>Proteoglycans/collagen*</td>
<td>1.69 (0.78) (0.94)</td>
<td>2.13 (1.19) (0.52)</td>
<td>2.19 (1.16) (0.52)</td>
<td></td>
</tr>
<tr>
<td>Calcification*</td>
<td>0.00 (0.00) (0.26)</td>
<td>0.00 (0.00) (0.26)</td>
<td>0.00 (0.00) (0.26)</td>
<td></td>
</tr>
<tr>
<td>Adventitial fibrosis*</td>
<td>0.44 (1.16) (0.67)</td>
<td>0.69 (1.69) (0.96)</td>
<td>1.06 (1.97) (0.96)</td>
<td></td>
</tr>
<tr>
<td>Medial injury*</td>
<td>0.75 (1.97) (0.5)</td>
<td>1.56 (1.63) (0.78)</td>
<td>1.38 (1.19) (0.78)</td>
<td></td>
</tr>
<tr>
<td>Injury index*</td>
<td>20.52±6.05 (0.29)</td>
<td>24.66±5.39 (0.29)</td>
<td>18.58±4.38 (0.82)</td>
<td></td>
</tr>
<tr>
<td>Adventitial RBCs*</td>
<td>0.00 (0.00) (0.16)</td>
<td>0.00 (0.00) (0.16)</td>
<td>0.06 (0.13) (0.16)</td>
<td></td>
</tr>
<tr>
<td>Medial RBCs*</td>
<td>0.00 (0.16) (0.49)</td>
<td>0.00 (0.28) (0.5)</td>
<td>0.00 (0.13) (0.5)</td>
<td></td>
</tr>
</tbody>
</table>

ZCB indicates zotarolimus-coated balloon; LD-ZCB, low-dose ZCB; HD-ZCB, high-dose ZCB; EEL, external elastic lamina; IEL, internal elastic lamina; and RBCs, red blood cells.

Data are expressed as mean±SD or median (interquartile range) with \( P \) vs controls shown for the ZCB groups.

*See “Histology Evaluation” in Methods for a description of the scoring system used for these parameters.
in the control group. In this subanalysis, only HD-ZCB was shown to be efficacious compared with the control group, which suggests a potential dose-dependent response (Figure 6). However, the resulting sample size was small and did not allow for comparison between the 2 ZCB groups.

The biological effect attributed to the delivery of zotarolimus consisted of medial smooth muscle cell loss with corresponding proteoglycan deposition. Medial areas in both ZCB groups (HD-ZCB 2.62±0.43 mm², LD-ZCB 2.56±0.37 mm²) were significantly less than in the control groups (HD-ZCB control 2.95±0.45 mm², P=0.01; LD-ZCB control 2.86±0.42 mm², P=0.002). The higher percentage of EEL disruption in the HD-ZCB group was associated with the greater fibrin deposition and higher number of adventitial red blood cells (Table 2). There was mild to occasionally moderate fibrin deposition in 9 (56%) of 16 vessels in the HD-ZCB group. The more regionally moderate deposition usually appeared in sections with large areas of medial and EEL injury. Mean fibrin score for this group was comparable to that of the control group. There was also mild to occasionally moderate fibrin deposition in 7 (44%) of 16 arteries treated in the LD-ZCB group and in 6 (37%) of 16 in the respective control vessels. In this group, the mean fibrin score for the treated group was comparable to that in the paired control arm. High-magnification qualitative analysis showed mature neointima and endothelial cells covering all analyzed vascular segments.

Discussion

Most of the data available today regarding DCB technologies are based on the use of paclitaxel as the antiproliferative

![Figure 4. Representative typical histological appearance of the treated superficial femoral artery segments with zotarolimus-coated balloon compared with contralateral controls at 28-day follow-up (magnification ×2, Movat pentachrome staining). LD-ZCB indicates low-dose zotarolimus-coated balloon; HD-ZCB, high-dose zotarolimus-coated balloon.](http://circinterventions.ahajournals.org/)}
peripheral vascular disease lesions.1,2 Zotarolimus has also been found to have in vivo chemical features that support its antiproliferative effect and prolonged tissue retention.18–21 In small in vitro studies have demonstrated that zotarolimus displays a high octanol:water partition coefficient compared with other antiproliferative agents.13 This property appears to enhance tissue uptake after delivery and facilitates its rapid crossing through cell membranes. There is recent evidence that suggests the biological efficacy of this delivery method. A recent publication demonstrated that the delivery of bare-metal stents premounted on a balloon coated with a specific ZCB formulation effectively reduced neointimal proliferation compared with a control group in healthy porcine coronary arteries.22

The aim of the present study was to test the safety, efficacy, and pharmacological profile of a different ZCB formulation in the SFA territory of the FHS model. The study showed that after short-term exposure, zotarolimus could be transferred efficiently into the arterial wall, and therapeutic levels can be maintained over time. The initial peak in arterial tissue concentration was achieved at 5 minutes after delivery. Then, zotarolimus tissue levels steadily declined within 24 hours and remained detectable up to 28 days. This pharmacokinetic profile displays a reduction in drug concentration in the arterial tissue during the first 24 hours after delivery appears to be typical for all DCB technologies, and it appears comparable to other paclitaxel-coated balloon technologies.23 Most DCB technologies have been tested with a healthy porcine model of restenosis. In addition, most of the DCB technologies used in human clinical trials among patients with SFA disease were tested in the normal porcine restenotic model, in which bare-metal stents were premounted on paclitaxel-coated balloons.7,8 Although well validated for evaluation of safety, those models are limited in the evaluation of efficacy and vessel healing after drug delivery, especially in the SFA territory.24 Therefore, in the present study, we decided to use the hypercholesterolemic swine (FHS) model, which displays a vascular territory similar in vascular diameters and anatomic configuration to the human SFA. The iliofemoral artery of the FHS, although slightly shorter than the human, has a vessel diameter that permits the testing and validation of peripheral devices. This strain of pigs maintains high cholesterol levels despite regular diet supplementation. Several validation studies have described the progression of disease over time.16,17,25 By 18 months, these animals develop complex atherosclerotic lesions with pathological features similar to human disease.16,17 Because of the relatively short follow-up in the present study, an additional low-grade diet supplementation was provided to slightly increase the cholesterol levels and to accelerate the disease process. At follow-up, the cholesterol levels increased in a stable fashion, and the levels remained similar between the 2 groups studied. We hypothesized that by 8 months (the age of animals included in the study), the nature of the disease found (fatty streaks and occasional pathological intimal thickening) would be sufficient to demonstrate an efficacy signal after single balloon injury.

The safety and efficacy arm of the present study was designed to evaluate 2 different drug doses (high and low) and an uncoated control group. Baseline vessel diameters measured by quantitative vessel angiography were identical for all treatment sites, which ensured the appropriate sizing of all devices. Initial injury for induction of neointimal proliferation was created by denudation of the endothelium with a Fogarty catheter according to a similar protocol previously published for rabbit models.26–28 Quantitative vessel angiography analysis revealed that the mean balloon overstretch (balloon-to-artery ratio) in all treated vessels was similar within all studied groups. This parameter indicates accurate and uniform balloon injury and reinforces the validity of comparisons between the groups. At 28 days, treatment of the SFA with ZCB was safe and effective in this model. At termination, there was no angiographic evidence of toxic

Figure 5. Histological evaluation of neointimal area in zotarolimus-coated balloon groups vs control noncoated angioplasty balloon groups at 28-day follow-up (paired t test). Error bars represent SD. LD-ZCB indicates low-dose zotarolimus-coated balloon; HD-ZCB, high-dose zotarolimus-coated balloon.

Figure 6. Relationship between vessel wall injury and percent- age area of stenosis by histology compared with controls. LD-ZCB indicates low-dose zotarolimus-coated balloon; HD-ZCB, high-dose zotarolimus-coated balloon; IEL, internal elastic lamina; and EEL, external elastic lamina.
vascular effects in any of the treated segments, such as vessel aneurysm or thrombus formation. Qualitative vascular angiography showed no differences with regard to minimal lumen diameters or percentage diameter stenosis in any of the ZCB groups compared with controls. In the HD-ZCB group, these angiographic variables did not change once the occluded artery in the control group was excluded. The lack of angiographic efficacy seen at follow-up is not a surprising finding because in the absence of stents, angiographic lumen loss over time tends to be small, and the potential for detection of small changes in neointimal formation is out of the resolution range of angiography.

Histomorphometric analysis at 28 days revealed that both ZCB formulations resulted in lower degrees of neointimal proliferation than their paired controls; however, a statistically significant decrease in the amount of neointimal formation and reduced area stenosis was only achieved in the HD-ZCB group. In addition, both ZCB formulations displayed a lower total medial area and increase of fibrin deposition in ZCB-treated arteries, which was more prominent in the ZCB-HD group. These findings suggest a dose-response behavior and are a typical response related to the antiproliferative effect of the drug, as previously described in several publications on drug-eluting stents. Although all of these parameters were higher in both ZCB groups, no statistical differences were found when both ZCBs were compared. However, a subanalysis of the data showed that arterial segments with the highest degree of injury (EEL disruption) displayed a higher proportion of neointimal inhibition, medial cell loss, and fibrin deposition. This delayed healing response appears to be the result of the delivery of antiproliferative drugs in vascular segments that had high degrees of injury and appears to be dose-dependent, because it was more commonly found in the HD-ZCB group (Figure 6). However, the resulting sample size was small and did not allow for statistical comparisons between the 2 ZCB groups.

At termination, the resulting neointima covering the analyzed segments was mature and showed complete endothelial coverage in all treated groups. This pattern of complete endothelialization in the setting of drug delivery has been reported previously in studies evaluating the safety of drug-eluting stents in the normal porcine artery. In the present study, this finding is not surprising, because the injury induced was relatively low (Table 2) and involved arteries with a low burden of disease. However, the resulting histological patterns of vascular healing were favorable and not particularly different from those described for other drug delivery–based technologies. However, the evaluation of surface endothelialization may be a challenging task, and although descriptive analysis showed complete vessel-surface coverage, the limitations of all potential assessments of mature endothelial coverage make it possible that the resulting endothelial lining was either lacking or dysfunctional.

It has been proposed that the overall effectiveness of any DCB technology depends on the particular drug formulation and the coating method. Although the present study supports the short-term safety and efficacy of this particular zotarolimus formulation in the FHS model, the long-term effects on healing and restenosis need to be further investigated. In addition, the impact of several atherosclerotic components on drug transfer and pharmacokinetics is still unknown. Despite the modest proliferative response seen in the control arm, an efficacy signal was achieved in both ZCB groups. A recent publication showed the efficacy of a different ZCB formulation by obtaining a higher degree of histological stenosis in the control arm by the use of stents in the healthy porcine coronary artery. In the present study, in the absence of a stent, the control group displayed a modest degree of histological stenosis, which makes it more challenging to evaluate the efficacy. It is plausible that if a more robust stenotic response could have been elicited in the control arm, more obvious differences in efficacy and healing could have been found between the doses tested.

In summary, the use of a ZCB in the SFA territory of the FHS was safe and effective at 28 days after initial injury. The ZCB technology efficiently delivered zotarolimus to the arterial wall, and therapeutic levels were maintained over time. Both ZCB formulations showed a positive vascular effect by reducing neointimal proliferation after vascular injury with no evidence of delayed healing or excessive fibrin deposition. The potential of this technology as a promising alternative for the treatment of peripheral vascular occlusive disease in humans needs to be determined.

Sources of Funding
This research was funded by Abbott Vascular (Santa Clara, CA).

Disclosures
Drs Zhao, Stankus, Schwartz, and Nikanorov are employees of Abbott Vascular, and Dr Virmani is a consultant to Abbott Vascular. The remaining authors report no conflicts.

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Vascular Response to Zotarolimus-Coated Balloons in Injured Superficial Femoral Arteries of the Familial Hypercholesterolemic Swine

Circ Cardiovasc Interv. 2011;4:447-455; originally published online September 27, 2011; doi: 10.1161/CIRCINTERVENTIONS.110.960260

Circulation: Cardiovascular Interventions is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-7640. Online ISSN: 1941-7632

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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