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Specific Coronary Drug-Eluting Stents Interfere With Distal Microvascular Function After Single Stent Implantation in Pigs

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Objectives The aim of this study was to compare the effects of single drug-eluting stents (DES) on porcine coronary function distal to the stent in vivo and in vitro.

Background The mechanism of endothelial dysfunction occurring in human coronary conduit arteries up to 9 months after DES implantation is unknown.

Methods A sirolimus-eluting stent (SES), paclitaxel-eluting stent (PES), and a bare-metal stent (BMS) were implanted in the 3 coronary arteries of 11 pigs. After 5 weeks, in vivo responses in distal coronary flow to different doses of bradykinin (BK) and nitrates were measured. In vitro, vasodilation to BK and nitrates, as well as vasoconstriction to endothelin (ET)-1 were assessed in both distal coronary conduit and small arteries. In addition, contributions of nitric oxide (NO) and endothelium-derived hyperpolarizing factors (EDHFs) and cyclic guanosine monophosphate (cGMP) responses to BK-stimulation were determined in vitro.

Results Both DES did not alter in vivo distal vasomotion. In vitro distal conduit and small arterial responses to BK were also unaltered; DES did not alter the BK-induced increase in cGMP. However, after NO synthase blockade, PES showed a reduced BK-response in distal small arteries as compared with BMS and SES ($p < 0.05$). The ET-1–induced vasoconstriction and vascular smooth muscle cell function were unaltered.

Conclusions In this study of single stenting in healthy porcine coronaries for 5 weeks, SES did not affect distal coronary vascular function, whereas PES altered distal endothelial function of small arteries under conditions of reduced NO bioavailability. Therefore, specifically the EDH-component of microvascular function seems affected by PES. (J Am Coll Cardiol Intv 2010;3:723–30) © 2010 by the American College of Cardiology Foundation

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Percutaneous coronary intervention with stenting is an established treatment in most patients with obstructive coronary artery disease. Clinical trials have shown that the 2 first-generation drug-eluting stents (DES), the sirolimus-eluting stent (SES) and paclitaxel-eluting stent (PES), reduce restenosis and revascularization rates by at least 50% as compared with bare-metal stents (BMS) (1). Despite beneficial effects of DES on restenosis, coronary endothelial dysfunction has been observed in areas adjacent to SES (2–4) and PES (5) versus BMS, on average 6 months after implantation. This evidence clearly points toward chronic coronary endothelial dysfunction in the peri-stent area of conduit arteries after both types of DES-implantation. In addition, 9 months after single SES and PES placement, conduit arterial dysfunction was shown predominantly in distal and far distal segments of the stent (6). This observation suggests that, after single DES, especially the distal coronary flow area is at high risk for adverse effects. However, the exact nature of this distal coronary dysfunction is difficult to study in clinical settings. Experimental studies might help to investigate the underlying mechanism(s) (7).

Overlapping PES implantation in a porcine model resulted in, besides distal conduit dysfunction, abnormal endothelium-dependent relaxation of distal small arteries (8). Currently, no data are available comparing the effects of single DES on endothelial function of the coronary microcirculation distal to the stent. In addition, it is presently unknown which specific components of endothelial function might be particularly affected. This information is important, because a functional microvascular endothelium plays a key role in the regulation of local vascular tone and myocardial perfusion by releasing vasodilating factors, including nitric oxide (NO) and endothelium derived hyperpolarizing factors (EDHFs) (9). The latter is believed to be more prominent in circumstances of impaired NO-mediated vasodilatation (10,11).

Consequently, the present study was undertaken to compare the effects of single BMS, SES, and PES on distal coronary function in healthy pigs (12) with focus on the microcirculation, in both in vivo and in vitro settings. We hypothesized that DES implantation would result in distal endothelium-dependent microvascular dysfunction, evidenced by decreased vasodilation and increased vasoconstriction. Specifically, we focussed on the NO and EDHF components of vascular function.

Abbreviations and Acronyms

BK = bradykinin
BMS = bare-metal stent(s)
cGMP = cyclic guanosine monophosphate
CRC = concentration-response curve
DES = drug-eluting stent(s)
EDHF = endothelium-derived hyperpolarizing factor
ET = endothelin
L-NAME = N-nitro-L-arginine methyl ester
NO = nitric oxide
pEC50 = concentration necessary to produce 50% of the maximal response
PES = paclitaxel-eluting stent(s)
SES = sirolimus-eluting stent(s)
SNAP = S-nitroso-N-acetylpenicillamine

Methods

Coronary intervention. Eleven Yorkshire-Landrace pigs of either sex (57 ± 4 kg at implantation; 75 ± 7 kg at death) entered the study. The study was performed in accordance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996), after approval of the local Animal Ethics Committee.

Animal preparation and stent implantation were performed as described previously (12). In short, 1 day before surgery animals received 300-mg acetylsalicylic acid and a loading dose of 300-mg clopidogrel. Under general anesthesia, left and right coronary angiography were performed to select arterial segments of 2.5 to 3.5 mm in diameter in the left anterior descending, left circumflex, and right coronary arteries. Three types of commercially available stents were randomly implanted in selected uninjured coronary segments with a balloon/artery ratio of 1:1, such that each animal received all stents (1 stent/coronary artery) in a random fashion: a BMS (R-stent Evolution/Prodigy, 316L stainless steel stent, 15-mm length, 3.0- to 3.5-mm diameter, Orbus MT, Fort Lauderdale, Florida), a SES (Cypher: DES with 1.4-$\mu g/mm^2$ sirolimus-loading, 316L stainless steel stent, 13-mm length, 3.0- to 3.5-mm diameter, Cordis, Miami, Florida), and a PES (Taxus: DES with 1.0-$\mu g/mm^2$ paclitaxel-loading, 316L stainless steel stent, 12-mm length, 3.0- to 3.5-mm diameter, Boston Scientific, Natick, Massachusetts). After repeat angiography, the animals were allowed to recover and followed for 5 weeks. This period is believed to result in maximal neointimal thickness after BMS implantation in animals, comparable to the response at 6 to 9 months in patients (13). During follow-up, the animals received 300-mg acetylsalicylic acid and 75-mg clopidogrel daily.

In vivo coronary function assessment. After 5 weeks, changes in coronary flow velocities distal to the stent induced by different agonists were measured with a Doppler tipped guidewire (FloWire, Volcano Therapeutics, Inc., Rancho Cordova, California). Intracoronary flow velocity measurements were performed continuously, both at stable baseline hemodynamic status and during intracoronary infusion of the endothelium-dependent vasodilator bradykinin (BK) (0.05 to 1.0 $\mu g/kg/min$), the endothelium-independent vasodilating agent sodium nitroprusside (5 to 20 $\mu g/kg/min$), and at maximal vasodilation to adenosine (100 $\mu g/kg/min$) (14,15). The alpha$_1$-adrenoceptor agonist phenylephrine was co-infused to maintain mean arterial pressure at approximately 90 mm Hg, while leaving coronary microvascular tone unperturbed (15). Each agonist

concentration was maintained for 3 min, followed by a 5-min washout period to allow for restoration of baseline flow. The peak-increase in distal coronary flow velocity was calculated for each concentration as maximum minus baseline.

Tissue collection. All pigs were killed after 5 weeks of follow-up. Hearts were immediately excised and collected in cold, oxygenated Krebs bicarbonate buffer (16,17). Segments of epicardial conduit arteries (>2 mm diameter), starting approximately 0.5 cm distal to the stent and segments of epicardial small arteries (approximately 300 μm diameter) in the specific distal flow area of the stented artery, were isolated and placed in buffer for in vitro functional studies. The stented segment was placed in 4% buffered formaldehyde for histology.

In vitro coronary conduit and small arterial function assessment. Segments of conduit arteries (approximately 4 mm length) were suspended on stainless steel hooks in organ baths. Segments of small arteries (approximately 2 mm length) were mounted in a Mulvany wire myograph (DMT A/S, Aarhus, Denmark). Vascular responses were measured as changes in isometric force (16,17).

Both conduit and small arteries were subjected to the same experimental protocol. After stabilization, segments were exposed to depolarization by 0.1 mol/l potassium chloride. Upon wash-out of potassium chloride, after equilibration, in a first set of segments, endothelium-dependent relaxation to BK (10^{-10} to 10^{-6} mol/l) was recorded upon pre-constriction with the thromboxane analogue U46619 (10^{-8} to 10^{-7} mol/l). To determine NO-dependent and -independent contributions to BK-induced relaxation, in a second set of pre-constricted segments, the concentration-response curve (CRC) for BK was constructed after 30 min of pre-incubation with 10^{-4} mol/l of the NO-synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME). From a third set, CRCs for endothelin (ET)-1 (10^{-10} to 10^{-7} mol/l) were constructed to study vasoconstriction, followed by endothelium-independent but NO-mediated vasodilation to S-nitroso-N-acetylpenicillamine (SNAP) (10^{-7} to 10^{-6} mol/l).

Cyclic guanosine monophosphate measurements. To study BK-induced cyclic guanosine monophosphate (cGMP) production, segments of small arteries distal to the stent of a subset of pigs (n = 5) were nonexposed (control subjects) or exposed to BK (10^{-6} mol/l) with or without pre-incubation with L-NAME (10^{-4} mol/l). The cGMP concentrations were determined by enzyme-linked immunoadsorbent assay and expressed as pmol $\times$ mg^{-1} protein (17).

Histology. Fixed stented segments were plastic embedded and stained with hematoxylin-eosin (routine stain) and resorcin-fuchsin (elastin stain) for quantitative and qualitative analysis. Neointimal thickness at the stent struts and inflammation scores directly adjacent to the struts were determined (12).

Data analysis. Differences between the stent-types of individual outcome variables (procedural, histology data, single vascular function data, and the like) were assessed with paired *t* tests with correction for multiple testing. Statistical analysis of logarithmic vascular function CRCs was performed with a nonlinear mixed-effects model fit by maximum likelihood with nested random effects (statistical program R, version 2.10.1, The R Foundation for Statistical Computing, Wien, Austria).

In vitro vascular relaxant responses to BK and SNAP were expressed as percentage of contraction to U46619 and ET-1, respectively. Constrictor responses were normalized to 0.1 mol/l potassium chloride. The concentration necessary to produce 50% of its maximal response (pEC_{50}) was determined for each CRC reaching a maximum, with logistic function (16,17). Data are given as mean ± SEM. A value of $p < 0.05$ was considered statistically significant.

Results

Procedural characteristics. Quantitative coronary angiography analysis of selected segments revealed no differences in mean coronary diameter of the 3 stent groups, both before and immediately after stenting. Also, procedural characteristics showed no differences in mechanical vessel injury at stent implantation (Table 1). Furthermore, in a subset of pigs (n = 5), quantitative coronary angiography analysis of the stented segments was performed 5 weeks after implantation. No significant differences in percentage late lumen loss were observed (BMS vs. SES, PES: 16.8 ± 3.4% vs. 17.7 ± 5.5%, 6.9 ± 1.5%; p = NS).

Effects of DES on distal coronary conduit and small arterial function. IN VIVO ASSESSMENT. During flow measurements distal to the stent, no significant changes occurred in heart

Table 1. QCA Assessment of Coronary and Procedural Characteristics at the Time of Stent Implantation

Group	Pre-Stenting (mm)	Post-Stenting (mm)	Balloon Size (mm)	Max Inflation Pressure (atm)	BA-Ratio
BMS (n = 10)	3.02 ± 0.09	3.15 ± 0.10	3.30 ± 0.09	13.4 ± 1.5	1.09 ± 0.01
SES (n = 10)	3.13 ± 0.11	3.29 ± 0.09	3.35 ± 0.10	14.4 ± 1.1	1.07 ± 0.03
PES (n = 11)	3.19 ± 0.10	3.37 ± 0.11	3.49 ± 0.11	14.9 ± 1.2	1.09 ± 0.01

Pre- and post-stenting: mean coronary lumen diameter at site of intervention before and directly after stent placement. Balloon size: mean diameter of contrast-filled balloon during maximal inflation. Max inflation pressure: maximal inflation pressure used for stent placement.

BA = balloon-to-artery; BMS = bare-metal stent(s); PES = paclitaxel-eluting stent(s); QCA = quantitative coronary angiography; SES = sirolimus-eluting stent(s).

rate (85 ± 2 beats/min) and mean arterial pressure (92 ± 3 mm Hg) at baseline or during infusion of BK, sodium nitroprusside, or adenosine. Also, no significant differences in distal vasodilatory responses to BK, sodium nitroprusside, or adenosine were reported after 5 weeks of BMS, SES, and PES implantation (Fig. 1).

IN VITRO ASSESSMENT. Contractile responses to potassium chloride and pre-constriction to U46619 were similar between stent types, both for conduit and small arteries (data not shown).

CONDUIT ARTERIES. Segments of coronary conduit arteries distal to the stent dilated to BK in a dose-dependent manner (pEC_{50} BMS vs. SES vs. PES: 8.2 ± 0.2 vs. 8.0 ± 0.3 vs. 8.0 ± 0.1). Relaxations corresponded with approximately 70% of the pre-constriction level by U46619, with no differences between the different stents (Fig. 2A). Pre-treatment with L-NAME greatly reduced dilation to BK ($p < 0.01$, L-NAME vs. no L-NAME for all stent-types). The effect of L-NAME was not different between the stents (Fig. 2B). The compound ET-1–induced vasoconstriction in a concentration-dependent manner to 100% of the response to 0.1 mol/l potassium chloride (pEC_{50} BMS vs. SES vs. PES: 7.5 ± 0.1 vs. 7.7 ± 0.1 vs. 7.5 ± 0.1). This effect was not significantly altered by either SES or PES (Fig. 2C). The exogenous NO-donor SNAP resulted in vasodilation similar for all stent types (Fig. 2D).

SMALL ARTERIES. Segments of coronary small arteries distal to the stent dilated to BK in a concentration-dependent manner (pEC_{50} BMS vs. SES vs. PES: 8.3 ± 0.2 vs. 8.2 ± 0.2 vs. 8.0 ± 0.1). Relaxations amounted up to 90% of the pre-constriction level and were influenced by stent-type (Fig. 2E), in that chronic PES implantation caused a modest rightward shift of the BK-CRC ($p < 0.01$, PES vs. SES; $p = 0.1$, PES vs. BMS) (Fig. 2E). After pre-incubation with L-NAME, the BK-CRC was clearly shifted to the right ($p < 0.01$, L-NAME vs. no L-NAME for all stent-types) (Fig. 2F). The effect of L-NAME in small arteries was more modest than in conduit arteries. This confirms that the NO-independent component of the BK response in small arteries is more pronounced than in conduit arteries (16,17). The PES implantation shifted the BK-CRC in the presence of L-NAME approximately 10 times to the right as compared with BMS and SES ($p = 0.02$, PES vs. BMS; $p = 0.02$, PES vs. SES) (Fig. 2F). Thus, small arteries distal of PES showed impaired vasodilation upon incubation with L-NAME (pEC_{50} BMS vs. SES vs. PES: 7.6 ± 0.3 vs. 7.9 ± 0.2 vs. 7.0 ± 0.3). The PES-type of stent specifically reduced the NO-independent component of the BK response. The compound ET-1–induced vasoconstriction in a concentration-dependent manner up to 150% of the response to 0.1 mol/l potassium chloride (pEC_{50} BMS vs. SES vs. PES: 7.7 ± 0.1 vs. 7.6 ± 0.1 vs. 7.6 ± 0.1), unaffected by stent-type (Fig. 2G). The exogenous NO-donor SNAP resulted in vasodilation of the small arteries similar for all stent-types, indicating intact vascular smooth muscle cell function (Fig. 2H).

Cyclic GMP measurements in distal small arteries. After BK-stimulation, distal microvascular cGMP levels were 1.95 (0.5 to 11.9) pmol × mg^{-1}, 1.01 (0.2 to 2.3) pmol × mg^{-1}, and 1.63 (0.6 to 7.7) pmol × mg^{-1} protein (geometric mean and range of BMS vs. SES vs. PES; p = NS). Pre-incubation with L-NAME reduced the microvascular cGMP content after BK-stimulation by 80.4 ± 7.7% vs. 61.8 ± 22.3% vs. 73.0 ± 6.3%, respectively (p = NS).

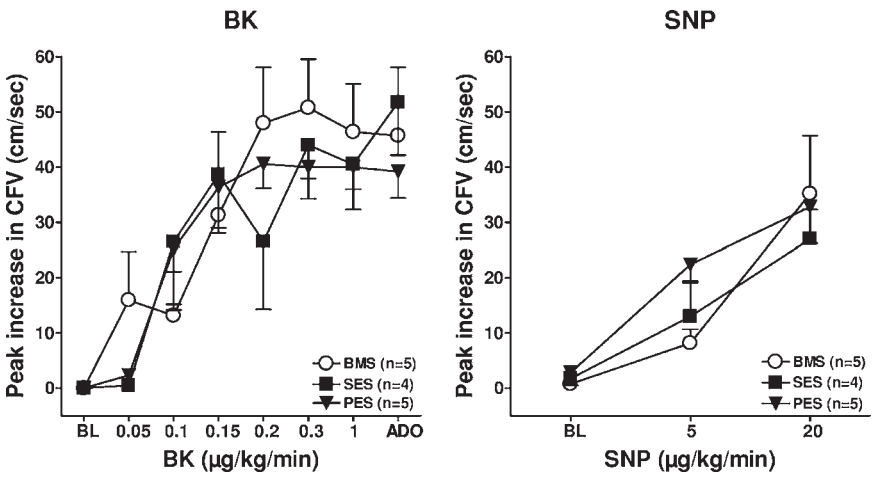

Figure 1. In Vivo Coronary Function

Effects of 5 weeks of single stents on baseline (BL), endothelium-dependent (bradykinin [BK]), adenosine (ADO) and endothelium-independent (sodium nitroprusside [SNP]) peak increases in coronary flow velocity (CFV) of the distal coronary circulation in vivo (n = 5 pigs). BMS = bare-metal stent(s); PES = paclitaxel-eluting stent(s); SES = sirolimus-eluting stent(s).

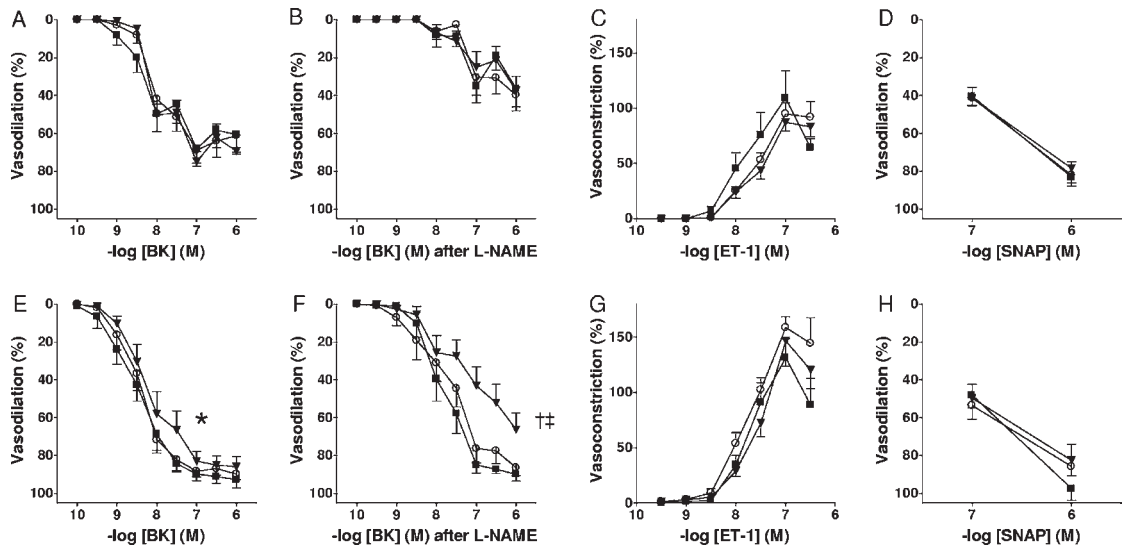

Figure 2. In Vitro Coronary Conduit Arterial Function and In Vitro Coronary Small Arterial Function

Effects of 5 weeks of single stents on distal endothelium-dependent (BK, BK after pre-incubation with N-nitro-L-arginine methyl ester [L-NAME]), endothelium-independent vasodilation (S-nitroso-N-acetylpenicillamine [SNAP]), and vasoconstriction (endothelin [ET]-1) of coronary conduit arteries and small arteries in vitro (n = 11 pigs). **Top panels:** conduit arteries; **bottom panels:** small arteries. Concentration-response curves (CRCs) to BK (**A, E**: *p < 0.01, PES vs. SES); CRCs to BK after pre-incubation with L-NAME (**B, F**: †p = 0.02, PES vs. BMS; ‡p = 0.02, PES vs. SES); CRCs to ET-1 **(C, G)**; CRCs to SNAP **(D, H)**. Abbreviations as in Figure 1.

These results agree with previously published data on BK-stimulated porcine coronary small arteries (17).

Histology of stented coronary arteries. Qualitative analysis showed that the vascular response after stenting followed patterns typical for BMS (Fig. 3A) and DES (Figs. 3B and 3C) in pigs (12). The neointima in BMS consisted of vascular smooth muscle-like cells within a collagenous matrix largely covered by endothelial cells. The neointima in DES was similar, including the presence of endothelial cells in all DES. Typically for DES, the neointima also contained fibrinoid deposition—a marker of delayed healing (18)—surrounding the stent struts (Figs. 3B and 3C). In PES, mitotic figures characteristic for paclitaxel were observed (Fig. 3C), whereas SES showed more inflammation resulting in increased neointimal formation on top of the struts (BMS vs. SES vs. PES: 313 ± 49.3 μm vs. 524 ± 82.9 μm vs. 268 ± 36.0 μm; p < 0.01 SES vs. PES). Neointimal thickness was not significantly different among the groups upon exclusion of stents with a high inflammation score (BMS vs. SES vs. PES: 313 ± 49.3 μm vs. 355 ± 58.2 μm vs. 235 ± 16.6 μm; p = NS).

Discussion

In the present study, we compared the effects of first-generation DES on endothelial function of the distal coronary vasculature with special focus on the microcirculation, in both in vivo and in vitro settings in healthy pigs. We hypothesized that DES implantation would result in distal endothelial dysfunction evidenced by decreased vasodilation and increased vasoconstriction. The main findings were that after 5 weeks of DES implantation, in vivo vascular function distal to DES was essentially unaffected. This was confirmed in vitro, because SES implantation did not alter BK-mediated vasodilation, whereas PES had only modest effects. However, under conditions of reduced NO bioavailability, PES-induced defects in distal microvascular function in vitro were clearly exposed. These defects were principally due to impairment of the NO-independent component of BK-induced vasodilation. Specifically, endothelial function was affected, because the vasodilation response to an exogenous NO donor was unaltered. The PES-type of stent did not alter the cGMP response upon BK stimulation in vitro, the key second messenger system of NO-dependent vasodilation, a finding in agreement with the vascular function results.

DES effects on distal coronary function. This study indicates for the first time that the effects of single DES might extend into the distal coronary microcirculation of healthy pigs. This conclusion is based on results obtained in vitro in isolated coronary small arteries. Five weeks after stenting, microvascular alterations are not primarily induced by circulating concentrations of DES drugs, because 30 days after DES placement, drug-release from the stent is minimal (1).

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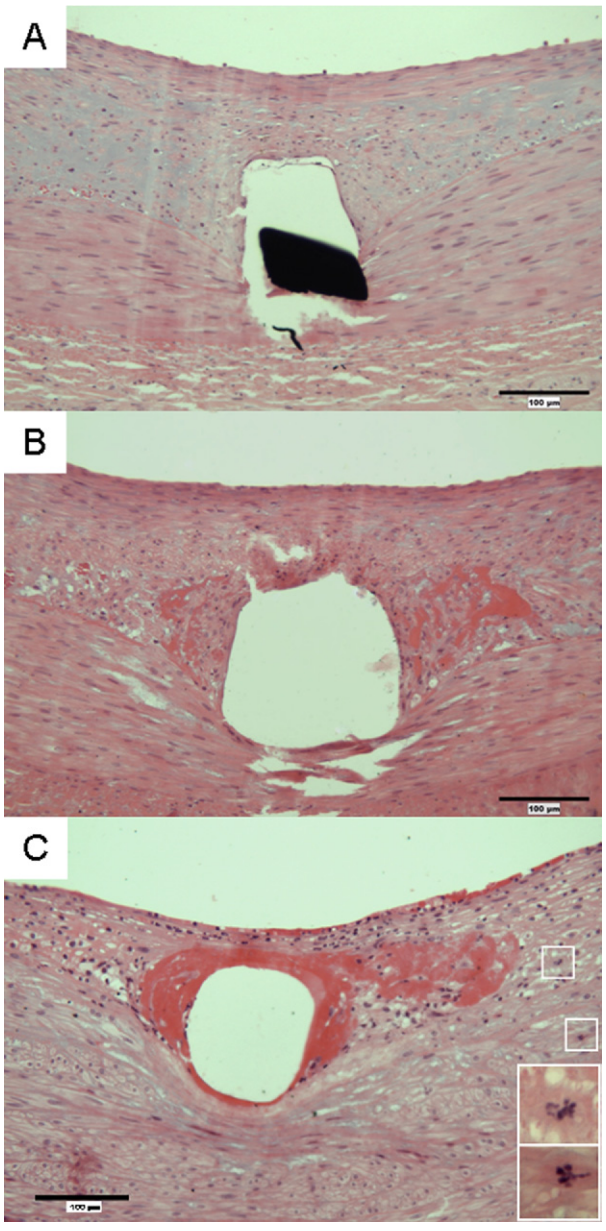

Figure 3. In Stent Histology

Representative in-stent histological sections. The BMS shows a well-healed neointima with vascular smooth muscle cells in a collagenous matrix **(A)**; SES shows less cellularity and moderate amounts of fibrinoid **(red) (B)**; PES also shows fibrinoid surrounding the stent struts as well as mitotic spindles (**C**, **magnifications**). All stents were largely covered by endothelium (hematoxylin-eosin stain; **bar** = 100 μm; **magnification inserts:** 2× original image; **white squares:** areas of magnification). Abbreviations as in Figure 1.

In addition, in-stent histology showed neointimal healing for all groups, thereby minimizing ongoing drug release in the circulation.

A recent pig study already showed abnormal endothelium-dependent relaxation of distal small arteries after 1 month of exposure to PES (8). However, in this study overlapping PES were implanted, creating a more profound local injury and inflammatory reaction contributing to the vasomotor dysfunction in the peri-stent area as compared with the present study. Furthermore, with overlapping DES implantation, additional drug concentrations will be eluted from the stents, aggravating local side effects. The authors reported a decreased NO-mediated conduit arterial response adjacent to overlapping PES, most likely due to locally enhanced oxidative stress. However, the mechanisms of the impaired small arterial responses of the distal perfusion bed were not examined. Of note, the reported abnormal endothelium-dependent relaxation of distal small arteries did not result in enhanced ET-1–induced vasoconstriction (8).

In the present study no clear differences were observed in distal microvascular functions with BK alone or with SNAP, either in vivo or in vitro. Only after NO synthase blockade in vitro, significant differences were demonstrated in microvascular responses of PES-treated coronary arteries. This suggests that PES specifically affected the NO-independent component of BK-induced microvascular endothelial vasodilation (i.e., EDHF) (17). Classically, this EDHF component can be blocked by applying inhibitors of small and intermediate conductance calcium-dependent potassium channels (apamin and charybdotoxin) (17). Preliminary data from our laboratory confirm that adding apamin and charybdotoxin on top of L-NAME fully blocked the microvascular BK-response after chronic DES-implantation (data not shown). The EDHFs are known to be of particular importance in small arteries, although their exact molecular mechanisms and pathways remain to be unravelled (16,17,19,20). In agreement with an alteration in EDHF and not in NO, the cGMP-response—the key second messenger system of NO—was unaltered by PES in vitro. The PES did not affect ET-1–induced microvascular constriction, possibly because NO and ET-1 are balanced (21).

The exact molecular mechanisms by which 5 weeks of single PES resulted in alterations of distal EDHF-mediated microvascular function cannot be derived from the present study. However, it is of interest to note that, although both DES reduce restenosis rates effectively (1), they have different mechanisms of action (18). Accumulation of extracellular reactive oxygen species is a crucial step for paclitaxel-induced cancer cell death (22). In addition, overlapping PES implantation was associated with enhanced local oxidative stress affecting NO-mediated vasodilation of, in particular, conduit arteries in the peri-stent area (8). However, reactive oxygen species increase—in particular, a rise in hydrogen peroxide—also has been reported to inhibit EDHF-synthesis by cytochrome P450 epoxygenases (17,23,24). Therefore, local vascular accumulation of paclitaxel might give rise to local oxidative stress, modifying

specific components of endothelial function, perhaps depending on the affected vessel size.

Clinical studies. The in vivo observations of the current study, made in conduit arteries immediately distal to both DES, showed unaffected vascular function. These observations contrast with clinical studies reporting coronary endothelial dysfunction up to 9 months after DES implantation (2–6). An explanation for this discrepancy could involve different study time-frames, protocols, and the use of healthy porcine vessels. First, stent healing in healthy pigs is much faster as compared with diseased patients. Although the results obtained in pigs after 5 weeks of stenting might correlate to patient results obtained 6 to 9 months after stenting, one should be cautious with extrapolation of DES-results (13). Second, in contrast to human coronary arteries, porcine coronaries respond poorly to acetylcholine as an agonist, due to limited presence of muscarinic receptors within the vascular wall. Although the acetylcholine response is a hallmark in the detection of impaired coronary endothelial function in patients, BK-induced endothelium-dependent vasodilation is clinically relevant as well (25). Finally, in the clinical setting severely diseased vessels are stented instead of the healthy, uninjured coronary arteries of the pigs of the present study. Obviously, in patients, endothelial vulnerability of conduit arteries might have existed before stenting, and this might explain why an impaired conduit endothelial response after DES implantation was already observed in humans without NO synthase blockade, whereas in healthy pigs it could only be observed in the more sensitive small arteries in the presence of L-NAME.

Study limitations. In the present study, part of the experiments were performed in an in vitro setting. Although in vitro not all the influencing factors of in vivo are present, this specifically allowed us to examine vascular responses with and without NO bioavailability in the DES-exposed coronary vessels of each animal. In the in vivo setting, such an extensive vasoactive protocol would be hampered both by the potential systemic effects of NO blockade as well as BK-induced tachyphylaxis, which needs to be taken into account when studying vasomotion with and without NO blockade.

Additionally, the present study reports the in vitro results of PES in a relatively limited number of pigs that was large enough to show clear changes like the microvascular response to BK after pre-incubation with L-NAME but might have been too low to draw conclusions on the basis of a small difference such as the microvascular response to BK.

Conclusions

We compared the functional effects of first-generation single DES on the distal coronary circulation. Particularly PES might show distal endothelium-dependent microvascular dysfunction, because the loss of local EDHF-mediated vasodilation eventually might alter the vasomotor balance and therefore local myocardial perfusion in the in vivo setting. Our results might be relevant for vulnerable coronary artery disease patients with severe microvascular endothelial dysfunction, given their already existing reduced microvascular NO-bioavailability. However, the in vitro observations of the present study warrant further studies to examine distal vascular alterations caused by PES over time in more detail as well as at longer follow-up.

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