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Time Course of Endothelium-Dependent and -Independent Coronary Vasomotor Response to Coronary Balloons and Stents

Comparison of Plain and Drug-Eluting Balloons and Stents

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Objectives This study sought to determine the time dependency of the endothelium-dependent and -independent vascular responses after percutaneous coronary intervention (PCI) with drug-eluting (DEB) or plain balloons, bare-metal (BMS), and drug-eluting (DES) stents, or controls.

Background Long-term endothelial dysfunction after DES implantation is associated with delayed healing and late thrombosis.

Methods Domestic pigs underwent PCI using DEB or plain balloon, BMS, or DES. The dilated and stented segments, and the proximal reference segments of stents and control arteries were explanted at 5-h, 24-h, 1-week, and 1-month follow-up (FUP). Endothelin-induced vasoconstriction and endothelium-dependent and -independent vasodilation of the arterial segments were determined in vitro and were related to histological results.

Results DES- and BMS-treated arteries showed proneness to vasoconstriction 5 h post-PCI. The endothelium-dependent vasodilation was profoundly ($p < 0.05$) impaired early after PCI (9.8 ± 3.7%, 13.4 ± 9.2%, 5.7 ± 5.3%, and 7.6 ± 4.7% using plain balloon, DEB, BMS, and DES, respectively), as compared with controls (49.6 ± 9.5%), with slow recovery. In contrast to DES, the endothelium-related vasodilation of vessels treated with plain balloon, DEB, and BMS was increased at 1 month, suggesting enhanced endogenous nitric oxide production of the neointima. The endothelium-independent (vascular smooth muscle–related) vasodilation decreased significantly at 1 day, with slow normalization during FUP. All PCI-treated vessels exhibited imbalance between vasoconstriction–vasodilation, which was more pronounced in DES- and BMS-treated vessels. No correlation between histological parameters and vasomotor function was found, indicating complex interactions between the healing neoendothelium and smooth muscle post-PCI.

Conclusions Coronary arteries treated with plain balloon, DEB, BMS, and DES showed time-dependent loss of endothelial-dependent and -independent vasomotor function, with imbalanced contraction/dilation capacity. (J Am Coll Cardiol Intv 2012;5:741–51) © 2012 by the American College of Cardiology Foundation

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The loss of intact arterial wall structure due to atherosclerosis is associated with impaired regulation of vascular tone, local thrombus formation or inflammation, and vessel wall remodeling, such as aneurysms or adaptive or constrictive remodeling (1–3). Despite immediate relief of the vessel obstruction by percutaneous coronary intervention (PCI), the disrupted endothelium and the mechanical tension of the media/adventitia result in adverse clinical outcomes due to vessel thrombosis, remodeling, and the development of neointimal hyperplasia (2–5). Delayed endothelialization after drug-eluting stent (DES) implantation is related to late vessel thrombosis, but paradoxically, lesser degree of neointimal hyperplasia and restenosis (6–8) due to endothelial flow-mediated focal heterogeneity of protein and gene expression and biochemical pathways (2).

Recent in vivo and in vitro studies have shown that first-generation DES implantation resulted in persistent endothelial dysfunction with resistance to vasodilator nitrate (9–15). By contrast, the endothelium-dependent coronary vasomotor function remained relatively preserved after placement of the latest generation of DES, such as biolimus-eluting stents, and may be related to better long-term clinical outcome (12,16), although this finding still remains to be confirmed. Comparative in vitro studies performed in porcine coronary arteries 1 month after bare-metal stent (BMS) or DES implantation consistently reported the loss of endothelium-dependent vasodilation, but heterogenous results regarding the vascular smooth muscle cell–related vasomotor response (10,17,18).

Abbreviations and Acronyms

BMS = bare-metal stent(s)
DEB = drug-eluting balloon(s)
DES = drug-eluting stent(s)
FUP = follow-up
LAD = left anterior descending coronary artery
LCX = left circumflex coronary artery
PCI = percutaneous coronary intervention
QCA = quantitative coronary angiography
RCA = right coronary artery

Interestingly, to date, no data are available on the effect of drug-eluting balloons (DEB) on coronary vasomotor reaction, even though the longitudinal and vertical diffusion of the antiproliferative drug into the endothelium and transmural layer, and the midterm persistence of the drug in the arterial wall have been shown (19,20). Furthermore, the time course of neoendothelial function and healing arterial wall layer in relation to the developing neointima and restenosis has not been not investigated, despite the fact that the timely vascular response to constrictors or endogenous and exogenous dilators (such as regular medicines in patients with coronary artery diseases) strongly influences the short- and long-term clinical outcomes (21).

Therefore, the aim of the present study was to determine the time-dependent progress of the contractile and endothelium-dependent and -independent vasodilation of coronary arteries after PCI with DEB or plain balloon, BMS, and DES in direct relation to the morphological injury in porcine coronary arteries.

Methods

Animal preparation. Domestic pigs (n = 27) received a loading dose of aspirin (100 mg) and clopidogrel (300 mg) per os 1 day before PCI. After overnight fasting, anesthesia was initiated with 12 mg/kg ketamine hydrochloride, 1 mg/kg xylazine, and 0.04 mg/kg atropine and maintained with isoflurane and oxygen via a mask. After intratracheal intubation, the anesthesia was continued with a gas mixture of 1.5 vol% to 2.5 vol% isoflurane, 1.8 vol% O_2, and 0.5 vol% N_2O. Blood pressure, O_2 saturation, and electrocardiogram were monitored continuously; serum electrolytes and blood gas (IL GEM Premier 3000, Bedford, Massachusetts) were measured before and after intervention. The experiments were conducted in the Institute of Diagnostic Imaging and Radiation Oncology, University of Kaposvar, Hungary. The investigations conform to the "Position of the American Heart Association on Research Animal Use," adopted by the American Heart Association on November 11, 1984, and the relevant specific Hungarian laws were followed.

Percutaneous coronary intervention. The right femoral artery was punctured under sterile conditions, and a 7-F intra-arterial sheath was placed. After a loading dose of unfractionated heparin (200 IU/kg), a guiding catheter of 7-F (Medtronic, Minneapolis, Minnesota) was introduced either into the left or right coronary artery ostium, and coronary angiography was performed using a nonionic contrast agent. A guiding wire (Cordis, Miami Lake, Florida) was introduced into the coronary arteries (left anterior descending [LAD], left circumflex [LCX], or right [RCA] coronary arteries, first diagonal branch of the LAD, and obtuse marginal branch of the LCX) (Fig. 1). Stents were implanted randomly into the LAD and the LCX. Plain balloon, DEB, BMS, or DES were chosen randomly.

In accordance with the guidelines of pre-clinical stenting experiments (22), stents were implanted with an inflation time of 30 s and 10 to 12 atm to reach a stent/artery ratio of 1.1:1. Due to the elasticity of the porcine coronary artery, a 1.3:1 balloon/artery ratio was used for balloon overstretch injury (19). Only 1 device was used for each artery. A maximum of 3 stents were implanted in 1 animal, whereas 5 different balloons were used in 1 animal (randomly chosen LAD, LCX, or RCA for stents, and additionally, diagonal branches and obtuse marginalis of LCX for balloons) (Fig. 1). In addition, the balance of the affected arteries, available devices, and follow-up (FUP) times were also considered. The number of arteries with PCI was 5-5-5-9 for plain balloon, 13-6-5-5 for DEB, 5-7-5-6 for BMS, and 11-6-5-5 for DES at 5-h, 1 and 7 ± 2 days, and 1-month FUP, respectively, in a total of 27 pigs (Fig. 1).

After control angiography, the guiding wire, the catheter, and the introducer sheath were removed, and the femoral arteries were closed with FemoSeal closure device (St. Jude

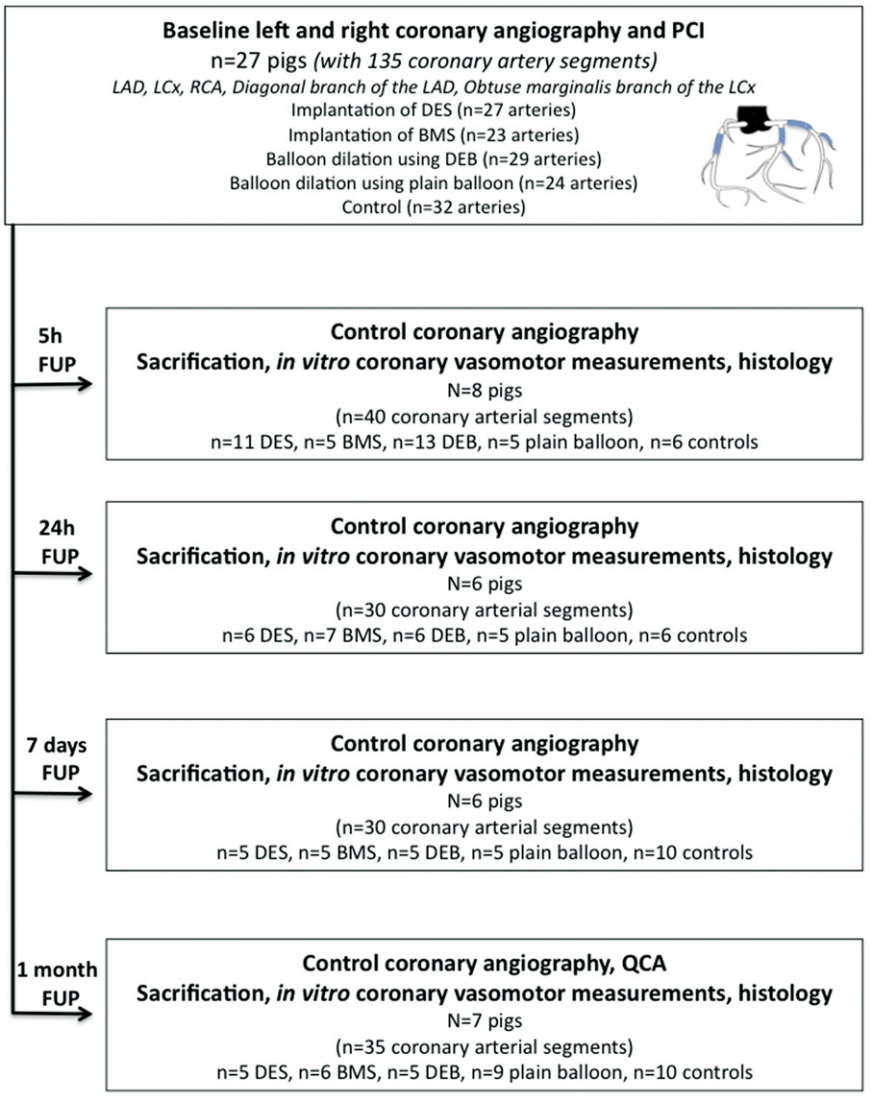

Figure 1. Flow Chart of the Experiments

BMS = bare-metal stent(s); DEB = drug-eluting balloon(s); DES = drug-eluting stent(s); FUP = follow-up; LAD = left anterior coronary artery; LCX = left circumflex coronary artery; PCI = percutaneous coronary intervention; QCA = quantitative coronary angiography; RCA = right coronary artery.

Medical, St. Paul, Minnesota). The animals were then allowed to recover from the anesthesia. Heart rate, oxygen saturation, blood pressure, and electrocardiography were monitored during the procedure.

Devices. The size and length of the plain balloon (test balloon, not for human use), DEB (test balloons, 3-μg paclitaxel/mm^2 balloon surface, not for human use), BMS (test stent, not for human use), or DES (test stent, 3-μg paclitaxel/mm^2 stent surface, not for human use) were 2.75 or 3.0 mm and 15 mm, respectively. All PCI devices were constructed for pre-clinical experiments.

FUP examinations. FUP times were selected to correspond to specific human findings. The 5-h and 1-day FUP reveal or exclude PCI-induced vessel thrombosis, whereas 1-week FUP is related to the active smooth muscle cell proliferation. Moreover, the 1-month FUP time in pigs matches 6-month FUP in humans (22–24). Thus, we have chosen these FUP time points for measurement of the vascular response to PCI accordingly.

During FUP, the animals were treated with a daily dose of clopidogrel (75 mg) and aspirin (100 mg).

The animals underwent control coronary angiography followed by euthanasia 5 h, 1 day, 7 ± 2 days, and 1 month (±3 days) after index PCI with 10 ml of saturated potassium chloride, and the hearts were explanted in toto (Fig. 1). The coronary arteries were prepared, and the site of balloon

dilation, the stented segments, and the proximal segments adjacent to stenting were cut and flushed with physiological saline solution, then the vasomotor response of the cut (stent-free) segments was measured. After completion of the in vitro measurements, the balloon-dilated segments, the segments with stents, and the proximal parts were fixed in 4% formalin. The stented arteries were embedded in Technovit 9100 (Heraeus Kulzer GmbH, Wehrheim, Germany), whereas the native coronary arteries were embedded in paraffin, cut into 4- to 6-μm-thick slices, and then stained with hematoxylin-eosin and MOVAT pentachrome stains (22,25).

Quantitative coronary angiography. Post-stent and FUP quantitative coronary angiography (QCA) parameters were measured by means of a computer-assisted quantitative arteriographic edge-detection algorithm (ACOM.PC, Siemens, Erlangen, Germany) (Online Methods 1).

Measurements of vasomotor responses. The dilated segment of the coronary arteries as well as the proximal segments adjacent to the stents were isolated, and after the removal of fat and connective tissue, 4-mm-long ring segments were cut and mounted in a temperature-controlled 15-ml tissue bath (37°C) containing a modified Krebs–Henseleit buffer solution (Fig. 2), as published previously (8) (Online Methods 2). Briefly, to measure the isometric circular wall tension of the vessels, each segment was suspended between 2 L-shaped metal pins (0.4 mm in diameter) in a myograph (8). After approximately 1 h, the vessels were maximally contracted with endothelin-1 (30 nmol/l).

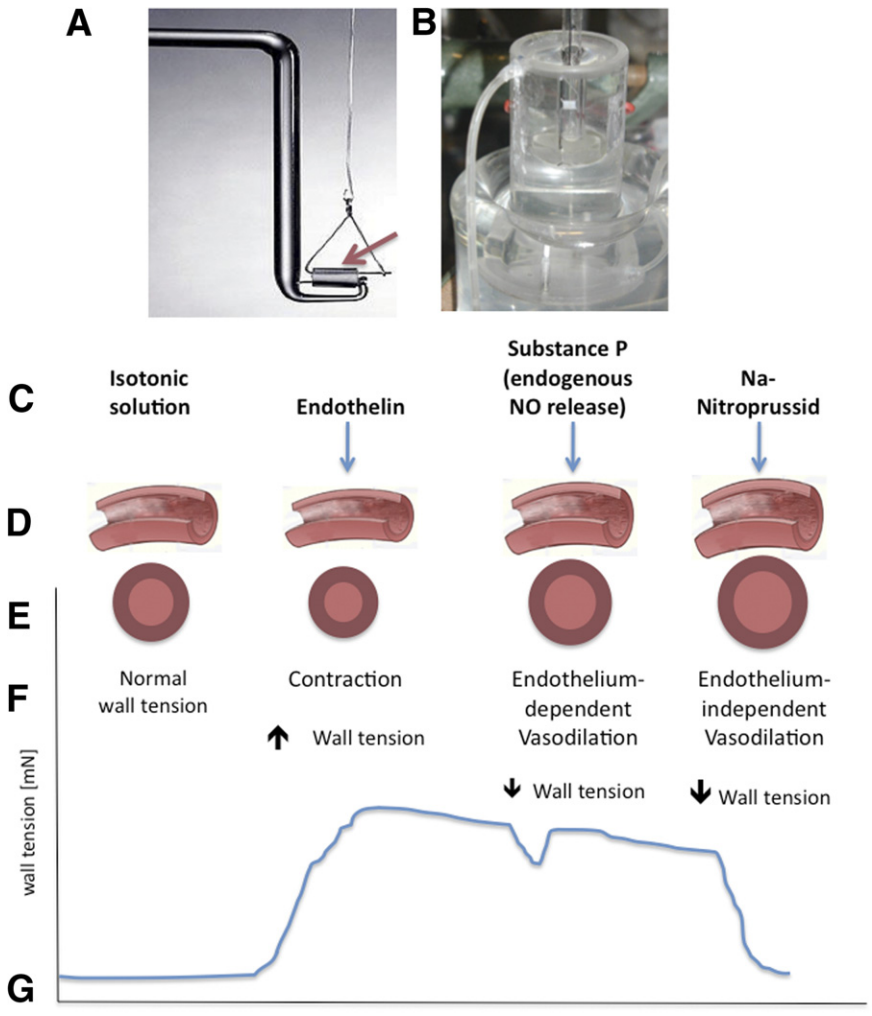

Figure 2. Schematic Illustration of the In Vitro Measurements of Vessel Vasomotor Reaction

(A) L-shaped metal pins in a myograph with a vessel **(arrow)**. **(B)** Bath chamber with the L-shaped pins. **(C)** Applied **(arrows)** solution and drugs (NO = nitric oxide). **(D)** Longitudinal section of the arteries and changes in response to the applied solution and drugs (schematic illustration). **(E)** Cross-sectional changes of the arteries in response to the applied solution and drugs (schematic illustration). **(F)** Pathophysiological mechanism of the changes of the arteries in response to the applied drugs. **(G)** Schematic illustration of the wall tension in response to the applied drugs **(blue line)**.

Maximum endothelium-dependent vasodilation was achieved by a bolus application of substance P directly into the organ bath with increasing concentration, up to a final concentration of 1 nmol/l. Sensitivity of smooth muscle to external nitric oxide (NO) was investigated by subsequent addition of sodium nitroprusside (4 mmol/l). The vasoconstriction, corresponding to media injury and/or endothelial dysfunction in regulation of vascular tone, was expressed as milliNewtons (mN). The endothelium-dependent and -independent vasodilation (stiffness of vascular smooth muscle after overstretch injury of PCI) were expressed in percent change of steady-state level contraction, and in mN/s/mN units, respectively.

Histopathology and histomorphometry. Each arterial segment (stented, balloon-dilated, and proximal to stents) was cut into 3 parts (proximal, middle, and distal) and investigated histopathologically and histomorphometrically. Stented segments were evaluated using recommendation of Schwartz et al. (22) and Virmani et al. (6) (Online Methods 3).

Dilated segments or the segments proximal to stents were analyzed similarly to the score system after stenting, adapted for balloon injury only (19,25) (Online Methods 4). Endothelial coverage was expressed as the percentage of the lumen circumference covered by endothelium, measured by computerized planimetry (ImageJ version 1.440, NIH, Bethesda, Maryland).

The following quantitative histomorphometric parameters were measured: lumen, internal and external elastic lamina area, and the maximal neointimal thickness. The calculated histomorphometric parameters included the neointima area (difference between internal elastic lamina and lumen area), media area (difference between external and internal elastic lamina area), and % area stenosis [(neointimal area/internal elastic lamina area) × 100].

Statistics. In the absence of normal values with standard deviation or expected significant intraindividual change and, therefore, no possible sample size calculation regarding the in vitro vasomotor function measurements, a minimum number of 5 samples per group and 1 FUP time were attempted. No adjustments were made for multiple comparisons or for multiple observations within animals.

Continuous parameters were tested for normal distribution and expressed as mean ± SD. Differences in variances were tested, and if the Levene test had a p value <0.05, a nonparametric test was used for comparison. In case of equal variances, the analysis of variance was supplemented with 2-sided Student *t* test.

Correlation between vessel constriction and endothelium-dependent and -independent vasodilation or between histological and vasomotor parameters was evaluated using linear regression analysis. A p value of <0.05 was considered significant. The statistical analyses were performed with SPSS for Macintosh version 17 (SPSS Inc., Chicago, Illinois).

Results

FUP angiography. Coronary angiography at 5 h, 1 day and 7 ± 2 days revealed no stenosis nor suspected thrombosis. At the 1-month FUP, no angiographic abnormalities of the dilated segments were seen. However, QCA documented 15 ± 10% and 8 ± 9% diameter stenosis of the stented segments with BMS and DES, with a late lumen loss of 0.78 ± 0.32 mm and 0.24 ± 0.21 mm, respectively. The arterial segments proximal or distal to the stents showed no angiographic abnormalities.

Time dependency of the vasomotor reaction post-PCI. Endothelin induced proneness to vasoconstriction in arteries with DES or BMS (22.6 ± 4.7 mN or 20.6 ± 4.6 mN) at the earliest time point (5 h) post-PCI, compared with control (12.2 ± 3.6 mN), or balloon-dilated arteries ($p < 0.05$) (Figs. 3 and 4). Interestingly, 1-day post-PCI, the vasoconstrictive response seemed to be normalized in DES and BMS arteries. At 7 ± 2 days post-PCI, the endothelin-induced vasoconstriction increased in drug-eluting device–treated arteries ($p < 0.05$ between controls and DEB/DES), which was normalized at 1 month, as the vessel repair was completed.

The endothelium-dependent vasodilation was significantly ($p < 0.05$) decreased in all treated arteries 5 h post-PCI (49.6 ± 9.5% vs. 9.8 ± 3.7% or 13.4 ± 9.2% or 5.7 ± 5.3% or 7.6 ± 4.7% in controls vs. plain balloon, DEB, BMS, or DES, respectively) compared with controls, as an obvious sign of immediate endothelial layer injury post-PCI, with slow recovery (Fig. 4). At 1-month FUP, a hypersensitivity of the neoendothelium-dependent vasodilation to substance P was observed in PCI-treated arteries (but not DES-treated vessels) as compared with controls ($p < 0.05$) (68.6 ± 10.0% or 76.0 ± 13.1% or 78.7 ± 18.3% in plain balloon, DEB, or BMS). By contrast, the endothelium-dependent vasodilation remained significantly depressed in DES-treated vessels (33.3 ± 7.4%).

The endothelium-independent (muscular layer–related) vasodilation was profoundly impaired 1 day post-PCI in DEB/BMS/DES-treated arteries (0.062 ± 0.045 mN/s/mN, 0.054 ± 0.041 mN/s/mN, and 0.023 ± 0.003 mN/s/mN, respectively) compared with controls (0.142 ± 0.047 mN/s/mN), but to a lesser extent in plain balloon arteries, indicating less media (muscular) damage with the plain balloon when compared with DEB, BMS, or DES (Fig. 4). At 7-day FUP, the dysfunction in endothelium-independent vasodilation still persisted in DEB and DES-arteries.

Vasoconstriction–vasodilation balance of the epicardial coronary vessels. A significant correlation was found between the vasoconstriction response and endothelium-dependent vasodilation of the noninstrumented arteries (Fig. 5), indicating a physiological balance between contraction–relaxation. However, after pooling the data for 1 device (independent from FUP time), no association could be

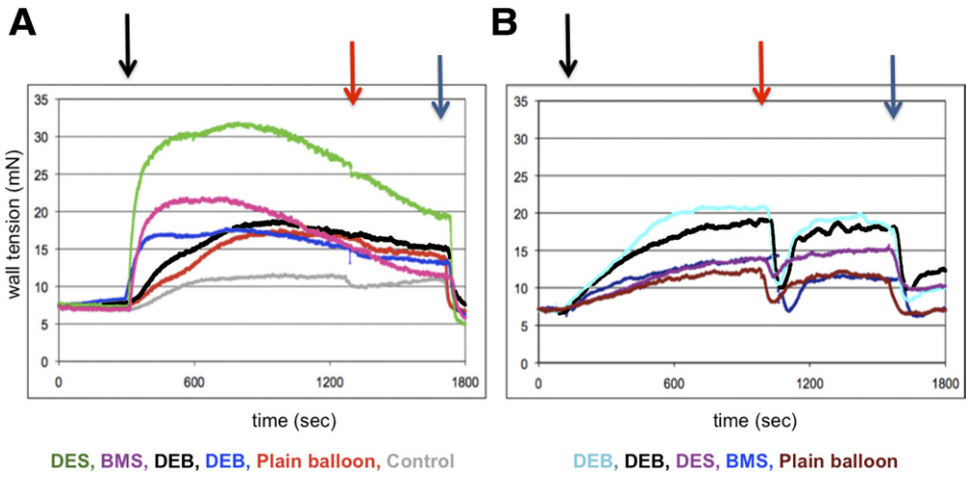

Figure 3. Vasomotor Reaction of the Treated Coronary Arteries After Coronary Intervention

Vasomotor response of the vessels **(A)** 5 h post-intervention and **(B)** at 1 month post-PCI with DES, BMS implantation, and after dilation with plain balloon or 2 different types of DEB and control. Vasoconstrictive response to endothelin **(black arrow)**, endothelium-dependent vasodilation to substance P **(red arrow)**, and endothelium-independent vasodilation to nitroprusside sodium **(blue arrow)**. Abbreviations as in Figure 1.

found between contraction and relaxation, suggesting an imbalance in vasomotor function caused by PCI, with more profound equilibrium disturbance if a stent was implanted.

No differences between the LAD, LCX, and RCA were observed regarding the vasomotor responses.

Histology. Figure 6 and Online Figure 1 summarize the main histopathological and histomorphometric parameters of the arteries at 5 h, 1 and 7 ± 2 days, and 1 month post-PCI. The injury score was significantly ($p < 0.05$) lower in the balloon groups (0.92 ± 0.31 and 0.90 ± 0.45 for plain balloon and DEB, respectively) compared with the stent groups (1.35 ± 0.32 and 1.41 ± 0.52 for BMS and DES, respectively). The fibrin and inflammation score increased in each group during the FUP (Online Fig. 1). Implantation of the stents interrupted the endothelial continuity in DES and BMS vessels 5 h post-PCI ($p < 0.05$ between BMS/DES vs. plain balloon/DEB). With the new endothelium partially covering the stent struts and the interstrut spaces in stents, the endothelialization was 98 ± 2% in BMS- and 87 ± 6% in DES-treated vessels at 1-month FUP (Figs. 6 and 7).

A gradual increase in neointimal area was measured in all groups during the FUP, with the highest degree of maximal neointima areas and % area stenosis in BMS-treated vessels (2.07 ± 0.72 mm^2 and 46 ± 14%) ($p < 0.05$ between BMS and balloons/DES). The media area increased nonsignificantly in DES-treated arteries at 1-month FUP (Figs. 6 and 7, Online Fig. 1).

Comparison of histological findings and vasomotor function. The proneness to vasoconstriction of the vessels at 5 h post-PCI with a pseudonormalization at 1-day FUP failed any histological correlate.

Although endothelialization was significantly better in balloon-dilated arteries early post-PCI as compared with stented vessels, the endothelium-dependent vasodilation was impaired similarly in all groups, suggesting an obvious discrepancy between morphology and functionality of the injured arteries. The endothelialization was almost complete in all PCI-treated arteries at 1-month FUP, but the PCI-treated arteries (except DES-treated vessels) exhibited a higher responsiveness to endogenous NO production of the new intimal layer when compared to controls.

The profound impairment of the endothelium-independent vasodilation did not correlate with the injury score or medial thickening at any time point.

Discussion

This is the first report on the time course of vasomotor reactions of coronary arteries in response to PCI with different coronary devices. The main findings of our study are:

1. DES-treated arteries showed increased vasoconstrictive response early post-DES implantation, together with profound impairment of the endothelium-dependent vasodilation and decrease in endothelium-independent vasodilation. During the FUP, the endothelium-dependent vasodilation response remained impaired.
2. BMS implantation led to an injured vasomotor reaction partially similar to DES, regarding the vasoconstriction, endothelium-dependent, and -independent vasodilation with slow, but complete recovery.

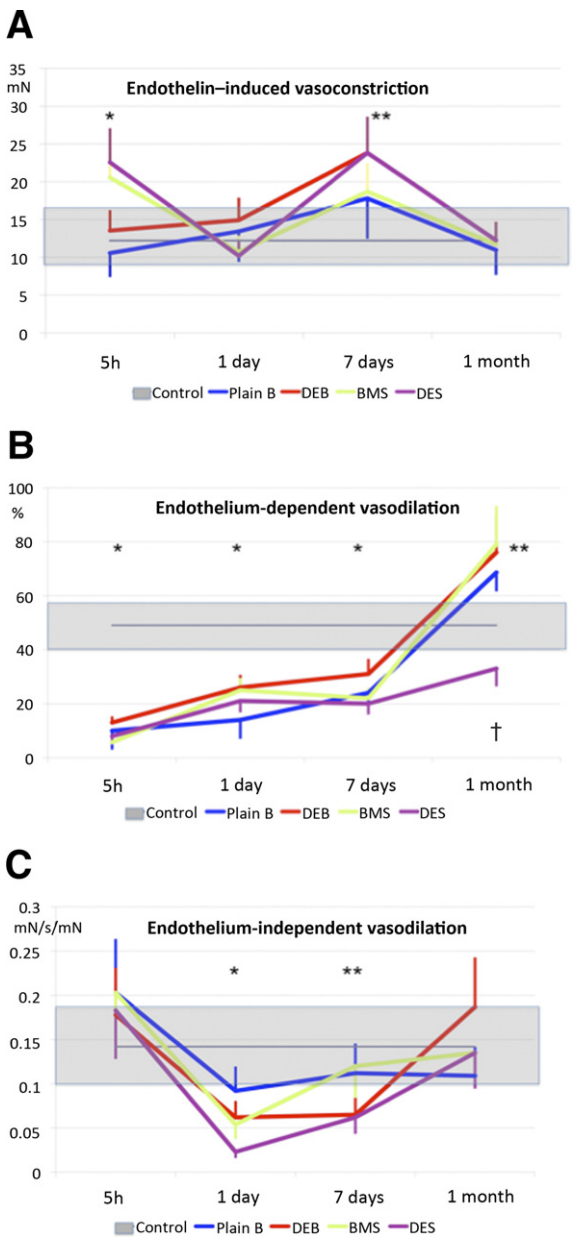

Figure 4. Time-Dependent Vasoconstriction and Dilation Post-PCI

Time dependency of the **(A)** endothelin-induced vasoconstriction (*$p < 0.05$ between control/Plain B/DEB vs. BMS/DES; **$p < 0.05$ between control vs. DEB/DES), **(B)** endothelium-dependent vasodilation to substance P (*$p < 0.05$ between control vs. Plain B/DEB/BMS/DES; **$p < 0.05$ between control vs. Plain B/DEB/BMS; and †$p < 0.05$ between DES vs. control/Plain B/DEB/BMS), and **(C)** endothelium-independent vasodilation to nitroprusside sodium (*$p < 0.01$ between control vs. DEB/BMS/DES; **$p < 0.05$ between control vs. DEB/DES) of the vessels post-PCI with DES or BMS implantation or dilation with plain balloon or DEB at 5 h, 1 and 7 days, and 1 month post-PCI. The **gray rectangle** represents the normal range (mean value ± 1 SD). Each mean value and ±SD represent the measurements of 5-5-5-9 segments with plain balloon, 13-6-5-5 with DEB, 5-7-5-6 with BMS, and 11-6-5-5 with DES at 5-h, 24-h, 1-week, and 1-month FUP, respectively. Plain B = plain balloon; other abbreviations as in Figure 1.

3. Plain balloon use influenced neither the vasoconstrictive, nor the endothelium-independent vasodilation response significantly, but similar to other PCI devices, induced a prompt decrease in endothelium-dependent vasodilation with slow normalization during the 1-month FUP.
4. DEB-treated coronary arteries behaved generally similarly to plain-balloon–treated vessels, regarding the endothelium-dependent vasodilation, but similarly to DES-treated arteries, and led to proneness to vasoconstriction at 7 days and slow normalization of endothelium-independent vasodilation.

The morphological (histological) parameters did not correlate with the vasomotor reactions, suggesting highly complex interactions between the neoendothelium or muscular layers, and endogenous or exogenous vasoreactive stimuli in coronary arteries post-PCI with an imbalance between constriction/dilation responses.

PCI-induced vasomotor dysfunction of the coronary arteries. Online Table 1 summarizes the published data on the PCI-related vasomotor responses, emphasizing the importance of the impaired vasomotor reaction in relation to adverse long-term events (9,14,15,26–28), such as in-stent restenosis or major adverse cardiac events (1,29). Human studies measured the vasomotor function mainly distal to the stented arteries (9,12,14,16,17,26,27) or the flow-mediated endothelium-dependent vasodilation induced either by exercise (13,15,28) or atrial pacing (9,12), with the consistent conclusion of loss of endothelium-dependent vasodilation 6 to 9 months after DES placement. Similar to our method, an organ-chamber model with modified myography was used to measure the endothelium-dependent and -independent vasodilation (1) with contractile reaction at the proximal and distal parts of the stented arteries at 1-month FUP in a pre-clinical porcine model. These results were comparable to humans regarding the endothelium-dependent vasodilation, but heterogenous findings were reported on impaired endothelium-independent vasodilation.

Both the in vivo and in vitro studies reported impaired vasomotor function at FUP, when the endothelialization should be almost complete. By contrast, we have measured the time dependency of the vasomotor response, and revealed important, albeit not completely explainable, mechanisms, such as profound impairment of the endothelium-dependent dilation and proneness to constriction to endothelin early after PCI, with a significantly different response between stents and balloons. The strong and permanent passive stretching of the endothelial and muscular layers by stenting elicits hypersensitivity of the adjacent arterial segments to endothelin, probably through endothelin-related contraction due to interaction with G protein–coupled receptors, or production of asymmetric dimethylarginine (30,31) increasing vascular

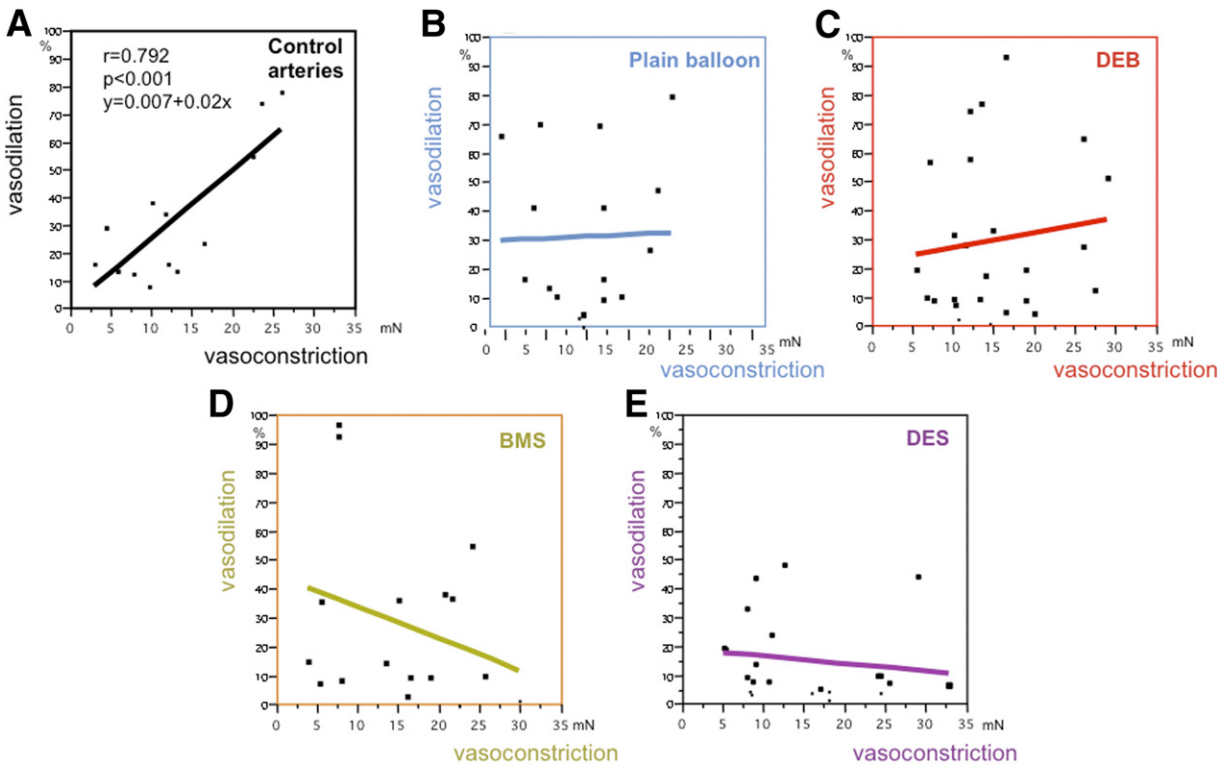

Figure 5. Association Between Endothelin-Induced Vasoconstriction and Endothelium-Dependent Vasodilation of the Vessels to Maintain the Normal Vascular Tone

Pooled data of measurements of 1 device-type–related artery, independently from the follow-up time. **(A)** Significant linear correlation between the contraction/dilation capacities of the normal (control) vessel (n = 32). No correlation between contraction and relaxation after implantation of **(B)** plain balloon (n = 24), **(C)** DEB (n = 29), **(D)** BMS (n = 23), and **(E)** DES (n = 27). Abbreviations as in Figure 1.

resistance. The application of DEB also sensitized the arteries to endothelin-induced vasoconstriction 7 days post-PCI, likely due to the accumulation of the drug within the vessel wall.

Interesting is the time dependency of the endothelium-dependent vasodilation without correlation with the re-endothelialization progress. Similar to our findings, Li et al. (10) and Pendyala et al. (5) showed an increased endothelium-dependent dilation of the coronary arteries at FUP, but without presenting comparative baseline data. By contrast, other authors described a decrease of endothelium-dependent vasodilation long after endothelial regrowth (17). In our experiment, the increased endothelium-dependent vasodilation at FUP can be assigned to the combination of the original and newly developed endothelium, exceeding the values of the native coronary arteries, indicating an altered phenotype of the newly developed endothelium with higher sensitivity to endogenous NO, with the exception of DES-treated vessels.

The DEB exposes the arterial wall to the drug for a limited time; usually, the drug is eliminated within days. By contrast, the mechanical characteristics of the plain balloon and DEB are similar. This might be the reason for the similarity of some functional and histological findings between the 2 balloon types.

The obvious mismatch between vasoconstriction and vasodilation found in all PCI-treated vessels suggests that PCI of any type disturbs the physiological harmony between the contractile and vasodilatory capacities to a much higher extent if stents are implanted. Moreover, the localized hypersensitivity or hyposensitivity of the newly developed neointima to endogenous NO might result in turbulence in the dilated and stented segment, further aggravating the local shear stress.

Endothelium-independent vasodilation of PCI-treated coronary vessels. The endothelium-independent vasodilation showed profound impairment at 1 day, with slow recovery during the FUP, suggesting a PCI-triggered time-dependent reorganization of the muscular (medial and adventitial) layer after arterial overstretch injury. Implantation of DES impaired the smooth muscle cell–related vasodilation to a larger extent than the other PCI device–treated arteries, indicating a stronger dysfunction of the thickened media and a pathological signaling pathway from the endothe-

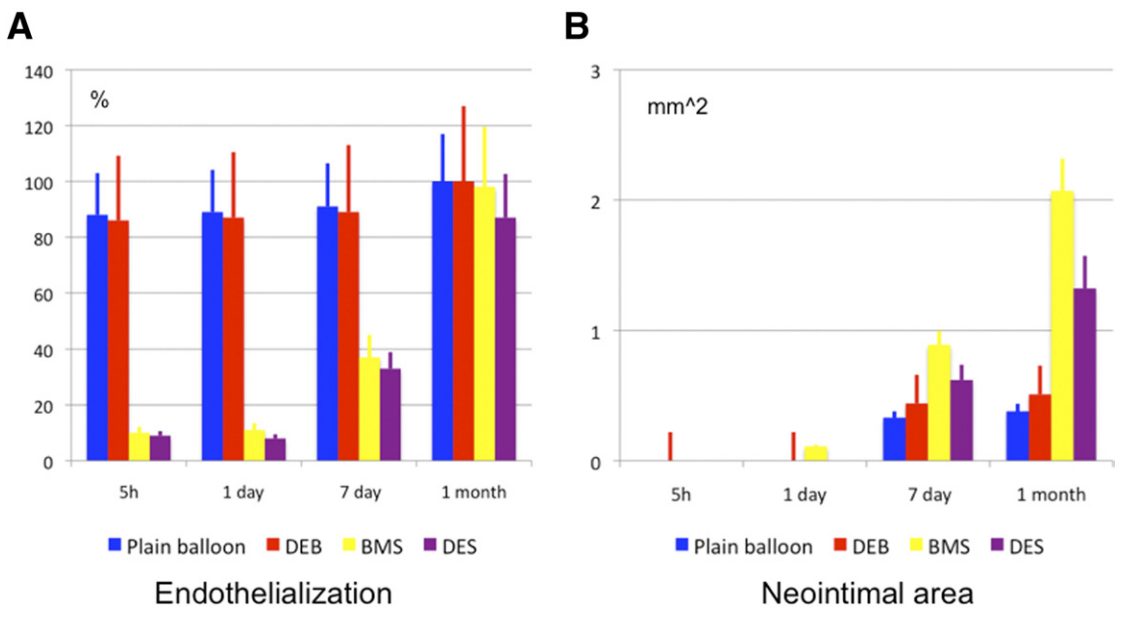

Figure 6. Time-Course of the Histopathological and Histomorphometric Changes Post-PCI

(A) Endothelialization and **(B)** neointimal area of the coronary arteries treated with either plain balloon, DEB, BMS, or DES. Each mean ± SD value represents the measurements of 5-5-5-9 segments with plain balloon, 13-6-5-5 with DEB, 5-7-5-6 with BMS, and 11-6-5-5 with DES at 5-h, 24-h, 1-week, and 1-month FUP, respectively. Abbreviations as in Figure 1.

lium to the medial smooth muscle cells, which may also be influenced by the storage of the drug within the vessel wall (1).

Association between vasomotor response and histological changes. Stent implantation resulted in a higher injury score than balloons, likely leading to a difference in vasomotor function between the stents and balloons. The difference is device-specific, as DEB use is associated with short-term overstretch with a short-term (days) high paclitaxel level in the tissue (9,10), in contrast to the DES with its permanent arterial stretch and long-term slow- or moderate release and penetration of drugs into the vessel wall. The differences in vasomotor response of the various drug-eluting PCI devices can be attributed to the altered drug release kinetics, activation of P2 receptors, and other flow-mediated mechanotransduction or cell-signaling processes (2,31). These local biological mechanisms in connection with the systemic inflammatory changes, proliferation, and migration of the smooth muscle cells or local NO bioavailability are thoroughly investigated in cell culture and in pre-clinical experiments (2,5,9,11,32), revealing device-specific profiles of the arteries.

Additionally, in our experiment, DES-treated arteries showed susceptibility to vasoconstriction at 7-day (time of smooth muscle cell activation) and incomplete recovery of the endothelium-dependent vasodilation at 1-month FUP, which might be due to the incomplete endothelialization (33). However, the time course of histopathological and histomorphometric changes was not completely in accord with the time course of the vasomotor reactions.

Study limitations. We have investigated paclitaxel-coated stents and balloons; a "limus" group–coated device was not available for this experiment. However, the endothelium-dependent vasodilation disturbances were reported to be similar in both drug-eluting groups.

We have only tested the proximal segment adjacent to the stent, not the distal part, although it has been shown that the distal part demonstrates inferior dilation capacity. However, such a comparison is already published (12) (Online Table 1) pointing out that the vasomotion of the nonstented reference segments reflects both the local and the systemic endothelial functions, influenced by the local antiproliferative drug storage in case of DES.

All PCI devices were constructed for pre-clinical experiments, not for human use. Using the test coronary devices of different companies, we must take confidentiality into consideration. However, we have given the size and length of the balloons and stents, and the paclitaxel concentration of the drug-eluting balloons and stents, which design and features are similar to the devices used in human PCI.

The coronary stents were implanted into the healthy arteries of pigs, which is not representative of the atherosclerotic human coronaries. Being similar to humans, the anatomy and pathophysiology of the coronary

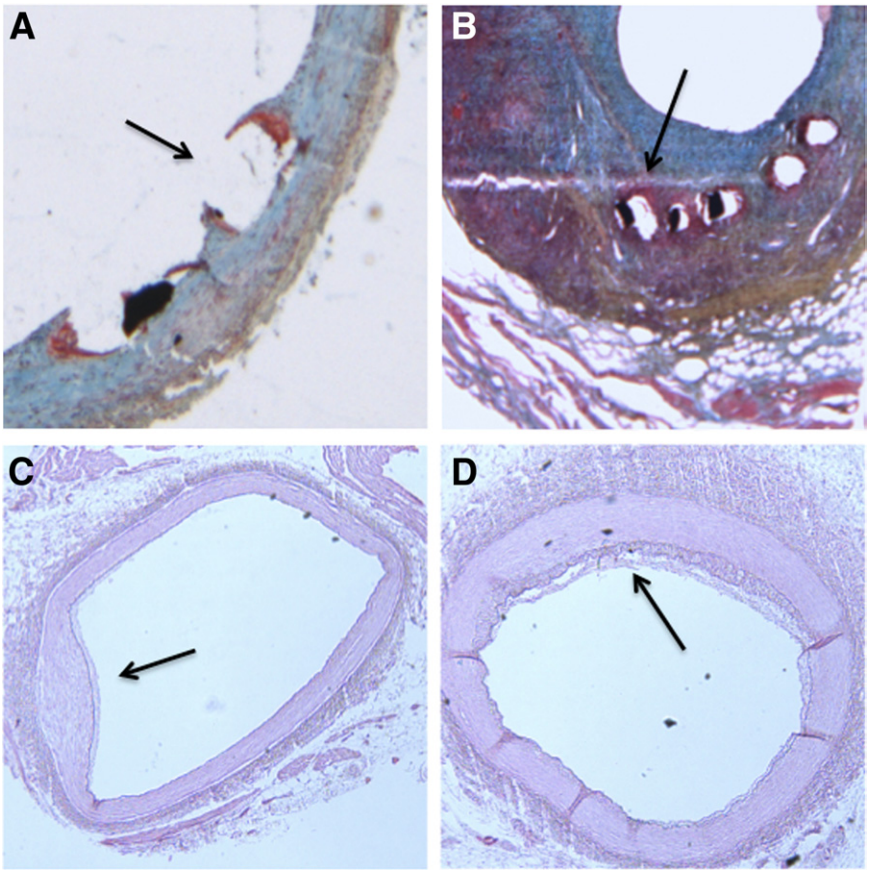

Figure 7. Histology of Coronary Arteries 1 Month Post-PCI

(A) Lack of endothelialization 1 month post-PCI in a vessel treated with DES **(arrow)** (MOVAT staining) (10× magnification). **(B)** Thick neointima of an artery 1 month post-BMS implantation (MOVAT) with fibrin deposition around the stent struts **(arrow)** (10× magnification). **(C)** Localized thickened neointima and media and inflammatory reaction of the adventitia 1 month after overstretch injury by plain balloon (hematoxylin and eosin [HE] staining) **(arrow)** (2× magnification). **(D)** Circular neointimal proliferation with thrombus adhesion 1 month after overstretch injury by DEB (HE staining) (2× magnification). Abbreviations as in Figure 1.

arteries of pigs make this model attractive for basic clinical translational research, and is well accepted as a pre-clinical model of PCI.

Conclusions

Coronary arteries treated with plain balloon, DEB, BMS, and DES showed time-dependent loss of endothelium-dependent vasodilation with imbalanced increases in endothelin-induced vasoconstriction and impaired endothelium-independent vasodilation post-PCI with slow recovery, which might influence the long-term outcome of PCI, regarding re-stenosis, vessel remodeling, and thrombosis. The novelty of our experiment is the serial observation, in parallel with the 4 most-used PCI devices, and the relationship between the functional and histological findings. Furthermore, the presented in vitro experiments have a potential to offer directions to the engineering of new intracoronary devices.

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Key Words: endothelium-dependent vasodilation ■ percutaneous coronary intervention ■ pre-clinical experiment ■ vasoconstriction.

APPENDIX

For supplementary methods, figures, and tables, please see the online version of this article.