Clinical research

Indication of long-term endothelial dysfunction after sirolimus-eluting stent implantation


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Aims
Endothelial dysfunction has been related both to progression of atherosclerotic disease and to future cardiovascular events. 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 (seven SES, five 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 (IVUS) was performed and coronary flow reserve (CFR) assessed in all patients. At follow-up significant vasoconstriction was seen in SES (median 32% diameter reduction from baseline) but not in BS (median 2% reduction) patients after acetylcholine infusion ($P = 0.03$ for SES vs. BS); endothelium-independent vasodilatation to nitrates did not differ significantly between groups (20% SES, 5% BS, $P = 0.14$). IVUS revealed no late unhealed dissections and CFR was comparable between groups (SES 3.1 vs. BS 3.2, n.s.).

Conclusion
SES implantation may have an adverse effect on local endothelium-dependent vasomotor responses compared with BS implantation at 6 months. Long-term clinical consequences of this observation are still unknown.

KEYWORDS
Coronary stents; Drugs; Endothelium

Introduction
Sirolimus-eluting stent (SES) implantation significantly reduces restenosis compared with 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 BS has been reported in porcine and human coronary arteries.4,5 Recently, exercise-induced coronary vasoconstriction was reported in the vessel segments adjacent to SES but not BS.6 We assessed endothelial-dependent and -independent vasomotion immediately after stenting and at 6 months after SES or BS implantation.

Methods
Patient selection
We prospectively studied 15 patients, nine with SES (Cypher, Cordis Co., Warren, NJ, USA) and six BS (DepoStent, Conor Medsystems, Inc., Menlo Park, CA, USA) patients. Stents were implanted for single de novo lesions. Most important exclusion criteria were: acute myocardial infarction within 72 h after onset of symptoms, angiographic evidence of fresh thrombus (filling defect proximal to or involving the stenosis), LVEF <30%, vessels with reference diameter <2.5 and >4.0 mm in diameter, and lesions treated with a balloon injury >10 or >40 mm in length.

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 BS 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).7 Allocation of patients to either group was dependent on availability of stents and patient informed consent.

One of the nine SES patients refused follow-up. One additional patient in each group was excluded because of in-stent restenosis. Thus, seven SES and five BS patients were analysed. Mean duration until follow-up studies was very similar (mean 189 ± 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.

Evaluation of endothelial function
Studies were performed directly after completion of the interventional procedure and at scheduled 6-month angiography. Long-acting vasoactive drugs were stopped for at least 24 h before
angiography. Endothelium-dependent and independent coronary vasomotion was studied using standard protocols. The intracoronary stent scaffolds the arterial wall and virtually abolishes vasomotion. For this reason, we analysed 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 the guide wire was subsequently withdrawn into the catheter.

Quantitative coronary angiography
Off-line quantitative analysis of coronary angiography was performed with the CAAS II system (Pie Medical Imaging, Maastricht, NL, USA), blinded to knowledge of stent type. Using the method of subsegmental analysis (as validated in brachytherapy trials), the mean lumen diameter was calculated of the segment starting 2 mm distal to the stent until 17 mm distal to the stent. 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} M).

Coronary flow reserve
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 in. 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 min through the femoral vein) was administered for assessment of hyperaemic flow velocity and CFR. Measurements were done in duplicate.

Intravascular ultrasound
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, 2.9F mechanical ultrasound catheter (UltraCross, Boston Scientific Scimed Inc., Fremont, CA, USA).

Statistics
Data are presented as median and interquartile range (25%; 75%) because of low number of patients and as mean ± SD where mentioned. For continuous variables of baseline characteristics Student’s t-test was used, for categorical data the Fisher’s exact test. Mann–Whitney U test was 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 hypercholesterolaemia 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 (mean 2.0 ± 0.2 mm vs. 2.2 ± 0.4 mm).

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics</th>
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<tbody>
<tr>
<td>BS</td>
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<td>------</td>
</tr>
<tr>
<td>Age (SD)</td>
</tr>
<tr>
<td>Male sex (%)</td>
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<tr>
<td>Current smoking (%)</td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>Type 1 (%)</td>
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<tr>
<td>Type 2 (%)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
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<tr>
<td>Hypercholesterolaemia (%)</td>
</tr>
<tr>
<td>Prior myocardial infarction (%)</td>
</tr>
<tr>
<td>Positive family history (%)</td>
</tr>
<tr>
<td>Statin therapy (%)</td>
</tr>
<tr>
<td>ACE-inhibitor (%)</td>
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<tr>
<td>Body surface area (m²)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Table 2 Mean coronary segment diameter change from baseline</th>
<th>SES (n = 7)</th>
<th>BS (n = 5)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ach Median (25%; 75%)</td>
<td>32; (48; 21)</td>
<td>-2; (-5; 0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Nitrates Median (25%; 75%)</td>
<td>20; 10; 23</td>
<td>5; 1; 13</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 2 shows results of maximal dose of acetylcholine of 10^{-6} M at follow-up. In the SES group, a 32% (−48; −21) reduction in mean coronary diameter compared with baseline was observed, after infusion of Ach 10^{-6} M, whereas there was no significant change in the BS group [−2% (5; 0)], (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 [−21% (−26; −8) vs. −8% (−13; −7) 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 median 3.1 (2.7; 3.7) vs. BS 3.2 (3.1; 3.6), n.s.]. Absolute flow increased from 16 ± 7 cm/s for SES and 16 ± 4 cm/s for BS to 52 ± 26 cm/s and 51 ± 15 cm/s for SES and BS, respectively (mean ± SD). 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.
Drug-eluting stents have shown a large reduction of restenosis compared with BS. However, their mechanism of action may have unwanted effects on vessel healing after stent implantation. The major finding of the present study was that SES implantation suggests an adverse effect on local endothelium-dependent vasomotor responses.

**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 neo-intimal 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 centimetres along the coronary artery wall. 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 with the distal edge.

Data on the effects of sirolimus on vasomotion is limited, not conclusive, but growing. Early reports showed vasodilatory effects of sirolimus after acute exposure to high doses in isolated rat aortic rings. More recent data showed severe impairment of endothelial function in the coronary segments of swine in vitro, after incubation for 48 h with sirolimus. 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. 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.

Recently, Togni et al. reported exercise-induced coronary vasoconstriction in the vessel segments adjacent to SES but not BS. In that study, no measurements were performed immediately after the intervention and, therefore, baseline differences in endothelial function cannot be excluded. However, their data and the results obtained in the present study both indicate that coronary segments distal to SES show paradoxical vasoconstriction when compared with BS.

The effects of sirolimus on vasomotion can hypothetically be caused by a direct effect on the endothelium, on the signalling 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 signalling pathway. A preserved medial vasomotion is also suggested by the preserved vasodilation 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 vs. balloon angioplasty in porcine coronary arteries. Caramori et al. demonstrated persistent vasomotor dysfunction distal to coronary stents implanted 6 months earlier.

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 double antiplatelet agent treatment 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. Pre-dilatation was done with a balloon 1 mm...
smaller in diameter than the subsequently implanted stent, and balloons were shorter than the stent. No BS were post-dilated, while three out of seven SES were post-dilated. 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 post-dilatation. Post-dilatations 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 vasomotor 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 centre, 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 [−8% (−13; −7) to −2% (−5; 0) P = 0.07], while a trend towards worsening was seen in the SES group [−21% (−26; −8) to −32% (−48; −21), P = 0.25]. This means that the large difference in vasomotion response to acetylcholine seen at follow-up between BS and SES 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 6 months after the intervention, the vasoconstrictive response to Ach, as seen in this study, would be the result of a persistent denuded 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. 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. However, our results do suggest that prolonged follow-up to exclude late adverse consequences is warranted in patients who receive a drug-eluting stent.

Mild vasodilatory response after nitroglycerin
Only a mild vasodilatation was seen in reaction to nitrates in both groups, mean: 15 ± 11% for SES and 7 ± 7% for BS, median (Q1, Q3): 20 (10, 23) for SES and five (1, 13) for BSs. These differences were not significant from baseline. However, these small diameter changes are in line with other studies. Sabaté et al. reported only a mean 7–9% vasodilatation of the studied segment 6 months after balloon angioplasty with or without additional radiation therapy. Treasure et al. reported a mean of 13% vasodilatation after nitrates in a non-treated control coronary artery at baseline in a study investigating endothelial function in patients treated with lovastatin or placebo.

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 BS in our centre at the time of the study on one hand, while on the other hand our centre 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 vasomotor 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 vasoconstrictor 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 BS 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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References


