Vascular response after implantation of coated and non-coated coronary stents

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Vascular response after implantation of coated and non-coated coronary stents

Vaatwandreactie na implantatie van metalen stents en medicijn-gecoate stents in de kransslagader

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Aan mijn ouders

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Chapter 1

General Introduction and Outline of the Thesis

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Vessel wall injury

September 16, 1977, Andreas Gruentzig performed the first balloon angioplasty of a stenotic coronary artery in a conscious human (1). Since then techniques have changed but the principle is still the same. During percutaneous coronary intervention (PCI) the atherosclerotic plaque causing the coronary stenosis is broken by high pressure balloon inflation in the coronary lumen and mainly pushed into the coronary artery wall.

As the coronary artery wall is elastic, the balloon will not only push the plaque material into the vessel wall, but also stretch the whole vessel wall increasing its diameter during dilatation. Substantial vessel wall injury can be inflicted by balloon oversizing (2).

The major drawbacks of balloon angioplasty were acute elastic recoil after balloon deflation, late constrictive vessel remodelling and neointimal proliferation as a reaction to the vessel wall injury (3-5). This led to a very high percentage of restenosis within 6 months after PCI.

To overcome these problems intracoronary stents were introduced. The stent struts prohibit early and late recoil, but the struts can cause considerable damage to the vessel wall and severity of vessel wall injury was correlated to restenosis by Schwartz et al. (2). The healing response to this vessel wall trauma, resulting in neointimal proliferation inside the stent is responsible for the in-stent restenosis.

Despite these drawbacks, coronary stenting has been shown to be superior to balloon angioplasty only (6, 7). Restenosis rates were reduced to 20- 30 % at 6 months.

A new promising technique emerged to treat the very therapy resistant in-stent restenosis, intracoronary irradiation therapy. Both gamma- and beta-emitters confirmed their effectiveness in randomized trials (8- 13). However, long-term results are disappointing, showing 60 % major adverse cardiac events at 4 years, mainly due to late occlusions (14-16). Delayed vascular wall healing after radiation therapy with long-lasting thrombogenicity is thought to be the main reason.

Beta-radiation as adjunctive therapy to balloon angioplasty or stenting of de novo lesions did not show reduction in clinical events either and again a high rate of late thrombotic occlusions (17, 18).

The major breakthrough in preventing in-stent restenosis was the introduction of the sirolimus-eluting stent and later the paclitaxel-eluting stent. Both drugs with different anti-proliferative mechanisms are released from a polymer stent coating and reduced in-stent

restenosis percentages to single digit numbers for non-complex lesions (19- 22). Despite the impressive results, in-stent restenosis still occurs, especially in challenging lesions. In the early days of sirolimus-eluting stents, 3.0 mm was the largest available diameter. This led to frequent post-dilatation to achieve a diameter of 1 mm larger or even more. In-stent restenosis has been reported after stent fracture of overdilated stents. This is probably caused by the combination of less local drug concentration because of overstretching of the stent, combined with polymer damage and direct vessel wall damage caused by the broken stent struts.

The aim of the first part of the thesis was to study vascular injury and healing response after stent implantation.
Chapter 2 describes the different phases of vessel wall injury and healing response after coronary stenting.
Chapter 3 quantifies vessel wall injury after implantation of different types of stents.
Chapter 4 shows a case-report of a patient with a very late occlusion, five years after balloon angioplasty and additional beta-radiation therapy.
Chapter 5 reports a case, were overstretching of a drug-eluting stent led to stent fracture, resulting in restenosis at follow-up.

Endothelial dysfunction after PCI.

Normal endothelial function is very important for optimal myocardial function and prevention of cardiac events. Endothelium has a key function in the regulation of vascular tone (23), anti-thrombogenicity and prevention of development of atherosclerotic lesions (24, 25). Endothelial dysfunction has been correlated with worse long-term outcome of coronary artery disease (26). Endothelial dysfunction results amongst others in a loss of vasodilatory capacity. This loss of endothelium-dependent vasodilatation has been found in patients with risk factors for atherosclerosis like smoking, aging, hypercholesterolemia, hypertension, hyperglycemia, a family history of premature atherosclerotic disease and obesity (24, 27-29) and reflects one of the earliest asymptomatic states of atherosclerotic disease.
PCI results in acute vessel wall injury and (partial) endothelial denudation of the treated segment. Within several weeks re-endothelialization is completed (30). However, a new endothelial confluent layer does not mean restoration of normal endothelial function (31, 32).

After PCI a vessel wall healing response is elicited. However, adjunctive radiation therapy to prevent restenosis, does not only decrease or delay neointimal proliferation, it also delays the healing response (33, 34). Caramori et al. showed long-term endothelial dysfunction of the coronary segment distal to the PCI site (35).
As mentioned above, drug-eluting stents like the sirolimus-eluting stent and the paclitaxel-eluting stent significantly decreased the rate of in-stent restenosis. However, some data suggest delayed wound healing together with delayed recovery of endothelial function after implantation of these stents (36- 38), exposing the patients to a higher risk of late stent thrombosis and delayed in-stent restenosis.
Endothelial cells are aligned along the flow direction in the coronary artery. Flow turbulence caused by a stenosis or branching of the coronary tree causes areas of low and high shear stress at the interface of the lumen and coronary artery wall and areas of low shear have been related to endothelial dysfunction in vitro (39-43). However, a relation between local endothelial dysfunction and low shear stress has never been shown in vivo, neither in animal models nor in humans.

The aim of the second part of the thesis was to study endothelial function early and late after different coronary interventions (balloon, bare stent, brachytherapy, DES), as well as to link endothelial dysfunction to shear stress.
In **Chapter 6** endothelial permeability as an indicator of abnormal function was investigated in porcine coronary arteries, late after balloon angioplasty injury or stenting.
In **Chapter 7** endothelium-dependent vasomotion of an atherosclerotic human coronary segment was studied directly and 6 months after intracoronary beta-irradiation therapy.
In **Chapter 8** endothelial functional recovery 6 months after stenting with sirolimus-eluting stents was evaluated compared to recovery after bare stent implantation.
Chapter 9 attempted to correlate local endothelial function with local shear stress in patients during 6 months follow-up coronary angiography after implantation of a sirolimus-eluting stent, using three-dimensional vessel reconstruction and coronary flow measurements during intracoronary acetylcholine infusions.

Coated stents and drug-eluting stents

Stent thrombosis is a serious event and was the reason for the dampened enthusiasm for stenting after the early attempts of 1986 (44) and the years after. In the early days of stenting, very rigorous anti-coagulant regimes were used including heparinization after the procedure, oral anticoagulant therapy and dextran infusions, to overcome the risk of early

stent thrombosis. The higher risk of bleeding complications with this regime led to attempts to make the stent less thrombogenic. Only after resolving the problem of stent thrombosis without significantly increasing risks of major bleeding by the BENESTENT pilot trial (45, 46) using heparin-coated stents and others (47-49), stenting gained popularity and by 2004 most lesions are stented in the Western world. Though excellent results on anti-thrombogenicity, heparin-coated stents did not fulfil the promise of reduction of in-stent restenosis. It was thought that reduction of stent-strut adherent thrombus in the early days after stenting would reduce late restenosis because the adherent thrombus could be a nidus of growth factors and inflammatory cytokines, stimulating neointimal proliferation.

Despite the excellent data on drug-eluting stents, concern has been raised regarding the risk of stent thrombosis in drug-eluting stents. Experimental data showing delayed vascular healing after PCI and reports of stent thrombosis have led to longer double anti-platelet therapy. In bare stents 1 month was the standard, in drug-eluting stent patients 6 to 12 months of double anti-platelet therapy has become the rule. Though concerns about stent thrombosis even led to an official FDA warning in 2003 (50), increased risk compared to bare stents could never be substantiated.
The general concern led to very cautious use in acute coronary syndromes, because of the higher “thrombotic state” of these patients, inherent to the acute coronary syndrome.

The aim of the last part of the thesis was to study safety and efficacy of withdrawing rigorous anticoagulant regimes when using heparin-coated stents as well as thrombotic risk and long-term outcome of drug-eluting stents.
Chapter 10 reports the findings of the use of a heparin-coated stent in porcine coronary arteries. This preclinical study led to the clinical BENESTENT 2 pilot and trial.
Chapter 11 gives an overview of subsequent research and trials with different heparin-coatings, conducted up till 2004.
Chapter 12 is a summary of the available data on drug-eluting stent studies by January 2004, written for the course book of “ The Paris Course on revascularization 2004”.
Chapter 13 and 14 investigated safety and efficacy of the use of sirolimus-eluting stents (**Ch 13**) and paclitaxel-eluting stents (**Ch 14**) in the percutaneous treatment of acute myocardial infarction patients.

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Part 1:

Vessel wall injury

Chapter 2

Pathobiology of coronary stents

Sjoerd H. Hofma, Heleen M.M. van Beusekom, Willem J van der Giessen

J Interv Cardiol 2001;14:597-600

Pathobiology of Coronary Stents

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The use of metal stents has significantly reduced the adverse event rates after percutaneous therapy of coronary artery disease.[1–3] Early risks associated with stent implantation, like subacute occlusion, have been reduced by improving stent expansion[4] and antiplatelet therapies.[5] Typically in recent trials the early occlusion rate is approximately 1% per stented lesion[6] compared with approximately 5%–10% in earlier series. However, more and more patients with multivessel disease will be treated percutaneously and the proportion of these patients with periprocedural enzyme elevations is far from negligible (6.2% after treatment of 2.6 lesions per patient in the ARTS trial).[6] Blockade of the platelet glycoprotein IIb/IIIa receptor is effective in reducing early complications after stent placement.[7] The combination of abciximab and percutaneous coronary angioplasty (PTCA) is, however, still associated with significant bleeding.

The successful reduction of early events has shifted attention towards late risks after coronary stent treatment, that is, the recurrence of stenosis (restenosis). The stent as mechanical support still fails in this respect in about 10%–25% of cases. Especially in high risk subsets (diabetics, saphenous vein grafts, small vessels < 2.5 mm in diameter, long lesions, etc.), restenosis may rate as high as 50%. In long lesions and small vessels, current stents are only marginally if at all superior to balloon angioplasty.

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Since the use of stents is standard practice, we must learn how to enhance their clinical performance. The main target for improvement is to limit the problem of neointimal growth, which has been identified as the main contributor of intrastent restenosis. Neointimal growth is a response to arterial damage and the introduction of a foreign body, the stent. Pathological studies, supported by results from experimental investigations, have revealed distinct stages describing the body's response to stent implantation: (1) The thrombotic response (days 0–3): upon placement of the stent circulating plasma proteins contact it's surface (Fig. 1).[8] This is followed by the rapid activation, adhesion, and aggregation of platelets and neutrophils (Fig 2).

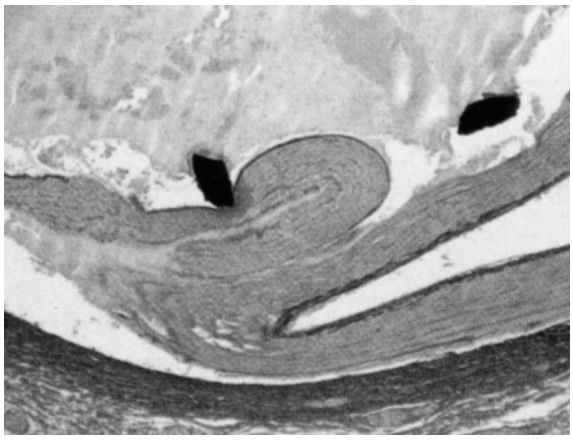


Figure 1. Acute thrombotic occlusion as the result of excessive vascular injury. Plasma proteins and a dense mass of platelets can be seen adherent to the media which is doubled up and folded over on itself as the result of a severe dissection.

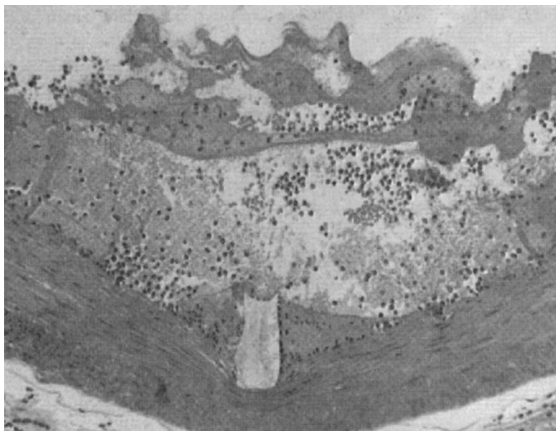


Figure 2. Detail of a stented coronary artery at 4 hours following stenting showing a dense thrombus mass directly covering the stent, overlaid by a loose mass consisting mainly of neutrophils and platelets, and finally covered by layers of intermingled fibrin and platelets.

(2) The recruitment phase (days 2–8): an intense cellular infiltration is induced by thrombus and the injury of stent implantation. The infiltration consists of monocytes that become macrophages as they migrate into the thrombus and injured artery. Lymphocytes are often also present. Both types of cells marginate from the bloodstream and from the adventitia. Myofibroblasts derived from intima and media migrate to the damaged areas of the vessel wall (Fig. 3) and start to colonize the thrombus.[8] (3) The proliferative phase (days 4–45): cells progressively proliferate and resorb residual thrombus. While myocytes populate the neointima, inflammatory cells remain typically associ-

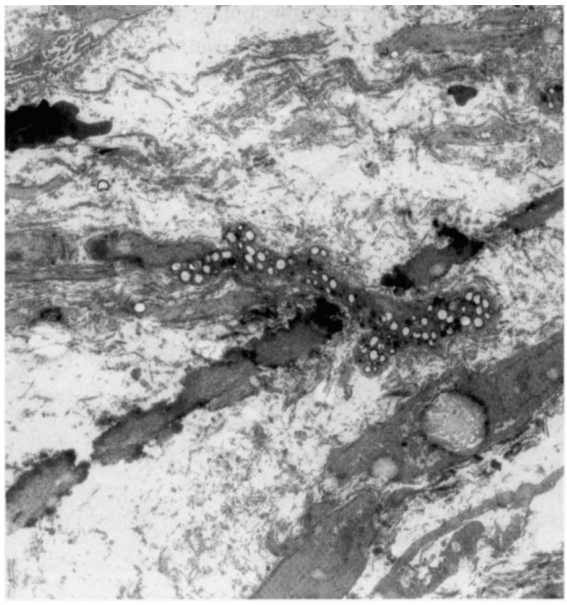
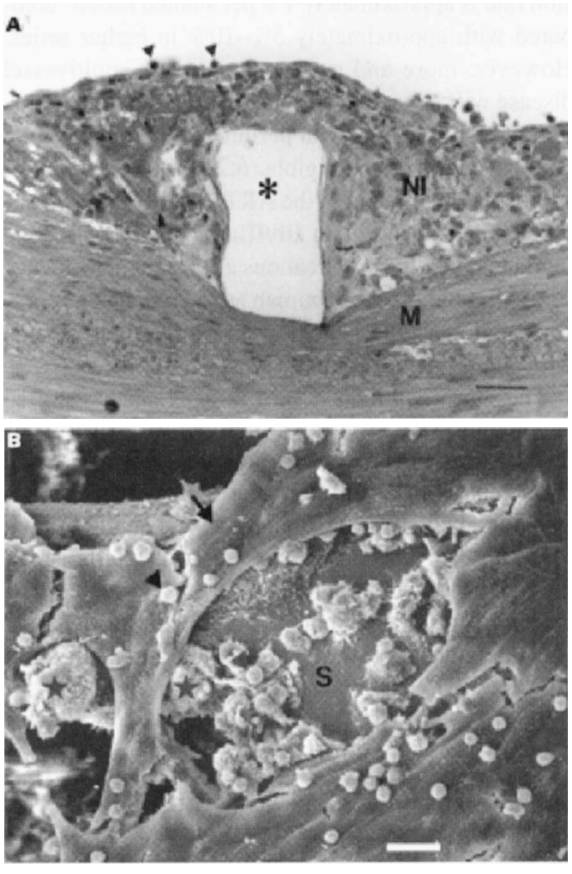


Figure 3. Transmission electron microscopy of a smooth muscle-like cell traversing the internal elastic membrane. The cell is laden with lipid particles.

Figure 4. Late stent thrombosis secondary to an intense inflammatory response 3 weeks following stenting of a porcine coronary artery.



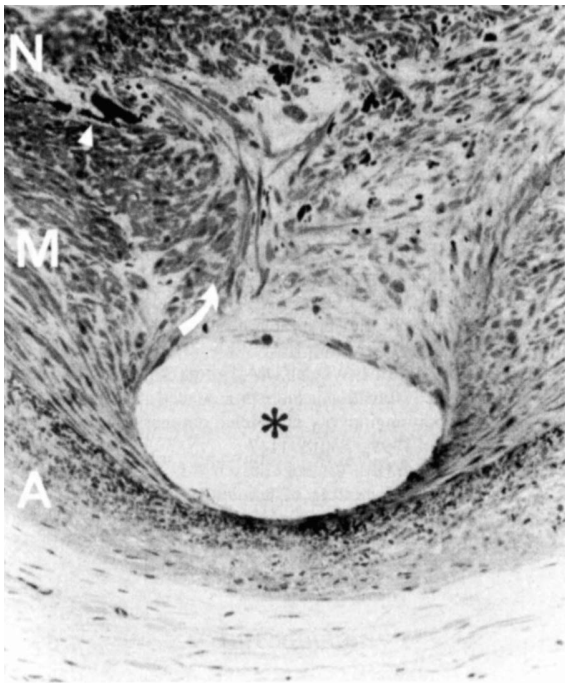


Figure 6. This stented porcine coronary artery shows abundant extracellular matrix in the tissue directly overlying the stent strut (*). A = adventitia; M = media; N = neointima.

ated with the stent struts. Thrombosis secondary to this inflammatory response may under certain circumstances grow to occlude the vessel (Fig. 4). All layers of the vessel wall seem active to repair the initial injury. Endothelial cells progressively cover the arterial wound (Fig. 5)[9] and with time, synthetic cellular phenotypes produce extracellular matrix material (Fig. 6). (4) The healing phase: the neointima, it's size in part determined by the extent of vascular injury (Fig. 7) will slowly reduce in size by cellular apoptosis and extracellular matrix remodeling. Furthermore, the initially dysfunctional endothelium (Fig. 8)[10,11] will in

Figure 5. Early healing response as illustrated by (A) light microscopy of the intimal thickening at 5 days postimplant showing granulation tissue over the stent wire void (*) with occasional leucocytes (arrowheads) attached to the endothelium (bar = 30 μm). (B) SEM of a stent strut (S) at 5 days showing an incomplete endothelial lining (arrow) with leucocytes (arrowheads) and macrophages (*) occupying areas devoid of endothelium (bar = 20 μm). NI = neointima; M = media; hematoxylin and eosin.

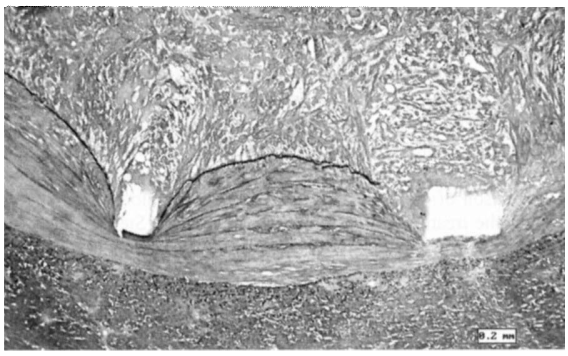


Figure 7. Detail of a stented porcine coronary at 4 weeks following stent placement. Rupture of the internal elastic membrane is clearly visible as the discontinuation of the black line (arrow) at the site of a stent strut void (*). Resorcin-Fuchsin stain.

time mature and may then provide a better functional barrier, depending on the underlying disease.

A better understanding of the role of these distinct, but in part simultaneous, processes will hopefully improve our hold on the process of restenosis.

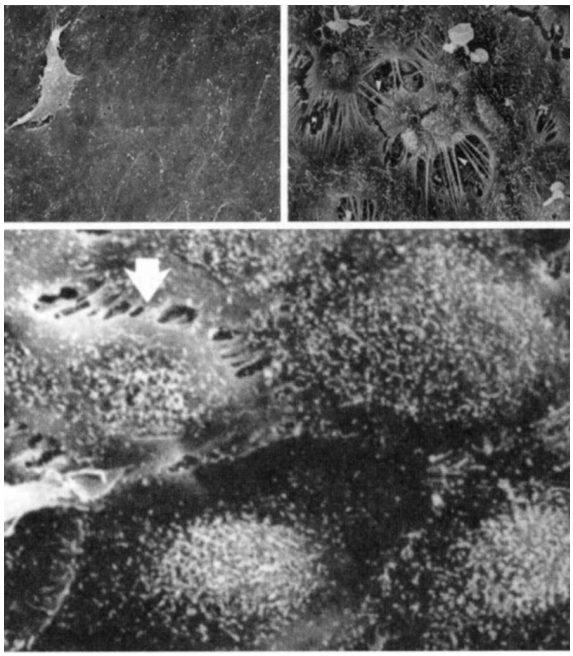


Figure 8. Endothelial dysfunction as shown by scanning EM (A) normal endothelium. (B) Endothelial cell retraction at 2 weeks following stenting showing fingerlike projections between adjacent endothelial cells (arrowhead). Some have broken during critical point drying (*). (C) Human vessel at 3 months following stenting still shows signs of endothelial dysfunction as the result of the intervention or the result of the underlying atherosclerotic disease. Modified from references 10 and 11.

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Chapter 3

Increasing arterial wall injury after long-term implantation of two types of stent in a porcine coronary model

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Eur Heart J 1998;19:601-609

Increasing arterial wall injury after long-term implantation of two types of stent in a porcine coronary model

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Aims There is increased late loss in luminal diameter following long-term coronary stenting, compared with balloon angioplasty. We evaluated short- and long-term vessel wall injury after experimental implantation of two stent designs as well as balloon angioplasty and their relationship to neointimal hyperplasia.

Methods and Results Wiktor stents and Palmaz-Schatz stents were implanted in normal coronary arteries of pigs (balloon/artery ratio: 0·9–1·1). In control coronary arteries, balloon angioplasty was performed. At 1, 4 and 12 weeks, the vessel injury score, neointimal thickness and inflammatory response were assessed by histology.The vessel injury score increased over time in both Wiktor and Palmaz-Schatz stents: $0·9 \pm 0·1$, $1·5 \pm 0·5$ and $1·7 \pm 0·6$ (mean ± SD) for Wiktor stents and $0·7 \pm 0·2$, $1·0 \pm 0·1$ and $1·2 \pm 0·3$ for Palmaz-Schatz stents at 1, 4 and 12 weeks follow-up, respectively. No increase in injury was seen in balloon angioplasty controls. Inflammation was seen in both stented groups but was absent 12 weeks after balloon angioplasty. No strong correlation between injury and neointimal thickness was apparent.

Conclusion Stents induce chronic injury in contrast to balloon angioplasty. Stent design (coil vs slotted tube) as well as inflammation may influence vessel response.
(Eur Heart J 1998; 19: 601–609)

Key Words: Stent, coronary arteries, vascular injury, pigs, angioplasty, histology.

Introduction

A greater acute gain in luminal diameter is the mechanism for favourable late restenosis rates in stenting compared to balloon angioplasty[1,2], despite increased late luminal loss[3]. With minimal stent recoil, and remodelling, this late loss is exclusively due to neointimal thickening[4–6], while elastic recoil and remodelling are both major components of restenosis after balloon angioplasty[7–13]. A correlation between neointimal thickening and arterial damage after balloon angioplasty and stenting has been reported[14–18].

Schwartz *et al.*[14] have developed a vessel injury score for stents enabling analysis of arterial damage and neointimal thickening. This score has been validated in porcine coronary arteries 4 weeks after implantation of tantalum coil stents, which were over-sized to create arterial injury. A high correlation was reported between vessel injury score and neointimal thickening. However, creating deep arterial injury by over-sizing will cause a non-specific tissue response which might blur more subtle changes in tissue reaction, making the comparison of different stents difficult. Whether vessel injury score at 4 weeks represents acute damage at implant or includes additional chronic damage by the presence of the stent in the vessel wall cannot be evaluated.

The importance of stent design in arterial injury and neointimal thickening was recently demonstrated by Rogers *et al.* in rabbit iliac arteries[19]. The goal of the present study was therefore to investigate the relationship between coronary arterial wall damage and neointimal hyperplasia in two different stent designs without over-sizing. Furthermore, several time points were studied, from 1 up to 12 weeks. We also evaluated the inflammatory response in each stented segment. To find out whether the observed arterial injury, neointimal thickening and inflammatory reaction are specific features after stenting, or a general healing response

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to the acute implantation trauma, additional animals underwent balloon angioplasty only.

Methods

Animal preparation

Domestic pigs (n=53, weight: 26–46 kg, HVC, Hedel, The Netherlands) underwent the experimental procedures according to the *Guide for the Care and Use of Laboratory Animals*[20], and after approval by the Committee on Experimental Animals of Erasmus University Rotterdam. Experiments were performed as previously described[21]. Briefly, after an overnight fast animals were sedated with ketamine hydrochloride (20 mg . kg^{-1}). Following endotracheal intubation, pigs were mechanically ventilated with 30% oxygen in nitrous oxide. Anaesthesia was maintained with 1–4 vol% enflurane. An intramuscular injection of procaine penicillin G (200 000 I.E./ml) and dihydrostreptomycin sulphate (200 mg per 10 kg body weight) was administered as antibiotic prophylaxis. Arteriotomy of the left carotid artery was performed under sterile conditions and a 9 F introduction sheath was inserted. Heart rate and arterial blood pressure were monitored and arterial blood was sampled to control blood gases and acid-base balance. After administration of 200 IU . kg^{-1} of heparin sodium and 250 mg acetyl salicylic acid, a 9 F guiding catheter was advanced into the ascending aorta. Left coronary angiography was performed using iopamidol (Iopamiro 370, Dagra, Diemen, the Netherlands) as contrast agent after injection of 1 mg of isosorbide dinitrate.

Stents

The Wiktor stent (Medtronic Inc., Minneapolis, Minn., U.S.A.) and the Palmaz-Schatz Coronary Stent (PS 153, Johnson & Johnson Interventional Systems Co., Warren, NJ, U.S.A.), were studied. The Wiktor stent consists of a single tantalum wire (0·127 mm diameter) formed into a sinusoidal wave and wrapped into a helical coil structure[21]. The Palmaz-Schatz Coronary Stent is composed of two segments (7 mm each) of slotted tubes (strut thickness: 0·064 mm), connected by a short (1 mm) coupler[22].

Stent implantation

Coronary angiograms were measured on-line, with a quantitative analysis system using the edge-detection method (CMS, Medis Inc., Nuenen, The Netherlands)[23]. A segment with a mean diameter of approximately 2·5 mm (for 3·0 mm balloon) or 3·0 mm (for 3·5 mm balloon) was selected from the left anterior descending or left circumflex coronary artery. The stent-mounted catheter was advanced to this pre-selected segment over a steerable guide-wire. A single 30 s inflation was performed at 6–8 atmospheres and the maximally inflated contrast-filled balloon was measured to determine the balloon/artery ratio. Angiography was repeated immediately after implantation for assessment of patency and acute result. Finally, the introduction sheath was removed, the carotid artery ligated, the skin closed and the animals were allowed to recover from anaesthesia.

Balloon angioplasty

In two groups of five animals, only balloon angioplasty was performed with a balloon inflation of 30 s at 6–8 atmospheres. The sites of balloon injury were chosen at anatomical landmarks (side branches) which could easily be identified at follow-up.

Follow-up procedure

At 1, 2, 4 or 12 weeks post-implant, animals were anaesthetized as described above. The thorax was opened by a mid-sternal split, and the ascending aorta was cross-clamped after injection of a lethal dose of sodium pentobarbital and fibrillation of the heart with a 9 V battery. Saline (300 ml) was infused, followed by 400 ml of buffered (pH 7·3) formaldehyde under a pressure of 120 mmHg just above the coronary ostia. Finally, the heart was excised, the coronary arteries dissected free from the epicardial surface and the stented or ballooned segments placed in 4% formaldehyde for at least 24 h in preparation for microscopy. After removal of the stent struts, the tissue was processed for paraffin embedding. Haematoxylin-eosin was used as a routine stain while resorcin-fuchsin was used as an elastin stain.

Morphometry and injury score

From each stented or ballooned coronary artery segment, three transverse sections from the proximal, middle and distal part were used for histological analysis. Neointimal thickness was measured on top of the stent struts using a calibrated microscope reticle, as used for standard microscopic measurements. Individual thickness at each stent strut from all three sections was averaged to obtain the mean neointimal thickening per stent. In the ballooned vessels, neointimal thickening was measured at areas of fragmentation of the internal elastic lamina or medial proliferation. The mean for all three sections was taken as mean neointimal thickening.

To evaluate the vessel wall damage caused by the stent, the same elastin stained transverse sections used for morphometry were used for analysis of injury. At each stent strut, damage was quantified by the vessel injury score, according to Schwartz *et al.*[14]. This score

Table 1 Values for vessel injury score according to Schwartz* et al.*[14] (with permission)

Score	Description of vascular injury
0	Internal elastic lamina intact; endothelium denuded; media compressed, not lacerated
1	Internal elastic lamina lacerated; media compressed, not lacerated
2	Internal elastic lamina lacerated, media visibly lacerated, external elastic lamina intact but compressed
3	External elastic lamina lacerated; large lacerations of media extending through external elastic lamina; coil wires sometimes residing in adventitia

grades wall damage from 0 when the internal elastic lamina is intact to 3 when even the external elastic lamina is disrupted (Table 1). Individual scores of the stent struts of the three sections of one stent were averaged to obtain the mean vessel injury score per stented segment. For the balloon angioplasty groups, the vessel injury score cannot be applied because the grading of injury is directly coupled to the presence of a stent strut. Therefore, we graded the injury in the balloon angioplasty groups in analogy to the fracture length method[24], but for a non-over-sized model. Fragmentation of the internal elastic lamina, often accompanied with some degree of medial hypertrophy occurred in one or more areas; it was rare to see one area with a totally ruptured and disintegrated internal elastic lamina. The circumferential lesion length (L_{lesion}) divided by the total circumferential internal elastic lamina length (L_{tot}) was used as a measure of magnitude of damage (L_{lesion}/L_{tot}).

Inflammatory response

Inflammation was assessed in the HE-stained sections corresponding to those used for analysis of vessel injury score and neointimal thickening measurements, according to the following semi-quantitative score: 0: non-existent inflammatory response; 1: inflammatory infiltrates in the adventitia; 2: diffuse, clearly recognizable inflammatory infiltrates in the adventitia; 3: severe, often granulomatous, inflammatory response in the adventitia, sometimes extending to the intima.

Statistical analysis

All data were expressed as mean ± SD. Differences in the balloon/artery ratio, vessel injury score and neointimal thickening between the different stent and balloon groups at the same point in time were evaluated with the non-parametric Wilcoxon Rank Sum Test. A P value $<0{\cdot}05$ (two-tailed) was considered statistically significant. To evaluate differences in vessel injury score within the same groups at different points in time, Kruskal–Wallis one way Analysis of Variance was used. Because of multiple testing, the Bonferroni correction was applied to correct for increasing type I error and significance was stated at the 0·025 level. After curve fitting, regression analysis was used to investigate progression over time of injury response and differences between stent types[25]. Regression analysis was also performed to describe the correlation between vessel injury score and neointimal thickening. (Statistical package: SPSS, release 6·0, SPSS Inc. Chicago, Illinois, U.S.A.).

Results

Systemic haemodynamics and blood gases during intervention

During interventions, heart rate (94 ± 14 beats . min^{-1}, 99 ± 14 beats . min^{-1} and 97 ± 14 beats . min^{-1}) and mean arterial blood pressures (82 ± 17 mmHg, 74 ± 14 mmHg and 73 ± 8 mmHg) were similar for the Wiktor stent, Palmaz-Schatz stent and balloon groups, respectively, while arterial blood gases remained within the normal range (pH: 7·35–7·45; Po_2: 120–160 mmHg; Pco_2: 35–45 mmHg).

Stent implantation

Twenty-one Wiktor stents were placed (one stent per artery) in 16 pigs. Three stented arteries were excluded from final analysis: one stent migrated during implant, a second was erroneously oversized in a small marginal branch, while a third stented animal died suddenly after 23 days without macroscopic evidence of stent occlusion. In total, 18 Wiktor stents were analysed.

Twenty-eight Palmaz-Schatz stents were implanted in 27 animals. Nine stents were excluded from analysis following the death of three animals from arrhythmia during the implantation procedure and six (six stents) from stent thrombosis within 48 h post-implantation. Thrombosis, confirmed by light microscopy, was not accompanied by vascular damage, and was therefore excluded from analysis. In total 19 Palmaz-Schatz stents were analysed.

Angiography during the implantation procedure showed that stents were properly sized, as demonstrated by balloon-artery ratios of 0·9–1·1 (Table 2(a)).

Balloon angioplasty

Ten coronary artery segments in 10 pigs underwent balloon angioplasty with a balloon/artery ratio of $1{\cdot}0 \pm 0{\cdot}1$ (Table 2(b)). Further augmentation of injury up to 12 weeks could not be demonstrated.

At 1 week, the vessel injury score was lower in the Palmaz-Schatz stent group despite a slightly higher mean balloon/artery ratio compared to the Wiktor

Table 2(a) Morphological parameters and balloon/artery ratios of the Wiktor and Palmaz-Schatz stent groups at 1, 4 and 12 weeks follow-up

Follow-up (weeks)	Wiktor Stent					Palmaz-Schatz stent				
	#	VIS	NT μm	Inflammation score	B/A	#	VIS	NT μm	Inflammation score	B/A
1	6	0·9 ± 0·1*	61 ± 10	0·6 ± 0·7	0·9 ± 0·1*	8	0·7 ± 0·2	61 ± 35	1·0 ± 0·9	1·0 ± 0·1
4	7	1·5 ± 0·5	151 ± 50	0·6 ± 0·6	1·1 ± 0·1	5	1·0 ± 0·1	103 ± 13	0·5 ± 0·5	1·0 ± 0·1
12	5	1·7 ± 0·6†	305 ± 155‡	1·6 ± 0·9	1·1 ± 0·1*	6	1·2 ± 0·3‡	198 ± 54†	0·2 ± 0·4	1·0 ± 0·1

Data are mean ± SD; #=number of animals; VIS=vessel injury score; NT=mean neointimal thickness; B/A=balloon/artery ratio. *=$P<0·05$ W vs PS for same time-point; †=$P<0·025$ vs other time-points of same stent design. ‡=$P<0·025$ vs 1 week of same stent design.

Table 2(b) Morphological parameters and balloon/artery ratios of the balloon angioplasty groups at 2 and 12 weeks follow-up

Follow-up (weeks)	(#)	Balloon angioplasty			
		L_{lesion}/L_{tot}	NT μm	Inflammation score	B/A
2	5	0·29 ± 0·29	23 ± 24	0·4 ± 0·5	1·0 ± 0·1
12	5	0·26 ± 0·23	12 ± 15	0 ± 0	1·0 ± 0·1

Data are mean ± SD; #=number of animals; L_{lesion}/L_{tot}=length of fragmented IEL (L_{lesion}) divided by total circumferential IEL length; NT=mean neointimal thickness at L_{lesion}; B/A=balloon/artery ratio.

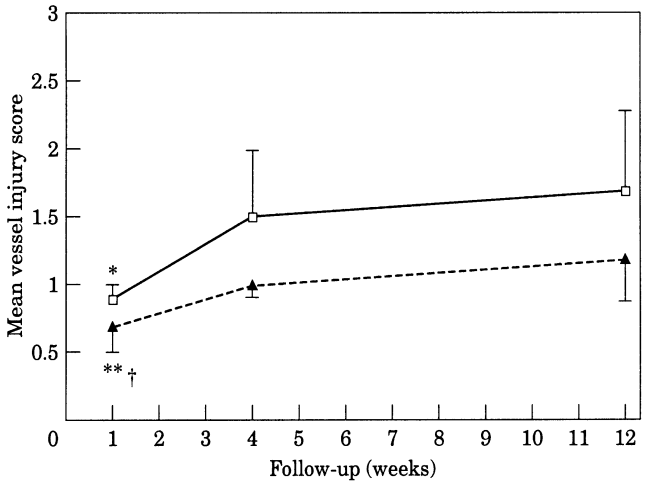

Figure 1 **Progression of vessel wall injury between 1 and 12 weeks for both stent designs. *$P<0·025$ vs 4 and 12 weeks Wiktor; †$P<0·05$ vs 1 week Wiktor; **$P<0·01$ vs 12 weeks Palmaz-Schatz. Linear regression showed a significant progression of vessel injury score between 1 and 12 weeks ($P=0·0004$) as well as a difference in vessel injury score between both stent designs ($P=0·0013$). □=Wiktor; ▲=Palmaz-Schatz.**

stents. Although the increase in the vessel injury score between 1 and 4 weeks in the Palmaz-Schatz stents showed only a trend, the progress of the vessel injury score between 1 and 12 weeks was significant in this analysis ($P=0·0004$) (Fig. 1). In both stent designs linear regression analysis revealed a continued difference in vessel wall injury at follow-up ($P=0·0013$) (Fig. 1). The slope of increase in the vessel injury score over time was not significantly different ($P=0·62$) between the stent types.

After balloon angioplasty, vessel wall damage was very mild, expressing itself at follow-up as

Table 2(a) Morphological parameters and balloon/artery ratios of the Wiktor and Palmaz-Schatz stent groups at 1, 4 and 12 weeks follow-up

Follow-up (weeks)	Wiktor Stent					Palmaz-Schatz stent				
	#	VIS	NT μm	Inflammation score	B/A	#	VIS	NT μm	Inflammation score	B/A
1	6	0·9 ± 0·1*	61 ± 10	0·6 ± 0·7	0·9 ± 0·1*	8	0·7 ± 0·2	61 ± 35	1·0 ± 0·9	1·0 ± 0·1
4	7	1·5 ± 0·5	151 ± 50	0·6 ± 0·6	1·1 ± 0·1	5	1·0 ± 0·1	103 ± 13	0·5 ± 0·5	1·0 ± 0·1
12	5	1·7 ± 0·6†	305 ± 155‡	1·6 ± 0·9	1·1 ± 0·1*	6	1·2 ± 0·3‡	198 ± 54†	0·2 ± 0·4	1·0 ± 0·1

Data are mean ± SD; #=number of animals; VIS=vessel injury score; NT=mean neointimal thickness; B/A=balloon/artery ratio. *=$P<0·05$ W vs PS for same time-point; †=$P<0·025$ vs other time-points of same stent design. ‡=$P<0·025$ vs 1 week of same stent design.

Table 2(b) Morphological parameters and balloon/artery ratios of the balloon angioplasty groups at 2 and 12 weeks follow-up

Follow-up (weeks)	(#)	Balloon angioplasty			
		L_{lesion}/L_{tot}	NT μm	Inflammation score	B/A
2	5	0·29 ± 0·29	23 ± 24	0·4 ± 0·5	1·0 ± 0·1
12	5	0·26 ± 0·23	12 ± 15	0 ± 0	1·0 ± 0·1

Data are mean ± SD; #=number of animals; L_{lesion}/L_{tot}=length of fragmented IEL (L_{lesion}) divided by total circumferential IEL length; NT=mean neointimal thickness at L_{lesion}; B/A=balloon/artery ratio.

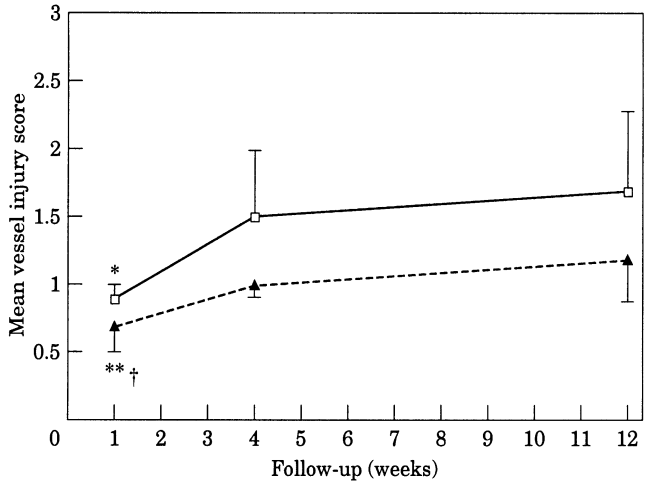

Figure 1 **Progression of vessel wall injury between 1 and 12 weeks for both stent designs. *$P<0·025$ vs 4 and 12 weeks Wiktor; †$P<0·05$ vs 1 week Wiktor; **$P<0·01$ vs 12 weeks Palmaz-Schatz. Linear regression showed a significant progression of vessel injury score between 1 and 12 weeks ($P=0·0004$) as well as a difference in vessel injury score between both stent designs ($P=0·0013$). □=Wiktor; ▲=Palmaz-Schatz.**

stents. Although the increase in the vessel injury score between 1 and 4 weeks in the Palmaz-Schatz stents showed only a trend, the progress of the vessel injury score between 1 and 12 weeks was significant in this analysis ($P=0·0004$) (Fig. 1). In both stent designs linear regression analysis revealed a continued difference in vessel wall injury at follow-up ($P=0·0013$) (Fig. 1). The slope of increase in the vessel injury score over time was not significantly different ($P=0·62$) between the stent types.

After balloon angioplasty, vessel wall damage was very mild, expressing itself at follow-up as

fragmentation of the internal elastic lamina accompanied by an increase in medial thickness. There was no increase in injury from 2 to 12 weeks (Table 2(b)).

Inflammatory response

Table 2(a) shows the inflammation score for both stent groups. Extensive inflammation was not observed in either group, with most scores being <1. The balloon angioplasty vessels showed a very mild inflammatory reaction at 2 weeks, but at 12 weeks the inflammatory response was absent in all vessels studied.

Morphometry

Mean neointimal thickening at the stent wires in the Wiktor stent groups increased from 61 ± 10 μm at 1 week to 151 ± 50 μm at 4 weeks and 305 ± 155 μm at 12 weeks ($P<0.025$). In Palmaz-Schatz stented coronary arteries, the neointimal thickening also increased significantly from 61 ± 35 μm at 1 week to 103 ± 3 μm at 4 and 198 ± 54 μm at 12 weeks respectively (Table 2(a)).

Table 2(b) shows that neointimal thickening at 2 weeks after balloon angioplasty was limited (23 ± 24 μm) and significantly less than after stent implantation. There was no progression of neointimal thickening between 2 and 12 weeks.

Correlation between injury and neointimal response

The correlation between vessel injury score and the neointimal thickening was poor in each of the stent groups (Fig. 2). The correlations for all Wiktor stents together ($y=155x-29$, $r=0.69$, $P=0.001$) and all Palmaz-Schatz stents together ($y=86x+47$, $r=0.36$, $P=0.14$) were not significantly different from each other. To increase the power of the analysis, all data were pooled. Even then correlation between vessel injury score and neointimal thickening remained weak (Fig. 3; $y=107x+17$, $r=0.49$, $P=0.002$).

Discussion

Background and purpose of study

The use of stents is increasing exponentially worldwide. However, concerns remain as regards thrombogenicity and vessel wall tissue response to the stent. Tissue response and thrombosis have been strongly related to acute vessel wall damage during the procedure[14,17,18,26]. Stent injury in the porcine model has therefore been used to study restenosis[27–31]. Schwartz *et al.*[14], by oversizing the stent (Wiktor at 4 weeks) and thereby creating deep arterial injury, showed a strong correlation between vessel injury score and neointimal thickening.

Rogers *et al.*[19] were able to reduce vessel wall injury and neointimal thickening after stenting by modifying the geometric configuration of the stent. However, their data derive from peripheral rabbit arteries and are also limited to one time point at 14 days. Colombo *et al.* have emphasized the importance of correct sizing of the stent using high-pressure inflation guided by intravascular ultrasound[32]. Applying these rules of stent deployment, Serruys *et al.* observed no subacute thrombosis and a restenosis rate of only 6% at 6 months after implantation of a heparin coated Palmaz-Schatz stent in 50 patients of the Benestent II pilot trial[33].

We investigated the vessel injury score concept in two different stent designs, at various time points and without deliberately creating deep arterial damage (mean balloon/artery ratio: 0·9–1·1). The data were compared with a control group that underwent balloon angioplasty alone.

Main findings

The major finding of the present study is that in properly sized stents, but not after balloon angioplasty alone, vessel wall injury increases over time. Although neointimal thickening increases concurrently, no strong correlation could be found between vessel injury score and neointimal thickening. In all stent groups the inflammatory response was very mild. However, in contrast to the balloon angioplasty group, inflammation was still visible after 12 weeks and may have influenced the progression of vessel injury score over time.

Acute vs chronic injury

Acute vessel wall damage is caused during the interventional procedure. If this damage is predominantly caused by stretching of the vessel wall, then this damage is comparable in balloon angioplasty and stenting (with a balloon-expandable stent), except for the profile of the stent on the outer surface of the balloon. For the Palmaz-Schatz stent this implies an extra profile of 2 times 64 μm (128 μm), and for the Wiktor stent 2 times 127 μm (254 μm). In stenting a 3·0 mm coronary artery with a 3·0 mm stent-mounted balloon, this would mean at 4 or 8% increase in diameter, respectively. However, in our data this increase in profile was not accompanied by a proportional increase in damage within the given ranges of balloon/artery ratios of 0·9 to 1·1 (± 8%). Stent strut geometry (round vs rectangular) is an additional factor which may modify vessel wall injury caused during stretching[19]. We found a significant lower mean vessel injury score in the Palmaz-Schatz stent compared to the Wiktor stent at 1 week follow-up, which might represent lower acute damage at

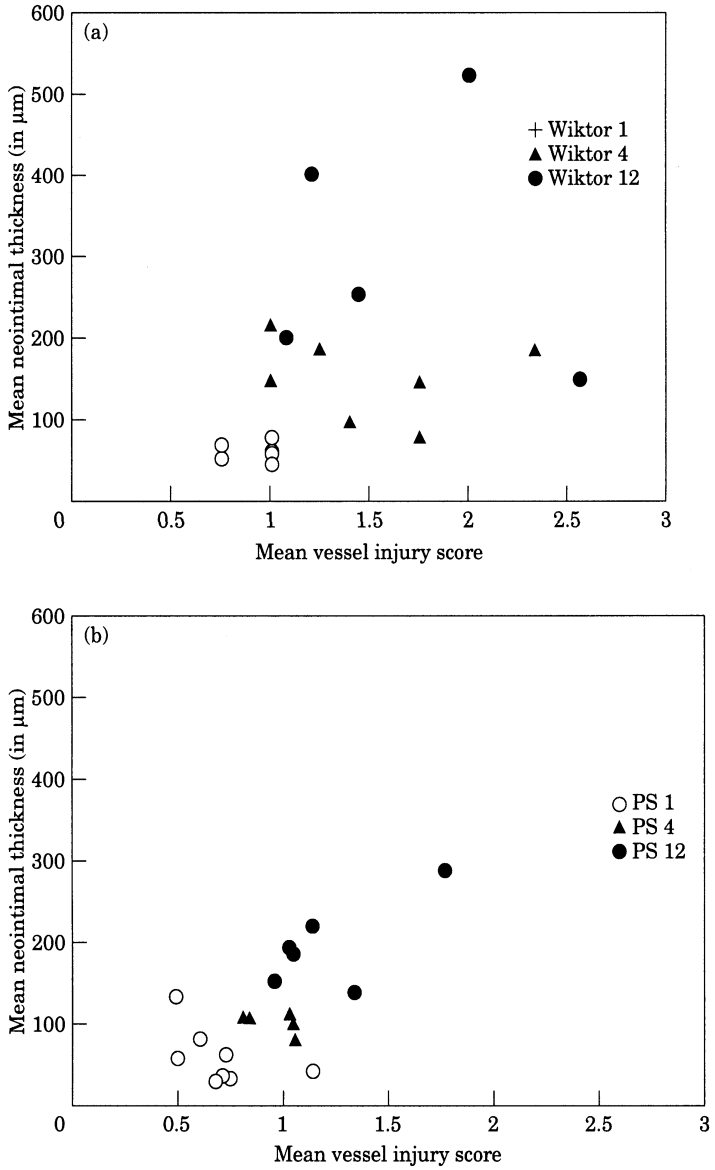

Figure 2 **(a) Correlation between mean vessel injury score and mean neointimal thickness per individual Wiktor stent at 1, 4 and 12 weeks follow-up. No significant correlation can be found in either group. (b) Correlation between mean vessel injury and mean neointimal thickness per individual Palmaz-Schatz (PS) stent at 1, 4 and 12 weeks. No significant correlation can be found in either group.**

implant. Further studies assessing damage directly after implantation might elucidate this further.

Chronic damage was defined as vessel wall damage occurring during follow-up. The present data show a significant increase in damage, as assessed by the vessel injury score, between 1 and 12 weeks post-stenting (P=0·0097). This was probably caused by the continued presence of the stents, as chronic damage was not observed after balloon angioplasty alone. Unfortunately, it was not possible to use the same injury scoring system in stented and ballooned arteries. However, we feel that the large difference in outcome in this study allows for the above conclusion.

Possible implications of chronic injury

In this study, the progressive damage caused by the stent in the first weeks after stenting may act as a direct stimulus for smooth muscle cell proliferation through the release of several growth factors and chemotactic agents from the damaged cells[34–37]. However, the

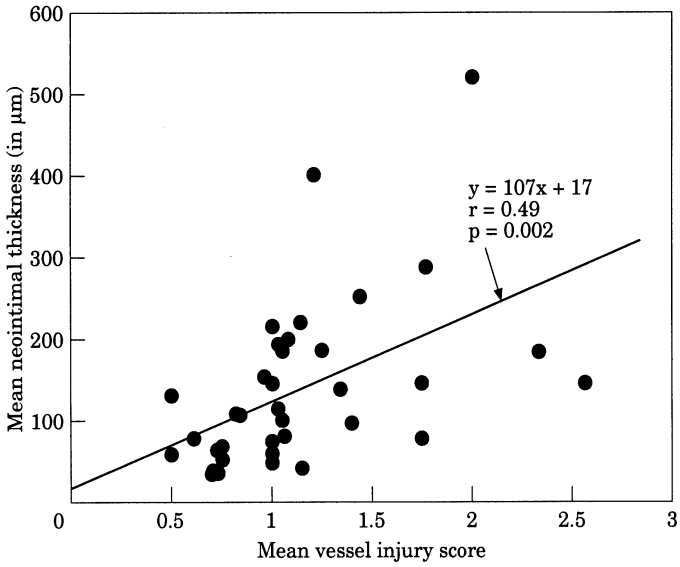

Figure 3 **Correlation between mean vessel injury score and mean neointimal thickness plotted for both stents in all pigs. (y=107x+17, r=0·49, *P*=0·002).**

resultant neointimal thickening is probably not influenced by damage alone as no strong correlation was evident between vessel injury score and neointimal thickening in the present study. Our data indicate that the largest increase in vessel injury score occurs between 1 and 4 weeks, while the largest increase in neointimal thickening occurs between 4 and 12 weeks.

The weak correlation between vessel injury score and neointimal thickening may also be due to the very low mean neointimal thickenings (151 µm for Wiktor and 103 µm for Palmaz-Schatz, both at 4 weeks). These values, however, are comparable to other studies by our group[21,38]. It is unlikely that an increase in the number of experiments in this study will result in a better correlation, as pooling all the data did not show a better correlation (Fig. 3). Therefore, in the present study, damage is probably only one of the contributing factors to the resultant neointimal thickening. The study by Schwartz *et al.* indicated that damage played a more important role than in ours, but this does not contradict our results. Their model was characterized by immense acute damage, resulting in neointimal thicknesses of up to 1400 µm.

Rogers *et al.*[19] have shown that the difference in geometry or surface characteristics between stents may be important in relation to the injury inflicted to the vessel wall. Our results show that this effect is most pronounced during the first weeks after stenting.

The role of inflammation

In all groups, mild inflammation was seen in the first weeks. Although a correlation between inflammation score and vessel injury score or neointimal thickening could not be observed, the persisting mild inflammatory response in the stent groups at 12 weeks may have contributed to the progression of vessel wall injury, as this was not observed in the group who received balloon angioplasty alone. Theoretically, persistent inflammation may have facilitated the increased morphological injury by allowing deeper stent strut penetration into the vessel wall, resulting in a higher vessel injury score. Furthermore, by releasing growth factors and cytokines, inflammatory cells may also influence neointimal thickening and chronic endothelial dysfunction[39].

Study limitations

Our first time point of follow-up was chosen at one week. We are aware that this does not represent true acute damage at implant. However, histological assessment of acute injury requires removal of stent struts from 'freshly' injured vessel wall tissue, which may induce more handling damage than removal after several days to weeks. After one week, a measurable neointima is present, which data could be included in this analysis.

In the Wiktor stent group, no early stent thrombosis was seen while six Palmaz-Schatz stents thrombozed in the first 48 h post implantation, causing the death of the animals. These six Palmaz-Schatz stents were not included in the analysis, because increased vessel wall damage could not be found in either of these cases and therefore bias was not likely to be introduced.

In this study, non-atherosclerotic coronary arteries of juvenile pigs were stented. Vessel wall injury and tissue response may be different when stenting atherosclerotic lesions. However, in the pig model, hypercholesterolaemic diets or endothelial abrasion before stenting do not significantly change the tissue

response[31]. Moreover, our model has extensively been used in pre-clinical stent testing, and it seems valid to assess vessel wall injury, tissue response, thrombotic response and restenosis in the same model.

Conclusions

This study shows progression of vessel wall injury up to 12 weeks after stenting, but not after balloon angioplasty alone. Different stent designs cause different degrees of acute injury and this difference persists at longer follow-up.

As (mild) inflammatory response was persistent in the stent groups in contrast to balloon angioplasty, this may be important in influencing progression of vessel wall injury subsequent to mechanical injury caused by stenting. In this study, no strong correlation between injury score and neointimal hyperplasia could be demonstrated.

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Chapter 4

Late-late occlusion after intracoronary brachytherapy

George Sianos, Nico Mollet, Sjoerd Hofma, Pim de Feyter, Patrick W. Serruys

Circulation 2003;108:e69-e70

Images in Cardiovascular Medicine

Late-Late Occlusion After Intracoronary Brachytherapy

Georgios Sianos, MD; Nico Mollet, MD; Sjoerd Hofma, MD;
Pim J. de Feyter, MD; Patrick W. Serruys, MD

A 57-year-old man with a history of anterior myocardial infarction in April 1997, initially treated with successful thrombolysis, underwent cardiac catheterization due to persistent postinfarction angina. A single-vessel disease, with a significant lesion in the left anterior descending coronary artery (LAD), was found. The patient was treated with balloon angioplasty followed by intracoronary beta radiation therapy according to the Beta Energy Restenosis Trial (BERT). He received 16 Gy at 2 mm from the centerline of the 90Sr/90Y source. He remained asymptomatic for 41/2 years. During this period, he underwent control angiography with the use of Intravascular Ultrasound (IVUS) at 6 months and 3 years, as mandated by protocol. After this period, he again developed angina, and an exercise test was positive for ischemia. Diagnostic coronary angiogram at almost 5 years revealed single-vessel disease, with totally occluded LAD with collateral filling from the right coronary artery. The angiographic sequence is presented in Figure 1. The IVUS images are presented in Figure 2. Before the reintervention, a 16-row, electrocardiographic-gated, cardiac, multislice spiral CT scan was also performed (Figure 3). An attempt at percutaneous recanalization was not successful, and the patient underwent coronary artery bypass surgery with implantation of the left internal mammary artery in the LAD.

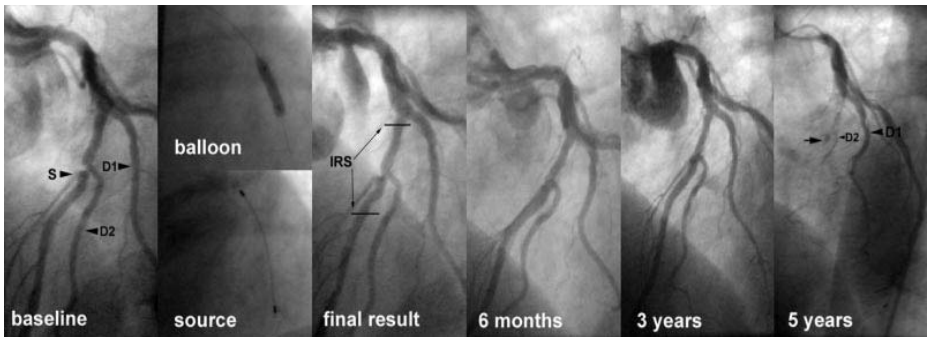

Figure 1. The LAD, filmed at the left anterior oblique and cranial projection. At baseline a severe focal lesion just before the bifurcation of the second diagonal branch (D2) with a septal perforator branch (S) was observed. A big first diagonal branch (D1) can also be seen. The patient was treated with balloon angioplasty (single dilatation with a 3×12-mm balloon at 12 atm) followed by catheter-based irradiation with the use of the 30-mm-long, 90Sr/90Y Beta-Cath source, with a good final result. The balloon and the radiation source were filmed in the right anterior oblique and cranial projection. The irradiated segment (IRS) is indicated by the 2 black horizontal lines in the "final result" frame. Control angiogram at 6 months and 3 years revealed well-preserved result without restenosis. At 5 years, there is severe lumen compromise throughout the length of the irradiated segment, with minimal contrast penetration up to the D2 and complete occlusion after its takeoff (arrow).

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Movies are available in the online-only Data Supplement at http://www.circulationaha.org.(*Circulation.* 2003;108:e69-e70.)

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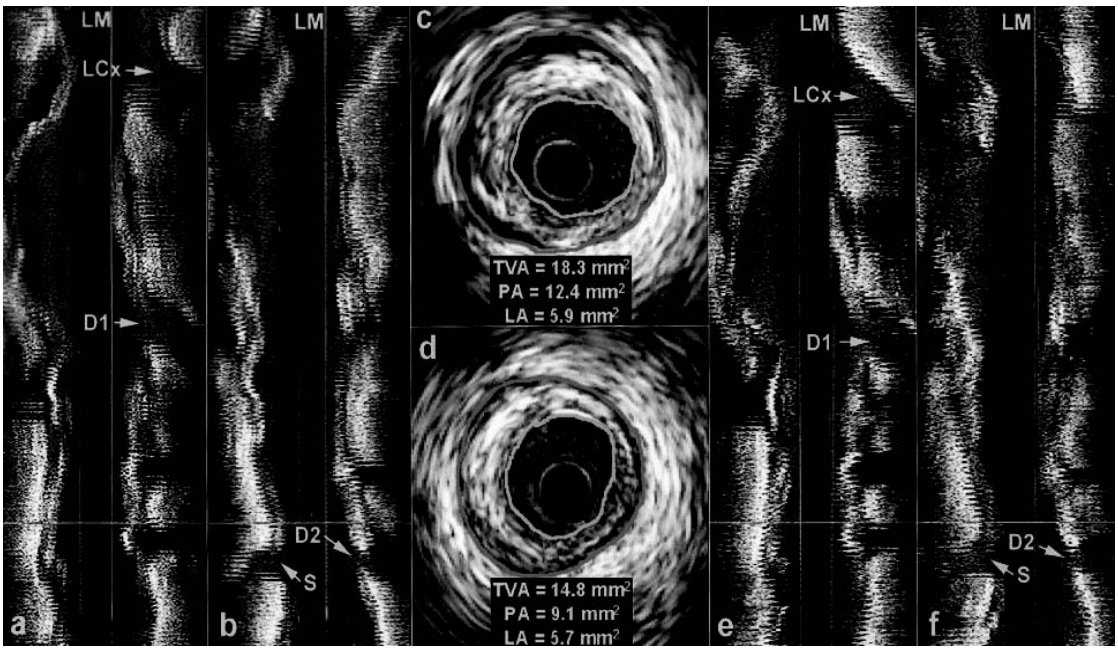

Figure 2. Left panels, Longitudinal intravascular ultrasound reconstruction of the LAD at 6 months follow-up, corresponding to the angiographic projection in Figure 1. The left main coronary artery (LM) is on top of the image and the distal LAD at the bottom. a, Slice showing the bifurcations with the left circumflex coronary artery (LCx) and the first diagonal branch (D1). b, Slice showing the bifurcations with the second diagonal (D2) and the septal (S) branches. There is a 60-degree difference between the planes of slices shown in panels a and b. Right panels, Longitudinal intravascular ultrasound reconstruction of the LAD at 3 years follow-up. Slice shown in panel e corresponds to that in panel a. Slice shown in panel f corresponds to that in panel b. Middle panel, Cross-sectional images corresponding to the site of the initial stenosis at baseline, just before the bifurcations of the LAD with the D2 and the septal branches, as indicated by the yellow and red horizontal lines at the longitudinal reconstructions shown in the lower half of the other panels, at 6 months (c) and 3 years (d). The red line delineates the external elastic membrane and the green line the lumen surface. Between 6 months and 3 years, a reduction in the total vessel area (TVA) can be observed (negative remodeling) with accompanying reduction in the plaque area (PA) (plaque regression). This results in an unchanged lumen area (LA) between 6 months and 3 years.

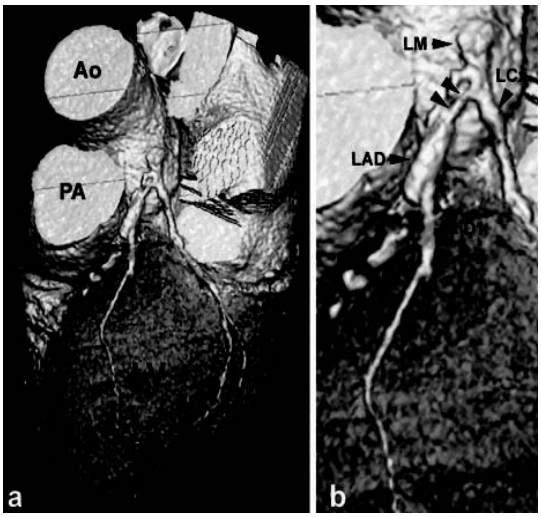

Figure 3. a, Electrocardiographic-gated multislice CT 3D volume-rendered image of the heart and the great vessels. The aortic root (Ao), the pulmonary artery (PA), and the left ventricle (LV) are clearly visible. b, Detailed picture of the left coronary artery corresponding to the angiographic frames in Figure 1. The left main coronary artery (LM) is divided into the left anterior descending (LAD) and the left circumflex (LCx) coronary arteries. A severe stenosis at the ostium of the LAD (double arrowhead) can be seen. This lesion was not obvious in the angiographic images presented, because of overlap of the ostium of the LAD with LCx. More distally, the LAD is occluded (arrowhead) after the takeoff of the first diagonal branch (D1), at the site of previous treatment with balloon dilatation and beta irradiation.

Chapter 5

Stent fracture and restenosis in the Drug Eluting Stent era

Georgios Sianos, Sjoerd Hofma, Jurgen MR Ligthart, Francesco Saia, Angela Hoye, Pedro A Lemos, Patrick W Serruys

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Stent Fracture and Restenosis in the Drug-Eluting Stent Era

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Key words: sirolimus-eluting stents; restenosis; stent fracture

INTRODUCTION

Coronary stents, initially reserved for bailout situations, are now used in more than 80% of all cases. However, their efficacy is limited by the occurrence of in-stent restenosis, ranging from 15% to 35% of cases, depending on lesion morphology [1–3]. Recently, drug-eluting stents have been proven very effective in suppressing neointimal proliferation and reduced restenosis to single digit numbers [4–6]. We report two cases of treatment failure with sirolimus-eluting stents (SESs) related to stent fractures.

CASE REPORTS

Case 1

A 45-year-old male with hypercholesterolemia and hypertension presented with stable angina class II–III according to the classification of the Canadian Cardiovascular Society (CCS) and a positive exercise test. In his past medical history, he had an inferior wall myocardial infarction in 1994, followed by elective balloon angioplasty of the right (RCA) and left circumflex (LCx) coronary arteries. Coronary angiography revealed a long complex lesion in the mid RCA.

The RCA was engaged with a 6 Fr guiding catheter (Mach1 FR 4.0, Boston Scientific Scimed, Maple Grove, MN) using the standard femoral approach. A 0.014″ PT Graphix Intermediate guidewire (Guidant, Santa Clara, CA) was used to cross the lesion. The vessel was treated with direct BX Velocity SES implantation (Cypher, Cordis Europe, Roden, The Netherlands), 2.75 × 8 mm distally, 3.0 × 33 mm in the middle part, and 3.0 × 8 mm proximally. All stents were deployed at 18 atm. The proximal stents were postdilated with a 3.5 mm balloon (Adante 3.5 × 20 mm; Boston Scientific Scimed) up to 20 atm with a good angiographic result (Fig. 1). Postprocedural intravascular ultrasound (IVUS; Avanar F/X, Jomed, Beringen, Switzerland) examination with automated pullback (Trak Back 2, Jomed) speed of 0.5 mm/sec revealed good stent apposition with 1 mm gaps between the three stents. The patient was enrolled in the Rapamycin-Eluting Stent Evaluated at Rotterdam Cardiology Hospitals (RESEARCH) registry and agreed to undergo control angiography at 6 months postprocedure. He was discharged next day on aspirin for life and clopidogrel for 6 months.

The patient remained asymptomatic for 6 months. The diagnostic angiogram revealed a very focal stenosis (4.95 mm long), 66% by quantitative coronary angiography (QCA), in the middle part of the 3 × 33 mm long stent (Fig. 1). IVUS investigation (Atlantis SR, 40 MHz; Boston Scientific) revealed significant neointimal formation at the point of the angiographic stenosis with lumen compromise [minimal luminal area (MLA), 2.8 mm^2] and absence of stent struts corresponding to fracture of the stent (Fig. 2). There was very effective suppression of the neointima formation throughout the rest of stent length and also in the stents deployed proximal and distally. Minimal formation of neointima hyperplasia was observed in the gap between the distal and the mid stent (MLA with IVUS measurements was 4.8 mm^2). During the examination, the IVUS catheter was occlusive and the patient experienced angina.

Based on these findings and despite the absence of symptoms until his presentation, the lesion was treated with implantation of 3.0 × 8 mm Cypher stent deployed at 16 atm and postdilated with a 3.5 mm × 20 mm balloon (Worldpass, Cordis Europe) up to 16 atm.

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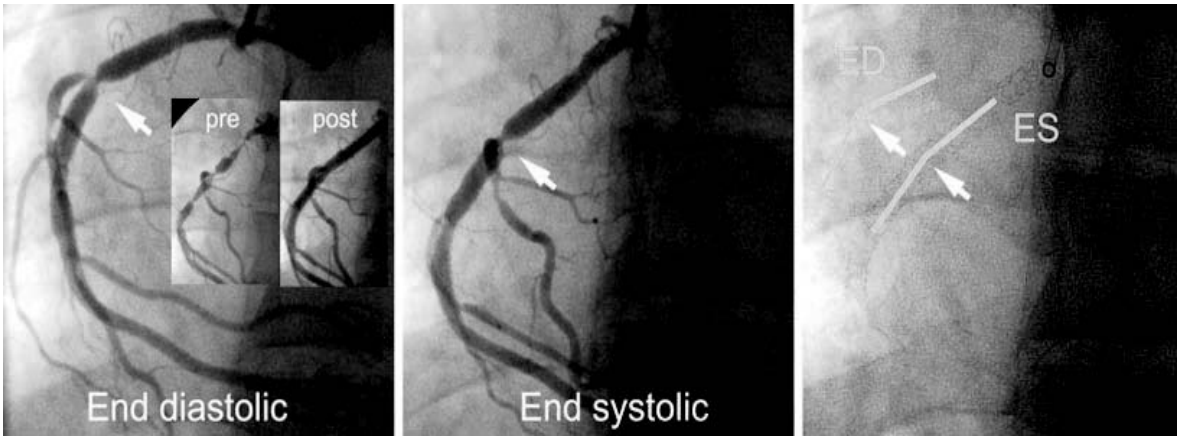

Fig. 1. Angiographic image of the right coronary artery at 6-month follow-up. Left: End-diastolic frame. Middle: End-systolic frame. The inserts in the left shows the RCA at baseline before (pre) and after (post) treatment. The arrow indicates the restenotic lesion. Right: Schematic diagram showing the difference in geometry of the Cypher 3 × 33 mm stent during the heart cycle. In end-diastole (ED), a much greater angle is formed between the two arms of the fractured stent compared to end-systole (ES). The arrowhead indicates the breakpoint of the stent and the position of the restenosis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

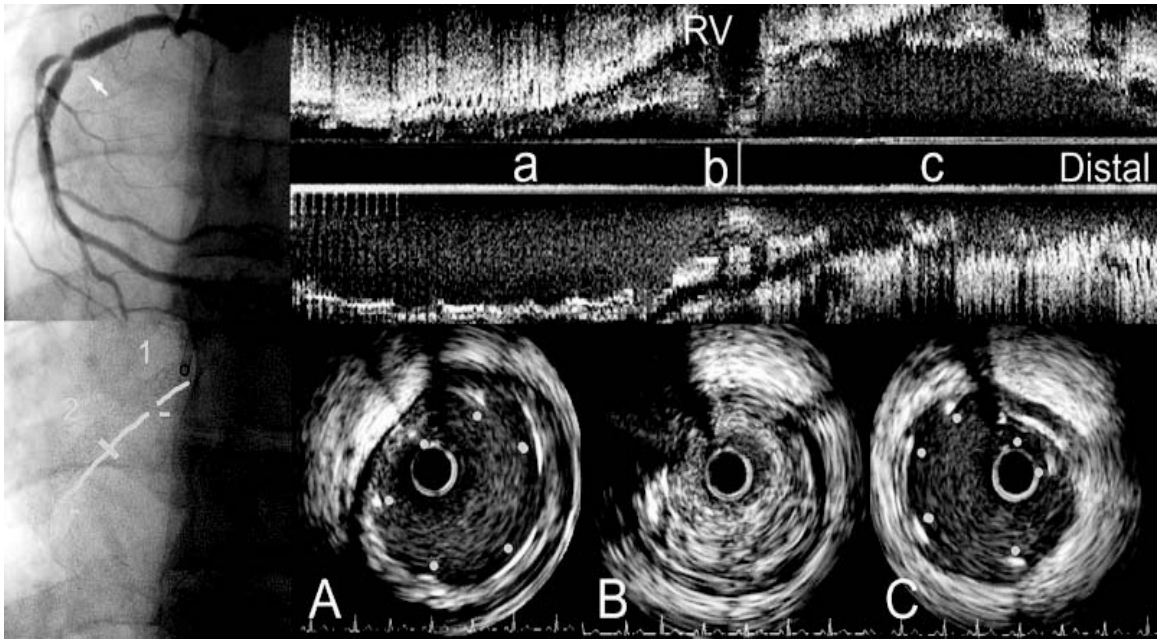

Fig. 2. Top left: Angiogram of the RCA at 6-month follow-up. The arrow indicates the restenotic lesion. Bottom left: View of the stents without contrast. The yellow lines represent a schematic diagram of the relative position of the stents: (1) Cypher 3.0 × 8 mm, (2) Cypher 3.0 × 33 mm, and (3) Cypher 2.75 × 8 mm. The gaps between the stents are clearly visible. The perpendicular yellow line in the middle of stent 2 corresponds to the point of the stent fracture and restenosis. Top right: Longitudinal IVUS reconstruction of the RCA; the yellow vertical line and b correspond to the point of the restenosis. The right ventricular branch (RV) is clearly visible. Bottom right: A, B, and C are cross-sectional images corresponding to the positions a, b, and c at the longitudinal reconstruction. In A and C, proximal and distal from the stenosis, respectively, there are six stent struts visible (yellow dots) with complete absence of neointimal hyperplasia. Cross-section B at the restenotic site shows a large concentric plaque, with the RV branch visible at 11 o'clock. No stent struts are visible at this cross-section, indicating the stent fracture. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

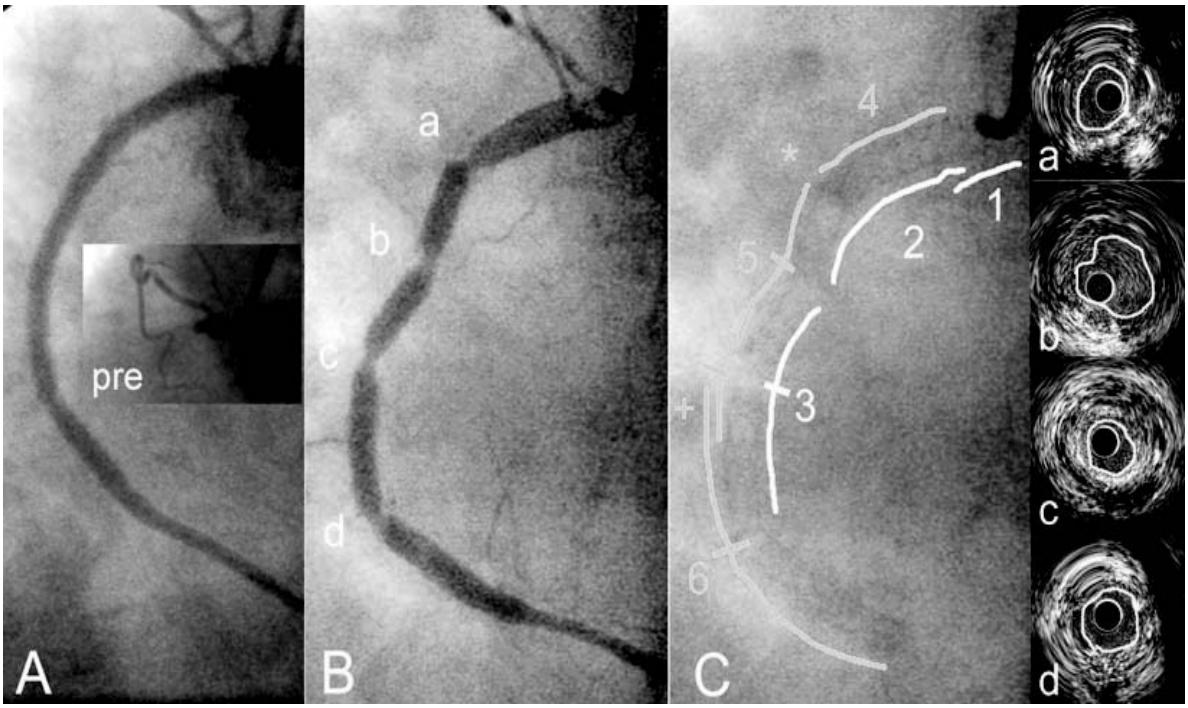

Fig. 3. The insert in A shows the totally occluded right coronary artery at baseline (pre). A: Final result after recanalization and stent implantation. B: Result at follow-up. There are four very focal lesions, a to d, from proximal to distal, giving the vessel a beaded appearance. The difference in vessel geometry from hemispherical postintervention to a wave shape at follow-up is clearly visible, indicative of stent fracture. C: View of the RCA without contrast in the same projection as in frame B with a schematic diagram of the stents implanted during the two interventions. The white lines correspond at the stents implanted during the first intervention: (l) Carbostent 3.0 × 9 mm, (2) Carbostent 3.0 × 25 mm, and (3) Carbostent 3.0 × 25 mm. There was an overlap between stents 1 and 2 and a gap between stents 2 and 3. The yellow lines correspond to the stents deployed during the second intervention. (4) Cypher 3.0 × 18 mm, (5) Cypher 3.0 × 33 mm, and (6) Cypher 3.0 × 33 mm. There was a gap between stents 4 and 5 (asterisk, corresponding to the proximal, a, restenotic lesion) and an overlap between stents 5 and 6 (cross). The three stent fractures are indicated by the small horizontal lines and they correspond anatomically to the restenotic lesions b, c, and d in frame B. It is important to note that the proximal stent fracture in stent 5 occurred at the gap between its overlap between stent 2 and 3 and the two distal fractures at both sides of overlap between stents 5 and 3. Right: IVUS cross-sectional images with the lumen plaque detail corresponding to the restenotic lesions in B. Note that the smallest lumen area is observed in frame c. The IVUS interpretation is described in detail in Figure 4. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Case 2

A 48-year-old male patient presented with unstable angina class IIB according to Braunwald classification. His medical history consisted of an inferior non-Q-wave myocardial infarction 2.5 years ago. Due to postinfarction angina, coronary angiography was performed, revealing an occluded right coronary artery (RCA). Successful recanalization followed during the same session with implantation of three Carbostents (Sirius, Sorin Biomedica, Saluggia, Italy) 3.0 × 25 mm distally, 3.0 × 25 mm in the middle, and 3.0 × 9 mm proximally (Fig. 3C). He remained asymptomatic for 2 years and then developed effort angina CCS class III. Repeat catheterization revealed ostial reocclusion of the RCA (Fig. 3A) that was treated with repeat percutaneous intervention.

The right coronary artery was cannulated with a 6 Fr Judkins Right 4.0 guiding catheter (Vista Brite, Cordis Europe) using the right radial artery approach. Crossing with a guidewire was difficult and was finally achieved with a 0.014″ Crosswire NT guidewire (Terumo, Tokyo, Japan). Afterward, predilatation was performed with a 2.5 × 30 mm balloon (Worldpass, Cordis Europe) followed by Cypher stent implantation. Three stents were deployed: 3.0 × 33 mm distally, 3.0 × 33 mm in the middle, and 3.0 × 18 mm proximally, with good final angiographic result (Fig. 3A). The 3.0 mm stents were postdilated with a 3.5 mm × 20 mm balloon (Adante, Boston Scientific Scimed) up to 22 atm. The patient was discharged on aspirin for life and clopidogrel for 6 months.

Five months after the index procedure, the patient was readmitted due to unstable angina. Coronary angiography

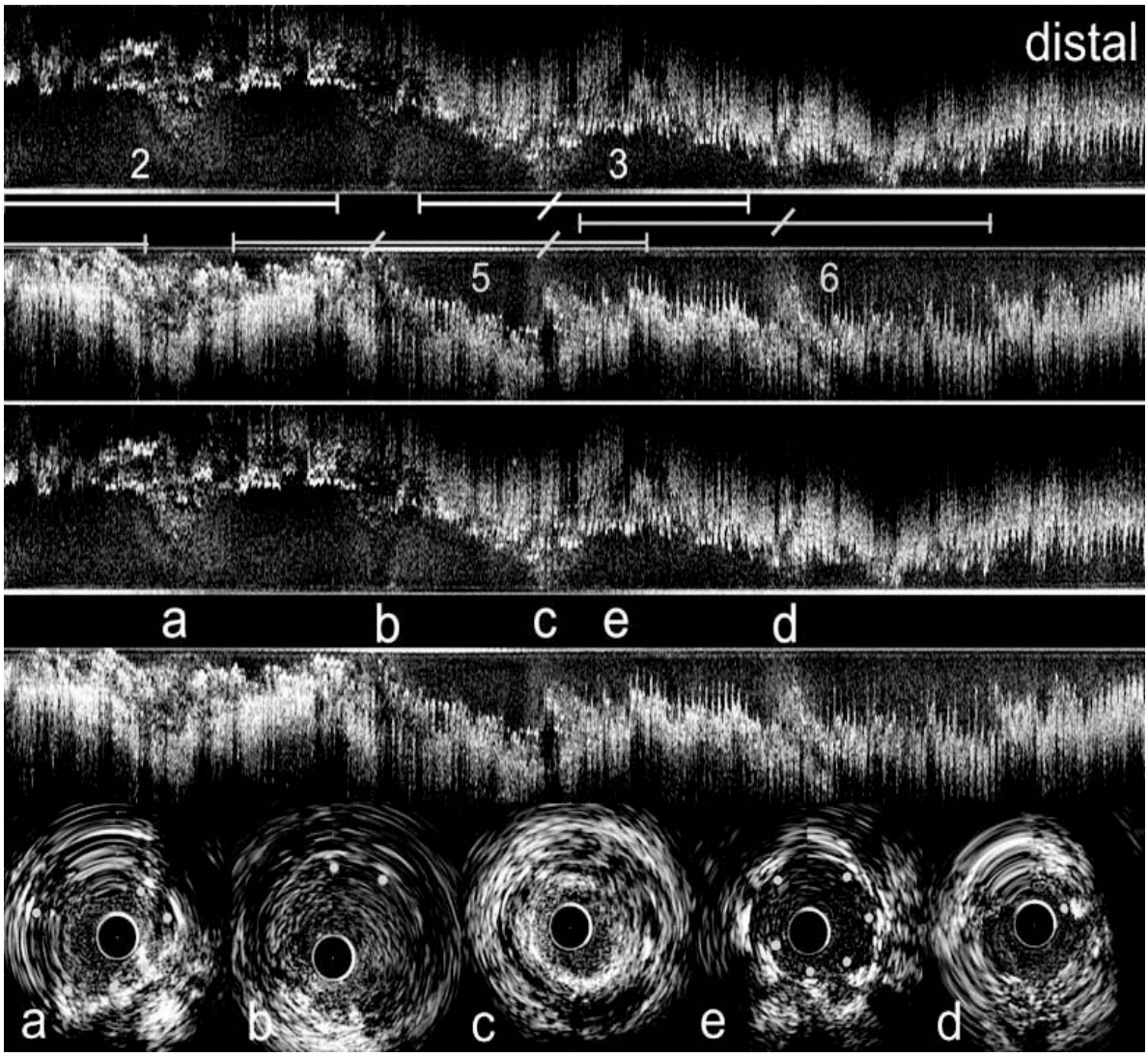

Fig. 4. Top: Longitudinal IVUS reconstruction of the right coronary artery with a schematic diagram of the stents corresponding to the description in Figure 3. The most proximal Carbostent 1 and part of the proximal Cypher stent 4 were not visualized due to selective position of the guiding catheter in the ostium of the RCA. Middle: The same longitudinal reconstruction. The focal formation of neointimal hyperplasia is visible and corresponds to the angiographic, identically lettered, restenotic lesions a to d described in Figure 3B. There is complete absence of tissue formation in between the lesions. Bottom: Cross-sectional IVUS images of the four restenotic lesions. The yellow dots correspond to the visible stent struts. In cross-section a, the tissue accumulation in the gap between the Cypher stents 4 and 5, one layer of struts is visible. They are the struts of the Carbostent 2. In cross-section b, only two stent struts can be observed, indicating partial fracture of the 33 mm long Cypher stent 5 in its proximal part. In cross-section c, no stent struts are visible, which means complete fracture of both the Cypher stent 4 at its distal part and complete fracture of the peripheral Carbostent 3. It is the restenotic lesion with the larger plague burden and smallest luminal area. The cross-section e, adjacent to cross-section c, shows the overlap of the two long Cypher stents and the Carbostent 3. Three overlapping layers of struts are visible without any neointimal hyperplasia. In cross-section d, only one strut can be seen, with moderate plaque formation, corresponding to the partial fracture of the distal 33 mm long Cypher stent 6. As indicated by the short inclined lines in the top, the two distal fractures were observed at the extremities of stent overlapping and the third in a gap of the previous noneluting stents. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

revealed an unusual pattern of restenosis with four discrete restenotic lesions throughout the length of the stents and complete absence of any restenotic tissue in between (Fig. 3B). IVUS examination (Atlantis SR, 40 MHz; Boston Scientific) revealed local neointimal formation with absence of metallic stent struts at the distal three restenotic lesions, corresponding to areas of stent fracture. The fourth most proximal one corresponded at the location of the known gap

between the proximal and mid sirolimus-eluting stents. There was complete absence of neointimal hyperplasia in between the restenostic lesions (Fig. 4).

The patient was treated with implantation of four 3.0 × 8 mm Cypher stents. During the procedure, intravenous IIb/IIIa glycoprotein inhibitor abciximab was administered and continued for 12 hr after the procedure. Clopidogrel medication was prescribed for another 6 months.

DISCUSSION

Drug-eluting stents, given their outstanding performance in controlled clinical trials, seem set to revolutionize the percutaneous treatment of coronary artery disease. Angiographic restenosis is well below the range of 10% and reported cases have generally involved portions of the vessel that have been subjected to balloon injury but were not covered by the stent, such as restenosis in an unintentional gap between adjacent stents or just proximal or distal to the stent. Given these results, SESs and lately taxol-eluting stents are the standard treatment in our institution for all the patients undergoing percutaneous interventions.

The two cases reported here suggest the existence of a new potential mechanism of restenosis after implantation of sirolimus-eluting stents for either de novo atherosclerotic lesions or for in-stent restenosis, namely stent fracture with restenosis in the unstented gap.

Stent fractures have been reported in both nonvascular settings, such as the biliary tract [7,8] and esophagus [9,10], and vascular settings, such as the iliac artery [11] and in the subclavian artery and vein [12]. Excessive mechanical stress due to extreme flexion of the vessel or compression by tissue (tumor growth in esophageal stents) is the proposed cause of these fractures.

Stent fracture has also been reported after pulmonary artery stenting for pulmonary artery stenosis in congenital heart disease [13]. For stents positioned near pulsatile structures such as the heart or the proximal great vessels, a fracture rate of 15–30% has been reported [14–16]. In a recent randomized trial that compared autoexpandable nitinol stents with or without a sirolimus coating for the treatment of diffuse superficial femoral disease, stent fractures occurred in 6 of 33 patients and were equally distributed in coated and noncoated stents. No clinical or angiographic consequences were reported [17].

A case of coronary stent fracture was recently reported in a venous bypass graft (18). In vein grafts, mechanical stresses can be very high, depending on the curvature of the graft, the presence of perigraft fibrosis, and the intrathoracic space available.

Recently, a warning was issued after seven cases of fractures of NIRoyal coronary stent, occurring between 3 weeks and 9 months after implantation, were reported. Five of them were implanted in native coronary arteries and two in vein grafts. All stents were long, 25 and 32 mm, and in four cases there were two or more overlapping stents. Six out of seven patients presented with recurrent symptoms related to restenosis and three of the fractured stents were totally occluded (www.medical-devices.gov.uk).

To our knowledge, this is the first report of fracture of SES in coronary arteries. This resulted in an unusual pattern of very focal in-stent restenosis with complete abolition of neointimal hyperplasia in the rest of the stent length. Since the effectiveness of the SES is related to the release of an active therapeutic compound, local underdosage due to the stent fracture in combination with increased mechanical irritation are the most probable causes of the very focal restenosis.

All fractured stents were long (33 mm) and were postdilated with larger balloons at high pressures. In the first case, the stent was fractured in its middle part at the point of maximal vessel curvature and movement. In the second case, two of the fractures were observed at the extremities of stent overlapping and the third in a gap of the previous noneluting stents. This means that all fractures occurred around areas of increased rigidity due to metal overlapping that may have acted as a fulcrum for metal deformation due to vessel movement. Longer stents covering longer vessel areas are subjected to higher radial forces compared to shorter stents and may be more prone to fracture, especially when placed in tortuous vessels or calcified lesions. Stent overexpansion could also have contributed to the stent fracture.

In conclusion, this report illustrates an unusual cause of restenosis with sirolimus-eluting stents, the stent fracture. The very effective suppression of neointimal formation within the stent causes an unusual pattern of very focal restenosis at the point of fracture. Further studies with intravascular ultrasound should be performed to clarify whether this is an important issue or a sporadic observation.

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Part 2:

Endothelial dysfunction after PCI

Chronic injury

Chapter 6

Long-term Endothelial Dysfunction Is More Pronounced After Stenting Than After Balloon Angioplasty in Porcine Coronary Arteries

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Long-Term Endothelial Dysfunction Is More Pronounced After Stenting Than After Balloon Angioplasty in Porcine Coronary Arteries

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Objectives. **To compare percutaneous transluminal coronary angioplasty (PTCA) and stent implantation with respect to the long-term changes they induce in the newly formed endothelium in porcine coronary arteries by studying both morphological and functional parameters of the endothelium at 2 weeks and 3 months after intervention.**

Background. **Problems affecting PTCA or stent implantation have been overcome to a large extent by means of better techniques and the availability of new drugs. Late problems, however, still exist in that restenosis affects a large number of patients. With an increasing number of patients being treated with stents, the problem of in-stent restenosis is of even greater concern, as this seems difficult to treat. A functional endothelial lining is thought to be important in controlling the growth of the underlying vascular tissue. We hypothesized that the enhanced neointimal hyperplasia observed after stenting is associated with a more pronounced and prolonged endothelial dysfunction.**

Methods. **Arteries were analyzed using a dye-exclusion test and planimetry of permeable areas. Thereafter, the arteries were processed for light and scanning electron microscopy for assessment of morphology and proliferative response.**

Results. **Leakage of the endothelium for molecules such as Evans blue-albumin as well as prolonged endothelial proliferation is observed as late as 3 months after the intervention, and is more pronounced after stenting. Permeability is associated with distinct morphologic characteristics: endothelial retraction, the expression of surface folds, and the adhesion of leukocytes.**

Conclusions. **Stenting especially decreases long-term vascular integrity with respect to permeability and endothelial proliferation, and is associated with distinct morphologic characteristics.**

(J Am Coll Cardiol 1998;32:1109–17)

Problems affecting percutaneous transluminal coronary angioplasty (PTCA) or stent implantation such as acute closure, stent thrombosis, or bleeding complications due to the stringent anticoagulation protocols from the early days of stent use have been overcome to a large extent by means of better techniques and the availability of new drugs (1–4). Late problems, however, still exist in that restenosis affects approximately 15–20% of patients after primary stenting and 30–50% after PTCA alone. With an increasing number of patients being treated with stents (up to 50%), the problem of in-stent restenosis is of even greater concern as this seems difficult to treat.

Every intervention aimed at increasing lumen size inevitably leads to damage of the vessel wall. The subsequent healing response, necessary to pacify the inflicted wound, triggers the growth of a neointimal thickening (NI). Excessive growth of this NI is only one of the contributors to restenosis after PTCA, but is likely the sole responsible factor when dealing with in-stent restenosis. PTCA mechanically damages the endothelial cells and induces endothelial dysfunction that persists for several weeks both in the laboratory animal and in patients (5–7). A functional endothelial lining is important in controlling the growth of the underlying vascular tissue (8), and it may well be that the enhanced neointimal hyperplasia observed after stenting is associated with a more pronounced and prolonged period of endothelial dysfunction.

The objective of the present study was, therefore, to compare PTCA and stent implantation with respect to the long-term changes they induce in the newly formed endothelium in porcine coronary arteries. We therefore studied both morphological (as assessed by general pathology, morphometry, histochemistry, electron microscopy [EM]) and functional parameters of the endothelium (barrier function and proliferative status).

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Abbreviations and Acronyms

BrdU = bromodeoxy uridine
EB = Evans blue
EM = electron microscopy
LM = light microscopy
NI = neointimal thickening
PTCA = percutaneous transluminal coronary angioplasty

Methods

Animal care. Experiments were performed under the regulations of the animal care committee of the Erasmus University Rotterdam and in accordance with the "Guide for the Care and Use of Laboratory Animals" (9).

Animal preparation. Experiments were performed in Yorkshire pigs (25–30 kg; HVC). After an overnight fast, the animals were sedated with 20 mg/kg ketamine hydrochloride. After induction of anesthesia with thiopental (12 mg/kg) and after endotracheal intubation, the pigs were connected to a ventilator that administered a mixture of oxygen and nitrous oxide (1:2 [vol/vol]). Anesthesia was maintained with 0.5–2.5 vol% isoflurane. Antibiotic prophylaxis was administered by an intramuscular injection of 1,000 mg of a mixture of procaine penicillin-G and benzathine penicillin-G.

Under sterile conditions, an arteriotomy of the left carotid artery was performed and a 9-F introduction sheath was placed. Then 10,000 IU heparin sodium were administered followed by left coronary angiography using the nonionic contrast agent iopamidol (Iopamiro 370) after intracoronary administration of 1 mg isosorbide dinitrate.

Coronary interventions. From the angiograms (analyzed on-line using a quantitative coronary angiography analysis system), arterial segments of 2.5–3.5 mm in diameter were selected in the left anterior descending and/or left circumflex coronary arteries. Typically, balloon sizes were chosen 0.2–0.5 mm larger than the recipient artery. The stents (PS 153, Palmaz-Schatz Coronary Stent; JJIS, and Wiktor stent; Medtronic) were placed as described before (10,11). PTCA was performed in a similar way, using identical inflation parameters. After repeat angiography of the treated coronary arteries, the guiding catheter and the introducer sheath were removed, the arteriotomy was repaired, and the skin was closed in two layers. The animals were then allowed to recover from anesthesia.

Experimental groups and follow-up. Interventions were performed in four groups of animals, as shown in Table 1. In groups 1 and 2 (a subset of animals from a previously published study [10]) the animals received a Palmaz-Schatz stent only and were followed for 4 and 12 weeks, respectively, to assess: 1) the "molecular window," i.e., to which extent the barrier function of the endothelial lining was impaired, and 2) whether this "window" of permeability changed in time. In groups 3 and 4, both PTCA and stent implantation were performed in each animal. These animals were followed for 2 and 12 weeks to assess the morphologic determinants correlating with the impaired barrier both in morphologically immature and mature endothelium, and to study differences between the two types of intervention.

Assessment of cell proliferation. To assess the proliferative response to stent implantation and PTCA in comparison with control coronary arteries, five animals each in groups 3A, 3B, 4A, and 4B were given three intramuscular injections of BrdU (Sigma Chemical Co.) at 100, 50, and 50 mg/kg at 8-h intervals, starting 24 h before sacrifice. Using light microscopy (LM), the total number and number of BrdU-positive cells were counted for each section in several high-power fields both proximal and distal in the treated arteries. The right coronary artery served as a control.

Assessment of intimal permeability at follow-up. In this test (Fig. 1, dye-exclusion test) Evans blue (EB) (Sigma Chemical Co.) was used in two configurations (12,13).

EB-albumin. To subject the arteries to the large molecular marker (70 kD), 300 mL of EB in saline (0.3% [wt/vol]) was administered intravenously, to allow for EB binding to albumin. The infusion was given for 30 min, and then 1 h was allowed for recirculation of the EB-albumin complex.

Binding control. During and after the EB infusion, arterial blood samples were taken, proteins precipitated with trichloric acid (final concentration 20%), and then spun down to check the supernatant for unbound dye.

EB-saline. To subject the arteries to the small molecular marker (1 kD), 300 mL of EB in saline (0.3% [wt/vol]) was administered directly into the coronary circulation after a saline flush. After completion of the EB infusions, the coronary arteries were flushed with approximately 300 mL saline

Table 1. Study Groups

Group	Intervention	Follow-up	n	Molecular Weight	Study Objective
1	Palmaz-Schatz stent	4 weeks	10	1 and 70 kD	Window of permeability
2	Palmaz-Schatz stent	12 weeks	10	1 and 70 kD	Window of permeability
3A	Wiktor stent	2 weeks	9	70 kD	Morphologic determinants
3B	PTCA	2 weeks	9	70 kD	Morphologic determinants
4A	Wiktor stent	12 weeks	5	70 kD	Morphologic determinants
4B	PTCA	12 weeks	5	70 kD	Morphologic determinants

n = number of arteries.

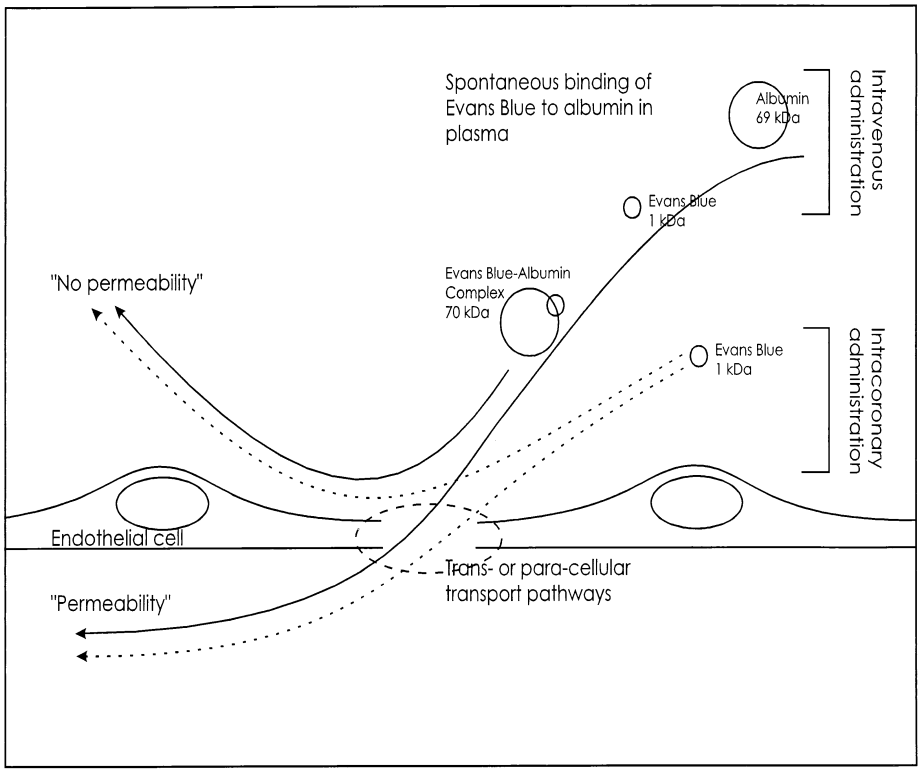

Figure 1. EB can be administered both intravenously and intracoronarily. Intravenous administration results in the spontaneous binding of EB to albumin, and subjection of the arterial wall to the 70-kD large complex. Intracoronary administration after a saline flush to remove serum proteins results in subjection of the arterial wall to the smaller 1-kD molecule. Blue staining of the arterial wall indicates a breach in the luminal barrier.

before pressure fixation in situ (approximately 100 mm Hg) with 500 mL 4% buffered formaldehyde. Then the heart was excised, and the treated and control coronary arteries (not treated with balloon or stent) were dissected from the epicardial surface.

Macroscopic assessment. The excised treated and control coronary arteries were opened longitudinally and checked under a dissection microscope for penetration of the blue dye. The arteries were documented on film and used for planimetric analysis of the permeable areas. Thereafter, both proximal and distal areas of the specimen were divided for EM and LM.

Routine histology. To check for abnormal vascular reactions to the interventions and for a general assessment of the histological appearance, all specimens were processed for routine histology as described before (10). Sections were stained with hematoxylin-eosin as a routine stain and resorcin-fuchsin as a collagen and elastin stain.

Histochemistry. *Lectin- and immunocytochemistry.* This was performed to confirm the identity of the endothelium and smooth muscle cells as described before (14).

Detection of BrdU incorporation. After acid DNA denaturation and elimination of endogenous tissue peroxidase activity, rehydrated paraffin sections were exposed to mouse anti–BrdU antibody (Becton Dickinson & Co.), dilution 1:80, to detect BrdU-positive cells. As a second antibody, HRP-labeled rabbit anti–mouse antibody (Dakopatts) was used, with 670 μg/ml Di Amino Benzidine (Sigma Chemical Co.) in phosphate-buffered saline as a detecting reagent.

Morphometry. Intimal and medial thickness was determined along the length of the treated arterial segments. A distinction was made between intimal and medial thickness within and outside the PTCA lesion area and the media underneath or between the stent struts. Data were analyzed using elastin-stained sections and assessed on a microscopy image analysis system (Impak C, Clemex vision Image analysis system; Clemex Technologies Inc.) as described before (11). In addition, lesion length was determined in the arteries treated with PTCA, which was defined as the percentage of the internal elastic lamina containing discontinuities or associated with an intimal and/or medial thickening (15).

Scanning and transmission EM. To study endothelial morphology (scanning EM) and to assess endothelial cell-cell contact (transmission EM), selected tissues were fixed with 2.5% glutaraldehyde in 0.15 M cacodylate buffer, postfixed with 0.1 M cacodylate buffer containing 1% OsO_4 and 50 mM ferricyanide ($K_3[Fe\{CN\}_6]$), and further processed as described before (18). Specimens were examined in a JSM25 scanning electron microscope (Jeol Ltd.) and a CM100 transmission microscope (Philips).

Statistical analysis. Analysis was performed using Sigmastat (versions 1.0 and 2.0, Jandel Scientific). Data are given as mean ± standard deviation. Morphometry was analyzed with a

Table 2. QCA Assessment of Arterial and Balloon Diameter at the Site of Intervention

Group	Pre	Balloon	Ratio	Post	Fu
1 (n = 10)	3.05 ± 0.28	2.98 ± 0.38	0.95 ± 0.07	3.06 ± 0.33	2.37 ± 0.39
2 (n = 10)	3.11 ± 0.21	3.03 ± 0.30	0.97 ± 0.06	3.02 ± 0.17	3.12 ± 0.27
3A (n = 9)	2.87 ± 0.27	3.02 ± 0.41	1.02 ± 0.09	2.74 ± 0.29	2.65 ± 0.28
3B (n = 9)	2.95 ± 0.31	2.85 ± 0.32	0.95 ± 0.05	2.66 ± 0.34	2.79 ± 0.48
4A (n = 5)	2.61 ± 0.46	2.92 ± 0.39	1.08 ± 0.06	2.67 ± 0.30	2.80 ± 0.25
4B (n = 5)	2.58 ± 0.25	2.65 ± 0.42	1.04 ± 0.06	2.28 ± 0.28	3.08 ± 0.42

Data are in mm and given as mean ± SD. Pre, Post, Fu: mean coronary lumen diameter at the site of intervention before, directly after, and at follow-up, respectively. Balloon: mean diameter of the contrast filled balloon during maximal inflation. Ratio: balloon to artery ratio.

one-way ANOVA, the planimetry and angiography with a one-way repeated measures ANOVA, and followed by an all-pairwise comparison in case of statistical significance using a Student Neuman Keuls or Tukey test. A p value of <0.05 was considered statistically significant.

Results

Procedural outcome. A total of 39 animals were enrolled in the study. In group 3, one animal died suddenly within 1 h after the procedure due to stent thrombosis. In group 4, four animals died: one animal died during the procedure due to ventricular fibrillation, one died within a few hours after the procedure due to stent migration and subsequent stent thrombosis, one animal due to respiratory problems during recovery from anaesthesia, and one animal died at 3 weeks after the procedure ex causa ignota (not stent related). The remaining 34 animals were used for analysis, as summarized in Table 1. Quantitative angiographic measurements are shown in Table 2. Quantitative coronary angiography (QCA) confirmed that all stent and balloon sizes closely matched coronary artery vessel size with a balloon-artery ratio between 0.95 and 1.1.

Intimal permeability. Binding control confirmed that complete binding of EB to the albumin was achieved.

The "window" of permeability (groups 1 and 2). Macroscopy (Fig. 2A and B) revealed that both the 1- and 70-kD markers were able to stain the vessel wall at 4 as well as at 12 weeks post stenting. This indicates that there is a wide window of permeability that does not change during the first 3 months.

The "extent" of permeability (all groups). Except for occasional small areas distal to side branches, where the endothelium is often subject to hemodynamic stress, the control arteries did not reveal staining (Fig. 2A). Both stent- and balloon-treated arteries, however, did reveal a distinct staining of the vessel wall (Fig. 2B–D). In the stented arteries staining of the intima was generally observed over the stent wires, while between the stent struts a much lower level of staining was seen (Fig. 2B and C). Both the Wiktor stent and the Palmaz-Schatz stent, however, revealed a specific staining pattern. In the Palmaz Schatz stent staining was seen over the stent struts in the area of the stent ends and in the area of the coupler (Fig. 2B), while in the Wiktor stents staining was observed over the wire along the whole length of the stent (Fig. 2C). These patterns did not change during the observation period. In group 3B (2 weeks after PTCA alone) there were also areas that revealed staining albeit less prominently (Fig. 2D). In group 4B (12 weeks after PTCA) the pattern was the same but the staining intensity was less.

Planimetry. Planimetry of the area permeable to EB at 2 weeks showed that the percentage was 35.4 ± 19.7% for the stented arteries ($p < 0.05$ vs balloon and control), 10.1 ± 6.8% for the PTCA treated arteries, and 2.5 ± 2.2% for the control arteries.

Electron microscopy and the endothelial barrier function. Both scanning and transmission EM were performed on groups 3 and 4. It confirmed that, in contrast to normal endothelium (Fig. 3A), at 2 weeks after either stent implantation or PTCA the endothelial covering was still incomplete. The areas covering the stent struts especially showed missing cells, often in association with adhesion of leukocytes and platelets. These areas were highly permeable to the EB dye. The more diffusely permeable areas were characterized by an endothelial layer where the cells appeared "retracted" (Fig. 3B). Transmission EM showed small or nonexistent intercellular junctional complexes (Fig. 3C) in the areas with retracted cells. Twelve weeks after the interventions, scanning EM showed that the endothelial covering was complete in all groups, and transmission EM now showed more extensive junctional complexes even with tight junctions (Fig. 3D). Permeability was now associated with a different phenomenon, namely an endothelial layer with adhesion of leukocytes with a rounded morphology that were also often seen penetrating the endothelium as well as surface folds (Fig. 3E), which indicates an increased endocytotic activity, although endothelial retraction was still observed occasionally.

Morphometry and assessment of cell proliferation. Morphometric analysis is summarized in Table 3. At 2 weeks after stenting (group 3A) there is a trend ($p > 0.05$) toward a larger NI over the stent struts than after PTCA at the site of the lesion (group 3B). At 12 weeks both the Wiktor stent (group 4A) and the Palmaz-Schatz stent (group 2) do induce a statistically significant larger NI than after PTCA. Also, the Wiktor stent induces a significantly more pronounced NI as compared with the Palmaz-Schatz stent. Morphometric analysis of the medial layers show that the stents significantly impress the media, whereas at the site of the PTCA lesion the media has thickened. At 4 and 12 weeks (groups 1, 2, and 4A) the media has thickened between the stent struts and is now

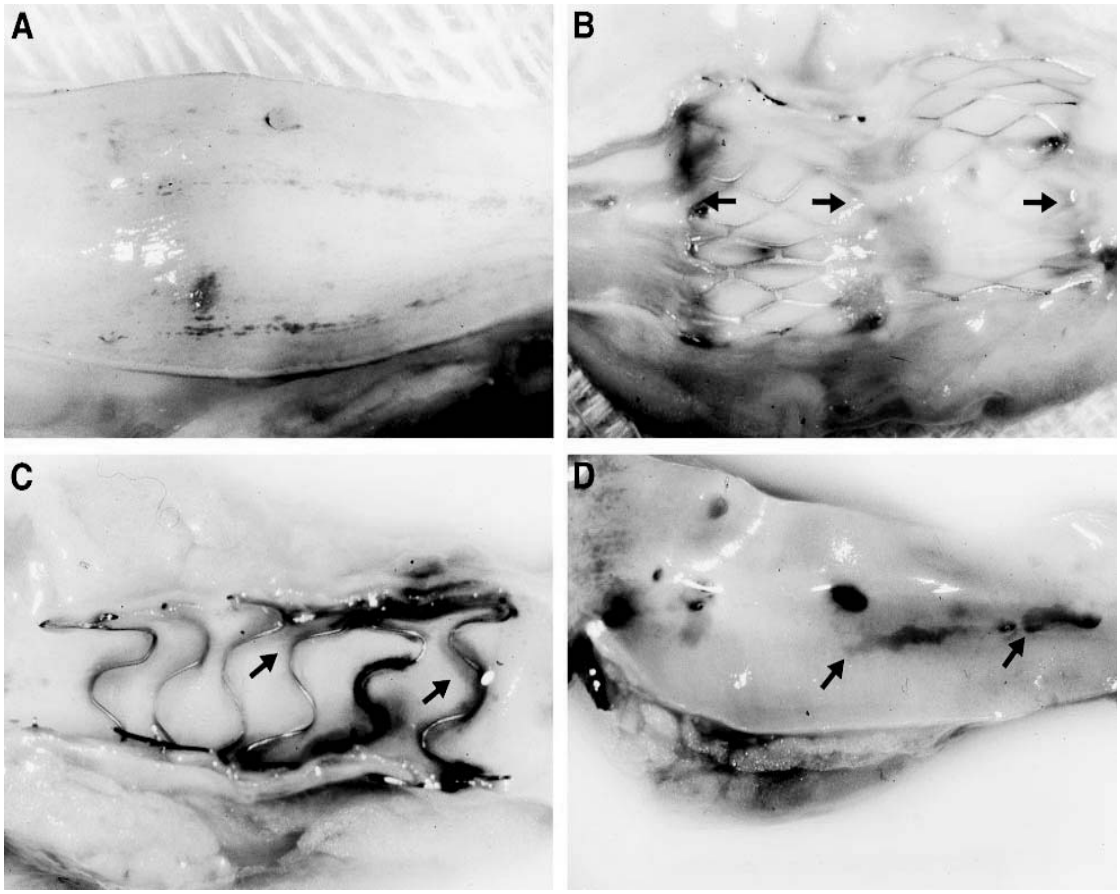

Figure 2. Macroscopy of the dye-exclusion test. **A,** Control right coronary artery, shows a clean surface with occasional small blue areas. **B,** Group 2, Palmaz-Schatz stent, 1 kD. For both molecular weights, blue staining is found mainly at the stent ends and in the area of the coupler (**arrows**). **C,** Group 3A Wiktor stent. Blue staining is seen especially in the region of the intimal tissue covering the stent struts (**arrows**). **D,** Group 3B, PTCA. Randomly stained areas (**arrows**) are found in the arterial segment treated with PTCA and are more apparent and intense in group 3B than 4B.

similar to the lesion area in the balloon-treated arteries at 2 and 12 weeks.

For assessment of cell proliferation, the BrdU-positive and total number of cells were counted in several sections. For each artery this amounted to 200–400 endothelial cells; 200–400 (balloon group) and 1500–5500 (stent group) intimal cells; 1000–4000 medial and adventitial cells. The percentage of BrdU incorporation is summarized in Table 4, and shows that at 2 weeks after intervention the stent (group 3A) induced a higher percentage of BrdU incorporation as compared with PTCA in all tissue layers ($p < 0.05$). In the PTCA vessels (both lesion area and nonlesion area) there was a trend ($p > 0.05$) toward a higher percent proliferating cells as compared with control (not injured) values. Twelve weeks after intervention the levels of BrdU incorporation had decreased in all groups. Only the endothelium overlying the stent struts was still significantly higher as compared with control values ($4.8 \pm 2\%$; $p < 0.05$).

Microscopy. There were no adverse or unexpected vascular reactions to the interventions as performed in groups 1–4, and in general the tissue response was as described before (10,11). In short, the stented arteries in groups 1, 2, and 4 were covered by a variable intimal thickness consisting of smooth muscle cells in a collagenous matrix and covered by endothelium. Inflammatory reactions were limited for groups 1 and 2. In group 4A, there were areas where the stent strut lacerated the media, a phenomenon associated with a diffuse inflammatory response. At 2 weeks (group 3A), the stent was embedded in a mass of organizing thrombus, containing leukocytes and macrophage giant cells, and was covered by an incomplete layer of endothelial cells. There was medial hyperplasia (medial thickening and longitudinal orientation of smooth muscle cells) in association with BrdU incorporation between the stent struts.

PTCA. At 2 weeks after PTCA (group 3B), there were focal areas with a limited amount of neointima, which consisted of smooth muscle cells in a collagenous matrix. In these areas we observed fragmentation of the Lamina Elastica Interna (LEI) and significant medial hyperplasia in association with incorporation of BrdU (Fig. 4A). These lesions encompassed approximately 30% of the measured circumference. The endothelial covering (as confirmed by lectin histochemis-

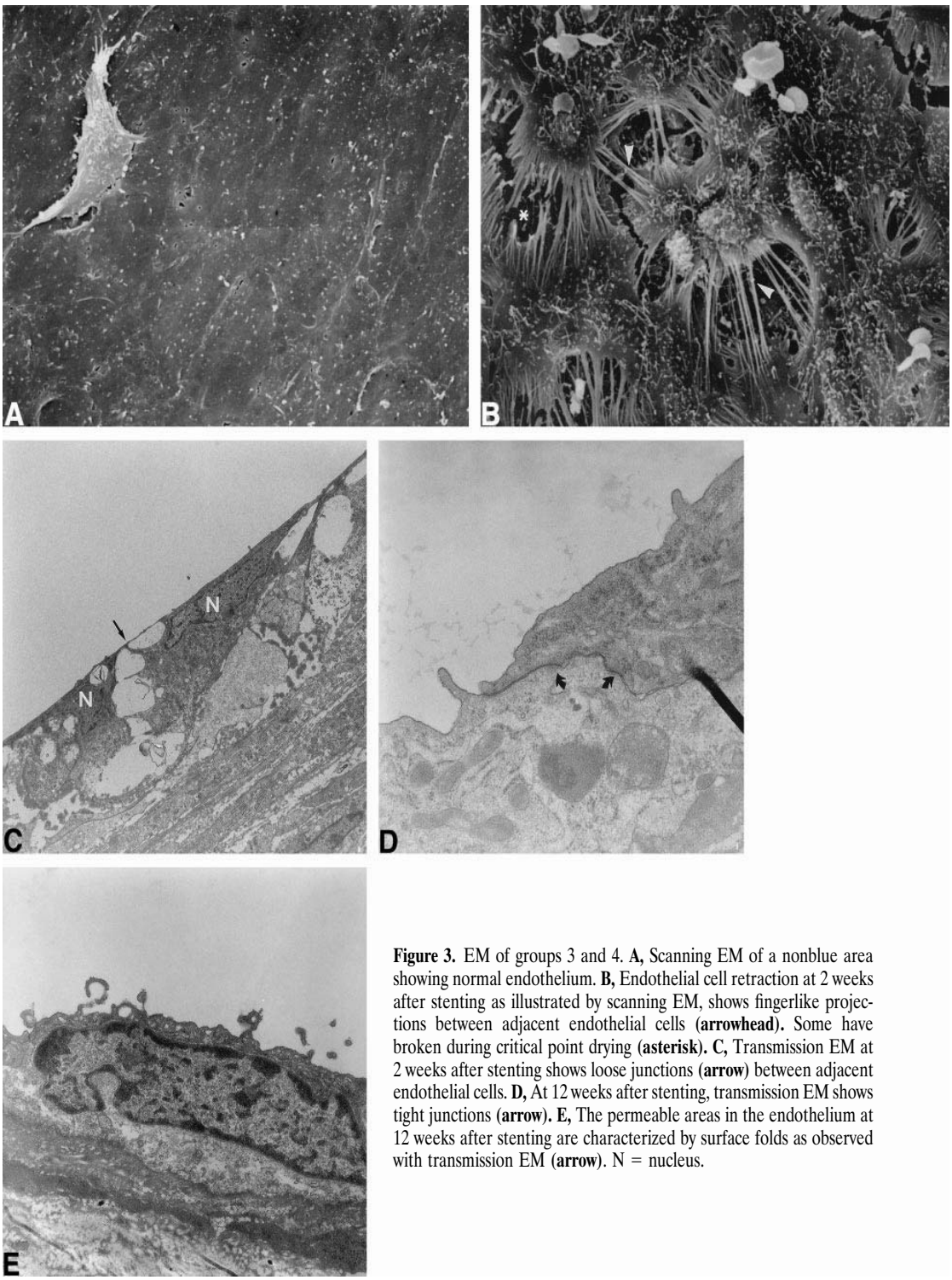

Figure 3. EM of groups 3 and 4. **A,** Scanning EM of a nonblue area showing normal endothelium. **B,** Endothelial cell retraction at 2 weeks after stenting as illustrated by scanning EM, shows fingerlike projections between adjacent endothelial cells **(arrowhead).** Some have broken during critical point drying **(asterisk). C,** Transmission EM at 2 weeks after stenting shows loose junctions **(arrow)** between adjacent endothelial cells. **D,** At 12 weeks after stenting, transmission EM shows tight junctions **(arrow). E,** The permeable areas in the endothelium at 12 weeks after stenting are characterized by surface folds as observed with transmission EM **(arrow)**. N = nucleus.

try) was incomplete and had a variable morphology. At 12 weeks after PTCA (group 4B), there was still a limited amount of intimal hyperplasia. Although the media was still thickened in these areas and the lesions still encompassed approximately 30% of the measured circumference, there was no incorporation of BrdU, nor a clear fragmentation of the LEI. On the contrary, we often observed an additional internal elastic membrane under the newly formed endothelium (Fig. 4B). The adventitia was unremarkable.

Discussion

In contrast to restenosis after PTCA, which is dictated both by constrictive remodeling and tissue growth (16,17), resteno-

Table 3. Morphometry

Group	NI	Media	
	Stent or PTCA Lesion	Underneath PTCA Lesion or Stent Struts	Between Stent Struts or PTCA Lesion/Nonlesion
1 (stent)	259 ± 104*3a,3b,4b	99 ± 22	187 ± 28*3a,3b,4a
2 (stent)	192.1 ± 63.3*3b,4b	109.8 ± 21.6	184.3 ± 16.3*3a,3b,4b
3A (stent)	114 ± 60	71 ± 40	122 ± 26
3B (PTCA)	33 ± 11	221 ± 77*1,2,3a,4a	119 ± 15
4A (stent)	305 ± 155*3a,3b,4b	73 ± 29	182 ± 29*3a,3b,4b
4B (PTCA)	19 ± 12	181 ± 53*1,3a,4a	128 ± 33

*$p < 0.05$ vs group . . ., ANOVA-Tukey test. Data are in μm and given as mean ± SD. The NI was measured on top of the stent struts and compared with the NI in the PTCA lesion. Media thickness was measured underneath and between the stent struts and compared with the media thickness in the PTCA lesion and nonlesion area.

sis after stenting is the result of tissue growth alone. With increasing numbers of patients being treated with stents (up to 50%), the problem of in-stent restenosis is becoming more and more of a problem, as this seems more resistant to effective treatment.

Vascular dysfunction and in particular endothelial dysfunction has been described after PTCA both in humans and animals (5–8). As one of the first changes in the etiology of atherosclerosis, endothelial dysfunction might also be involved in the ongoing tissue growth after angioplasty procedures (8,18). The aim of our study, therefore, was to investigate endothelial function after stenting and compare this with PTCA alone by assessing both morphologic and functional parameters.

The main finding in our study is that both PTCA and stent implantation result in an impairment of the vascular barrier function at least up to 3 months after the procedure as evidenced by the uptake of the EB dye. This loss of barrier function is more pronounced after stenting than after PTCA, and showed a stent-specific pattern. This breach was characterized by specific endothelial morphologic correlates: in the early phase by incomplete endothelialization and endothelial retraction or loose intercellular connections. The late phase was characterized by the expression of surface folds and the adhesion of leukocytes. Both phenomena were also observed in stented human vein grafts (19).

Transport routes across the endothelial lining. The extravasation of (macro)molecules, such as EB bound to albumin, proceeds mainly by two routes. One is through diffusion via the cellular junctions, i.e., paracellular exchange (20,21), and the other is by vesicle-mediated transport, i.e., transcellular transport (22).

Paracellular exchange is morphologically associated with small interendothelial gaps caused by contractile forces in the cell and by disintegration of cell–cell junctions (23,24). This process is regulated by actin fibers, which are connected to other proteins anchoring the cells to their neighbors and to the extracellular matrix (25,26). Vasoactive agents and thrombin can affect the integrity of the endothelium through phosphorylation of specific target proteins. As a consequence, actin reorganization may occur through RhoA– and protein kinase C–activated pathways. The interaction between actin and nonmuscle myosin, activated by phosphorylation of the myosin light chain, subsequently causes contraction and gap formation (24,27). Interaction of leukocytes with the endothelium, which is enhanced by inflammatory mediators, can enhance this response (28). In addition to the effects of vasoactive agents, paracellular permeability can also be enhanced by the lingering proliferative response of the endothelium itself. Cell retraction associated with cell mitosis causes paracellular gaps in arterial endothelial cells in vivo (29). Because cell division is found until at least 3 months after intervention, particularly in areas overlying stent struts, it may contribute to the observed leakage of EB-albumin complex. Both endothelial barrier function and proliferation are under control of the extracellular matrix. Remodeling of the extracellular matrix by proteases can re-

Table 4. Assessment of Cell Proliferation (% BrdU-Positive Cells)

Group (n)	EC	NI	M	ADV
Control (n = 6)	0.4 ± 0.1	Nonexistent	0.9 ± 0.1	1.07 ± 0.1
3A (n = 6)	22.2 ± 7.5*	18.8 ± 2.7*	6.5 ± 1.2*	7.9 ± 2.1*
3B: lesion area (n = 5)	5 ± 3.8	6.14 ± 8.2	2.66 ± 2.62	3.98 ± 4.41
3B: non-lesion area (n = 6)	2.64 ± 2.65	Nonexistent	0.86 ± 0.43	0.98 ± 0.78
4A (n = 5)	3.5 ± 1.1	2.7 ± 1.1	1.6 ± 1	2.3 ± 1.1
4B: lesion area (n = 4)	1.3 ± 1.5	1.2 ± 0.91	1.5 ± 0.67	0.75 ± 0.76
4B: non-lesion area (n = 5)	0.3 ± 0.6	Nonexistent	0.99 ± 0.91	0.64 ± 0.54

EC = endothelial cells; M = media; ADV = adventitia. *$p < 0.05$ vs. all groups, ANOVA-student neuman keuls test. Data are mean ± SEM.

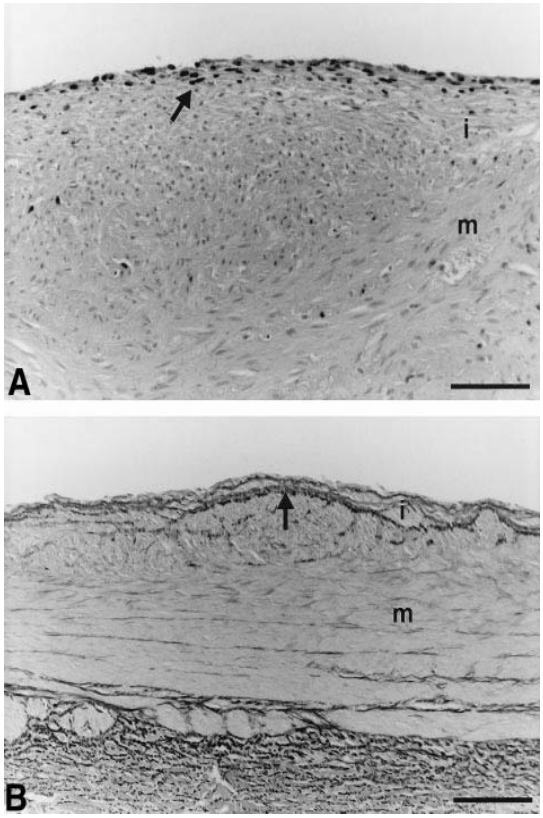

Figure 4. LM. **A,** Group 3B. At 2 weeks after PTCA, focal lesions can be found with BrdU incorporation **(arrow),** especially underneath the endothelial lining but also elsewhere in the intima **(i)** and media **(m).** HRP-DAB with hematoxylin counterstain. Bar = 50 μm. **B,** Group 4B. At 12 weeks after PTCA, there is still a limited NI, sometimes with an additional elastic membrane underneath the endothelium **(arrow).** Resorcin-Fuchsin, i = intima, m = media. Bar = 50 μm.

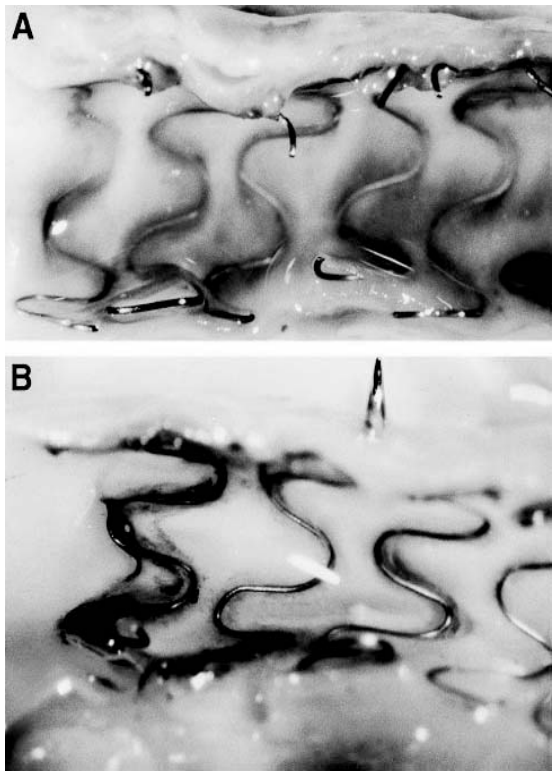

Figure 5. Macroscopy c-AMP–treated arteries. Macroscopy of the coronary arteries at 2 weeks after implantation of a Wiktor stent. While a "control" artery **(A)** was not exposed to db-c-AMP, but only to EB, the c-AMP-treated artery was first exposed to 1 mM db-c-AMP and then to EB with a molecular weight of 1 kD **(B),** showing an improvement especially in the areas between the stent struts.

duce the firm interaction between cell and matrix. This reduction may enhance the efficacy of endothelial permeability-increasing agents (25,30).

Transcellular (vesicle-mediated) transport is the second major route for macromolecular exchange across the endothelial lining. Both paracellular and transcellular exchange of albumin are increased by VEGF (31), a growth factor that is induced in injured arteries (32,33). Further studies have to elucidate whether and for how long VEGF is induced in stented coronary arteries.

In vivo (34,23) and in vitro studies (35,36) have shown that an increase in endothelial permeability in the microcirculation can be reversed by exposing the endothelium to cAMP-elevating agents. In preliminary experiments we found that intracoronary administration of 1 mM dibutyryl-cAMP within 10 min indeed partly normalizes the barrier function in areas of increased permeability covering and adjacent to the implanted stents (Fig. 5). The areas that were permeable because of missing endothelial cells were not affected by this treatment. A reduced cellular cAMP concentration/content has been reported in arterial cells in intimal tissue affected by atherosclerosis (37). However, it remains to be established whether this also occurs in the new layer of endothelial cells in stented arteries. The reduction in endothelial permeability by elevation of cellular cAMP levels indicates that a major part of the leakage of EB-albumin occurs via impaired junctional complexes between endothelial cells. However, the current state of knowledge does not permit exclusion of a minor contribution by vesicular transport.

Permeability after PTCA takes place preferentially in the area designated as the lesion area containing intimal hyperplasia. Clearly these are areas where the endothelium is attached to a changed basement membrane or extracellular matrix. In the stented segments, the intima covering the stent struts is also constantly changing during the process of scar maturation, which might explain permeability over the stent struts in general but not the differences between stent designs. Whereas the Wiktor stent shows preferential dye-uptake over all of the stent wires throughout the whole stent, the Palmaz-Schatz stent shows dye-uptake preferentially over the stent struts at both stent extremities and over the area of the coupler. It would seem that these specific patterns of dye-uptake are a reflection of the design of the stent. The Wiktor stent has a more open design than the Palmaz-Schatz stent, and as permeability seems to occur in areas where theoretically movement between the tissue and the stent struts may occur,

this may be a factor influencing the chronic vascular irritation by the stents. From the literature it is known that mechanical instability of healing bone fractures, for instance, affects the composition of the extracellular matrix with respect to the level of sulfate incorporation in the glycosaminoglycans (38). If these processes also take place in the vessel wall it would certainly influence endothelial function and permeability.

Conclusions. This study indicates that especially stenting decreases long-term vascular integrity with respect to permeability. Leakage is observed with molecules such as EB and EB-albumin complex, and is associated with prolonged endothelial proliferation and distinct morphologic characteristics such as endothelial retraction, the expression of surface folds, and the adhesion of leukocytes.

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Irradiation injury

Chapter 7

No change in endothelial- dependent vasomotion late after coronary irradiation

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No change in endothelial- dependent vasomotion late after coronary irradiation

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Running title: endothelial function after radiation

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Abstract

Purpose

Mechanical injury from balloon angioplasty and stenting is known to cause prolonged endothelial dysfunction, even distal to the injured segment. Intravascular irradiation therapy is associated with delayed healing response and may therefore also impede endothelial functional recovery. This study was conducted to assess endothelial function late after irradiation of atherosclerotic coronary arteries.

Methods and Materials

In 15 patients (8 with additional radiation and 7 patients with stenting only) directly after the intervention and at 6 months follow-up, endothelial function of the distal segment was studied by assessment of coronary diameter after intracoronary acetylcholine. Coronary flow reserve and intravascular ultrasound investigation was performed for unequivocal interpretation of angiographic data.

Results

No significant different response to acetylcholine could be detected at baseline nor at follow-up (-17 ± 14 % vs. - 17 ± 15 % for radiation vs. non-radiation at baseline, p = 1.0; - 8 ± 11 % vs. - 9 ± 13 % at follow-up, p = 0.8). IVUS data revealed more constrictive remodeling in the non-radiation patients, but minimal increase in mean plaque area in the radiation patients compared to significant decrease in non-radiation patients (+ 4 % vs. – 25 %, p = 0.02).

Conclusions

Irradiation of atherosclerotic coronary arteries doesn't affect endothelium-dependent vasodilatation acutely or at 6 months. Irradiated segments demonstrated less negative remodeling but higher plaque burden than controls.

Introduction

Vascular endothelium plays a crucial role in normal vasomotor function (1). Next to this, it is of importance in anti-thrombogenicity of the blood vessel wall as healthy endothelium produces for example nitric oxide and prostacyclin with anti-platelet aggregation activity, as well as pro- and anti-fibrinolytic factors (2).
Mechanical injury by balloon angioplasty but especially stenting has been shown to cause prolonged injury and endothelial dysfunction up to 6 months after the procedure (3-7). Also intracoronary irradiation to treat or prevent in-stent restenosis has been associated with a delayed healing response of the vessel wall after the intervention (8, 9).
As late effectiveness of intracoronary radiation is hampered by the high occurrence of late thrombotic occlusions and excess of adverse events (10-12), it is hypothesized that the trauma of stenting with additional irradiation therapy may cause severe long-lasting endothelial dysfunction. This may be caused by the chronic trauma combined with radiation-dependent delayed healing and re-endothelialization.
The purpose of this study was to assess whether coronary irradiation alone, could be responsible for endothelial dysfunction, leading to late sequelae of therapy. Endothelial function was studied distal to the implanted stent in patients who received stenting with additional intracoronary irradiation therapy, compared to patients who received a stent without additional irradiation therapy. Studies were performed directly after the procedure and at 6 months follow-up.

Methods

Patient selection
23 patients were prospectively included in the study, of which 16 patients were randomized to stenting followed by intracoronary irradiation or stenting alone in de-novo lesions in the BRIDGE trial. BRIDGE (Beta-Radiation Investigation with Direct stenting and Galileo in Europe) is a randomized multi-center study, evaluating the effect of additional beta-irradiation with a P-32 source after successful stenting of a de-novo coronary lesion, less than15 mm in length (13). Two patients received irradiation therapy without stenting for in-stent restenosis and 5 patients received stents without irradiation for de-novo lesions outside the BRIDGE protocol. Of the 23 patients initially included, 3 patients refused follow-up studies, 4 patients were excluded at follow-up because of in-stent restenosis and 1 patient received follow-up angiography in another center, without study protocol.

In total 15 patients could be included in the analysis, with both angiographic studies at baseline as well as at 6 months follow-up: 8 patients receiving a stent or balloon angioplasty with additional irradiation and 7 patients receiving a stent only.
The study was approved by the Medical Ethics Committee of the Erasmus Medical Center and written informed consent was obtained from all patients.

Procedure

Direct stenting was performed in all patients, except for 2 patients who were treated with balloon angioplasty to treat in-stent restenosis. After the procedure was completed, patients received additional irradiation therapy as part of the BRIDGE protocol (n=6) or were treated with compassionate use irradiation therapy (n=2) or no additional therapy (n=7). Then endothelial function testing of the coronary segment distal to the stent was performed, followed by measurement of coronary flow reserve (CFR). Finally intravascular ultrasound (IVUS) evaluation was performed.
All patients received dual platelet therapy consisting of acetylsalicylic acid 80 mg once daily and clopidogrel 75 mg once daily for the duration of the follow-up.

Angiography was repeated followed by endothelial function testing, CFR and IVUS at 6 months follow-up study.

Radiation specification

Beta-irradiation to the treated segment of the coronary artery was delivered by the Galileo^{TM} Intravascular Radiotherapy System (ACS/Guidant, Houston, TX, USA). This system comprises a 32P 0.018" source wire, a centering catheter and the GALILEO TM Source Delivery Unit. The Source Delivery Unit is a high dose rate afterloader, designed specifically for coronary radiotherapy. The centering balloon catheter is 32 mm long and the source wire was automatically advanced first to the distal position and then more proximal, radiating a total length of 40 mm. Radiopaque markers (proximal and distal to the balloon) allowed for precise positioning of the centering catheter at the lesion site. In total, 20 Gy was delivered at 1 mm depth in the vessel wall and dwell time to do so was automatically computed based on the average Reference Lumen Diameter. Full dose radiation was received by a segment length of 32 mm; with a total radiation length of 40 mm. Fall-off dose was received by 4 mm of vessel length at both sides.
In the BRIDGE protocol, 18 mm stents were used, which means that approximately 11 mm of vessel segment on both sides of the stent received radiation, of which 7 mm on both sides received full dose (figure 1). In this way geographical miss (when the length of the

stent and the peri-stent segments are not covered by radiation therapy) (14) could be avoided.

Evaluation of endothelial function

Long-acting vasoactive drugs had been stopped for > 24 hours. However, restrictive use of intracoronary nitrates was allowed during the procedure.

Endothelium-dependent and independent coronary vasomotion was studied as described in detail by Ludmer (15). The segment distal to the stent receiving radiation was studied.

A selective intracoronary infusion catheter (Multi-functional Probing Catheter, Boston Scientific, Galway, Ireland) was advanced over the guidewire and positioned in the stented segment. To avoid wire-induced coronary spasm, the wire was retracted within the catheter. The region of interest was the first 11 mm distal to the stented segment and care was taken to select an angiographic view without fore-shortening of this segment. To determine baseline angiographic diameter, an initial infusion of vehicle solution, normal (0.9%) saline, was administered at an infusion rate of 2 ml/min for 2.5 minutes, followed by angiography. Endothelium-dependent vasomotion was studied by infusion of incremental doses of acetylcholine (Ach), 10^{-8}, 10^{-7} and 10^{-6} Molar, 2.5 minutes at each concentration followed by angiography. Subsequently, endothelium-independent vasomotion was studied after an intracoronary administration of Isosorbidedinitrate (ISDN) (2-3 mg). Throughout each infusion heart rate, systemic arterial blood pressure and electrocardiogram were monitored continuously.

Quantitative Coronary Angiography

Off-line quantitative analysis of the effect of Ach on coronary diameter was performed as described previously with a validated computer-based edge detection system (CAAS II, Pie Medical Imaging, Maastricht, NL) (16).Angiograms were performed in a way to ensure minimal fore-shortening of the coronary segment distal to the stent. During analysis, the distal end of the stent was marked. The coronary segment distal to the stent was analyzed, beginning at two mm from the stent edge, to minimize effects of restrictive movement of the vessel wall due to constraints of the scaffolding stent. The next 9 mm was analyzed. With a zone of 5 mm of full-dose radiation distal of the stent and a 4 mm zone of fall-off radiation dose, this covered the total radiated area distal to the stent. (see figure 1).

Coronary Flow Reserve

CFR measurement was performed to evaluate microvascular resistance in both groups (17). In short, a 0.014-inch Doppler tipped guidewire (FloWire, Volcano Therapeutics Inc., Rancho Cordova, CA, USA) was positioned in the coronary artery, just distal to the

implanted stent. After baseline flow velocity measurement, adenosine was administered in a dose of 140 mcg/kg/min by i.v. infusion for 2 minutes for assessment of hyperemic flow velocity and CFR. Measurements were done in duplicate.

Intravascular Ultrasound

As structural changes, like unhealed dissections, may influence the ability to vasodilate, IVUS study was performed in all patients following endothelial function testing and after CFR measurements.

IVUS was performed according to standard techniques, using a 30Mhz, 2.9 F mechanical ultrasound catheter (UltraCross, Boston Scientific Scimed Inc, Fremont, CA,USA) using automated pullback at 0.5 mm/sec. Images were analyzed off-line from recorded video-tape by two blinded observers (JL, JA).

Statistical Analysis

Data are presented as mean ± SD. Unpaired student-*t* tests were used to compare diameter changes between groups and paired student-*t* tests within groups and between baseline and follow-up. For non-continuous variables Pearson Chi-Square test was used. ANOVA analysis of variance with correction for difference in baseline values was used for the change in IVUS measured mean plaque area between irradiated patients and non-irradiated patients. A value of $P < 0.05$ (two-tailed) was considered to indicate statistical significance. (SPSS; release 11.0.1).

Results

Patient characteristics were very similar between the groups, but only the irradiated group contained also female patients (table 1).

Endothelial function

At baseline, vasoconstrictive reaction to maximal dose of acetylcholine of 10^{-6} Molar was observed (-17 ± 14 % vs. -17 ± 15 % respectively, $p = 0.98$), in both irradiated and non-irradiated groups (table 2).

A trend to less vasoconstriction to Ach can be seen in both groups at FU (-8 ± 11 % for irradiated patients; $p = 0.06$ vs. baseline, and -9 ± 13 % for non-irradiated patients; $p = 0.15$ vs. baseline; $p = 0.84$, and $p = 0.88$ for the difference in percentage change between groups).

CFR

Coronary flow reserve (CFR) did not differ between groups and between baseline and follow-up: (2.9 ± 0.6 vs. 3.0 ± 0.9 for irradiated and non-irradiated groups at baseline, and 3.1 ± 1.2 vs. 3.0 ± 0.9 at follow-up*).*

IVUS

IVUS investigation discovered two short distal stent edge dissections (max 2 mm in length, < 20 degrees circumference) directly after the procedure in the group without additional irradiation. Both were resolved at follow-up studies.

Quantitative IVUS data could be obtained from 7 patients with additional irradiation and 5 patients without irradiation (table 3). The 2 mm immediately distal to the stent was excluded from analysis. The segment studied had a mean segment length in the irradiation group of 9.1 ± 1.8 mm and 9.2 ± 1.7 mm at baseline and follow-up respectively, and in the non-irradiation group of 9.6 ± 1.6 mm at baseline and 9.6 ± 1.5 mm at follow-up.

At follow-up a non-significant, less reduced vessel area was seen in the irradiated group than in the non-irradiated group (-6 % vs. -15 %, p = 0.6) (fig. 2). Despite this a larger luminal area reduction was measured in the irradiation group (-14 % vs. -7 %, p = 0.6), due to minimal increase in plaque area in the irradiation group as opposed to a large decrease in the non-irradiation group (+ 4 % vs. – 25 %, p = 0.02).

Discussion

The present study compared the vasodilatory response to the endothelium-dependent vasodilator acetylcholine of atherosclerotic coronary segments, receiving irradiation or no irradiation.

The main result of the study is that endothelial dysfunction at 6 months did not differ after intracoronary irradiation compared to no radiation. However, the change in plaque area over time was significant between groups (p = 0.02), with minimal increase in plaque area in the irradiated segments and a decrease in non-irradiated segments.

Radiation and endothelial function

Intracoronary radiation therapy is an effective treatment of in-stent restenosis. Radiation blocks cell proliferation, induces apoptosis and inhibits cell migration, resulting in reduction of neointimal accumulation. Radiation also prevents constrictive remodeling after balloon angioplasty, probably mediated by reduction of healing response together with radiation

induced fibrosis (18). Despite favorable mid-term results and acceptable long-term results (19, 20), late thrombotic occlusions, edge restenosis and delayed in-stent restenosis have severely mitigated the enthusiasm for intracoronary radiation therapy. At 4 years follow-up MACE of up to 60 % has been reported in unselected patients (10). An unusual high percentage up to 9 % of late thrombotic events was seen, from 2 to 15 months after the intervention (11, 12). Without radiation, thrombotic events are extremely infrequent beyond 2 weeks after the intervention.

Late thrombosis and neointimal proliferation have been related to delayed healing response and incomplete endothelialization after radiation therapy. Farb et al. demonstrated delayed healing of the intimal surface after implantation of radioactive stents in normal rabbit iliac arteries, with incomplete endothelialization up to 12 months after stenting (8). Cheneau et al. showed only 40 % endothelial cell coverage of rabbit iliac arteries 6 months after stenting and gamma-irradiation. No increase in endothelial coverage was seen between 1 and 6 months, while control stented segments were almost fully covered with endothelial cells after 1 month (9). Incomplete endothelialization associated with increased recruitment of platelets 1 month after balloon angioplasty and beta-radiation of pig coronary arteries was shown by Salame et al. while complete endothelial coverage without increase in platelet recruitment was present in the non-radiated ballooned arteries (21).

Recovery of endothelial function after radiation therapy has been studied by Menendez et al. showing impaired endothelium-dependent and independent vasomotion response of the abdominal aorta of rats, 6 months after external beam radiation therapy. Vascular sclerosis with medial fibrosis was present, typical for radiation vasculopathy and the possible explanation for the impaired non-endothelium-dependent vasomotion (18). Impaired endothelium-dependent vasomotion response in humans has also been reported, several weeks after external beam radiation of cervical arteries (22) and in axillary arteries, more than 3 years after external beam radiation for breast carcinoma (23).

Data on endothelial functional recovery after intracoronary radiation is, however, equivocal. Li et al. reported impaired endothelium-dependent vascular response of normal non-injured porcine coronary arteries, 1 month after 20 Gy ^{32}P beta radiation, using the same delivery device as in our study (24). Surprisingly, Sabate et al. reported restored endothelium-dependent vasomotion 6 months after balloon angioplasty and additional beta-radiation with a ^{90}Sr/^{90}Y source delivering 12 to 16 Gy at 2 mm from the source (25).

The present study was conducted to study the endothelium-dependent vasomotion response after irradiation of non-injured atherosclerotic coronary arteries.

At the index procedure, moderate vasoconstriction could be seen in both groups, with or without additional irradiation. As radiation was only administered a few minutes before vasomotion studies, no acute effect of radiation was expected and vasoconstriction in both groups was indeed comparable. At 6 months follow-up, persistent but less vasoconstrictive reaction was present in both groups. No significant indication was seen of worse endothelium-dependent vasomotion in the irradiated group. In our study the segment was distal of the interventional trauma, but long-term endothelial dysfunction of this segment distal to the stent was already shown by Caramori et al.(6). This lack of late effects of irradiation is in agreement with the above mentioned study by Sabate et al., though they reported even complete restoration of endothelial function with a vasodilatation reaction to Ach. Sabate et al. however, investigated vasomotion of the treated segment itself and only at 6 months follow-up, not excluding possible differences in vasomotion at the index procedure. In the present study baseline data is available, showing comparable vasoconstriction in both groups directly after the procedure, suggesting no substantial difference in endothelial dysfunction in these distal coronary segments without angiographic disease. Moreover, CFR was comparable in both groups excluding major differences in microvascular disease.

IVUS and radiation

Complete IVUS studies were possible in most patients and analysis showed less decrease in vessel area over 6 months in the irradiated patient group. Despite the larger loss in vessel area in the non-irradiated patient group, loss of mean lumen area was less because of a significant reduction in plaque area. This was not seen in the irradiated group. Despite this reduction in plaque area distal to the 6 months earlier treated lesion, endothelial function did not improve compared to the irradiated patient group. It may be hypothesized that in the irradiated patients endothelial function did not improve, but rather that reduced vasoconstrictive remodeling with vessel wall sclerosis limits constrictive vasomotion after acetylcholine stimulation.

Study limitations

This study included only a limited number of patients. Because intracoronary irradiation therapy was substituted by the use of drug-eluting stents in our center, no additional irradiation patients could be included after the completion of inclusion of patients for the BRIDGE trial. However, based on the current results and earlier studies by Sabate et al., it

is very unlikely that we would have found an opposite outcome by increasing the number of patients.

Conclusions

6 Months after irradiation of atherosclerotic coronary arteries with 20 Gy, no evidence could be found of worse endothelial function compared to patients without additional irradiation therapy.

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Table 1. Patient baseline characteristics

		Irradiation (N=8)	Bare stent (N = 7)	p- value
Age in years (SD)		66 (12)	65 (11)	0.93
Male Sex		5 (63%)	7 (100%)	0.07
Coronary Risk Factors	Smoking	1 (13%)	1 (14%)	0.92
	Diabetes	1 (13%)	1 (13%)	0.92
	Hypertension	1 (13%)	2 (29%)	0.44
	Hypercholesterolemia	3 (38%)	3 (43%)	0.83
	Family History	5 (63%)	3 (43%)	0.45
Prior Myocardial Infarction		2 (25%)	1 (14%)	0.61
No. Diseased Coronary Vessels	One	4 (50%)	4 (57%)	0.78
	Two	4 (50%)	3 (43%)	0.78
Statin use		5 (63%)	4 (57%)	0.83
ACE inhibitor use		1 (13%)	1 (14%)	0.92
Mean diameter of analyzed coronary segment in mm (SD)	At index procedure	2.5 (0.6)	2.5 (0.4)	0.99
	At follow-up	2.3 (0.5)	2.4 (0.3)	0.80

Data are presented as number of patients (%).
SD = Standard deviation

Table 2.

Mean percentage coronary segment diameter change from baseline (± SD) after maximal Ach dose (10^{-6} molar) and endothelium independent vasomotion (intracoronary nitrates), at index procedure and 6 months follow-up.

		irradiation N = 8	no irradiation N = 7	p- value
Index procedure	Ach	- 17 ± 14 %	-17 ± 15 %	0.98
	nitrates	0 ± 12 %	-4 ± 7 %	0.35
Follow-up	Ach	-8 ± 11 %	-9 ± 13 %	0.84
	nitrates	1 ± 11 %	2 ± 8 %	0.86

Ach: acetylcholine

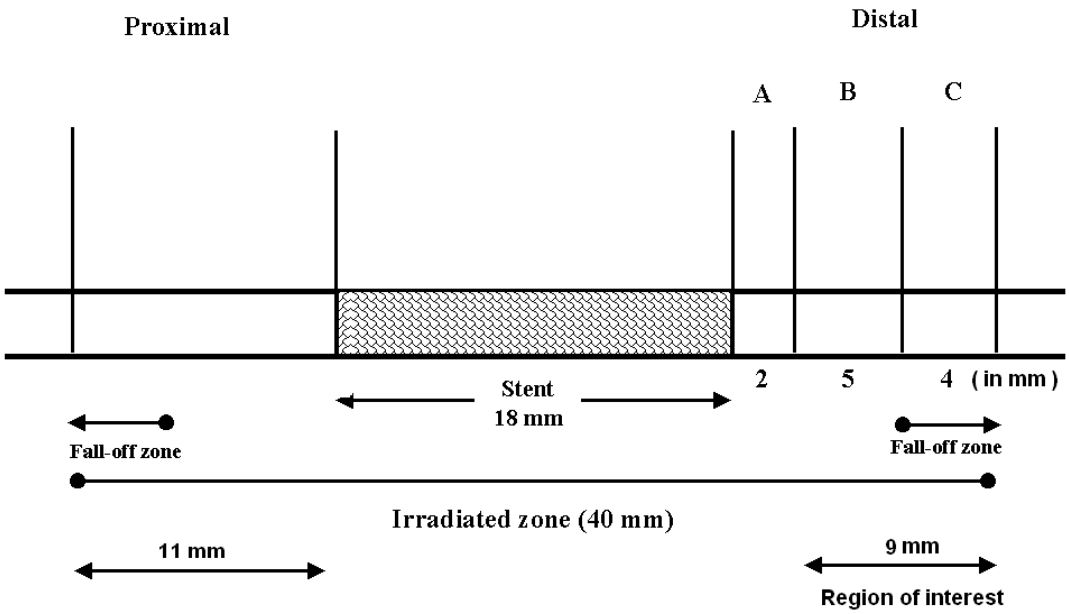
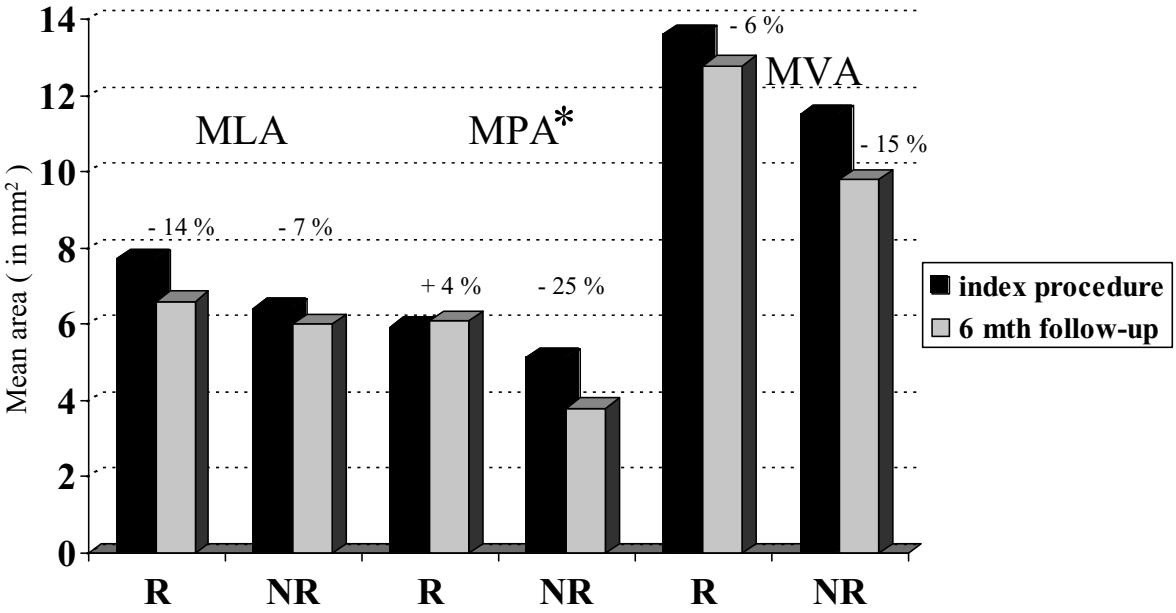


Figure 1.

A schematic diagram, depicting the stent and the different zones. **A: Transitional zone:** diameter change is believed to be (restrictively) influenced by stent margin. **B: Full dose zone:** In radiation group full dose irradiation is received, without mechanical balloon damage. **C: Fall-off dose zone:** In radiation group progressive fall-off of irradiation dose to distal, without mechanical balloon damage. The segment used in the current analysis is depicted as the 9 mm region of interest, distal to the stent.



Quantitative IVUS measurements in patients with additional radiation therapy(R) and without additional radiation(NR). Black bars represent data at index procedure, grey bars data from 6 months follow-up studies.

Numbers represent mean cross-sectional area of the investigated coronary segment in mm^2.

* p = 0.02 for difference in Mean Plaque Area change between groups.

MLA: mean lumen area; MPA: mean plaque area; MVA: mean vessel area.

Drug-eluting stents

Chapter 8

Impairment of Distal Endothelium-Dependent Coronary Vasomotion after Sirolimus-Eluting Stent Implantation

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Submitted

Impairment of Distal Endothelium-Dependent Coronary Vasomotion after Sirolimus-Eluting Stent Implantation

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Abstract:

Aim

Endothelial dysfunction has been related both to progression of atherosclerotic disease and to future cardiovascular events. No study has addressed the potential impact of drug-eluting-stent implantation on endothelial function. We assessed local epicardial endothelial function 6 months after sirolimus-eluting stent (SES) or bare metal stent (BS) implantation.

Methods and Results

In 12 patients (7 SES, 5 BS) endothelium-dependent vasomotion of a coronary segment 15 mm in length, starting 2 mm distal to the stent, was assessed, with quantitative coronary angiography immediately after the procedure and at 6 months follow-up, after intracoronary infusion of acetylcholine. Intravascular ultrasound was performed and coronary flow reserve (CFR) assessed in all patients. At follow-up significant vasoconstriction was seen in SES (32% diameter reduction from baseline) but not in BS (no change) patients after acetylcholine infusion (p=0.03 for SES vs. BS); endothelium-independent vasodilatation to nitrates did not differ significantly between groups (15% SES, 7% BS, p=0.14). IVUS revealed no late unhealed dissections and CFR was comparable between groups (SES 3.2 ± 0.6, BS 3.2 ± 0.5, n.s.).

Conclusion

SES implantation may have an adverse effect on local endothelium-dependent vasomotor responses compared to BS implantation at six months. Long-term clinical consequences of this observation are yet unknown.

Introduction

Sirolimus-eluting stent (SES) implantation significantly reduces restenosis compared to bare stents (BS) (1,2). Inhibition of restenosis by sirolimus is related to cell-cycle arrest in the late G1-phase. This salutary effect on restenosis may be accompanied by delayed healing of the traumatized vessel wall (3). Long-term endothelial dysfunction after bare stents has been reported in porcine and human coronary arteries (4,5). No data exists on endothelial function after implantation of SES. We assessed endothelial-dependent and -independent vasomotion after stenting and at 6 months after SES or BS implantation.

Methods

Patient selection

We prospectively studied 15 patients, 9 with a SES (Cypher, Cordis Co, Warren, NJ, USA) and 6 BS (DepoStent, Conor Medsystems, Inc., Menlo Park, CA, USA) patients.

Stents were implanted for single de novo lesions. In the Thoraxcenter, the SES was the default stent at the time of the study. Six patients receiving a DepoStent with comparable lesion characteristics and vessel size were included in this study. This stent was the only bare metal stent implanted at the Thoraxcenter during the study period. Except for a different stent design, both stents are made of 316L stainless steel and stent strut thickness is identical (140 µm) (6). Allocation of patients to either group was dependent on availability of stents and patient informed consent.

One of the 9 SES patients refused follow-up. One additional patient in each group was excluded because of in-stent restenosis. Thus 7 SES and 5 BS patients were analyzed. Mean duration until follow-up studies was very similar (188 ± 4 days for BS and 191 ± 8 days for SES group, n.s.). The Erasmus MC Ethics Committee approved the protocol and written informed consent was obtained from all patients.

Evaluation of endothelial function

Studies were performed directly after completion of the interventional procedure and at scheduled six-month angiography. Long-acting vasoactive drugs were stopped for at least 24 hours before angiography. Endothelium-dependent and independent coronary vasomotion was studied using standard protocols (7). The intracoronary stent scaffolds the arterial wall and virtually abolishes vasomotion. For this reason, we analyzed the 17 mm segment immediately distal to the stent, excluding the first 2 mm. A selective intracoronary

infusion catheter (Multi-functional Probing Catheter, Boston Scientific, Galway, Ireland) was advanced over the guide wire and positioned in the stent. To avoid wire-induced coronary spasm, the guide wire was subsequently withdrawn into the catheter. As baseline, an infusion of vehicle solution, normal (0.9%) saline, was performed for 2.5 minutes at an infusion rate of 2 ml/min, followed by baseline angiography. Thereafter, endothelium-dependent vasomotion was studied by infusion of incremental doses of acetylcholine (Ach), 10^{-8}, 10^{-7} and 10^{-6} Molar, for 2.5 minutes at each concentration. Subsequently, endothelium-independent vasomotion was assessed after an intracoronary bolus of nitrates (2-3 mg).

Quantitative Coronary Angiography

Off-line quantitative analysis of coronary angiography was performed with the CAAS II system (Pie Medical Imaging, Maastricht, NL), blinded to knowledge of stent type. Endothelial dysfunction was defined as abnormal vasoconstriction of ≥ 3% mean vessel diameter change from baseline (saline infusion), beyond the variability of the method of analysis, of the segment studied after the maximal dose of Ach (10^{-6} Molar).

Coronary Flow Reserve

CFR measurement was performed to exclude differences in microvascular coronary resistance between groups. This was performed after completion of endothelial function testing. A 0.014-inch Doppler tipped guidewire (FloWire, Volcano Therapeutics Inc., Rancho Cordova, CA, USA) was positioned in the coronary artery just distal to the implanted stent. After baseline flow velocity measurement, adenosine (140 mcg/kg/min for 2 minutes through the femoral vein) was administered for assessment of hyperemic flow velocity and CFR. Measurements were done in duplicate.

Intravascular Ultrasound

IVUS was performed in all patients at the end of the study protocol, as unhealed dissections may influence late recovery of endothelial function. IVUS was performed according to standard techniques, using a 30 MHz, 2.9 F mechanical ultrasound catheter (UltraCross, Boston Scientific Scimed Inc, Fremont, CA, USA).

Statistics

Data are presented as mean ± SD and as median and range as appropriate. For continuous variables of baseline characteristics Student-t test was used, for categorical data the Fisher's exact-test. Mann Whitney U test were performed to analyse diameter

changes between groups. A value of $P < 0.05$ (two-tailed) was considered to indicate statistical significance.

Results

SES patients were younger and more often female whereas hypercholesterolemia and a family history of coronary artery disease were more common in the BS group (Table 1).
Mean coronary diameters at follow-up were similar between SES and BS (2.0±0.2 mm vs. 2.2± 0.4 mm).
Table 2 shows results of maximal dose of Acetylcholine of 10^{-6} Molar at follow-up. In the SES group, a 32±23 % reduction in mean coronary diameter compared to baseline was observed, after infusion of Ach 10^{-6} Molar, whereas there was no significant change in the BS group (0±7%), (p=0.03 SES vs. BS, Figure 1; Table 2). Endothelium-independent dilatation to nitrates did not differ significantly between groups (Table 2).
Figure 2 highlights the vasomotion reaction of the coronary segment to incremental doses of acetylcholine and nitrates in both groups.
Endothelial function studies were also performed directly after implantation to exclude major differences between groups at baseline. Constrictive response was seen in both groups (-20 ± 14 % vs. -11 ± 10 % for SES and BS groups respectively; p=0.25). However, absolute values are not comparable between index procedure and follow-up, because all patients in both groups did receive intracoronary nitrates during the interventional procedure, i.e. before endothelial function study.
CFR at follow-up did not differ significantly between groups (SES 3.2 ± 0.6 vs. BS 3.2 ± 0.5, n.s.). Absolute flow increased from 16 ± 7 cm/sec for SES and 16 ± 4 cm/sec for BS to 52 ± 26 cm/sec and 51 ± 15 cm/sec for SES and BS respectively. CFR at baseline is not included in the data as CFR immediately after the intervention can greatly be influenced by the procedure itself, which was not the purpose of the measurements in this study.
On IVUS no stent-edge dissections were seen in either group.

Discussion

Drug eluting stents have shown a large reduction of restenosis compared to bare metal stents (1,2). However, their mechanism of action may have unwanted effects on vessel healing after stent implantation (3,8). The major finding of the present study was that SES implantation suggests an adverse effect on local endothelium-dependent vasomotor

responses six months after SES compared to BS implantation distal to the interventional segment.

Possible mechanisms for endothelial dysfunction after SES implantation

Direct drug effect?

Sirolimus (rapamycin) is a potent immunosuppressive agent, which has anti-proliferative capacity through cell-cycle arrest in the late G1-phase. It has been shown to be effective in inhibiting in-stent neointimal hyperplasia. The amount of drug on the stent is extremely small (< 3%) compared to the doses employed systemically in kidney transplant patients to treat rejection. Around 80% of the drug is released within 30 days of implantation. It is, therefore, unlikely that the drug can affect vasomotion in the distal segment by diffusion from the blood stream late after implantation. However, it cannot be excluded that the drug can reach the vessel wall directly distal to a drug-eluting stent, for example by diffusion through the tissue and through the vasa vasorum. As recently shown, the vasa vasorum interna in porcine coronary arteries originating directly from the lumen of the artery can extend over several centimeters along the coronary artery wall (9). Clinical data support the hypothesis of drug elution distal to the stent. In recent trials SES stent implantation resulted in higher restenosis rates at the proximal edge of the stent compared to the distal edge (2).

Data on the effects of sirolimus on vasomotion is limited and not conclusive. Early reports showed vasodilatory effects of sirolimus after acute exposure to high doses in isolated rat aortic rings (10). More recent data showed severe impairment of endothelial function in the coronary segments of swine in vitro, after incubation for 48 hours with sirolimus (11). Guba et al. reported anti-angiogenic activities of sirolimus, linked to a decrease in production of vascular endothelial growth factor (VEGF) and to a marked inhibition of the response of vascular endothelial cells to stimulation by VEGF (12). The authors suggested that this might reduce the chance of recurrent or de novo cancer in organ transplant patients. However, this might also delay endothelial recovery after vascular injury.

The effects of sirolimus on vasomotion can hypothetically be caused by a direct effect on the endothelium, on the signaling pathway of the endothelium to the medial smooth muscle cells or a direct effect on the media. The data of Guba et al. suggest a direct effect on the endothelium and/or the signaling pathway. A preserved medial vasomotion is also suggested by the preserved vasodilatation after intracoronary nitrates in the current study.

General effect caused by delayed healing response?

A delayed healing response associated with prolonged endothelial dysfunction is shown after stenting versus balloon angioplasty in porcine coronary arteries (4). Caramori et al. demonstrated persistent vasomotor dysfunction distal to coronary stents impanted 6 months earlier (5).

The anti-proliferative activity of sirolimus may merely cause a prolonged healing response with concomitant delayed recovery of endothelial function. In this respect it would be very interesting to repeat the vasomotion studies at later timepoints to evaluate the timeframe of endothelial functional recovery, if present. In future studies this should be incorporated in the protocol as this may have implications for the duration of anti-platelet regime and other possible interventions to improve endothelial function such as ACE-inhibitors and high dose cholesterol lowering medication.

Procedural differences between groups?

In both groups direct stenting was performed in all but one patient. Predilatation was done with a balloon 1 mm smaller in diameter than the subsequently implanted stent and balloons were shorter than the stent. No bare stents were postdilated, while 3 out of 7 SES were postdilated. As clearly visible in figure 1, in all but one SES patient, vasoconstrictive response to Ach was seen. The one patient not showing significant constriction was treated with direct stenting without postdilatation. Postdilatations were within the stent at all times. These implantation characteristics together with the fact that measurements started 2 mm distal to the stent make significant differences in the vessel wall trauma distal to the stent very unlikely.

The coronary segments distal to the stent had a normal angiographic appearance in both groups. IVUS study revealed no unhealed distal stent edge dissections, which could have influenced endothelial recovery.

Though our main interest was the vasomotory response at 6 months follow-up, studies were also performed immediately after the intervention itself to exclude major difference in baseline vasomotion between groups. However, in our center interventions are performed with liberal use of intracoronary nitrates to appreciate vessel diameter and reduce spasm. This means that acetylcholine studies were performed after the use of nitrates and results between baseline and follow-up are not comparable. Despite this, significant vasoconstrictive response to acetylcholine could be seen in both groups. Though a trend to more vasoconstrictive response was seen in the SES group at baseline, response had a tendency to improve in the BS group at follow-up (-11 ± 10 % to 0 ± 7 %, p= 0.07) while a trend towards worsening was seen in the SES group (-20 ± 14 % to -32 ± 23 %, p = 0.25). This means that the large difference in vasomotion response to acetylcholine seen

at follow-up between bare stent and sirolimus-eluting stent patient groups could not be explained by a difference in vasomotion at baseline.
During the intervention, the interventional guide wire is distal to the treated segment. Endothelial damage or even local endothelial denudation distal to the implanted stent due to wire manipulation cannot be excluded. However, it is very unlikely that six months after the intervention, the vasoconstrictive response to Ach, as seen in this study, would be the result of a persistent denudated coronary artery, instead of a regenerated but still dysfunctional endothelial layer.

Studies were performed 6 months after SES implantation. Persistent drug elution from the stent is unlikely. If present, the drug concentration would be extremely low. If the alteration in endothelial response we observed is a consequence of SES implantation, it likely represents the late sequelae of earlier high dose exposure. The clinical relevance of this is unclear as even 3 year clinical follow-up in the first SES treated patients does not show delayed restenosis or vessel closure, though some late catch-up effect was recently reported (13). Moreover, we report a localized endothelial dysfunction, while generalized coronary endothelial vasodilator dysfunction has been linked to long-term atherosclerotic disease progression and cardiovascular event rates (14). However, our results do suggest that prolonged follow-up to exclude late adverse consequences is warranted in patients who receive a drug-eluting stent.

Study limitations

The baseline characteristics of both groups were not completely balanced. However, in the BS group, patients were older, more often men and more often suffered from hypercholesterolemia, all of which are known to predispose to endothelial dysfunction. Despite this, endothelial dysfunction was significantly more marked in the SES patient group.

Our patient groups were small, due to the very limited number of patients receiving a bare stent in our center at the time of the study on one hand, while on the other hand our center changed from SES to a paclitaxel-eluting stent as the new default stent during recruitment of our study patients, preventing us from inclusion of more SES patients. Despite the finding of a statistically significant difference in vasomotory response to Ach, these results should be confirmed in larger studies to overcome the limitations of statistics on small sample sizes.

The stent design was not the same in both groups. Ideally, the BS and SES should have had the same design. However, both stents were made from 316L stainless steel and strut thickness was identical. Moreover, the segment studied extended 17 mm distal to the stent, making an effect of stent design 6 months after implantation and without restenosis unlikely.

No vasomotion study was performed in a control vessel of the SES patients to rule out a stronger vasoconstrictory response to Ach in this patient group, not related to sirolimus. However, as the trend to vasoconstriction in the SES patients was very uniform compared to the reaction in the bare stent patients (figure 1), an intrinsic stronger response of the vessel wall to Ach is not very likely.

Conclusions

SES implantation may have an adverse effect on local endothelium-dependent vasomotor responses six months after SES compared to BS implantation. The long-term clinical consequences of this observation are yet unknown.

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Table 1. Patient characteristics

	Bare stent (N=5)	SES (N=7)	p-value
Age (SD)	65 (9)	52 (5)	0.01
Male Sex	80%	43%	0.20
Current smoking	40%	43%	0.91
Diabetes			
Type 1	0%	0%	1.00
Type 2	0%	14%	0.38
Hypertension	40%	43%	0.92
Hypercholesterolemia	80%	43%	0.41
Prior myocardial infarction	20%	29%	0.74
Positive family history	80%	57%	0.51
Statin therapy	100%	100%	1.00
ACE-inhibitor	20%	14%	0.80
Body surface area (m^2)	1.8 ± 0.1	1.8 ± 0.1	0.91

Table 2.

Mean coronary segment diameter change from baseline after maximal endothelium-dependent vasomotion (10^{-6} molar acetylcholine) and endothelium-independent vasodilation (2-3 mg i.c. nitrates).

		SES N=7	Bare stent N=5	p- value
Ach	Mean ± SD	- 32 ± 23 %	0 ± 7 %	0.03
	Median (25 %; 75 %)	- 32 (-50; -9)	-2 (-5; 6)	
Nitrates	Mean ± SD	15 ± 11 %	7 ± 7 %	0.14
	Median (25 %; 75 %)	20 (8; 23)	5 (0;15)	

Ach: Acetylcholine; SES: sirolimus-eluting stent

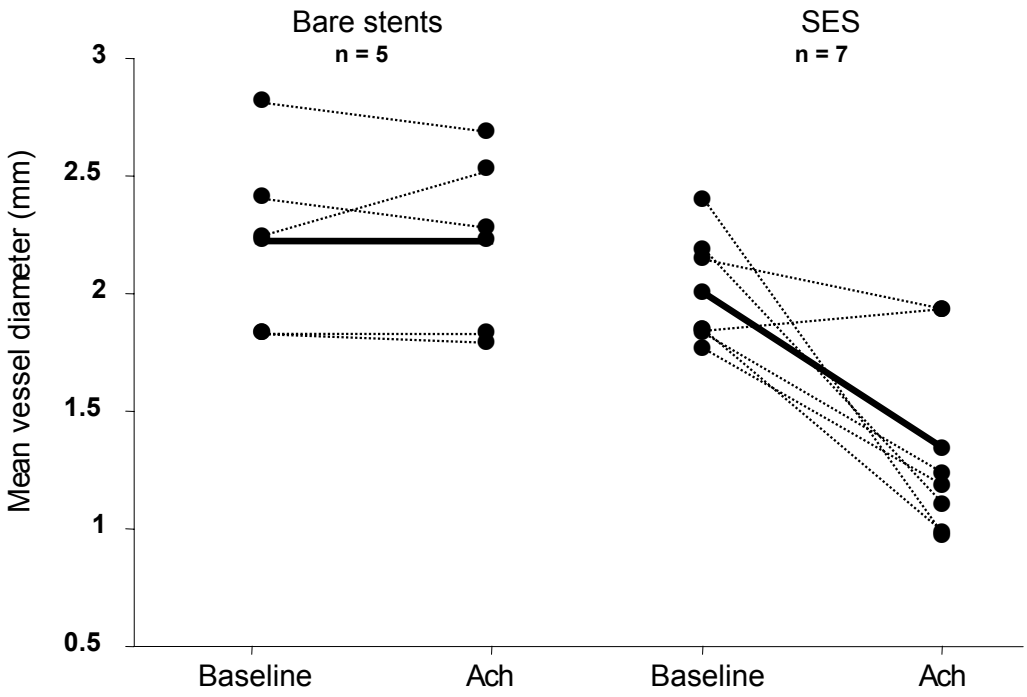

Figure 1.

Mean coronary artery segment diameter at 6 months FU at baseline (saline infusion) and after maximal acetylcholine infusion (10^{-6} Molar) for individual patients. Thick line is mean diameter per group. SES: Sirolimus-eluting stent.

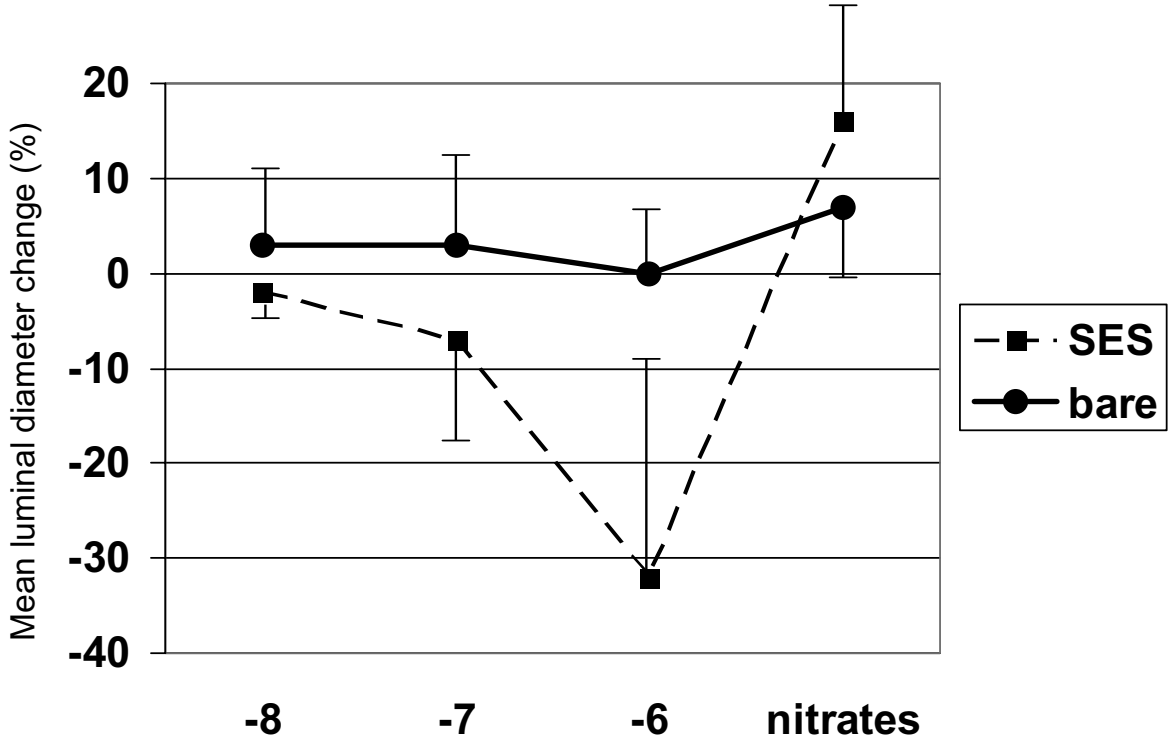

Figure 2.

Mean coronary artery segment luminal diameter change at 6 months follow-up after intra-coronary infusion of acetylcholine with incremental concentrations of 10^{-8}, 10^{-7}, and 10^{-6} Molar. Comparison of patients with sirolimus-eluting stent(SES) and patients with bare stent implantation.

Chapter 9

Endothelial dysfunction is located at low shear stress areas in human coronary arteries in vivo

J.J. Wentzel, S.H. Hofma, J.C.H. Schuurbiers, F.J.H. Gijsen, A. Thury, W.J. van der Giessen, P.W. Serruys, C. J. Slager

Submitted

Endothelial dysfunction is located at low shear stress areas in human coronary arteries in vivo.

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Abstract

Atherosclerotic plaques occur at low shear stress areas in the arterial tree. Endothelial dysfunction is presumed to be a precursor of atherosclerotic plaque formation. The aim of our study was to investigate in patients whether local endothelial dysfunction is related to low shear stress (SS).

Materials

In 7 patients treated 6 months earlier for coronary artery stenosis using sirolimus eluting stent implantation, endothelium dependent vasomotion was assessed by acetylcholine (ACh) provocation using a selective intracoronary infusion of 10^{-8} and 10^{-7} M. The arterial response distal from the stent was measured by biplane contrast angiography. The lumen was 3D reconstructed using the lumen contours in the angiograms of the arteries distal from the stent. Computational fluid dynamics in these 3D reconstructions applying patient specific flow and viscosity delivered local SS before and after ACh provocation. Relative changes in local SS were used to determine the local response of the artery to ACh.

Results

Average SS was 1.4±0.6 Pa. Administration of ACh led to an increase in SS by 32% and 50% for 10^{-8} and 10^{-7} M ACh respectively. Administration of ACh to local arterial sites exposed to low SS (<1.3 Pa, being the median of all SS values) showed an increase in shear stress of 52% and 73% for 10^{-8} 10^{-7}M ACh respectively, while local arterial sites exposed to normal to high SS this SS increase was much lower 11% and 26% for 10^{-8} 10^{-7} M respectively. These data imply higher degree of endothelial dysfunction at arterial sites exposed to low SS.

Conclusions

3D reconstruction of human coronary arteries from biplane contrast angiograms allows 3D study of local endothelial function. For the first time, we showed in human coronary arteries in vivo that endothelial dysfunction is most severe at low SS areas.

Introduction

Atherosclerotic plaques occur at specific locations in the arterial tree. For instance close to side branches (1) and at inner curves of coronary arteries (2,3). As these locations coincide with zones of low shear stress and flow separation, low and oscillating shear stress is thought to play a key role in the atherosclerotic plaque formation (2,3). The earliest appearance of atherosclerotic disease is local endothelial dysfunction(4) and has been shown to reflect the propensity to further develop atherosclerotic plaques and cardiovascular events (5,6). Indeed, endothelial dysfunction is initially mainly observed at coronary branch points (7).

Several in vitro studies showed a close correlation between the functionality of the endothelium and the local shear stress conditions (8,9). However, no in vivo data are available confirming a direct relationship between endothelial function and shear stress in human coronary arteries.

A common method to determine local endothelial function is the acetylcholine provocation test(10). In this test the vessel response to increasing concentrations of acetylcholine is measured by angiography. Functional endothelium will respond to incremental concentrations of acetylcholine by inducing increased vasodilation, while dysfunctional endothelium is associated with vasoconstriction(11). Traditionally, the vessel response is expressed as average diameter change of a certain segment. As diameter measurements do not allow discriminating the local response over the circumference of the artery(Figure 1), this measure is not suitable for comparison with local shear stress. The latter has shown to be heterogeneous over the circumference of a curved coronary artery.

To enable the study of shear stress related endothelial function in human coronary arteries in vivo, in which the circumferential heterogeneity is considered, we developed a 3D reconstruction technique based on biplane angiography and combined that with computational fluid dynamics to obtain the local shear stress. Using this method we tested for the first time in human coronary arteries the hypothesis that local endothelial dysfunction is located at low shear stress areas.

Methods

Patient selection

9 patients being treated for single vessel disease using a sirolimus eluting stent (Cypher, Cordis) 6 months before, were eligible for this study. Of the 9 patients, 1 patient refused

follow-up and 1 patient was excluded from follow-up studies because of asymptomatic in-stent restenosis. In total in 7 patients vasomotion studies could be performed in the arterial segment distal from the stent. This study was approved by the Medical Ethics Committee of our institution and written informed consent was obtained from all patients in accordance with the guidelines established by the Committee for the Protection of Human Subjects.

Evaluation of endothelial function

Before vasomotion studies were performed, as described in detail previously(12), long-acting vasoactive drugs had been stopped for at least 24 hours. Because of severely restricted vasomotion at the stented coronary segment, the segment distal to the stent was studied, excluding the first proximal 2 mm. Percutaneous femoral access and a 7 French guiding catheter was used in all patients. A multi-functional probing catheter (Boston Scientific) was advanced over the guidewire and positioned in the stent. To avoid wire-induced coronary spasm, the wire was removed. At the start of the vasomotion study, being 6 months after stent implantation, baseline angiography was performed after an initial infusion of saline solution through the intracoronary multi-functional probing for 2.5 minutes. Then endothelium-dependent vasomotion was studied by infusion of incremental concentrations of acetylcholine (ACh) for 2.5 minutes at concentrations to result in final intracoronary concentrations of 10^{-8} M, 10^{-7}M and 10^{-6}M, assuming a mean coronary blood flow of 80 ml/min as described in detail before (10,12,13). Throughout each infusion heart rate, systemic arterial blood pressure and electrocardiogram were monitored continuously. Because of a significant generalized vasoconstrictive response at the highest concentration of 10^{-6}M ACh, only the lower concentrations of 10^{-8} M and10^{-7}M Ach were used to investigate the relationship between local vasomotion response and shear stress.

3-D lumen reconstruction derived from angiography

Care was taken to visualize the segment distal to the stent in 2 views, with projections differing by more than 60 degrees and ensuring minimal fore-shortening. Angiography was performed in biplane and settings were not changed throughout the study. ECG gated biplane angiograms were selected for further analysis. 3D-lumen reconstruction was performed after tracing the contours of the coronary arteries distal to the stent in biplane contrast angiograms for baseline and the 2 acetylcholine provocation geometries (Figure 2). After 3D reconstruction of the centerline of the contours (Figure 3A), the local cross sectional lumen dimensions were obtained from the perpendicular distance between centerline and contour at both angiograms (Figure 3B). Application of a calibration cube ensured the correct size, spatial location and direction of the vectors determining the local

vessel diameter in 2 directions. Finally, ellipses where fitted encompassing the 2 vectors per cross section (Figure 3B). Combining the local ellipses resulted in a 3D lumen reconstruction of the geometry of the coronary segment (Figure 3C).

Doppler flow studies

Coronary Doppler flow was measured just proximal to the region of interest using a 0.014-inch Doppler tipped guidewire (FloWire, Endosonics, Rancho Cordova, CA, USA). Doppler recordings were taken 1 and 2.5 min after the start of each infusion. A blood sample of 5 cc was drawn for viscosity measurements using a capillary viscometer.

Computational fluid dynamics

Shear stress was determined applying a well-validated finite element software package (Sepran, Sepra, Leiden, the Netherlands). This software solved the non-linear incompressible fluid flow Navier-Stokes equations in a multitude of nodes contained in brick-like elements (axial resolution 0.5 mm, circumferential resolution approximately 0.5 mm, being 1/16 of the length of the circumference), which filled the luminal space of the 3D reconstruction. Blood was modeled as a Newtonian fluid using patient specific viscosity and a density of 1050 kg/m^3. Entrance flow was determined from the Doppler measurements taken at 2.5 min after ACh infusion, using a parabolic inflow profile. At the wall no slip was assumed and at the outflow zero stress conditions were applied. Convergence was reached when differences in velocity between iterations fell below 0.1 mm/s(3). In order to study only how changes in geometry relate to shear stress distribution, the shear stress after provocation was computed applying the same baseline flow for all the 3 geometries, i.e. baseline and 10^{-8} M and 10^{-7}M ACh geometries.

Analysis and statistics

The studied segment length per patient was dependent on the quality of the angiograms at baseline and after acetylcholine provocation. Per patient, the final studied segment length was determined by the segment length being appropriately visualized in all the three angiograms (baseline, 10^{-8}M 10^{-7}M ACh). Anatomical landmarks and the location of the stent were used to match the baseline geometry and the geometries observed after acetylcholine provocation in both axial and circumferential direction, to allow local comparison between the 3 geometries. The local spatial discrimination is determined by the local resolution of the mesh, being approximately 0.5 x 0.5 mm.

The baseline shear stress distribution observed in all the patients was used to subdivide the data points into 10 categories being each 10^{th} percentile of that distribution. For each

patient, in each category, the average of the baseline shear stress (SSbas), shear stress after administration of acetylcholine 10^{-8} M (SS8) and 10^{-7}M (SS7) and relative change was determined: RSS8 = (SS8-SSbas)/SSbas*100% and RSS7 = (SS7-SSbas)/SSbas*100%. The relative change in shear stress (RSS8,RSS7) was used to derive changes in geometry. RSS7(8)>0% means relative increase in shear stress attributed to acetylcholine provocation, which implies local vasoconstriction representing endothelial dysfunction. Likewise, RSS7(8)<0% implies vasodilation and thus healthy endothelium.

The data from local arterial sites exposed to low shear stress at baseline, being the average of the data points from category I-V (Group A) were compared to local arterial sites exposed to high shear stress, being the average of data points from category VI-X (Group B) applying paired t-test (N=7). Per group one sample t-test was applied to evaluate differences from baseline (N=7).

To evaluate whether the response of the endothelium was dependent on the distance from the stent, we investigated whether the axial local distance contributed significantly to the observations. Therefore, the cross sections were subdivided into 3 groups based on the axial distance to the stent (I:2-7 mm, II: 7-12 mm and III: 12-17mm). Subsequently, a 2-way ANOVA was performed in which the shear stress group (A or B) and location (I,II or III) were included in the model. A p-value of <0.05 was considered significant. All values were expressed as mean ± SD. The software package SPSS 11.0.1 (SPSS Inc. Chicago, Illinois, USA) was used for all statistical calculations.

Results

Patient population and hemodynamics

Patients were 52 ±5 years old. Risk factors for atherosclerosis were distributed over the studied patient group as follows: 57% of the patients had a positive family history, 43% hypertension, 43% hypercholesterolemia, 14% Diabetes. The flow in the studied coronary arteries changed from 30 ml/min to 45 ml/min and 60 ml/min respectively, after administration of 10^{-8}M and 10^{-7}M acetylcholine.

Geometry

The length of the studied segments ranged from 7.5 mm to 31.5 mm (17±9 mm). The baseline vessel diameter was 2.4±1.3 mm and showed a trend towards vasoconstriction with increasing acetylcholine concentration: 2.3±1.3 mm 2.2±1.3 mm for 10^{-8}M and 10^{-7} M acetylcholine concentration respectively. Because Ach 10^{-6}M induced a significant

generalized vasoconstrictive response, only the lower concentrations of 10^{-8} M and10^{-7}M ACh were used to investigate the relationship between local vasomotion response and shear stress.

Shear stress

Average shear stress in the coronary arteries at baseline was 1.4±0.6 Pa. In general, acetylcholine provocation induced an increase in shear stress by 32% and 50% for 10^{-8}M and 10^{-7}M ACh respectively ($p<0.05$).

The borders of the 10 categories based on the 10^{th} percentile of all baseline shear stress values range from 0.16-8.49 N/m^2 (figure 4). In Figure 4A, B the relative shear stress increase attributed to 10^{-8} and 10^{-7}M ACh is depicted dependent on the 10 SSbas categories for each patient individually. In general, the arterial sites exposed to the lowest baseline shear stress showed the highest RSS8 or RSS7 (maximal 300%), which gradually decreased with rising SSbas.

Figure 5 shows the significant differences in relative shear stress for local arterial sites experiencing low (Group A being 0.16-1.3 Pa) versus normal to high SSbas (Group B being 1.3 –8.5 Pa). RSS8 was 52% ($p<0.05$) for group A, while RSS8 was not different from 0% (11%±3%, N=7, p=NS) for group B. Administration of 10^{-7}M ACh resulted in an increase in shear stress for both groups (A:RSS7=73±64% B:26±23%), with a similar trend for group A versus group B (Figure 5). This data imply that sites exposed to low shear stress constrict more after administration of ACh than high shear stress areas suggesting variations in local endothelial dysfunction related to shear stress. We found no differences in shear stress related endothelial dysfunction between the 3 different axial locations. (N=30, 2-way ANOVA RSS7: p=0.004; RSS8: p=0.034).

Discussion

The main finding of this study is an enhanced endothelially mediated vasoconstrictive reaction to acetylcholine at local arterial sites exposed to low shear stress (below 1.3 N/m^2). This is the first time an in vivo correlation between low shear stress and endothelial dysfunction could be demonstrated in human coronary arteries.

Determination of local endothelial function versus shear stress

Because of the localized appearance of atherosclerotic plaques known to be related to low shear stress areas(2) and because endothelial dysfunction precedes plaque formation(4), we aimed to investigate in human coronary arteries the relationship between *local* shear

stress and *local* endothelial function such that the circumferential heterogeneity is considered. Acetylcholine is a vasoactive agent commonly used to assess the endothelial function in human arteries in vivo(10). Administration of a vasoactive agent to the circulation shifts the existing balance between vasodilator and vasoconstrictor agents produced by the endothelium and results in an arterial diameter change. If the endothelium is intact ACh works at endothelial muscarinic receptors, evoking additonal production of NO, which causes smooth muscle cell relaxation. However if the endothelium looses its integrity, ACh can work directly, with declining interference of the endothelial cells, at the smooth muscle cells causing vasoconstriction(11). For this in vivo testing incremental concentrations of ACh are used that increasingly shift the balance between vasodilation and vasoconstriction.

Although biplane angiography permits optimal assessment of the full 3D response of the coronary artery to ACh, current analysis methods to assess endothelial function are based on measuring changes in local diameter(12). Diameter measurements do not include any information on the spatial 3D orientation. Moreover, it excludes the measurements of local arterial response at 1 side of the artery (Figure 1). For instance, a vasoconstriction exclusively in the inner curve of a coronary artery (Figure 1C) would result in the same change in diameter as a local vasoconstriction in the outer curve (Figure 1D). Therefore, this approach does not allow determination of endothelial function considering circumferential heterogeneity. Furthermore, this 2D approach hinders the computation of local shear stress requiring a 3D geometry. Hence, we developed a method based on biplane angiography to 3D reconstruct the coronary arteries. Using the 3D reconstruction, local shear stress could be determined applying computational fluid dynamics at baseline and after administration of ACh. In order to assess the local response of the artery to acetylcholine administration, we opted for studying relative changes in shear stress rather than local changes in geometry. Increase in local shear stress by ACh administration was associated with vasoconstriction and thus endothelial dysfunction.

Endothelial function versus shear stress

In the present study we showed a close relationship between enhanced endothelial dysfunction and low shear stress <1.3 N/m^2 distally from a 6 months prior implanted sirolimus eluting stent (Figure 5). This observation is very much in line with the known association between low shear stress and parameters of endothelial dysfunction observed in in vitro and animal studies, including the diminished eNOS expression(14) and NO production, increased endothelin synthese expression (14), increased expression of adhesion molecules (15) and decreased production of antioxidant enzymes (16).

In another study Hofma et al. (17) observed that the arterial segments distally from a sirolimus eluting stent 6 months after implantation responded to 10^{-6} M ACh with significant constriction compared to bare metal stents. In the present study we analyzed the arterial response to 10^{-7} and 10^{-8} M ACh, which were not different from the response to bare metal stents using the angiographic method. However, since shear stress changes are very sensitive to small and local changes in diameter, we were able to identify local regions with endothelial dysfunction. If the sirolimus effect also manifests itself at low doses ACh, we suppose a diminished effect going from proximal to distal. Therefore, we investigated whether the observed relationship between endothelial function and shear stress was still present after accounting for the axial location. Since we did not find differences in the observed shear stress related response between proximal and distal arterial segments, it is most likely that the shear stress was responsible for the circumferential heterogeneity.

Conclusion

This study demonstrates the 3D local assessment of endothelial function in human coronary arteries based on biplane contrast angiograms. Moreover, for the first time, we showed in human coronary arteries in vivo that local endothelial dysfunction is enhanced at low SS areas.

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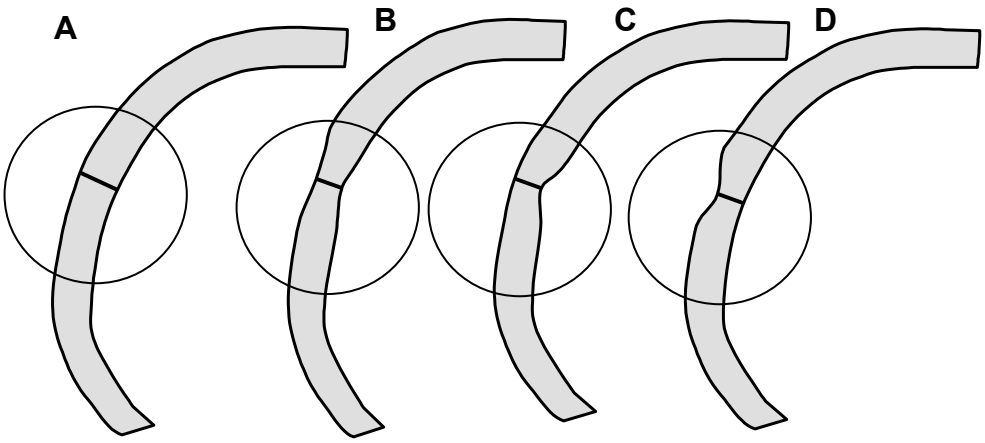

Figure 1: Cartoon of an angiogram of a coronary artery A) before administration of acetylcholine and after administration of acetylcholine showing 3 different possible arterial responses of endothelial dysfunction resulting in an equal diameter decrease, in which B) shows a combined arterial constrictive response at inner and outer curve, while C and D exclusively show a constrictive response at the inner or outer curve respectively.

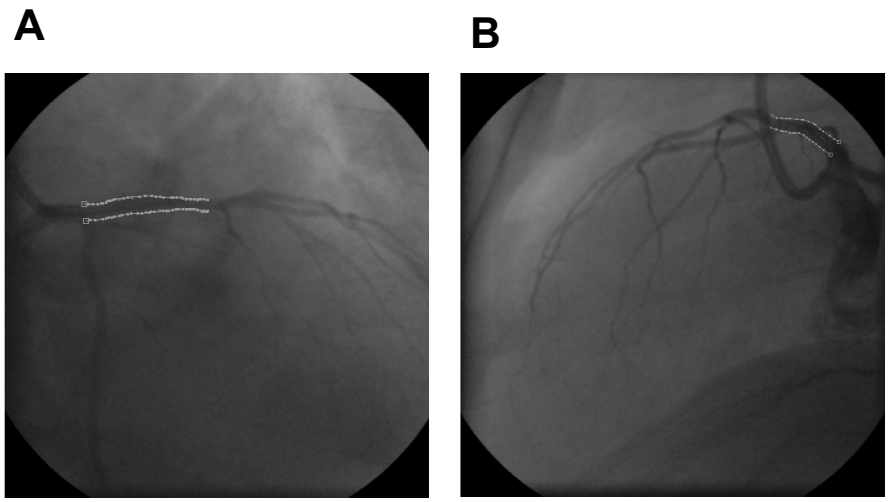

Figure 2: Biplane angiogram of coronary artery with contours delineated

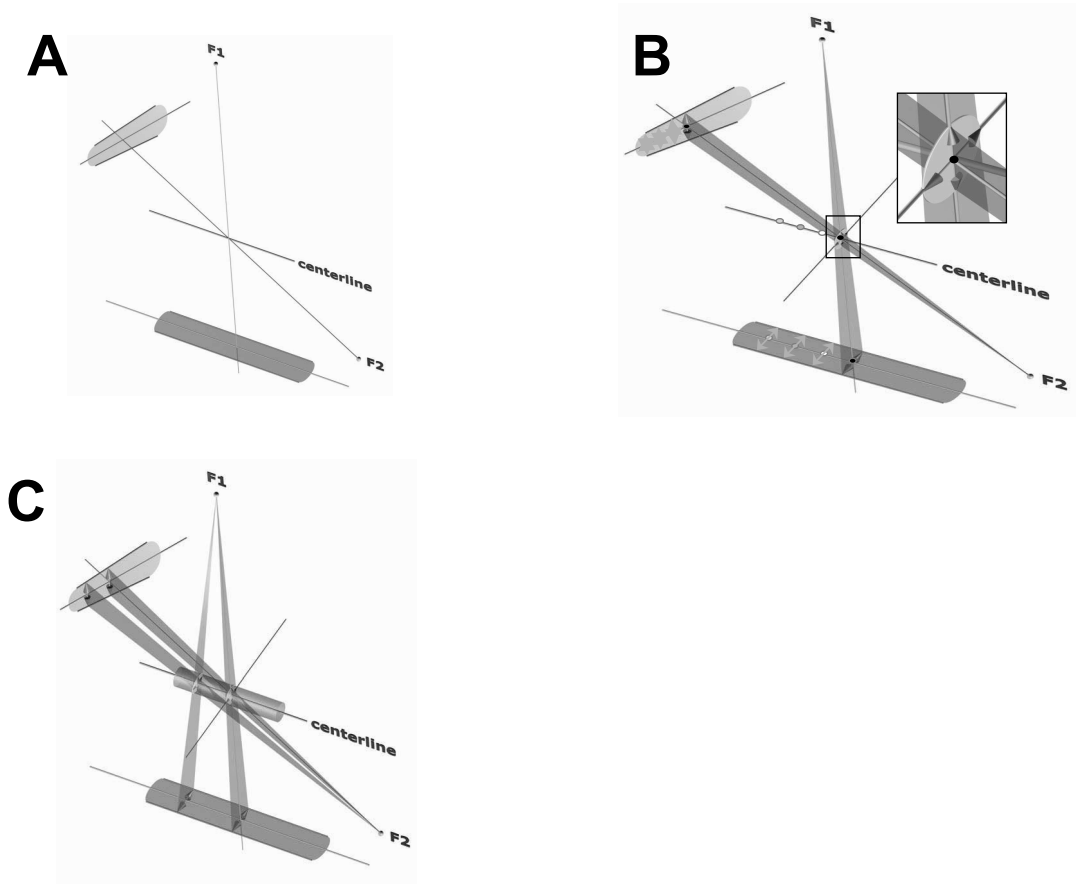

Figure 3: Principle of 3D reconstruction from biplane angiography. A) the centerline is 3D reconstructed from the center of the two angiograms B) at each location of the centerline, 2 vectors are spanned such that the projection of them on the respective two X-ray intensifiers, is perpendicular to the centerline and correct in size. The 2 vectors form the basis for an ellipse C) to obtain the ellipses at each location along the centerline, which delivers the 3D object.

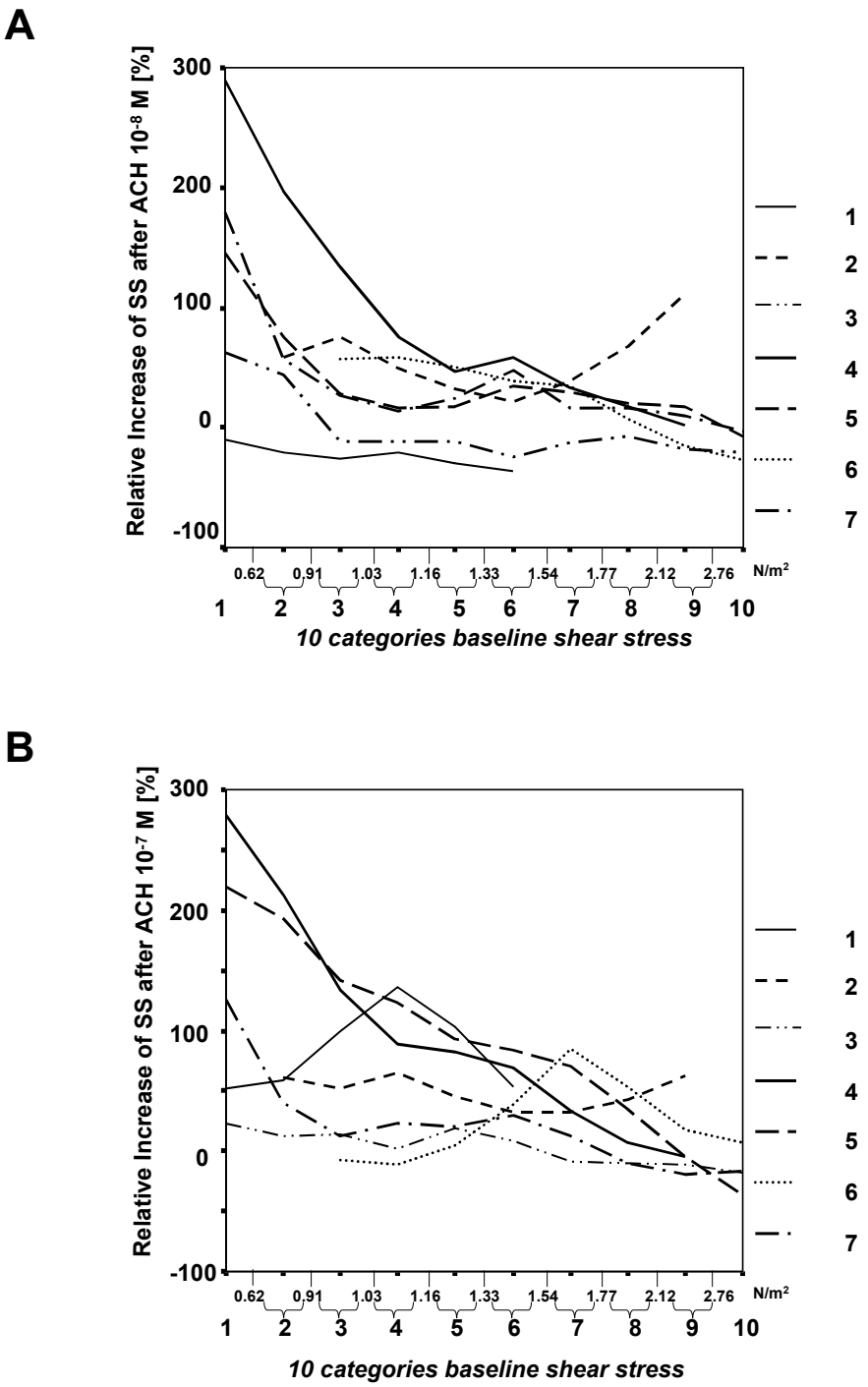

Figure 4:

Relative increase in shear stress attributed to acetylcholine provocation (A: 10^{-8}M; B: 10^{-7}M) for each patient individually related to the 10 baseline shear stress categories based on the 10^{th} percentile of the shear stress distribution.

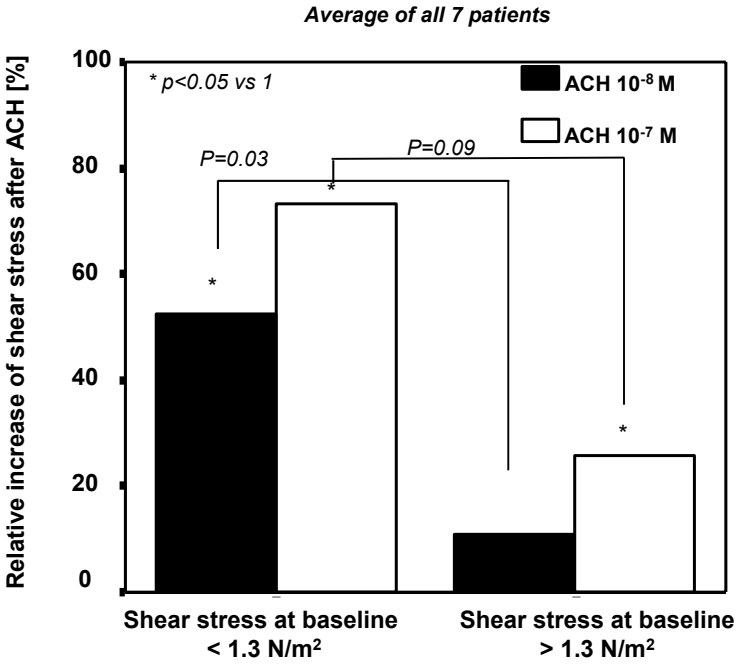

Figure 5: Relative increase in shear stress attributed to acetylcholine provocation for group A (<1.3 Pa baseline shear stress) and group B (>1.3 Pa baseline shear stress)

Part 3:

Coated stents and drug-eluting stents

Chapter 10

Reduction in thrombotic events with heparin-coated Palmaz-Schatz stents in normal porcine coronary arteries

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Circulation 1996;93:423-430

Reduction in Thrombotic Events With Heparin-Coated Palmaz-Schatz Stents in Normal Porcine Coronary Arteries*

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Background The use of stents improves the result after balloon coronary angioplasty. Thrombogenicity of stents is, however, a concern. In the present study, we compared stents with an antithrombotic coating with regular stents.

Methods and Results Regular stents were placed in coronary arteries of pigs receiving no aspirin (group 1; n=8) or aspirin over 4 weeks (group 2, n=10) or 12 weeks (group 3, n=9). Stents coated with heparin (antithrombin III uptake, 5 pmol/stent) were placed in 7 pigs that did not receive aspirin (group 4). The other animals received aspirin and coated stents with a heparin activity of 12 pmol antithrombin III/stent (group 5, n=10) or 20 pmol/stent (group 6, n=10; group 7, n=10). Quantitative arteriography was performed at implantation and after 4 (groups 1, 2, and 4 through 6) or 12 weeks (groups 3 and 7). In an additional 5 animals, five regular and five coated stents (20 pmol/stent) were placed and explanted after 5 days for examination of the early responses to the implants. Thrombotic occlusion of the regular stent occurred in 9 of 27 in groups 1 through 3. However, in 0 of 30 of the animals receiving high-activity heparin-coated stents (groups 5 through 7), thrombotic stent occlusion was observed ($P<.001$). Histological analysis at 4 weeks showed that the neointima in group 6 was thicker compared with its control group 2 (259±104 and 117±36 μm, $P<.01$), but at 12 weeks the thickness was similar (152±61 and 198±49 μm, respectively). Comparison at 5 days suggested delayed endothelialization of the coating.

Conclusions High-activity heparin coating of stents eliminates subacute thrombosis in porcine coronary arteries. **(*Circulation*. 1996;93:423-430.)**

Key Words • stents • thrombosis • heparin

Over the past 15 years, the operator experience and equipment involved in PTCA have improved. Nevertheless, acute or subacute occlusion of the dilated artery occurs in 3% to 8% of cases within hours to days, requiring an immediate repeat procedure or emergency coronary bypass graft surgery.[1] Another unresolved issue of PTCA is late restenosis, which occurs in 30% to 50% of cases, predominantly after 3 to 6 months.[2,3] There is no effective pharmacological prevention of the restenosis process.[4-6]

Aspirin can reduce the incidence of acute occlusion to a limited extent.[7] High-dose systemic antiplatelet drug therapy may be more effective as it reduces early complications after PTCA by approximately 35%—at the expense, however, of more bleeding complications.[8] This beneficial effect appears to be sustained, as a reduction in the need for later revascularization has also been observed.[9]

The only proven approach to reduction of the incidence of late restenosis (by 25% to 31%) is the use of coronary stents.[10,11] The use of stents, however, is not free from complications because of the risk of stent thrombosis and bleeding or vascular complications, requiring both costly monitoring and a prolonged hospital stay.[10-19] Therefore, a combination of drugs and stents has been proposed to overcome both early and late complications of PTCA.[16-19]

Several approaches have been introduced to improve the surface properties of vascular prostheses.[20] Heparin coating of stents is an attractive method because the anticoagulant properties of heparin,[21] its inhibitory effect on mesenchymal cell growth and differentiation,[22-24] and extracellular matrix formation[25] are well established. The aim of the present study was to compare the thrombogenicity and histological features of stents with and without heparin coating after implantation in the coronary circulation in pigs.

Methods

Balloon-Expandable Intracoronary Stent

The stent used in the present study (Palmaz-Schatz coronary stent, Johnson & Johnson Interventional Systems Co) is composed of two segments (7 mm each) of slotted tubes connected by a short (1-mm) coupler, and it is mounted coaxially over the balloon of an angioplasty catheter.[26] When expanded, the metallic contact surface area is 10% to 15%.

Heparin Coating of Palmaz-Schatz Stent

The coating applied to the stents consists of heparin molecules that have been end point covalently coupled to an underlying polymer matrix (a modification of the CBAS[27] [Carmeda AB]). The efficacy of this coating is based on the continuous and repeated interaction between the active site of the immobilized heparin and circulating antithrombin III. The

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Selected Abbreviations and Acronyms

CBAS = Carmeda Bioactive Surface
LAD = left anterior descending coronary artery
PTCA = percutaneous transluminal coronary angioplasty

coating consists, in principle, of four layers. A first layer on top of the steel is a polyamine layer; a dextran sulfate layer is applied on top of that. The third base layer is polyamine. Finally, these functional amino groups are covalently coupled to the aldehyde groups of partially degraded heparin molecules (Fig 1). The heparin activity of the coated stent is measured according to its ability to bind antithrombin III with high affinity and expressed in picomoles (of antithrombin III) per stent.

Approximately 15% of the end point–attached heparin molecules will carry the high-affinity antithrombin III–binding site, which is responsible for the anticoagulant action of the compound. Modifications of the surface chemistry and selection of heparin molecules with binding sites for antithrombin III were used to increase the antithrombin III–binding activity of three coatings of incremental activity (onefold to fourfold higher activity per surface area than the conventional CBAS). Pilot in vitro studies have shown that (1) mounting and expansion of the stent did not affect the integrity of the coating, as demonstrated with a colorimetric assay using toluidine blue[28]; and (2) sterilization with heat or ethylene oxide reduced the antithrombin III–binding activity considerably (50% to 70%). Consequently, the stents used in this study were initially coated under clean room conditions but not sterilized (group 4; Table 1); coated stents for later groups were sterilized and therefore initially coated with a higher heparin content to compensate for the loss during ethylene-oxide sterilization (groups 5 through 7). At the end of the study, the remaining heparin activity at the surface of explanted stents (both coated and controls) was assessed with the use of an antithrombin III–binding assay.

Animal Preparation

Experiments were performed in cross-bred Landrace-Yorkshire pigs (20 to 28 kg in weight; HVC) as described previously.[29] The investigations were carried out according to a protocol approved by the Committee on Experimental Animals of Erasmus University. After an overnight fast, the animals were sedated with 20 mg/kg ketamine hydrochloride. After endotracheal intubation, the pigs were connected to a ventilator that administered a mixture of oxygen and nitrous oxide (1:2, v/v). Anesthesia was maintained with 1 to 4 vol% enflurane. Antibiotic prophylaxis was administered by an intramuscular injection of 1000 mg of a mixture of procaine penicillin G and benzathine penicillin G. Under sterile conditions and after additional local anesthesia of the skin with lidocaine 2%, an arteriotomy of the left carotid artery was performed, and a 9F introduction sheath was placed. Heparin sodium (10 000 IU) was administered, and a 9F guiding catheter was advanced to the ascending aorta. After measurement of arterial blood pressure and heart rate and after withdrawal of an arterial blood sample for the measurement of blood gases and acid-base balance (settings of the ventilator were corrected if necessary), coronary angiography was performed with iopamidol (Iopamiro 370) as contrast agent. Seventy-eight animals underwent the catheterization procedures. Of these, 9 animals were excluded from final analysis due to the following reasons. In 3 animals, a stent was not implanted due to a coronary artery anomaly, air embolism at baseline angiography, and spontaneous arrhythmias, respectively. Complications during stent implantation (ventricular fibrillation or arrest during balloon inflation or balloon rupture) occurred in 3 other animals. Postoperative problems (aspiration hypoxia, reanesthesia for postoperative bleeding, and a leg problem) were the reason for premature withdrawal in 3 additional animals and were considered to be unrelated to stent placement.

Final analysis was performed for 69 animals, in which stent implantation was successful and no adverse events were observed.

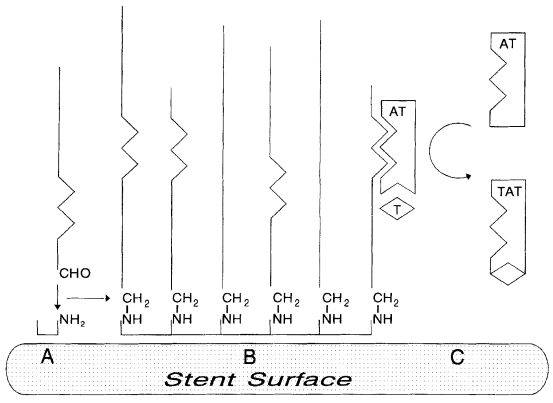

FIG 1. Principle of heparin coating and mechanism of antithrombotic action of heparin-coated stent surface. A, The metal surface has been conditioned with functional amino groups that can bind covalently with the aldehyde group of fragmented heparin molecules. B, Thus, end point–attached heparin molecules form a heterogeneous population, some without and some with the epsilon-shaped antithrombin III (AT)–binding region. C, Circulating antithrombin can bind to the active site, which catalyzes the inhibition of activated coagulation factors, eg, thrombin (T). The resultant inactive antithrombin/thrombin complex (TAT) is released into the bloodstream, thereby enabling the active site on the heparin to repeat interaction with AT and TAT.

TABLE 1. Experimental Groups, Treatment, and Size of Implanted Stents

Group	Stents, No. of Animals	Heparin, pmol/stent	Aspirin, 300 mg/d	Stent Size, 3.0/3.5/4.0 mm	Follow-up, wk
1	8	–	–	4/3/1	4
2	10	–	+	5/5/0	4
3	9	–	+	1/8/0	12
4	7	5	–	4/2/1	4
5	10	12	+	6/4/0	4
6	10	20	+	3/7/0	4
7	10	20	+	2/8/0	12

Stent Implantation

Based on the angiograms and with the diameter of the guiding catheter used as a reference, a segment with a diameter of 2.5 to 3.5 mm was selected in the proximal LAD using online quantitative coronary arteriography after intracoronary injection of 1 mg isosorbide dinitrate. Side branches were not avoided, but stents were not placed at curved coronary artery segments. Then, a heparin-coated or a regular stent (in alternate order) crimped on its deflated balloon was advanced over a 0.014-inch steerable guide wire to the preselected site for implantation. The balloon was inflated to a pressure of 6 atm for 30 seconds and then deflated, and negative pressure was maintained for 20 seconds. The catheter was then slowly withdrawn while leaving the stent in place. After repeat angiography of the stented coronary artery, the guiding catheter and the introducer sheath were removed, the arteriotomy was repaired, the skin was closed in two layers, and the animals were allowed to recover from anesthesia. Animals receiving the control stents and the three types of heparin-coated stents were assigned to seven groups (Table 1). Fifteen animals did not receive antithrombotic prophylaxis after the procedure (groups

1 and 4). Forty-nine animals received 300 mg acetylsalicylic acid/day PO, starting the day before implantation; this treatment was continued daily during the follow-up period.

Follow-up Angiography and Quantitative Analysis

The anesthesia and catheterization procedures at 4- or 12-week follow-up were similar, as described above; coronary angiography was performed in the same projection, and identical settings of the x-ray equipment were used during implantation. All coronary angiograms were measured on-line using a personal computer-based system for quantitative angiographic analysis with the edge-detection method (Cardiovascular Measurement System, Medis Inc).[30]

Microscopic Examination

After angiography at follow-up, the thorax was opened by a midsternal split, and a lethal dose of sodium pentobarbital was injected intravenously, immediately followed by cross-clamping of the ascending aorta. After the aortic root was punctured above the coronary ostia, 300 mL saline followed by 500 mL buffered 4% formaldehyde were infused under a pressure of 150 cm H_2O. The heart was then excised, and the coronary arteries were dissected from the epicardial surface. The stented and adjacent unstented segments were placed in 4% formaldehyde in phosphate buffer, pH 7.3, for at least 48 hours in preparation for microscopy. After further fixation for at least 48 hours, the tissue was processed for light microscopic examination as described previously.[29] Hematoxylin and eosin was used as a routine stain, and resorcin-fuchsin was used as an elastin stain.

Morphometry

For measurement of the thickness of the various layers of the arterial wall, at least six resorcin-fuchsin–stained sections of each stented coronary segment were examined from the proximal, mid, and distal portions of the stent. With a calibrated eyepiece, the neointimal and medial thicknesses were measured on top of and between the stent struts. The distance between the endothelial lining and the stent strut or internal elastic lamina was taken as the thickness of the intima.[31] The media was defined as the layer between the internal and external elastic laminae.

Assessment of Stent Thrombosis

Immediately after the animals were sacrificed and the arteries underwent fixation or after autopsy, the stented coronary artery was opened lengthwise using a pair of fine scissors and examined under a dissection microscope. Low-power photomicrographs were taken from each coronary artery, and the presence or absence of stent occlusion was assessed by two observers. In addition, light microscopical examination was used to confirm the thrombotic origin of the occlusion, as demonstrated by the presence of a platelet-rich, layered thrombus.

Assessment of the Early Response to Stent Implantation

In an additional group of five animals, one regular stent (in the left circumflex coronary artery) and one high-activity heparin-coated stent (in LAD) were placed per animal. These animals received procedural heparin during the implantation plus 300 mg acetylsalicylic acid during 5 days of follow-up. At 5 days, repeat angiography was performed, followed by excision of the stented coronary arteries for light microscopy and morphometry, as described. In addition, lectin cytochemistry and scanning electron microscopy were performed to study and compare the early blood and tissue responses to the regular and coated stents, by using previously described methods.[32]

TABLE 2. Experimental Groups, Outcome, and Size of Stents With Complications

Group	No. of Animals	Heparin Coating, pmol/stent	Stent Occlusion	Stent Size, mm
1	8	...	2	2×3.0
2	10	...	4	2×3.0
				?×3.5
3	9	...	3	3×3.5
4	7	5	3	3×3.0
5	10	12	0*	...
6	10	20	0*†	...
7	10	20	0*†	...

*$P<.001$ groups 5 through 7 vs groups 2 plus 3.
†$P<.01$ groups 6 plus 7 vs groups 2 plus 3.

Statistical Analysis

All data are expressed as mean±SD. The occurrence of thrombotic events between the groups was compared by Fisher's exact test. A two-tailed P value of $<.05$ was considered statistically significant. The significance of the changes in the angiographic and morphometric data were evaluated by unpaired t test when ANOVA indicated that the groups belonged to different populations. Because of repeated comparisons for these two parameters, only $P<.01$ (two-tailed) was considered statistically significant.

Results

Systemic Hemodynamics and Blood Gases During Angiography

During implantation, heart rate and mean arterial blood pressure were similar for all groups (99±14 beats per minute and 74±14 mm Hg, respectively). At restudy after 4 weeks (groups 1 through 5), heart rate (105±18 beats per minute) and mean arterial blood pressure (85±17 mm Hg) were comparable, but when restudied after 12 weeks, groups 6 and 7 showed an increase in these parameters (to 112±25 beats per minute and 101±22 mm Hg, respectively; $P<.01$). However, at no time was there any difference between the groups with coated or control stents. The oxygenation of arterial blood and acid-base balance were in the normal range during stent placement and follow-up angiography.

Follow-up Evaluation

In 64 animals, the stent could be placed successfully, and these were included in the final analysis. Eight of the animals that received a noncoated stent died suddenly within 48 hours, whereas a ninth pig survived 4 weeks with an infarction of the LAD perfused myocardium (Table 2).

In the animals receiving the 5 pmol/stent heparin coating without aspirin (group 4), three cases of sudden death occurred within 48 hours. However, all animals survived that received a stent with 12 or 20 pmol/stent heparin coating in combination with oral aspirin (groups 5 through 7). The data in Table 2 also show that problems were not associated with smaller-diameter stents.

Quantitative Angiographic Measurements

Quantitative analysis of the baseline coronary angiograms showed that luminal diameters of the proximal LAD were similar for all groups (range, 2.9 to 3.2 mm; Table 3). The diameters of the stent-mounted angioplasty balloons at maximal inflation pressure also did not

TABLE 3. Quantitative Angiographically Assessed Mean Diameters of Arteries and Balloon at the Site of Stent Implantation at Baseline, Immediately After Placement, and After 4 or 12 Weeks of Follow-up

Group	No. of Animals	Baseline, mm	Balloon, mm	Stent, mm	Follow-up 4 wk, mm	Follow-up 12 wk, mm
1	8	2.9±0.4	3.2±0.5	3.2±0.3	2.7±0.6	...
2	10	2.9±0.3	2.9±0.3	2.8±0.3	2.7±0.3	...
3	10	3.2±0.2	3.1±0.3	3.1±0.2	...	2.8±0.2
4	7	3.2±0.6	2.9±0.4	3.1±0.5	2.4±0.4	...
5	10	2.9±0.3	2.8±0.3	2.8±0.2	2.6±0.3	...
6	10	3.0±0.3	3.0±0.4	3.1±0.3*	2.4±0.4*	...
7	10	3.1±0.2	3.0±0.3	3.0±0.3	...	3.2±0.4†

Values are mean±SD.
*$P<.01$ vs group 2.
†$P<.01$ vs groups 3 and 6.

differ between the groups (range, 2.8 to 3.2 mm). The measured balloon-to-artery ratio was 1.0, demonstrating precise matching of balloons and recipient arteries. Implantation of the stents did not change the arterial diameters of the groups (range, 2.8 to 3.2 mm).

After 4 weeks of follow-up, the average luminal diameter showed no change in both control groups as well as in the groups receiving the low- or intermediate-activity heparin-coated stent. However, in the highest-activity heparin-coated stent group (group 6), the diameter had decreased by 0.7±0.6 mm ($P<.01$).

After 12 weeks of follow-up, the group receiving the control stents did not show a decrease in luminal diameter compared with the 4-week data for groups 1 and 3. However, the group receiving the high-activity coated stents (group 7) now showed no change in luminal diameter compared with its baseline and immediately poststent angiograms, and an actual increase occurred compared with the 4-week high-activity stented group.

Stent Thrombosis

On postmortem examination, all stents retrieved from animals that received a regular stent and died suddenly demonstrated stent occlusion (Fig 2A and 2B) in several cases accompanied by myocardial infarction of the corresponding anterior wall of the left ventricle. Major disruption of the vessel wall or incomplete expansion or marked overdilatation of the stent was not observed in these specimens. Microscopical examination confirmed the presence of a layered, platelet-rich thrombus in all occluded stents. In the group receiving the low-activity coated stents (group 4), all three cases of sudden death proved to be caused by stent thrombosis. In none of the animals receiving a stent with 12 or 20 pmol heparin/stent was partial or occlusive stent thrombosis observed.

Therefore, (sub)acute stent thrombosis was observed in 37% in the groups receiving regular noncoated stents ($P<.01$), and the only factor that could be identified as responsible for stent occlusion was the absence of the high-dose heparin coating.

Light Microscopy

Examination of the seven groups showed that all stents were covered by a neointima of variable thickness (Fig 3A through 3D), ranging from only several cell layers to a collagen- and elastin-rich tissue up to 500 μm in thickness. Typically, such a neointima contained a large amount of extracellular matrix with smooth muscle cells in disarray near the intimal/medial border. Toward the lumen, the tissue was more dense and cellular, containing macrophages and a few lymphocytes but mainly smooth muscle cells oriented in a circular fashion.

Medial impression by the stent struts was variable. Some metal struts lacerated only the internal elastic lamina. A minority of the struts, however, penetrated the medial layer, resulting in either a clean cut into the media or actual dissection, sometimes with damage to the external elastic lamina. Even in those cases, inflammatory changes were minimal and discrete (Fig 3). An active inflammatory reaction was rarely observed, and only in two (control stents) was a cellular response with

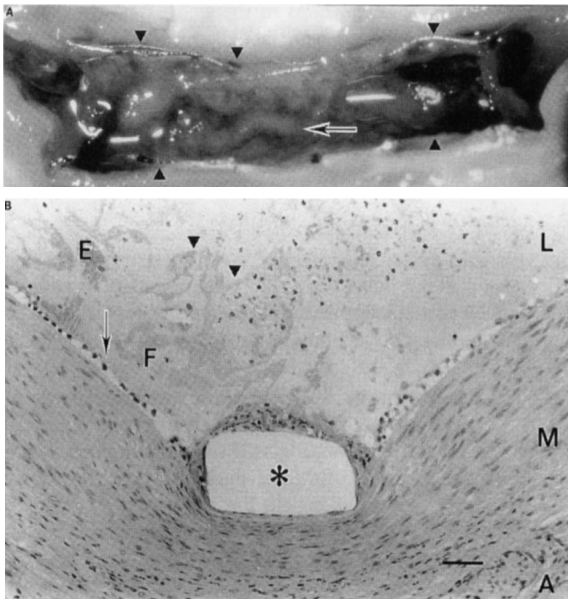

FIG 2. A, Photomacrograph of a control stent (group 3) occluded with a platelet-rich thrombus (arrow) causing sudden death approximately 27 hours after implantation. The stent has been opened longitudinally (arrowheads indicate struts). B, Photomicrograph of the thrombosed stent shown in A. Both the stent wire (* indicates stent wire void) and the luminal border are lined with a single layer of leukocytes (arrow). The lumen (L) is obstructed by a platelet-rich thrombus, showing the typical layered appearance (arrowhead) of an in vivo thrombotic occlusion. M indicates media; A, adventitia; E, erythrocytes; F, fibrin; and bar, 50 μm.

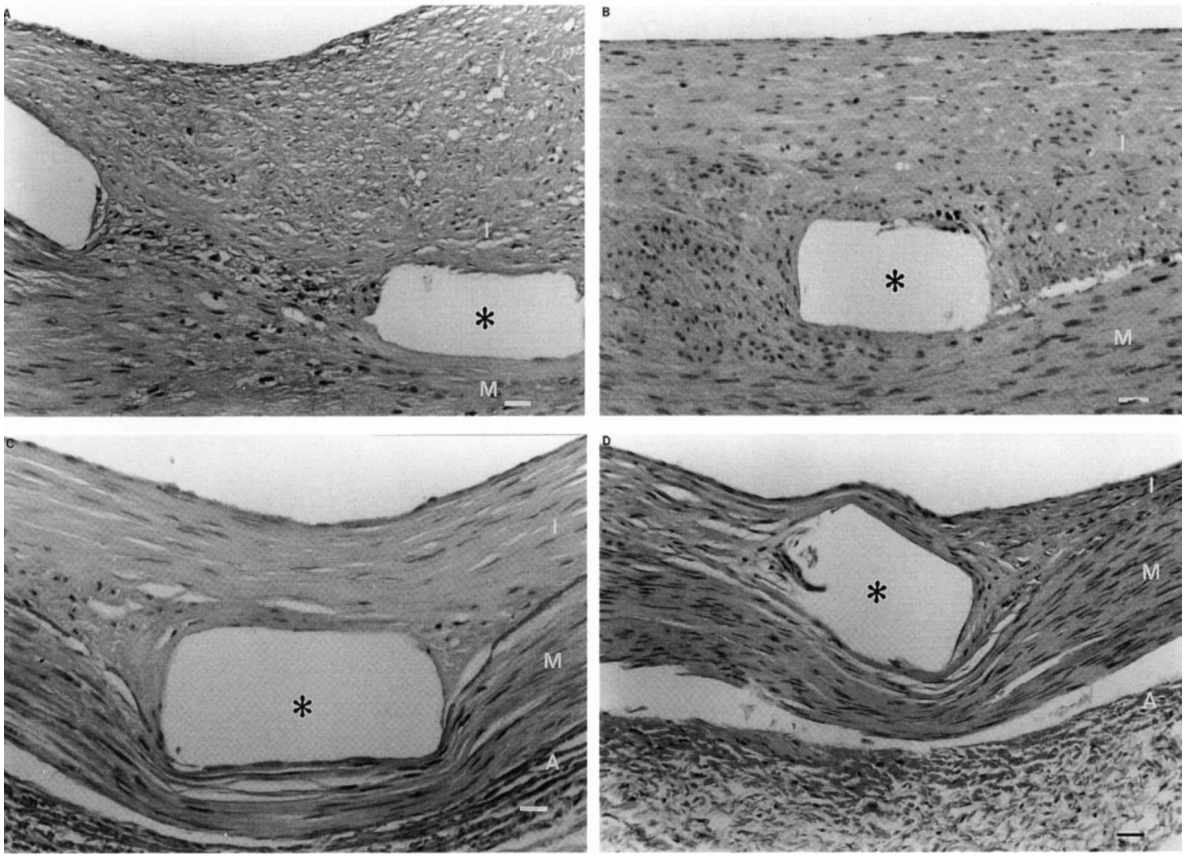

FIG 3. Composition of the light microscopy of the control stent (A, group 2) and the heparin-coated stent (B, group 6) at 4 weeks after implantation and of the control stent (C, group 3) and the heparin-coated stent (D, group 7) at 12 weeks after implantation. In all specimens, the tissue response consisted of a neointimal thickness containing smooth muscle cells within a collagenous matrix. Although the thickness varied among the groups, the overall reaction was limited in its nature. I indicates intima; M, media; A, adventitia; *, stent wire void; and bar, 25 μm. Hematoxylin azophloxin stain.

monocytes and macrophages more prominently associated with the stents.

The only difference between the 4- and 12-week groups was an increase in neovascularization from adventitia toward the intima in the later group, in both coated and control stents. The only late features exclusively seen in some coated stents was an occasional spot of calcification (in two coated stents) and swollen appearance of the overlying endothelium (three coated stents).

Morphometry

Comparison after 4 weeks of follow-up showed that there was no difference in neointimal thickening between groups 1 and 4 (both received no aspirin after the procedure) and between groups 2 and 5 (Table 4). However, a comparison of groups 2 and 6 and of groups 5 and 6 showed that the increased thickening of the neointima in the group with the highest heparin activity was significant ($P<.01$). The thickness of the media under the metal struts did not differ between the groups.

After 12 weeks of follow-up, the difference between coated and noncoated stents was no longer observed as the thickness in the high-activity heparin-coated group was significantly smaller after 12 weeks (group 7) compared with at 4 weeks (group 6; $P<.01$). Along the length of the stent, from proximal to distal, there was an observed increase in intimal thickening for both the coated and the regular stent groups. The measurements

TABLE 4. Morphometry

Group	No. of Animals	NS, μm	N, μm	MS, μm	M, μm
1	6	263±93	301±127	120±66	195±58
2	6	117±36	100±43	90±19	156±16
3	6	198±49	185±33	110±26	187±20
4	4	166±35	175±30	90±13	174±45
5	10	109±55	98±51	90±21	154±26
6	10	259±104*	210±82	99±22	187±28
7	10	152±61	156±51	113±16	172±20

Values are mean±SD. NS indicates neointima covering stent struts; N, neointima between stent struts; MS, media under stent struts; and M, media between stent struts.
*$P<.01$ vs groups 2, 5, and 7.

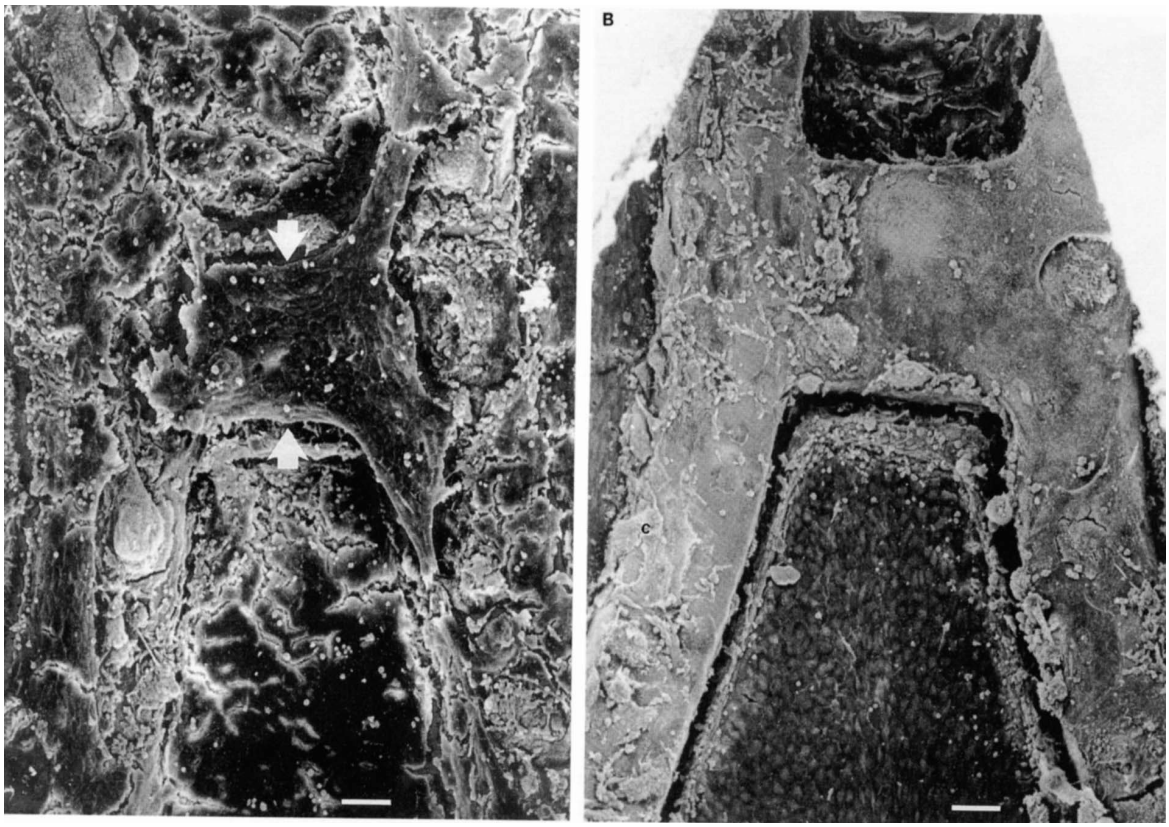

FIG 4. A, Scanning electron microscopy of the regular Palmaz-Schatz stent at 5 days after implantation. The photograph, taken at the level of an intersection between two stent struts (arrows), shows advanced endothelial covering but marked leukocyte adhesion. Bar indicates 57 μm. B, Scanning electron microscopy of the highest-activity heparin-coated Palmaz-Schatz stent at 5 days after implantation. The photograph, again taken at the intersection between two stent struts, clearly shows the absence of endothelial cells. Only a variable number of leukocytes and a protein layer cover the struts, whereas in-between the endothelial layer appears to be intact. Bar indicates 57 μm.

of other vessel wall layers did not differ between the groups.

Assessment of the Early Response to Stent Implantation

In the additional group of five animals, five coated stents (20 pmol antithrombin III uptake) and five regular stents were placed (balloon-to-artery ratio, 1.0±0.1). Angiographically measured coronary diameters before implantation, immediately after implantation, and after 5 days of follow-up for the heparin-coated stents were 2.9±0.2, 3.0±0.2, and 3.0±0.3 mm, respectively; and for the regular stents, diameters were 2.7±0.2, 2.9±0.2, and 2.8±0.5 mm, respectively (P=NS). Morphometrical assessment of the thickness of the layer covering the stent struts showed no differences between the two types of stents (57±17 μm for the heparin-coated stents and 62±47 μm for the regular stents). Light microscopy demonstrated that the early local reactions to both types of stent were similar, showing a proteinaceous adherent layer with adherent leukocytes. However, both the lectin cytochemical identification of endothelium and the scanning electron microscopy demonstrated a decreased endothelial cell covering of the heparin-coated stents (Fig 4A and 4B).

Heparin Activity of the Stents After Explantation

The control stents showed no detectable antithrombin III–binding activity after 4 weeks of follow-up (n=2). However, measurement of the coated stents explanted at the same time revealed that 20% to 50% of this activity was still detectable at 4 weeks (n=2).

Discussion

Metal Stents Reduce Restenosis but Are Thrombogenic

PTCA with balloon dilatation is a good alternative treatment to aortocoronary bypass surgery for patients with symptomatic coronary heart disease. Surgery offers longer symptomatic relief but is more invasive and requires a longer rehabilitation period.[33] PTCA allows patients to regain active life earlier, but in many patients the duration of symptomatic relief is shorter due to the restenosis process. Recently, two randomized clinical trials that compared balloon angioplasty with stent implantation were completed.[10,11] Both trials showed that stent implantation reduced the need of revascularization due to restenosis by approximately 30%. These new data will influence the choice of therapy in patients eligible for both surgery and PTCA.

The favorable outcome of stent implantation, however, has its price: a longer hospital stay and a 15% vascular complication rate, both caused by the use of an extensive anticoagulant regimen to prevent subacute thrombosis of the stent. Stent thrombosis has been recognized as a problem inherent to all metal stents in

animal experiments as well as in patients with coronary heart disease.[10-19,32,34-36]

Heparin Coating

Stents with improved surface characteristics may further enhance the clinical results of coronary stenting by reducing the risk of thrombotic stent occlusion. The same techniques that have been applied to improve the blood compatibility of vascular grafts may also enhance the quality of stents.[20] A widely used technique is coating of the cardiovascular implant surface with heparin.[37,38] In the present study, we tested an established heparin coating (CBAS)[27] and subsequent modifications of this coating applied the stainless steel Palmaz-Schatz coronary stent.

Reduction in Stent Thrombosis by High-Activity Heparin Coating

In the present study, the standard CBAS coating (5 pmol/stent) did not reduce the incidence of early thrombotic complications (Table 2). Subsequently, 300 mg aspirin was administered daily to the animals to reduce the background thrombogenicity in the animal stent model. The use of stents coated with higher heparin activity subsequently groups eliminated thrombotic events compared with a new appropriate control group also receiving aspirin. The incidence of stent thrombosis in the control groups (25% to 33%) in the present study is similar to that observed earlier with a self-expanding metallic stent in the same model.[35] This high incidence of stent thrombosis of regular stainless steel stents is not an artificial feature of this swine model. During the initial clinical experience with the Palmaz-Schatz stent, an 18% incidence of subacute closure was observed when warfarin or Coumadin treatment was withheld.[39] Very recently, it has been reported that noncoated slotted tube stents show a 42% thrombotic occlusion rate in the rabbit iliac model.[40]

We did not include an additional experimental group receiving the standard coating and aspirin. Therefore, we cannot exclude a significant contribution of the surface conditioning inner layers of the coating (to which the heparin molecules were attached) to the overall thromboresistance of the stent. However, evidence in favor of an active role for heparin may be provided by studies that showed that thrombin inhibitors reduced platelet and fibrinogen deposition during arterial injury or stent placement in the pig.[41,42] However, other studies have shown that coating metal stents with only a passive polymer layer also reduces local platelet deposition or thrombotic occlusion in experimental animals.[35,43,44] Nevertheless, the results of the present study are consistent with the antithrombotic action of the CBAS coating in extracorporeal systems.[45,46]

Effect of Heparin Coating on Neointimal Hyperplasia

Heparin has been shown to reduce smooth muscle cell proliferation, an important component of the restenosis process, in injured arteries of experimental animals.[22-24,47-49] This property of heparin may be unrelated to its anticoagulant effect.[23] In the present study, we did not observe a reduction in the thickness of the neointimal layer due to the heparin coating. On the contrary, a temporary increase occurred in the group with the highest activity of the coating. Results for the early-response (5-day) group of the present study showed delayed wound healing, which indicates that the well-known action of heparin to reduce endothelial cell attachment and growth[50] is most likely responsible for the reduced endothelial control of smooth muscle cell growth.

However, the increased neointimal response with the highest activity heparin coating at 4 weeks proved to be only temporary as after 12 weeks the tissue response was similar for coated and noncoated stents.

Conclusions

This study demonstrates that heparin coating of metal stents reduces thrombotic events associated with their deployment in normal coronary arteries of pigs. If confirmed in clinical studies, this coated stent may permit reduction in the systemic anticoagulation responsible for vascular complications and longer hospital stay.

Acknowledgments

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Chapter 11

Heparin-coated stent trials

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Heparin-coated stent trials

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Effects of heparin on blood coagulation and cellular proliferation

Heparin is a highly sulphated linear polysaccharide that was discovered in 1916 as an anticoagulant. Heparin binds to antithrombin III, thereby inducing a conformational change. This results in inhibition of thrombin and other serine proteases involved in the blood clotting cascade immediately after administration. The actions of heparin on neointimal proliferation are complex. This was demonstrated by Edelman et al. (1). They found an increase of intimal hyperplasia after arterial injury in the rat model when heparin was administered once daily. Twice daily had different effects depending on time-interval of dosing but very significant reduction in intimal hyperplasia was seen with continuous administration. Clowes et al. showed that heparin should be administered for 4 to 7 days to have an antiproliferative effect after injury (2).
Therefore, the need for continuous heparin administration for 7 days after stent implantation provides the rationale for a heparin-coated stent.

Methods of heparin-coating

There are several ways to attach heparin to the stent surface. These include: adsorption of benzylkonium alcohol solution (3,4), ionic bonding (5,6), dispersion in polymer (7-9), surface grafting (10-12), covalent coupling of functionalized surface with mid- or end-point attachment (13-17) and heparin-polymer block copolymers (18).
The first methods result in a weak binding and heparin will be lost within a short time span. Most research and all clinical trials have been performed with covalently bound heparin-coated stents.

The Palmaz-Schatz- and BX-stent with Hepacoat™
In the early development of heparin coatings, a reduction of heparin activity was frequently seen after covalent attachment of the molecules to the surface. Larm et al (19) immobilized

heparin fragments with end-point attachment on materials coated with polyethylamine. By this method heparin activity was preserved after attachment. Based on this principle the Carmeda BioActive Surface coating (CBAS, Carmeda AB, Stockholm) was developed and applied to extracorporeal systems. This coating was used as a base for the heparin-coated Palmaz-Schatz stent. However, in a demanding environment, like the porcine coronary model, this coating was not sufficient to eliminate stent occlusion. Elimination of subacute stent thrombosis could be shown with higher-activity heparin coating (12 to 20 pmol anti-thrombin III binding activity per stent;Hepacoat™, J & J Cordis, Warren, NJ)(20).
In the early 90's, stents were still implanted with a strict anti-coagulant regime and prolonged heparinization. In four phases of Benestent-II pilot, using this heparin-coated PS stent, anti-coagulant regime was reduced and finally coumadin and postprocedural heparin were replaced by aspirin and ticlopidin. Stent thrombosis did not occur and bleeding complications dropped to 0 % (21).
The BENESTENT-II trial randomized 827 patients to heparin-coated stent implantation or standard balloon angioplasty. The patients were more challenging with 45 % unstable angina pectoris. Stent thrombosis occurred in only one patient (0.2 %) (22). This is well below the 1- 2 % of stent thrombosis seen in contemporary trials. Restenosis rates at 6 months were 16 % in the heparin-stent group. Similar restenosis rates have been achieved with non-coated stents. Data suggest no reduction of neointimal hyperplasia within the stent in comparison to uncoated stents. (20-22).
The heparin-coated Palmaz-Schatz stent has also been used in chronic total occlusion (TOSCA) and acute myocardial infarction (Stent-PAMI trial). In the TOSCA trial 410 patients with chronic total occlusion of a native coronary artery were randomized to heparin-coated stent vs. balloon angioplasty. The incidence of MACE after 6 months was similar with both strategies (PTCA:23.6 vs. stent: 23.3 %) but restenosis rate was reduced from 70 % in the PTCA group to 55 % in the stent group (23). Evaluating the 721 patients with stable and unstable angina pectoris as well as acute myocardial infarction who have been treated with a heparin-coated Palmaz-Schatz stent in the Benestent-II pilot, the Benestent-II trial and PAMI trials, the incidence of subacute stent thrombosis (SAT) was extremely low (incidence of 0.12 %!!).
A recent registry from a single, large cardiac center evaluated primary thrombotic outcome (defined as angiographically documented SAT and/or sudden unexplained cardiac death (SCD)) in 337 patients receiving 543 BX velocity Hepacoat stents and 939 patients receiving 1688 bare-metal stents. SAT or SCD was seen in 3.03 % of procedures in the bare-metal stent group and 0.58 % in the heparin-coated stent group. 96 % of SAT within 30 days occurred in patients with an acute coronary syndrome (24).

Hep@net *Registry and HOPE*

This internet-based registry compares the Hepacoat BX-Velocity stent with the bare BX-velocity stent. Last data available on www.TCTMD.com show 0.5% stent thrombosis with the Hepacoat (n=1062) vs 1.2% with the bare stent (n=1301) P= 0.14. Enrollment in this registry is continuing. In another registry, the HOPE Registry (Hepacoat and an antithrOmbotic regimen of asPirin alone) 200 patients were stented with the BX Velocity stent with Hepacoat. SAT rate was acceptable with 1 % at 30 days despite only aspirin use post procedure and no ticlid or clopidogrel. However, IIb/IIIa – inhibitor use was 55% in this study (25).

The Hepamed™-coated Wiktor stent and beStent

The conditioning layer of the coating of the Hepamed coating (Medtronic Bakken Research Center, Maastricht, NL) is completely covalently coupled. (26). This makes this coating potentially more stable than Hepacoat™.In addition, the heparin molecules are neither fragmented nor enriched with the active antithrombin binding site, resulting in a predictable heparin layer on the stent, but with a lower antithrombin binding capacity per picomole of attached heparin. Therefore, additional heparin has been added.

In the MENTOR trial theWiktor stent with Hepamed coating was implanted in 132 patients. A subacute occlusion rate of 0.8 % was seen despite the fact that 43 % of patients had unstable angina. The 6 month event-free survival was 85 % and angiographic restenosis rate was 22 % (27). The Wiktor Stent with Hepamed coating has also been studied in saphenous vein bypass grafts. In 50 patients 55 stents were placed in lesions in very old vein grafts (11.7 +/- 3.9 years). MACE free survival was 86% at 6 months and angiographic restenosis rate was 22%. This is a favourable result for this challenging patient subset. (28).

A randomized , multicenter trial has been initiated in Scandinavia (Stents in Small Coronary Arteries, SISCA), comparing the efficacy of the Hepamed coated beStent (Medtronic Instent, Minneapolis MN, USA) with balloon angioplasty in 145 patients with stable angina pectoris due to a de novo lesion with a reference diameter between 2.1 and 3.0 mm. There were no differences between the groups regarding early events (mean reference diameter 2.4 mm). Event-free survival at 6 month follow-up was significantly better with the heparin-coated stent than with balloon angioplasty (90.5% vs 76.1%, respectively; P = 0.016). (29).

This clinical benefit was maintained at 1 year follow-up with no change in eventfree survival between 6 months and 1 year. (30).

The Corline™ coated Jostent

In the Corline coating a single modified polymeric amine conditioning layer is used. Unfractionated heparin is linked with specific covalent bounds to form a macromolecular heparin conjugate. The anti-thrombin activity per cm2 may be lower than in the Hepacoat and Hepamed coatings. However, in the Corline coating unfractionated heparin is used as opposed to fragmented heparin in the Hepacoat. Theoretically, important properties of the heparin molecule, like antiproliferative actions, which are not necessarily associated with the antithrombin binding site, may be retained.

A small pilot study using this stent was performed in Russia. The stent was implanted in 46 patients with 93.4% acute angiographic succes. However, follow-up was limited to the in-hospital phase (31).

The Corline coating has been used in the only trial that has been initiated thusfar to compare heparin-coated with bare stents(COAting of STents : COAST trial). In this study the Jomed stent was compared side-to-side with the Corline Jo stent in a three-armed study of 588 patients. Patients with stable or unstable angina pectoris due to single or multiple stenoses were included when the reference diameter of the coronary artery was between 2.0 and 2.6 mm, thus providing additional information on performance of coated and non-coated stents in small arteries. COAST, however, lacks the power to discriminate between the (sub)acute thrombosis rates, even when the incidence of stent thrombosis in these small vessels would have been as high as 5 %. In COAST, patients were randomized to balloon angioplasty (n= 195), bare stent (n=196) or heparin-coated stent (n=197). In the balloon angioplasty arm 27% (n=53) of patients crossed over to recieve a stent, which is a considerably higher percentage than usually seen in trials in larger (mean diameter 3.0 mm) vessels. Only 3 patients in the bare stent arm and four patients in the heparin-coated stent arm crossed over to recieve balloon angioplasty. In a per-protocol analysis 6 month restenosis rates were 32%, 27% and 30%, for balloon, stent and heparin-coated stent groups respectively. The “intention to treat”results are summarized in the table (table 78-1). There were no statistical differences between the groups. The only suspected stent thrombosis occurred in the heparin-coated arm. (32).

Wőhrle et al. reported a non-randomized study in which 368 stents were implanted in 303 lesions from 278 patients, comparing uncoated and Corline heparin-coated Jomed stents. More patients included in this study were high risk than in any of the studies cited above, since > 60% had multivessel disease, 8% total occlussion, 17 % of patients had acute infarction and 19 % of lesions were restenotic lesions, equally divided over both groups. No benefit of heparin coating could be detected on the incidence of stent thrombosis, myocardial infarction, restenosis rate or re-intervention rate (33).

Table 51-1: Clinical trials with heparin-coated stents (references: see text)					
	Coating	Study	Patients	6 or 7 month MACE	6 months Restenosis
Benestent II pilot 1996 (21)	Hepacoat	4-phased registry; AP/UAP	N=207; angio success 98%	14 %	13 %
Benestent II randomized 1998 (22)	Hepacoat	Balloon vs HC-stent; AP/UAP	Balloon n=410 Stent n=413	19 % 13 % p=0.013	31 % 16 % p< 0,001
Stent-PAMI pilot 1999	Hepacoat	Registry; acute MI	N=101; angio success 96%	19 %	18 %
Stent-PAMI randomized 1999 (34)	Hepacoat	Balloon vs. HC-stent; acute MI	Balloon n=448 Stent n=452	20 % 13 % p< 0,01	34 % 20 % p< 0,001
TOSCA 1999 (23)	Hepacoat	Randomized; Total occlusion	Balloon n=208 Stent n=202	24 % 23 % ns	70 % 55 % p= 0,001
Mentor 2000 (27)	Hepamed coating	Registry AP/UAP	N=132	15 %	22 %
Wőhrle 2001 (33)	Corline coating	Alternating cohorts of 50 coated or uncoated	Bare 133 HC 144	25.7 25.2 ns	30.3 33.1 ns
SISCA 2001 (29)	Hepamed coating	Randomized; AP, small vessels	Balloon n= 71 Stent n= 74	23.9 9.5 p= 0.016	18.8 9.7 p= 0.15
COAST 2003 (32)	Corline coating	Randomized; AP/UAP, small vessels	Balloon n=195 Coated Stent n=197 Bare Stent n=196	15.4 11.7 ns 11.7	32.2 29.6 ns 24.8
Russian Pilot 1998 (31)	Corline coating	Feasibility	Stent n=46 93.4 % angio succes	No follow-up	No follow-up
South Korean study 1999 (35)	Corline coating	Registry Acute MI	Stent n= 102	13.7%	17.2 %
Lev 2004 (36)	Hepacoat	Registry, AMI primary and rescue	Bare n=114 HC n=124	21.1% 16.1%	14.9% 10.5%

Heparin coated stents in acute myocardial infarction

The heparin-coated Palmaz-Schatz stent was used in the Stent-PAMI trial(34). In this trial patients with acute myocardial infarction (AMI) were randomized to angioplasty alone (448

patients) or angioplasty with stenting (452 patients). Abciximab was only used in 5.8 % of stented patients and 4.5 % in the angioplasty group. The combined primary endpoint at 6 months of death, reinfarction, disabling stroke, or target-vessel revascularization was significantly lower in the stent group (12.6 % vs. 20.1 % for PTCA, p<0.01). At 1 month and at 6 months no significant difference was seen in reinfarction. This suggests no lower thrombotic occlusion rate with a heparin-coated stent. Restenosis rate at 6.5 months was 20.3 % for the stent group vs. 33.5 % for the PTCA alone group. (P<0.001)

Recently, a study comparing 124 patients receiving a heparin-coated stent (HepaCoat BX Velocity, Cordis, NJ) for acute myocardial infarction with 114 patients receiving a bare stent showed reductions in stent thrombosis (0.8% vs 6.1%, p=0.03) and recurrent MI (4% vs 10.5%, p=0.05) at 30-day, but no significant effect on 180-day outcome (composite uotcome death/MI/TVR 16.1% vs 21.1%) (36).

A South-Korean group reported a registry of 102 patients with acute MI treated with 111 Corline heparin-coated stents(35). Postprocedural heparin or abciximab was not used. Mortality at 6 months was 6.0 %, 1 patient with recurrent infarction was seen and revascularisation rate was only 8 %. Restenosis rate at 6 months was 12.7 %. This registry lacked a control group, which is especially regretted since the outcome was quite good, even in the absence of abciximab.

Conclusions

The high thrombotic occlusion rate of stents in the early nineties prompted research to develop a heparin-coated stent.

The very low incidence of less than 0.2 % subacute thrombosis after implantation of a heparin-coated stent in the setting of stable angina, unstable angina and even acute myocardial infarction holds promise. However, whether this is truly the result of the heparin coating or of improved interventional techniques is difficult to distinguish. Though apparent in ‘real-world registries’, a trial directly comparing Corline-coated versus non-coated stents showed no benefit in small (mean diameter 2.4 mm) arteries (COAST). In the meantime it seems justified to use a heparin-coated stent in situations of high thrombus burden or high platelet reactivity. Adverse effects of this choice have at least never been seen. Another niche may be treatment of patiens with known aspirin or clopidogrel intolerance.

No significant reduction of in-stent restenosis has been demonstrated compared to bare metal stents.

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Chapter 12

Drug-eluting stents

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Drug-Eluting Stents

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Introduction

In-stent restenosis has long been considered the main complication limiting the long-term efficacy of coronary stenting. Although stent implantation itself has been shown to reduce restenosis compared to balloon angioplasty, *in-stent* restenosis still occurs in 10-40% of the patients. Although a number of "predictors" have been described and are helpful in characterizing "high-risk" populations, the occurrence of restenosis remains largely unpredictable for a particular patient.[1-3] Moreover, *in-stent* restenosis in its more complex forms may re-occur in up to 80% of patients following percutaneous re-treatment with conventional techniques.[4] Although intracoronary brachytherapy has been proven effective in reducing the recurrence rate of *in-stent* restenosis, treatment failure still frequenlty occurs. In a recent study, 60% of patients have been reported to experience at least one major cardiac event up to 4 years after endovascular irradiation (unpublished data).

A large body of evidence has been accumulated in an attempt to understand the processes involved in restenosis. The initial injury caused by the mechanical dilatation and stent implantation triggers a "normal" healing vascular response that ultimately leads to neointimal formation, which, when excessive, may re-narrow the vessel lumen (restenosis). An array of local reparative processes have been shown to occur after the initial vascular trauma, involving platelets, inflammatory cells, smooth muscle cells, endothelial cells, and the secretion of a number of growth factors and cytokines.[5,6]

Based on the accumulated knowledge about the cellular and molecular mechanisms of restenosis, an endless list "concepts", "strategies", devices, and drugs have been tested, and failed, to decrease restenosis.[7] More recently, however, drug-eluting stents have emerged as an effective therapeutic option to reduce the incidence of *in-stent* restenosis. The present chapter will focus on describing the current clinical and pre-clinical information available for drug-eluting stents, as well as the limitations and future research directions for these devices.

Rationale

A proposed explanation for the repeated failure of clinical pharmacological studies with systemically administered drugs is that these agents cannot reach sufficient tissue levels at the site of dilatation without increasing the risk of systemic side effects. In this regard, local administration offers advantages by applying the drug to the precise site of injury, therefore yielding very high concentration of the active agent with low or negligible systemic concentrations.

Utilizing the stent itself as the platform for local drug delivery is an appealing approach. Coronary stents have been extensively proven to be safe and effective in mechanically alleviating coronary obstructions, with predictable and stable short-term results in a wide range of clinical situations. By combining an agent with antiproliferative properties to a "conventional" metallic stent, one is able to preserve the mechanical scaffolding properties of stenting while the active agent is administered to the very spot of vascular injury, with no time delay, with high local doses, and with the potential to control the time (short- vs. long-course) and site (mural vs. luminal) of drug release, among other characteristics.

The delivery vehicles

Coated stents have been subjected to extensive investigations long before being available for implantation in humans. The process of binding pharmacological agents to a metallic mesh has been early recognized to be challenging. The coating should be resistant to mechanical abrasion during the frequently laborious process of stent implantation, and should comply with a number of pharmacological practical requirements, such as drug release in a predictable (dose and time) way and suitability for sterilization. Furthermore, the coating itself should not induce an increased vascular reaction. It should be noted in addition that a potential universal coating is unlikely, and that different pharmacological agents may require different delivery vehicles. Currently, a variety of different formulations have been developed that provide appropriate stent coating for clinical use, including direct drug binding, and coatings with phosphorylcholine, nonerodable or bioabsorbable polymers, or ceramic layers.

The agents

The local agent should be one that inhibits the complex cascade of events that leads to neointimal formation after stent implantation. The inflammatory and proliferative mechanisms of the general tissue healing response and the specific role of blood and vessel wall components on the vascular reparative processes are all potential targets for therapeutic approaches aiming at reducing neointimal proliferation. A variety of potential candidates are available (Table 1) and an increasing number of clinical studies have been conducted to evaluate the efficacy of different eluting-stents.

It is important to recognize that the clinical effect of these devices is highly dependent on each one of the components of the complex platform/vehicle/agent, as well as the interactions among these elements. It is therefore unlikely that a drug-eluting stent "class-effect" might exist, due to the myriad of possible therapeutic combinations. Indeed, different drug-eluting stents have been shown to significantly vary in their ability to reduce restenosis. Indeed, several drug-eluting stents have been already shown ineffective, as summarized in Table 2.

Table 1. Overview of possible anti-restenotic approaches for stent-based strategies

Anti-proliferative	Anti-thrombins	Immunomodulators	Migration inhibitors/ ECM modulators	Promote healing/ endothelialization
Paclitaxel	Hirundin	sirolimus and analogs	Halofuginone	VEGF
QP-2	Iloprost	tacrolimus	Propyl hydroxylase inhibitors	17-β estradiol
Vincristin	Abciximab	Biorest	C-proteinase inhibtors	Tkase inhibitors
Methotrexate		Mizoribine	Metalloproteinase inhibitors	BCP 671
Angiopeptin		Cyclosporin	Batimastat	Statins
Mitomycin		Biorest	Probucol	NO donors
BCP 678		Interferon γ1b		EPC antibody
Antisense c-myc		Leflunomide		
ABT 578		Tranilast		
Actinomycin-D		Cyclosporin		
RestenASE		Corticosteroids		
1-chloro-deoxyadenosine		Micophenolic acid		
PCNA ribozyme		Biphosphonates		
Celecoxib				

Table 2. Failed pharmacologic stent-based strategies to prevent restenosis

Trial	Agent	Vehicle	Stent platform	Reason of clinical failure
SCORE[47]	Taxol derivative QP2 (4000γg)	polymer sleeves	QuaDS-QP2 stent	excessive incidence of stent thrombosis and myocardial infarction possibly due to the polymer sleeves
DELIVER[38]	Paclitaxel (3μg/mm^2)	direct binding	Multi-link penta	lack of efficacy
ACTION[48]	Actinomycin-D (10 and 2.5μg/mm^2)	Polymeric coating	Multi-link tetra	lack of efficacy
BRILLIANT-EU[49]	Batimastat	Phosphorylcholine coating	BiodivYsio stent	lack of efficacy
PRESENT trials[50]	Tacrolimus (60 and 230μg)	Nanoporous ceramic coating	FlexMaster ceramic stent	lack of efficacy
EVIDENT[50]	Tacrolimus (352μg)	PTFE	PTFE-covered stent graft	lack of efficacy
IMPACT[51]	Micophenolic acid (14-day or 45-day release 3.3 (μg/mm^2)	"Unicoat" polymer	Duraflex stent	lack of efficacy

Polymer-coated sirolimus- and paclitaxel-eluting stents have the largest clinical experience to date, with a total of 8 already completed randomized trials comparing the effect of these devices against conventional stents. Sirolimus-eluting stents are available for clinical use in Europe, Asia, and South America since 2002 and in the US since 2003. Polymer-coated paclitaxel-eluting stents have been commercialized in Europe, Asia, and South America since 2003 and are expected to be launched in the US in the beginning of 2004. The main clinical information derived from randomized trials and from other clinical studies including post-marketing registries are detailed below. In addition to sirolimus and paclitaxel stents, an increasing number of other drug-eluting stents have been tested in preliminary clinical trials with promising results and are summarized in the sections below.

Sirolimus

Sirolimus (Rapamycin; Rapamune®) is a naturally occurring macrocyclic lactone with a potent immunosuppressive action, which was approved by the US Food and Drug Administration for use in renal transplant recipients in September 1999. Sirolimus inhibits cellular proliferation by blocking cell cycle progression at the G1 to S transition. Its action is mediated by binding to an intracellular receptor, the FK506 binding protein (FKBP12). The complex rapamycin-FKBP12 then inhibits the activity of a key regulatory kinase denominated mammalian Target Of Rapamycin (mTOR). The inhibition of mTOR suppresses cytokine-driven (IL-2, IL-4, IL-7 and IL-15) T-cell proliferation. Its inhibition has several important effects, including the inhibition of translation of a family of mRNAs

that code for proteins essential for cell cycle progression. The inhibition of mTOR prevents mitogen-induced downregulation of p27Kip1.[8,9] In addition, smooth muscle cell proliferation is shown to be inhibited by rapamycin-FKBP12 in p27Kip1(-/-) knockout mice, suggesting that neointimal inhibition may also operate via a pathway that is independent of p27Kip1.[8] Human neointimal tissue extracted during atherectomy exhibited a peculiar upregulation of FKBP12 at mRNA and protein levels, indicating the potential for sirolimus in preventing coronary restenosis.[10]

Systemic and local administration of sirolimus in porcine models of restenosis have been shown to significantly reduce neointimal hyperplasia.[11,12] Sirolimus-eluting stents have been first implanted in patients with coronary disease in the pioneer First-In-Man experience, which included 45 patients with relatively non-complex *de novo* lesions treated between December 1999 and February 2000.[13-18] Patients were treated in São Paulo, Brazil (n=30), and Rotterdam, The Netherlands (n=15) and received sirolimus-eluting stents in 2 formulations. Both formulations contained 140 µg of sirolimus per cm^2, but with different delivery kinetics (fast-release formulation [<15-day drug release], or slow release formulation [>28-day drug release]). Angiographic *in-stent* restenosis was not detected in any case up to 2 years and only one case had a 52% diameter stenosis proximal to the stent.[13,18] Two-year intravascular ultrasound examination showed only minimal neointimal proliferation, with 6.3±5.5% percent intimal hyperplasia within the stent in the fast release group (São Paulo), 7.5±7.3% in the slow-release group (São Paulo), and 4.4±3.1% in the slow-release group Rotterdam.13,18 Moreover, the 2-year event-free survival was 90.1%, with no extra major events occurring between 2 and 3 years.[16]

Four randomized trials comparing the outcomes of patients treated with sirolimus-stents and conventional bare stents have been concluded to date, and are summarized in Tables 3, 4, 5, 6, and 7).[19-23] The Randomized Study with the Sirolimus-Eluting Bx Velocity Balloon-Expandable Stent in the Treatment of Patients with *de novo* Native Coronary Artery Lesions (RAVEL) trial included 238 patients with single non-complex *de novo* lesions. At six months follow-up, the angiographic restenosis rate of the treated group was zero, the loss in minimal lumen diameter was zero. The clinical outcomes were significantly better among patients treated with sirolimus stents, with 94% of patients being free of any major cardiac events at 1 year (compared to 71% in the bare stent group; p<0.01).[20] Recently, data from the RAVEL trial with a more prolonged follow-up period have shown maintenance of the initial results, with a 2-year event-free survival of 90%.[19]

The subsequent SIRolImUS-Eluting Bx Velocity™ Balloon-Expandable Stent (SIRIUS) trial, which randomized 1101 patients with *de novo* lesions to sirolimus or bare stents, confirmed the clinical efficacy of sirolimus-eluting stents.[21] *In-stent* binary restenosis (within the margins of the stent) was reduced by 91% (3.2% vs. 35.4%; p<0.01) and in-segment restenosis (including the stented portion and the 5mm segments proximal and distal to the stent) was reduced by 75% (8.9% vs. 36.3%; p<0.01).21 At 9 months, the incidence of major adverse events was significantly lower in the sirolimus group (7.1% vs. 18.9%; p<0.01), mainly due to a decrease in the need of target lesion revascularization (4.1% vs. 16.6%; p<0.01). Prolonged follow-up data (up to 2 years) were recently presented and showed sustained benefit of sirolimus-eluting stent implantation in the SIRIUS trial.[24] The recently published E-SIRIUS trial has enrolled 352 patients with longer lesions and smaller vessels than the RAVEL and SIRIUS trials.[23] Nevertheless, the 8-month *in-stent* restenosis rate was 3.9% in the sirolimus and 41.7% in the bare stent group (p<0.01). Similarly, the incidence of in-segment restenosis (5-mm edges included) was significantly reduced (5.9% vs. 42.3%; p<0.01). The 9-month incidence of major cardiac events was 8% vs. 22.6% in the sirolimus and bare groups (p<0.01). Similarly, in the C-SIRIUS trial, which randomized 100 patients to sirolimus or conventional stenting, in-segment restenosis was not detected in any patient after sirolimus-eluting stent implantation.[22] Differently from the previous trials, in the C-SIRIUS direct stenting was allowed (at the discretion of the operator), which did not affect the incidence of restenosis (i.e. zero restenosis rate in patients treated with pre-dilatation or direct stenting). Intravascular ultrasound examination at follow-up further confirmed the marked neointimal inhibition after sirolimus-eluting stent implantation. In the RAVEL trial, the percent neointimal obstruction at 8 months was 1 ± 3% in the sirolimus group versus 29 ± 20% in the bare group (p<0.001).[25] Also, in the SIRIUS study, sirolimus-eluting stents were associated with a significant reduction of *in-stent* percent obstruction (3.1 % vs. 33.4%; p<0.001).[21]

Subgroup analysis of patients included in the RAVEL and SIRIUS have shown that the overall benefit of sirolimus-eluting stents was also observed across many subsets of patients and lesion types.[21,26] However, in the SIRIUS trial, post-sirolimus restenosis was significantly increased in diabetics, long lesions, and small vessels. Indeed, post-sirolimus restenosis has been shown to frequently occur in association with higher complexity characteristics.[27] The impact of sirolimus-eluting stents for the treatment of more complex lesions has been addressed in recently released studies evaluating some patient subgroups not enrolled in the early randomized trials (Table 8).

The Rapamycin Eluting Stent Evaluated At Rotterdam Cardiology Hospital study (RESEARCH) was a single-center registry which included patients treated with SES according to a non-restrictive inclusion criterion. Virtually all consecutive patient subsets were considered eligible. Long-term (12-month) outcomes of the first 508 patients with *de novo* lesions treated exclusively with SES

Table 3. Randomized trials comparing bare metal stents with polymer-coated sirolimus- or paclitaxel-eluting stents - Study characteristics

	Study groups	Design	Inclusion criteria	Exclusion criteria	Antiplatelet
SIROLIMUS					
RAVEL[19,20,26]	Polymer-coated sirolimus-eluting stent (n=120 pts) Bare stent (n=118 pts)	randomized double-blind	*de novo* lesion native vessel single lesion lesion length < 18 mm vessel diameter 3 - 3.5mm	total occlusion ostial thrombus containing lesion unprotected LMC with >50% stenosis evolving myocardial infarction left ventricular ejection fraction <30%	Aspirin lifelong Clopidogrel for 2 months
SIRIUS[21]	Polymer-coated sirolimus-eluting stent (n=533 pts) Bare stent (n=525 pts)	randomized double-blind	*de novo* lesion native vessel single lesion lesion length 15 - 30 mm vessel diameter 2.5 - 3.5mm	total occlusion ostial thrombus containing lesion unprotected LMC with >50% stenosis myocardial infarction < 48 hours left ventricular ejection fraction <25% bifurcation multivessel stenting	Aspirin lifelong Clopidogrel for 3 months
E-SIRIUS[23]	Polymer-coated sirolimus-eluting stent (n=175 pts) Bare stent (n=177)	randomized double-blind	*de novo* lesion native vessel single lesion lesion length 15 - 32 mm vessel diameter 2.5 - 3.0mm	total occlusion ostial thrombus containing lesion unprotected LMC with >50% stenosis evolving myocardial left ventricular ejection fraction <25%infarction bifurcation multivessel stenting	Aspirin lifelong Clopidogrel for 2 months
C-SIRIUS[22]	Polymer-coated sirolimus-eluting stent (n=50 pts) Bare stent (n=50 pts)	randomized double-blind	Single *de novo* coronary lesion Stable or unstable angina or documented silent ischemia Lesion length 15 - 32 mm Vessel diameter 2.5 - 3.0 mm	total occlusion Recent MI (< 24 hours) Unprotected LM disease Ostial location Angiographic evidence of thrombus Pretreatment with devices other than balloon LV ejection fraction < 25%	Aspirin lifelong Clopidogrel for 2 months
PACLITAXEL (polymer-coated)					
TAXUS I[32]	Polymer-coated slow-release paclitaxel-eluting stent (n=31 pts) Bare stent (n=30 pts)	randomized double-blind	Single lesion, single stent Restenotic or *de novo* lesions lesion length ≤12 mm vessel diameter 3.0 - 3.5mm	total occlusion acute myocardial infarction left ventricular ejection fraction <30% stroke < 6 months	Aspirin for at least 12 months Clopidogrel for 6 months
TAXUS II[31]	Polymer-coated slow-release paclitaxel-eluting stent (n=131 pts) Bare stent control for SR-paclitaxel (n=136 pts) Polymer-coated moderate-release paclitaxel-eluting stent (n=135 pts) Bare stent control for MR-paclitaxel (n=137 pts)	randomized double-blind	Single lesion, single stent Native vessel *De novo* lesions lesion length ≤12 mm vessel diameter 3.0 - 3.5mm	total occlusion evolving myocardial infarction unprotected LMC with >50% stenosis left ventricular ejection fraction <30% coronary intervention < 30days	Aspirin lifelong Clopidogrel for at least 6 months
TAXUS III[33]	Polymer-coated slow-release paclitaxel-eluting stent (n=28 pts)	series of cases	Single lesion, single stent Native vessel *In-stent* restenosis lesions lesion length ≤30 mm	total occlusion evolving myocardial infarction left ventricular ejection fraction <30% stroke < 6 months	Aspirin lifelong Clopidogrel for 6 months
TAXUS IV[34,35]	Polymer-coated moderate-release paclitaxel-eluting stent (n=662 pts) Bare stent control for MR-paclitaxel (n=652 pts)	randomized double-blind	Single *de novo* lesions Length 10 - 28 mm vessel diameter 2.5 -3.75mm	total occlusion Prior intervention in the target vessel < 9 months myocardial infarction < 72 hours ostial bifurcation	Aspirin lifelong Clopidogrel for 6 months

NA=not available; pts=patients

Table 4. Randomized trials comparing bare metal stents with polymer-coated sirolimus- or paclitaxel-eluting stents - Patient characteristics

	diabetics (%)	AMI admission (%)	Multivessel disease (%)	LAD (%)	LM (%)
SIROLIMUS					
RAVEL[19,20,26]					
Sirolimus	16	0	NA	49	0
Bare stent	21	0	NA	51	0
SIRIUS[21]					
Sirolimus	25	0	42	44	0
Bare stent	28	0	41	43	0
E-SIRIUS[23]					
Sirolimus	19	0	NA	57	0
Bare stent	27	0	NA	56	0
C-SIRIUS[22]					
Sirolimus	24	0	NA	36	0
Bare stent	24	0	NA	40	0
PACLITAXEL (polymer-coated)					
TAXUS I[32]					
Paclitaxel	23	0	NA	55	0
Bare stent	13	0	NA	27	0
TAXUS II[31]					
Paclitaxel-SR	11	0	NA	40	0
Bare stent-SR	16	0	NA	44	0
Paclitaxel-MR	17	0	NA	42	0
Bare stent-MR	14	0	NA	52	0
TAXUS III[33]	14	0	25	36	4
TAXUS IV[34,35]					
Paclitaxel	23	0	NA	40	0
Bare stent	25	0	NA	41	0

Table 5. Randomized trials comparing bare metal stents with polymer-coated sirolimus- or paclitaxel-eluting stents - Procedural characteristics

	IIbIIIa (%)	ACC/AHA Lesion type C (%)	ISR Mehran Class III or IV[4] (%)	Chronic total occlusion (%)	Stents/pt (%)	Bifurcation stenting (%)
SIROLIMUS						
RAVEL[19,20,26]						
Sirolimus	10.1	0	-	0	1.0 ± 0.3	0
Bare stent	9.5	0	-	0	1.1 ± 0.3	0
SIRIUS[21]						
Sirolimus	60	26	-	0	1.4 ± 0.7	0
Bare stent	59	21	-	0	1.4 ± 0.6	0
E-SIRIUS[23]						
Sirolimus	14	NA	-	0	*	0
Bare stent	18	NA	-	0	*	0
C-SIRIUS[22]						
Sirolimus	58	30	-	0	1.38 ± 0.57†	0
Bare stent	48	16	-	0	1.66 ± 0.94	0
PACLITAXEL (polymer-coated)						
TAXUS I[32]						
Paclitaxel	NA	0	NA	0	NA	0
Bare stent	NA	0	NA	0	NA	0
TAXUS II[31]						
Paclitaxel-SR	12	NA	0	0	‡	0
Bare stent-SR	13	NA	0	0	‡	0
Paclitaxel-MR	21	NA	0	0	‡	0
Bare stent-MR	24	NA	0	0	‡	0
TAXUS III[33]	NA	-	18	4	1.5 ± 0.5	0
TAXUS IV[34,35]						
Paclitaxel	58	20	-	0	1.08 ± 0.29	0
Bare stent	57	22	-	0	1.09 ± 0.36	0

ISR=*in-stent* restenosis; MR=moderate release; NA=not available; SR=slow-release

* overlapping stents in 34% (sirolimus) vs. 31% (bare stent), † p<0.05 vs. control , ‡ One study stent was implanted in 93% of pacltaxel-SR patients and in 94% of the remaining groups

Table 6. Randomized trials comparing bare metal stents with polymer-coated sirolimus- or paclitaxel-eluting stents - Quantitative Angiography Analysis

	Pre-procedure		Post-procedure		Follow-up				
	RD (mm ± SD)	length (mm ± SD)	DS (% ± SD)	MLD (mm ± SD)	time (months)	DS (% ± SD)	MLD (mm ± SD)	Restenosis (%)	Late loss (mm ± SD)
SIROLIMUS									
RAVEL[19,20,26]*									
Sirolimus	2.60±0.54	9.56±3.33	11.9±5.9‡	2.43±0.41	6	14.7±7.0 ‡	2.42±0.49 ‡	0 ‡	-0.01±0.33 ‡
Bare stent	2.64±0.52	9.61±3.18	14.0±6.8	2.41±0.40	6	36.7±18.1	1.64±0.59	26.6	0.80±0.53
SIRIUS[21] †									
Sirolimus	2.79±0.45	14.4±0.58	16.1±9.7	2.38±0.45	8	23.6±16.4 ‡	2.15±0.61 ‡	8.9 ‡	0.24±0.47 ‡
Bare stent	2.81±0.49	14.4±0.58	16.2±8.5	2.4±0.46	8	43.2±22.4	1.60±0.72	36.3	0.81±0.67
E-SIRIUS[23] †									
Sirolimus	2.60±0.37‡	14.9±5.4	18.2±9.6	2.17±0.39	8	24.7±14.7 ‡	1.97±0.48 ‡	5.9 ‡	0.19±0.39 ‡
Bare stent	2.51±0.37	15.1±6.5	18.0±9.1	2.10±0.39	8	48.3±23.4	1.29±0.61	42.3	0.80±0.57
C-SIRIUS[22]*									
Sirolimus	2.65±0.30	14.5±6.3	6.1±9.1	2.53±0.30	8	20.5±10.3	2.15±0.35‡	2.3‡	0.09±0.31‡
Bare stent	2.62±0.35	12.6±5.2	5.6±10.6	2.50±0.28	8	47.8±24.5	1.39±0.69	52.3	0.79±0.74
PACLITAXEL (polymer-coated)									
TAXUS I[32]*									
Paclitaxel	2.99±0.46	10.70±3.27	6.12±9.49	2.95±0.34	6	13.56±11.77‡	2.60±0.49‡	0	0.36±0.48‡
Bare stent	2.94±0.52	11.89±4.93	9.84±7.06	2.87±0.43	6	27.23±16.69	2.19±0.65	10	0.71±0.47
TAXUS II[31] †									
Paclitaxel-SR	2.78±0.44	10.6±3.9	23.1±9.3	2.15±0.37	6	26.8±12.8‡	2.01±0.46‡	5.5‡	0.31±0.38‡
Bare stent-SR	2.77±0.49	10.5±4.1	21.2±8.4	2.23±0.43	6	35.1±15.1	1.70±0.49	20.1	0.79±0.45
Paclitaxel-MR	2.72±0.46	10.2±4.8	21.5±8.0	2.20±0.39	6	26.8±13.1‡	2.00±0.48‡	8.6‡	0.30±0.39‡
Bare stent-MR	2.73±0.45	10.7±4.1	22.0±9.0	2.20±0.40	6	37.1±17.8	1.66±0.56	23.8	0.77±0.50
TAXUS III[33]*§	2.84±1.25	13.61±6.36	16.9±7.6	2.41±0.46	6	26.9±18.6†	1.93±0.61	4.5	0.47±0.48
TAXUS IV[34,35] †									
Paclitaxel	2.76±0.48	14.4±6.7	19.1±9.5	2.26±0.48	9	26.3±15.5‡	2.03±0.55‡	7.9‡	0.23±0.44‡
Bare stent	2.79±0.48	14.4±7.1	19.1±9.9	2.29±0.49	9	39.8±18.5	1.68±0.61	26.6	0.61±0.57

* *In-stent* quantitative coronary angiography, † In-segment quantitative coronary angiography (includes the 5-mm proximal and distal edges)
‡ p<0.05 vs. control, § excludes patients with restenosis in a bare stent or in a gap between the paclitaxel-eluting stents

Table 7. Randomized trials comparing bare metal stents with polymer-coated sirolimus- or paclitaxel-eluting stents - Clinical Outcomes

Study	Follow-up (months)	Death (%)	Myocardial infarction (%)	Repeat revascularization (%)	Any event (%)	Stent thrombosis (%)
SIROLIMUS						
RAVEL[19,20,26]	12					
Sirolimus		1.7	3.3	0*	5.8*	0
Bare stent		1.7	4.2	22.9	28.8	0
SIRIUS[21]	9					
Sirolimus		0.9	2.8	3.8*	7.1*	0.4
Bare stent		0.6	3.2	15.8	18.9	0.8
E-SIRIUS[23]	9					
Sirolimus		1.1	4.6	4.0*	8.0*	1.1
Bare stent		0.6	2.3	20.9	22.6	0
C-SIRIUS[22]	9					
Sirolimus		0	2	4	4	0
Bare stent		0	4	18	18	2
PACLITAXEL (polymer-coated)						
TAXUS I[32]	24					
Paclitaxel		0	0	3	3	0
Bare stent		0	0	10	10	0
TAXUS II[31]	12					
Paclitaxel-SR		0	2.3	10.1*	10.9*	0.7 †
Bare stent-SR		1.5	5.1	15.9	22.0	0
Paclitaxel-MR		0	3.7	6.9*	9.9*	0 †
Bare stent-MR		0	5.2	19.1	21.4	0
TAXUS III[33]	12	0	3.6	21.4	29.0	0
TAXUS IV[34,35]	12					
Paclitaxel		1.4	3.5	6.8*	10.6*	0.6
Bare stent		1.2	4.6	16.7	19.8	0.8

* p<0.05 vs. control, † rates of angiographically documented stent thrombosis

Table 8. Sirolimus-eluting stents for complex subsets

	Design	Restenosis (%)	Late loss (mm)	TVR (%)
SIRIUS bifurcation[52]*§	Randomized: MV stenting + SB stenting (n=43 pts) vs. MV stenting + SB balloon (n=43 pts)	Sirolimus+sirolimus (MV): 6.0 Sirolimus+sirolimus (SB): 24.0 Sirolimus+balloon (MV): 6.2 Sirolimus+balloon (SB): 18.7	0.27±0.47 0.52±0.60 0.14±0.24 0.27±0.38	Sirolimus+sirolimus: 11.1 Sirolimus+balloon: 4.5
Degertekin[53] † ||	Series of cases with ISR (n=16 pts)	13.3	0.26±0.67	6.3
Sousa[54] ‡ ¶	Series of cases with ISR (n=25 pts)	4.0	0.16±0.42	0
Saia 55 * ||	Series of cases with post-brachytherapy ISR (n=12 pts)	40	0.68±1.20	33.3
RESEARCH AMI[56] *§	Series of cases with ST elevation AMI (n=96 pts)	0	-0.04±0.25	0
RESEARCH *de novo*[28] ¶	Consecutive cases treated in 2 phases: bare stents (n= n=450 pts) vs. sirolimus (508 pts)	-	-	Bare stent: 10.9 Sirolimus: 3.7

ISR=*in-stent* restenosis; MI=myocardial infarction; MV=main vessel; SB=side branch; TVR=target vessel revascularization
* angiographic follow-up at 6 months, † angiographic follow-up at 4 months, ‡ angiographic follow-up at 12 months, § clinical follow-up at 6 months, || clinical follow-up at 9 months, ¶ clinical follow-up at 12 months.

were compared with 450 patients treated with bare stents in the period just prior to the introduction of drug-eluting stents.[28] Only 2 (0.4%) presented with thrombotic stent occlusion in the first month after the procedure, while the stent thrombosis rate in the bare stent group was 1.6% (p=0.1). There were no further thrombotic events up to one year. Sirolimus-eluting stents reduced by 38% the 1-year risk of major cardiac events (9.7% vs. 14.8%; p<0.01), mainly due to a risk reduction of 65% in clinically driven repeat intervention (3.7% vs. 10.9%; p<0.01). Importantly, approximately 68% of patients included in the registry would have been excluded from the earlier clinical trials (e.g. patients with previous coronary surgery, patients admitted with acute myocardial infarction, and those with multivessel stenting, among other characteristics).

Paclitaxel

Paclitaxel was originally isolated from the bark of the Pacific Yew. It is an antineoplastic agent that is currently used to treat several types of cancer, most commonly breast and ovarian cancer. Paclitaxel exerts its pharmacological effects through formation of numerous decentralized and unorganized microtubules. This enhances the assembly of extraordinarily stable microtubules, interrupting proliferation, migration and signal transduction.[29,30] Unlike other anti-proliferative agents of the colchicine type, which inhibit microtubuli assembly, paclitaxel shifts the microtubule equilibrium towards microtubule assembly. It is highly lipophylic, which promotes a rapid cellular uptake, and has a long-lasting effect in the cell due to the structural alteration of the cytoskeleton.

Stent-based paclitaxel has been investigated by several groups, using different stent types and preparations (copolymer coatings for paclitaxel elution[31-35] or direct dip-coating of paclitaxel on a stainless steel stent).[36-39] Clinical studies utilizing direct dip-coating of paclitaxel stents are summarized in Tables 9 and 10. Contradictory clinical results have been obtained with these devices. While the European Evaluation of Paclitaxel Eluting Stent trial (ELUTES)[36] and the ASian Paclitaxel-Eluting stent Clinical Trial (ASPECT)[37] have shown a significant, dose-dependent reduction in restenosis with paclitaxel stents, the larger RX Achieve™ Drug-Eluting Coronary Stent System In the Treatment of Patients With *de novo* NativE CoronaRy Lesions (DELIVER-I) study failed in demonstrating the beneficial effect of these devices.

Clinical studies utilizing polymer-coated paclitaxel-eluting stents are summarized in Tables 3, 4, 5, 6, and 7.[31-35] In total, more than 1,900 patients with *de novo* lesions have been enrolled in the TAXUS I,32 II,31 and IV trials[34,35] and randomized to paclitaxel or bare stents. A marked reduction in neointimal proliferation and binary restenosis was observed in the active groups, leading to 12-month target lesion revascularization rates that ranged from 0 to 6.8% with paclitaxel stents, which was significantly lower than in controls (TLR rates from 10.0 to 16.7%).[31,32,34,35] Multivariate analysis from patients included in the TAXUS IV trial[34,35] have identified several multivariate predictors of 9-month target lesion revascularization. Apart from utilization of bare stents (OR 4.58 [95% CI: 2.64, 7.95]; p<0.0001), other independent predictors were: diabetes (1.78 [95% CI: 1.10, 2.88]; p=0.02), increase in stent length (1.04 [95% CI: 1.01, 1.07]; p=0.006), decrease in acute gain (2.08 [95% CI: 1.11, 3.88]; p=0.02), and lesion angulation (0.98 [95% CI: 0.97, 0.99]; p<0.03). Polymer-coated paclitaxel-eluting stents were shown to be safe, with rates of subacute stent thrombosis similar to those seen in the bare stents (Table 7).

Table 9. Clinical Studies with Dip-Coated Paclitaxel-eluting Stents - Study Design

	Study groups	Design	Inclusion criteria
ELUTES[36]	2.7 µg/mm^2 paclitaxel- (n=37 pts) 1.4 µg/mm^2 paclitaxel (n=39 pts) 0.7 µg/mm^2 paclitaxel (n=39 pts) 0.2 µg/mm^2 paclitaxel (n=37 pts) Bare stent (n=38 pts)	randomized	*de novo* lesion native vessel single lesion
ASPECT[37]	3.1 µg/mm^2 paclitaxel (n=60 pts) 1.3 µg/mm^2 paclitaxel (n=58 pts) Bare stent (n=59 pts)	randomized	Single lesion lesion length <15 mm vessel diameter 2.5 - 3.5mm
DELIVER I [38]	3.0 µg/mm^2 paclitaxel (n=522 pts) Bare stent (n=519 pts)	randomized	Up to 2 native vessels treated (1 target and 1 non-target, with only 1 *de novo* lesion per vessel) lesion length <25 mm vessel diameter 2.5 - 4.0 mm
DELIVER II[39]	3.0 µg/mm^2 paclitaxel (n=1531 pts)	series of cases	One of the following: - 1 target lesion: length < 25 mm, chronic total or subtotal occlusion, restenotic, or in a bifurcation site - 1 target lesion: length > 25 mm, *de novo*, chronic total or subtotal occlusion, restenotic, or involving a bifurcation site - 2 target lesions: length < 25 mm, *de novo*, chronic total or subtotal occlusion, restenotic, or in a bifurcation site

CTO=chronic total occlusion; NA=not available; pts=patients

Table 10. Clinical Studies with Dip-Coated Paclitaxel-eluting Stents - Angiographic and Clinical Outcomes

	Restenosis (%)	Late loss (mm ± SD)	Clinical follow-up (months)	Death (%)	Myocardial infarction (%)	Target vessel revascularization (%)	Any event (%)	Stent thrombosis (%)
ASPECT[37]* †			6					
3.1 µg/mm^2 paclitaxel	4‡	0.29±0.72‡		0	3.4	1.7	10	5.1
1.3 µg/mm^2 paclitaxel	12‡	0.57±0.71‡		1.7	1.7	1.7	7	1.7
Bare stent	27	1.04±0.83		0	1.7	1.7	5	0
ELUTES[36] §			12					
2.7 µg/mm^2 paclitaxel	3.1	0.10±0.12 ‡		2.7	2.7	5.4	14	2.7
1.4 µg/mm^2 paclitaxel	13.5	0.47±0.11		0	0	10.3	10	0
0.7 µg/mm^2 paclitaxel	11.8	0.47±0.12		0	0	7.7	10	0
0.2 µg/mm^2 paclitaxel	20.0	0.72±0.12		0	0	5.4	5	0
Bare stent	20.6	0.73±0.12		0	0	15.8	18	2.6
DELIVER I[38] * ||			12					
3.0 µg/mm^2 paclitaxel	16.7	0.43‡		0.2	1.4	6.2	7.5	0.4
Bare stent	22.4	0.56		0.8	1.0	6.8	9.4	0.4
DELIVER II[39]	-	-	6	2.3	4.9	9.6	15.7	NA

NA=not available

* In-segment quantitative coronary angiography (includes the 5-mm proximal and distal edges)

† all stent thrombosis events occurred in patients on aspirin and cilostazol (instead of aspirin and ticlopidin/clopidogrel)

‡ p<0.05 vs. control

§ *In-stent* quantitative coronary angiography

|| available for 228 pts in the paclitaxel group and fro 214 controls

The WISDOM registry is a post-marketing registry conducted with the objective of evaluating unselected patients treated with paclitaxel stents in the "real world". Preliminary results of this multinational registry on approximately 1,000 patients have been presented in the AHA annual meeting in November 2003 and confirmed the 30-day safety of paclitaxel-eluting stents in more complex patients, with only 0.4% of patients presenting stent thrombosis.

Other Drugs

To date, sirolimus- and paclitaxel-eluting stents have the most extensive accumulated clinical experience and are the only drug-eluting stents commercially available for clinical use. However, a myriad of new devices have been recently tested and are currently in various stages of development for clinical use. New drug-eluting stents that have been already evaluated in preliminary clinical studies are summarized in Table 11. Several analogues of sirolimus have been investigated.

Limitations

A late "catch up" phenomenon has been described after implantation of a high dose (800 µg) paclitaxel derivative QP2-eluting stent, with the restenosis rate increasing from 13% at 6 months to 62% at 12 months.[40,41] However, long-term efficacy should be evaluated separately for each drug-eluting stent assembly, since a "class-effect" is unlikely for these devices and each platform/vehicle/agent complex should be evaluated separately. To date, the sirolimus-eluting stent has the largest body of long-term data available. The First In Man study has shown persistent positive results up to 2 and 3 years, without any evidence of late catch-up restenosis.[16,18] In the RAVEL[19] and SIRIUS[24] trials, no further events due to restenosis were observed up to 2 years. With paclitaxel, no rebound effect was seen from 6 to 12 months in TAXUS-I,[32] TAXUS II,[31] TAXUS IV,[34,35] ELUTES,[36] and ASPECT[37] trials.

In the RAVEL trial, stent malapposition (as observed by intravascular ultrasound) was more frequent at 6 months in

Table 11. Clinical studies with new drug-eluting stents

	design	inclusion criteria	Study groups	Restenosis (%)	Late loss (mm)
EVELOLIMUS					
FUTURE I trial[57]	randomized (2:1) single-blind	Single *de novo* lesions Length <18 mm vessel diameter 2.75 -4.0mm	Bioabsorbable polymer-coated everolimus-eluting stent (n=27 pts)	0	0.11*
			Bare stent control for MR-paclitaxel (n=15 pts)	9.1	0.85
FUTURE II trial[57]	randomized (1:2) single-blind	Single *de novo* lesions Length <18 mm vessel diameter 2.75 -4.0mm	Bioabsorbable polymer-coated everolimus-eluting stent (n=21 pts)	0	0.12*
			Bare stent control for MR-paclitaxel (n=43 pts)	19.4	0.85
17β-ESTRADIOL					
EASTER trial	series of cases	Single, *de novo* lesions Vessel size 3.0 mm to 4.0 mm	Phosphorylcholine-coated estrogen-eluting stent (2.54 µg/mm^2) (n=30 pts)	6.6	0.31
DEXAMETHASONE					
STRIDE trial	series of cases	Single, *de novo* lesions Vessel size 2.75 mm to 4.0 mm	BiodivYsioMatrix LO stent immersed in dexamethasone solution (15mg/ml) (n=71 pts)	13.3	0.45
ABT-578					
ENDEAVOR trial	series of cases	Single, *de novo* lesions Vessel size 3.0 mm to 3.5 mm	Phosphorylcholine-coated Driver cobalt alloy stent (n=100 pts)	NA	0.20
Nitic Oxide drug-elution					
NOBLESSE trial	series of cases	Single, *de novo* lesions Vessel size 2.75 mm to 3.5 mm	Bioabsorbable polyesteramide-coated Genic stent with oxigen free scavenger covalently bounded (n=45 pts)	9.5	0.69

*p<0.05 compared to controls

sirolimus-eluting stent patients than in the control arm[25]. Moreover, in SIRIUS[42] late acquired stent malapposition was more commonly observed in the sirolimus group. However, in TAXUS-II[31] patients treated with bare stents or paclitaxel-eluting stents there were similar rates of late-acquired malapposition. Nevertheless, these observations of late malapposition by ultrasound have not been associated with any adverse events throughout the follow-up period in any of these studies.[25,31,42-44] Also, late thrombotic stent occlusion was not seen to be more frequent in patients treated with sirolimus- or paclitaxel eluting stents, even after clopidogrel discontinuation.

The costs of currently marketed drug-eluting stents (i.e. sirolimus- and paclitaxel-eluting stents) have been perceived as a major limitation for a more widespread use of these devices. In an analysis from the RAVEL trial, the utilization of the sirolimus-eluting stent resulted in a mean additional procedural cost of 1,286 €, as compared to the control group based on costs in the Netherlands.[45] However, due to the decrease in re-interventions attributable to the sirolimus-eluting stent at the end of the first year of follow-up the estimated cost difference had decreased to 54 €. In other words, in the RAVEL trial the reduction of major event risk from 28.8% to 5.8% after sirolimus-eluting was accomplished at an extra cost of 54 € per patient. Moreover, data from the the SIRIUS trial have shown that at 1 year the costs of sirolimus-eluting stent implantation were approximately US$ 300 higher per patient. In the SIRIUS, investigators have reported a ratio of approximately US$1700 per repeat revascularization avoided, which has been considered to compare favorably with other medical treatments for patients with cardiovascular disease.[46] Obviously, the cost and effect estimations derived from the RAVEL and SIRIUS trial cannot be directly extrapolated to other situations and formal analyses from other clinical scenarios are warranted.

Future Directions

As restenosis rates are still not "zero" in the real world of interventional cardiology, the search for and testing of new drugs will continue. Next to newer drugs and optimalization of the drug carrier, other methods are currently being investigated to locally treat the injured vessel wall. For example, stent-based delivery of adenoviral gene vectors was recently achieved *in vitro* and in carotid arteries of rats. Though stent-based local gene delivery has not entered the clinical arena yet, it will be possible very soon and give a lot of new opportunities to attack coronary artery disease and the problems of restenosis. Another possibility to contain the detrimental effects of vessel wall injury after percutaneous interventions is to restore the integrity of the endothelial cell lining as soon as possible. This way, the attraction of inflammatory cytokines as well as activated platelets and macrophages can be limited.

The most logical way to accomplish this is endothelial cell seeding of the stent. The problems of sterilization, mechanical stretch during implantation and endothelial cell viability have been the major limitations in this approach. Recently, a method has been developed to coat a stent with antibodies to CD-34 receptors on progenitor endothelial cells. In this way circulating endothelial cells can be captured from the circulation and provide an early re-endotheliazation on the stent surface. After promising animal data showing a confluent layer of endothelial cells very early after stenting, clinical pilot trials are on-going.

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Chapter 13

Short- and long-term clinical benefit of sirolimus-eluting stents compared to conventional bare stents for patients with acute myocardial infarction

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EXPRESS PUBLICATION

Short- and Long-Term Clinical Benefit of Sirolimus-Eluting Stents Compared to Conventional Bare Stents for Patients With Acute Myocardial Infarction

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OBJECTIVES This study investigated the clinical outcomes of patients with ST-segment elevation myocardial infarction (MI) treated with sirolimus-eluting stents (SESs) or with conventional bare stents.

BACKGROUND The clinical impact of SES implantation for patients with ST-segment elevation MI is currently unknown.

METHODS Primary angioplasty was performed with SESs in 186 consecutive patients with acute MI who were compared with 183 patients treated with bare stents. The incidence of death, reinfarction, and repeat revascularization was assessed at 30 and 300 days.

RESULTS Postprocedure vessel patency, enzymatic release, and the incidence of short-term adverse events were similar in both the sirolimus and the bare stents (30-day rate of death, reinfarction, or repeat revascularization: 7.5% vs. 10.4%, respectively; p = 0.4). Stent thrombosis was not diagnosed in any patient in the sirolimus group and occurred in 1.6% of patients treated with bare stents (p = 0.1). At 300 days, treatment with SESs significantly reduced the incidence of combined adverse events (9.4% vs. 17%; hazard ratio [HR] 0.52 [95% confidence interval (CI) 0.30 to 0.92]; p = 0.02), mainly due to a marked reduction in the risk of repeat intervention (1.1% vs. 8.2%; HR 0.21 [95% CI 0.06 to 0.74]; p = 0.01).

CONCLUSIONS Compared to conventional bare stents, the SESs were not associated with an increased risk of stent thrombosis and were effective in reducing the incidence of adverse events at 300 days in unselected patients with ST-segment elevation acute MI referred for primary angioplasty. (J Am Coll Cardiol 2004;43:704–8) © 2004 by the American College of Cardiology Foundation

Routine stent implantation has been advocated for patients with acute myocardial infarction (MI) referred for primary angioplasty, with superior results compared to balloon dilation (1–3). However, the late clinical efficacy is still hampered by the occurrence of in-stent restenosis and the need for repeat intervention.

Sirolimus-eluting stents (SESs) have proven to be effective in reducing late restenosis compared to conventional stenting in elective patients (4–6). We have recently shown in a relatively small consecutive series of cases that SES implantation in patients with acute MI was safe and associated with an extremely low (zero) incidence of angiographic restenosis at six months (7). However, the clinical benefit of SESs in comparison to conventional stent implantation remains currently unknown. Therefore, we evaluated the long-term clinical outcomes of a large series of patients with acute MI treated with primary angioplasty utilizing either SESs or conventional metal stents.

METHODS

Since April 2002, SES implantation (Cypher, Johnson & Johnson-Cordis unit, Cordis Europa NV, Roden, The Netherlands) has been utilized as the strategy of choice for patients treated with percutaneous intervention in our institution (8). Up until January 2003, a total of 186 consecutive patients with ST-segment elevation acute MI have been treated with primary angioplasty utilizing exclusively SESs and were included in the present report. The first 89 patients of the present series were included in an angiographic substudy, of which the results have been reported previously (7). A control group for comparison was composed of 183 consecutive patients with ST-segment elevation acute MI treated with conventional bare stents in the period immediately before the introduction of SESs. The following bare metal stents were used: BX Sonic or BX Velocity in 53% (Cordis, Johnson & Johnson, Warren, New Jersey); Multi-Link Penta in 22% (Guidant Corp., Santa

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Abbreviations and Acronyms

CI = confidence interval
CK = creatine kinase
HR = hazard ratio
MI = myocardial infarction
SES = sirolimus-eluting stent
TIMI = Thrombolysis In Myocardial Infarction

Clara, California); Multi-Link Tetra in 6% (Guidant Corp.); R-Stent in 6% (Orbus Medical Technologies, Fort Lauderdale, Florida), and other stents in 12%. In both study phases, all patients were enrolled regardless of the clinical or anatomical presentation, including patients admitted with cardiogenic shock (defined as persistent systolic blood pressure <90 mm Hg, or the need of vasopressors or intra-aortic balloon pumping required to maintain blood pressure >90 mm Hg with evidence of end-organ failure and elevated left ventricular filling pressures). Therefore, the total study population comprised all 369 consecutive patients with ST-segment elevation acute MI undergoing primary angioplasty with either bare stents or SESs in the two study phases, respectively. Patients with angioplasty after failed thrombolytic therapy were excluded from the present analysis. This study protocol was approved by the local ethics committee, and written informed consent was given by every patient.

The final interventional strategy, as well as the utilization of periprocedural glycoprotein IIb/IIIa inhibitors and antithrombotic medications, was entirely left to the discretion of the operator. Baseline and postprocedure anterograde flow were evaluated off-line according to the Thrombolysis In Myocardial Infarction (TIMI) criteria (9) by cardiologists blinded to both the stent group and to the clinical outcomes. Clopidogrel was recommended for at least one month in the control group. In the SES group, clopidogrel was prescribed for three months, unless one of the following was present (in which case clopidogrel was maintained for at least six months): multiple SES implantation (>3 stents), total stented length >36 mm, bifurcation stenting, and in-stent restenosis.

Patients were prospectively followed for the occurrence of major adverse cardiac events: 1) all-cause death, 2) nonfatal MI, or 3) target vessel revascularization. Reinfarction was diagnosed by recurrent symptoms and/or new electrocardiographic changes in association with re-elevation of the creatine kinase (CK) and CK-MB levels of >1.5 times the previous value, if within 48 h, or >3 times the upper normal limit, if after 48 h from the index infarction (1,7). Target vessel revascularization was defined as a repeat intervention (surgical or percutaneous) driven by any lesion located in the same epicardial vessel treated at the index procedure. Thrombotic stent occlusion was angiographically documented as a complete occlusion (TIMI flow grade 0 or 1) or a flow-limiting thrombus (TIMI flow grade 1 or 2) of a previously successfully treated artery. Routine angiographic follow-up was obtained only for patients treated with SESs enrolled during the first six months; results of this subanalysis have been previously reported (7).

Continuous variables were presented as mean ± standard deviation, and were compared using the Student unpaired *t* test. Categorical variables were presented as counts and percentages and compared with the Fisher exact test. Survival free of adverse events was estimated using the Kaplan-Meier method and differences between curves were evaluated by the log-rank test. Cox proportional hazards survival models were used to assess risk reduction. Multivariate analyses were performed to identify independent predictors of long-term major adverse cardiac events. Baseline and procedural characteristics associated with the incidence of adverse events at univariate analysis (p value for selection ≤0.2) were tested for their multivariate predictive value (tested variables: SES utilization, diabetes, cardiogenic shock, multivessel disease, culprit vessel, pre-procedure TIMI flow, postprocedure TIMI flow, current smoking). The final model was built by backward stepwise variable selection with an entry and exit criteria set at the $p = 0.05$ and $p = 0.1$ levels, respectively.

RESULTS

Baseline characteristics were similar between both study groups, except by an older age and a lower incidence of previous MI in the sirolimus group (Table 1). Procedural characteristics differed between both groups in terms of the utilization of glycoprotein IIb/IIIa inhibitors (sirolimus: 37% vs. bare stents: 56%; $p < 0.01$) and the number of stents implanted (sirolimus: 1.9 ± 1.2 vs. bare stents: 1.7 ± 1.0; $p = 0.03$). As defined by the study protocol, the duration of clopidogrel prescription was longer for patients with sirolimus stents (Table 1).

No significant differences existed in the 30-day outcomes between patients treated with sirolimus or bare stents (Table 2). Stent thrombosis was diagnosed in three patients (1.6%) treated with bare stents and was not detected in the SES group ($p = 0.1$) (Table 2).

At 300 days, no differences were noted between both study groups in the incidence of death and death or reinfarction (Table 2). However, the incidence of 300-day major adverse events was significantly lower in the sirolimus stent group compared to the bare stent group (9.4% vs. 17%, respectively; hazard ratio [HR] 0.52 [95% confidence interval (CI) 0.30 to 0.92]; $p = 0.02$) (Table 2, Fig. 1), mainly due to a marked reduction in the risk of repeat intervention (1.1% vs. 8.2%, respectively; HR 0.21 [95% CI 0.06 to 0.74]; $p = 0.01$). A multivariate analysis was performed to adjust for baseline and procedural imbalances between the study groups (Table 3). Sirolimus-eluting stent utilization was identified as an independent predictor of 300-day death, reinfarction, or repeat revascularization (HR 0.53 [95% CI 0.29 to 0.95]; $p = 0.03$).

Table 1. Baseline and Procedural Characteristics of Patients Treated With Bare Stents or SES Implantation

	Bare Stents (n = 183)	SES (n = 186)	p Value
Male (%)	79	75	0.4
Age, yrs ± SD	57 ± 12	60 ± 12	0.04
Diabetes (%)	12	11	0.9
Current smoking (%)	47	46	0.8
Previous myocardial infarction (%)	24	14	0.03
Previous angioplasty (%)	9	7	0.4
Previous bypass surgery (%)	3	2	0.3
Coronary disease			0.3
Single-vessel (%)	48	55	
Double-vessel (%)	29	27	
Triple-vessel (%)	24	18	
Cardiogenic shock (%)	10	13	0.3
Time from symptom onset to angioplasty, h ± SD	3.0 ± 2.7	3.2 ± 1.9	0.6
Infarct-related vessel			0.3
Right coronary artery (%)	30	37	
Left anterior descending (%)	57	53	
Left circumflex artery (%)	10	8	
Left main coronary artery (%)	1	2	
Bypass graft (%)	2	—	
TIMI flow baseline			0.7
Grade 0/I (%)	73	73	
Grade II (%)	15	17	
Grade III (%)	13	10	
TIMI flow after angioplasty			0.5
Grade 0/I (%)	4	2	
Grade II (%)	17	15	
Grade III (%)	79	83	
Number of stents ± SD	1.7 ± 1.0	1.9 ± 1.2	0.03
Glycoprotein IIb/IIIa inhibitor (%)	56	37	<0.01
Clopidogrel prescription, months ± SD	2.1 ± 1.5	3.7 ± 2.1	<0.01
Peak CK, IU/l ± SD*	3,957 ± 5,135	3,126 ± 3,126	0.1
Peak CK-MB, IU/l ± SD†	319 ± 230	296 ± 255	0.5

*Upper limit of normal 199 IU/l. †Upper limit of normal 23 IU/l.
CK = creatine kinase; SD = standard deviation; SES = sirolimus-eluting stents; TIMI = Thrombolysis In Myocardial Infarction.

DISCUSSION

The main finding of the present study was that SES implantation was effective in reducing the incidence of adverse events at 300 days in unselected patients with ST-segment elevation acute MI, compared to conventional bare stenting. Furthermore, the risk of subacute thrombosis within the first 30 days did not appear higher compared with bare metal stents. Sirolimus-eluting stents were asso-

Table 2. Kaplan-Meier Estimates of Adverse Events at 30 Days and at 300 Days

	Bare Stents (n = 183)	SES (n = 186)	p Value
30-Day outcomes			
Death (%)	5.5	5.9	1.0
Death or nonfatal reinfarction (%)	7.1	6.5	0.8
Target vessel revascularization (%)	4.4	1.1	0.1
Any event (%)	10.4	7.5	0.4
Stent thrombosis (%)*	1.6	0	0.1
300-Day outcomes			
Death (%)	8.2	8.3	0.8
Death or nonfatal reinfarction (%)	10.4	8.8	0.5
Target vessel revascularization (%)	8.2	1.1	<0.01
Any event (%)	17.0	9.4	0.02

*Angiographically documented stent thrombosis.
SES = sirolimus-eluting stents.

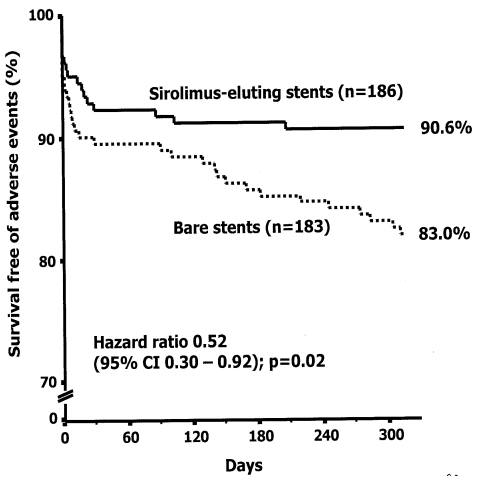

Figure 1. Survival free of reinfarction or target vessel revascularization in the sirolimus-eluting stent and conventional stent groups. CI = confidence interval.

Table 3. Multivariate Predictors of 300-Day Major Adverse Cardiac Events

	Hazard Ratio	95% Confidence Interval	p Value
SES utilization	0.53	0.29–0.95	0.03
Cardiogenic shock	3.31	1.72–6.34	<0.01
Culprit vessel left main coronary	6.05	1.60–22.87	<0.01
Culprit vessel left anterior descending	2.02	1.10–3.71	0.02
Postprocedure TIMI flow grade			<0.01
Grade 0/I (reference)	1.00	—	
Grade II	0.29	0.11–0.76	
Grade III	0.17	0.07–0.40	
Current smoking	0.57	0.31–1.02	0.06

SES = sirolimus-eluting stents; TIMI = Thrombolysis In Myocardial Infarction.

ciated with a relative reduction of 48% in the risk of death, reinfarction, or repeat intervention and a relative reduction of 79% in the risk of repeat intervention at 300 days.

In our series, reperfusion treatment with SESs was associated with similar rates of vessel patency, enzymatic release, and 30-day complications compared to bare stents. The death rate and the incidence of death or reinfarction were similar in both study groups, but somewhat higher than those reported in randomized trials with selected patients (1,2). These findings most probably reflect the unrestrictive inclusion criteria of our series (10), which frequently enrolled patients not included in randomized studies, as, for instance, cardiogenic shock, multivessel disease, and unprotected left main lesions. Importantly, stent thrombosis has not been identified in any patient treated with sirolimus stents and occurred in three controls (1.6%), with no statistical difference between the groups. Although the incidence of stent thrombosis in the bare stent group was at a somewhat higher range, our results in this group were not discrepant from historical series with conventional stents (1,2,11–13).

Coronary stenting for the treatment of acute MI has been limited by the need of late repeat intervention, which has been reported to occur in approximately 9% of cases at six months, ranging from 3.6% to 22.7% (1–3). The incidence of repeat intervention after conventional stenting in our series (8.2%) was in line with these previous figures. Conversely, patients treated with SES implantation clearly had a reduced risk of reintervention at 10 months. Of note, between 30 days and 10 months, no additional patient was referred for repeat revascularization, which is consistent with the lack of angiographic restenosis after sirolimus stent implantation, as previously shown in a subset of patients from the present population (7).

The peri- and postprocedural antiplatelet therapeutic scheme differed between patients treated with either bare or sirolimus stents in our series. Patients in the sirolimus group received fewer glycoprotein IIb/IIIa inhibitors but had a longer clopidogrel prescription time. However, none of these characteristics were identified as independent predictors influencing the outcomes of patients. The impact of clopidogrel and glycoprotein IIb/IIIa inhibitors on the long-term clinical outcomes of patients with ST-segment elevation acute MI remains to be established (2,14,15).

Conclusions. Sirolimus-eluting stent implantation for unselected patients with ST-segment elevation acute MI was associated with similar procedural and 30-day outcomes compared to bare stents, but markedly reduced the risk of major adverse events and repeat intervention at 10 months. By providing effective mechanical reperfusion with similar results to the current therapeutic standard, and decreasing the incidence of late complications, SESs appeared as an attractive approach for patients admitted with acute MI. The promising results of the present study warrant further confirmation in the context of a randomized trial.

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Chapter 14

One Year clinical follow-up of Paclitaxel-eluting stents for Acute Myocardial Infarction compared to Sirolimus-eluting stents

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Submitted

One Year clinical follow-up of Paclitaxel-eluting stents for Acute Myocardial Infarction compared to Sirolimus-eluting stents

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Brief Title: Paclitaxel-eluting stents in myocardial infarction

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Abstract

Objective

Comparison of clinical outcome of paclitaxel-eluting stents (PES) versus sirolimus-eluting stents (SES) for the treatment of acute ST-elevation myocardial infarction.

Design and Patients

The first 136 consecutive patients treated exclusively with PES, in the setting of primary PCI for acute myocardial infarction in our single centre registry were prospectively clinically assessed at 30 days and 1 year follow-up and compared with 186 consecutive patients treated exclusively with SES, in the preceding period.

Setting

Academical tertiary referral center.

Results

At 30 days, all-cause mortality and or re-infarction was similar between groups (6.5% vs. 6.6% for SES and PES respectively, p = 1.0). A significant difference in target vessel revascularization (TVR) was seen in favour of SES (1.1% vs. 5.1% for PES, p = 0.04). This was driven by stent thrombosis (n=4), especially in bifurcation stenting (n=2). At one year, no significant differences were seen between groups, with no late thrombosis and 1.5 % in-stent restenosis (needing TVR) in PES, versus no re-interventions in SES (p = 0.2). One-year survival free of major cardiac events (MACE) was 90.2 % for SES and 85 % for PES (p = 0.16).

Conclusions

No significant differences were seen in MACE-free survival at one year between SES and PES for the treatment of acute myocardial infarction with very low rates of reintervention for restenosis. Bifurcation stenting in acute myocardial infarction should, if possible, be avoided due to the increased risk of stent thrombosis.

Introduction

The efficacy of drug-eluting stents to treat coronary artery stenosis in stable patients has been proven in recent trials with single digit restenosis rates for non-complex lesions[1] [2] [3] [4].

The potential risk of higher thrombogenicity however, has led to prolonged anti-platelet therapy and cautious use in acute coronary syndromes. We have recently shown that the use of sirolimus-eluting stents (SES) for acute myocardial infarction is safe and not associated with higher thrombogenicity[5]. The safety and efficacy of paclitaxel-eluting stents (PES) in this setting has not been reported yet.

A recent meta-analysis clearly showed the benefit of primary percutaneous coronary intervention (PCI) over thrombolytic therapy for the treatment of acute myocardial infarction[6] and the superiority of (bare metal) stenting over balloon angioplasty has been well documented in acute myocardial infarction[7].

We report the one year clinical outcome of a consecutive patient cohort treated solely with PES in the setting of primary PCI for acute ST-elevation myocardial infarction and compared this with an earlier published patient population treated with SES.

Methods

Patients

Since February 16, 2003, PES (Taxus, Boston Scientific, Galway, Ireland) has been implemented in our hospital as the default stent for all patients. Data was collected for the Taxus-Stent Evaluated At Rotterdam Cardiology Hospital (T-SEARCH) Registry. This is a prospective single-centre registry set up with the main purpose of evaluating the safety and efficacy of PES implantation for patients treated in daily practice. Up until September 2003, 134 consecutive patients received exclusively PES in the setting of primary PCI for acute myocardial infarction. All patients were enrolled in the analysis including patients in cardiogenic shock (defined as persistent systolic blood pressure< 90 mm Hg, or the need for vasopressors or intra-aortic balloon pumping required to maintain blood pressure > 90 mm Hg with evidence of end-organ failure and elevated left ventricular filling pressures). Patients who underwent rescue PCI after failed thrombolysis were not included in this study.

One year clinical outcome was compared with the one year data from the first 186 patients treated exclusively with SES in the setting of primary PCI for acute myocardial infarction in 2002, when SES was the default stent in our center[5].

This study protocol was approved by the local ethics committee, and written informed consent was obtained from every patient.

Treatment strategy and definitions

The interventional strategy and use of IIb/IIIa inhibitors was entirely left to the discretion of the operator. Clopidogrel was recommended for 6 months, in addition to lifelong acetylsalicylic acid 80 mg (ASA). The loading dose of 300 mg clopidogrel was given prior to the intervention. If the patient was not on ASA, 250 mg of intravenous ASA was given at the start of the procedure.

The occurrence of major adverse cardiac events (MACE) was evaluated at 1 year. MACE were: a) all-cause mortality, b) non-fatal myocardial infarction c) target lesion and/or target vessel revascularization.

Re-infarction was defined as new symptoms and/or new electrocardiographic changes in association with re-elevation of the CK-MB levels of 1.5 times the previous value, if within 48 hours, or > 3 times the upper normal limit, if > 48 hours from the index infarction. Target lesion revascularization(TLR) was defined as a repeat intervention (surgical or percutaneous) to treat a luminal stenosis within the stent or in the 5-mm distal or proximal segments adjacent to the stent. Target vessel revascularization(TVR) was defined as a repeat intervention, driven by any lesion located in the same epicardial vessel treated at the index procedure. Thrombotic stent occlusion was angiographically documented as a complete occlusion (TIMI flow 0 or 1) or a flow-limiting thrombus (with TIMI flow 1 or 2) of a previously successfully treated artery.

Follow-up

Clinical follow-up was performed for all patients, while repeat angiography was clinically driven by symptoms or signs of ischemia. Information about in-hospital outcomes was obtained from our institutional electronic clinical database and by review of the hospital records for those discharged to referring hospitals (patients were referred from a total of 14 local hospitals). Post-discharge survival status was obtained from the Municipal Civil Registries at 1, 6 and 12 months. All repeat interventions (surgical and percutaneous) and re-hospitalizations were prospectively collected during follow-up. Questionnaires regarding anginal status and medication use were sent to all living patients at 6 and 12 months. Referring physicians and institutions were contacted for additional information if required.

Statistical Analysis

Continuous variables are presented as mean ± SD and were compared using Student's unpaired *t*- test. Categorical variables are presented as counts and percentages and compared using Fisher's exact test. All statistical tests were 2-tailed. The cumulative incidence of adverse events was estimated according to the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards survival models were used to assess risk reduction. Multivariate analyses were performed to identify independent predictors of long-term major adverse cardiac events. Significant baseline and procedural characteristics at univariate analysis (tested variables: age, diabetes, cardiogenic shock, multi-vessel disease, left main stem as the infarct related artery, post procedure TIMI flow, bifurcation treatment, multi-vessel treatment, duration of pain); gender and stent type were tested for their multivariate predictive value. The first model was built by backward stepwise variable selection with the exit criteria set at the p=0.1 level; the final model built by forcing stent type together with all significant predictors.

Results

In total 136 patients were treated with PES only, in the setting of primary PCI for acute myocardial infarction in the study period. These patients were compared to 186 patients treated with SES for the same indication in the period before our centre switched to PES as the default strategy. Follow-up of the 186 patients with SES from our earlier report[5] was extended from 300 days to 1 year for the comparison.

Baseline characteristics are depicted in table 1. PES patients had less diabetes (3.7 vs. 10.8 %, p = 0.02), larger nominal stent size (3.11 vs. 2.89 mm, p <0.001), and a higher percentage of peri-procedural glycoprotein IIb/IIIa inhibitor use (55.1 vs. 36.6 %, p = 0.001). Despite inclusion of consecutive patients in both SES and PES groups, there was a significant difference in incidence of diabetes. This does not reflect selection bias. The smaller nominal stent size in the SES group reflects the unavailability of SES > 3.0 mm at the time of the study.

MACE was analysed at 1 month and 1 year. Results are shown in table 2. No significant difference was seen in death and death or re-MI between the two groups neither in the first month nor at late follow-up. However, a significant difference in TVR was seen in favour of SES, which was already apparent at 30 days driven by stent thrombosis.

Six out of seven patients with TVR within 30 days in the PES group received target lesion re-intervention. Of these, 4 interventions were necessary because of subacute stent

thrombosis (ST). Only one of these patients had been treated with peri-procedural GP IIb/IIIa inhibitor during the index procedure.
Two out of 4 ST's were in patients treated with bifurcation lesions (one patient with crush bifurcation stenting without kissing balloon postdilatation but with peri-procedural GP IIb/IIIa inhibitor and one patient with T-stent bifurcation stenting without kissing balloon postdilatation and without GP IIb/IIIa inhibitor). The two remaining cases were due to stent underexpansion, diagnosed after intravascular ultrasound investigation at re-intervention.
Bifurcation stenting percentage was not significantly different between groups (8.6 % vs. 9.6 % for SES and PES respectively, p = 0.8). Although across all strategies no significant difference was found in bifurcation lesion treatment between SES and PES patients, a trend was seen towards more crush stenting and less T-stenting in PES patients (table 3).
MACE-free survival at 12 months was 90.2 % for SES and 85 % for PES patients (p = 0.16) (by Kaplan-Meier estimate)(figure 1).
On multivariate analysis, stent type was not an independent predictor of MACE at one year, and when forced into the model of significant predictors, remained non-significant (p=0.14). (Table 4). However, independent predictors were TIMI flow 0 or 1 (HR 10.2), cardiogenic shock (HR 4.4) and diabetes mellitus (4.8).

Discussion

The main finding of this paper is that patients treated with drug-eluting stents for acute myocardial infarction have a very low rate of repeat revascularization for restenosis at one year follow-up.
Although no significant difference in MACE at 1 year was found between the different drug-eluting stents, a trend to worse outcome was seen in the patients treated with PES, despite more favourable baseline characteristics like less diabetes, higher use of GP IIb/IIIa inhibitors and larger nominal stent diameter.

Short-term follow-up
The largest difference between the groups consists of target vessel re-interventions in the first 30 days. These were mainly driven by stent thrombosis.
In respect to these observations with 2/13 ST in bifurcation lesions and 2/123 in non-bifurcation lesions, it seems prudent to try to avoid using two stents for bifurcation treatment in acute myocardial infarction. If unavoidable, the risk for ST may be reduced by kissing balloon postdilatation and peri-procedural GP IIb/IIIa inhibitor. In a separate report

of 2500 patients we have confirmed that bifurcation stenting in acute myocardial infarction was a significant predictor of stent thrombosis and conferred a 13 fold increase in risk[8]. Overall rate of ST was 1.2% (4/322) for DES use in acute myocardial infarction. This is comparable to ST rate in bare stents[8,9] and DES[8] in patients treated for stable coronary lesions.

Long-term follow-up

Between 30 days and 1 year two patients treated with PES were referred for target vessel revascularization, both for in-stent restenosis (1.5 %), compared to no additional interventions in the SES patients.

No late stent thrombosis was diagnosed in either group. In this study, the risk for late stent thrombosis after stopping of clopidogrel, which was prescribed for 6 months in PES and 3-6 months in SES, does not seem to be increased for treatment of patients with acute coronary syndromes with drug-eluting stents. It is important that in order to conclusively address this potential problem, larger studies specifically looking at this endpoint should be performed. Furthermore, until more is known, complete cessation of anti-platelet therapy should be avoided if possible, to avoid the risk of late thrombosis, as recently pointed out by McFadden et al [10].

Early and 1- year mortality was identical in both groups (5.9% at 30 days and 8.1 % at 1 year for SES and PES). This is very comparable to earlier studies[6] despite the presence of cardiogenic shock in 12% of patients and multi-vessel disease in almost half. As shown before, mortality is not changed by the use of drug-eluting stents[5]. Its benefit is reduction of re-intervention as in elective PCI.

The recommendations in the most recent NICE guidelines explicitly exclude acute myocardial infarction and lesions with visible thrombus as an indication for DES[11]. Our results are reassuring and do not indicate that patients with acute myocardial infarction should be denied the benefit of very low re-intervention rates of drug-eluting stents.

Conclusions

The use of PES for the treatment of acute myocardial infarction seems safe and no significant differences were seen with the results of SES at 1 year follow-up with a very low rate of reintervention for re-stenosis. However, a trend towards more early re-interventions was visible, mainly due to stent thrombosis. Bifurcation stenting should be avoided in the setting of primary PCI, if possible.

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Figure 1. Kaplan-Meier estimate of MACE-free survival at 360 days.

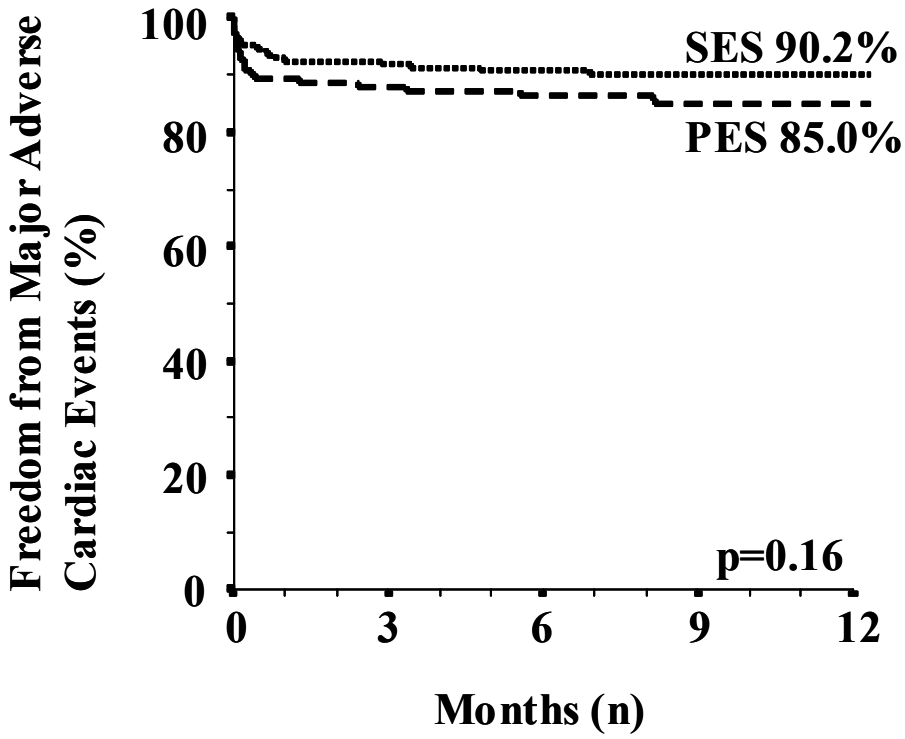

Figure1

Survival free of death, re-infarction or TVR in SES patients vs. PES patients by Kaplan-Meier estimate. TVR: target vessel revascularization SES: sirolimus-eluting stent; PES: paclitaxel-eluting stent.

Table 1: Baseline Characteristics

	SES (n=186)	PES (n=136)	p Value
Male gender, %	74.7	83.8	0.06
Age, years (SD)	59.7 (11.7)	59.2 (12.1)	0.7
Diabetes, %	10.8	3.7	0.02
Current Smoking, %	45.7	44.9	0.9
Hypercholesterolemia, %	33.9	30.1	0.5
Hypertension, %	24.2	20.6	0.5
Previous Myocardial Infarction, %	14.4	10.6	0.4
Previous PCI, %	6.5	5.9	1.0
Previous CABG, %	1.6	2.2	0.7
Coronary Artery Disease			0.9
1-vessel, %	54.8	52.2	
2-vessel, %	27.4	28.7	
3-vessel, %	17.7	19.1	
Cardiogenic Shock, %	13.4	11.8	0.7
Time from Symptom Onset to PCI, hours (SD)	3.2 (1.9)	3.1 (2.4)	0.7
Infarct-related Vessel			0.6
LAD	52.7	51.5	
LCx	8.2	8.8	
RCA	37.4	36.0	
LMS	1.6	2.2	
SVG	0	1.5	
Bifurcation lesion	8.6	9.6	0.8
Vessels Treated			1.0
1	84.9	86.0	
>1	15.1	14.0	
TIMI flow Baseline			0.4
Grade 0/I	73.1	78.7	
Grade II	16.5	11.0	
Grade III	10.4	10.3	
TIMI Flow Final			0.7
Grade 0/I	2.1	2.2	
Grade II	14.8	11.8	
Grade III	83.0	86.0	
Number of stents (SD)	1.9 (1.2)	1.8 (1.1)	0.4
Total stented length, mm (SD)	34.7 (23.5)	35.9 (22.9)	0.6
Mean nominal stent diameter (SD)	2.89 (0.16)	3.11 (0.33)	<0.001
Glycoprotein IIb/IIIa inhibitor, %	36.6	55.1	0.001
Peak CK, IU (SD)	3126 (3126)	3234 (2567)	0.8
Peak CK-MB, IU (SD)	296 (255)	359 (330)	0.2

PES: paclitaxel-eluting stent; SES: Sirolimus-eluting stent

Table 2: MACE at 30 days and 1 year

	SES (n=186)	PES (n=136)	p Value *
0 – 1 month			
Death (%)	5.9	5.9	1.0
Death or re-MI (%)	6.5	6.6	1.0
TLR (%)	1.1	4.4	0.07
TVR (%)	1.1	5.1	0.04
Death, re-MI or TVR (%)	7.5	10.3	0.4
Stent thrombosis (%)	0	2.9	0.03
0 – 12 months			
Death (%)	8.1	8.1	1.0
Death or re-MI (%)	9.2	10.3	0.7
TLR (%)	1.1	5.9	0.02
TVR (%)	1.1	6.6	0.01
Death, re-MI or TVR (%)	9.7	14.7	0.22
Stent thrombosis (%)	0	2.9	0.03

* by Fisher's exact test

re-MI: re-infarction; PES: paclitaxel-eluting stent; SES: Sirolimus-eluting stent; TLR: target lesion revascularization; TVR: target vessel revascularization

Table 3. Bifurcation lesions: treatment strategy

	SES (n=16)	PES (n=13)	p Value
Main branch stent only	3 (18.8%)	3 (23.1%)	0.4 *
Crush	2 (12.5%)	4 (30.8%)	
Culotte	0	1 (7.7%)	
T-Stent	9 (56.3%)	3 (23.1%)	
V-Stent	2 (12.5%)	2 (15.4%)	
Final Kissing balloon	6 (42.9%)	8 (61.5%)	0.3

* Across all strategies by Chi Square test

PES: paclitaxel-eluting stent; SES: Sirolimus-eluting stent

Table 4: Final Cox regression model of independent predictors of MACE at one year with stent type forced in

Independent predictor	Hazard ratio	95% Confidence Interval	P value
TIMI flow			
Grade 3 (reference)	1.00		
Grade 2	2.90	0.86-9.77	0.09
Grade 0 or 1	10.24	2.61-40.13	0.001
Cardiogenic shock	4.38	1.63-11.8	0.003
Diabetes mellitus	4.77	1.61-14.1	0.005
Duration of pain (per hour increment)	1.17	0.98-1.39	0.08
Multi-vessel disease	2.13	0.79-5.78	0.14
Use of paclitaxel-eluting stent	2.07	0.78-5.48	0.14

Chapter 15

Summary and Conclusions

Samenvatting en Conclusies

Dankwoord

Curriculum Vitae

List of Publications

Summary and Conclusions

The aim of this thesis was to study vascular response to coronary interventions. This involved study of vessel wall injury, both acute and chronic, endothelial dysfunction and the performance of drug-eluting stents in clinical practice, as they are associated with delayed vascular healing due to their anti-proliferative capacity.

The **first part** of the thesis is focussing on the vascular wall trauma, caused by the interventions.

Chapter 2 describes the four phases of wound healing after intracoronary stenting, i.e. the early thrombotic response, followed by the recruitment phase with cellular infiltration, the proliferative phase and the final healing phase. In **chapter 3** it is shown that the vessel wall injury inflicted in the acute phase by stenting is even increasing in the weeks after the intervention. Two very different stent types were studied in the porcine coronary model and the difference in response suggests that persistent inflammatory response and stent design may influence the chronic injury and healing response.

Intracoronary irradiation therapy (brachytherapy) was the most effective therapy for treating in-stent restenosis until the recent favourable outcomes with drug-eluting stents. Brachytherapy was also investigated as adjunctive therapy directly after stenting or balloon angioplasty to prevent the occurrence of restenosis. However, long-term results are disappointing and a high percentage of late thrombotic occlusion has been reported. In **chapter 4** a case-report is presented with late occlusion five years after balloon angioplasty and adjunctive brachytherapy, while 3 year follow-up angiography had shown a patent vessel! Impaired healing response of the vessel wall after radiation therapy is thought to be the cause of these late (thrombotic) occlusions.

Chapter 5 shows the sequelae of the early drug-eluting stent period, when the largest available stent size was 3.0 mm. To achieve acceptable stent apposition in large coronary vessels, stents were up-sized with larger balloons. This can lead to stent strut fracture and as shown in this case the combination of less local drug elution and the direct trauma of the broken stent struts as well as damage to the polymer can lead to restenosis, even in large vessels and with the use of drug-eluting stents.

The **second part** of this thesis is focussing on endothelial dysfunction after percutaneous coronary interventions (PCI).

In **chapter 6** vascular permeability as a parameter of regeneration of a mature endothelial lining after balloon angioplasty or stenting was investigated, both early and late after the intervention. Dye-exclusion tests showed persistent vascular permeability up to 3 months

after the intervention, being the most pronounced in the stented vessels. This indicates immature endothelial functional recovery and this was correlated with distinct morphologic characteristics, such as endothelial retraction, the expression of surface folds and the adhesion of leukocytes.

Brachytherapy is associated with a delayed vascular healing response. In **chapter 7** we investigated whether more endothelial dysfunction could be demonstrated in patients treated 6 months before with stenting and adjunctive brachytherapy or no brachytherapy. In both groups, endothelial function was still abnormal after 6 months, like shown before by Caramori et al. in bare stents, but no difference could be demonstrated. Investigating endothelial function 6 months after drug-eluting stent implantation in **chapter 8** led to the remarkable observation that endothelial function was severely disturbed, while in the control group no significant paradoxal vasoconstriction (as a sign of endothelial dysfunction) could be demonstrated. This is not in accordance with the excellent clinical results up to three year after drug-eluting stent implantation. This outcome should be investigated in a larger patient population, but is reason for some concern and may be related to the slight increase in stent thrombosis seen after drug-eluting stent implantation, particularly late stent occlusions. If confirmed, adjunctive medical therapy like ACE-inhibitors as well as already advised cholesterol-lowering therapy for all patients after drug-eluting stents should be considered, next to the already implemented prolonged use of double anti-platelet therapy.

The subset of patients from the study reported in chapter 8 was used to study the relation between local endothelial function and shear stress in **chapter 9**. Low shear stress has been correlated to endothelial dysfunction but this has only been shown in vitro. Using sophisticated mathematical calculations, 3D reconstructions of the coronary segments distal to the implanted stents were made. Incorporating coronary flow velocity data and studying the patients after intracoronary acetylcholine infusions, correlations between local shear stress and local endothelial dysfunction (vasoconstriction) could be found. In regions with a shear stress of less than 1.3 Pa, a significantly larger vasoconstrictive response to acetylcholine could be seen compared to areas with shear stress of 1.3 Pa or more (normal to high shear stress). It is for the first time that such a correlation could be demonstrated in vivo.

The **third part** of this thesis is focussing on coated and drug-eluting stents.

Chapter 10 reports the findings of the use of a heparin-coated stent in porcine coronary arteries. This preclinical study led to the clinical BENESTENT 2 pilot and trial, showing a very low risk of stent thrombosis, despite a progressively milder anti-coagulant regime,

ending with dual-platelet therapy, without post-procedural heparin. Today this is still the clinical practice.

Over the last 10 years further attempts have been undertaken with different heparin stent-coatings to minimize the risk of stent thrombosis and reduce the incidence of in-stent restenosis. **Chapter 11** provides an update on those attempts, highlighting three different heparin-coatings, the Hepacoat™, the Hepamed™ and the Corline™ coating. Clinical trials have shown very low rates of subacute stent thrombosis of 0.2 %. The average incidence of subacute stent thrombosis in bare metal stent is around 1 %. However, the other goal to reduce the rate of in-stent restenosis has not been reached and no significant difference has emerged from the different trials. There is a nice for heparin-coated stents in highly thrombotic subsets of patients or patients with a contraindication for prolonged antiplatelet therapy, but availability of the stents has become a problem.

After attempts with passive stent coatings newer concepts with drug-elution from a stent-based polymer, or even without polymer were investigated. This is a fast evolving field of research which has led sofar to two commercially available drug-eluting stents with excellent clinical results in multiple large trials, the sirolimus-eluting stent (SES) (Cypher, Cordis Co, Warren, NJ, USA) and the paclitaxel-eluting stent (PES) (Taxus, Boston Scientific, Galway, Ireland). Next to these stents, multiple drug-eluting stents are in the pre-clinical or clinical testing phase. **Chapter 12** is providing an update up to February 2004 of this field.

In **chapter 13** the first registry results are reported of the use of SES in the highly thrombotic environment of patients with an acute myocardial infarction. Concerns of higher risk of stent thrombosis in drug-eluting stents have been expressed multiple times and though it never has been substantiated, this fear has led to restrictive use in acute myocardial infarction. In this series of 186 consecutive patients no stent thrombosis was diagnosed and only 1.1 % of patients needed target vessel reintervention at 300 days follow-up compared to 8.2 % in the bare stent group in this study.

Chapter 14 investigated the safety of the PES for the same indication of primary PCI for acute myocardial infarction. PES is also effective, but the slightly higher risk of subacute thrombosis of 2.9 % raises some concern. Half of these stent thromboses were seen in stented bifurcation lesions. This suggests that bifurcation stenting should be avoided in the setting of an acute myocardial infarction if possible and bifurcation treatment should be kept as simple as possible. Late stent thrombosis after 30 days was not seen, which is very reassuring.

In Conclusion this thesis demonstrated that vessel wall injury occurs during coronary intervention, that this vessel wall injury can even increase after the acute injury in the case

of stenting and that this injury leads to neointimal proliferation. Endothelial dysfunction is still present months after the intervention, but no worse endothelial function could be demonstrated late after brachytherapy compared to no brachytherapy. In contrast, after SES implantation severe endothelial dysfunction was still detectable after 6 months.
A correlation could be found between local endothelial dysfunction and areas of low shear stress.
Despite this, SES and PES show excellent intermediate-term clinical results and no late stent thrombosis could be found in patients treated during acute myocardial infarction.
However, delayed vascular healing with prolonged endothelial dysfunction is reason for some concern and despite extended duration of double anti-platelet therapy, stent thrombosis rates are similar to those in bare stents and the timing is less predictable. More attention towards improving endothelial function after intervention of the culprit lesion seems advisable. May be this can be achieved by adjunctive medication like ACE-inhibitors and aggressive cholesterol lowering.

Samenvatting en Conclusies

Het doel van dit proefschrift was het bestuderen van de vaatwandreactie na interventies in de kransslagader. Hierbij werd gekeken naar acute en chronische vaatwandbeschadiging, endotheeldysfunctie en naar het gedrag van de nieuwste generatie stents, de zogenaamde “drug-eluting stents” (DES), waarbij na implantatie gedurende een bepaalde tijd langzaam een medicijn diffundeert uit de stentcoating ter voorkoming van in-stent restenose. Deze medicijnen worden namelijk gerelateerd aan vertraagde genezing van de beschadigde vaatwand, wegens de sterke anti-proliferatieve werking van deze medicijnen.

Het **eerste deel** van het proefschrift concentreert zich op de vaatwandbeschadiging die ontstaat door de interventie.
Hoofdstuk 2 Beschrijft de vier fasen van de wondgenezing na het plaatsen van een stent, nl. de vroege trombotische respons, gevolgd door de recruteerfase gekenmerkt door het aantrekken van cellen, vervolgens de proliferatieve fase en tot slot de helingsfase. In **hoofdstuk 3** wordt aangetoond dat de initiele vaatwandschade de eerste weken na de interventie zelfs nog toeneemt. In een varkensmodel worden twee verschillende stenttypes onderzocht in de kransslagader. Persisterende ontstekingsreactie en het verschil in stentontwerp lijken van invloed op de vaatwandschade en de helingsrespons.
Voor de goede resultaten van de nieuwe DES was intracoronaire bestralingstherapie (brachytherapy) de meest effectieve therapie voor de behandeling van restenose in de stent. Daarnaast werd ook onderzocht of brachytherapy deze in-stent restenose zou kunnen voorkomen. De langetermijn resultaten bleken teleurstellend en vooral late stenttromboses werd frequent gezien, resulterend in een afsluiting van het vat. **Hoofdstuk 4** beschrijft een patient waarbij 5 jaar na de behandeling en preventieve brachytherapie het bloedvat alsnog afgesloten raakt, terwijl het bij de 3-jaarscontrole nog fraai open was. De sterk vertraagde wondgenezing door de bestraling speelt waarschijnlijk een rol in deze erg late complicaties.
In de begintijd van de DES waren er alleen maar relatief kleine maten beschikbaar. Om toch iedereen te kunnen laten profiteren van deze goede stents werden ze ook gebruikt in grotere vaten. Vervolgens werd de stent met een grotere balloon nagerekt. **Hoofdstuk 5** laat zien dat dit kan leiden tot een breuk in de stent en re-stenose. Door de scherpe gebroken stentstruts, beschadigde polymeercoating en ter plekke minder effectieve dosering van het medicament dat re-stenose tegen moet gaan, kan deze stentbreuk alsnog leiden tot re-stenose, zelfs in grote vaten waar dit normaliter zelden voorkomt.

Het **tweede deel** van dit proefschrift bestudeert de endotheelfunctie na interventies in de kransslagader.

In **hoofdstuk 6** werd vaatwandpermeabiliteit as marker van herstel van een volgroeide endotheellaag bestudeerd, zowel na ballonangioplastiek als na stenting, zowel vroeg als laat na de behandeling. Abnormale permeabiliteit kon worden aangetoond tot 3 maanden na de behandeling, waarbij dit meer uitgesproken was na stenting dan na ballonangioplastiek. Dit impliceert incompleet herstel van de endotheellaag. Hierbij konden karakteristieke morfologische veranderingen van de endotheelcellen worden aangetoond.

Zoals boven reeds genoemd is de wondgenezing vertraagd na brachytherapie. In **hoofdstuk 7** werd onderzocht of ernstiger endotheeldysfunctie aangetoond kon worden 6 maanden na stenting en brachytherapie dan na stenting zonder brachytherapie. In beide groepen was na 6 maanden de endotheelfunctie nog abnormaal, zoals ook reeds eerder aangetoond door Caramori et al. na stenting, maar een verschil tussen stent of stent en brachytherapie kon niet aangetoond worden. In **hoofdstuk 8** werd de endotheelfunctie onderzocht 6 maanden na implantatie van de nieuwe generatie stents, de DES, en in dit geval de sirolimus-gecoate stent (SES). Dit leidde tot de opmerkelijke bevinding dat 6 maanden na de behandeling de endotheelfunctie ernstig gestoord was na implantatie van de SES, terwijl dit veel minder het geval was na een ongecoate stent. Dit is opvallend aangezien de klinische resultaten met inmiddels een follow-up van ruim 3 jaar uitstekende resultaten laten zien van deze stent. Deze bevinding zal moeten worden bevestigd in een veel grotere groep patienten, maar het roept wel op tot waakzaamheid en langdurige follow-up. Hoewel in grote studies nooit hard aangetoond, is er toch een vermoeden op een iets verhoogde kans op stenttrombose bij het gebruik van DES, vooral na het voortijdig stoppen van bloedplaatjesaggregatieremmers. Het langdurig gebruik van dubbele plaatjesaggregatieremmende medicatie is inmiddels reeds gebruikelijk, maar misschien zou een ACE-remmer na stenting ook standaard moeten worden net als cholesterolverlagende medicatie. Beide medicijnen hebben bewezen gunstig effect op atheroscleroseprogressie en een gunstig effect op het endotheel.

In **hoofdstuk 9** werd de relatie bestudeerd tussen locale endotheelfunctie en shear stress (schuifspanning). Tot nu toe is alleen in “in vitro” onderzoeken een relatie tussen low shear stress en endotheeldysfunctie aangetoond. Door gebruik te maken van wiskundige rekenmodellen konden 3D reconstructies worden gemaakt van de coronairvaten uit angiografische plaatjes en door middel van correlatie met gemeten bloedstroomsnelheden tijdens acetylcholineinfusie in de kransslagaderen, kon de relatie tussen shear stress en endotheeldysfunctie (vaatvernauwing op acetylcholine) worden bestudeerd. Gebiedjes met een lage shear stress van < 1,3 Pa gaven een significant grotere vaatvernauwing na

acetylcholine vergeleken met gebiedjes met een hogere shear stress. Het is voor de eerste keer dat dit in vivo is aangetoond.

Het **derde deel** van dit proefschrift concentreert zich op gecoate stents en DES.
Hoofdstuk 10 beschrijft de resultaten van het gebruik van heparine-gecoate stents in kransslagaders van biggen. Deze preklinische studie leidde tot de bekende klinische studies, de BENESTENT 2 pilot en trial. Hierbij werd de veiligheid van de heparine-gecoate stent aangetoond zonder een verhoogd risiko op stenttrombose met alleen nabehandeling met twee verschillende plaatjesaggregatieremmende medicamenten. Dit is 8 jaar later nog steeds de gebruikelijke therapie.
De afgelopen 10 jaren zijn nog diverse studies verricht met verschillende soorten heparine-gecoate stents om het risiko op stenttrombose te minimaliseren en het percentage in-stent restenose te verminderen. Dit eerste was succesvol met zelfs trombosepercentages ver onder de 0,5 % ten opzichte van ongeveer 1 % in metalen stents. Helaas is een significant reductie van in-stent restenose nooit aangetoond. **Hoofdstuk 11** geeft een overzicht van deze studies.
De boven reeds genoemde DES hebben inmiddels zeer goede resultaten laten zien in grote studies en worden in snel toenemende mate gebruikt in plaats van metalen stents. Tot nu toe zijn twee van dit soort stents in grote trials onderzocht en commercieel op grote schaal verkrijgbaar, nl. de sirolimus-eluting stent (SES) (Cypher, Cordis Co, Warren, NJ, USA) en de paclitaxel-eluting stent (PES) (Taxus, Boston Scientific, Galway, Ireland). **Hoofdstuk 12** geeft een overzicht van de preklinische en klinische studies met diverse DES tot februari 2004. **In Hoodstuk 13** worden de eerste 186 patienten beschreven die in het ErasmusMC werden behandeld met een SES voor een acuut hartinfarct. Hoewel een patient met een acuut hartinfarct een duidelijk verhoogde stollingsneiging van zijn bloed heeft in de acute fase, bleek het gebruik van de SES ook in deze setting veilig en zonder verhoogde risiko's op stenttrombose. Ook bleek dat patienten die met een SES behandeld werden vrijwel nooit terug hoefden te komen voor een herinterventie in het behandelde vat gedurende een follow-up van 300 dagen (1,1% vs. 8,2 % voor patienten behandeld met een metalen stent).
In **hoofdstuk 14** werd de andere DES nl. de PES geevalueerd voor dezelfde indicatie. Ook deze stent liet goede resultaten zien, echter er werden enkele patienten teruggezien met stenttrombose (2,9 %). De helft van deze patienten waren in de acute fase behandeld voor een afsluiting op een vertakking in de kransslagader. Hierbij waren beide takken gestent, en hoewel deze getallen erg klein zijn, zou dit mogelijk een verhoogde stenttrombose kans geven. Late stenttromboses werden niet gezien en na 1 jaar was er geen significant verschil in effectiviteit tussen de SES en de PES.

Conclusie: Dit proefschrift laat zien dat de vaatwand beschadigd raakt tijdens de behandeling van vernauwingen in de kransslagader met ballonangioplastiek of stentplaatsing. Deze beschadiging kan zelfs nog toenemen in de eerste weken na stentplaatsing. Dit wordt gevolgd door een genezingsreactie met vorming van een neointima. Bij excessieve schade is de kans groter op in-stent restenose door meer neointimaproliferatie. Endotheeldysfunctie is nog maanden na de interventie in het bloedvat aantoonbaar. Hoewel deze genezingsfase wordt vertraagd door bestralingstherapie kon na 6 maanden geen verschil in endotheeldysfunctie worden aangetoond in bestraalde patienten tov niet-bestraalde patienten. Daar stond tegenover dat in patienten die een SES ontvingen de endotheelfunctie na 6 maanden nog sterk gestoord was. Ook kon in deze patientengroep een relatie aangetoond worden tussen gebieden van lage schuifspanning (shear stress) en locale endotheeldysfunctie.
Ondanks dit zijn de middellange termijn resultaten van SES en PES uitstekend en kon zelfs in de zeer trombogene setting van het acuut hartinfarct geen verhoogde neiging tot late stenttrombose aangetoond worden, als uiting van vertraagde vaatwandgenezing. Desalniettemin is vertraagde wondgenezing, gepaard gaand met langdurige endotheeldysfunctie reden tot enige zorg. Ondanks de forse verlenging van de behandelduur met twee bloedplaatjesaggregatieremmers is de incidentie van stenttrombose vergelijkbaar met ongecoate stents en het optreden ervan minder voorspelbaar. Het lijkt verstandig na het opheffen van de belangrijke vernauwing meer aandacht te besteden aan de kwaliteit van het endotheel. Dit zou misschien kunnen door een groter gebruik van ACE-remmers na de PCI alsmede door aggresieve cholesterolverlaging.

Dankwoord

Het voltooien van dit proefschrift is tevens een afsluiting van een periode van 11 jaren verbondenheid aan het Erasmus Medisch Centrum. Het begon eind 1993 als “inval” angio om tijdelijk een zwangere agio te vervangen, daarna als arts-onderzoeker op de “Experimentele Cardiologie” in de witte toren tot het geld op was en er geen onderzoekersplaats meer was, vervolgens opnieuw als agnio en tot slot de fel begeerde opleidingsplaats. Een vrij gebruikelijk scenario in die tijd.
Gedurende mijn kliniekjaren heb ik mijn onderzoekstijd en onderwerpen nooit uit het oog verloren en konden mijn “biggenstudies” gecontinueerd worden met humaan onderzoek op het “ echte cathlab”.
Tot mijn grote vreugde kreeg ik de mogelijkheid om na mijn opleiding tot cardioloog, mijn grote passie, de interventiecardiologie te bedrijven en daarnaast de lopende cathlab studies af te ronden. Uiteindelijk resulteerde dat in het nu voor u liggende boekje.
Het bovenstaande was nooit gelukt zonder de hulp, begrip en geduld van vele mensen. Vooral de cathlab studies van de BRIEF studie (door mij verzonnen acronym voor **BR**achytherapy **I**nduced **E**ndothelial **F**unction study) (hoofdstuk 7,8 en 9) waren zeker niet “brief” en behoorden tot de langste cathlabprotocollen in Rotterdam, en dat wil heel wat zeggen in deze kliniek. Dit vergde veel geduld van de uitvoerende cardiologen, verpleegkundigen, technici en de patienten.

Allereerst wil ik mijn promotor, Professor Serruys bedanken. Beste Patrick, ik ken geen mensen die meer gedreven zijn op het gebied van interventiecardiologie dan jij. Schijnbaar onvermoeibaar, altijd op de hoogte van de nieuwste data en plannen, zelfs voordat ze op papier staan en altijd bereid je in te zetten om nieuwe ideeën te onderzoeken en concepten te testen. Daarnaast herinner ik me de complexe en soms langdurige procedures waarin ik mocht assisteren en ook de complicaties, die met jouw hulp goed afliepen.
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Vanaf 1994 tot de dag van vandaag kon ik altijd bij jou terecht met mijn vragen en twijfels en lukte het jou meermalen mijn pessimisme over de voortgang van de studies te veranderen in optimisme over nieuwe mogelijkheden om de studies te continueren.
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Curriculum Vitae

Sjoerd Hepke Hofma was born on Januari 27th, 1965, in Sneek. He spent the first five years of his life in Balk and then moved to Leeuwarden. He attended " Het Christelijk Gymnasium" for 6 years and studied medicine at the " Rijksuniversiteit Groningen". After 4 years of basic training combined with a lot of climbing adventures in between, he spend 15 months as a research fellow at the University of Utah, under direct supervision of prof. dr W. J. Kolff. Playing with the artificial hearts and learning all about endothelial function from dr Fazal Mohammad, " this was the place " were he became convinced to become a cardiologist. After reluctantly returning to the Netherlands, he performed his clinical rotations in " Het Medisch Spectrum Twente". Convinced already that interventional cardiology should be his future, he finished his rotations with 3 months of cardiology in "De Weezenlanden " hospital in Zwolle, where the later famous study of primary PCI versus thrombolysis for acute myocardial infarction had just started. He pulled some of the envelopes for the patient randomization procedure. After he fulfilled his mandatory military service in Deventer, he started as a junior physician at the Thoraxcenter in Rotterdam in 1993. There was no vacancy, but he was allowed to fill a gap of a few months pregnancy leave of one of the residents. During this months he received a phone call from prof. dr Piet Verdouw, head of the Department of Experimental Cardiology, which led to a two year stay at his lab, working closely with dr Wim van der Giessen and dr Heleen van Beusekom, performing preclinical interventional studies in pigs and receiving very valuable exposure to new interventional techniques.

In 1996 he was a ward physician in the Thoraxcenter again, now mainly working in peri-operative care under supervision of dr Meindert Taams, an excellent bed-side teacher and later colleague in Leeuwarden.

In 1997 he started his training in cardiology, first spending 2 years at the Albert Schweitzer hospital in Dordrecht for his training in internal medicine (Head: dr J. van der Meulen). From 1999 till 2002 he finished his training in cardiology at the Thoracenter (Head: prof. dr J.R.T.C. Roelandt). From 2002 till june 2004, he worked at the catheterization laboratory under supervision of prof. dr Patrick Serruys, first as a fellow and later as a senior interventionalist. In June 2004, he went back to his Frysian roots to work as a cardiologist in the new emerging " Thoraxcenter of the North", Medisch Centrum Leeuwarden, where half of his time is dedicated to interventional cardiology.

List of Publications

1. J.J. Wentzel, **S.H. Hofma**, J.C.H. Schuurbiers, F.J.H. Gijsen, A. Thury, W.J. van der Giessen, P.W. Serruys, C. J. Slager. Endothelial dysfunction is located at low shear stress areas in human coronary arteries in vivo. Submitted.
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3. **Sjoerd H. Hofma**, Andrew TL Ong, Jiro Aoki, Gaston A Rodriguez Granillo, Marco Valgimi-gli, Evelyn Regar, Peter PT de Jaegere, Eugene P McFadden, Georgios Sianos, Willem J van der Giessen, Pim J de Feyter, Ron T Van Domburg, Patrick W Serruys. One Year clinical follow-up of Paclitaxel-eluting stents for Acute Myocardial Infarction compared to Sirolimus-eluting stents. Submitted.
4. **Sjoerd H. Hofma**, Wim J. v.d. Giessen, Bas M. van Dalen, Pedro A. Lemos, Eugene P. McFadden, Georgios Sianos, Jurgen MR Ligthart, Jiro Aoki, Dirk van Essen, Pim J. de Feijter, Patrick W. Serruys, MD Ph. No change in endothelial-dependent vasomotion late after coronary irradiation. Cardiovasc Radiat Med 2005; In press.
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