Prophylaxis of Restenosis With \(^{186}\)Re-Labeled Stents in a Rabbit Model

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Background—Intraluminal \(\beta\)-irradiation has been shown to decrease neointimal proliferation after angioplasty in experimental models. The purpose of this study was to test the technical feasibility and biological effects of \(^{186}\)Re-labeled stents.

Methods and Results—Thirty-four New Zealand White rabbits were fed a 0.5% cholesterol diet before balloon angioplasty and insertion of Palmaz stents in the infrarenal aorta. The animals were killed 7 weeks after stent implantation. Two of 34 animals died prematurely (aortic leak, pneumonia). Control stents (n=7) were compared with \(^{186}\)Re stents (2.6 MBq [n=6], 8.1 MBq [n=5], 16.0 MBq [n=6], and 25.3 MBq [n=8]). Stent application was successful in all cases. No thrombus occlusion was observed. After 7 weeks, neointima formation was 2.2 ± 0.2 mm² in the control group. In the treatment groups, a dose-dependent neointima reduction was detectable (0.5±0.5 mm² [2.6 MBq], 0.4±0.4 mm² [8.1 MBq], and 0 mm² [16.0 MBq, 25.3 MBq]). No induction of neointimal formation was observed at the edges of the stents. Radiation resulted in delayed reendothelialization.

Conclusions—\(^{186}\)Re stents were capable of reducing neointima formation in a dose-dependent fashion. \(^{186}\)Re stents did not cause late thrombosis or neointimal induction at the stent margins in the observation period of 7 weeks. (Circulation. 2001;104:480-485.)

Key Words: atherosclerosis ■ angioplasty ■ hypertension ■ restenosis ■ radioisotopes ■ stents

Both \(\gamma\)- and \(\beta\)-irradiation delivered via a radioactive catheter-based line source have been shown to reduce restenosis in various animal models and in clinical trials.\(^{1-3}\) An alternative and perhaps simpler approach to intravascular radiation is the use of a stent itself as a platform for local radiation delivery.\(^{4}\)

Studies in animals about the effects of \(\beta\)-emitting stents have shown variable effects on neointimal formation.\(^{5-7}\) Clinical studies of >260 implants of \(^{32}\)P stents with activity levels between 0.75 and 20 \(\mu\)Ci have been analyzed.\(^{8}\) It has become obvious, however, that the major problem of this technology is the edge effect, which is essentially a new neointimal buildup at the stent margins.\(^{9}\) There are several hypotheses about the undesired clinical effects of \(^{32}\)P stents. In general, the success of \(\beta\)-particle–emitting stents as a method of reducing restenosis is dependent on the balance between suppression of cellular proliferation and migration and radiation-related cellular injury. Compared with \(^{32}\)P, \(^{186}\)Re is characterized by a shorter half-life (3.8 versus 14.3 days) and a lower mean \(\beta\)-energy (330 versus 645 keV). \(^{186}\)Re is predominantly a \(\beta\)-emitter (92.2%). The maximum energy of \(\beta\)-particles is 1.077 MeV. It also has a limited \(\gamma\)-ray emission, the most abundant (9.5%), with an energy of 137 keV, allowing dosimetry with a gamma camera. The purpose of this study was to test the dose effect of \(^{186}\)Re stents on restenosis in a rabbit model.

Methods

This protocol was approved by the Institutional Laboratory Care and Use Committee and was conducted in accordance with regulatory guidelines for the care of laboratory animals. Stainless steel stents (2 cm long, Palmaz P204, Cordis) were made radioactive with \(^{186}\)Re by a process performed in a 2-mL vial filled with \(^{186}\)Re dissolved in hydrochloric acid. The vial with the stent was placed into a sonification bath. Then the stent was washed in pure ethanol. The activity of each stent was determined by its \(\gamma\)-component in a scintillation chamber. The \(^{186}\)Re stents implanted had activities ranging from 2.6 to 25.3 MBq. The stents were divided into 5 groups: (1) control, nonradioactive (n=7), (2) low activity (2.6±0.2 MBq [n=6]), (3) intermediate activity (8.1±0.5 MBq [n=5]), and (4 and 5) high activity (16.0±0.6 MBq [n=6] and 25.3±2.6 MBq [n=8]). The delivered lifetime radiation dose (0.5 mm from a stent wire of a 4-mm stent) was 38 Gy in the low-activity group, 121 Gy in the intermediate-activity group, and 250 and 377 Gy in the high-activity groups, respectively.
Animal Model
Thirty-four male New Zealand White rabbits (3.0 to 3.5 kg body weight [BW]) underwent the placement of radioactive stents in the infrarenal aorta. Interventions and imaging procedures were done under general anesthesia. All animals were first fed a 0.5% cholesterol diet for 28 days. A 3F sheath was placed in the right common femoral artery, and heparin 50 U/kg BW was administered. After balloon denudation with a 2F Fogarty arterial embolectomy catheter (Baxter), Palmaz stents mounted on a balloon (diameter 4 mm, length 2 cm, Savvy, Cordis) were deployed in the infrarenal aorta. Before the onset of the study, angiograms of New Zealand White rabbits were acquired. The size of the infrarenal aorta did not differ significantly between the animals (3.4 to 3.8 mm). Therefore, a balloon diameter of 4 mm was used in all animals without an preexisting angiogram. Single balloon inflation at a pressure of 6 to 8 atm was performed. At the end of the procedure, the common femoral artery was ligated. One day before stent implantation, the animals received aspirin 7 mg/kg BW IM. After stent implantation, the animals were treated with heparin 400 U/kg BW SC twice daily and aspirin 7 mg · kg BW$^{-1} · d^{-1}$ IM for the following 3 days and were then put on aspirin 15 mg/kg BW IM every third day until completion of the study. After stent implantation, the animals received standard diet. At day 49, the animals were euthanized with a lethal dose of barbiturate.

Activity Measurement
Planar whole-body scintigrams were obtained (acquisition time 5 minutes) with an Apex SP4 HR gamma camera (Elscint) immediately after the intervention and 1, 2, 7, and 14 days after stent implantation to determine the activity of the stents in vivo.

Histology
The infrarenal aorta was perfusion-fixed with 4% neutral buffered formalin. The stented arterial segments were removed and sectioned transversely into 2 parts. One part with the remaining stent struts was embedded in methylmethacrylate and cut with a carbide knife (slice thickness 50 μm). The other part was sawed into 2-mm segments. The stent wires were removed under a binocular microscope. The sections were embedded in paraffin and cut into slices 4 to 5 μm thick.

Histological Analysis
All histological analyses were performed with the investigator blinded to the stent characteristics. Histological measurements were done from the proximal, mid, and distal regions of the stent. Adjacent, nonstented arterial segments were examined for any adverse effects, such as neointimal formation at the edges. Morphometry of the in-stent area was performed on cross sections. To investigate the edges of the stent, a longitudinal section from the transition of the stented to the nonstented artery was done from the ends embedded in methylmethacrylate. At 4 different positions, the thickness of the neointima and the media were measured (a) in the stent 1.5 mm from the edge, (b) in the stent 0.5 mm from the edge, (c) in the nonstented aorta 0.5 mm from the edge, and (d) in the nonstented aorta 1.5 mm from the stent. All morphometric measurements were performed on elastica–van Gieson–stained segments.

Immunohistochemical staining by the avidin-biotin method was carried out for the visualization of the endothelium (factor VIII, Incstar), macrophages (RAM 11, Dako), and smooth muscle cells (α-actin, Sigma). For the description of the pathohistological effects of irradiation, the segments were analyzed by a pathologist blinded to the stent characteristics (A.G.) for fibrin (location [A] near the stent struts, [B] in the neointima, [C] media, and [D] adventitia), necrosis (locations b to d), edema (locations a to d), inflammation (locations a to d), and endothelialization. The effects were classified semiquantitatively (range 0 to ++ +). Cell density analysis was carried out on hematoxylin-eosin–stained cross sections with digital photographs. The injury score was assessed semiquantitatively, adapted from Schwartz et al.\textsuperscript{10}

Figure 1. Measured activity (% of initial activity) of $^{186}$Re stents (half-life–corrected) after implantation in rabbits. Washoff rate of rhenium within 14 days was < 10%. Stents were labeled with 25.3 MBq (n = 8). Values are mean ± SD.

Statistical Analysis
Values are expressed as mean ± SD. Mean values for histological variables were compared between groups by ANOVA or test for analysis of paired or unpaired data as appropriate (JMP 3.1, SAS). A value of $P < 0.05$ was considered statistically significant.

Results
All stents were successfully implanted in the infrarenal aorta. Two of 34 animals died prematurely. One animal (control group) died 1 day after the surgical procedure of a leak in the aorta. One animal from the 8.1-MBq group developed pneumonia and died at day 5. No acute or subacute stent thrombosis was found in any of the rabbits.

Stability of $^{186}$Re-Labeled Stents
Stents were labeled within 15 minutes. Half-life–corrected activity measurements obtained by region-of-interest analysis of whole-body scintigrams are shown in Figure 1. After 14 days, >90% of the rhenium could be detected at the stented site in vivo (25.3-MBq group).

Morphometry
Figure 2A illustrates the neointimal area after 49 days in the different groups. Seven weeks after stent implantation, a neointima was found in the control group that was significantly different from that of the treatment groups ($P < 0.05$ control group versus each of the radioactive stent groups). A dose-dependent reduction of neointimal formation was observed. Neointimal growth was completely suppressed in the highest-activity groups (16.0 and 25.3 MBq). With increasing radioactivity, an increasing atrophy of the media occurred. The area of the media (differences not significant, $P = $NS; Figure 2B) and the cell density of the media ($P < 0.05$ control versus treatment groups; Figure 2C) decreased. With higher activities, an opposite phenomenon was seen in the adventitia, where cell density increased (Figure 2D).

Longitudinal sections, which showed the stented vessel and the adjacent nonstented area, were used to investigate possible edge effects and allowed an exact analysis of the region. There was no evidence of intimal hyperplasia of the edges in any of the stent groups. In the 2.6-MBq group, only a slight increase of neointimal thickness from 0.24 ± 0.08 mm (non-
stented aorta, point d) to 0.27±0.12 mm (transition area, c) and 0.34±0.06 mm (stent exit, b) was seen. This increase of neointimal thickness was not significant between the areas investigated and did not result in any luminal stenosis. The data of the neointimal thickness of the edge area are summarized in Table 1.

**Histology and Immunohistology**

To assess the effects of radiation, the vessel together with the surrounding tissue was analyzed for fibrin, necrosis, edema, inflammation, thrombosis, and endothelialization in 4 different locations: neointima, near the stent struts, media, and adventitia. The segments were rated semiquantitatively by a pathologist blinded to the treatment groups as previously proposed by Brehme et al.11 The results are summarized in Tables 2 and 3. The data shown are limited to fibrin and endothelialization. The numbers show the numbers of animals (frequency of criteria), which were rated from 0 to ++++. In general, a dose-dependent reaction was found. With higher radiation doses, the degree of fibrosis, necrosis, and edema increased. Inflammation was seen mainly near the stent struts (dose-dependent), with only small numbers of granulocytes and monocytes in the neointima, media, and adventitia, which were independent of the amount of exposure to radiation. Fibrin-rich, acellular regions were found in particular near the stent struts and in the neointima in the mid- and high-dose stents. The adventitia was α-actin–negative in the control group and turned α-actin–positive in the 8.1-, 16.0-, and 25.3-MBq groups. Reendothelialization after stent implantation was found in the control group, whereas in mid- and high-dose stents, the absence of an endothelial layer suggests a delayed healing. The injury score was not found to be different between the control group and the treatment groups. Representative hematoxylin-eosin–stained sections from the control stent and the different treatment groups are shown in Figure 3.

**Discussion**

The aim of this study was to test the feasibility of 186 Re stents to reduce restenosis in an animal model. Therefore, a method was developed to label stents reproducibly and stably with 186 Re. Less than 10% of the rhenium was washed off the stent struts in vivo. The labeling procedure for 186 Re stents is completely different from the activation process for 32 P stents. 32 P stents are manufactured by ion implantation and have to be activated in a reactor. This method is complex and

<table>
<thead>
<tr>
<th>Location of Measurement</th>
<th>In-Stent, Point a, mm</th>
<th>Stent Exit, Point b, mm</th>
<th>Transition Area, Point c, mm</th>
<th>Nonstented Aorta, Point d, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.172±0.051</td>
<td>0.150±0.025</td>
<td>0.143±0.085</td>
<td>0.174±0.077</td>
</tr>
<tr>
<td>2.6 MBq</td>
<td>0.079±0.095</td>
<td>0.344±0.062</td>
<td>0.269±0.122</td>
<td>0.237±0.080</td>
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<tr>
<td>8.1 MBq</td>
<td>0.067±0.082</td>
<td>0.064±0.023</td>
<td>0.219±0.056</td>
<td>0.254±0.089</td>
</tr>
<tr>
<td>16.0 MBq</td>
<td>0±0</td>
<td>0.199±0.112</td>
<td>0.145±0.021</td>
<td>0.216±0.190</td>
</tr>
<tr>
<td>25.3 MBq</td>
<td>0±0</td>
<td>0.047±0.049</td>
<td>0.225±0.094</td>
<td>0.172±0.101</td>
</tr>
</tbody>
</table>

The neointimal thickness was measured in the stented aorta (point a), at the stent exit (point b), in the transition area (point c), and in the nonstented aorta (point d). No significant increase of neointimal thickness at the margins of the stent was observed. Values are mean±SD. For numbers, see Figure 2.
time-consuming, and therefore, the stents have to be ordered well in advance of implantation. 186 Re stents were produced by use of a kit-like procedure allowing rapid, reproducible, and flexible labeling within 15 minutes. Therefore, labeling with the desired activity is possible immediately before or even during the intervention. The main γ-emission energy from 186Re is 137 keV, which is comparable to 99mTc (140 keV), the widely used isotope for scintigraphy, allowing in vivo dosimetry. Only 10% of the rhenium was washed off after 14 days. Because of the short half-life, most of the 186Re that is washed off will already have decayed at this point in time. Because of the low activity of 186Re needed to reduce restenosis, a low equivalent dose for human beings is expected. Even if the whole dose were to be washed off immediately after stent placement, no significant equivalent dose in patients is to be expected.

Implantation of radioactive stents resulted in a dose-dependent reduction of neointima formation, with a total inhibition in the high-dose groups (16.0 and 25.3 MBq). No major limitations of radiation therapy for the prevention of restenosis occurred, such as late thrombosis (mainly after-loading therapy with stent administration) or, especially with radioactive stents, an edge effect with increased neointima formation at the end of the stented area. These positive findings could be due to the isotope used in our study.

The “candy-wrapper” effect has been described as one of the major problems that became evident after a clinical trial with 32P stents reported by Albiero et al.9,12 The mechanisms of this phenomenon are still unclear. Several pathohistological findings and reasons have been discussed: neointima formation versus late lumen loss, or a combination of the 2; balloon injury at the stent edges; low-dose radiation at the margins of the stent; and side effects of the low radiation over a long time period.13,14 Thus far, the edge effect has not been investigated very explicitly in animals. Hehrlein et al.4 using the rabbit iliac model, noted that the maximum cross-sectional neointimal area was found predominantly at the ends of all stents (32P stent activity levels of 0.5, 4, 6, and 13 μCi plus control stent). In comparison, after 186Re stent implantation, an increase of neointimal thickness was observed only in the 2.5 MBq group at the edges of the stented aorta. This increase, however, was not significant. In the other groups, no increase of neointimal thickness occurred.

<table>
<thead>
<tr>
<th>Group/Location</th>
<th>Fibrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>2.6 MBq</td>
<td>+</td>
</tr>
<tr>
<td>8.1 MBq</td>
<td>++</td>
</tr>
<tr>
<td>16.0 MBq</td>
<td>+++</td>
</tr>
<tr>
<td>25.3 MBq</td>
<td>++++</td>
</tr>
</tbody>
</table>

**Table 2. Frequency of Pathological Findings in the Stented Aorta From Each Treatment Group**
must be emphasized that our study was not conducted to investigate the reasons for the candy-wrapper effect. Because long-axis sectioning was performed at the margins of the stent, the role of a possible late lumen loss cannot be described. All rabbits were pretreated with a 0.5% cholesterol diet and balloon denudation, and therefore, the nonstented aorta showed neointima formation as well. The edge effects observed with radioactive stents are an illustration of species differences between animals and humans. Taylor et al7 did not observe any edge effect in the canine model after deployment of 32P stents but did in a rabbit model that was also investigated in their laboratory. Reasons for this difference are unknown but may relate to differences in the technique of stent administration (multiple, high-pressure balloon inflations in human stenting), the differences in normal versus atherosclerotic vascular wall in the zones adjacent to the radioactive stent, or the more rapid healing seen in animal models. In our study, the administration pressure was 6 to 8 atm only, which may have resulted in a smaller vessel trauma at the edges. The major finding of our study is that neointima formation was not significantly increased in the edge region and was totally inhibited in the in-stent area of the high-dose group. This may be due to the different properties of 186Re (shorter half-life, lower β-energy emitted) compared with 32P.

The Isostent trial, which investigated radioactive 32P stents in patients, showed only a very rare incidence of thrombosis. The problem of late thrombosis might be more a problem of afterloading therapy in patients with stent administration than with radioactive stents alone. Stent thrombosis has not been quantified in our model. In further studies, the rate of thrombosis will have to be investigated, eg, by labeling of platelets with 111In.

With increasing doses, 186Re stents led to a higher incidence of fibrosis and inflammation. Such effects as increased fibrosis, inflammation, and cellular proliferation after 32P stent administration were also described by others. Whether this may cause late side effects is still unclear and has to be investigated. The most striking point in our investigations was the thickening of the adventitia with increasing activity, which has not been seen with 32P stents. Adventitial effects were described by Taylor et al7 after implantation of 32P stents in coronary arteries in a canine model. In contrast to our results, arteries treated with 32P stents showed a significant, dose-dependent reduction in adventitial thickness. Because of the lower β-energy of 186Re compared with 32P, the deposition of 60% of the energy is obtained at a depth of 1.85 mm with 32P and at 0.92 mm with 186Re stents. Therefore, most of the energy applied in 186Re stents is deposited in the neointima and media, whereas the adventitia receives only a low amount of radiation. Interestingly, the adventitia stained α-actin–negative in the control group and α-actin–positive in the high-dose groups. This may be due to a transformation into myofibroblasts related to the low radiation in this area. Except for this finding, the highest dose investigated in our study has not been favored for clinical investigation. The most promising doses seem to be 8.1 or 16 MBq. Before clinical application, further studies on the long-term effects of these doses and investigations seem to be mandatory.

The clinical effect of a radioactive stent may be modulated by the use of radioisotopes with different half-lives, energies, activities, and penetration properties. According to the half-lives of 32P (14.3 days) and 186Re (3.8 days), the dose rate for the same total dose in 32P stents is much lower. A comparison of 32P and 186Re is summarized in Table 4. With high-dose-rate brachytherapy, doses of ≈20 Gy targeted at a distance of 2 mm from the source appear to be efficacious in lowering or preventing restenosis. Generally, the biological effect of a dose is lowered if the dose rate is reduced and the overall exposure time is increased. Therefore, with low-dose-rate radiation, the applied dose has to be higher to achieve similar radiation effects. On the basis of our dose measurements, we applied a dose of 377 Gy at 0.5 mm from the stent wires in our highest-activity group (25.3 MBq).

### Study Limitations

The results observed in this animal model may not reflect the pathological mechanisms that occur in human restenosis after angioplasty. The effects of radioactive stents in the “atherosclerotic” infrarenal aorta of rabbits after cholesterol diet and stent application are not able to predict their effects in heavily atherosclerotic human arteries. The present study was not designed to detect possible edge effects of radioactive stents. There are differences between edge effects that are illustrated in different animal studies and in humans. The edge restenosis problem is the major limitation of radioactive stents in patients. Further animal studies are necessary to define the vascular injury at the stent edges evoked by higher balloon inflation pressure.

### Conclusions

This is the first report on a new process of labeling radioactive stents with 186Re. Reproducible on-site labeling in 15 minutes was feasible. There was a low washoff rate and no acute thrombosis in rabbits. 186Re stents were able to inhibit neointimal proliferation in a dose-dependent manner with no

### Table 3. Frequency of Endothelialization

<table>
<thead>
<tr>
<th>Control</th>
<th>2.6 MBq</th>
<th>8.1 MBq</th>
<th>16.0 MBq</th>
<th>25.3 MBq</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
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</table>

### Table 4. Comparison of 32P and 186Re

<table>
<thead>
<tr>
<th></th>
<th>32P</th>
<th>186Re</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-life, d</td>
<td>14.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Maximum β-energy, MeV</td>
<td>1.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Mean β-energy, MeV</td>
<td>0.69</td>
<td>0.33</td>
</tr>
<tr>
<td>Depth for deposition of 60% of the energy, mm</td>
<td>1.85</td>
<td>0.92</td>
</tr>
</tbody>
</table>
in-stent neointima formation. No significant neointimal proliferation at the stent edges was observed.

Acknowledgments

The authors are very grateful for the help of Dirk Fluehs (Department of Medical Physics, University of Essen, Germany) for the dosimetry of the $^{186}$Re-labeled stents.

References

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