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ORIGINAL ARTICLE

Vascular response and mechanical integrity of the new biodegradable polymer coated sirolimus-eluting PROLIM stent implanted in porcine coronary arteries

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Abstract

Background: Although durable polymer coated drug-eluting stents (DES) are standard care in percutaneous coronary interventions, new stent platforms employing biodegradable polymer based drug delivery are increasingly being used in clinical practice.

Aim: To evaluate the short- (28 days) and medium-term (90 days) vascular effects of the new biodegradable polymer coated sirolimus-eluting stent — the PROLIM stent.

Methods: The objectives of the study were evaluated using standard angiographic and histological methods. In addition, the mechanical integrity of tested stents was assessed using Faxitron imaging. A total of 12 PROLIM stents, 11 biodegradable polymer only coated stents (BPCS), and 12 bare metal stents (BMS) were implanted in the coronary arteries of 16 female non-atherosclerotic domestic swine using an overstretch of 1.1:1.0.

Results: At 28 days, neointimal proliferation was significantly lower in the PROLIM and BMS stents compared to the BPCS stents ($p \leq 0.05$). Interestingly, despite thin neointima found at this time in the PROLIM group, there was a further significant decrease in neointimal formation between 28 and 90 days ($p = 0.04$). Although a statistically bigger neointima was found in BPCS stents at 28 days compared to the PROLIM and BMS stents, there was a 50% decrease in the neointimal area at 90 days follow-up ($p = 0.02$) which reached the level seen in other groups. The endothelialisation was completed in all tested stents after 28 days. There was a significant increase of fibrin depositions in the PROLIM treated arteries at 28 days which were resorbed nearly completely at 90 days follow-up. At 28 days, the inflammatory response was found to be numerically higher in the BPCS stents ($p = NS$) compared to other tested groups. On the contrary, at 90 days follow-up when the degradation process of the polymer had been completed, the inflammatory reaction decreased substantially to the level seen in the PROLIM and BMS stents. Faxitron analysis of the stented arteries revealed no major abnormalities except for isolated strut fractures observed in the mid portions of two BMS stents and one BPCS stent.

Conclusions: The PROLIM — a biodegradable polymer coated sirolimus-eluting stent — demonstrates very good short-term and medium-term angiographic and histological results. The lack of 'catch-up phenomenon', fast endothelialisation process, and minimal inflammatory reaction may contribute to favourable clinical outcomes using PROLIM stents.

Key words: stent, biodegradable polymer, sirolimus, porcine

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INTRODUCTION

The efficacy of drug-eluting stents (DES) in preventing restenosis and reducing the need for repeat revascularisation has been proven in numerous clinical trials including practically all lesion and patient subsets [1]. Although durable polymer coated DES are the standard of care in percutaneous coronary interventions, the use of biodegradable polymer based DES has been shown to be non-inferior in terms of clinical and angiographic outcomes [2]. Therefore, new stent platforms employing biodegradable polymer based drug delivery are increasingly being used in clinical practice. Several recent studies have shown that limus derivatives are superior to paclitaxel delivered from a durable polymer platform [3–5]. A combination of biocompatible, biodegradable polymer and potent antiproliferative drug seems to confer a particular benefit. Therefore a new biodegradable polymer coated sirolimus-eluting stent called PROLIM has been designed by the Balton Company (Warsaw, Poland).

The aim of this study was to assess 28 and 90 day safety, biocompatibility, feasibility and structural integrity of the new PROLIM stent implanted in overstretched non-atherosclerotic porcine coronary arteries. The objectives of the study were fulfilled using standard angiographic and histological methods. In addition, the structural integrity of tested stents was assessed by high resolution radiography. The control and reference devices consisted of bare metal stents (BMS) made of stainless steel 316 L and biodegradable polymer coated stents without sirolimus (BPCS).

METHODS

Device description

The PROLIM is a balloon expandable stent dedicated for coronary lesions. The stent platform (Flexus, Balton, Poland) is made of a laser-cut 316 L stainless steel tube with a wall thickness of 0.115 mm. It has a closed cell design for uniform drug distribution and a metal-to-artery surface ratio of 19%. The profile of the whole implantation system, including stent, is 0.038 inches (0.9652 mm). The PROLIM is covered with a copolymer of lactic and glycolic acid mixed with sirolimus. This combined solution is then sprayed onto the stent. There is no primer or topcoat layer. *In-vitro* studies have shown that the coating degrades entirely within eight weeks [6]. The biocompatibility, feasibility and safety of an identical stent model coated with polymer releasing paclitaxel have been confirmed in preclinical and clinical studies [6, 7]. Laboratory tests have shown that approximately 90% of sirolimus is released from the stent surface during the first 20 days. After 30 days, the drug is no longer detectable. The delivery system used for the PROLIM stent (the River balloon catheter; Balton, Poland) is a rapid exchange system compatible with 0.014 inch (0.36 mm) guide wires and 5 F guiding catheters with a high pressure balloon.

Study design

This study was conducted at AccelLAB Inc (Boisbriand, Quebec, Canada). The study protocol was reviewed and approved by AccelLAB's Institutional Animal Care and Use Committee. The review insured compliance with the regulations of the Canadian Council on Animal Care.

A total of 12 PROLIM, 11 BPCS and 12 BMS stents were implanted in 16 female non-atheroslerotic domestic swine weighing 38–48 kg, using a target overstretch of 1.1:1.0. All stents were 3.0 × 15 mm or 3.5 × 15 mm. Sacrifice occurred at either 29–30 days or 86–90 days post-implantation. All animals received dual antiplatelet therapy consisting of oral acetylsalicylic acid (325 mg) and clopidogrel (300 mg initial dose and 75 mg subsequently), starting three days prior to intervention and continuing until sacrifice. After anaesthesia had been inducted using propofol, the animals were intubated and supported with mechanical ventilation. Isoflurane in oxygen was administered to maintain a surgical plane of anaesthesia. Subsequently, an arterial sheath was introduced into the left or right femoral artery through an inguinal skin incision. An initial bolus of heparin (~ 400 U/kg) was administered and activated clotting time (ACT) was measured every 30 minutes thereafter to maintain an ACT of at least 300 seconds. Coronary angiograms were performed after administration of intracoronary nitroglycerin (500 μg). The selection of the target site was made based on a visual assessment of the anatomy and quantitative coronary angiography (QCA) analysis. These sites were selected to avoid side branches and segments with tapering greater than 10%, in order to ensure uniform interaction of the stent coating with the arterial wall. The balloon was then inflated at a steady rate to a pressure sufficient to achieve a target stent-to-artery ratio of 1.1:1 (acceptable range of 1.05:1 to 1.15:1). After the last angiogram was obtained, the delivery system was removed, the femoral artery was ligated, and inguinal incision layers were sutured. All animals were injected with a long-lasting antibiotic. At termination, all animals were anaesthetised as described above and a final angiography was performed. The animals were then sacrificed and subsequently gross examination of the heart, stented vessels, thoracic and abdominal cavities was performed. The hearts were excised and perfused with lactated Ringer's solution followed by neutral-buffered formalin, then immersed in neutral-buffered formalin for further histological analysis.

Angiographic analysis

All treated arteries were qualitatively evaluated for stent migration, dissection and aneurysms. The Medis QCA-CMS 6.0 system was used for QCA analysis. Of two angles available for analysis, the image with the minimal foreshortening and maximal stenosis was chosen. Balloon diameter was measured at baseline. In-segment minimal lumen diameter (MLD) and reference vessel diameter (RVD) were measured at base-

line and at terminal angiogram. From these measurements, the following parameters were calculated: balloon-to-artery ratio (defined as balloon/pre-stent mean luminal diameter), stent-to-artery ratio (defined as post-stent/pre-stent mean luminal diameter) and late lumen loss (LL; defined as post-stent MLD — final MLD). Diameter stenosis was calculated based on the following formula (1 – [MLD/RVD]) × 100%.

Stent radiography

High resolution radiographs of fixed whole hearts and explanted stented arteries were captured at two perpendicular angles using Faxitron MX-20 apparatus (Faxitron, Tucson, AZ, USA). Radiographs of explanted stented vessels were examined to evaluate stent expansion, morphology, stent continuity and/or other abnormalities.

Device acute performance

To evaluate acute performance of the PROLIM stents, a special 'Device Acute Performance' form was created. During the introduction and deployment of the devices, the operator evaluated the function and performance of the devices including, but not limited to: (1) guide catheter and guidewire compatibility; (2) trackability/flexibility; (3) radiopacity; (4) inflation/deflation times; (5) angiographic appearance; and (6) overall device performance. Success was defined as the complete delivery of the stents and the absence of any major or sub-acute complications during the procedures. Device acute performance characteristics were subjectively evaluated based on the judgment and experience of the operator according to the following rating scale: 1 = poor; 2 = below average; 3 = average; 4 = good; or 5 = excellent.

Histology evaluation

All stented segments were embedded in methyl methacrylate and cut to obtain approximately 8 μm sections. Subsequently, these sections were stained with haematoxylin and eosin (H&E) and Verhoeff-van Gieson (VVG) stains. All sections were examined by an experienced veterinary pathologist for semi-quantitative and descriptive histopathology. In addition, a comprehensive histomorphometry analysis was performed.

Histomorphometry analysis

VVG-stained sections of stented arteries were examined using light microscopy and quantitative morphometric computer-assisted methods with Image Pro Plus 6.1.0.346 software. For each section, the cross-sectional areas (external elastic lamina [EEL area], internal elastic lamina [IEL area] and lumen area) were measured and then the following parameters were calculated: medial area (defined as: EEL area – IEL area); intimal area (defined as IEL area – luminal area); area stenosis (calculated as: {1 – (luminal area/IEL area)} × 100); and mean intimal thickness (calculated as follows: $\sqrt{\text{IEL area}/\pi}^{\circ} - \sqrt{\text{lumen area}/\pi}$).

Semi-quantitative and descriptive histopathology

All sections were evaluated using semi-quantitative scoring criteria. To evaluate the amount of injury, a scoring system defined by Schwartz et al. [8] was used: 0 = IEL intact; 1 = IEL lacerated; 2 = media completely lacerated; and 3 = EEL lacerated. Each strut in the section was scored and the mean injury score for each section was calculated and reported. To evaluate the extent of inflammatory reaction, the following grade was used: 0 = no or very few (≤ 3) inflammatory cells around strut; 1 = few (~4–10 inflammatory cells around strut); 2 = many (> 10) inflammatory cells around strut, can extend into but does not efface surrounding tissue; 3 = many (> 10) inflammatory cells, effacing surrounding tissue. Each strut in the section was scored, and the mean inflammation score for each section was calculated and reported. The predominant inflammatory cell type(s) was described, per stent section. In addition, when peri-strut granulomas were present, their incidence was graded (as a percentage of affected struts), per section and per stent segment. The extent of fibrin deposits was assessed as follows: 0 = absent or rare minimal spotting around strut; 1 = fibrin in small amounts, localised only around strut; 2 = fibrin moderately abundant or denser, extending beyond strut; 3 = abundant, dense fibrin, bridging between strut. Each strut in the section was scored; the mean fibrin score for each section was calculated and reported. The endothelialisation score was based on the following criteria: 0 = < 25% of artery circumference covered by endothelium; 1 = 25–75% of artery circumference covered by endothelium; 2 = 76–99% of artery circumference covered by endothelium; 3 = complete endothelial coverage. The neointimal immaturity which estimates the proportion of neointimal areas containing a few or no myofibroblasts, a high proportion of mucinous matrix, oedema or fibrin, and undifferentiated mesenchymal cells or inflammatory cells (areas are interpreted as less mature) 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.

Statistical analysis

Statistical analyses were performed using Sigma Stat 3.1 software. Selected continuous data is expressed as mean ± standard deviation. For continuous data, equal variance and normality tests were initially performed. When equal variance and normality were observed, one-way analysis of variance (ANOVA) with Dunnett's post-ANOVA tests were used to test for differences in variables between stent types. When either equal variance test or normality test failed, and for the histologic semi-quantitative scores, a Kruskal-Wallis test (with Dunn's method for post-hoc group comparison) was conducted. An appropriate t-test was used to calculate the statistical significance of differences between two studied time points, within the same tested group. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Procedural results

A total of 17 stents (six PROLIM, six BMS and five BPCS) were implanted in eight animals designated for 28 days, and 18 stents (six PROLIM, six BMS and six BPCS) were placed in eight animals selected for 90 days. All stents were successfully implanted, kept their dedicated shape, and conformed well to the vessel anatomy at implantation. There were no cases of thrombus formation, slow-flow or any other coronary or access site complications. All stents were easy to track and implant with quick deflation time and excellent angiographic visibility.

Stent performance

Among all stents implanted, dissection occurred once, following a balloon burst in the left circumflex artery after BPCS placement. A BMS stent was used to repair the dissection and the vessel was excluded from the analysis. A balloon burst was also noted in the right coronary artery after a BMS stent implantation, but the vessel was not damaged and therefore was kept for analysis. Guide catheter and guidewire compatibility, device trackability/flexibility, radiopacity, balloon inflation/deflation times and device angiographic appearance were rated as excellent for all stents. Overall device performance was rated as excellent in all stents except for the two in which balloon burst occurred, which were rated as below average.

High resolution radiography

High resolution radiography (Faxitron) analysis of the stented arteries revealed no major abnormalities except for isolated strut breaks observed in one BMS stent of the D28 cohort, and one BMS and one BPCS stent of the D90 cohort. In all these stents, struts broke in links located in the middle portion of the devices. In all cases of strut breaks, the stents retained their normal tubular shape; there was no stent fracture or segmentation. Stents implanted in curved coronary segments showed good conformability to the shape of the vessel.

Angiographic results

The angiographic results are summarised in Table 1. There was no aneurysm formation, filling defects, stent migration or malposition at terminal angiography in any cohort. Mean stent-to-artery ratio did not differ among stent groups at both time points. At 28 days, the mean diameter stenosis ranged from 15.0––21.1%, with the lowest value observed for PROLIM and the highest for BMS stents. Similarly, late lumen loss showed the lowest value in PROLIM stent and the highest in BMS. However, there were no statistically significant differences among stent types. The mean diameter stenosis at 90 days ranged from 7.0–10.3%, with the lowest value observed for BMS and the highest for BPCS stents. The mean lumen loss at this time point was practically identical among all tested groups. There were no statistically significant differences among stent types.

Histomorphometry

The histomorphometry data is summarised in Table 2 and representative microscopic sections of the vessels at 28 days are presented in Figure 2. At 28 days, mean percentage area stenosis was between 29.8% (for PROLIM stents) and 41.5% (for BPCS stents). Intimal area and mean intimal thickness were significantly lower in BMS and PROLIM stents (Figs. 1, 2) compared to BPCS stents ($p < 0.050$). Similarly to QCA analysis, there was a decrease in area stenosis and a significant decrease ($p = 0.04$) in mean intimal thickness at 90 days compared to the 28 days cohort. There was no late catch-up effect in any of the parameters analysed. At 90 days, the percentage area stenosis was between 21.7% (PROLIM) and 27.5% (BPCS). Intimal area and mean intimal thickness were similar among groups.

Table 1. Summary of quantitative coronary angiography at baseline, 28 and 90 days follow-up

	PROLIM	BMS	BPCS
28 days follow-up	N = 6	N = 6	N = 5
Balloon-to-artery ratio	1.10 ± 0.03	1.09 ± 0.04	1.09 ± 0.05
Stent-to-artery ratio	1.05 ± 0.03	1.07 ± 0.04	1.04 ± 0.03
Diameter stenosis [%]	15.0 ± 11.2	21.1 ± 8.6†	15.4 ± 3.0
Minimal lumen diameter [mm]	2.34 ± 0.54	2.34 ± 0.54	2.18 ± 0.41†
Late loss [mm]	0.35 ± 0.31	0.49 ± 0.25	0.55 ± 0.31
90 days follow-up	N = 6	N = 6	N = 5
Balloon-to-artery ratio	1.12 ± 0.03	1.10 ± 0.04	1.10 ± 0.06
Stent-to-artery ratio	1.09 ± 0.04	1.08 ± 0.02	1.08 ± 0.07
Diameter stenosis [%]	9.7 ± 3.6	7.0 ± 5.0†	10.3 ± 3.2
Minimal lumen diameter [mm]	2.79 ± 0.57	3.06 ± 0.43	2.75 ± 0.04†
Late loss [mm]	0.19 ± 0.22	0.20 ± 0.20	0.22 ± 0.11

No significant difference among groups; †significant difference ($p \leq 0.05$) between 28 and 90 days parameters within the same tested group

Table 2. Histomorphometric analysis at 28 and 90 days

	PROLIM	BMS	BPCS
28 days	N = 6	N = 6	N = 5
EEL bounded area (mm^2)	9.20 ± 2.10	10.45 ± 2.00	10.37 ± 1.56
IEL bounded area [mm^2]	7.87 ± 1.86	8.82 ± 1.68	8.58 ± 1.31
Medial area [mm^2]	1.34 ± 0.25	1.62 ± 0.33	1.79 ± 0.31
Intimal area [mm^2]	2.30 ± 0.40*†	2.76 ± 0.35*	3.61 ± 0.88†
Luminal area [mm^2]	5.57 ± 1.64	6.06 ± 1.90	4.98 ± 0.78
Area stenosis [%]	29.8 ± 5.8	32.7 ± 9.6	41.5 ± 6.3
Mean intimal thickness [mm]	0.26 ± 0.04*	0.30 ± 0.07*	0.40 ± 0.08†
90 days	N = 6	N = 5	N = 5
EEL bounded area [mm^2]	9.27 ± 1.79	10.61 ± 1.48	8.51 ± 1.56
IEL bounded area [mm^2]	7.83 ± 1.60	8.93 ± 1.31	6.94 ± 1.62
Medial area [mm^2]	1.44 ± 0.32	1.68 ± 0.25	1.57 ± 0.15
Intimal area [mm^2]	1.70 ± 0.71†	2.04 ± 0.55	1.81 ± 0.25†
Luminal area [mm^2]	6.13 ± 1.50	6.89 ± 1.25	5.14 ± 1.57
Area stenosis [%]	21.7 ± 7.9	23.1 ± 6.9	27.5 ± 7.3
Mean intimal thickness [mm]	0.18 ± 0.08	0.21 ± 0.06	0.22 ± 0.04†

*Indicates significant difference *vs* BPCS; †significant difference ($p \leq 0.05$) between 28 and 90 days parameters within the same tested group

Histopathology

A summary of pathological changes at 28 and 90 days is presented in Table 3. At 28 days, injury and inflammation scores were similarly low (means < 1) in PROLIM and BMS stents, but tended to be higher in BPCS stents (Table 3, Figs. 2, 3). The inflammatory infiltrates in most stents were composed predominantly of macrophages and occasional multinuclear giant cells. In 1/6 PROLIM stents, rare peri-strut granulomas were also observed, affecting 4% of struts. In 1/5 BPCS stents, frequent peri-strut granulomas were present (46% of struts). Fibrin scores were significantly higher in the PROLIM group than in the others. Endothelialisation was complete in all stent sections examined. Neointimal immaturity scores were moderate (means 1–2) in all groups, although they tended to be slightly lower in the PROLIM and BMS groups. At 90 days, injury and inflammation scores were similarly low in all groups (means < 1). Fibrin scores were very low in all stents, essentially absent or present in insignificant amounts. Endothelialisation appeared complete in all stents. Neointimal immaturity scores were overall similar among groups, and tended to be lower than at 28 days.

DISCUSSION

The introduction of first generation DES revolutionised interventional cardiology by reducing restenosis and the need for target lesion revascularisation [1]. Unfortunately, the safety of first generation DES has been limited by their suboptimal polymer biocompatibility, mechanical issues of stents and polymers, and local drug toxicity [9].

Regardless of drug properties, it has been suggested that the main cause of adverse clinical events found in first generation DES could be the durable polymers used as stent coatings and drug reservoirs. The durable polymeric coatings impair endothelialisation, cause excessive and prolonged inflammation and late hypersensitivity reactions, as well as induce necrosis within vascular smooth muscle cells, thus influencing the rate of restenosis and stent thrombosis [10–12]. In addition, mechanical complications of durable polymers have been reported (e.g. delamination and 'webbed' polymer surface, non-uniform coating integrity) which lead to stent expansion issues and uneven drug distribution [9]. Many approaches have been proposed to modify, eliminate or replace polymeric coatings with more biocompatible substances. These have included direct drug deposition on the modified metal surface, selective coating of certain stent elements and/or non-polymeric coatings which derived from, or adequately mimic, substances endogenous to the body and which have known biocompatibility, for instance phosphorylocholine or hydroxyapatite [13, 14]. Among these solutions, there is an advantage in the use of biodegradable polymeric coatings which may fully control elution of an antiproliferative compound over the period of time required to inhibit neointimal proliferation [9, 10]. After that time, the polymer is fully degraded to carbon dioxide and water, leaving only BMS in the vessel wall. This eliminates any possible adverse reactions related to durable polymers and minimises the risk of late stent thrombosis and restenosis.

The utility of this technology has been already tested in several clinical trials [2, 7, 15, 16]. The present study aimed to evaluate the 28 day and 90 day vascular effects of biodegradable polymer-coated drug-eluting PROLIM stents implanted in normal non-atherosclerotic porcine coronary arteries. Due to

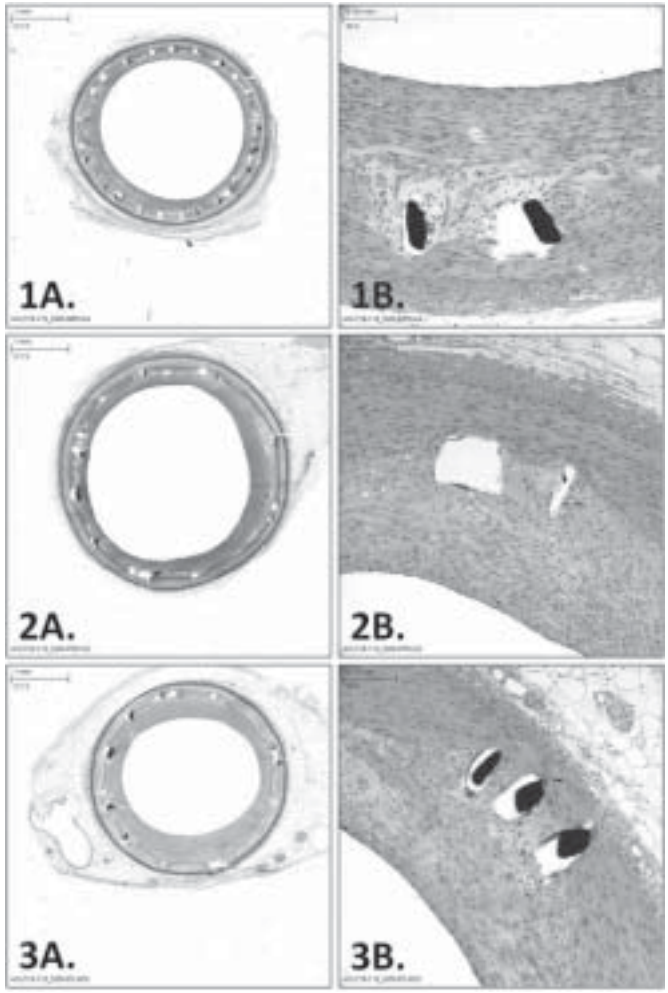

Figure 1. Representative microscopic sections of vessels at 28 days treated with PROLIM stent (**1**), BMS stent (**2**), and BPCS stent (**3**) (left column: Verhoeff-van Gieson stain; right column: haematoxylin and eosin stain)

the fact that several papers published recently have suggested the superiority of limus analogues over the widely used paclitaxel, sirolimus was chosen as an antiproliferative compound to minimise neointimal hyperplasia after stent placement. QCA analysis revealed that the mean balloon overstretch (balloon-to-artery ratio) in all treated vessels was similar within all studied groups, indicating accurate and uniform balloon injury and supporting the validity of comparisons between the groups.

At 28 days, the overall histological parameters of neointimal proliferation were significantly lower in the PROLIM and BMS stents compared to the BPCS stents. The endothelialisation was completed in all tested stents. There was a significant increase of fibrin deposition in the PROLIM treated arteries and a trend towards a decreased medial area. This is a typical biological finding related to the anti-proliferative effect of the drug in the arterial wall and has been reported in several publications that included pre-clinical evaluation of DES [17]. Importantly, at 90 days follow-up, fibrin was resorbed nearly completely.

Interestingly, the relatively thin neointimal layer observed at 28 days in the PROLIM group was further decreased at 90 days. This is in contrast to several publications describing some DES technologies, where late increase of neointimal formation was found between 28 and 90 days [18–20]. The same trend was also found in our previous work where biodegradable polymer-coated paclitaxel-eluting stents were tested in porcine coronary arteries [6]. It is assumed that this finding, known as 'late catch-up phemonenon', may be related to the effect of a drug and/or polymer combination and persistent vascular inflammation.

In the current study, the combination of sirolimus with a biodegradable polymer that fully degrades into carbon dioxide and water over approximately eight weeks eliminated the risk for persistent polymer-induced inflammation and 'catch-up phenomenon'. In fact, although a statistically larger neointima was found in BPCS stents at 28 days compared to the other groups, a 50% decrease in neointimal area was found at 90 days.

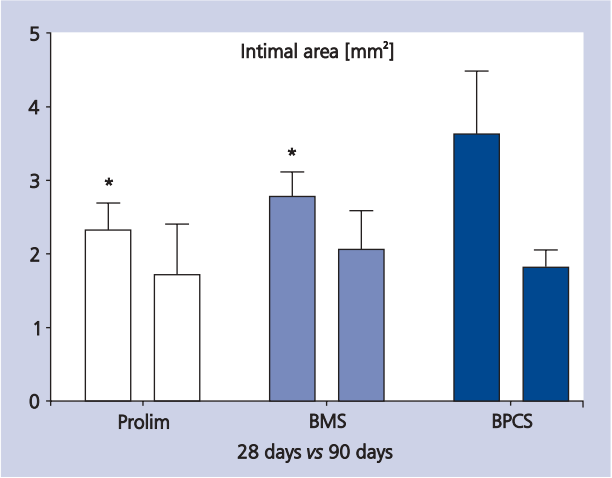

Figure 2. Pattern of neointimal formation between 28 and 180 days follow-up; *indicates significant difference *vs* BPCS

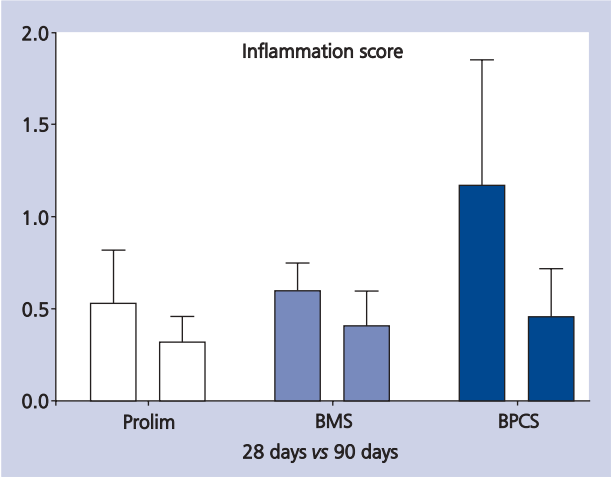

Figure 3. Comparison of inflammatory response between 28 and 90 days follow-up

Table 3. Histopathology data at 28 and 90 days follow-up

	PROLIM	BMS	BPCS
28 days follow-up	N = 6	N = 6	N = 5
Injury	0.15 ± 0.11	0.16 ± 0.20	0.28 ± 0.33
Inflammation	0.52 ± 0.30	0.59 ± 0.16	1.16 ± 0.69
Fibrin	0.49 ± 0.23*,&,†	0.13 ± 0.11	0.13 ± 0.11
Endothelialisation	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
Neointimal immaturity	1.11 ± 0.40	1.22 ± 0.27	1.73 ± 0.28
90 days follow-up	N = 6	N = 5	N = 5
Injury	0.38 ± 0.23	0.34 ± 0.18	0.35 ± 0.28
Inflammation	0.31 ± 0.15	0.40 ± 0.20	0.45 ± 0.27
Fibrin	0.01 ± 0.02†	0.00 ± 0.00	0.01 ± 0.02
Endothelialisation	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
Neointimal immaturity	0.50 ± 0.35	0.53 ± 0.65	0.33 ± 0.41

*Indicates significant difference *vs* BPCS; &indicates significant difference *vs* BMS; †significant difference ($p \leq 0.05$) between 28 and 90 days parameters within the same tested group

At 28 days, at a time where the polymer degradation process is ongoing, the inflammatory response tended to be higher in the BPCS stents (although no statistical difference was found) compared to the other groups. It is possible that polymer degradation products may cause local tissue irritation and increase the inflammatory reaction [11, 21]. On the contrary, at 90 days, when the polymer degradation process is considered complete, the inflammatory reaction was substantially lower in the BPCS stents, similar to that seen in the PROLIM and BMS stents. The lower level of inflammation in the PROLIM stents at 28 days can be explained by the anti-inflammatory properties of sirolimus [22], which could attenuate the potential pro-inflammatory effects of polymer degradation.

Another important factor evaluated in the current study which may potentially affect the clinical outcomes of DES technology is the performance and structural integrity of the stent. It has been shown that stent fractures may increase the risk of restenosis [23–25] and stent thrombosis [26, 27]. In our study, high resolution radiography of stented arteries revealed no major abnormalities except for isolated strut breaks in the mid portions. Although this finding did not affect the stent structure and did not cause its full fracture, it led the producer to improve the stent design. Such improvements seem necessary in terms of clinical practice where the same stent must withstand significantly larger and longer strains compared to porcine arteries. Overall, the device performance was rated as excellent in all stents except the two where balloon burst occurred, for which it was rated as below average.

Limitations of the study

Although the porcine coronary implantation model is well established for safety evaluation of new devices, its value in

predicting efficacy in the clinical setting is limited. In this regard, caution must be shown in drawing any conclusions about PROLIM efficacy in relation to clinical restenosis and revascularisation rates. We focused on QCA and histopathology without immunohistochemistry analysis. In addition, no *in vivo* pharmacokinetics analysis was performed.

CONCLUSIONS

The PROLIM — biodegradable polymer coated sirolimus-eluting stent — demonstrates very good short-term and medium-term angiographic and histological results. The lack of 'late catch-up phenomenon', fast endothelialisation, and minimal inflammatory reaction may contribute to the favourable clinical outcomes of the PROLIM stent.

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Conflict of interest: *Krzysztof Milewski and Robert J. Gil: consultancy for Balton; Piotr Buszman and Wojciech Wojakowski: lecture honoraria from Balton; Louis-Georges Guy, Jean-Martin Lapointe, Guy Leclerc, Diane Beaudry: employee of Accellab who received grant funding from Balton.*

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Odpowiedź naczyniowa i integralność mechaniczna nowego stentu PROLIM pokrytego polimerem uwalniającym sirolimus po jego wszczepieniu do tętnic wieńcowych świni

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Streszczenie

Wstęp: Stenty uwalniające leki z powierzchni polimerów trwałych (niebiodegradowalnych) stanowią obecnie standard w przezskórnych interwencjach wieńcowych. Jednak coraz częściej wykorzystuje się również platformy wykonane z polimerów biodegradowalnych. Polimery ulegające całkowitej degradacji w kilka lub kilkanaście tygodni po implantacji stentu mogą przyczyniać się do eliminacji występowania przedłużonego odczynu zapalnego, ograniczając tym samym ryzyko późnych i bardzo późnych zakrzepic, jak również możliwości opóźnionego w czasie przerostu neointimy i tym samym restenozy.

Cel: Celem badania była ocena krótko- i średnioterminowych efektów naczyniowych nowych stentów PROLIM, pokrytych polimerem biodegradowalnym, uwalniającym sirolimus, implantowanych do tętnic wieńcowych świń domowych.

Metody: Po uzyskaniu zgody lokalnej Komisji Etycznej badaniu poddano 12 stentów PROLIM, 12 stentów metalowych (BMS; grupa kontrolna) oraz 11 stentów pokrytych polimerem biodegradowalnym bez leku (BPCS; grupa referencyjna). Do badania włączono łącznie 16 świń domowych, którym implantowano 17 stentów na okres 28 dni (6 stentów PROLIM, 6 BMS i 5 BPCS) oraz 18 stentów na okres 90 dni (6 stentów PROLIM, 6 BMS i 6 BPCS). Wszystkie stenty implantowano tak, aby średnica balonu po rozprężeniu przekraczała średnicę referencyjną naczynia w stosunku 1.1:1.0 (*oversize*). Po okresie odpowiednio 28 i 90 dni każdorazowo wykonano kontrolną koronarografię, a następnie badane zwierzęta uśpiono w celu wykonania analizy histologicznej oraz histomorfometrycznej. Każdy badany segment naczynia odwadniano i odtłuszczano, zatapiano w żywicy (metyl methacrylate), cięto na skrawki o grubości ok. 8 μm oraz wybarwiano hematoksyliną-eozyną oraz barwnikiem Verhoeff-van Giesona. Ponadto, w celu oceny kształtu i struktury badanych stentów, wykonywano badanie z wykorzystaniem promieniowania rentgenowskiego o wysokiej rozdzielczości (*faxitron*).

Wyniki: Analiza wykonana po okresie 28 dni od implantacji wykazała istotnie mniejszy przerost neointimy w grupie stentów PROLIM i BMS w porównaniu ze stentami BPCS ($p \leq 0,05$). Co ciekawe, zaobserwowano dalszą redukcję powierzchni neointimy w stentach PROLIM między 28. a 90. dniem badania ($p = 0,04$). Chociaż po 28 dniach od implantacji stentów BPCS wykazano istotnie grubszą neointimę w porównaniu ze stentami PROLIM i BPCS, w 90. dniu jej powierzchnia została zredukowana o 50% ($p = 0,02$) do wartości podobnej jak w pozostałych grupach w tym punkcie czasowym. Analiza histologiczna wykazała kompletną endotelializację we wszystkich badanych grupach już po 28 dniach od implantacji. Chociaż po 28 dniach w grupie stentów PROLIM wykazano istotnie zwiększoną ilość włóknika, to analiza w grupie 90-dniowej wykazała jego praktycznie całkowitą resorpcję. W 28. dniu stenty BPCS cechowały się największym stopniem zapalenia (chociaż bez znamienności statystycznej), który po okresie degradacji polimeru został zredukowany do wartości identycznych jak w przypadku pozostałych grup. Analiza rentgenowska o wysokiej rozdzielczości (*faxitron*) nie uwidoczniła żadnych istotnych nieprawidłowości, z wyjątkiem izolowanych pęknięć przęseł w środkowej części 2 stentów BMS oraz 1 BPCS.

Wnioski: Badany stent PROLIM, pokryty polimerem biodegradowalnym uwalniającym sirolimus, wykazał bardzo dobre efekty angiograficzne i histologiczne zarówno w obserwacji krótko-, jak i średnioterminowej. Brak efektu przyrostu neointimy w okresie 28–90 dni (*catch-up phenomenon*), szybka endotelializacja i minimalna reakcja zapalna mogą przyczynić się do uzyskania korzystnych efektów klinicznych.

Słowa kluczowe: stent, polimer biodegradowalny, sirolimus, świnia

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