Augmentation of Wall Shear Stress Inhibits Neointimal Hyperplasia After Stent Implantation
Inhibition Through Reduction of Inflammation?

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Background—Low wall shear stress (WSS) increases neointimal hyperplasia (NH) in vein grafts and stents. We studied the causal relationship between WSS and NH formation in stents by locally increasing WSS with a flow divider (Anti-Restenotic Diffuser, Endoart SA) placed in the center of the stent.

Methods and Results—In 9 rabbits fed a high-cholesterol diet for 2 months to induce endothelial dysfunction, 18 stents were implanted in the right and left external iliac arteries (1 stent per vessel). Lumen diameters were measured by quantitative angiography before and after implantation and at 4-week follow-up, at which time, macrophage accumulation and interruption of the internal elastic lamina was determined. Cross sections of stent segments within the ARED (S+ARED), outside the ARED (S[minus]ARED), and in corresponding segments of the contralateral control stent (SCTRL) were analyzed. Changes in WSS induced by the ARED placement were derived by computational fluid dynamics. Computational fluid dynamics analysis demonstrated that WSS increased from 0.38 to 0.82 N/m² in the S+ARED immediately after ARED placement. This augmentation of shear stress was accompanied by (1) lower mean late luminal loss by quantitative angiography (−0.23±0.22 versus −0.58±0.30 mm, P=0.02), (2) reduction in NH (1.48±0.58, 2.46±1.25, and 2.36±1.13 mm², P<0.01, respectively, for S+ARED, S[minus]ARED, and SCTRL), and (3) a reduced inflammation score and a reduced injury score. Increments in shear stress did not change the relationship between injury score and NH or between inflammation score and NH.

Conclusions—The newly developed ARED flow divider significantly increases WSS, and this local increment in WSS is accompanied by a local reduction in NH and a local reduction in inflammation and injury. The present study is therefore the first to provide direct evidence for an important modulating role of shear stress in in-stent neointimal hyperplasia. (Circulation. 2003;107:2741-2746.)

Key Words: shear stress ▪ restenosis ▪ stents ▪ cells ▪ inflammation

Although stents are responsible for a clear reduction in the restenosis rate,1 stents exacerbate the normal proliferative reaction because of a variety of factors related to stent design.2 In addition to conventional risk factors for stent restenosis, the restored level of blood flow seems to be a crucial factor in the development of neointimal hyperplasia (NH).3 It has been postulated that wall shear stress (WSS) is an important causative factor. Indeed, other studies4 have demonstrated that low WSS is associated with and even predicts NH in either bypass graft or stents. However, these earlier studies could not exclude the possibility that other factors in addition to WSS explained the observations. This hypothesis is not unrealistic, because it is known that the strain and lipid distribution are different in inner and outer curves of blood vessels.5,6

In view of the above-mentioned arguments, we devised an experiment to evaluate the role of shear stress on NH with a more direct approach. To that end, we induced a local augmentation in WSS with a new device, the Anti-Restenotic Diffuser (ARED) flow divider (Figure 1; European patent number EP0989830), which is positioned in 1 of the 2 stents placed at similar locations in the external iliac arteries of rabbits fed a hypercholesteremic diet. With this approach, we were able to exclude the effect of 2...
potential confounding factors, spatial differences in hypercholesteremia and in endothelial function.

The role of inflammation in atherosclerosis was recently demonstrated.7 Macrophages play a key role in inflammation,8 and accumulation of macrophages depends on shear stress. Furthermore, activated macrophages produce metalloproteinase-12 and cathepsin K and S,9 which may dissolve elastic membranes, including the internal elastic membrane. This membrane is known to act as a diffusion barrier, and disruption of this membrane is associated with more neointimal hyperplasia (NH).2 Hence, to evaluate whether this mechanism underlies shear stress–mediated reduction in NH, we evaluated elastic membrane interruption and macrophage accumulation in the vicinity of the stent struts.

Methods

Instrumentation

A total of 9 New Zealand White rabbits (weight, 3.4±0.3 kg) were fed a high-cholesterol diet (2% wt/wt) for 2 months before intervention and during follow-up to induce atherosclerotic plaques. On the day of experimentation, the animals were premedicated with fentanyl (infusion rate, 0.2 mg·kg⁻¹·h⁻¹) and propofol (Diprivan; 10 mg/mL at an infusion rate of 10 mL/h) and a 2:1 mixture of NaO and O₂ after intubation. The respirator was adjusted to achieve and maintain physiological blood gas levels (7.35<pH<7.45, 35<PCO₂<45 mm Hg; PO₂>100 mm Hg).

The reduction in mean arterial blood pressure induced by the anesthetics was compensated for by an infusion of epinephrine, titrated (rate, 2 to 12 mL/h; 1 mg/mL; Centralfarm) to achieve a mean arterial blood pressure of ~70 mm Hg. The marginal ear artery was cannulated with a fluid-filled catheter, and the lateral ear vein was cannulated for the infusion of propofol and epinephrine. Subsequently, one of the carotid arteries was dissected, and a 5F introducer sheath (Fast Cath, Daig Inc) was positioned for the advancement of guidewires and guiding catheters.

All experiments were performed in accordance with institutional regulations and the “Guiding Principles for the Care and Use of Animals” as approved by the Council of the American Physiological Society.

Stent and ARED Implantations

A 5F guiding catheter was advanced into the aortic bifurcation over a 0.25-inch guidewire. After angiography had been performed, a PTCa balloon (length, 13 or 18 mm; diameter, 2.5 mm) was advanced into one of the external iliac arteries. A balloon-to-vessel ratio of 1.5 was selected by angiography, and the balloon was inflated 3 times at a pressure of 9 atm. One stent (MultiLink; length, 13 or 18 mm; diameter, 3 mm; Guidiant) was then implanted at nominal pressure (9 atm). Subsequently, the other femoral artery was similarly dilated, denuded, and stented. After randomization, the ARED flow divider (diameter, 1 mm; length, 10 [n=6] or 15 [n=2] mm) (Figure 1) was implanted inside 1 of the 2 stents. Care was taken to have an adequate stented segment upstream and downstream of the flow divider.

Finally, the rabbits were injected with acetylsalicylic acid (10 mg/kg IM; Aspegic, Loxo Synthelabo) and amoxicillin (25 mg/kg IM; Clamoxyl, SmithKline Beecham) to prevent thrombocyte aggregation and wound infection, respectively. During the 4-week follow-up period, clopidogrel (25 mg PO; Plavix, Sanofi Winthrop) and acetylsalicylic acid (10 mg/kg PO) were given daily.

Angiography and Quantitative Analysis

Before and after stent implantation and at follow-up, angiography of the left and right iliac arteries was performed, with a commercially available x-ray system (Siemens) and contrast medium (Iomeuron350, Bracco-BykGulden). Images were digitized with a Movie Grabber (Rubo Medical Imaging). Offline measurements were performed with an automated edge-detection system (Pie Medical Imaging) that had been validated previously,10 using as reference for the calibration the known length of the ARED device itself, to decrease measurement errors using a small reference object.10 The mean diameter of the complete stented segment was compared after the procedure and at 4-week follow-up.

Follow-Up and Recatheterization

After a 4-week follow-up period, the contralateral carotid artery was dissected for the introduction of a 4F sheath. An angiographic overview image of both femoral arteries was taken with a 4F guiding catheter located in the abdominal aortic region. Subsequently, the rabbit was euthanized (Euthasate, 20 mL/kg), and perfusion fixation (formaldehyde 4% for 30 minutes) was performed in the aorta under controlled arterial pressure of 70 mm Hg. Next, both iliac arteries, including the stents and the ARED, were dissected and stored in 4% formaldehyde.

Histological Analysis

The tissue was embedded in methyl methacrylate and sectioned. Cross sections were obtained at 7 distinct locations, 2 outside and 5 inside the stent, of which 3 were located inside the segment containing the ARED flow divider. For the control vessel, similar locations were obtained. Hematoxylin-eosin staining was used for vessel morphology. Lumen, neointimal, and tunica media areas were measured (Clemex Technologies Inc). From each blood vessel, multiple histological cross sections of the stent including the ARED (S+ARED), of the stent outside the ARED (S[minus]ARED), and of corresponding regions in the contralateral control stent (SCTRL) were analyzed.

An elastin staining (Verhoeff staining) and autofluorescence were used to identify the internal elastic membrane. Cross sections within the middle of the ARED, at similar locations in the control stent, and at the edge of the ARED were analyzed. Injury score was analyzed according to a modified scheme proposed by Schwartz et al.11 In brief, the number of stent struts with properties according to score 3 of Schwartz et al were counted and divided by the number of stent struts studied. This number was reported as percentage. Macrophages were identified in standardized regions near the stent struts and quantified according to Kornowski et al.12

Computational Fluid Dynamics

To estimate the augmentation in WSS distribution after ARED placement and at follow-up, in the intervention vessel and in the control vessel, 3D computational fluid dynamics was used. For a detailed description, see Reference 4. Briefly, the geometry for the S+ARED vessel consisted of a 5.5-cm straight tube with a centrally located cylinder 15 mm in length and 1 mm in diameter, simulating the flow divider. The geometry of the control vessel consisted of a straight tube only. The diameters of these tubes were derived from the average stent measurements obtained from quantitative coronary arteriography (QCA) (Table 1). Both the mesh geometry and boundary conditions were used to solve the Navier-Stokes equations.
TABLE 1. Systemic Hemodynamic and Quantitative Luminal Diameters Obtained Before Intervention, After Stent Implantation, and at 4-Week Follow-Up

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After Stent</th>
<th>Follow-Up</th>
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<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>181±8</td>
<td>191±42</td>
<td>182±41</td>
</tr>
<tr>
<td>Systolic arterial pressure, mm Hg</td>
<td>80±11</td>
<td>79±10</td>
<td>85±20</td>
</tr>
<tr>
<td>Diastolic arterial pressure, mm Hg</td>
<td>62±13</td>
<td>58±12</td>
<td>52±15</td>
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Angiographically derived mean diameters of 9 rabbits, mm

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After Stent</th>
<th>Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARED</td>
<td>1.9±0.3</td>
<td>2.8±0.2</td>
<td>2.6±0.1†</td>
</tr>
<tr>
<td>Control</td>
<td>1.9±0.3</td>
<td>2.8±0.2</td>
<td>2.2±0.4</td>
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</tbody>
</table>

Angiographically derived mean diameters of 6 rabbits, mm

<table>
<thead>
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<th></th>
<th>Before</th>
<th>After Stent</th>
<th>Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARED</td>
<td>1.9±0.3</td>
<td>2.9±0.1</td>
<td>2.6±0.1†</td>
</tr>
<tr>
<td>Control</td>
<td>2.0±0.3</td>
<td>2.9±0.2</td>
<td>2.2±0.4</td>
</tr>
</tbody>
</table>

ANOVA with repeated measures: *P<0.05, sections with ARED (n=8) vs control (n=10) at follow-up. Subanalysis was performed on 6 rabbits with ARED (n=6) and controls (n=6): †P=0.053.

by use of a well-validated finite-element software package (Sepran, Sepra). The accuracy of the calculations was set at 10⁻² m/s, which resulted in an error of WSS of ~1%.

Analysis and Statistics

The 3D velocity field and 3D shear stress distribution obtained from CFD were displayed with software developed in-house (MatLab, The Mathworks Inc). The pre-ARED situation was taken as a reference, and each shear stress value was normalized with respect to that condition.

One-way ANOVA was applied to the QCA measurements, taking time as a fixed factor, and to the histological measurements comparing the stents with an ARED (S+ARED), a stent without an ARED at the same vessel segment (S[minus]ARED), and vessels containing a stent only (SCTRL). A subset analysis was performed on 6 animals that had both a stent and a stent plus ARED placed in their iliac arteries, applying ANOVA with location and presence of the ARED as fixed factors. For all ANOVAs, dummies were introduced for each rabbit. Regression analysis was performed on NH (mm²) versus injury score (%) and on NH (mm²) versus inflammation score (arbitrary units) from cross sections located within the ARED and immediately outside the ARED. Similar locations in the control vessel were analyzed, and ANCOVA was used to test for differences. Data are presented as mean±SD. All tests were 2-tailed, and a value of P<0.05 was considered statistically significant.

Results

After randomization, 8 stented arteries received an ARED, whereas 1 ARED could not be delivered and remained safely in the distal aorta. To compensate for this delivery failure, we implanted 2 AREDs in 1 animal. For the entire group, the average mean, systolic, and diastolic arterial pressures were 68±12, 80±11, and 62±13 mm Hg, respectively, which remained unchanged during instrumentation and at 4-week follow-up.

Shear Stress Distribution

The shear stress distribution within the flow divider increases with respect to the region upstream and downstream of the flow divider (Figure 2B). In the control artery, an average reduction of the WSS to 33% of baseline was induced by the increments in vessel diameter related to the oversized implantation of the stents. In the S+ARED, WSS decreased to only 69% of baseline (Figure 3, left). The additional pressure gradient across the stent including the flow divider was only 0.17±0.5 mm Hg. At 4-week follow-up, WSS was 68% of baseline in S[minus]ARED, whereas it was restored in the S+ARED (Figure 3, left).

Quantitative Coronary Angiography

All vessels were patent at follow-up. Table 1 summarizes the QCA measurements. In the control group, mean stent diameter decreased by 21%, whereas it decreased by only 8% in the stents with the flow divider (Figure 3, right). A significantly lower mean late lumen loss was observed in the stents with the ARED ([minus]0.23±0.22 versus [minus]0.57±0.30 mm, P=0.02).

Figure 2. 2D projection of 3D velocity profiles in a circular blood vessel without (A) and with (B) a flow divider. After vessel was opened lengthwise, a 2D projection of 3D shear stress field on vessel wall could be displayed (C and D). E and F display 1D shear stress value in a cylinder without (E) and with (F) a flow divider. Note localized effect of flow divider.
Figure 3. Diameters obtained by QCA for 18 vessels (left) and shear stress distribution within ARED for 18 vessels (right). Because no absolute flow was measured in all animals, shear stress data were normalized to their respective baseline values. fu indicates follow-up.

Morphometric Analysis

Figure 4 demonstrates cross sections obtained in the control vessel (A), at the edge of the ARED (B), and within the stent including the ARED (C). Comparison of B and C demonstrates the local effect of the flow divider, and comparison of A and C shows the effect of the flow divider at a similar location in the vascular tree. After pooling of the data, a significantly lower NH area was observed in the stent cross location in the vascular tree. After pooling of the data, a stratifies the local effect of the flow divider, and comparison of sections including the ARED (C). Comparison of B and C demon-

Discussion

The major findings of the present study may be summarized as follows: (1) WSS is increased by placing a flow divider in a stent without introducing large pressure gradients; (2) the locally increased shear stress induces a reduction in NH, because segments upstream and downstream of the flow divider and at corresponding locations did not show a reduction of NH; and (3) a reduced injury score and a reduced inflammation score accompany the reduction of NH. Because the relationships between these variables and NH were unchanged, the reduction in NH by shear stress was a result of the reduction of the injury and/or inflammation score.

In the present study, we placed a flow divider randomly in 1 of 2 stents placed in the left and right external iliac arteries of rabbits. Known confounding factors, such as the degree of hypercholesteremia and blood pressure, could therefore be excluded. Furthermore, because similar locations within the arterial tree were compared, a difference in endothelial function related to anatomic location could be excluded as a potential explanation. Finally, because similar stents were used for comparison, a geometrical factor related to stent design could also be excluded as a confounding factor. Consequently, the present experiments indicate that augmentation of shear stress directly reduces in-stent NH.

The effect of alterations in blood flow and shear stress on NH has been studied predominantly in vein grafts and after balloon angioplasty. However, the influence of alterations in shear stress on NH formation in stented segments has not gained much attention. One experimental study in normocholesteremic dogs showed that a reduction in blood flow to an entire arterial segment augments NH after stent implantation. In the present study, we evaluated the effects of local augmentation of shear stress on in-stent NH formation after endothelial dysfunction. The existence of endothelial dysfunction and hypercholesteremia are of importance, because it has been associated with a reduced response of endothelial cells to shear stress and because of the association with inflammation.

The present study confirms the hypothesis that shear stress reduces the accumulation of macrophages, thereby preventing the dissolution of the internal elastic membrane and migration of smooth muscle cells and NH formation. A reduced

$\rho=0.78, P<0.05$, which was also not different between the 3 vessel segments.
accumulation of macrophages may be a result of reduced expression of vascular cell adhesion molecule-1 or monocyte chemotactic protein-1 or an increased NO production.\textsuperscript{16,17}

The present data imply that high shear stress is protective against NH, which is in accordance with a recent study in human coronary arteries by Wentzel et al.\textsuperscript{3}

**Limitations**

We applied a well-validated numerical package to calculate the shear stress distribution and pressure drop over the ARED flow divider.\textsuperscript{18} The present method relies on several assumptions. We used parabolic inflow as entrance and constant zero stress as outflow conditions. To exclude an effect of these boundary conditions on the velocity profiles inside the vessel segment of interest, we used an entrance and outlet length of 10 times the diameter. Further lengthening of these entrance and outlet lengths did not influence the outcome of the calculations. We used a noncompliant wall, because the stent inhibits radial expansion. Finally, we applied Newtonian conditions for blood. This assumption generally holds at a shear rate of $>100\text{ s}^{-1}$. The calculations in the regions of the flow divider showed that this condition was fulfilled. No systematic Doppler measurements were available at baseline or at follow-up. However, comparison of Doppler measurement in the ARED with our numerical calculations in a single animal revealed a close similarity between the 2 methods (data not shown). Computational fluid dynamics calculations including the nitinol arms were also performed. These calculations showed a minimal effect of the wires on the velocity and the shear stress field, underscoring the importance of the simpler calculations.

Because of technical failure, 2 animals did not receive any ARED, which was compensated for by the placement in 2 other animals of 2 AREDs. Because our arguments rely in part on the existence of a stent without an ARED and a stent with an ARED in a single animal, we performed a subanalysis on 6 animals fulfilling this criterion. All statistical results were similar for the entire group and the subanalysis, as shown in Tables 1 and 2, confirming our earlier conclusions.

In conclusion, the present study shows that locally increasing WSS within a stent by means of the ARED flow divider device reduces NH in an animal model with endothelial dysfunction. Because systemic effects and location could be excluded as confounding factors, the results indicate that doubling of shear stress may reduce in-stent NH by $>50\%$. The mechanism of NH reduction involves a reduced accumulation of macrophages and internal elastic membrane proteolysis. Thus, shear stress could well be one of the most important factors to modulate in-stent restenosis.

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**References**


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