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Original Studies

Experimental Evaluation of Pharmacokinetic Profile and Biological Effect of a Novel Paclitaxel Microcrystalline Balloon Coating in the Iliofemoral Territory of Swine

Piotr P. Buszman,[1,2*] MD, PhD, Krzysztof Milewski,[1] MD, PhD, Aleksander Żurakowski,[1] MD, PhD, Jacek Pajak,[3] MD, PhD, Michał Jelonek,[1] MD, Paweł Gasior,[1,3] Athanasios Peppas,[2] MS, Armando Tellez,[2] MD, Juan F. Granada,[2] MD, and Paweł E. Buszman,[1] MD, PhD

Background: New paclitaxel coated balloons (PCB) developments have been proposed to maintain therapeutic levels of drug in the tissue while decreasing particle release. In this series of studies, we evaluated the pharmacokinetic profile and biological effects after paclitaxel delivery via novel microcrystalline PCB coating (mcPCB, Pax®, Balton) in porcine iliofemoral arteries. Methods: Ten domestic swine were enrolled yielding 24 iliofemoral segments for evaluation. In the pharmacokinetic study, nine mcPCBs were dilated for 60 sec and animals sacrificed after 1 hr, 3 and 7 days. Studied segments were harvested and tissue paclitaxel concentration was analyzed utilizing HPLC. In the biological response evaluation, self-expandable stents were implanted followed by post dilation with either mcPCB ($n = 10$) or POBA ($n = 5$). After 28 days, angiography was performed, animals were sacrificed and stented segments harvested for histopathological evaluation. Results: The 1-hr, 3 and 7 days vessel paclitaxel concentrations were 152.9 ± 154.5, 36.5 ± 49.5, and 0.9 ± 0.7 ng/mg respectively. In the biological response study, stents in the mcPCB group presented lower angiographic measures of neointimal hyperplasia as expressed by late loss when compared to POBA (-0.43 ± 0.9 vs. 0.23 ± 1.2; $P = 0.24$) at 28 days. In the histopathological evaluation, percent area of stenosis (%AS) was reduced by 42% in the mcPCB group ($P < 0.05$). The healing process in mcPCB group was comparable to POBA with regard to fibrin deposition (0.7 vs. 0.7; $P =$ ns), neointimal maturity (1.97 vs. 1.93; $P =$ ns), inflammation score (0.92 vs. 1; $P =$ ns) and endothelialization score (1.77 vs. 1.73; $P =$ ns). The mcPCB group did however display a greater tendency of medial cell loss and mineralization (60% vs. 0; $P = 0.08$).

[1]American Heart of Poland, Center for Cardiovascular Research and Development, Katowice, Poland
[2]Cardiovascular Research Foundation, The Skirball Center For Cardiovascular Research, Orangeburg, New York
[3]Medical University of Silesia, Katowice, Poland

*Correspondence to: Piotr P. Buszman, Center for Cardiovascular Research and Development, American Heart of Poland Inc, 41 Czajek str., Katowice. E-mail: p.buszman@ahp-ccrd.org

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Conclusions: Delivery of paclitaxel via a novel mcPCB resulted in low long-term tissue retention of paclitaxel. However, this technological approach displayed reduced neointimal proliferation and favorable healing profile. © 2013 Wiley Periodicals, Inc.

Key words: peripheral vascular disease; drug delivery; restenosis; paclitaxel coated balloon

INTRODUCTION

Paclitaxel coated balloon (PCB) technologies have been clinically introduced as an alternative therapeutic option to bare metal (BMS) and drug eluting stents for the revascularization of in stent restenosis (ISR) [1,2] and de novo peripheral artery disease [3,4]. Following these promising results, utilization of PCB has been further extended to other applications such as de novo coronary lesions with or without stent support [5–8]. Conversely, the routine use of PCB with BMS is debatable and requires further investigation [8], leaving the field for the improvement in current technology. Although clinically effective, the manufacturing process of first generation PCB coatings contributed to inconsistent drug concentrations, particulate formations on the balloon surface and their shedding during the interventional procedures. As a consequence developments of new PCB coatings have been proposed to address consistency, uniformity, small particle drug coverage, which may potentially contribute to improved vessel healing profile and improved clinical outcomes. Nevertheless, data on the safety and efficacy of this novel coating developments both in preclinical and clinical setting remain limited. Therefore in a series of studies, we evaluated tissue paclitaxel uptake, retention and vascular response to a novel microcrystalline PCB in porcine iliofemoral arteries.

METHODS

Device Description

The test article evaluated in this study was an over-the-wire balloon catheter coated with a microcrystalline form of paclitaxel at a dose of 3 μg/mm^2 and a proprietary excipient (PAX®, Balton, Warsaw, Poland). The coating of this device is applied by semiautomatic microsyringe surface drug deposition and a proprietary drying process which allows for more consistent, uniform and microparticle paclitaxel coverage (Control articles were identical uncoated angioplasty over-the-wire balloons (Neptun®, Balton Warsaw, Poland). Nitinol self-expandable stents (Jaguar®, Balton, Warsaw, Poland) were used in the vascular response study to induce injury and neointimal proliferation. All devices were 5–7 mm in diameter and 20 mm in length.

Study Schematics

Study scheme is presented in Fig. 1. In total 10 animals of either gender ranging between 35 and 40 kg were enrolled. In the paclitaxel tissue transfer study, three domestic swine (DS) of either gender were enrolled in which nine iliofemoral segments were tested. In the following vascular response experiment, seven DS were enrolled yielding 15 iliofemoral segments.

Experimental Procedures

All experiments were approved by the local bioethical committee for animal research and animals received a standard of care in accordance with the act of animal welfare and the "Principles of Care of Laboratory Animals" [9].

Prior to intervention each animal received oral 325 mg aspirin and 150 mg clopidogrel bisulfate 1 day prior to the start of the study. Antiplatelet therapy was continued with 75 mg aspirin and 75 mg clopidogrel bisulfate administered orally before anesthesia. Animals were sedated with intramuscular ketamine hydrochloride (20 mg/kg) and xylazine (2 mg/kg), and anesthesia was induced with an intravenous administration of propofol (20–40 mg). After reaching an adequate anesthetic level, the animals were intubated and maintained with intravenous continuous propofol infusion (2–4 mg/kg/hr). A vascular sheath (6 or 7 F) was placed in the right or left superficial femoral artery. Before catheterization, heparin (5,000–10,000 U) was injected to maintain an activated clotting time $>$250 sec. Nitroglycerin was administered intra-arterially to prevent and relieve vasospasm, and the iliofemoral arteries were subject to quantitative vascular angiography (QVA). Experimental procedures were performed with intention to test two contralateral iliofemoral arteries and one access site iliac segment (three sites per animal). At termination the animals were humanely euthanized with an approved euthanasia solution and the peripheral vessels were carefully harvested for paclitaxel or histopathological analysis.

Pharmacokinetic Study

In the pharmacokinetic study, a total of nine Iliofemoral arterial segments from three animals (three per

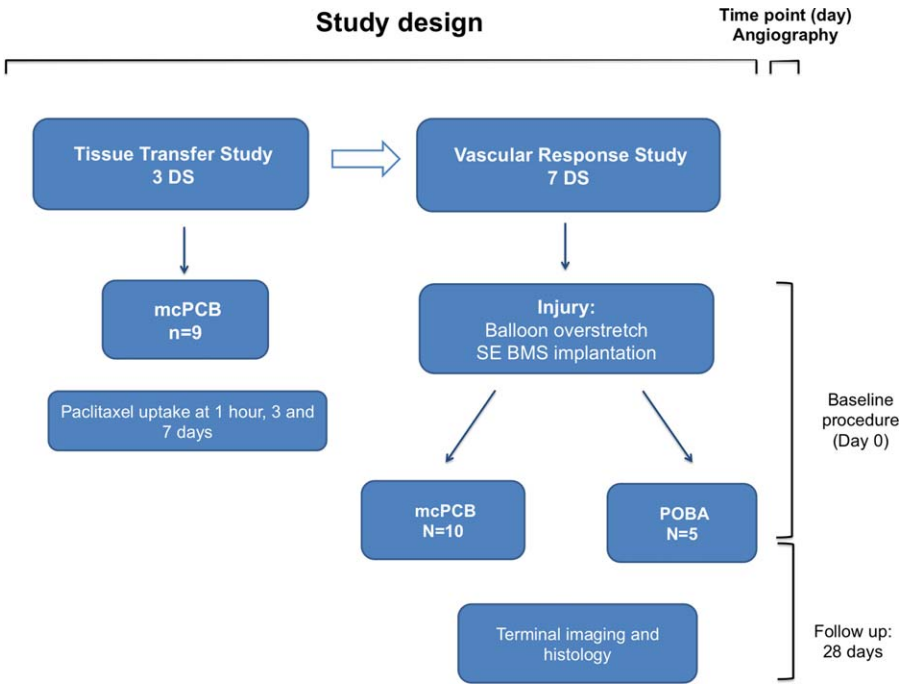

Fig. 1. Figure depicting study flow. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

animal) were mapped using QVA to correctly size the studied segment for mcPCB dilatation. Inflations sites in each iliofemoral artery were targeted utilizing the take-off vessels as reference points. Devices were deployed at appropriate inflation pressures resulting in an optimal balloon-to-artery (BAR) ratio of 1.2:1.0 (120% overstretch). Angiographic evaluation was repeated on all vessels after device deployment. All QVA evaluations were subject to core laboratory analysis. The treatment balloons were inflated for 60 sec, deflated, and removed. Animals were then sacrificed at 1 hr, 3 and 7 days (one animal per time point).

Quantification of Paclitaxel

The paclitaxel concentrations within the iliofemoral arteries were measured using high-performance liquid chromatography (AnaKat Institut für Biotechnologie GmbH, Berlin, Germany, analysis blinded to sample origin). Briefly, after thawing, the tissue samples were weighed at ambient temperature and ethanol was added to completely cover the tissue. The samples were then treated with ultrasound for 15 min (45 kHZ). About 200 ml samples were centrifuged. A standard curve was produced in the ranging from 50 and 2,000 g/ml. The samples for the standard curve were prepared by diluting a stock solution with a concentration of 1,000 μg/ml. Aliquots of all samples (samples from tissue and standard curve) were transferred into autosampler vials and an equal volume of 0.1% formic acid was added. The flow rate of the high performance liquid chromatography system was 0.2 ml/min through a column of ODS Hypersil (ThermoElectronCorporation, Thermo Scientific, Waltham, Massachusetts, USA), particle size 5 μm, pore size 120A. The isocratic mobile phase consisted of 70% methanol containing formic acid (0.1%). Paclitaxel was detected by mass spectrometry in multiple reaction-monitoring mode with a transition of paclitaxel from 854.4 to 105.1 AMU. The tissue paclitaxel concentration was expressed in ng/mg. Drug elimination (k_{elimin}) was calculated based on the following formula $k_{\text{elim}} = [\ln(\text{C-peak}) - \ln(\text{Ctrough})]/\text{time interval}$. Paclitaxel half-life was also calculated as $t^1/_2 = 0.693/k_{\text{elim}}$

Vascular Response Study

Fifteen iliofemoral sites of seven DS were evaluated. Six of the total 21 available sites were excluded due to inadequate size or anatomy. The target sites were injured by 120% overstretch utilizing a plain balloon followed by implantation of self-expandable nitinol stent at 110% overstretch. To account for animal disparities and potential confounders, the stented segments, not animals were randomized in 2:1 fashion to mcPCB or plain balloon (POBA) postdilation for 60 sec (±1) using pressure to achieve the balloon to stent ratio of 1:1. Following the procedure, final QVA was

performed, the sheath was removed and animals recovered and survived for 28 days. Upon completion of the survival period, animals underwent QVA of the treated vessels followed by euthansia. The peripheral vasculature was then completely exposed and after the precise identification of the stent locations, the arterial tree containing stents was extracted, flushed with and immersed in normal buffered formalin 10% for subsequent histological analysis.

Histopathological Evaluation

Histological analysis was conducted by an independent pathology laboratory (AccelLAB, Boisbriand, Quebec, Canada). The stented arterial segment was embedded in methyl methacrylate (MMA) then cut into, three blocks (proximal, mid and distal). Each block was then cut into two sections, ground and polished to a final section thickness of 60-μm thick or less. One section was stained with Verhoeff-van Gieson [VVG] for elastin and the other with hematoxylin and eosin (H&E). Stented sections stained with VVG were examined by a trained operator for histomorphometry using light microscopy, image capture, and quantitative morphometric computer-assisted methods with Image Pro Plus 6.1.0.346 software. All H&E and VVG stained artery sections were examined by the Study Pathologist for semiquantitative and descriptive histopathology. For each section, the operator delineated the external elastic lamina (EEL—at the junction of the medial and adventitial layers), the internal elastic lamina (IEL—at the junction of the medial and neointimal layers), and the luminal border. Neointimal thickness was an average of the multiple distances between the IEL and the luminal border tracings provided by the software. In addition, the neointimal area, the media area and % stenosis were calculated using the following formulae: Media = EEL − IEL; Neointima = IEL − Lumen; % Stenosis = [1 − (Lumen Area/IEL Area)] × 100. To evaluate the amount of injury and inflammation, criteria defined by Schwartz et al. [10] and Kornowski et al. [11] respectively were utilized. Morphological assessment of endothelialization was described as percentage endothelial coverage of the arterial circumference: 0 = <25%, 1 = 25–75%, 2 = 76–99%, 3 = complete. The extent of fibrin deposition was assessed as follows: 0—absent, or rare minimal spotting around strut, 1—fibrin in small amounts, localized only around strut, 2—fibrin moderately abundant or denser, extending beyond strut, 3—abundant, dense fibrin, bridging between strut. The neointimal immaturity was defined as: 0 = no immature areas; 1 = <25% of neointima containing immature areas; 2 = 25–75% of neointima containing immature areas; 3 ≥ 75% of neointima containing immature areas. The media hypocellularity was defined as the result of the severity of cellular loss and its extension within arterial circumference and graded from 0 to 3. Other observations considered to be significant by the Study Pathologist were described and when applicable received a subjective grade (1 = minimal, 2 = moderate, 3 = marked) and the percentage of struts affected was enumerated.

Angiographic Evaluation

QVA analysis was done using QAngio XA Software™ version 7.1.14.0 (Medis Medical Imaging Systems®). The baseline and 28-days follow-up artery minimal lumen diameters (MLD) within the treated segments were measured from nonsuperimposed and nonforeshortened views using the guiding catheter as a standard for calibration. Reference vessel diameters (RVD) were taken from the proximal and distal portions of the vessel at the treated sites and the balloon-to-artery ratio was calculated. Percent diameter stenosis (%DS) at follow-up was calculated as: 1 − (MLD/RVD) × 100% and the late lumen loss (LLL) was calculated as the difference between MLD at follow-up minus MLD at baseline.

Statistical Analysis

Parametric data with a normal distribution were expressed as mean ± standard deviation (SD). The distribution of data was analyzed using the Shapiro–Wilk test. Data falling in a skewed distribution were expressed as median and interquartile range (IQR). Parametric data were compared either with the Student's *t* test for normal distribution or the *U*-Mann–Whitney test for a skewed distribution. *P*-values of ≤0.05 were considered statistically significant. All tests and graphs were performed using Statistica 8.0 (Stat Soft, Tulsa, OK).

RESULTS

Paclitaxel Tissue Uptake and Retention

The arterial tissue was harvested at different times following mPCB and POBA dilatation The temporal tissue paclitaxel concentrations are presented in Fig. 2. The initial uptake of the drug by the vessel wall was 5.8% of the total amount of paclitaxel on the balloon surface. The analysis of residual paclitaxel on the balloons after intervention showed that concentration decreased from 3.0 to 0.7 μg/mm^2 in which 32% of the initial drug amount remained on the balloon surface and 39% of the drug was lost to the blood stream. At 3 and 7 days, the tissue retained 23.9 and 0.6% of initial drug uptake. The calculated half-life for paclitaxel delivered via mcPCB was nearly 1 day (22.7 hr).

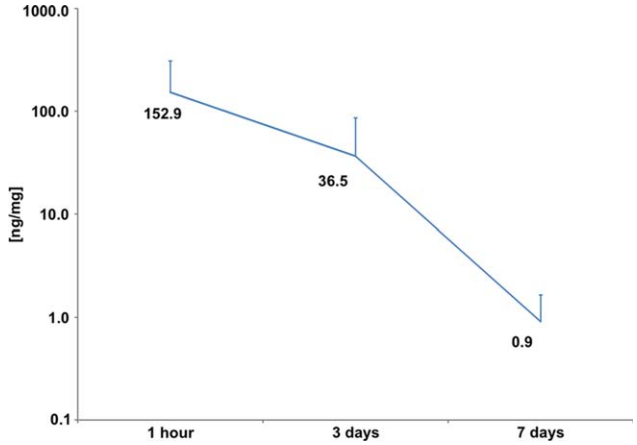

Fig. 2. Tissue transfer arm of the study. Paclitaxel vessel baseline uptake and temporal retention [ng/mg]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Vascular Response Study

Quantitative angiographic analysis. The baseline and follow up angiographic analyses are summarized in Table I. Prior to injury, the vessels were comparable between groups with regard to baseline average reference diameter and minimal lumen diameter. Similarly, the balloon to artery ratio and stent to artery ratio were similar.

At terminal follow up (28 days) animals increased body weight by 24.3 ± 3 kg, angiographic surrogates of neointimal hyperplasia were reduced in the mcPCB group as expressed by percent diameter stenosis (standardized difference = 39.3%) and late lumen loss (standardized difference = 62.5%), however, these differences were not statistically significant.

TABLE I. Summary of Quantitative Vascular Angiography in all Treated Vessels

	mcPCB ($n=10$)	POBA ($n=5$)	P
Baseline			
MLD [mm]	4.15 ± 0.8	4.47 ± 1.2	0.53
RD [mm]	4.34 ± 0.8	4.62 ± 1.2	0.59
B:A	1.16 ± 0.2	1.22 ± 0.2	0.62
S:A	1.08 ± 0.1	1.11 ± 0.3	0.81
Post-stent MLD [mm]	4.51 ± 0.6	4.78 ± 0.7	0.44
Post-stent RVD [mm]	4.65 ± 0.6	4.93 ± 0.7	0.41
Acute gain	0.36 ± 0.7	0.3 ± 1.2	0.91
Follow up (28 days)			
MLD [mm]	4.94 ± 0.6	4.54 ± 0.8	0.31
RD [mm]	5.23 ± 0.5	5.12 ± 0.5	0.68
% AS	11.02 ± 10.0	19.55 ± 22.8	0.32
% DS	5.83 ± 5.7	8.78 ± 9.3	0.45
Late loss [mm]	-0.43 ± 0.9	0.23 ± 1.2	0.24

MLD—minimal lumen diameter, RD—reference diameter, %DS—percent diameter stenosis, S:A—stent to artery ratio.

Histopathological Analysis

A summary of histomorphometry results is presented in the Table II. The vessels and stents in both groups were comparable with regard to sizes, as expressed by the EEL and IEL area. The lumen area was significantly larger by 52% in the mcPCB group. In the accordance with the angiographic outcomes, the neointimal formation was significantly reduced represented by 42.1% decrease in percent area stenosis after mcPCB dilation and tendency toward reduction of neointimal thickness (38.3% decrease, $P = 0.1$) when compared to POBA (Fig. 3).

The qualitative histopathological analysis (Fig. 4) showed that the neointimal proliferation was induced with the same amount of injury in both groups. The healing process expressed as neointimal maturity, fibrin deposition and completeness of endothelialization was advanced and comparable in both groups. Similarly, the biocompatibility defined as neointimal, peri-strut and adventitial inflammation was low and equivalent in the control and mcPCB group. Conversely, there was a tendency toward higher loss of medial cellularity and significantly higher mineralization within the medial layer after paclitaxel delivery. Representative histological stent cross sections and peri-strut magnifications are presented in Fig. 5.

DISCUSSION

This series of studies showed that local paclitaxel delivery via a novel microcrystalline balloon coating is feasible and effective in the iliofemoral arteries of domestic swine [12]. The paclitaxel vascular uptake and retention study revealed high levels of paclitaxel within the arteries soon after delivery via mcPCB dilation followed by a rapid decrease over time yet still detectable at 7 days albeit 1 ng of drug per 1 mg tissue. In the vascular response study, mcPCB stent post dilation displayed an average of 40% reduction in neointimal proliferation at 28 days when compared to POBA control, yet a favorable healing and biocompatibility profile with low inflammation and fibrin deposition was reported in the histopathological evaluation.

TABLE II. Histomorphometric Analysis of all Stents Explanted at 30 Days

	mcPCB $n=10$	POBA $n=5$	P
EEL area [mm^2]	27.60 ± 6.5	22.55 ± 2.6	0.13
IEL area (stent area) [mm^2]	24.58 ± 6.8	19.37 ± 2.1	0.13
Medial area [mm^2]	3.03 ± 0.4	3.19 ± 0.8	0.6
Lumen area [mm^2]	19.32 ± 4.6	12.65 ± 2.8	0.01
Area of stenosis [%]	19.8 ± 8.7	34.2 ± 15.7	0.03
Neointimal thickness [mm]	0.29 ± 0.15	0.47 ± 0.26	0.1

Analysis includes means of all sections.

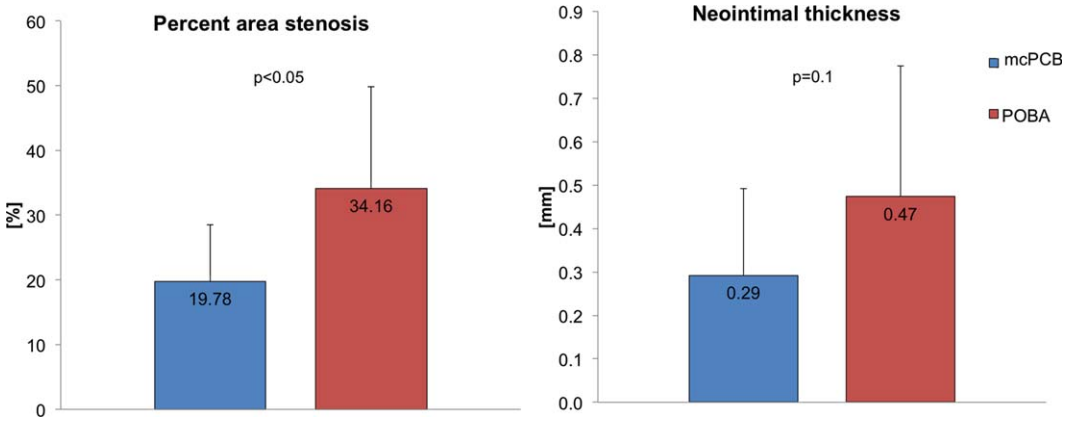

Fig. 3. Vascular healing response. Histomorphometric results representing device efficacy. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The domestic porcine model chosen in our study for preclinical PCB testing has been previously described and validated [13–15]. We chose the ilio-femoral territory, as the size, distribution and morphology is very similar to those of human vessels. Moreover, this vascular territory seems to have a histological composition comparable to human peripheral arteries. It has been shown that this mode allows not only for assessing PCB safety, but additionally evaluating efficacy signal is also feasible [12,13].

Additional novelty of this study arises from the procedural strategy chosen to assess the vascular response. In previous reports, stenting of peripheral arteries was performed after PCB dilation to simulate a typical bailout as it occurs in the clinical setting [15]. In our experiment, we first stented the target arterial segment which

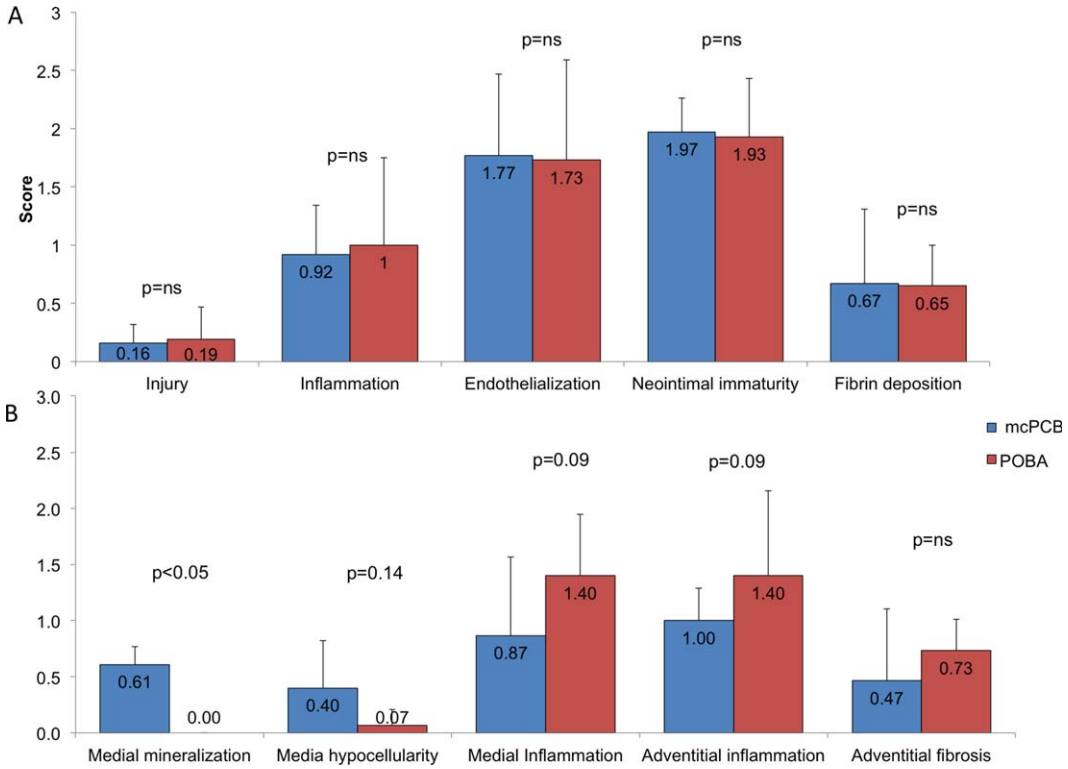

Fig. 4. Vascular healing response. Healing profile and biocompatibility of studied vessel segments. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

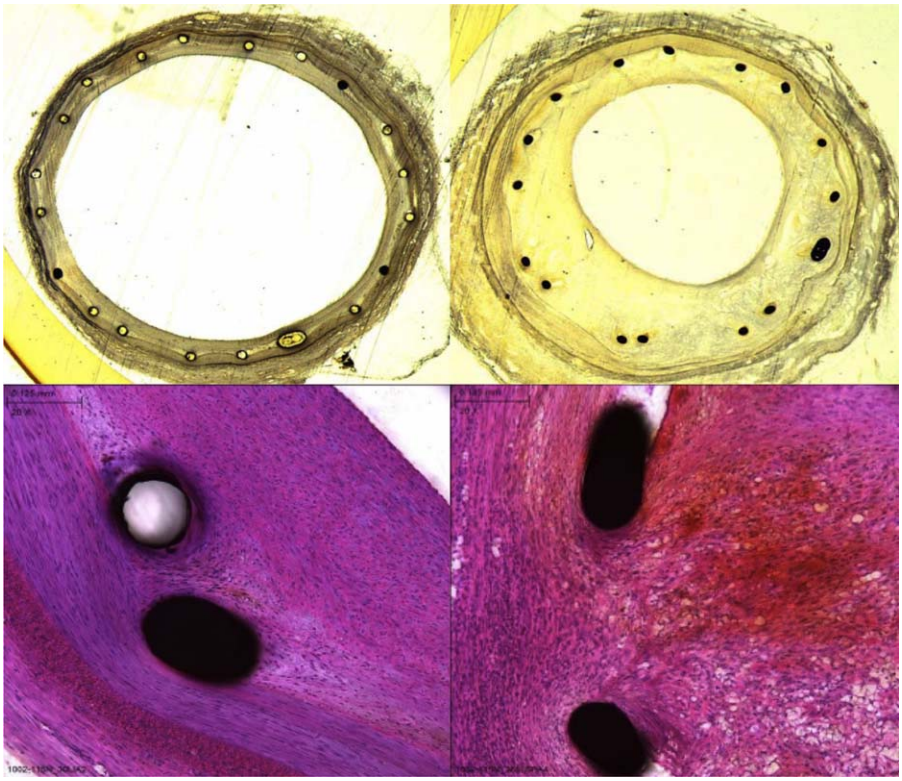

Fig. 5. Representative vessel cross sections and peri-strut magnification. Verhoeff-van Gieson staining—upper panel, Hematoxylin and Eosing staining—lower panel. mcPCB—left panel. POBA—right panel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

was followed by 60-sec mcPCB post dilation. This sequence seems to be more appropriate when stenting is intended as the primary mode of action, that is, iliac or coronary artery disease, not excluding the possibility of post dilation of a stent implanted as a bailout. Conversely, the most appropriate strategy in this scenario for treatment with PCB (predilation or postdilation) remains debatable

Vascular Paclitaxel Uptake and Retention

In the pharmacokinetic study, we reported high initial iliofemoral vessel uptake of paclitaxel following delivery via mcPCB (~50 ng/mg) and sustained vessel paclitaxel retention up to 7 days. The levels of paclitaxel found in the arterial wall at 1 hr after delivery are very similar to those found after inflations with clinically established paclitaxel-urea coated balloons (Invatec, FreePac coating) in the coronary arteries at this time point [16]. Conversely, the drug levels we report at 7 days (~1 ng/mg) are lower when compared to the same paclitaxel-urea coated balloons mentioned above. Otherwise, this concentration is similar to the one observed after paclitaxel—shellac coated balloons inflations in the coronaries, which have proven efficacy in the clinical setting [17,18]. Based on these observations, it seems that the range of therapeutic concentration for inhibition of neointimal proliferation is wide; however, the vessel healing profile may vary. It also appears that the drug concentrations in the peripheral arterial walls are similar to those observed in coronary arteries.

Neointimal Inhibition Efficacy

In the following, vascular response study, at 28 days, the angiographic surrogates of neointimal proliferation expressed as late loss and percent diameter stenosis were reduced by 64 and 38% respectively, however, due to the high variation in the results, the differences were not found to be statistically significant. In the histomorphometric analysis, the neointimal proliferation was significantly reduced on average by 40% when compared to POBA control. These results are consistent with the pre clinical study by Milewski et al. [15] in which similar efficacy was observed in the paclitaxel–iopromide coated balloon (PACCOCATH®, Bayer Schering Pharma/MEDRAD) which has been well-studied and is commonly used in clinical practice.

Vascular Healing and Biocompatibility Profiles

The mean injury scores were minimal and not significantly different between treatments indicating homogeneity in the methodology of the stent implantation and balloon dilation. The biocompatibility of mcPCB was confirmed by low inflammatory scores comparable to the control group and the lack of granulomas and giant cells. Similarly, fibrin deposition, a typical biological response to paclitaxel, was low and comparable between the two groups. Endothelial coverage of 75–99% of the stent luminal areas in both groups was observed. The incomplete endothelialization at 28 days is somewhat abnormal but may have been secondary to endothelial injury from preterminal catheterization procedures or increased mobility of the femoral arteries. Furthermore, this finding was similarly observed in both mcPCB and POBA, therefore we concluded that it was not contributable to the treatment.

Some additional observations were noted at greater frequency or mean severity in the mcPCB group, and therefore were likely related to drug effects: in particular, peri-strut, intimal and/or medial mineralization, and medial hemorrhage and/or devitalization (cell loss). Medial hemorrhage and devitalization are known effects of paclitaxel within the media of the artery and may lead to medial loss and fibrosis in the long term [19].

Study Limitations

The main limitation of this study derives from its experimental nature and the analytical methods used. First, the normal peripheral arteries of domestic swine lack the biological components seen in atherosclerotic vascular disease; therefore, the results found in our studies may not correlate with the diseased vessels potentially found in humans. Also, the significant amount of variability observed in pharmacokinetic studies using animal models is well known.

This study focused in assessing the short-term efficacy and the local tissue response, thus particle release and long-term vascular effects are currently unavailable.

CONCLUSIONS

Based on the results of our study, we conclude that the delivery of paclitaxel via a microcrystalline balloon coating results in sustained vessel drug retention up to 7 days in porcine iliofemoral arteries resulting from a single drug delivery. Although the retained concentrations were low (~1 ng/mg) at 7 days, a significant reduction of neointimal proliferation was reported in the vascular response study utilizing the same technology. Furthermore, contrary to paclitaxel delivered via durable platform and stent matrix, a preferable healing profile was observed which could be attributable to the low vessel paclitaxel levels. Therefore, it appears there is a very broad therapeutic range of locally delivered paclitaxel, which results in different vessel healing patterns.

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