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Preclinical evaluation of a novel polyphosphazene surface modified stent

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ABSTRACT

Background: Treatment options for patients with coronary artery disease at high risk for bleeding complications are limited. The aim of the current preclinical study was to evaluate neointimal coverage, endothelial recovery, inflammation and thrombogenicity in a novel thin-strut (71 μm thickness) Cobalt Chromium (CoCr) stent modified with a nano-thin Polyzene®-F (PzF) surface coating.

Methods and results: Twenty-eight single PzF nano-coated stents and 20 bare metal control stents (BMS) were implanted in the coronary arteries of 24 pigs, with scheduled 5- (n = 5), 28- (n = 13), and 90-day (n = 6) follow-up in addition to overlapping configuration (n = 6 each), examined at 28-days. Histomorphometric analysis showed significantly lower neointimal thickness in PzF nano-coated stents than BMS controls at both 28- and 90-days (p = 0.023 and 0.005) and reduced inflammation (p = 0.06 and 0.13). Endothelial coverage over luminal surfaces at all time points was similar between nano-coated stents and BMS controls. We conducted supplementary in-vitro experiments using human monocytes and an ex-vivo swine carotid-jugular arterio-venous shunt model to better understand the healing properties afforded by the PzF nano-coating. Overall, the PzF-nano-coating showed reduced monocyte adhesion and thrombus formation compared to the un-coated controls.

Conclusions: Stents modified with a nano-thin PzF-coating implanted in healthy swine indicate favorable vascular healing properties shown by reduced neointimal hyperplasia and inflammation, along with resistance to thrombus formation in an ex-vivo shunt model over unmodified stents.

1. Introduction

Drug eluting stents (DES) have become the gold standard for the treatment of coronary artery disease [1,2]. Particularly with second-generation DES both a reduction in early stent thrombosis and a slower rate of late catch-up promoted their wide use even in ST-elevation myocardial infarction [3]. Despite the tremendous improvement in the management of patients suffering an acute coronary syndrome, the need for strict adherence to dual anti-platelet therapy (DAPT) to avoid ischemic complications often poses patients at risk for subsequent bleeding events, which have also been shown to contribute to cardiovascular mortality after stent implantation [4]. Complications of stent thrombosis and bleeding remain a considerable concern in high risk patients and those in need of surgery [5]. A recent meta-analysis of short-and long-term DAPT therapy after DES implantation has shown that shorter DAPT (3 or 6 months) is associated with low rates of bleeding but a higher rate of stent thrombosis, compared with longer-DAPT (≥12 months), which is somewhat attenuated with the use of 2nd generation DES [6].

Usage of BMS, particularly for coronary indication has decreased dramatically, because of the need for repeat revascularization and higher rates of early stent thrombosis. Newer developments in surface modifications however, may represent a viable strategy to improve the performance of BMS, as constructing a buffer layer at the interface of the organic and inorganic materials may further support long-term biocompatibility. The COBRA-PzF™ coronary stent system (CeloNova BioSciences, San Antonio, TX) applies a novel surface modification consisting of a thin nano-coating (Polyzene-F, ≤0.050 μm thickness) consisting of poly[bis(trifluoroethoxy) phosphaszene (PTFEP), a soft pliable inorganic polymer with a –[P═N]n– backbone and trifluoroethanol side groups. Application of PzF to an ultra-thin (71 μm) CoCr stent results in hydrophobic surface properties causing high human serum albumin and low fibrinogen and fibronectin adsorption. Thus, resistance to thrombus formation and early endothelialisation may indicate greater biocompatibility in the absence of anti-proliferative drugs [7–12] possibly allowing for ultra-short duration of DAPT. The aim of the current study was to investigate the safety profile of the

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COBRA PzF stent in comparison to BMS using established preclinical models and subsequent testing employing in-vitro and ex-vivo assays.

2. Methods

The study protocol was approved by the Institutional Animal Care and Use Committee of the Medstar Research Institute and Synecor Labs and conforms to the position of the American Heart Association on use of animals in research and the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health [13].

3. Experimental set-up

3.1. Test devices and grouping

Polyzene-F (PzF) is a proprietary formulation of poly [bis (trifluoroethoxy)-phosphazene], which is an ultrapure polymer applied at an approximate thickness of 50 nm. The test device was a Cobalt Chromium (CoCr) PzF surface modified stent (3.0 × 15 mm, COBRA PzF, CeloNova BioSciences, San Antonio, TX) with 71 μm strut thickness. Commercially available CoCr BMS (3.0 × 15 mm Multilink Vision (Vision-BMS), Abbott Vascular, Santa Clara, CA) with 81 μm strut thickness served as control. An un-modified COBRA stent (3.0 × 15 mm, COBRA-BMS, CeloNova BioSciences, San Antonio, TX) with an identical platform served as second control in the porcine shunt model to evaluate the specific impact of PzF coating on acute thrombogenicity.

4. Experimental models

Vascular healing after implantation of Cobra PzF stents was investigated in a healthy porcine model of single coronary stent implantation (n = 24 animals) and compared to Vision BMS with scheduled 5, 28-and 90-day follow-up, while animals receiving overlapping stent configurations were survived for 28-days. In separate studies, monocyte adherence and cytokine production were assessed in culture as further detailed in the supplemental methods while an ex-vivo arterio-venous porcine shunt model served to assess acute thrombogenicity as previously described (Supplemental Fig. S1) [14].

4.1. Porcine coronary stent implantation model

A total of 24 healthy domestic Yorkshire cross swine (12–13 weeks, Palmetto Research Swine, Reevesville, SC) were included in the study with random allocation of COBRA PzF or Vision BMS. Stents were deployed using nominal pressure in two or three of the major coronary arteries: Left anterior descending (LAD), left circumflex (LCX), and right coronary artery (RCA) with an oversize ratio of 1.1:1.0 and an inflation duration of 20 s. Dual anti-platelet medication (aspirin 81 mg/day and clopidogrel 75 mg/day) were administered three days prior to catheterization and maintained until termination.

At the conclusion of the in-life phase, animals were euthanized under deep anesthesia. Whole hearts were flushed with Ringer's Lactate and then gravity perfused at 80 to 100 mmHg with formalin. The specimens were then prepared for light microscopy (LM) by staining with Hematoxylin and Eosin or Movat pentachrome stain. Morphometric analysis was performed using morphometry software (IP Lab for Mac OS X, Scanalytics, Rockville, MD). A vessel injury score was calculated according to the Schwartz method [15]. Overall, neointimal inflammation (score 0–4), adventitial inflammation (score 0–3), and fibrin (score 0–3) were scored for each section as previously described [16].

5. In vitro models

5.1. Monocyte adherence assay

To further understand the mechanism of inflammation in the arteries with COBRA PzF implants we assessed the interaction of human elutriated monocytes on thin CoCr coupons modified with PzF while unmodified coupons served as a control as detailed in the supplemental methods. Monocytes (4×10^5 cells/well) were seeded in 24-well plates containing PzF modified and un-modified CoCr coupons. Adherent cells were then investigated after 48 h with respect to number, viability, differentiation, and cytokine production.

5.2. Cytokine content in monocyte supernatant

The supernatants from cells cultivated for 48 h were carefully harvested, centrifuged free from cells, frozen and maintained at −80 °C and then analyzed. Cytokines were measured using an array-based multiplex ELISA system (Human Inflammation Array Q3 kit, Rey Biotech, Inc., Norcross, GA) for simultaneous detection of 40 selected inflammatory markers, as detailed in the supplemental methods.

5.3. Porcine ex vivo arterio-venous shunt model

Platelet adherence and thrombus formation were assessed using an ex vivo arteriovenous shunt model in the pig involving a circuit loop consisting of Sylgard elastomer tubing with three in-line test and control stents. The circuit was run for 60 min as previously described [17] or until flow rate was reduced ≥50% resulting from blockage. Bolus and maintenance low dose intravenous heparin (100 IU/kg) was used to achieve blood activated clotting times (ACT) between 150 and 200 s with minimal interference on platelet activation and aggregation, as previously confirmed by light transmission aggregometry [14,18]. At the conclusion of each run, stents were gravity perfused with Ringer's Lactate until cleared of blood and then fixed in 10% neutral buffered formalin. The stents were then gently removed from the tubing and bisected longitudinally where one half underwent immunofluorescent staining with an anti-CD61/CD42b platelet marker cocktail and confocal microscopy imaging while the remaining half was processed for SEM as previously described [17]. The experimental set-up is detailed in the supplemental methods.

5.4. Statistical analysis

Results for continuous variables with normal distribution were expressed as mean ± standard deviation. Normality of distribution was tested with the Wilk–Shapiro test. Variables with non-normal distribution were expressed as median with 25% and 75% percentiles. The Kruskal-Wallis test with Dunnett's post hoc correction was used for comparison of non-normally distributed data, while an ANOVA with Dunnett's post hoc correction was used for normally distributed data. A value of $p \leq 0.05$ was considered statistically significant. For the porcine ex vivo arterio-venous shunt model, nested generalized linear mixed models (GLMM) with Dunnett's correction for multiple testing were employed in order to investigate group differences in consideration of multiple measurements per individual. Within these models, stent type was considered as fixed effect, while the experimental factor variables animal, shunt position and duration of the shunt run were considered as nested random effects. The analyses were performed with SPSS Advanced Statistics Version 22 (IBM, Armonk, New York). The statistical tests were 2-tailed and a p value of <0.05 was considered to indicate statistical significance.