S irolimus- and paclitaxel-eluting stents are routinely used in coronary revascularization because drug-eluting stents (DES) significantly reduce rates of restenosis and target lesion revascularization compared with bare metal stent (BMS). However, available DES have limitations, such as late thrombosis because of delayed healing with poorer endothelialization and persistent local inflammation. Statins can inhibit cell proliferation, inflammation, and restore endothelial function. The present study evaluated the ability of stent-based cerivastatin delivery to reduce stent-induced inflammatory responses and adverse effects on endothelial function, and to inhibit neointimal hyperplasia in a porcine coronary model.

**Methods**

Pigs were randomized into groups in which the coronary arteries (9 pigs, 18 coronaries in each group) had either a cerivastatin-eluting stent (CES) or a BMS. All animals survived without any adverse effects. Inflammatory cell infiltration evaluated using scanning electron microscopy on day 3 after stenting was significantly decreased in the treated vessels (inflammation score: 1.15±0.12 vs 2.43±0.34, p<0.0001). At day 28, endothelial function with intracoronary infusion of bradykinin was preserved in both the CES and BMS groups. Volumetric intravascular ultrasound images revealed decreased intimal volume in the CES group (28.3±5.4 vs 75.9±4.2 mm³, p<0.0001). Histomorphometric analysis showed reduced neointimal area (1.74±0.45 vs 3.83±0.51 mm², p<0.0001) in the CES group despite similar injury scores (1.77±0.30 vs 1.77±0.22, p=0.97).

**Conclusion**

In porcine coronary arteries CES significantly decreased neointimal hyperplasia with a decreased early inflammatory response and without endothelial dysfunction. (Circ J 2008; 72: 832–838)

**Key Words:** Angioplasty; Restenosis; Statin; Stents
In Vivo Study
The stents were sterilized with ethylene oxide before implantation. Pigs (Shiraishi, Tokyo, Japan; aged 2–3 months, weighing 30–35 kg) were randomized into 2 groups of 9 each. One group was implanted with a BMS (control) and the other with a stent loaded with 300 μg of cerivastatin. The CES (18 stents in 9 pigs) were mounted with 20 mm balloons and deployed in the left anterior descending (LAD) or right coronary artery. The controls comprised 18 BMS balloons and deployed in the left anterior descending (LAD) CES (18 stents in 9 pigs) were mounted with 20 mm balloons. Stent implantation was as described by Schwartz et al.9 The stent balloons were inflated for 30 s to achieve a 1.1:1 to 1.2:1 stent-to-artery ratio. Aspirin (326 mg) was administered daily during follow-up.

For the early study, 3 pigs in each group were euthanized 3 days after stenting to observe the inflammatory reaction in 6 coronary arteries after coronary angiography (CAG). In the chronic study (28 days after stenting), the remaining 6 pigs in each group were killed for histological examination after coronary physiological tests and intravascular ultrasound (IVUS). The pigs were fed with standard laboratory chow. Blood was collected from all animals on the day of implantation, and in the chronic study on day 28 after stenting. All procedures involving stent implantation, IVUS and histological analysis were performed in a blinded manner.

Early Study: Scanning Electron Microscopy (SEM)
Inflammatory cell infiltration in the 6 coronary arteries from the 3 pigs of each group was evaluated by SEM 3 days after stenting. We established the early histological end-point based on a previous study that had shown that inflammatory cell adhesion peaks at 3 days after stenting.10 The stent-injury segment was cut longitudinally and then opened along the longitudinal cut. SEM proceeded as described.11,12 Ten sites were randomly selected near the struts and inflammatory cells were counted in each high-power field.

Chronic Study: Coronary Physiology and Tissue Morphometric Analysis
Evaluation of Endothelial Function in LAD at 28 Days After Stenting We examined endothelium-dependent and -independent coronary vasomotion near the stent, as described by Miyata et al.13 in 12 coronary arteries from 6 pigs per group. The intracoronary stent scaffolds the arterial wall and prevents vasoconstriction. We therefore analyzed the 5-mm segment immediately distal to the stent, which was injured by the stent balloon. A selective intracoronary infusion catheter (Multi-functional Probing Catheter; Boston Scientific, Galway, Ireland) was advanced over the guidewire and positioned in the stent. To avoid wire-induced coronary spasm, the guidewire was subsequently withdrawn into the catheter. After baseline CAG was recorded, bradykinin acetate (0.1 μg/kg) was administered through an infusion catheter into the LAD to examine endothelium-dependent coronary vasomotion. Nitroglycerin (10 μg/kg) was injected as an intracoronary bolus to assess endothelium-independent coronary vasoreactivity. CAG was performed 2 min after infusion with either bradykinin or nitroglycerin. We quantified CAG using the CAAS II system (Pie Medical Imaging, Maastricht, NL, USA) in the off-line mode while being blinded to knowledge of stent type. The mean lumen diameter of the segment starting immediately distal until 5 mm distal to the stent was calculated. The degree of the coronary vasoinhibitory response is expressed as % change in lumen diameter from baseline values.

Volumetric Assessment Using IVUS We performed IVUS using 2.9F 40-MHz transducers (Atlantis SR plus, Boston Scientific Scimed, Maple Grove, MN, USA) with a motorized retraction speed of 0.5 mm/s (12 coronary arteries from 6 pigs per group). The results were recorded on ½-inch high-resolution s-VHS tapes for off-line volumetric assessment and sent to our IVUS laboratory for offline analysis. Intimal volume at the stent site was assessed by volumetric analysis with a Netra 3D IVUS system (Schmage) as described Okazaki et al.14 We also calculated the maximum % area of stenosis as: 100x[(stenotic lumen area/in-stent area).

Histological Examination Coronary arteries were harvested and perfused with 10% buffered formalin. The stented segments were processed for plastic embedding and for staining with hematoxylin-eosin, as well as with elastica von Gieson. Sections 5-μm thick from the proximal and distal extrastent segments and from the proximal, mid, and distal stented artery were prepared in the standard manner for histomorphometry. These sections were examined under a microscope and areas were measured using a KS-400 image analysis system (Carl Zeiss Vision GmbH, Halbermoss, Germany). Lumen, intimal and vessel areas were measured using computer-assisted digital planimetry. The injury score and the neointimal thickness were determined on an individual strut basis for all stents to evaluate the effectiveness of CES on the reduction of neointimal thickness at different injury scores. We averaged the injury scores for each strut to obtain the mean score for each of the 246 histological sections from 24 coronary arteries of 12 animals.

Semiquantitative histopathological evaluations included endothelialization, inflammation and fibrin scores according to published methods.15 Stent endothelialization was defined as the ratio (%) of the arterial lumen circumference covered by endothelium: 0, absent; 1, <25%; 2, 25–75% and 3, 75–100% coverage. The inflammation and fibrin scores were assessed as follows: 0, absent; 1, focal findings involving any portion of the artery; 2, moderate accumulation involving <25% of circumference of the artery and 3, heavy deposition involving >25% of the arterial circumference.

Statistical Analysis All data are presented as means±SD. We used Student’s unpaired 2-tailed t-test to compare the treated and control groups. Simple linear regression analysis accounted for the relationship between the injury and injury-dependent neointimal response in each group. The relationship between mean injury score and neointimal thickness was evaluated as previously described. Discrete variables were percentages and compared using the χ² test. Significance was established at a value of p < 0.05.

Results
In Vitro Cerivastatin Release Kinetics Drug release curves showed that 50% of the cerivastatin was released from the stent over the first 24 h. Thereafter, the release slowed to 80% at 74 h and was completed after 28 days of incubation in 70% acetonitrile at 37°C (Fig 1).

In Vivo Studies Each group of animals was matched for age and weight at the start of the experiment. The weight gain and final weight did not differ between the groups. All animals survived with-
Cerivastatin group          Control group
(n=6)                      (n=6)

Day 0* Day 28** Day 0* Day 28**

TC (mg/dl) 74.5±7.7 72.7±6.0 74.3±6.9 71.3±4.4
HDL-C (mg/dl) 28.7±5.6 27.7±4.6 29.1±5.0 28.0±6.2
TG (mg/dl) 26.1±6.1 23.1±4.8 27.6±7.4 25.0±5.4
GOT 32.4±5.5 31.0±3.1 28.6±5.5 29.3±3.3
GPT 30.2±5.4 30.2±5.1 29.8±5.9 26.8±3.3
r-GTP 29.9±4.0 29.3±3.2 29.3±3.7 29.8±2.3
CK 634±226 397±167 711±178 411±176
Body weight (kg) 29.4±3.9 32.4±5.9 30.4±4.1 33.2±3.2

*No significant differences between groups in all variables at baseline.
**No significant differences between groups in all variables at the end of
the study (day 28).

TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; TG, tri-
glycerides; GOT, glutamate oxaloacetate transaminase; GPT, glutamate-
pyruvate transaminases; r-GTP, glutamy transpeptidase; CK, creatine kinase.

Fig 1. In vitro release kinetics of cerivastatin-eluting stent in 1 ml NaCl at 37°C.

Fig 2. Scanning electron microscopy of vessel from cerivastatin-eluting stent (CES:
A; ×2,000) and the bare metal stent (BMS: B; ×2,000) 3 days after stenting. The globular
cells are monocytes. (C) The number of monocytes in the 6 coronary arteries in 3 pigs
each group. Ten sites were chosen at random as near the stent struts and the number
of monocytes was counted in each site.

Fig 3. Coronary endothelial vasodilator response to bradykinin (BK) and nitroglycerin
(chronic study at day 28: 6 left anterior descending coronary arteries in 6 pigs of each
group). BK caused a comparable extent of coronary vasodilatation in both groups. Re-
results are mean±SEM.
out evidence of stent thrombosis, infection, rhabdomyolysis or any other side-effects (Table 1). All stents remained angiographically open at 3 and 28 days. The lipid profiles were similar in both groups (Table 1).

Early Study: SEM
SEM revealed monocytes attached to the luminal surface as described in our previous report.11 Strut-associated monocytes were significantly reduced by the CES compared with the BMS (Fig 2A). Ten sites were chosen at random as the near-stent struts and the number of monocyte was counted in each. The number of monocytes in the high-power field (×2,000) was significantly lower in the CES than in the BMS group (Fig 2B; mean cell number: 13.2±3.3 vs 42.1±6.6, p<0.0001).

Chronic Study: Coronary LAD Response to Bradykinin
Mean reference coronary lumen diameters at follow-up were similar in both the CES and BMS groups (mean 2.94±0.22 vs 2.81±0.16 mm, p=0.13). Intracoronary bradykinin caused a slight vasodilator response in both groups. Histological examination showed that the target site appeared mildly injured with minimal intimal thickness. Endothelial dysfunction was defined as abnormal vasoconstriction of 3% mean vessel diameter change from baseline after bradykinin administration, and endothelium-dependent vasodilatation did not significantly differ between groups (Fig 3). Nitroglycerin (10μg/kg, IC) also caused comparable coronary vasodilatation.

Volumetric Analysis of IVUS Measurements (Chronic Study at Day 28: 12 Coronary Arteries in 6 Pigs of Each Group)
A typical volumetric IVUS image is shown in Fig 4 and IVUS parameters are summarized in Table 2. The neointimal volume within the stent was significantly reduced in the CES group compared with the BMS group (28.3±5.4 vs 75.9±4.2 mm³, p<0.0001). Stent volume did not significantly differ between the 2 groups. The minimal lumen cross-

### Table 2 Analysis of Quantitative IVUS at Day 28

<table>
<thead>
<tr>
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<th>Cerivastatin-eluting stent group</th>
<th>Control group</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>Cross-section analysis</td>
<td></td>
<td></td>
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<tr>
<td>Stent area (mm²)</td>
<td>8.82±0.97</td>
<td>8.78±0.79</td>
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<tr>
<td>Lumen area (mm²)</td>
<td>6.83±0.85</td>
<td>3.99±0.47</td>
<td>&lt;0.0001</td>
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<tr>
<td>Neointimal (mm²)</td>
<td>1.99±0.32</td>
<td>4.79±0.49</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% Area stenosis</td>
<td>22.6±3.2</td>
<td>54.3±3.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Volumetric analysis</td>
<td></td>
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</tr>
<tr>
<td>Stent volume (mm³)</td>
<td>133.2±7.2</td>
<td>134.3±6.7</td>
<td>0.68</td>
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<tr>
<td>Lumen volume (mm³)</td>
<td>104.9±5.4</td>
<td>58.5±6.5</td>
<td>&lt;0.0001</td>
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<tr>
<td>Neointimal volume (mm³)</td>
<td>28.3±5.4</td>
<td>75.9±4.2</td>
<td>&lt;0.0001</td>
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IVUS, intravascular ultrasound.
sectional area was significantly greater in the CES than in the BMS group (6.83±0.85 vs 3.99±0.47 mm², p<0.0001), whereas stent area did not significantly differ (8.82±0.97 vs 8.78±0.79 mm², p=0.90). Therefore, the %area stenosis in the CES was significantly smaller than that in the control group (22.6±3.2 vs 75.9±4.2%, p<0.0001). The intraobserver and interobserver correlations were r=0.95 and r=0.92, respectively.

Chronic Study: Pathological Measurements
A total of 12 coronary lesions were generated in 6 pigs implanted with CES and 12 in 6 control animals deployed with BMS. Morphometric analysis confirmed the IVUS data, demonstrating that the CES caused less neointimal thickening after 28 days than the BMS (Fig 5A; 1.74±0.45 vs 3.83±0.51 mm², p<0.0001), whereas the mean injury score did not differ between the 2 groups (1.77±0.30 vs 1.77±0.22, p=0.97). The relationship between depth of arterial injury and neointimal thickness was determined for both groups (Fig 5B). The results indicated that the intercepts significantly differed between the 2 groups whereas the slopes did not. The amount of neointimal thickness was lower in the CES group at the same degree of injury, indicating a true effect of this treatment. Consequently, CES increased the lumen area compared with control (5.88±0.61 vs 3.56±0.86 mm², p<0.0001). Injury to the unstented arterial wall immediately proximal to the stent and to the distal stent injured by stent balloons and intimal thickness appeared minimal in all pigs in both groups.

Endothelialization scores were comparable between the CES (2.66±0.19) and control (2.68±0.18) groups, and endothelialization was almost complete in both groups. In our preliminary study, we performed immunostaining (von Willebrand factor) to detect endothelial cell in stented sections, in which we confirmed that the inner cells of stented vessels were endothelial cells 28 days after stenting. Those data suggest that CES does not negatively interfere with intimal thickness. In the present study strut-associated

![Figure 5](https://example.com/f5.png)

**Figure 5.** Cross sections of a stented coronary artery. The stented coronary segments from cerivastatin-eluting stents (A) and bare metal stent (B) were prepared for morphometric analysis. Cross sections were stained with hematoxylin/eosin (×25), (C) Scatter plots of mean neointimal area vs mean injury score for the 2 groups. There is no difference between the 2 groups in the slopes, but neointimal thickness is less in the cerivastatin-eluting group.

<table>
<thead>
<tr>
<th>Table 3 Analysis of Quantitative Pathological Measurements at Day 28</th>
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<tr>
<td><strong>Cerivastatin-eluting stent group</strong></td>
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<tr>
<td>-------------------------------------</td>
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<tr>
<td>Intimal area (mm²)</td>
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<tr>
<td>Lumen area (mm²)</td>
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<tr>
<td>Stent area (mm²)</td>
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<td>% Lumen stenosis</td>
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<td>Injury score</td>
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<td>Endothelialization score</td>
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<tr>
<td>Fibrin deposition</td>
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<td>Inflammation score</td>
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*Fig 5.* Cross sections of a stented coronary artery. The stented coronary segments from cerivastatin-eluting stents (A) and bare metal stent (B) were prepared for morphometric analysis. Cross sections were stained with hematoxylin/eosin (×25).
fibrin deposition and inflammation scores were similar in both groups (fibrin score: cerivastatin 0.14±0.04 vs control 0.16±0.06, p=0.81; inflammation score: 0.41±0.29 vs 0.57±0.32; p=0.189). All arteries in both the CES and BMS groups were almost completely healed. Pathological parameters are summarized in Table 3.

Discussion

The results of this study demonstrate that CES significantly inhibits neointimal formation in association with significant reduction of the early inflammatory response in a porcine coronary model. Furthermore, CES resulted in almost complete healing with low toxicity. These findings are the first to show that stent-based cerivastatin delivery is effective and thus represents a promising approach for the prevention of restenosis without adverse effects.

Data from the present study showing an increase in the number of monocytes around the stent strut are similar to those of others who have demonstrated an increase in circulating monocytes after coronary stenting. A recent study has found that circulating mononuclear cells (namely primary inflammatory cells) adhere and migrate to vessel walls and differentiate into neointimal SMCs. Furthermore, the correlation between the number of monocytes and neointimal thickness is linear. Therefore, the early inflammatory reaction plays a critical role in the development of in-stent neointimal hyperplasia, and the anti-inflammatory effect observed in the current study is a potential mechanism through which CES attenuates neointimal hyperplasia.

Statins directly bind to receptors on the surface of inflammatory cells, which prevents these cells binding to the counterreceptor on the vessel surface. Moreover, statins diminish the production of chemoattractants by the vessel wall, which is mediated by a reduction in the expression of adhesion molecules such as ICAM-1, VCAM-1 and E-selectin on the vessel surface. These direct effects of statins reduce the net number of inflammatory cells on the vessel surface after stenting.

In addition to the effectiveness of CES in the present porcine model, several of the histological findings also suggest that this approach would be clinically safe. Endothelialization was largely complete by 28 days and acute or subacute thrombosis did not develop. Clinically available DES cause significant inhibition of endothelial cell proliferation and thrombosis. Clinically available DES cause almost complete healing with low toxicity. These findings are the first to show that stent-based cerivastatin delivery is effective and thus represents a promising approach for the prevention of restenosis without adverse effects.

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In addition to the effectiveness of CES in the present porcine model, several of the histological findings also suggest that this approach would be clinically safe. Endothelialization was largely complete by 28 days and acute or subacute thrombosis did not develop. Clinically available DES cause significant inhibition of endothelial cell proliferation and function. In fact, recent clinical studies have demonstrated that acetylcholine or exercise-induced vasodilation responses at the stent edge are disturbed when a SES is implanted. Furthermore, in an in vivo porcine model, coronary arteries exposed to sirolimus had a severely impaired relaxation response to bradykinin, which is a major limitation because delayed stent endothelialization and endothelial dysfunction might increase the risk of late thrombosis. Thus, a stent-based compound that inhibits SMC proliferation, but does not suppress endothelial cell proliferation while restoring endothelial function, is central to the new concepts aimed at developing the next generation of DES. From this perspective, statins are ideal because they have anti-proliferative properties on SMC but also protect the endothelium and improve endothelial function even in the setting of vascular injury. In particular, cerivastatin promotes coronary artery endothelial cells through increased eNOS levels via the PI3K/Akt dependent signaling pathway. In the present study we tested local endothelium-dependent vasomotor responses and found no adverse effect of cerivastatin on local endothelium-dependent vasomotor responses at the distal stent edge.

The systemic use of statins is limited because clinical trials have shown that statins do not decrease restenosis rates after stent deployment. One explanation for this result might be insufficient local drug concentration at the injury site. In addition, drug passage through the liver might result in metabolic changes of the observed properties of statins. Stents eluting lipophilic drugs have recently resulted in high drug levels around the stent struts. Cerivastatin is a lipophilic statin that passes easily through cell membranes, enabling intramural distribution and prolonged arterial tissue retention. Furthermore, cerivastatin is the most pharmacologically potent statin in animal studies. Thus, cerivastatin should elicit more pleiotropic effects than hydrophilic statins, but cause more side-effects. The potential for troublesome side-effects, such as muscle weakness, rhabdomyolysis and acute renal failure, limits the applicability of systemic cerivastatin administration to prevent in-stent restenosis. However, our study showed that local delivery using a stent platform might allow for deposition of a therapeutic concentration in the arterial wall without inducing systemic toxicity.

Stents that elute sirolimus and paclitaxel are currently available and both remarkably inhibit intimal hyperplasia and the restenosis rate. In the present study porcine coronary arteries treated with CES showed a significant reduction in neointimal thickness compared with BMS and the degree of effectiveness was equivalent to that obtained with stents eluting paclitaxel or sirolimus in pig coronary arteries. However, we can not directly compare our results to those reports because of differences in the methods. Nevertheless, our preclinical data are useful for clinical trials. Furthermore, the effectiveness of CES was consistent with the findings of Jaschke et al who demonstrated that cerivastatin elution led to significant inhibition of neointima formation in a rat carotid stent model, but the present study differs in some respects from theirs. We are the first to use a large animal model to test the efficacy of CES. The pig coronary artery stent model is a suitable predictor of not only restenosis but also thrombosis after stenting. In the context of aspirin monotherapy, none of the stents in the present study caused thrombosis in either group, indicating that the CES is safe in this model. In addition, we also used IVUS measurements to determine intimal volume throughout the length of the stent. This method was the same as the clinical endpoints.

Study Limitations

The porcine coronary model and the human coronary arteries critically differ. Stents are implanted into normal non-atherosclerotic arteries of the porcine model whereas in humans stents are deployed on extant atherosclerotic plaque. However, similar reductions of restenosis in native pig coronary arteries by stents eluting paclitaxel and sirolimus have been successfully transferred into clinical trials. Secondly, the temporal course of healing differs between pigs and humans. Neointimal growth peaks at 28 days in pigs, compared with 6–12 months in humans. Thirdly, long-term studies are required to evaluate whether the beneficial effects of cerivastatin exceed the period of stent-related drug release and whether the drug is simply delaying neointimal formation. Indeed, the beneficial effects observed at 4 weeks after sirolimus delivery from eluting stents in porcine coronary arteries were not maintained at 6 months of
follow-up, suggesting a possible “catch-up” phenomenon with DES as reported for brachytherapy. Whether or not CES can contribute to restenosis cannot yet be defined, but the beneficial effect of the CES in the present study suggests that this approach should be further tested in human restenosis.

Conclusion

The results of this study demonstrated that CES achieved a delicate balance between preserved safety and improved efficacy and thus have the potential to alter the course of future coronary intervention.

Disclosure

There are no conflicts of interest.

References


