Angiotensin-(1–7) Attenuates Neointimal Formation After Stent Implantation in the Rat

Bas Langeveld, Wiek H. van Gilst, René A. Tio, Felix Zijlstra, Anton J.M. Roks

Abstract—Angiotensin-(1–7) is an endogenous, biologically active peptide of the renin-angiotensin system with vasodilatory, antithrombotic, and antiproliferative properties. This study examined the effects of angiotensin-(1–7) infusion on neointimal formation after stent placement in the rat. Male Wistar rats underwent stent implantation in the abdominal aorta or sham surgery. Subsequently, an osmotic minipump was placed for angiotensin-(1–7) (24 μg/kg per hour) or saline administration. After 4 weeks, histomorphometric and histological analyses were performed, and the endothelial function was measured in isolated thoracic aortic rings. Stent implantation resulted in equal mean injury scores within the groups. The angiotensin-(1–7)–treated group displayed a significant reduction in neointimal thickness (112±8 versus 141±11 μm; P<0.05), neointimal area (0.51±0.05 versus 0.70±0.07 mm²; P<0.05), and percentage stenosis (10.4±1.0 versus 14.0±1.3%; P<0.05) compared with the saline-treated group. Furthermore, angiotensin-(1–7) infusion attenuated the stenting-induced impairment in endothelium-dependent relaxation (42.6±3.0 versus 64.5±6.0% of phenylephrine maximal contraction; P<0.001). In conclusion, angiotensin-(1–7) treatment attenuates neointimal formation after stent implantation in the rat, combined with an improvement of endothelial function.

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Key Words: angioptasy ■ angiotensin ■ aorta ■ hyperplasia ■ rats ■ vasodilation

Recent advances in the development of drug-eluting stents have led to a tremendous reduction in restenosis rates after stent implantation. Stents coated with rapamycin and paclitaxel inhibit the persistent smooth muscle cell proliferation after stenting.1,2 However, recently, some potential drawbacks of these stents have emerged. Paclitaxel-eluting stents show delayed re-endothelialization and rapamycin inhibits endothelial cell proliferation.3,4 Consequently, refinement of antirestenotic therapies remains mandatory. Particularly, repair of the normal biology of the vessel wall to prevent restenosis by means of re-endothelialization deserves special attention.5

Angiotensin-(1–7) [Ang-(1–7)] is an endogenous, biologically active peptide of the renin-angiotensin system, it is formed out of angiotensin I and II by several endopeptidases, among which is the recently identified angiotensin-converting enzyme-2.6–8 During angiotensin-converting enzyme inhibition and angiotensin II type I receptor blockade, Ang-(1–7) plasma levels are elevated. It has been suggested that part of the effects of these drugs are mediated through Ang-(1–7).9,10 Ang-(1–7) has vasodilatory actions.11,12 Furthermore, it inhibits thrombosis and smooth muscle cell proliferation, two major vascular responses after stent implantation.13,14 Moreover, Ang-(1–7) attenuates neointimal formation and smooth muscle cell proliferation after vascular injury.15 Considering these vascular-protective properties, Ang-(1–7) could play a role in attenuation of neointimal formation after stenting by repair of the normal biology of the arterial wall. Therefore, we studied the effects of continuous intravenous Ang-(1–7) infusion on neointimal formation in a rat model of in-stent restenosis.

Methods

Animal Protocol
Twenty-eight male Wistar rats (Harlan) weighing 450 to 520 g were anesthetized with O₂, N₂O, and isoflurane (Abbot B.V.). A pre-mounted 2.5×9 mm BeStent 2 (Medtronic) was implanted in the abdominal aorta as described previously, or a sham operation was performed.16 Subsequently, an osmotic minipump with a pumping rate of 0.25 μL per hour lasting for 28 days (Model 2004; Alzet, Charles River Netherlands), was implanted subcutaneously for drug delivery via a catheter in the jugular vein. Stented rats received Ang-(1–7) (Bachem; 24 μg/kg per hour; n=7) or saline (0.25 μL per hour; n=10). Sham-operated rats received saline infusion (n=6). With this method, Ang-(1–7) plasma levels of ≈917.8±194.1 pmol/L are reached.17 At this concentration, Ang-(1–7) binds to the Mas receptor and has subsequent functional effects.18 Five rats died perioperatively because of rupture of the aorta.

After 28 days, animals were anesthetized and heparinized with 500 IU intravenously (Leo Pharma B.V.). Abdominal aortas were subsequently harvested, fixed, embedded in methylmetacrylate, sec-
tioned, and stained for histological analysis.\textsuperscript{19} The endothelial function was tested in isolated thoracic aortic rings.\textsuperscript{20} This study was approved by the animal care and use committee of the University of Groningen and performed in accordance with the Guide for the Care and Use of Laboratory Animals.\textsuperscript{21}

**Histology**

Histomorphometrical analysis was performed on elastica van Gieson–stained sections by measurements of the proximal, middle, and distal parts of each stent. To assess neointimal formation, areas within the external elastic lamina, internal elastic lamina, and lumen were measured using digital morphometry. The neointimal area, media area, lumen area, and the percentage of stenosis were calculated.\textsuperscript{22}

The injury and inflammation scores were assessed as described by Schwartz et al\textsuperscript{22} and Kornowski et al.\textsuperscript{23} Briefly, each strut was assigned a nominal score from 0 to 3 dependent on the severity of the injury or inflammation. The average score is calculated by dividing the sum of scores by the number of struts.\textsuperscript{22,23} Total cell density and polymorphonuclear leukocyte density were determined in hematoxylin-eosin–stained sections at \( \times 400 \) magnification and expressed as \( 100/ \text{mm}^2 \). To assess a single measurement for each stent, the mean values of the proximal, middle, and distal parts were calculated.

**Organ Bath Studies With Isolated Aortic Rings**

Periarterial tissue was removed from the aorta, and rings of \( \sim 2 \text{ mm} \) were cut. Rings were connected to an isotonic displacement transducer at a preload of 14 mmol/L in an organ bath containing Krebs solution, pH 7.5, containing (in mmol/L): 120.4 NaCl, 5.9 KCl, 2.5 CaCl\(_2\), 1.2 MgCl\(_2\), 1.2 NaH\(_2\)PO\(_4\), 11.5 glucose, and 25.0 NaHCO\(_3\) at 37°C and continuously gassed with 95% O\(_2\) and 5% CO\(_2\). After stabilization, during which regular washing was performed, rings were checked for viability by stimulation with phenylephrine (1 mmol/L).

Rings were washed and restabilized. Sets of rings were precontracted with phenylephrine (1 mmol/L). The endothelium-dependent vasodilation was assessed by a cumulative dose of metacholine (10 nmol/L to 10 mmol/L). Subsequently, rings were dilated maximally by means of the endothelium-independent vasodilator sodium nitrite (10 mmol/L). Drugs were purchased from Sigma-Aldrich.

**Statistics**

Data are expressed as mean value\(\pm\)SEM. Statistical analysis between groups was performed by a Student \( t \) test. Differences in dose-response curves between groups were tested by ANOVA for repeated measures using Greenhouse–Geisser correction for asphericity. Values of \( P\leq0.05 \) were considered statistically significant. For statistical analysis, SPSS software was used.

**Results**

**Histological Analysis**

In all stented animals, a neointima was present after 28 days, on which histological analysis was performed. Histomorphometric measurements are presented in Table 1. Stent expansion, expressed as the internal elastic lamina area, was equal to...
in the saline- and Ang-(1–7)–treated groups. Accordingly, the mean injury score also did not show a difference between the groups. Furthermore, no differences were observed in the media areas. Neointimal thickness, neointimal area, and percentage stenosis were significantly decreased in the Ang-(1–7)–treated group, with 21%, 27%, and 26%, respectively. Representative photomicrographs of stented abdominal aortas of the saline- and Ang-(1–7)–treated animals are shown in Figure 1.

Histological measurements are presented in Table 2. The cellular density in the media of the Ang-(1–7)–treated group was diminished compared with the control group. No difference was observed in the cellular density in the neointima. The number of surface-adherent leukocytes appeared to be decreased in the Ang-(1–7) group, almost reaching level of significance ($P=0.06$). The neointimal density of polymorphonuclear leukocytes and the mean inflammation score, which represent the infiltrated inflammatory cells, did not differ between groups.

### Endothelial Function

The effects of stent implantation in the rat abdominal aorta and subsequent Ang-(1–7) infusion on endothelial function were examined in thoracic aortic rings. We investigated the endothelium-dependent vasodilatory effects of metacholine on phenylephrine-precontracted rings (Figure 2A). The contraction on phenylephrine was similar in the sham, control, and Ang-(1–7) group (329 ± 26, 297 ± 20, and 254 ± 29 μm, respectively; $P=1.00$ and $P=0.20$ for sham versus control and Ang-(1–7), respectively). Stenting resulted in a significant decline of 13% in endothelium-dependent relaxation compared with the sham-treated animals. In the Ang-(1–7)–treated group, we observed a significant improvement of 21% in vasodilatory response to metacholine compared with the saline-treated group. The vasodilatory response in the Ang-(1–7) group seemed to exceed the response in the sham animals; however, this was not significant ($P=0.952$; Figure 2A). The relaxation on endothelium-independent vasodilator sodium nitrite was equal in the sham, control, and Ang-(1–7) groups (Figure 2B).

### Discussion

In the present study, we examined the effect of Ang-(1–7) infusion on neointimal formation in a rat stenting model. We observed a significant reduction in neointimal thickness, neointimal area, and percentage stenosis after Ang-(1–7) treatment of 21%, 27%, and 26%, respectively. Additionally, we found an attenuation of the stent-induced impairment of endothelium-dependent relaxation after Ang-(1–7) administration. Ang-(1–7) treatment resulted in an improvement of 39% of endothelium-dependent relaxation in aortic rings. No differences in endothelial-independent relaxation were observed. These results indicate a strong improvement of endothelial function.

Restenosis after stent implantation ensues from focal thrombus formation, inflammation, and smooth muscle cell proliferation after deep injury to the vessel wall and de-endothelialization. Thrombus formation and smooth muscle cell proliferation are diminished by Ang-(1–7).13,14 Moreover, Ang-(1–7) infusion reduces neointimal formation and smooth muscle cell proliferation after vascular injury in the rat carotid artery.15 Our results are in line with these findings. Ang-(1–7) might inhibit neointimal formation after stenting through either of these mechanisms.

A reduction in neointimal formation would suggest a decrease in inflammatory responses. However, no differences in the inflammatory responses were observed. As seen in other models, the inflammatory response is nearly extinguished after 28 days.24 The extinguished inflammatory
response, and the rather small number of animals used, might explain why there was not a difference in the inflammation scores.

Ang-(1–7) has vasodilatory effects on the vascular system that are largely mediated through NO release. Furthermore, Ang-(1–7) stimulates NO release from endothelial cells. Moreover, continuous treatment with Ang-(1–7) improves endothelial function in a rat model of heart failure. Hence, Ang-(1–7) has a functional improving effect on the endothelium. Accordingly, the functional improvement of the endothelium after stent implantation with Ang-(1–7) infusion might indicate a structural repair of the endothelium. On the other hand, accelerated re-endothelialization inhibits neointimal formation after stent implantation in hypercholesterolemic rabbits. Possibly, Ang-(1–7) treatment also plays a role in attenuation of neointimal formation by structural recovery of the endothelium.

Perspectives
This study shows that continuous Ang-(1–7) treatment after stent implantation in the rat abdominal aorta results in attenuation of neointimal formation, combined with an improvement of endothelial function. Ang-(1–7) may be an important alternative to the presently available aggressive antiproliferative drug-eluting stents.

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References
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